A study on the Pentastomida parasitising crocodilian and chelonian final hosts, with special emphasis on the South African pentastome fauna

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Für meine Oma, Mathilde Flaskamp

DECLARATION / ERKLÄRUNG

I herby declare that the present investigation is my own work, excepting the assistence and sources acknowledged in the text. No unauthorized means were used. The work on which this thesis is based is original and neither the whole work nor any part of it has been submitted for another degree at this or any other university.

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig verfasst und keine unerlaubten oder ungenannten Hilfsmittel verwendet habe. Weder die gesamte Dissertation noch Teile daraus wurden bisher anderweitig als Prüfungsarbeit verwendet oder einer anderen Fakultät als Dissertation vorgelegt.

Pretoria, 28. September 2002 Kerstin Junker

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SUMMARY

Despite their discovery more than a century ago, knowledge on the Pentastomida is still scant and this is especially true for the family Sebekidae parasitising crocodilian and chelonian final hosts. In this study 15 Nile crocodiles from different localities in the Kruger National Park and an adjacent game reserve, as well as 20 terrapins from the Arabie Dam, Mpumalanga, South Africa were obtained between 1997 and 2002. During this period a total of 609 fish were caught at the same localities. The pentastomid parasites of the final and intermediate hosts were collected, described and analysed according to host parasite relationships and the influence of various ecological factors. The results of the South African investigation were compared with information on chelonian and crocodilian pentastomes throughout their range and, based on literature reviews and the examination of additional material from South American chelonians, a phylogenetic analysis of the Sebekidae was made.

The taxonomic part of this study resulted in the description of the males of *Leiperia cincinnalis* from South African Nile crocodiles and additional morphological data of the females. A new monospecific genus of chelonian pentastomes *Pelonia africana* n. g., n. sp. was described, which represents the first record of pentastome infections in chelonian hosts on the African continent. The morphology of *P. africana* is reminiscent of all the other genera included in the family Sebekidae. The similarity to the crocodilian pentastome *Sebekia wedli* suggests a close relationship between these two pentastomes and there is a strong possibility that *P. africana* developed from *S. wedli* captured by chelonian hosts. Nevertheless, the unique combination of diagnostic criteria seen in the specimens from the South African terrapins validate the erection of a new genus. Slide-mounted specimens of *Diesingia megastoma* from the Brazilian terrapin, *Hydromedusa tectifera*, are described in detail and the often incomplete and sometimes misleading descriptions of previous authors amended. Based on the results of this study, the confusion regarding the systematic status of the genus *Diesingia* is resolved and the validity of the genus, as well as its inclusion in the family Sebekidae is confirmed.

The family Sebekidae created by Sambon in 1922, to accommodate the crocodilian pentastomes *Sebekia*, *Alofia* and *Leiperia*, was reassessed, including the recently described genera, *Agema*, *Pelonia* and *Selfia*, as well as the genus *Sambonia*, which has only recently been included in the Sebekidae. Hypotheses on phylogenetic relationships amongst the currently known sebekiid genera are discussed. Own data and an extensive literature review are combined into a check-list of the sebekiid and subtriquetrid pentastomes of crocodilians and chelonians, providing a reference to the original records of these pentastomes world-wide. A total of 34 pentastome species belonging to eight genera and two families were recorded from 14 crocodilian and four chelonian species. All synonyms for the pentastomids and their hosts are provided.

Published data on the phylogenetic origin of the Pentastomida, as gained from Cambrian fossils, combined with the current knowledge of the phylogeny of crocodilians, are related to the evolution and current geographic distribution of the extant genera of the Sebekidae. *Sebekia* is the genus with the widest host spectrum, including hosts from both crocodilian subfamilies, and geographical range, and has reached the highest species diversity within the Sebekidae. It is assumed that representatives of the genus *Sebekia* and *Leiperia* evolved as early as 80 million years ago, while *Alofia*, *Selfia* and *Diesingia* seem to have emerged more recently from a common ancestor. The genus *Agema*, endemic to the African continent, presumably evolved after its host had diverged from the remaining crocodilian stock.

Morphological data obtained during the course of this study and data taken from the literature were used to expand on the phylogenetic system of the Sebekidae resulting in a cladogram of the family comprising all the currently known genera. The in-depth comparison of the diagnostic characteristics of the various sebekiids was refined into a key to the genera of this family, and the systematic relationships between sebekiid pentastomes occurring in the African corocodilians were analysed more closely.

Pentastomosis is common and wide-spread in Nile crocodiles from the KNP and adjacent areas with a prevalence of 93% and an intensity of infection ranging from 2 to 109. The species diversity is high, and six pentastome species representing three genera, all belonging to the family Sebekidae, were recovered from the crocodilian hosts. In 71% of the infected hosts more than two species were present.

Based on their high prevalence and intensity of infection, *S. wedli*, *Sebekia cesarisi* and *L. cincinnalis* were classified as core species of the pentastome communities of Nile crocodiles. *Sebekia okavangoensis* and *Alofia nilotici* are secondary species, while *Alofia simpsoni* is considered a satellite species. The various pentastome species form isolationist communities in individual crocodilian hosts and interspecific interaction is no longer an organizing factor in pentastome infracommunities.

The species distribution at different localities within the KNP is relatively homogenous, but the pentastome fauna of the recently created PMC Dam consists largely of accidental pentastomes.

Little variation was found in the prevalence of pentastome infections in different sized crocodiles, but a shift in the growing crocodile's diet results in a greater probability of exposure to infective pentastome larvae and thus the intensity of infection. The longevity of pentastomes facilitates accumulative infections.

The ratio between infective larvae, immatures and mature pentastomes recovered from Nile crocodiles gradually shifts during the seasons. The percentage of infective larvae was highest in the crocodiles examined during spring and declined to reach a low in the crocodiles examined during winter.

This coincides with the period of minimum feeding activity on the part of the crocodilian hosts. To the contrary, the highest percentage of mature pentastomids was recovered from crocodiles examined during winter, representing the advancing development of infective larvae acquired during previous seasons.

The pentastome fauna of South African Nile crocodiles is distinct from that of the other two African crocodiles, *Crocodylus cataphractus* and *Osteolaemus tetraspis* and only *S. okavangoensis* occurs in all three hosts.

Pelonia africana was the only pentastome recovered from the South African terrapins and the prevalence and intensity of infection were generally low (29% and 1.1), with a slightly higher prevalence in *Pelusios sinuatus* than in *Pelomedusa subrufa*.

Field data combined with the results of the experimental infections of Nile crocodiles gave new insight on the life cycle of *L. cincinnalis*, which includes an obligatory, gregarious phase in the cardiovascular system of the host. Copulation takes place in the trachea.

Experimental infections of Nile crocodiles with *Subtriquetra rileyi* were unsuccessful and it was not possible to confirm Nile crocodiles as the final host for this parasite.

Serranochromis meridianus from the KNP was recorded for the first time as intermediate host for *L. cincinnalis*. *Subtriquetra rileyi* is a common parasite of *Oreochromis mossambicus* and *Tilapia rendalli* larger than 200 mm. While *O. mossambicus* is the preferred host of *L. cincinnalis*, other sebekiids had a low prevalence. This picture was reversed in *T. rendalli*. The intensities of infection were generally low in all the hosts examined. The gender of the host has no influence on the prevalence and intensity of pentastome infections, but both variables increased with host size. It is probable that an immuno-incompatibility prevents *Leiperia* from sharing the same host species with other sebekiid genera and vice versa. Also, the long intestine, an adaptation to a predominantly vegetarian diet, possibly makes *O. mossambicus* a more suitable host for *L. cincinnalis.*

Fish caught in the Arabie Dam harboured no pentastomes, although terrapins originating from this reservoir were infected. Thus, especially in large and recently established systems, such as the Arabie Dam, the final hosts are better indicators of the presence of pentastomid infections.

Ovoid, haematin stained suck-marks indicate the presence of *S. rileyi* in the swim bladder of infected fish. Small haemorrhages underneath the basement membrane were caused by the pentastomes' feeding activity, the hooks and/or the body spines. A thickening of the basement membrane is associated with severe infections. Cellular debris in the lumen of infected swim bladders consists of heterophils, macrophages and some lymphocytes embedded in an amorphous matrix that often contained haematin. Cast pentastome cuticles were enclosed in connective tissue of host origin with a loose to heavy infiltration with macrophages. In general, the histopathological picture of the swim bladders is one of mild changes that would not influence the organ's normal functioning.

ZUSAMMENFASSUNG

Der Tatsache zum trotz, dass ihre Entdeckung bereits mehr als ein Jahrhundert zurück liegt, sind die Pentastomida noch stets wenig erforscht. Dies gilt insbesondere für die Krokodile und Schildkröten parasitierende Familie der Sebekidae. In der vorliegenden Arbeit wurden im Zeitraum von 1997 bis 2002 15 Nilkrokodile aus verschiedenen Gegenden des Krüger Nationalparks und aus einem angrenzendedn Wildreservat, sowie 20 Wasserschildkröten aus dem Arabie Stausee, Mpumalanga, Südafrika untersucht. Innerhalb des gleichen Zeitraums wurden im Krüger Nationalpark und dem Arabie Stausee insgesamt 609 Fische gefangen. Die Pentastomiden der End- und Zwischenwirte wurden gesammelt, beschrieben und im Hinblick auf das Wirts-Parasit-Verhältnis und den Einfluss verschiedener ökologischer Faktoren analysiert.

Der taxonomische Teil dieser Studie lieferte die Beschreibung der *Leiperia cincinnalis* Männchen und zusätzliches Datenmaterial über die Morphologie der Weibchen. Eine neue monospezifische Gattung von Chelonia parasitierenden Pentastomiden, *Pelonia africana* n. g., n. sp., wurde beschrieben und somit das Vorkommen von Pentastomiden in Schildkröten des afrikanischen Kontinents erstmalig dokumentiert. Die Morphologie von *P. africana* stimmt im wesentlichen mit der anderer Sebekiden Gattungen überein, und die Ähnlichkeit mit dem Krokodilpentastomiden *Sebekia wedli* deuted auf eine nahe Verwandschaft zwischen diesen beiden Pentastomiden hin. Es lässt sich vermuten, dass *P. africana* ursprünglich aus Vertretern von *S. wedli* hervorging, die Wasserschildkröten als Endwirt erobert hatten. Nichtsdestotrotz rechtfertigt die einzigartige Kombination der diagnostischen Merkmale in den Pentastomiden der südafrikanischen Wasserschildkröten die Errichtung einer neuen Gattung.

In Hoyer's Medium eingebettete *Diesingia megastoma* aus brasilianischen Wasserschildkröten, *Hydromedusa tectifera*, werden innerhalb dieser Studie detailliert beschrieben und die unvollständigen, oft missverständlichen

Beschreibungen voriger Autoren ergänzt. Hierdurch konnte die systematische Stellung der Gattung *Diesingia* geklärt und ihre Zugehörigkeit zur Familie Sebekidae befestigt werden.

Die Sebekidae, von Sambon im Jahr 1922 für die Krokodilpentastomiden *Sebekia*, *Alofia* und *Leiperia* errichtet, wurde einschließlich der neuen Gattungen *Agema*, *Pelonia* und *Selfia* sowie *Sambonia* revidiert. Die Gattung *Sambonia* wurde erst kürzlich zu den Sebekiden gestellt. Hypothesen bezüglich der phylogenetischen Verwandschaft innerhalb der derzeit bekannten Sebekiden Gattungen werden diskutiert. Unter Verwendung eigener Daten und einer umfangreiche Literaturstudie wurde eine Checkliste mit Verweisen zu den ursprünglichen Literaturstellen der weltweit in Krokodilen und Schildkröten vorkommenden Sebekiden und Subtriquetriden erstellt. Insgesamt 34 Pentastomidenarten, die acht Gattungen und zwei Familien zuzuordnen sind, wurden von 14 Krokodilspezies und vier Arten von Wasserschildkröten aufgezeichnet. Eine vollständige Liste der Synonyme für sowohl die Pentastomiden als auch ihre Wirte ist eingeschlossen.

Veröffentlichungen bezüglich des phylogenetischen Ursprungs der Pentastomiden, die sich auf Fossilfunde aus dem Kambrium stützen, und der heutige Wissensstand über die Phylogenese der Crocodylia werden zur Evolution und aktuellen geographischen Verteilung der heute lebenden Gattungen der Sebekidae in Bezug gesetzt. *Sebekia* ist die Gattung mit dem größten Wirtsspektrum und parasitiert beide Unterfamilien der Crocodylia. Ebenso ist *Sebekia* auch die Gattung mit dem weitesten geographischen Verbreitungsgebiet und der größten Artenvielfalt innerhalb der Sebekidae. Die Schlussfolgerung wird gezogen, dass die Gattungen *Sebekia* und *Leiperia* bereits vor 80 Millionen Jahren entstanden, wohingegen *Alofia*, *Selfia* und *Diesingia* erst spätter aus einem gemeinsamen Vorfahren hervorgingen. Die Gattung *Agema* ist endemisch auf dem afrikanischen Kontinent und erschien vermutlich erst nach der Abspaltung der Endwirte von den übrigen Vertretern der Crocodylia.

Morphologische Daten, die im Verlauf dieser Arbeit gesammelt wurden und solche, die Veröffentlichungen entnommenen wurden, wurden phylogenetisch ausgewertet und bilden die Grundlage zu einem erweiterten, sämtliche heute bekannten Gattungen umfassenden Stammbaum der Sebekidae. Der detaillierte Vergleich der diagnostischen Merkmale der verschiedenen Sebekiden wurde in einen Bestimmungsschlüssel zu den Gattungen dieser Familie umgesetzt, und die systematische Verwandschaft zwischen den in afrikanischen Krokodilen vorkommenden Sebekiden wurde genauer analysiert.

Pentastomose ist weit verbreitet unter den im Krüger Nationalpark und angrenzenden Gebieten vorkommenden Nilkrokodilen. Die Befallshäufigkeit beträgt 93% und die Befallsintensität variiert zwischen 2 und 109. Mit sechs Spezies, die drei verschiedenen Gattungen der Sebekidae angehören, war der Artenreichtum an Krokodilpentastomiden groß. Insgesamt 71% der infizierten Wirte beherbergten mehr als zwei Arten.

Aufgrund der hohen Befallshäufigkeit und –stärke wurden *S. wedli*, *Sebekia cesarisi* und *L. cincinnalis* als Kernspezies der Pentastomidengemeinschaft von Nilkrokodilen klassifiziert. *Sebekia okavangoensis* und *Alofia nilotici* sind Sekundärspezies, während *Alofia simpsoni* als Satellitenspezies eingestuft wurde. In den Endwirten formen die unterschiedlichen Pentastomidenarten jeweils Isolationsgemeinschaften, und interspezifische Wechselwirkungen haben ihren Einfluss auf die Organisation der Pentastomiden innerhalb eines einzelnen Wirtes verloren.

Die Artenzusammensetzung in den unterschiedlichen Untersuchungsgebieten des Krüger Nationalparks ist relativ einheitlich, wohingegen sich die Pentastomidenfauna des PMC Absetzteichs im wesentlichen aus zufällig mit den Endwirten eingetragenen Pentastomiden zusammensetzt.

Es ergab sich kein nennenswerter Unterschied in der Befallshäufigkeit bei unterschiedlich großen Krokodilen. Allerdings ändert sich das Beutespektrum der Krokodile mit zunehmender Größe. Dies erhöht die Wahrscheinlichkeit einer Aufnahme von Infektionslarven, was in einer steigenden Befallsstärke resultiert. Die hohe Lebensdauer von Infektionslarven erleichtert die Akkumulation von Pentastomiden im Wirt.

Die Alterszusammensetzung von Pentastomideninfektionen ist saisonal veränderlich. Der Prozentsatz an Infektionslarven war in den im Frühling untersuchten Krokodilen am höchsten und nahm zu einem Minimum im Winter hin ab, was mit einer temperaturbedingten Periode verringerter Nahrungsaufnahme seitens der Endwirte zusammenfällt. Im Gegensatz hierzu war der Anteil an adulten Pentastomiden im Winter am höchsten, was sich durch die fortschreitende Entwicklung der in vorangegangenen Jahreszeiten aufgenommenen Infektionslarven erklären lässt.

Die Pentastomidenfauna der südafrikanischen Nilkrokodile unterscheidet sich deutlich von der der beiden anderen afrikanischen Krokodile, *Crocodylus cataphractus* und *Osteolaemus tetraspis*, und nur *S. okavangoensis* parasitiert jeden der drei Wirte.

Aus den südafrikanischen Wasserschildkröten konnte nur *Pelonia africana* gesammelt werden. Die Befallshäufigkeit sowie die Befallsintensität waren relagiv gering (29% and 1.1), wobei die Prevalenz in *Pelomedusa subrufa* etwas geringer war als in *Pelusios sinuatus*.

Die Daten der Freilanduntersuchungen und der experimentellen Infektion der Nilkrokodile werfen neues Licht auf den Lebenszyklus von *L. cincinnalis*, der eine obligatorische, gregäre Phase im kardiovaskulären System der Endwirte beinhaltet. Die Kopulation erfogt in der Trachea.

Die experimentelle Infektion von Nilkrokodilen mit *Subtriquetra rileyi* war erfolglos und Nilkrokodile konnten nicht als Endwirt für diesen Pentastomiden bestätigt werden.

Serranochromis meridianus aus dem Krüger Nationalpark konnte erstmalig als Zwishenwirt für *L. cincinnalis* identifiziert werden. *Subtriquetra rileyi* ist ein häufiger Parasit in *Oreochromis mossambicus* und *Tilapia rendalli* mit einer Länge über 200 mm. Während *L. cincinnalis O. mossambicus* als Zwischenwirt bevorzugt, war die Prevalenz anderer Sebekiden gering. Bei *T. rendalli* ergab sich ein umgekehrtes Bild. In allen untersuchten Zwischenwirten war die Befallsintensität generell gering. Während das Geschlecht der Zwischenwirte keinen Einfluss auf die Befallshäufigkeit und –stärke hatte, stiegen beide mit zuhnemender Größe der Wirtstiere an. Möglicherweise führt eine Immununverträglichkeit dazu, dass *Leiperia* selten die gleiche Zwischenwirtsspezies mit den übrigen Sebekiden Gattungen teilt. Darüberhinaus bietet der lange Darm, der in *Oreochromis* eine Anpassung an seine vorwiegend vegetarische Lebensweise darstellt, möglicherweise günstigere Lebensbedingungen für *L. cincinnalis*.

Fische aus dem Arabie Stausee erwiesen sich in Bezug auf Pentastomiden als negativ, obgleich Wasserschildkröten aus dem gleichen Gewässer infiziert waren. Dies zeigt, dass Endwirte die geeigneteren Indikatoren für das Vorhandensein dieser Parasiten sind, vor allem, wenn es sich um große, kürzlich geschaffenen Systeme, wie z.B. den Arabie Stausee, handelt.

Die Schwimmblasen mit *S. rileyi* befallener Fische zeichnen sich durch ovale, Hämatin-gefärbte Saugmale aus. Hämorrhagien unter der Basalmembran zeugen von durch Saugvorgänge oder die Haken und/oder Stacheln der Pentastomiden verursachten Verletzungen. Bei schwerem Befall ist eine Verdickung der Basalmembran zu verzeichnen. Zellulärer Debris im Lumen befallener Schwimmblasen beinhaltet heterophile Leukocyten und Makrophagen sowie in eine amorphe, oft Hämatin-haltige Matrix eingebettete Lymphocyten. Bei der Häutung abgestoßene Chitinhüllen waren in locker mit Makrophagen infiltrierte Bindegewebsschichten der Wirte eingebettet. Generell weisen die befallenen Schwimmblasen nur geringe histopathologische Veränderungen auf, die die Funktion dieser Organe nicht beeinträchtigen.

CHAPTER 1

INTRODUCTION

The Pentastomida is a relatively uniform group of obligatory endoparasites with currently approximately 131 described species (Almeida & Christofferson, 1999). The true phylogenetic position of the Pentastomida has as yet remained unresolved, but lately consensus on their arthropod nature seems to have been reached (Walossek & Müller, 1994). Nevertheless, pentastomes display an intriguing mixture of annelid and arthropod characteristics. This, together with severe reductions in organ systems and appendages as adaptation to a strictly parasitic life style, conspired to obscure their systematic affinities (Self, 1969; Bosch, 1987). Hence, in the past pentastomes were included with the Annelida, assigned the status of an independent phylum between the former and the Arthropoda and placed in relationship with any of the extant arthropod taxa (Leuckart, 1860; Heymons & Vizthum, 1936; Osche, 1963; Doucet, 1965; Wingstrand, 1972; Riley, Banaja & James, 1978; Böckeler, 1984; Walossek & Müller, 1994).

The vermiform abdomen of the Pentastomida is usually contiguous with the cephalothorax, and in most species it is distinctly annulated. This annelid-like appearance is, however, not matched by an inner segmentation. The cephalothorax carries a sucking mouth supported by a chitinous oral cadre which is situated between two pairs of retractable hooks. Adult pentastomids suck blood from ruptured capillaries using their hooks to anchor themselves to the host tissue (Riley, 1986). Mainly occurring in tropical regions they nevertheless have a worldwide distribution and utilize a wide spectrum of tetrapod hosts such as amphibians, reptiles, birds and mammals (Walossek & Müller, 1994) including humans. Infections in humans are mainly caused by *Armillifer armillatus* and *Linguatula serrata*, but infections with *Leiperia* and *Sebekia* have also been recorded (Baird, Kassebaum & Ludwig, 1988; Mairena, Solano & Venegas, 1989). It has been estimated that reptiles account for roughly 90% of the hosts (Baer, 1952). Pentastomids reach maturity in the respiratory tracts of their final hosts and are sexually dimorphic. Their life cycles are complex and usually involve at least one intermediate host, but direct life cycles have been recorded (Banaja, James & Riley, 1975; Riley, 1986). According to Bosch (1987) the life-cycles of approximately 10% of the known pentastomid species have been experimentally elucidated.

Since the late seventies and early eighties a number of important taxonomic revisions were published, further knowledge on ultrastructural aspects gained and Riley (1986) compiled his extensive review on pentastome biology. The discovery of late Cambrian pentastomid fossils shed some light on the phylogenetic origin of the Pentasomida and emphasized their arthropod affinities (Walossek & Müller, 1994).

Despite this renewed interest in the Pentastomida, surprisingly little information is available concerning crocodilian and, even more so, chelonian pentastomes worldwide. These are generally limited to a few isolated reports of new species from usually a single or few host specimens and epidemiological investigations on the pentastome communities of these two groups of final hosts in South Africa have so far never been done.

GOALS AND OBJECTIVES OF THIS STUDY

The emphasis of this study was placed on the pentastome fauna of South African crocodiles and chelonians, which consists of genera and species of only the family Sebekidae. However, throughout the thesis an attempt was made to integrate the South African findings into the worldwide picture, so as to provide a synoptic overview of this family throughout their range rather than concentrating on isolated selected representatives.

As regards the crocodilian final hosts, the objectives were:

- to expand on the data available on the Pentastomida of the endangered Nile crocodile and the possible impact of these parasites on crocodiles in the wild as well as under farming conditions;
- to determine the extent of the infection in the Kruger National Park and to assess whether pentastomosis extends beyond the Park's borders;
- to investigate the pentastome species diversity and to elucidate the structure of pentastome communities in Nile crocodiles;
- to determine the influence of host sex and host size (age) on pentastome infections and to determine whether pentastome infections fluctuate seasonally in Nile crocodiles;
- to elucidate the life-cycles of *Leiperia* cincinnalis and *Subtriquetra rileyi* and attempt to confirm Nile crocodiles as final hosts for the latter pentastome.

For the chelonian final hosts the objectives were:

- to augment the dearth of existing data on African chelonian pentastomes by collecting basic information on the pentastome species diversity and the prevalence and intensity of infections.

For the fish intermediate hosts the objectives were:

- to expand on data on pentastome infections in fish in the Kruger National Park concerning host preference, prevalence and intensity of infection;
- to determine the presence of pentastomes in fish in watersheds outside the KNP;
- to assess the histopathological changes associated with infective pentastome larvae in the swim bladder of infected fish.

CHAPTER 2

GENERAL MATERIAL AND METHODS

2.1 STUDY LOCALITIES AND CLIMATOLOGICAL DATA

The Republic of South Africa is part of the Afrotropical (sub-Saharan) region but being to the south of the continent, four distinct seasons are present. Spring lasts from August to October, summer from November to January, autumn extents from February to April and May to July are classified as the winter months.

Nile crocodiles were obtained from different localities within the Kruger National Park and on two occasions from a locality just outside the Park, at Phalaborwa. Fish were collected either at the Phabeni Dam inside the KNP or were caught in the Arabie Dam. The latter was also the origin of the chelonians examined in the present study. All study areas are located in the north-eastern part of South Africa, namely the Northern Province and Mpumalanga, and are illustrated in Figure 1.

2.1.1 The Kruger National Park

The Kruger National Park (22º31' – 25º30' S, 30º45' – 32º05' E; Altitude 170 – 800 m; KNP) is the largest wildlife reserve in South Africa, covering an area of approximately 1 948 528 ha. From north to south it extends over almost 350 km, while its width averages a mere 60 km (Anon., 1988). The KNP shares the western border with the Northern Province and Mpumalanga, its northern boundary with Zimbabwe and its eastern one, the Lebombo mountains, with Mozambique.

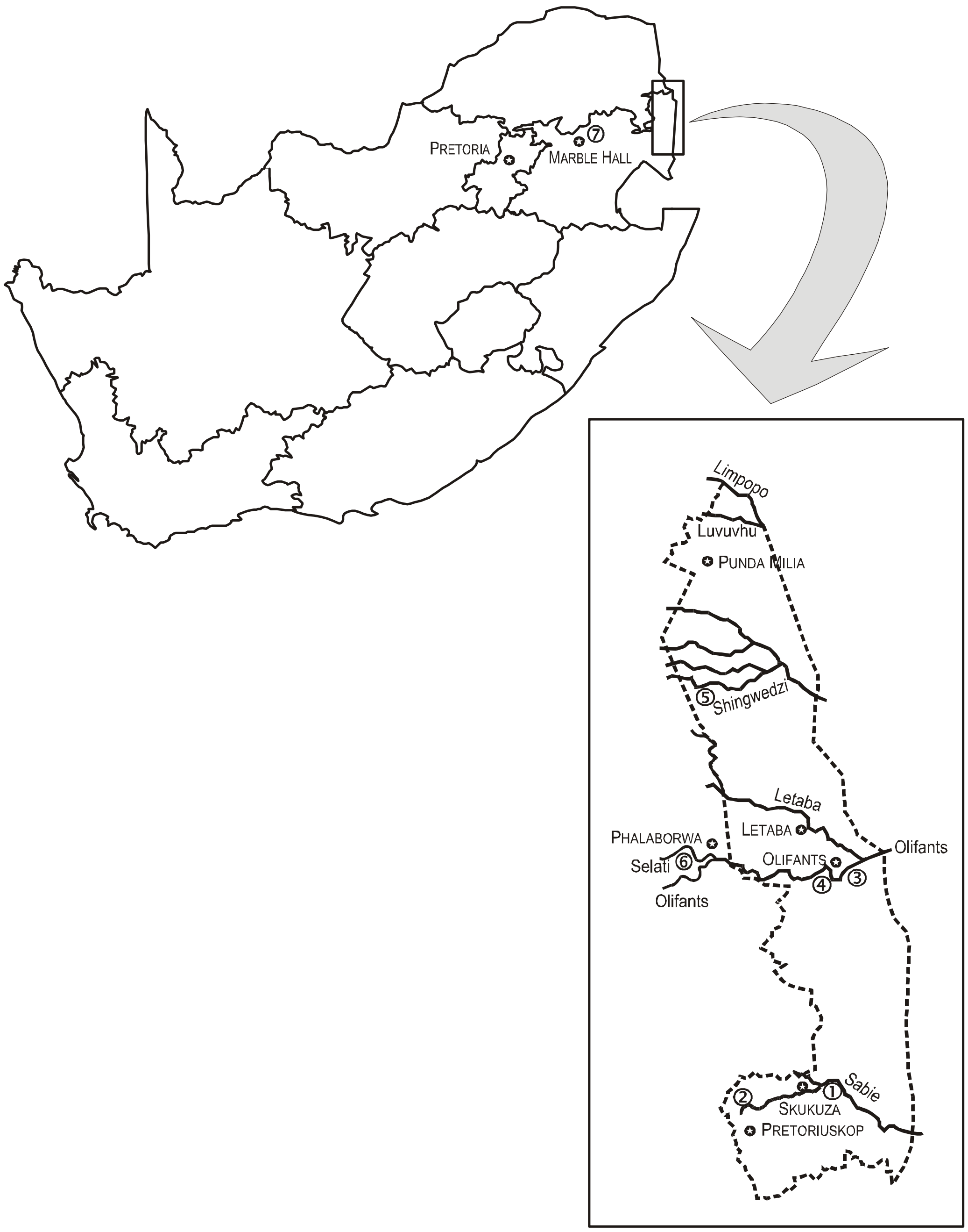

Figure 1. Map of South Africa indicating the localities at which final and intermediate hosts were collected. The area of the Kruger National Park is enlarged. ① Sabie River, ② Phabeni Dam, ③ Hardekooldraai, ④ Balule, ⑤ Silwervis Dam, ⑥ Phalaborwa Mining Corporation Game Reserve, ⑦ Arabie Dam.

Two perennial rivers, the Limpopo River and the Crocodile River form the natural northern and southern boundaries of the KNP, respectively. Other perennial rivers are the Sabie River, the Olifants River, the Letaba River and the Levuvhu River. Numerous smaller rivers and streams are present, but flow only during the wet seasons. Despite their inconsistent flow, several of these form perennial pools. All rivers, with the exception of the Olifants River that originates in the Mpumalanga Highveld, originate in the escarpment, which is formed by the Drakensberg, to the west of the KNP or in the Lowveld and all flow into the Indian ocean (Paynter & Nussey, 1986).

The average annual temperature ranges from a minimum of +4ºC to a maximum of +34ºC and the climate is generally characterized by mild winters, although isolated areas might experience frost during the winter months, and warm to hot summers. Precipitation usually occurs in summer with effective rains during the months October to April (Gertenbach, 1983; Zambatis & Biggs, 1995).

The KNP is one of the main refuges for wild Nile crocodiles in South Africa, and supports viable breeding populations (Swanepoel, personal comm., 2000).

2.1.1.1 The Sabie River

The Sabie River originates on the escarpment in the Mpumalanga Drakensberg near the town of Sabie. It passes through the southern third of the KNP and together with the Crocodile River, it defines a landscape of low lying areas called the thickets of the Sabie and Crocodile Rivers (Gertenbach, 1983). Both rivers are perennial.

The altitude of the landscape varies between 200 and 350 m and the climate is characterized by greater extremes than experienced in adjacent landscapes. The difference between day and night temperatures is pronounced, and while the average daily temperature exceeds 31ºC from November to March, sporadic frosts can occur during winter, when absolute minimum temperatures of –2,5ºC can be reached. From June to August the average daily minimum is below 10ºC and during May and September it is below 15ºC. Throughout the year, however, the average daily maximum stays above 25ºC. The rainfall varies between 500 and 550 mm per year with an annual average of 546 mm for Skukuza (24º9' S, 31º5' E) (Gertenbach, 1980; 1983). Table 1 shows the average maximum and minimum temperatures for each month as recorded at Skukuza.

Table 1. Average monthly minimum and maximum temperatures from 1965 to 1983 recorded at Skukuza (after Gertenbach, 1983).

Month	Average Daily Maximum (ºC)	Average Daily Minimum (ºC)
January	32,3	19,6
February	32,2	19,4
March	31,2	17,9
April	29,8	14,8
May	27,4	10,2
June	25,6	6,1
July	25,4	5,6
August	27,2	7,6
September	29,4	11,6
October	30,8	15,1
November	31,8	17,5
December	32,3	19,2

2.1.1.2 The Phabeni Dam

The Phabeni Dam (25º1' S, 31º15' E) is one of the reservoirs constructed in the KNP to ensure a permanent supply of drinking water for wildlife and refuge for aquatic animals. It has a surface area of roughly 3 ha. The Phabeni Dam is fed by the Phabeni River as well as the Sabie River, the latter which also drains the dam.

The reservoir is situated in the vicinity of Pretoriuskop in the south-western corner of the KNP in the Lowveld sour Bushveld of Pretoriuskop. The altitude in this part of the Park varies between 550 and 650 m above sea level (Gertenbach, 1983).

The annual rainfall averages about 740 mm. Daytime temperatures of 35ºC during summer are common, but at the same time the temperature during winter is very moderate and no frost occurs (Gertenbach, 1983). The main temperature data are displayed in Table 2.

In addition to a number of fish species, the Phabeni Dam harbours a population of about 20 Nile crocodiles and two species of terrapins, *Pelomedusa subrufa* and *Pelusios sinuatus* (personal observation, 1999).

Table 2. Average monthly minimum and maximum temperatures from 1981 to 1983 recorded at Pretoriuskop (after Gertenbach, 1983).

Month	Average Daily Maximum (ºC) (	Average Daily Minimum (ºC)
January	29,2	18,8
February	31,0	18,3
March	32,1	17,8
April	27,8	15,1
May	25,1	12,7
June	23,9	8,8
July	24,8	9,3
August	26,7	11,0
September	26,9	14,4
October	23,7	13,2
November	30,1	17,6
December	28,6	17,4

Figure 2. A. A stretch of the Sabie River. B, C. Phabeni Dam: B. Visitors. C. Dam wall. D, E. Intermediate hosts: D. *Tilapia rendalli*. E. *Oreochromis mossambicus*. F, G, H. Silwervis Dam: F. Crocodile enclosure baited with zebra carcass. G. Measuring of immobilized Nile crocodiles. Eye patches for protection of the retina. H. Working set up. I. Parasite collection from experimental crocodile.

2.1.1.3 *The Olifants River*

The Olifants River flows through the central part of the KNP, roughly dividing it into a northern and southern half. It is one of the main perennial rivers and together with the Timbavati River it drains the Combretum spp./Acacia spp. rugged veld whose altitude varies between 180 and 300 m (Gertenbach, 1983).

The rainfall is low and averages between 450 and 500 mm per year. High temperatures are experienced during summer months and from November to April the average daily maximum stays over 30ºC. Only in June and July does the average minimum temperature drop below 10ºC. Frost occurs rarely and is limited to low lying areas. Table 3 indicates the temperatures measured at Letaba camp which are representative of the climate at Olifants camp (Gertenbach, 1983).

Crocodiles were shot in the Balule vicinity (24º5' S, 31º45' E) and at Hardekooldraai (24º2' S, 31º40' E).

Table 3. Average monthly minimum and maximum temperatures from September 1981 to 1983 recorded at Letaba (after Gertenbach, 1983).

Month	Average Daily Maximum (ºC)	Average Daily Minimum (ºC)
January	34,0	22,0
February	34,9	21,5
March	34,3	20,5
April	33,4	19,4
May	28,6	12,6
June	26,7	8,0
July	26,7	10,4
August	28,9	11,3
September	27,8	12,9
October	27,3	14,0
November	33,3	19,0
December	32,4	19,1

2.1.1.4 The Shingwedzi River – Silwervis Dam

The Shingwedzi River is the southernmost river of the northern third of the KNP and its headwaters lie about 50 km outside the Park. It is a typical seasonal Lowveld river and two dams, the Silwervis Dam (23º15' S, 31º10' E) and the Kanniedood Dam, have been built in its course (Swanepoel, Boomker & Kriek, 2000).

The Shingwedzi River and its tributaries drain the Tsende Sandveld, a landscape with an altitude that varies between 300 and 450 m. According to Gertenbach (1980) this area receives between 450 and 550 mm rain per annum, and frost is exceptional. Temperature data for Shingwedzi camp are presented in Table 4.

Twenty-six Nile crocodiles from the Silwervis Dam had to be relocated in June 1998 because of extremely low water levels in the Shingwedzi River.

Table 4. Average monthly minimum and maximum temperatures from September 1981 to 1983 recorded at Shingwedzi (after Gertenbach, 1983).

Month	Average Daily Maximum (ºC)	Average Daily Minimum (ºC)
January	34,3	21,7
February	33,9	21,2
March	33,2	20,0
April	30,4	17,4
May	26,8	11,5
June	25,8	6,7
July	25,9	9,2
August	28,3	10,6
September	27,8	12,9
October	27,3	14,0
November	33,3	19,0
December	32,4	19,1

2.1.2 The Phalaborwa Mining Corporation Game Reserve

The Phalaborwa Mining corporation (PMC), situated in the town of Phalaborwa (24º55' S, 31º10' E) just outside the KNP, has several large sedimentation dams, in which water is purified before being re-used or released into the Selati River. The latter is one of the tributaries that join the Olifants River shortly before it enters the KNP. A number of crocodiles have migrated from the Olifants River into the Selati River and subsequently into the sedimentation dams where they now permanently reside (Swanepoel *et al.*, 2000). The temperatures taken at Letaba camp (Table 3) are a good reflection of the seasonal conditions at Phalaborwa.

2.1.3 The Arabie Dam

The Arabie Dam (24º53' S, 29º22' E), Northern Province and Mpumalanga, is fed mainly by the Olifants River and the Elands, Moses and Motsiphiri Rivers also contribute to it. It is a vast dam extending over several kilometres and, apart from being the main supply of drinking water for the region, its water is also used for irrigation and recreation. A number of ponds and marshy areas in its vicinity are temporarily or permanently inhabited by chelonians and catfish that have moved from the dam itself. The number of Nile crocodiles inhabiting the dam is estimated to be close to 120 (A. Hoffmann, personal communication, 2001), and it is one of the largest viable, breeding populations outside the KNP.

2.2 COLLECTION OF NATURALLY INFECTED HOSTS

2.2.1 Naturally infected crocodiles

From 1997 to 1999 fifteen Nile crocodiles were obtained from four different localities within the KNP and one locality just outside the Park’s borders. The data of these crocodiles are presented in Table 15 (see Chapter 7).

All Nile crocodiles collected from the Olifants River in 1997 were shot at the water and examined immediately upon death. Due to technical circumstances the crocodiles from the Sabie River and those from the Silwervis Dam were caught in baited traps and immobilized with gallamine triethiodide (Flaxedil™) before being shot. Crocodile 1/99 from the Olifants River and Crocodile 2/99 from the PMC Dam were found on land, immobilized with Flaxedil™ and shot the following morning. Crocodile 3/99 was caught in a baited cage and subsequently shot.

The length of the crocodiles was measured from the tip of the tail to the tip of the snout. The overall condition was judged by the amount of body fat on the tail and back, which, apart from the mesenteric organs of the abdomen, are the main storage areas for body fat in crocodilians.

2.2.2 Naturally infected terrapins

During an unrelated study in March 2000 three serrated hinged terrapins *P. sinuatus* (host numbers Psin1-3) were netted from small waterbodies in the immediate vicinity of the Arabie Dam and made available to the author for pentastome recovery. During the course of the same study, pentastome specimens were recovered from another species of southern African terrapins, the Cape terrapin *P. subrufa* (hosts number Psub2 and Psub3), as well as from a single *P. sinuatus* (host number WIII), and given to the author for morphometric analysis. In September 2000 two *P. sinuatus* (Psin 4 and 5) and P.subrufa (Psub1) were netted at the same locality. The data on the pentastome specimens recovered from the above hosts were published by Junker & Boomker (2002).

In February 2002 an additional six *P. sinuatus* (Psin6-11) and five *P. subrufa* (Psub4-8) were collected at the above locality and examined for the presence of pentastomes.

With the exception of hosts Psub2, Psub3 and WIII, which could not be measured, the length of the terrapins was determined by running the tape from the front end of the carapace across the dome to the caudal rim of the carapace. The carapace of *P. sinuatus* is more domed than that of *P. subrufa*, but both are sufficiently flattenend to allow comparison with straight lengths found in literature. The terrapins' condition was evaluated by the amount of visceral fat as well as the appearance of the limbs. Greater details concerning the terrapin hosts are provided in the ensuing chapters.

Terrapins were either killed by interperitoneal injection with sodium pentobarbitone (Eutha-naze™) or decapitated.

2.2.3 Naturally infected fish

From January to June 1999, 464 cichlids and a single barb were caught with baited hand-lines in the Phabeni Dam in the KNP. Using the same technique 20 cichlids were caught in Lake Panic, Skukuza, in the KNP in March 1999.

Between March 1999 and September 2000 114 fish were caught with seine nets in the Arabie Dam. During the same period 10 *Clarias gariepinus* were netted from the same small pond in the vicinity of the Arabie Dam from which the terrapins for this study were collected.

The total length of fish was measured from the tip of the snout to the most distal part of the caudal fin (Skelton, 1993).

2.3 COLLECTION OF PENTASTOMES

The carcass of each final host was opened and the trachea, the lungs and the heart were removed. The various organs were separated from each other and from the suspensory ligaments and placed individually into shallow plastic trays.

After removal of the trachea and the oesophagus, the nasopharynx was visually checked for subtriquetrids. The trachea was opened lengthwise and examined for the presence of *Leiperia* cincinnalis. The heart was inspected, exposing the different chambers, and special attention was paid to the aortic arches and the pulmonary artery. The liver was cut into 0.5 cm wide strips and the exposed surface checked for pentastomid larvae. Subsequently, the slices were transferred into tap water or, where the latter was not available, into river water.

The lungs were opened along the bronchi and bronchioles with a pair of scissors, exposing as much surface as possible, and subsequently placed into tap or river water, depending on where the necropsies were performed. Pentastomes were either removed directly from the respective organs or collected from the water after they had migrated out of the tissues.

With the exception of Crocodiles 5/97, 6/97 and 7/97 of which only the right lungs were examined, pentastomes were recovered from both lungs of a given host. For comparison the numbers of pentastomes recovered from the lungs of the former hosts have thus been multiplied by two. The resulting figures, as well as figures obtained by using these extrapolated results are marked with an asterisk (*) in the following chapters.

The body cavity of fish was opened by ventral incision and the viscera and tissues surrounding the gastro-intestinal tract were examined macroscopically for the presence of pentastome larvae. The swim-bladders were removed and placed into separate vials in tap water. Within 4 hours of collection, the swim bladders were examined under a stereoscopic microscope between two Perspex slides while applying gentle pressure. Pentastomes were removed from the respective tissues by blunt dissection.

All pentastome material was transferred into tap water and used for experimental infections or fixed and stored in cold 70% ethanol.

2.4 IDENTIFICATION AND MORPHOLOGICAL ANALYSIS OF PENTASTOMES

As many structures important for identification are difficult to view in alcohol material, specimens were mounted on microscope slides in Hoyer's medium, which clears the pentastomes.

The thickness of the abdomen can interfere with the orientation of morphologically relevant structures of the cephalothorax on the slide, especially in gravid females, where the egg-filled uteri cause distension of the abdomen. For detailed observation the cephalothorax was thus removed from the abdomen under a stereo-microscope using a razor blade, and the two parts of each specimen mounted under separate cover-slips on the same slide. When positioning the cover-slip over the embedded cephalothorax, light pressure was exerted in order to flatten and separate the hooks and the oral cadre. Separation of the cephalothorax of male pentastomes from the abdomen, was carefully done so as not to damage the copulatory spicules and to include these structures with the anterior part of the parasite.

Measurements of the body length and annulus counts were obtained from whole specimens fixed in 70% ethanol prior to bisection. The rows of chloride cell pores disposed on each abdominal annulus proved a valuable aid when establishing the number of annuli.

Drawings of whole structures or single measuring points were made using a Nikon Optiphot microscope with a drawing tube and the UFX II camera attachment for photography. Hook, oral cadre and copulatory spicule dimensions were measured as illustrated in Figure 3. Riley (1986) gives an excellent summary of the diagnostic value and limitations of the various morphologic criteria used in pentastome taxonomy and was extensively used to establish the diagnostic characteristics.

Female pentastomes were considered mature when their uteri contained eggs with fully developed primary larvae. Females with unembryonated eggs or without eggs were judged to be pre-patent. Males were classified as mature when the copulatory spicules were fully formed and the elongated, paired penises were coiled within the cirrus sacs (see Riley, Spratt & Winch, 1990).

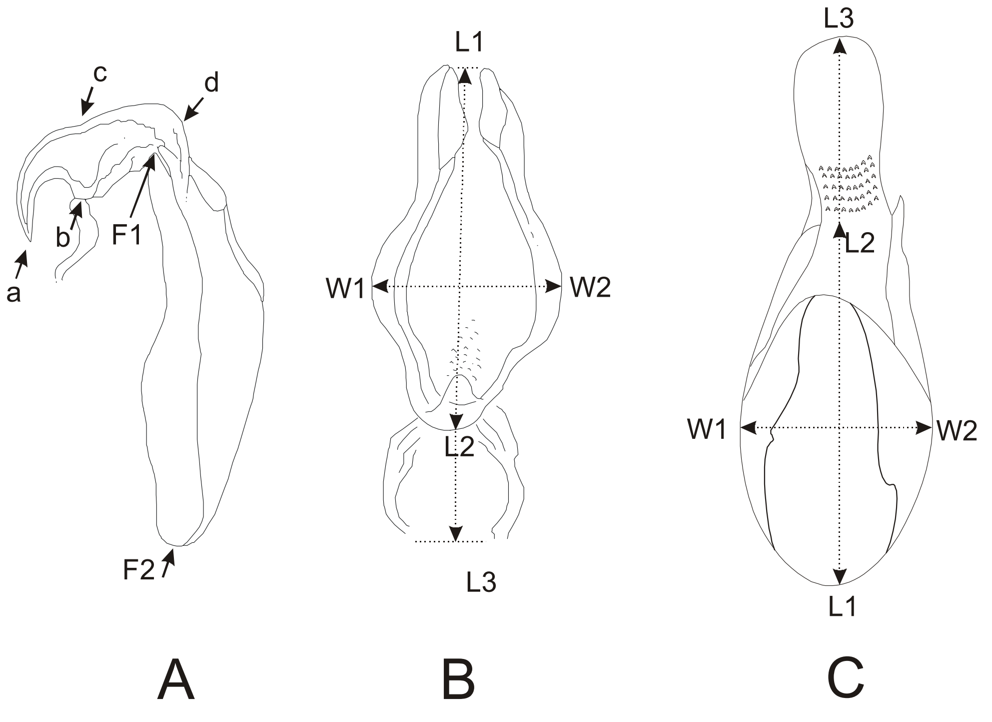

Figure 3. A. Schematic pentastome hook. Points d and b are the points of insertion of the extensory and flexory musculature, respectively, c demarcates the notch in the dorsal hook surface. ac = blade length, ad = hook length, bc = base length, cd = plateau length, F1F2 = fulcrum length. B. Schematic oral cadre. L1L2 = cadre length, L1L3 = overall length, W1W2 = (maximum) width. C. Schematic copulatory spicule. L1L2 = cowry shell length, L1L3 = total length, W1W2 = (maximum) width.

2.5 EXPERIMENTAL INFECTIONS

2.5.1 Experimentally infected crocodiles

Ten third generation (F3), female Nile crocodiles, about 0,8 to 1m in length and 1 to 2 years old, were obtained from a local crocodile farmer. The farming conditions were such that the crocodiles had no access to any natural river systems and the breeding stock had been bred in captivity to the second generation. Rather than using fish as a source of food, the rearing diet consisted of a pelleted concentrate and/or chicken meat. Thus, the possibility of a previous natural infection of the experimental hosts with pentastomes was effectively excluded.

The experimental crocodiles were housed in an outside enclosure, 2 x 7 m and 2 m high. The back third of the enclosure was roofed and the sides closed off. A pond, approximately 1 x 2 m and 0,5 m deep, was situated in the roofed area and equipped with a heater to ensure a minimum water temperature of 15ºC during the night and cold days. Infrared lights provided extra heat during winter. The surface of the cage consisted of river sand, with a patch of Kikuyu lawn in the front. The crocodiles were fed once a week on whole chicken sprinkled with a vitamin and mineral supplement for dogs.

The crocodiles were randomly split into two groups. The first group (B) was marked with respectively 1, 2, 3, 4 or 5 black cable ties fastened through holes punched with a leather punch through the lateral scales on the tail. Crocodiles of the second group (W) were marked in the same manner using white cable ties.

Crocodiles W1 to W5 were selected for infection with the pentastomes L. cincinnalis, the adults of which inhabit the trachea of the host, and S. *rileyi*, which is thought to occur in the nasopharynx.

Crocodiles B1 to B5 were to be infected with nymphs of *Alofia* sp. upon recovery of the latter from experimentally infected fish. Since the experimental infection of intermediate hosts did not produce infective larvae, this was not possible. Thus, Crocodile B1 was used as additional experimental host for L. cincinnalis and *Subtriquetra rileyi* (see below), but kept on standby together with Crocodiles B2, B4 and B5, which remained uninfected.

In order to use the experimental hosts to maximum effect, it was decided to infect each host twice, spacing the experimental infections 35 days apart. Thus each host would yield information of two developmental periods of their respective pentastomid parasites. For the infection of the experimental hosts with L. cincinnalis and S. *rileyi* pieces of chicken breast, approximately 2 cm long, were filled with the infective material and the incision closed with cotton thread. Subsequently, the meat was fed to the crocodiles using long-handled barbeque tongs. This method was chosen to avoid the extremely high stress levels experienced by the animals during force feeding. However, the crocodiles had not yet sufficiently settled down to accept hand-feeding freely and it was necessary to starve the crocodiles for some time prior to infection. The resulting increased aggression at feeding time led to Crocodile B3 taking the meat from Crocodile W3 during the second infection of Crocodiles W1 to W5.

Due to difficulties in obtaining sufficient infective material and the fact, that the experimental crocodiles were kept at a long distance from the KNP it was necessary to store collected infective larvae for up to 6 days prior to infection. Only infective larvae of S. *rileyi* which at the date of infection were still moving were used for the first and second infections of the experimental hosts. The same was applied for the excysted infective larvae of L. cincinnalis used during the first infection of the crocodiles. No encysted forms of L. cincinnalis were available. For the second infection, excysted as well as encysted L. cincinnalis material was available. In order to use the maximum number of infective stages, excysted larvae that showed no movement were included regardless.

Table 5. Infection regime of the experimental infection of *Crocodylus niloticus* with *Leiperia cincinnalis* and *Subtriquetra rileyi*.

Host	Pentastome burden of first infection	Pentastome burden of second infection	Age of infection at necropsy
W1	*L. cincinnalis*: 9, excysted *S. rileyi*: 22	*L. cincinnalis*: 7, excysted, moving 9, excysted, not moving 3, encysted *S. rileyi*: 18	138 and 98 days p.i.
W2	*L. cincinnalis*: 29, excysted *S. rileyi*: 54	*L. cincinnalis*: 7, excysted, moving 9, excysted, not moving 3, encysted *S. rileyi*: 18	138 and 98 days p.i.
W3	*L. cincinnalis*: 9, excysted *S. rileyi*: 22	Not infected	72 days p.i.
W4	*L. cincinnalis*: 9, excysted *S. rileyi*: 22	*L. cincinnalis*: 9, excysted, moving 15, excysted, not moving 7, encysted *S. rileyi*: 25	38 and 3 days p.i.
W5	*L. cincinnalis*: 9, excysted *S. rileyi*: 22	*L. cincinnalis*: 7, excysted, moving 9, excysted, not moving 3, encysted *S. rileyi*: 18	102 and 67 days p.i.
B3	Not infected	*L. cincinnalis*: 7, excysted, moving 9, excysted, not moving 3, encysted *S. rileyi*: 18	36 months p.i.
B1	*L. cincinnalis*: 60, excysted *S. rileyi*: 40	Not infected	32 months p.i.

Table 5 summarizes the infection regime followed during the first and second infections of the individual hosts and indicates the age of the two infections at the time of necropsy. While Crocodile B1 initially accepted the prepared meat, it did not swallow immediately but carried the meat into the water and began toying with it. In the process the meat was spat out repeatedly and some infective larvae of L. cincinnalis were seen floating on the water surface. The exact dose of infective material received by Crocodile B1 is thus uncertain.

Crocodiles B2, W1 and W2 were found dead in the enclosure, and Crocodile B2 was necropsied about 24 h after death, but the latter two hosts died during the author's absence on a field trip and the carcasses were stored in the freezer for two days before necropsy.

Crocodiles W3, W4 and W5 were euthanazed and transported to the laboratory where they were shot within the hour and subsequently necropsied, whereas Crocodiles B3 and B1 were shot directly in their enclosure, taken to the laboratory and necropsied within 4 h of death.

Crocodiles B4 and B5 were not infected, but sacrificed in March 2002 and examined within 4 hours after death to exclude the possibility of accidental infection. Like the previous crocodiles that were shot, these were also killed with a single shot from a small caliber firearm through the brain. The procedure is the standard method used by many crocodile farmers.

The necropsies were performed following the procedures described under "Collection of parasites". The stomach of Crocodile W4 was opened and rinsed in running water. The washings were collected over a sieve with 0.038 mm apertures and subsequently examined in a Perspex counting chamber under a stereo microscope for residues of pentastomid larvae.

The viability of recovered pentastomes was assessed under a dissecting microscope by the presence or absence of movement. Histopathologic examination was not attempted due to the destructive nature of the recovery procedure.

2.5.2 Experimentally infected fish

Platys, Xiphophorus maculatus, according to Axelrod, Emmens, Sculthorpe, Vorderwinkler, Pronek & Burgess (1981) "an old scientific standby", were chosen as experimental intermediate host for its excellent properties as a community fish. Furthermore, Xiphophorus helleri, another member of the same genus, is a proven intermediate host for *Sebekia* mississippiensis from the American alligator (Overstreet, Self & Vliet, 1985).

Platys (35 to 50 mm long) were kept in glass tanks filled with water from the regular water supply at Onderstepoort, University of Pretoria, at a density of about 1 fish in 2 to 2.5 l water. All tanks were equipped with heaters and the water kept at a constant temperature of 22 to 24ºC. Aquarium wastes were removed using the AquaClear® 150 dual filtration system. To provide additional aeration air stones were placed at the bottom of each tank, and the air pump set to produce medium large bubbles. The fish were fed once daily with a commercial maintenance food for tropical fish.

To recover pentastome eggs, the terminal parts of the uteri of patent females were removed and carefully teased apart in tap water. Time was allowed for pentastome eggs to sediment and the top layer of tap water and floating tissue particles were decanted. This process was repeated several times. Subsequently the concentrated the eggsuspension was swirled thoroughly to ensure an even distribution of eggs and one chamber of a McMaster counting slide filled. The number of eggs in the chamber was counted under a stereomicroscope. The number of eggs present in a single counting chamber equals the number of eggs present in 0.15 ml of egg-suspension.

In order to control the quality of the infective material a drop of the egg-suspension was placed on a slide under cover-slip pressure and examined under a microscope. The number of eggs containing fully developed primary larvae, as indicated by their well-developed penetration apparatus and distinct pairs of hooks, was counted. Movement of the hooked limbs can be detected in viable primary larvae under a microscope (Bosch, 1987).

The intermediate hosts were infected by placing them into 2 l beakers (a maximum of four fish per beaker) filled with 1000 ml of tap water, heated to the same temperature as the tanks and equipped with air stones. Subsequently, the eggsuspension was added to the water and the fish left in the infection units over night.

Fish were examined for pentastomes at certain intervals after the infection according to the procedures described above under "Collection of parasites".

2.6 HISTOPATHOLOGICAL EXAMINATIONS

The swim-bladders of some of the infected Oreochromis mossambicus and *Tilapia rendalli* were transferred into 70% ethanol and fixed with the parasites *in situ*. Subsequently, tissue blocks were embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin.

2.7 TERMINOLOGY

The ecological terms prevalence and (mean) intensity of infection used to describe the epidemiology of pentastome infections in their various hosts were used in accordance with the definitions given by Margolis, Esch, Holmes, Kuris & Schad (1982).

a) Prevalence:	Number of individuals of a host species harbouring pentastomid parasites divided by the total number of hosts examined. Expressed as percentage.
b) Intensity:	Number of pentastomid parasites recovered from a single infected host.

c) Mean intensity: Total number of pentastomid parasites recovered from all the hosts examined divided by the number of infected hosts.

The terminology regarding pentastome community patterns is used as defined by Esch, Bush & Aho (1990):

d) Parasite infrapopulation: Includes all members of a given species of parasite within a single host.

e) Parasite infracommunity: Includes all infrapopulations within a single host.

2.8 STATISTICAL ANALYSIS

Since the numbers of final hosts at our disposal were limited and fewer than ten hosts were usually examined per event only the following basic descriptive statistical functions were used to evaluate the data collected during the present investigation:

a) Arithmetic mean: $\bar{x} = 1/n * \sum_{i=1}^{n} x_i$

b) Standard deviation: $s = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{(n-1)}}$

CHAPTER 3

NEW OR REVISED TAXA OF CROCODILIAN AND CHELONIAN PENTASTOMES

During the course of these investigations the hitherto undescribed male of the pentastome *Leiperia cincinnalis* from the Nile crocodile and a new genus of chelonian pentastomes from South African terrapins were recovered. Because of its close relation to the new genus and other sebekiid pentastomes, the revision of *Diesingia megastoma* from South American terrapins is included in this chapter. The descriptions of these have already been published (Junker, Boomker, Swanepoel & Taraschewski, 2000; Junker & Boomker, 2002; Junker, Riley & Boomker, in press), but for completeness' sake their descriptions and redescription are presented below.

3.1 DESCRIPTION OF THE MALES OF *LEIPERIA CINCINNALIS* (VANEY & SAMBON, 1910) SAMBON, 1922 WITH ADDITIONAL DATA ON THE FEMALES

Introduction

In 1995 and during the present investigation field studies were conducted on pentastome infections of fish and crocodiles in the KNP (Junker, 1996; Junker, Boomker & Booyse, 1998a, b). Males of *L. cincinnalis* were recovered from hosts examined in 1998 and their morphology described for the first time (Junker *et al*., 2000). At the same time additional morphometric data on the females were obtained.

Leiperia cincinnalis (Vaney & Sambon, 1910) Sambon, 1922 (syn. *Reighardia cincinnalis* Vaney & Sambon, 1910) (Pentastomida) is common in Nile crocodiles on the African continent (Sambon, 1922; Heymons, 1940a; Fain, 1961; Junker, 1996) and has been known for a long time. Nevertheless, the descriptions of *L. cincinnalis* given were often inadequate. Riley & Huchzermeyer (1996) re-assessed the genus *Leiperia* Sambon, 1922 and re-examined material of *L. cincinnalis* collected by various authors. None of the collections included mature males, but the morphology of the females, nymphs and pre-adults were described in considerable detail (Riley & Huchzermeyer, 1996).

Material and methods

The collection data of the crocodiles examined are listed in Table 6. The crocodiles from the Phabeni and Silwervis Dams were caught in a baited cage and immobilised with gallamine triethiodide (Flaxedil™) before they were shot. The specimen from the Shimuwini Dam was killed with a single shot through the brain.

Table 6. Collection data of Nile crocodiles from the Kruger National Park.

Host Number	Date collected	Locality		Sex	Length (m)	Condition
A/95	22/2/95	Phabeni Dam	(25° 01' S, 31° 15' E)	M	3.2	good
B/95	27/6/95	Shimuwini Dam	(23° 42' S, 31° 17' E)	M	3.3	emaciated
1/98	10/6/98	Silwervis Dam	(23° 13' S, 30° 12' E)	F	2.8	good
2/98	10/6/98	Silwervis Dam	(23° 13' S, 30° 12' E)	M	2.4	good
3/98	10/6/98	Silwervis Dam	(23° 13' S, 30° 12' E)	F	3.2	good
4/98	10/6/98	Silwervis Dam	(23° 13' S, 30° 12' E)	F	2.7	good

F = female, M = male

The pentastomes were collected and mounted according to the procedures described in Chapter 2 "General Material and Methods".

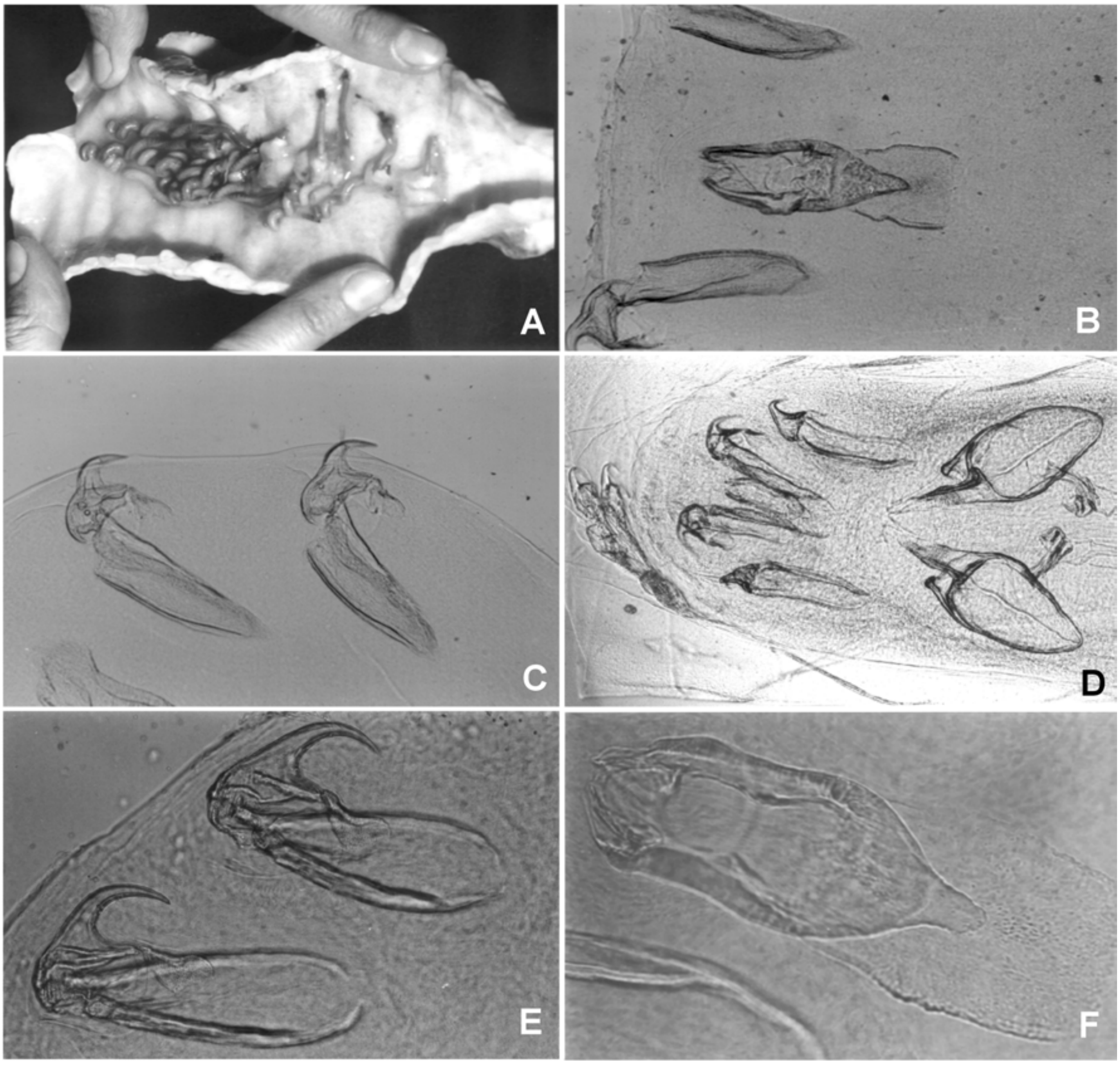

Figure 4. *Leiperia cincinnalis.* A. Mature females attached to a bronchus of the crocodile from the Shimuwini Dam, Kruger National Park. B. The oral cadre (overall length 925 µm) of a mature female recovered from the same crocodile. The anterior hook carries a distinct dorsal notch. C. Detail of the left hooks (hook length 304 µm) of another mature female from the Shimuwini-crocodile, possessing a prominent dorsal notch. D. The anterior part of a male (No. 4.18/98) showing the hooks, the oral cadre and the copulatory spicules. The elaborate cirrus tips as well as the chitinized armoured tubes that form part of the cirri are visible furthest right. E. Detail of the left posterior and anterior hook (hook length 273 µm) of the male recovered from the trachea (No. 2.3/98). The hooks are flat-topped with only a slight indent in the dorsal margin. The anterior apodemes are lobe-like and permeated by pores. F. Oral cadre of specimen No. 2.3/98 (overall length 405 µm) showing the numerous pores around the pharynx as well as the large anterior flanges.

Results

Leiperia cincinnalis is a common pentastome in the Nile crocodile and occurred in five out of six crocodiles examined in this study. Table 7 summarises the numbers and developmental stages recovered from the respective hosts.

Table 7. The numbers and developmental stages of *Leiperia cincinnalis* recovered from Nile crocodiles in the Kruger National Park.

Host number	Developmental stage				Total
	Females	Males	Pre-adults	Infective larvae	
A/95	3	0	0	3	6
B/95	15	0	7	57	79
1/98	0	6	30	12	48
2/98	2	1	0	0	3
3/98	0	0	0	0	0
4/98	7	10	22	33	72

Three adult *L. cincinnalis* females were attached to the trachea and three infective larvae recovered from the pulmonary artery of Crocodile A/95 while Crocodile B/95 harboured 15 patent females in the trachea and bronchi (Figure 4A). A total of 64 immature instars were recovered from the lungs, the heart and mainly the aorta pulmonalis, where the immatures occurred in two clusters.

A single infective larva of *L. cincinnalis* was found in the lungs of Crocodile 1/98, and a cluster of 47 specimens, representing different developmental stages together with cast cuticles, were recovered from the aorta pulmonalis. Six of the *Leiperia* were fully-developed males. In two other specimens well sclerotized cirrus tips were visible, but the single hooks, the oral cadre and the copulatory spicules were hardly chitinized. Eleven of the parasites were infective nymphs carrying double hooks. Many of the specimens with simple hooks were still surrounded by the old cuticula of the infective larva.

Crocodile 2/98 harboured two female *L. cincinnalis* in the trachea and a single male was found attached next to one of the females. The cirrus tips were absent and the anterior part of the spatulate extension of both the copulatory spicules projected through the anterior genital opening. One of the cirrus tips was seen in the anterior third of the vagina of female No. 2.1/98 (Figure 5F) and the vaginal lips of the latter specimen were still everted (Figure 5E). The female was 4.5 cm long and 1 mm wide, and the abdomen was only just beginning to coil. No *Leiperia* were found in the pulmonary artery or the lungs.

No *L. cincinnalis* were recovered from Crocodile 3/98, but Crocodile 4/98 harboured 72 specimens. Eleven females, ranging in body length from 4.5 to 10.5 cm, were attached to the trachea and the uteri of four, all of them less than 6 cm long, contained no eggs, but the latter were present in the remainder. Two prepatent *Leiperia* females were found in the lungs but were damaged in the recovery process. The remaining 59 specimens were collected from the pulmonary artery were they formed a cluster similar to that found in Crocodile 1/98. A total of 15 males were isolated from this cluster; 10 of these were fully mature, but in the remainder the copulatory spicules were not yet fully developed. One of the mature males was still within the cuticula of the infective larva and the simple hooks of another preceding instar were visible. The sex of another 11 *L. cincinnalis*, with simple hooks, remained undetermined. In addition, 33 infective larvae were present.

DESCRIPTION OF *LEIPERIA CINCINNALIS* (VANEY & SAMBON, 1910) SAMBON, 1922

(Tables 8, 9)

Females (Table 8)

The general morphology of the females of *L. cincinnalis* examined in this study conformed largely to that described by Riley & Huchzermeyer (1996). Additional and comparative data are presented in Table 8.

Every fully-mature, gravid female examined (n = 6) possessed hooks with a prominent dorsal notch (Figure 4B,C). Two females recovered from the trachea

(No. 2.1/98 and No. 4.7/98, 5.5 cm long) had hooks marked by only a slight indentation in the dorsal surface. These specimens were considerably shorter and had not reached patency. The hooks, as well as measurements of the oral cadre of one specimen (No. 4.7/98), were slightly smaller than those of the patent females. An immature female from the aorta pulmonalis had flat-topped hooks, while those of another specimen (No. 2.1/98) could not be measured.

Males (Table 9)

Well-fixed male specimens possessed a straight, cylindrical abdomen, tapering slightly to a rounded end. The male recovered from the trachea was 2.0 cm long while the body length of 3 males taken from the aorta ranged from 1.8 to 2.0 cm.

Heavily chitinized fulcra supported the prominent hooks (Figure 4E). In comparison to the length of the hooks, the fulcra appeared rather broad and compact. The anterior apodeme of the hooks widened into a lobe-like structure permeated by numerous pores (Figure 4E).

A certain degree of variabilty was observed in the hook morphology of male *L. cincinnalis* (n = 17). In some specimens the hooks were flat-topped, in others only slight depressions were visible and in yet others, hooks had a distinct dorsal notch.

Flat-topped hooks and hooks with a dorsal notch could at times be seen in the same specimen. The hooks and fulcra of the males were considerably shorter than those of the females and the measurements were actually nearer to those of the infective larvae (Junker, 1996). However, their gross morphology closely resembled that of females. This was true of the oral cadre with its characteristic *Leiperia* shape (Figure 4F). Starting in the posterior third of the oral cadre, the lateral prongs of the oral cadre converged gradually, giving it a V-shaped profile (Riley & Huchzermeyer, 1996).

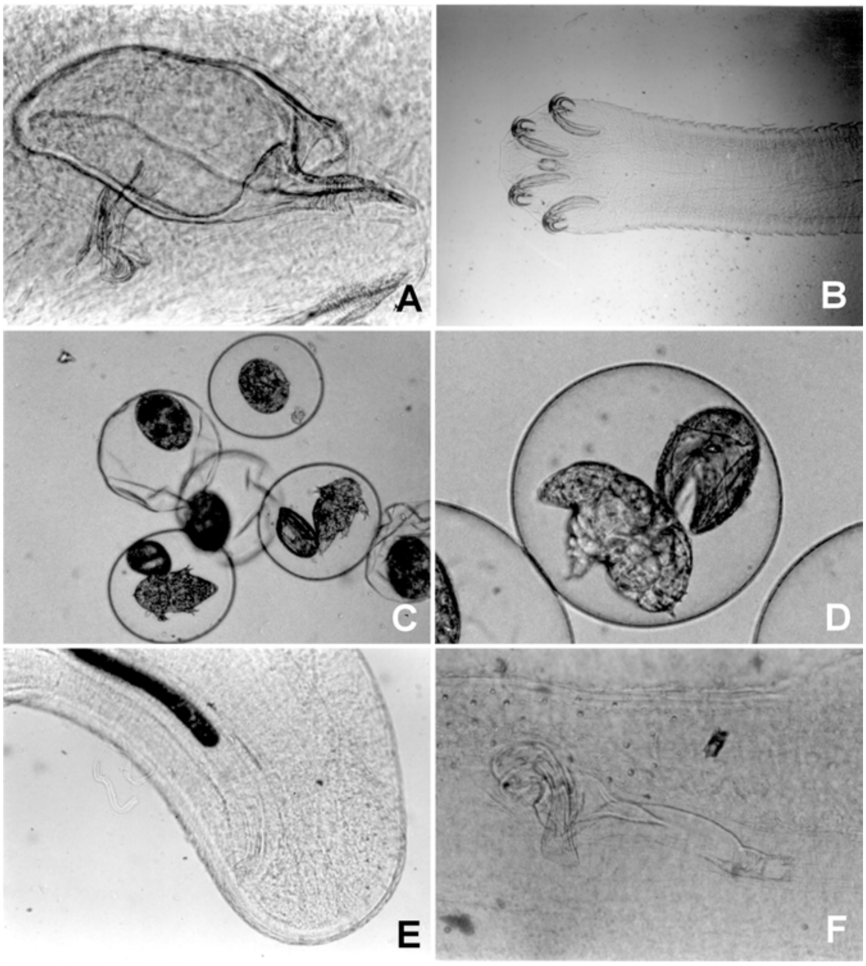

Figure 5. *Leiperia cincinnalis*. A. Right copulatory spicule (No. 4.19/98, total length 757 µm). The collar around the spatulate extension is heavily chitinized and carries rings of chitin. The cirrus tip is flared and accompanied by a strongly-chitinized scoop-like structure. B. The anterior part of an infective larva, showing the large double-hooks and the comparatively small oral cadre. C. Eggs of *L. cincinnalis* (outer shell diameter 233 µm) and two hatching primary larvae. D. Detail of a primary larva (length 101 µm) and split inner egg membrane. E. Posterior end of female (No. 2.1/98) found *in copula* showing the eversed vaginal lips. Part of the ruptured cirrus, visible as a transparent thread (left), is next to the abdomen. F. Cirrus tip of the male located in the vagina of No.2.1/98. Note the prominent chloride cell pore caps on the cuticula of the female.

Table 8. The main characteristics of female *Leiperia cincinnalis* recovered from Nile crocodiles in the Kruger National Park in 1995 and 1998. For comparative purposes data of Riley & Huchzermeyer (1996) and Junker (1996) are included. All measurements are in micrometres unless otherwise indicated.

Source	Specimen number	Number of annuli	Body length (mm)	Mouth dimensions			Hook dimensions			
				Overall length	Cadre length	Width	Hook length	Base length[b]	Hook depth	Fulcrum length
This paper	4.1/98	NM	105	920	727	285	NM	NM	NM	NM
	4.3/98	NM	100	925	750	322	313	259	NM	NM
	4.7/98[a]	NM	55	690	552	212	262	203	86	528
	4.9/98	NM	95	NM	NM	NM	353	267	94	644
	2.1/95	NM	NM	925	736	308	NM	NM	NM	NM
	2.2/95	NM	NM	925	745	281	NM	NM	NM	NM
Junker 1996	CWT2	NM	131	NM	NM	NM	354	NM	NM	645
	CWT3	100	110	824	689	275	348	NM	NM	651
	Lei3	100	120	915	750	NM	355	NM	NM	703
Riley & Huchzermeyer 1996	1947.12.1.57-59	NM	NM	NM	NM	NM	480	220	NM	810
	1927.11.15.28-30	NM	NM	NM	NM	NM	463	218	NM	705
	1932.7.22.1	NM	85?	1020	850	375	400	180	NM	640

[a] = uterus devoid of eggs, NM = not measured, [b] = The term "base length" in this paper refers to the same structure as the term "hook depth" used by Riley & Huchzermeyer (1996).

Table 9. The main characteristics of the males of *Leiperia cincinnalis* recovered from Nile crocodiles in the Kruger National Park. For comparative purposes data of three immature males (1927.**III**.10-6-11) examined by Riley & Huchzermeyer (1996) are included. All measurements are in micrometres.

Specimen number	Mouth dimensions			Hook dimensions				Copulatory spicules				
	Overall length	Cadre length	Width	Hook length	Base length	Hook depth	Fulcrum length	Total length	Cowry shell length	Width	Length of armoured tube	Number of grooves
4.18/98	432	340	106	194 b	150 b	90 b	420 b	782	607	NM	104	14
4.19/98	405	306	120	NM	NM	NM	NM	757	612	NM	113	13
4.20/98	426	338	113	232 a	166 a	87 a	437 a	782	605	207 a	108	14 a
4.21/98	474	363	113	232 a	163 a	90 a	426 b	814	637	NM	115 a	11
4.22/98	465	366	NM	240 b	166 b	78 b	447 b	817	621	202 a	124 a	13
4.23/98	460	343	124	216 a	161 a	NM	431 c	780	614	NM	115 a	13
4.24/98	458	336	129	196 b	153 b	NM	411	821	623	NM	127	12
4.25/98	451	331	120	189 a	145 a	NM	423 c	819	621	NM	133 a	13
4.26/98	449	343	133	NM	NM	NM	432 b	803	616	NM	120	14
4.27/98	446	331	120	244 b	174 b	NM	407 a	798	623	NM	133 a	16
1.16/98	426	317	104	NM	NM	NM	428 b	NM	637	NM	110 a	NM
1.17/98	NM	NM	NM	216 a	152 a	90 a	NM	791 a	607	NM	104	NM
1.18/98	NM	NM	NM	255 a	182 a	90 a	437 a	805 a	649 a	NM	NM	NM
1.19/98	462	340	110	245 c	171 c	87 c	NM	837	637	NM	108	14
1.20/98	497	386	115	206 b	158 b	93 b	NM	787 a	616 a	NM	101 a	NM
1.6/98	492	386	136	245 c	169 c	82 c	453 a	844	628	NM	129	12
Average	453	345	119	224	162	87	429	802	622	205	116	13
SD	26	23	10	23	11	5	14	24	13	NM	11	1
2.3/98	405	320	127	273	170 c	81 c	NM	780	614	NM	NM	13 a
1927.III.10-6.11	NM	NM	NM	290	NM	115	645	NM	NM	NM	NM	NM
	NM	NM	NM	280	NM	120	600	NM	NM	NM	NM	NM
	430	280	125	NM	NM	120	580	NM	NM	NM	NM	NM

[a] = only one hook/copulatory spicule measured, [b] = only two hooks measured, [c] = only three hooks measured, NM = not measured.

Both the large anterior flanges, as well as the area between the sclerotised supports of the pharynx, possessed the same pores as females (Riley & Huchzermeyer, 1996) (Figure 4B,F).

The paired copulatory spicules were strongly chitinised and the spatulate anterior extension carried an average of 13 rings of chitin folds while the collar around the neck of latter extension was marked by heavy chitinous rugosities (Figures 4D, 5A, 6A,B). According to Riley & Huchzermeyer (1996) the cirrus tip was “a flattened trumpet of longitudinally-striated chitin” (Figures 5A, 6A,C) and the whole structure was demarcated from the remaining unmodified section of the cirrus by “a short armoured tube supported by rings of chitin”, as described for *Leiperia australiensis* Riley & Huchzermeyer, 1996 by the latter authors. Parallel to and in close association with the trumpet, a second chitinous structure was visible (Figures 5A, 6C). This structure gave the impression of a scoop with serrated sides rolled up towards the mid-line. It was very similar in shape to the cirrus tip of *Sambonia lohrmanni* (Sambon 1910) Noc & Giglioli, 1922 (see Fain, 1961). While often separated under cover slip pressure, in some specimens the trumpet ran through the scoop and the latter served as a support.

Discussion

Rodhain & Vuylsteke (1932) described tufts of double- as well as single-hooked larvae of *L. cincinnalis* attached to the aorta of a crocodile from the Congo. Their reference to the genital apparatus being more developed in the single-hooked specimens suggested the presence of males, but, unfortunately, these authors did not describe them. Thus it was only recently that males of the genus *Leiperia* were described and those belonged to the Australian species *L. australiensis* (Riley & Huchzermeyer, 1996).

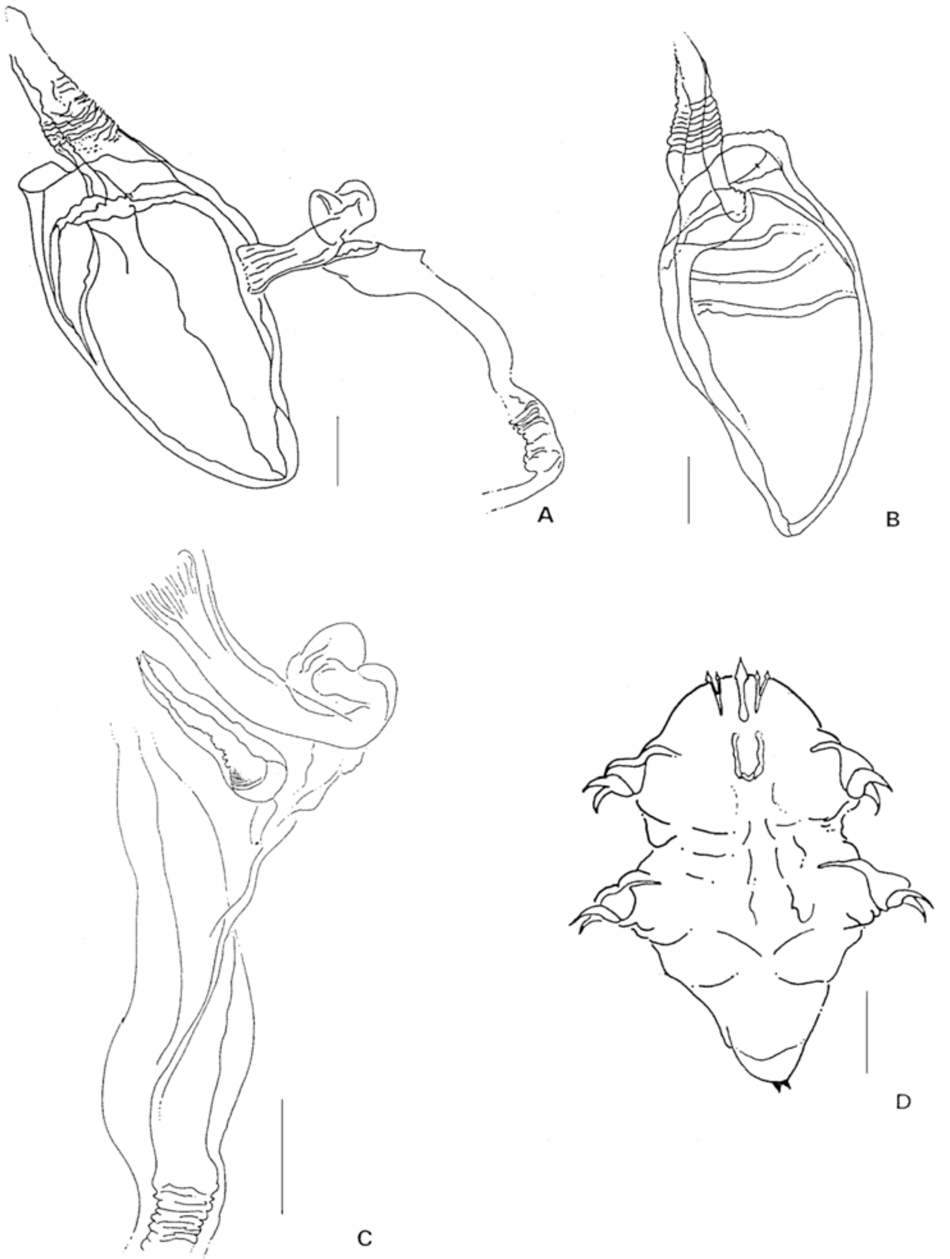

Figure 6. *Leiperia cincinnalis* A. Right copulatory spicule and cirrus tip (No. 4.18/98). B. Left copulatory spicule (No. 2.3/98). C. Detail of cirrus tip (No. 4.27/98). D. Primary larva showing the penetration apparatus, the U-shaped oral cadre, the clawed limbs and the tail carrying two minute terminal thorns. *Scale-bars*: A-C, 100 μm; D, 25 μm.

In terms of gross morphology, the copulatory spicules of *L. cincinnalis* males examined during this study and those of *L. australiensis* are very similar (Riley & Huchzermeyer, 1996). The cirrus tip in particular is very distinctive and links the genus *Leiperia* closely to the genus *Sambonia* Noc & Giglioli, 1922 in which the anterior part of the cirrus is similarly formed (Fain, 1961). The collar around the spatulate extension of the copulatory spicules of *L. cincinnalis* does not extend as far to the anterior tip of the spicules as the double collar found in *L. australiensis*. Although the copulatory spicules of *L. cincinnalis* are larger than the ones of *L. australiensis,* the hook and mouth dimensions are quite similar.

Riley & Huchzermeyer (1996) compared the hook morphology of females of *L. cincinnalis* from Uganda and the Congo. Both Ugandan specimens had flat-topped hooks, but the hooks of the two Congo specimens, one of them described by Fain (1961), were characterised by a dorsal notch. Riley & Huchzermeyer (1996) speculated that the difference in hook morphology might indicate geographical variation, but pointed out that the data set was too small to draw any final conclusions.

The present collection comprises male and female *Leiperia*, some of which carry flat-topped hooks and some of which possess hooks with a dorsal notch; sometimes both hook types occur in the same specimen. Visibility of the dorsal notch in the hooks can be influenced by the orientation of the hooks under cover-slip pressure and, especially in females, it becomes more prominent as the specimens grow and mature. Measurements of the males and females were relatively uniform, especially the dimensions of the copulatory spicules, and gave no indication of the presence of two species. Our findings suggest that all the specimens are *L. cincinnalis*.

This, however, does not exclude the existence of a second *Leiperia* species in Africa, especially in view of the fact that the flat-topped hooks of the Ugandan specimens were considerably larger than the notched hooks of specimens collected from South Africa. The size of the hosts, as well as the intensity of infection, may influence the final size reached by the parasites, but our data do not support this speculation. Morphometric analysis of mature females taken from the heavily infected crocodile from the Shimuwini Dam, from the moderately infected Crocodile 4/98, and from the lightly infected Crocodile 2/98 did not differ substantially. Unfortunately, the oral cadres of the *Leiperia* from Uganda could not be measured and no male specimens were available for comparison.

3.2 DESCRIPTION OF *PELONIA AFRICANA* N. G., N. SP.

Introduction

During the course of this study the two South African terrapins, *Pelusios sinuatus* and *Pelomedusa subrufa*, were obtained from pans or marshy areas near the Arabie Dam, Northern Province and a pentastome from the lungs of these hosts was described (Junker & Boomker, 2002). Specific data on collection dates and details on the hosts are provided in Chapter 6. Three more pentastomid specimens, WIII/1, and Psub2/1 and Psub3/1 from the lungs of *P. sinuatus* and *P. subrufa* respectively, were collected during another unrelated study at the same locality and made available to the author.

The majority of the six genera comprising the family Sebekidae Fain, 1961 occur exclusively in crocodilians. However, it has been speculated that a single species of the genus *Sebekia* Sambon, 1922 may also reach maturity in piscivorous turtles (Dukes, Shealy & Rogers, 1971). Until now, only the South American genus *Diesingia* Sambon, 1922 has been known to be exclusive to a chelonian definitive host (Sambon, 1922; Heymons, 1941a; Overstreet, Self & Vliet, 1985; Riley, 1994) and, generally speaking, information on the pentastome fauna of tortoises, terrapins and turtles is scarce.

Fain (1961) refers to a nymphal pentastome found encysted in the liver of *Kachuga lineata*, a semi-aquatic oriental tortoise, which Hett (1924) assumed to be the infective larva of *Subtriquetra megacephala* (Baird, 1853) Sambon, 1922. The latter genus belongs to the family Subtriquetridae Fain, 1961, which is also believed to be exclusive to crocodilians (Riley, 1986; Winch & Riley, 1986a; Junker *et al*., 1998a). In addition, some five genera of terrapins from North America have been reported to harbour nymphs of *Sebekia mississippiensis* Overstreet, Self & Vliet, 1985, a crocodilian pentastome described from the American alligator, *Alligator mississippiensis*, (Dukes *et al*., 1971; Boyce, 1985;

Overstreet *et al*., 1985). Significantly, all the literature cited above pertains to nymphal developmental stages of pentastomes.

It would appear that *Diesingia megastoma* (Diesing, 1836) (Sambon, 1922) is currently the only pentastome of which mature specimens have been recovered from the chelonian hosts, *Hydromedusa tectifera* and *Phrynops geoffroanus* (= *Hydraspis geoffroyana*) from Brazil (Diesing, 1836; Heymons, 1941a; Da Fonseca & Ruiz, 1956; Self & Rego, 1985). The author is not aware of any publications dealing with adult pentastomid parasites of chelonians from any other continent.

Pelonia africana n. g., n. sp. shares morphological similarities with all the other genera of the family Sebekidae, but possesses a unique combination of diagnostic criteria. Slide-mounted specimens of *D. megastoma* were re-examined and found to be distinctly different from the pentastomes recovered from the South African terrapins. It was thus considered appropriate to erect a new genus and species to accommodate these specimens.

Results

DESCRIPTION OF *PELONIA AFRICANA* N. G., N. SP. (Tables 10, 11)

TYPE HOSTS AND LOCALITY

Pelusios sinuatus and *Pelomedusa subrufa* from the Arabie Dam (24°53' S, 29°22' E), Northern Province, South Africa.

TYPE MATERIAL

Holotype male, no. T 2186 from *Pelomedusa subrufa*, allotype female, no. T 2187 from *Pelomedusa subrufa* and paratypes (immature) from *Pelomedusa subrufa* and *Pelusios sinuatus*, no. T 2188. All specimens are mounted in Hoyer's medium and deposited in the National Collection of Animal Helminths at the ARC-OVI, Onderstepoort, South Africa.

ETYMOLOGY

Pelonia has been named after its two host species that belong to the family Pelomedusidae which comprises freshwater chelonians from Africa, Madagascar and southern Australia.

DESCRIPTION

The body is claviform, the abdomen being widest in the anterior third and tapering to a bluntly rounded caudal tip. Ventrally the small trapezoid cephalothorax is continuous with the ventrally flattened abdomen but dorsally demarcated from the remainder of the body. A small number of wide annuli are present.

Females

The strongly chitinised oral cadre is more or less U-shaped, the gap between the lateral prongs only slightly narrowing anteriorly. Muscle contraction or the amount of pressure applied when mounting, can result in a more ovoid profile. The oral cadre appeared to be open anteriorly as the delicate chitinous fibres connecting the two sides were difficult to see. A heavily chitinised, bowl-shaped base unites the two lateral prongs posteriorly (Figure 7B, C). The oral cadre of the allotype female was slightly larger than that of the two immature specimens, WIII/1 and Psin2/1.

The smooth hooks are dorsally convex with a slight dorsal notch where the strongly curved blade emerges from the base (Figure 7F). The configuration of the posterior and anterior hooks appear to be identical and are supported by strong fulcra. Unfortunately, measurements could only be made from a single hook from an immature female.

It was not possible to decide whether the females were heterogynous, with the utero-vaginal pore being situated one or two annuli anteriorly from the anus, or ophistogynous. The eggs of *P. africana* consist of a spherical outer membrane, 183 ± 8 μm in diameter that surrounds a hyaline substance and an ovoid inner

Table 10. The main diagnostic characteristics of *Pelonia africana* n. g., n. sp. and comparative measurements of *Diesingia megastoma* and *Sebekia wedli*. All measurements are given in micrometres unless otherwise stated.

Specimen number	Body length (mm)	Number of annuli	Mouth dimensions			Hook dimensions		Copulatory spicules		
			Overall length	Cadre length	Width	Hook length	Fulcrum length	Total length	Cowry shell length	Width
WIII/1 (Paratype F, T 2188)	15	28	322	248	127	NM	NM	NA	NA	NA
Psin2/1 (Paratype F, T 2188)	13	27	313	216	133	115	239	NA	NA	NA
Psub2/1 (Allotype F, T 2187)	27	30	380	301	182	NM	NM	NA	NA	NA
Psub3/1 (Holotype M, T 2186)	9	27	265	207	150	NM	NM	515	324	214
Diesingia megastoma M (After Heymons 1941)	7	70	NM	524	205	NM	NM	NM	NM	NM
Diesingia megastoma M (After Heymons 1941)	6	70	NM	496	180	140	NM	NM	NM	NM
Diesingia megastoma F (After Self & Rego 1985)	10	65	NM	670	380	140*	520	NA	NA	NA
Sebekia wedli F (After Riley & Huchzermeyer 1995a)	15 - 19	NC	355	229	121	80	176	NA	NA	NA
Sebekia wedli M (After Riley & Huchzermeyer 1995a)	8	NC	212	136	76	59	134	310	213	115

F = female, M = male, NA = not applicable, NC = not counted, NM = not measured, * = only the length of the blade was measured eggshell, 96 ± 7 µm long and 70 ± 4 µm wide (Figure 7G), that encloses the primary larva.

Males

Although the oral cadre of the male is slightly smaller than those of the two immature females (Figure 7A), its general morphology, as well as that of the hooks and the fulcra, is similar to that of the females. The paired copulatory spicules are heavily sclerotised and cowry-shell shaped (Figure 7D). The anterior spatulate extension carries rows of rounded, chitinous teeth, which become progressively indistinct towards the tip (Figure 7E).

Table 11. The main diagnostic criteria of additional males of *Pelonia africana* n. g., n. sp. collected from *Pelusios sinuatus* and *Pelomedusa subrufa* from the Arabie Dam. All measurements are in micrometres. The top figures as regards the copulatory spicules refer to the left, the bottom figures to the right spicules.

Specimen number	Mouth dimensions			Hook dimensions				Copulatory spicules		
	Overall length	Cadre length	Width	Hook length	Blade length	Base length	Fulcrum length	Total length	Cowry shell length	Width
Psin7/1	271	202	129	95	59	38	NM	492 520	331 363	NM NM
Psin8/1	290	219	136	116	63	45	NM	515 529	345 354	NM 193
Psin9/1	265	205	115	98	61	40	NM	483 451	350 350	NM 166
Psub4/1	327	239	133	104	61	44	222	538 561	363 382	NM 189
Psub4/2	285	212	122	113	63	41	NM	547 529	354 331	NM NM

NM = not measured

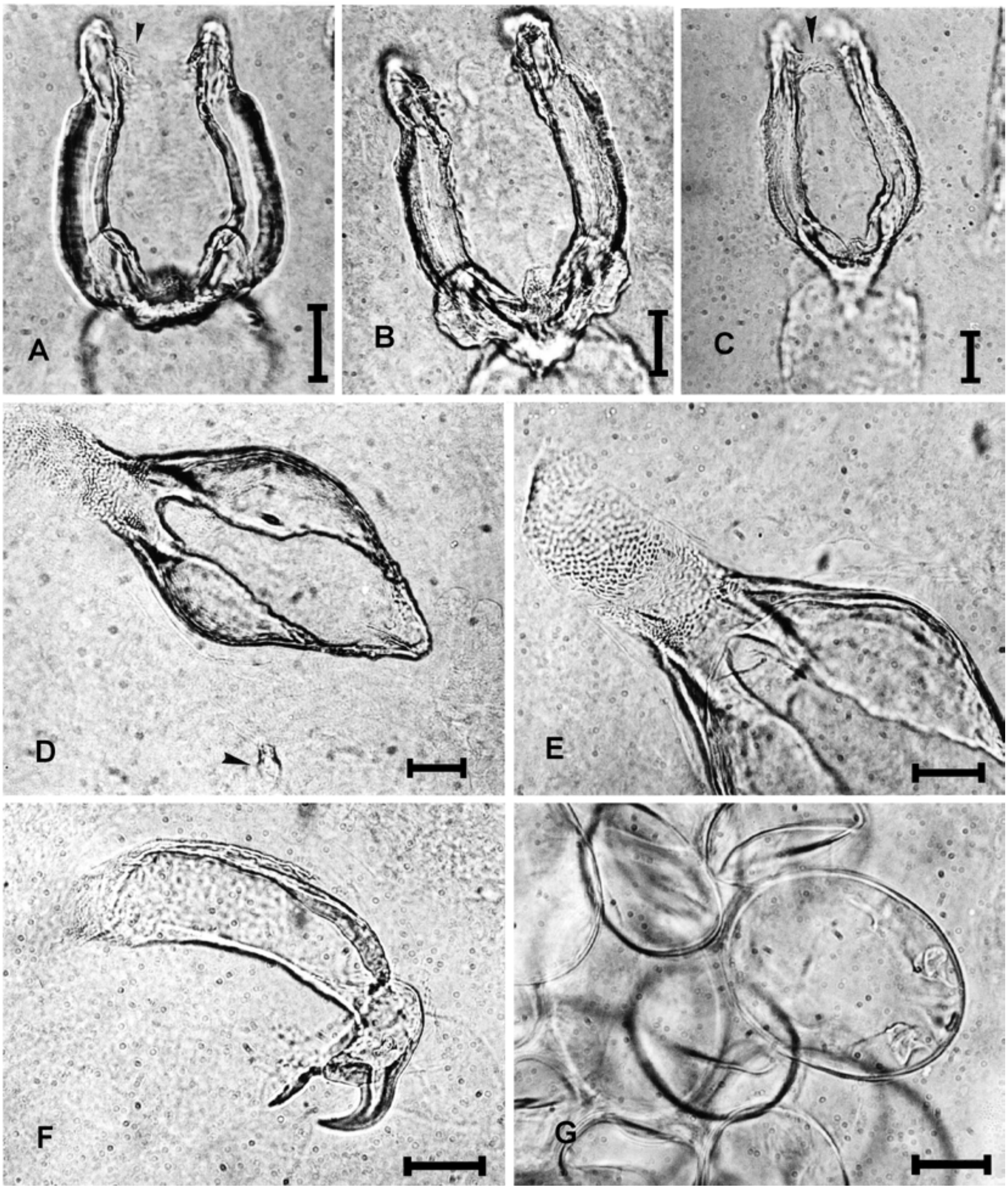

Figure 7. *Pelonia africana* n. g., n. sp.. A. Oral cadre of holotype male. The delicate chitinous fibres connecting the lateral prongs of the oral cadre anteriorly are partly visible (arrow). Scale bar: 50 μm. B. Oral cadre of the allotype female. The anterior chitinous bridge is not visible in this photograph. Scale bar: 50 μm. C. Oral cadre of an immature female. The chitinous fibres connecting the lateral prongs are clearly visible (arrow). Scale bar: 25 μm. D. Ventral view of the right copulatory spicule of the holotype male. It is obpyriform and the spatulate extension carries small chitinous teeth. The arrow marks a chitinous part of the cirrus. Scale bar: 50 μm. E. Detail of the left copulatory spicule. F. Right posterior hook of an immature female. Scale bar: 50 μm. G. Egg with fully developed primary larva. Scale bar: 50 μm.

Discussion

The body-shape of *P. africana* corresponds closely to the illustration of a mature female of *D. megastoma* from *H. tectifera* (Self & Rego, 1985). One of the main differences between *P. africana* and *D. megastoma* lies in the oral cadre. That of *D. megastoma* is more than twice the length and width than that of the African species. Own observations show the oral cadre of *Diesingia* to carry a small peg-like extension into the oesophagus, not unlike that of the genus *Alofia* Giglioli, 1922, which is absent in specimens of *Pelonia*. Furthermore, the prominent bowl-shaped chitinous structure at the base of the oral cadre of the latter genus is absent in *Diesingia*.

Both genera of chelonian pentastomes possess smooth hooks. However, the fulcra of *Pelonia* are devoid of any extensions, while the fulcra of *Diesingia* are furnished with cowl-like extensions, similar to those seen in the genus *Selfia* (Riley, 1994). The hooks of *D. megastoma* appear to be flat-topped with a sharply curved blade, demarcated from the shank by a single notch (Self & Rego, 1985), while those of *P. africana* are dorsally convex and marked by a slight dorsal notch.

So far, no conclusive description of the copulatory spicules of *Diesingia* has been given, and the two male specimens we examined, possess copulatory spicules that are unique among the members of the family Sebekidae. The cowry-shell shaped base and the long spatulate extension of the open side are reminiscent of other sebekiid genera (Riley *et al*., 1990), but the shorter of the two anterior extensions has been transformed into a tiller-like, chitinous spike.

The genus *Pelonia* is distinct from the genus *Diesingia*, and represent the first record of a new genus of pentastomes exclusive to chelonian final hosts from the African continent.

Pelonia africana is similar to the African crocodilian pentastome *Sebekia wedli* Giglioli, 1922 in Sambon, 1922. This is especially true for the oral cadre, which in the latter species is also approximately U-shaped, and because of an almost invisible, very delicate anterior bridge of chitin, it seems to be open anteriorly (Riley & Huchzermeyer, 1995a). A comparison of measurements, however, shows the buccal complex of *S. wedli* to be slightly smaller than that of *P. africana* (Riley & Huchzermeyer, 1995a, Junker *et al*., 1998b). The copulatory spicules of male *P. africana* are strongly reminiscent of *S. wedli*, and they could easily be confused, in that both are obpyriform and carry chitinous teeth on the spatulate extensions. Nevertheless, the spicules of *P. africana* are markedly larger than those of *S. wedli* (Riley & Huchzermeyer, 1995a).

The main distinguishing character between *P. africana* and *S. wedli* is the absence of the prominent spines on the dorsal hook surface. The lack of anterior extensions to the fulcra further serves to separate *Pelonia* from the other species of the genus *Sebekia* as defined by Riley *et al*. (1990).

Superficially, the aspinose hooks, the curve of the blade and the shape of the oral cadre might lead to confusion of *P. africana* with the *Alofia* spp.. The copulatory spicules of *P. africana*, however, lack the double-hooked collar diagnostic for *Alofia* and the genus *Selfia* Riley, 1994 (Riley, 1994). Moreover, the oral cadre neither possesses the distinct, open alofian U-shape nor the peg-like extension into the oesophagus (Riley & Huchzermeyer, 1995a, b; Junker, Boomker & Bolton, 1999).

Recently the new pentastomid genus *Agema* Riley, Hill & Huchzermeyer, 1997 has been described from African dwarf crocodiles, *Osteolaemus tetraspis osborni*, and slender-snouted crocodiles, *Crocodylus cataphractus* (Riley, Hill & Huchzermeyer, 1997). While the hooks of *P. africana* exhibit the already mentioned abrupt right-angle bend near the base, those of *Agema* are very smoothly curved and the ovoid oral cadre of the latter genus is closed anteriorly by prominent chitinous crescents (Riley *et al*., 1997).

Pelonia africana morphologically resembles especially the genus *Sebekia* and to a lesser extent the genus *Alofia*. Heymons (1941b) pointed out the similarity between *D. megastoma* and its South American *Sebekia*n and alofian counterparts. Therefore there is a strong case for the inclusion of the genera *Pelonia* and *Diesingia* into the family Sebekidae, as was suggested for the latter genus by Riley (1993). The fact that all sebekiid genera have a similar life-cycle using fish as intermediate hosts and semi-aquatic definitive hosts (Fain, 1961; Riley, 1986, 1994; Riley *et al*., 1997) indicates a close relationship between the two genera parasitising chelonians and those of crocodilians.

3.3 REDESCRIPTION OF *DIESINGIA MEGASTOMA* (DIESING, 1836) SAMBON, 1922 FROM THE SOUTH AMERICAN TERRAPIN *HYDROMEDUSA TECTIFERA*.

Introduction

Despite their discovery more than one-and-a-half centuries ago, the present knowledge on pentastomes parasitising chelonian final hosts is scant. A single species, *Diesingia megastoma* (Diesing, 1836) Sambon, 1922, from South American terrapins was recognized, and it was first described from two adult males recovered from the lungs of a Geoffrey's side-necked turtle, *Phrynops geoffroanus* (syn. *Hydraspis geoffroyana*), in Brazil by Diesing in 1836 as *Pentastoma megastomum* (Sambon, 1922; Fain, 1961). Since then, it has caused much confusion. The two original specimens, kept at the Museum of Natural History at Vienna, have been renamed and re-examined by several authors. Leuckart (1860) referring to Diesing's description only changed the name to *Pentastomum megastomum*, while Shipley (1898) examined the Vienna specimens himself. Without adding much detail to the initial, very superficial description, Shipley (1898) redescribed them as *Porocephalus megastomus*, a name previously given to them by Stiles (1893, in Sambon 1922) in accordance with the then common trend to include the majority of the exotic pentastomes in the genus *Porocephalus* Humboldt, 1811 (Heymons, 1941a). When Sambon (1922) reviewed the known pentastomid genera, Diesing's type specimens no longer fitted the diagnostic criteria of the genus *Porocephalus* and he suggested the inclusion of *P. megastomum* in the new genus *Diesingia*. This genus had been created to accommodate *Diesingia kachugensis* (Sambon, 1922) from the liver of *Kachuga lineata*, a semi-aquatic oriental tortoise. Riley (1986) pointed out that Hett (1924) considered *D. kachugensis* to be the larval stage of *Subtriquetra megacephala* (Baird, 1835) Sambon, 1922, a crocodilian pentastome. Heymons (1941a) was the first author to provide a more detailed description of the type specimens for which the name *D. megastoma* had become accepted, and included measurements and drawings of the hooks and the oral cadre. Da Fonseca & Ruiz (1956) created the genus *Butantanella* to accommodate *D. megastoma* and the specimens of a collection of pentastomes recovered from the lungs of 15 *Hydromedusa tectifera* from Brazil.

Self & Rego (1985) examined pentastome specimens from collections from the Instituto Oswaldo Cruz and from the British Museum. These included fully mature males and females from the lungs of the Brazilian terrapins, *H. tectifera* and *P. geoffroanus*, and Self & Rego (1985) concluded that these were identical with Diesing's specimens. On morphological grounds, Self & Rego (1985) reclassified *D. megastoma* as *Sebekia megastoma*, dismissing Da Fonseca & Ruiz's (1956) previous classification.

The uncertainty surrounding its type species led to considerable confusion as regards the systematic status of the genus *Diesingia*. Fain (1961) included it in the family Sebekidae, whereas Riley (1983), in an outline classification of the pentastomes, placed the genus separately, directly following the genera of the family Sambonidae, substituting the family name by a question mark. Subsequently, Self & Rego (1985) abandoned *Diesingia* in favour of the genus *Sebekia* Sambon, 1922, a view that was not adopted by Riley (1986) who created the monogeneric family Diesingidae. In a later publication, Riley (1994) placed *Diesingia* back into the family Sebekidae.

In this paper slide-mounted specimens of *D. megastoma*, collected from *H. tectifera* from Brazil are described. Based on our findings we support the validity of the genus *Diesingia* as well as its inclusion in the family Sebekidae.

Material and Methods

All specimens described here were originally recovered from *H. tectifera* from Brazil by an unknown collector. The collection comprises slide-mounted specimens as well as specimens that had previously been fixed in formalin (Da Fonseca & Ruiz, 1956), but are now preserved in alcohol. These are, however, in an extremely poor condition and very brittle. The anterior and posterior hooks had been removed and mounted in Hoyer's medium several years ago and the bottle with alcohol specimens now contains only the abdomina of the specimens F2463-5, F2463-6, and KI-3. These are mature females, as evidenced by hook measurements and the egg-filled uteri of two specimens.

The *D. megastoma* material studied comprises the following:

F2463-2. A male specimen, mounted whole and stained with a chromatin stain. The slide was originally labelled "*Butantanella megastoma*, from the Instituto do Butantan ex *Hydromedusa tectifera*" (see below).

F2463-3. A young female, mounted whole and originally identified as "Butantan F 5960, *Butantanella megastoma* of the lung of *H. tectifera*, from the Paraná State, Brazil". The slide is dated 7 December 1951 and carries the names Da Fonseca & Ruiz.

We conclude that F2463-2 and F2463-3 are two of the specimens on which Da Fonseca & Ruiz (1956) based their description of *B. megastoma*. In fact, Da Fonseca & Ruiz (1956) state, that specimen 5960 forms part of a collection of male and female pentastomes taken from the lungs of 15 *H. tectifera* in August 1951 at Tranqueira, Paraná State.

F2463-4. A male specimen. The abdomen, including the copulatory spicules, is mounted on one slide and a second slide contains the posterior and anterior hooks, as well as the oral cadre. The latter, however, is severely damaged.

F2463-5 and F2463-6. The hooks of two females were dissected out and the posterior and anterior hooks are mounted under separate cover slips on the same slide. The oral cadres of both specimens have apparently been lost.

KI-3. The whole slide-mounted cephalothorax of a mature female.

Results

DESCRIPTION OF *DIESINGIA MEGASTOMA* (DIESING, 1836) SAMBON, 1922

(Table 12, 13)

The abdomina of two alcohol preserved females are slender and elongated with pointed caudal tips that appear slightly curled ventrally. Since the only intact specimens are slide-mounted, little else can be said about the body shape. In the male specimens F2463-4 and F2463-2, as well as in the female F2463-3, a single row of chloride cells with prominent pore caps is present on the anterior border of each annulus.

Females

According to its slide label specimen F2463-6 was 1,5 cm in length prior to the dissection of hooks from its cephalothorax. The single prepatent female, F2463-3, has 55-60 annuli, but since the caudal tip is slightly damaged accurate counting is difficult. The same specimen possesses smooth hooks with long, slender, canaliculated blades that are sharply curved. A slight, but distinct, notch demarcates the blade from the flat dorsal hook surface. While the anterior and posterior hooks are equal in size, the morphology of their fulcra differs in that all possess a cowl-like anterior extension, but that the cowl of the anterior fulcra is spinose, while those of the posterior ones are smooth (Figure 8E, F; 2C, D). The hooks of three mature females are distinctly larger but otherwise similar to those described above. The cowl of the fulcrum is not always readily visible. The posterior hooks of specimen F2463-6 clearly show the cowl (Figure 9D) and a structure next to one of the anterior hooks is assumed to be the broken off spinose extension of the fulcrum. The second anterior hook of specimen F2463-6 and the hooks of specimens F2463-5 and KI-3 are in too poor a condition to observe any extensions to the fulcra.

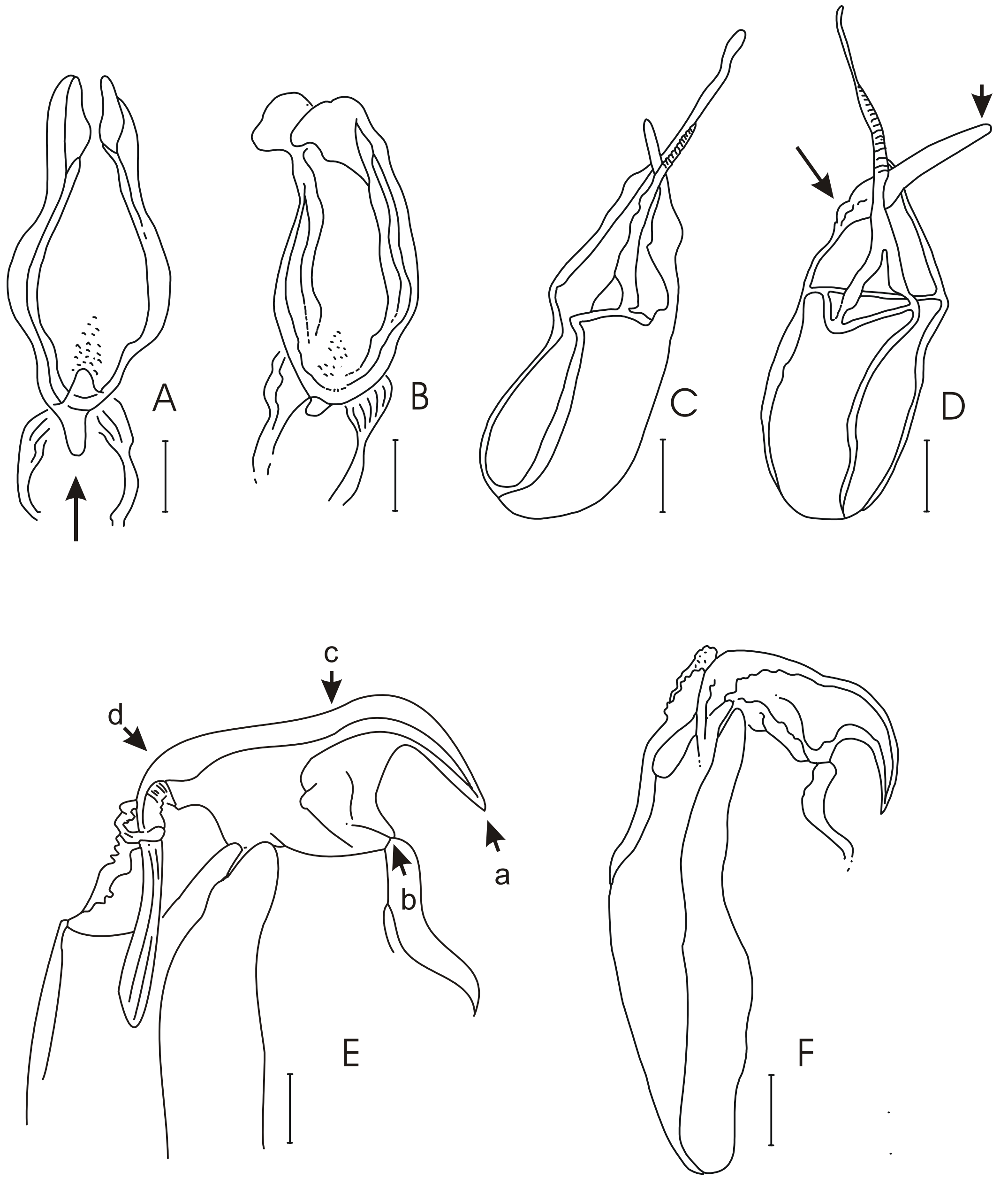

Figure 8. *Diesingia megastoma*. A. Ventral view of the oral cadre of male F2463-2 depicting the oesophageal peg (arrow). Scale bar: 100 μm. B. The oral cadre of female F2463-3 in ventrolateral view. The anterior chitinous lobes are prominent. Scale bar: 100μm. C. Left copulatory spicule of F2463-4 seen ventrolaterally. Scale bar: 100μm. D. Lateral view of the right copulatory spicule of the same male as in C. Note the cuticular ridges on the long spatulate extension. The shorter, fulcrum-like extension is jointed (large arrow), its length is the distance between the large and the small arrow. Scale bar: 100μm. E. Posterior hook of female F2463-6 depicting the aspinose cowl-like extension of the posterior fulcrum. Points D and B are the points of insertion of the extensory and flexory musculature, respectively, Point C demarcates the notch in the dorsal hook surface. AC = blade length, AD = hook length, BC = base length, CD = plateau length. Scale bar: 50μm. F. Anterior hook of female F2463-3. Note the spines on the cowl of the anterior fulcrum. Scale bar: 50μm.

The oral cadre of the prepatent female is about half the size of that of KI-3, but otherwise similar in shape. Seen from a slightly lateral view, the overall profile is ovoid and the oral cadre is open anteriorly. A small peg-like extension into the oesophagus measures 23 μm in the young female (F2463-3) and 92 μm in specimen KI-3. The lateral prongs widen into chitinous lobes anteriorly (Figure 8B, 9A).

Males

Specimen F2463-2 is 1,1 cm long and 58 annuli are present. The hooks of both males are more or less the same size as those of the young female and are morphologically similar. One of the anterior hooks of specimen F2463-4 has a spinose cowl of the fulcrum but no extensions could be seen in the remaining hooks. The hooks of specimen F2463-2 were too obscured by the dark stain to observe much detail.

The oral cadre of the male F2463-2 is slightly larger than that of the young female, F2463-3, and the peg-like extension into the oesophagus measures 60 μm (Figure 8A, 9B). Although seen ventrally, the shape of the oral cadre corresponds well with that of the female. Starting with a narrow base, the middle section of the oral cadre is slightly expanded, and narrows again towards the anterior end.

The copulatory spicules of the males consist of a cowry shell-shaped base from which two extensions protrude anteriorly (Figure 8C, 9F). The longer of the two extensions emerges ventrally, i.e. from the open side of the base. It consists of two lateral prongs that unite in the anterior half of the extension, to form a spatula. The distal half is reinforced by transverse cuticular ridges. The short extension emerges from an articulated joint at the closed dorsal side of the base. It is strongly reminiscent of the fulcrum found in collembolans and runs through the gap formed by the lateral prongs of the ventral extension (Figure 8D, 9E).

Table 12. The main diagnostic characteristics of *Diesingia megastoma* (Diesing, 1836) Sambon, 1922 from *Hydromedusa tectifera* (Chelonia) from Brazil. All measurements are in micrometres. In the case of the hooks and copulatory spicules, the figures on top refer to the structures on the left and the bottom figures to the corresponding feature on the right side.

Specimen No.	Sex	Oral cadre			Anterior Hooks					Posterior hooks					Copulatory spicules		
		Overall length	Cadre length	Width	Hook length	Blade length	Base length	Plateau length	Fulcrum length	Hook length	Blade length	Base length	Plateau length	Fulcrum length	Cowry-shell length	Total length	Spike length
F2463-2	M	653	488	239	NM	NM	NM	NM	NM	166 NM	94 NM	60 NM	83 NM	281 NM	345 345	773 750	248 258
F2463-4	M	NM	NM	NM	184 NM	113 NM	58 NM	83 NM	373 NM	202 207	108 108	67 67	106 106	329 NM	303 363	754 741	184 235
F2463-3	F	570	446	207	163 NM	106 NM	62 NM	92 NM	334 NM	177 NM	99 NM	67 NM	104 NM	NM NM	NA	NA	NA
F2463-5	F	NM	NM	NM	260 258	143 36	108 97	145 154	442 51	244 246	147 140	99 94	133 147	NM NM	NA	NA	NA
F2463-6	F	NM	NM	NM	246 NM	136 NM	97 NM	133 NM	NM 506	255 253	124 131	87 101	147 145	NM NM	NA	NA	NA
KI-3	F	1081	814	NM	251 248	147 147	99 87	126 127	483 511	258 NM	138 NM	97 NM	145 NM	398 NM	NA	NA	NA

F = female, M = male, NA = not applicable, NM = not measured

Table 13. Comparative measurements of *Diesingia megastoma* (Diesing, 1836) Sambon, 1922 found in the literature.

Species	Body length (mm)	Number of annuli	Mouth dimensions		Hook dimensions				Source
			Cadre length	Width	Total length (AD)	Blade length	Base length	Fulcrum length	
Diesingia megastoma M	7	70	524	205	NM	NM	NM	NM	Heymons (1941a)
Diesingia megastoma M	6	70	496	180	140	NM	NM	NM	Heymons (1941a)
Diesingia megastoma F*	11 - 20	ca. 70	770	440	140?	140?	NM	550	Da Fonseca & Ruiz (1956)
Diesingia megastoma F*	10	65	670	380	NM	140	110	520	Self & Rego (1985)

* = combined data of several females, ? = it is not clear which of the two measurments the authors refer to, F = female, M = male, NM = not measured.

Discussion

Comparative measurements of *D. megastoma* from the literature are presented in Table 13. The number of annuli counted by Heymons (1941a) and Da Fonseca & Ruiz (1956) are slightly higher than those of Self & Rego (1985) and the present authors. We assume this variance to be a result of counting techniques as well as differences in the quality of the material. Heymons (1941a) indicates, that the chloride cell pores of Diesing's two original specimens had become difficult to discern. Our counts exclude the tip of the tail as well as cephalothoracal annuli with incomplete rows of chloride cells.

According to Self & Rego (1985) the oral cadre of *D. megastoma* is closed anteriorly by fibres to form a ring. The same authors describe sharp anterior and posterior spurs that extend inwardly from the lateral prongs. Such structures were absent in the *Diesingia* material in the present study. Heymons (1941a) describes the oral cadre of *D. megastoma* as being U-shaped and open anteriorly, which is more in accordance with our findings. We believe the difference in shape of the oral cadre in Self & Rego's (1985) specimens to be due to a distortion caused by pressure while mounting the specimens.

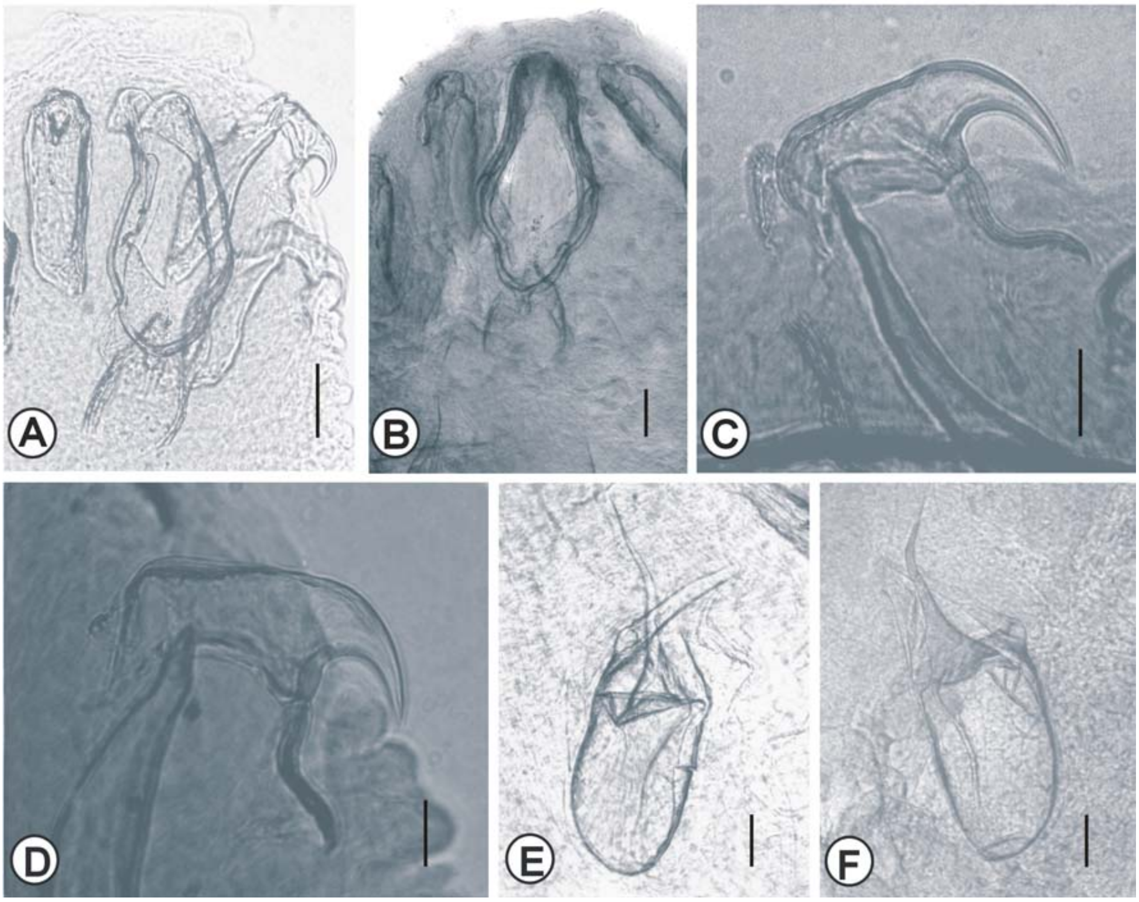

Figure 9. *Diesingia megastoma*. A. The oral cadre of female F2463-3. B. The oral cadre of male F2463-2. C. Close up on the left anterior hook of female F2463-3. D. Detail of the posterior hook of female F2463-6. E. Lateral view of the right copulatory spicule of male F2463-4. F The left copulatory spicule of male F2463-4. S*cale-bars:* A, 120 µm; B,E,F, 100 µm; C,D, 50 µm.

None of the previous descriptions (Heymons, 1941a; Da Fonseca & Ruiz, 1956; Self & Rego, 1985) mention the peg-like extension of the oral cadre into the oesophagus despite its being quite an obvious feature.

There is relatively little discrepancy in the description of the hooks of *D. megastoma* by earlier authors, all of which describe them as smooth, single and equal (Heymons, 1941a; Da Fonseca & Ruiz, 1956; Self & Rego, 1985). Two shallow dorsal notches are visible in Heymon's (1941) drawing of a hook and otherwise it conforms well to that of Rego & Self (1985). In our experience, slight differences in the orientation of specimens on the slide on which they are mounted can interfere with the visibility of certain structural characteristics and we attribute the fact that none of the above authors described the cowl-like anterior extensions of the fulcra to this.

Heymons (1941a) as well as Self & Rego (1985) omit the description of the copulatory spicules of the males, despite considering them fully mature specimens. This might be explained insofar as the true taxonomic value of these structures was only subsequently recognized (Riley 1986). Unfortunately, Da Fonseca & Ruiz (1956) refer only briefly to the copulatory spicules of the males. However, their illustration of a slide mounted and stained male specimen depicts the characteristic outline of the copulatory spicules well. The latter authors provide a detailed description of the female reproductive organs and state that the vulva is situated on a mammilliform protuberance on the 15th caudal annulus. Similarly, Self & Rego (1985) describe the utero-vaginal pore to be sub-terminal on a prominent papilla. These structures were not visible in neither the slide-mounted material nor the alcohol-fixed remains of the female specimens.

The measurements of the oral cadre of one of the males in the present study and those of the two males examined by Heymons (1941a) correspond well. The hooks of both males examined by us, however, are larger than the single measurement of Heymons (1941a), although the author specifies that the hook length was measured from the tip of the blade to the insertion of the *musculus extensor unci*. There is a good correlation between the length of the blade and the length of the base in the hooks of mature females as determined by Self & Rego (1985) and our own data. Despite slight deviations the measurements taken of the fulcra also appear uniform.

The oral cadre of the single female measured in this study appears considerably larger than recorded by Self & Rego (1985) and Da Fonseca & Ruiz (1956). It is difficult to interpret the hook dimensions provided by the latter authors, as their terminology is not in accordance with that later defined by Fain (1961) and there are no explanatory illustrations. We do, however, believe, that the length of the anterior and posterior hook refers to measurements of the blade only and we deduce that the term '*Bügel*' actually refers to the term fulcrum as it is used nowadays. We are in doubt as to the meaning of the term '*fulcrum*' as used by Da Fonseca & Ruiz (1956).

In addition to *Diesingia*, the crocodilian pentastome genera *Alofia* Giglioli, 1922 and *Sebekia*, represented by *Alofia platycephala* (Lohrmann, 1889) Giglioli, 1922 and *Sebekia microhamus* Self & Rego, 1985, are also present in Brazil (Heymons, 1941a; Self & Rego, 1985). The anteriorly open oral cadre of *D. megastoma* and especially its oesophageal peg is reminiscent of that of *Alofia*, but it is too oval in profile. The flat-topped, smooth, fang-like hooks are similar to alofian hooks, and it is mainly the presence of the anterior extensions of the fulcra which sets *Diesingia*'s hooks apart from the generic characteristics of *Alofia* (Fain, 1961; Riley, 1994), although *Alofia parva* Riley & Huchzermeyer 1995 has been described as possessing a spinose cowl-like extension to the anterior fulcrum. *Sebekia microhamus* is atypical for the genus since it possesses aspinose hooks that are too small to be confused with those of *D. megastoma* (Self & Rego, 1985).

The dorsally convex hooks of *Pelonia africana* Junker & Boomker, 2002 are smooth and the fulcra are without anterior extensions, and its oral cadre lacks the oesophageal peg. However, the single most distinguishing characteristic of the genus *Diesingia* from other sebekiid genera is the unique configuration of its copulatory spicules. The copulatory spicules of the genera *Alofia* and *Selfia* Riley, 1994 are marked by the double-hooked collar terminating the shorter of the two anterior extensions originating from the base (Riley, 1994; Junker *et al.*, 1999) and in the genera *Agema* Riley, Hill & Huchzermeyer, 1997, *Sebekia* and *Pelonia* only the longer ventral extension may be present or the shorter extension ends in a smooth collar (Riley *et al.*, 1990, 1997).

For the above reasons we do not concur with Self & Rego's (1985) abandonment of the genus *Diesingia* and the placement of *D. megastoma* into the genus *Sebekia*. Based on the morphological similarities between *D. megastoma* and its (South American) sebekiid counterparts, however, it is reasonable to include the genus *Diesingia* into the family Sebekidae, as suggested by Riley (1994).

CHAPTER 4

A CHECK-LIST OF THE PENTASTOMID PARASITES OF CROCODILIANS AND CHELONIANS

Published records and own data have been evaluated in order to determine the geographical distribution of the currently known species of pentastomid parasites infecting crocodiles and alligators, as well as chelonian definitive hosts.

The pentastomid parasites of chelonians and crocodilians are currently divided into two families, the Sebekidae and the Subtriquetridae. The family Sebekidae comprises the seven genera, *Agema, Alofia, Selfia, Sebekia*, *Leiperia*, *Diesingia* and *Pelonia*. The first four genera inhabit the lungs and bronchioles of the crocodilian host, whereas the genus *Leiperia* is found in the trachea, bronchi, heart and the pulmonary artery of crocodiles. The genera *Diesingia* and *Pelonia* parasitize chelonian final hosts, occurring in the lungs. Members of the monogeneric family Subtriquetridae inhabit the nasopharynx of their final hosts, but *Subtriquetra rileyi*, of which currently only infective larvae have been recovered, needs verification.

During the past ten years new progress has been made as regards the taxonomy of crocodilian pentastomes. The older genera *Alofia*, *Sebekia* and *Leiperia* have been revised and examination of new material has led to the description of several new genera and species (Riley, 1994; Riley *et al*., 1990; 1997; Riley & Huchzermeyer, 1996; Junker *et al*., 1998a). However, there is a dearth of data concerning the chelonian pentastomids.

In order to provide a quick reference tool, this check-list consists of two parts, following the example of Sambon (1922). The first part lists the parasites under their scientific names, their synonyms and their authorities. A short generic diagnosis is provided for each genus and the parasites are grouped according to the geographic distribution of their respective hosts. The list starts with the African species of each genus, followed by the Australian and Asian ones, and then the South and North American members.

The second part of the check-list alphabetically lists the hosts and their synonyms, and, also in alphabetical order, their respective parasites. The nomenclature and synonyms of the crocodilian and chelonian hosts are according to Getz (2002).

Only references dealing with mature pentastomes are included in the check-list, but for completeness' sake the intermediate hosts of pentastome species of which only larval forms are known are listed.

4.1 PARASITE/HOST CHECK-LIST OF THE PENTASTOMIDA

Family SEBEKIDAE Sambon, 1922

GENUS *AGEMA* RILEY, HILL & HUCHZERMEYER, 1997

GENERIC DIAGNOSIS: Overall shape typical of smaller members of the Sebekidae; males claviform, females with more uniform diameter and conical posterior terminating in small blunt point; adult hooks smooth; blades smoothly curved without abrupt right angled bend near to base; blade on anterior hook pair larger then that of posterior pair; fulcrum without cowl; mouth ovoid and sides of cadre united anteriorly and posteriorly by segments of chitin which appear as two crescents; copulatory spicules delicate and elongate; basal section without a hooked collar (Riley *et al*., 1997).

ETHIOPIAN REGION

1. *Agema silvaepalustris* Riley, Hill & Huchzermeyer, 1997

Crocodylus cataphractus

Riley, Hill & Huchzermeyer (1997), Republic of the Congo

Osteolaemus tetraspis

Riley, Hill & Huchzermeyer (1997), Republic of the Congo

GENUS *ALOFIA* GIGLIOLI, 1922

GENERIC DIAGNOSIS: Size small; body banana-shaped; hooks large with long, narrow, slightly curved blade and a slender base; absence of chitinous formation at the base of the anterior hook; chitinous buccal cadre large and U-shaped; intestine as in *Sebekia*; found in crocodiles (Fain, 1961 in Riley, 1994); caudal extremity of female bluntly rounded, often swollen into a bulb (Riley, 1994); hooks usually smooth (rarely with patches of minute spines (see *Alofia nilotici* and *Alofia parva* (Riley & Huchzermeyer, 1995a,b)), blades finely canaliculated, bent through almost a right angle at the base; peg-like extension of oral cadre projects into oesophagus; copulatory spicules with double-hooked collar on the shorter of the two anterior extensions (Riley & Huchzermeyer, 1995a,b; Junker *et al.*, 1999).

ETHIOPIAN REGION

1. *Alofia nilotici* Riley & Huchzermeyer, 1995

Crocodylus niloticus

Riley & Huchzermeyer (1995a), Botswana

Junker, Boomker & Bolton (1999), South Africa

2. *Alofia parva* Riley & Huchzermeyer, 1995

Crocodylus cataphractus

Riley & Huchzermeyer (1995b, 2000), Republic of the Congo

Osteolaemus tetraspis osborni

Riley & Huchzermeyer (1995b, 2000), Republic of the Congo

3. *Alofia simpsoni* Riley, 1994

Unknown crocodilian

Riley (1994), Ghana

Crocodylus niloticus

Riley & Huchzermeyer (1995a), Botswana

Junker, Boomker & Bolton (1999), South Africa

AUSTRALASIAN REGION

4. *Alofia ginae* Giglioli, 1922

Unknown crocodilian

Sambon (1922), Samoa

Most probably *Crocodylus porosus*, as it is the only crocodilian whose range extends as far as Fiji in the Pacific ocean and it is known to have colonized many small islands as far as nearly 1000 km from land (Ross, 1989).

Crocodylus porosus (?)

Riley (1994), Philippines

While the collector did not specify the host, Riley (1994) concludes it to be *C. porosus,* as the only other Philippinian crocodile, *Crocodylus mindorensis*, does not occur in the region the parasites were recovered from.

5. *Alofia merki* Giglioli, 1922

Sebekia merki Heymons, 1941

Unknown crocodilian

Sambon (1922), Samoa

Most probably *Crocodylus porosus*, as it is the only crocodilian whose range extends as far as Fiji in the Pacific ocean and it is known to have colonized many small islands as far as nearly 1000 km from land (Ross, 1989).

Crocodylus porosus

Riley (1994), Northern Territory, Australia

Riley (1994), Philippines

6. *Alofia indica* (Von Linstow, 1906) Hett, 1924, *species inquirenda*

Gavialis gangeticus

Hett (1924), India

SOUTH & NORTH AMERICAN REGION

7. *Alofia platycephala* (Lohrmann, 1889) Giglioli, 1922

Pentastomum platycephalum Lohrmann, 1889; *Porocephalus platycephalus* Shipley, 1898; *Reighardia platycephala* Sambon, 1910

Unknown crocodilian

Lohrmann (1889), South America

Caiman crocodilus

Self & Rego (1985), Brazil

Caiman latirostris

Heymons (1941b), Paraguay

8. *Alofia adriatica* (Hirst, 1922) Giglioli, 1922, *species inquirenda*

Unknown host

Hirst (1922), Adriatic

GENUS *LEIPERIA* SAMBON, 1922

GENERIC DIAGNOSIS: Female with spirally coiled abdomen; broad bands of chloride cells; hooks smooth, flat-topped with sharply curved blade; oral cadre V-shaped with large anterior flanges, numerous pores around the pharynx; copulatory spicules heavily chitinised with complex internal supporting structures, shorter of the two anterior extensions forms a smooth collar (double in *L. australiensis*) around the longer spatulate extension; cirrus tip modified into a flattened trumpet of longitudinally-striated chitin (Riley & Huchzermeyer, 1996; Junker, Boomker, Swanepoel & Taraschewski, 2000).

ETHIOPIAN REGION

1. *Leiperia cincinnalis* (Vaney & Sambon, 1910) Sambon, 1922

Reighardia cincinnalis Vaney & Sambon, 1910; *Porocephalus nematoides* Beauchamp, 1918

Crocodylus cataphractus
Fain (1961), Central Africa: infective larva
Crocodylus niloticus
Vaney & Sambon (1910), Uganda
Sambon (1922), Zimbabwe
Rodhain & Vuylsteke (1932), Democratic Republic of the Congo
Junker, Boomker, Swanepoel & Taraschewski (2000), South Africa

AUSTRALASIAN REGION

2. *Leiperia australiensis* Riley & Huchzermeyer, 1996

Crocodylus johnstoni
Riley & Huchzermeyer (1996), Northern Territory, Australia

Crocodylus porosus

Riley & Huchzermeyer (1996), Northern Territory, Australia

SOUTH & NORTH AMERICAN REGION

3. *Leiperia gracilis* Diesing, 1936, *species inquirenda*

Pentastoma gracile Diesing, 1836 (*partim*); *Pentastomum gracile* Leidy, 1856; *Pentastoma gracilis* Parona, 1891; *Porocephalus gracilis* Shipley, 1898; *Porocephalus crocodili* Wheeler, 1915 (*partim*); *Leiperia neotropica* Heymons & Vitzthum, 1935

Immature and larval forms were recovered from the following hosts, but adults have not been collected.

Alligator mississippiensis

Leidy (1856, in Sambon 1922), locality unknown, North America

Crocodylus acutus

Heymons (1935), South America

Caiman crocodilus

Heymons (1935), Brazil

GENUS *SEBEKIA* SAMBON, 1922

GENERIC DIAGNOSIS: Body short and squat with 58-94 compressed annuli; lateral lines conspicuous; cephalothorax small, wedge-shaped and projecting nipple-like from the abdomen, ventral surface continuous with that of the abdomen; mouth subterminal and shaped like an inverted ‘U’; oral cadre oval to elongate, highly variable in shape, without long, parallel sides and generally united anteriorly; hooks small, equal or subequal, claw-shaped, with convex or flat dorsal surface; all hooks spinous (rarely only the anterior pair); all fulcra often with spinous anterior extension (rarely only the anterior pair); hook barb curved, strongly united and continuous with shank; spicules generally obpyriform, with one or two fine sclerotized rods supporting membranous region distally. Parasites of the lungs of crocodilians, rarely of turtles (Riley *et al*., 1990).

ETHIOPIAN REGION

1. *Sebekia cesarisi* Giglioli, 1922

Crocodylus sp.
Sambon (1922), Africa
Crocodylus niloticus
Riley & Huchzermeyer (1995a), Botswana
Junker, Boomker & Bolton (1999), South Africa

2. *Sebekia okavangoensis* Riley & Huchzermeyer, 1995

Sebekia cesarisi Riley, Spratt & Winch, 1990

Crocodylus cataphractus
Riley & Huchzermeyer (2000), Republic of the Congo
Crocodylus niloticus
Riley & Huchzermeyer (1995a), Botswana
Junker, Boomker & Bolton (1999), South Africa
Osteolaemus tetraspis
Riley & Huchzermeyer (2000), Republic of the Congo

3. *Sebekia wedli* Giglioli, 1922

Pentastoma oxycephalum var. *minor* Wedli, 1861; *Sebekia oxycephala* Self & Rego, 1985

Crocodylus niloticus
Devos (1939), Democratic Republic of the Congo
Riley, Spratt & Winch (1990), Uganda
Riley & Huchzermeyer (1995a), Botswana
Junker, Boomker & Bolton (1999), South Africa

AUSTRALASIAN REGION

4. *Sebekia johnstoni* Riley, Spratt & Winch, 1990

Crocodylus johnstoni

Riley, Spratt & Winch (1990), Northern Territory, Australia

Crocodylus porosus

Riley, Spratt & Winch (1990), Northern Territory, Australia

5. *Sebekia multiannulata* Riley, Spratt & Winch, 1990

Crocodylus johnstoni

Riley, Spratt & Winch (1990), Northern Territory, Australia

Crocodylus porosus

Riley, Spratt & Winch (1990), Northern Territory, Australia

6. *Sebekia purdieae* Riley, Spratt & Winch, 1990

Crocodylus porosus

Riley, Spratt & Winch (1990), Northern Territory, Australia

7. *Sebekia jubini* (Vaney & Sambon, 1910) Sambon, 1922, *species inquirenda*

Porocephalus jubini Vaney & Sambon, 1910

Crocodylus siamensis

Sambon (1922), locality unknown, south-east Asia

8. *Sebekia novaeguineae* Riley, Spratt & Winch, 1990

Crocodylus novaeguineae

Riley, Spratt & Winch (1990), Papua New Guinea

SOUTH & NORTH AMERICAN REGION

9. *Sebekia acuminata* Travassos, 1924, *species inquirenda*

Unknown crocodilian

Travassos (1924), Brazil

10. *Sebekia divestei* Giglioli, 1922

Crocodylus acutus

Sambon (1922), locality unknown, Neotropical region

11. *Sebekia microhamus* Self & Rego, 1985

Caiman crocodilus

Self & Rego (1985), Brazil

12. *Sebekia mississippiensis* Overstreet, Self & Vliet, 1985

Pentastoma oxycephalum Diesing, 1836 (*partim*); *Pentastomum gracile* (syn. *Leiperia gracilis*) Leidy, 1856

Alligator mississippiensis

Deakins (1971), USA

Hazen, Aho, Murphy, Esch & Schmidt (1978), USA

Overstreet, Self & Vliet (1985), USA

13. *Sebekia oxycephala* (Diesing, 1836) Sambon, 1922, *species inquirenda*

Pentastoma proboscideum Rudolphi, 1819 (*partim*); *Pentastoma oxycephalum* Diesing, 1836 (*partim*); *Pentastoma gracile* Diesing, 1836 (*partim*); *Pentastomum oxycephalum* Diesing, 1850 (*partim*); *Pentastomum gracile* Diesing, 1850 (*partim*); *Pentastomum heterodontis* Leuckart, 1860; *Pentastomum oxycephalum* Chatin, 1882; *Porocephalus oxycephalus* Stiles, 1893; *Pentastoma proboscideum crocodili scleropis* Rudolphi (Shipley in Sambon 1922); *Reighardia oxycephala* Vaney & Sambon, 1910;

Porocephalus crocodili Wheeler, 1913 (*partim*); *Sebekia oxycephala* Sambon, 1922 (*partim*); *Bdukus ichthyius* Holl, 1929; *Leiperia heterodontis* Heymons & Vitzthum, 1935; *Sebekia crocodili* Heymons & Vitzthum, 1935

Alligator mississippiensis
  Sambon (1922), locality unknown
Caiman crocodilus
  Sambon (1922), locality unknown
Caiman latirostris
  Heymons (1941a), locality unknown
Crocodylus acutus
  Sambon (1922), locality unknown

14. *Sebekia samboni* Travassos, 1924, *species inquirenda*

Unknown crocodilian
  Travassos (1924), Brazil

15. *Sebekia trinitatis* Riley, Spratt & Winch, 1990

Caiman crocodilus
  Riley, Spratt & Winch (1990), Trinidad, South America

GENUS *SELFIA* RILEY, 1994

GENERIC DIAGNOSIS: Size small, cephalothorax minute in comparison with diameter of the abdomen; 78-82 well defined annuli; abdomen strongly curled ventrally; caudal extremity of female abruptly tapered to blunt point; hooks very small, with tiny blade only slightly offset from transversely creased and folded shank; rear of anterior hooks enveloped by soft, spinous cowl which forms an extension of the fulcrum; buccal cadre somewhat variable in shape, being oval to more U-shaped, but lacking parallel sides; copulatory spicule of male like that of *Alofia* (Riley, 1994).

AUSTRALASIAN REGION

1. *Selfia porosus* Riley, 1994

Crocodylus porosus
Riley (1994), Northern Territory

GENUS *DIESINGIA* SAMBON, 1922

GENERIC DIAGNOSIS: Hooks smooth, flat-topped, with sharply curved blades; fulcra with anterior cowl-like extension, extension smooth in posterior and spiny in anterior fulcra; oral cadre open anteriorly with an oesphageal peg similar to that in *Alofia*; copulatory spicule with cowry shell-shaped base, the short, ventral extension is transformed into a structure resembling the collembolan fulcrum, and is connected to the base by a joint (Junker, Riley & Boomker, in press).

SOUTH & NORTH AMERICAN REGION

1. *Diesingia megastoma* (Diesing, 1836) Sambon, 1922

Pentastoma megastomum Diesing, 1836; *Pentastomum megastomum* Leuckart, 1860; *Porocephalus megastomus* Shipley, 1898; *Sebekia megastoma* Travassos, 1923; *Sebekia crocodili* Heymons & Vitzthum, 1935; *Diesingia megastoma* Heymons; 1941a; *Butantanella megastoma* Da Fonseca & Ruiz, 1956; *Sebekia megastoma* Self & Rego, 1985

Phrynops geoffroanus
Diesing (1836), Brazil
Hydromedusa tectifera
Da Fonseca & Ruiz (1956), Brazil

GENUS *PELONIA* JUNKER & BOOMKER, 2002

GENERIC DIAGNOSIS: Hooks smooth, dorsally convex, with sharply bent blades, fulcra without extensions; oral cadre more or less U-shaped, closed anteriorly by delicate chitinous fibres; copulatory spicules almost identical to those of *Sebekia wedli*, with cowry shell-shaped base and the short anterior extension ending in a smooth collar, the long spatulate extension carries small chitinous teeth (Junker & Boomker, 2002).

ETHIOPIAN REGION

1. *Pelonia africana* Junker & Boomker, 2002

Pelomedusa subrufa
Junker & Boomker (2002), South Africa
Pelusios sinuatus
Junker & Boomker (2002), South Africa

Family SUBTRIQUETRIDAE Fain, 1961

GENUS *SUBTRIQUETRA* SAMBON, 1922

GENERIC DIAGNOSIS: Body elliptical, ventrally flattened and dorsally dome shaped with flattened margins; hooks simple, slender and sharply pointed, disposed in a curved line; oral cadre rounded (Fain, 1961; Winch & Riley, 1986; Junker *et al*., 1998a).

ETHIOPIAN REGION

1. *Subtriquetra rileyi* Junker, Boomker & Booyse, 1998

Infective larvae:

Tilapia rendalli swierstrae

Junker, Boomker & Booyse (1998a), South Africa

Oreochromis mossambicus

Junker, Boomker & Booyse (1998a), South Africa

AUSTRALASIAN REGION

2. *Subtriquetra megacephala* (Baird, 1853) Sambon, 1922

Pentastoma megacephalum Baird, 1853; *Porocephalus megacephalus* Shipley, 1898

Crocodylus palustris

Sambon (1922), Sunderbunds, India

Crocodylus palustris or *Gavialis gangeticus*? ("Sangor crocodile")

Sambon (1922), Bengal, India

3. *Subtriquetra shipleyi* Hett, 1924

Crocodylus palustris or *Gavialis gangeticus* ? ("Indian crocodile")

Hett (1924), India

SOUTH & NORTH AMERICAN REGION

4. *Subtriquetra subtriquetra* (Diesing, 1836)

Pentastoma proboscideum Bresmer, 1824 (partim); *Pentastoma subtriquetrum* Diesing, 1836; *Pentastomum subtriquetrum* Diesing, 1850; *Pentastomum pusillum* Diesing, 1856; *Linguatula* *subtriquetra* Raillet, 1883; *Linguatula pusilla* Shipley, 1898

Caiman crocodilus
  Sambon (1922), South America
  Winch & Riley (1986), Trinidad
Melanosuchus niger
  Sambon (1922), South America

4.2 HOST/PARASITE CHECK-LIST OF THE PENTASTOMIDA

Crocodylia

Family ALLIGATORIDAE (Cuvier, 1807) (Alligators and caimans)

GENUS *ALLIGATOR* CUVIER, 1807

1. *Alligator mississippiensis* (Daudin, 1801 [1802]) (American alligator)

Crocodilus mississipiensis Daudin, 1801 [1802]; *Alligator mississippiensis* (Daudin, 1802); *Alligator mississippiensis* Conant & Collins, 1991

Leiperia gracilis, *species inquirenda*, larval forms only
Sebekia mississippiensis
Sebekia oxycephala, *species inquirenda*

GENUS *CAIMAN* SPIX, 1825

1. *Caiman crocodilus* (Linnaeus, 1758) (Common caiman, Spectacled caiman)

Lacerta crocodilus Linnaeus, 1758; *Caiman sclerops* Schneider, 1801 (fide Medem 1981); *Caiman crocodilus* Conant & Collins, 1991; *Caiman crocodilus* Schwartz & Henderson, 1991; *Caiman crocodilus* Gorzula & Señaris, 1999

Subspecies *Caiman crocodilus apaporiensis* Medem, 1955
(= *Caiman sclerops apaporiensis* Medem, 1955)

Subspecies *Caiman crocodilus chiapasius* Bocourt, 1876
(= *Alligator* (*Jacare*) *chiapasius* Bocourt, 1876)

Subspecies *Caiman crocodilus fuscus* Cope, 1868
(= *Perosuchus fuscus* Cope, 1868; *Perosuchus fuscus* Gray, 1869)

Subspecies *Caiman crocodilus crocodilus* Linnaeus, 1758

Alofia platycephala
Leiperia gracilis, *species inquirenda*, larval forms
Sebekia microhamus
Sebekia oxycephala, *species inquirenda*
Sebekia trinitatis
Subtriquetra subtriquetra

2. *Caiman latirostris* (Daudin, 1801 [1802]) (Broad-snouted caiman)

Crocodilus latirostris Daudin, 1802; *Caiman fissipes* Spix, 1825; *Champsa fissipes* Wagler, 1828 (*fide* Hoogmoed & Gruber, 1983); *Alligator cynocephalus* Duméril & Bibron, 1836; *Champsa fissipes* Gulliver, 1846; *Jacare latirostris* Gray, 1862; *Alligator latirostris* Strauch 1866 (*partim*); *Alligator latirostris* Boulanger, 1886; *Caiman latirostris* Boulanger, 1889 (*partim*); *Jacaretinga latirostris* Vaillant, 1898

Subspecies *Caiman latirostris chacoensis* Freiberg & Carvalho, 1965

Subspecies *Caiman latirostris latirostris* Daudin, 1802

Alofia platycephala
Sebekia oxycephala, *species inquirenda*

GENUS *MELANOSUCHUS* GRAY, 1862

1. *Melanosuchus niger* (Spix, 1825) (Black caiman)

Caiman niger Spix, 1825; *Melanosuchus niger* King & Burke, 1989

Subtriquetra subtriquetra

Family CROCODYLIDAE (Cuvier, 1807) (Crocodiles)

Subfamily Crocodylinae (Cuvier, 1807)

GENUS *CROCODYLUS* LAURENTI, 1768

1. *Crocodylus acutus* (Cuvier, 1807) (American crocodile)

Crocodilus acutus Cuvier, 1807; *Crocodylus acutus* King & Burke, 1989; *Crocodylus acutus* Schwartz & Henderson, 1991; *Crocodylus acutus* Conant & Collins, 1991

Leiperia gracilis, *species inquirenda*, larval forms only
Sebekia divestei
Sebekia oxycephala, species inquirenda

2. *Crocodylus cataphractus* Cuvier, 1825 (Slender-snouted crocodile)

Cocodilus cataphractus Falconer, 1846; *Crocodylus cataphractus* Schmidt, 1919; *Crocodylus cataphractus* King & Burke, 1989

Agema silvaepalustris
Alofia parva
Leiperia cincinnalis, infective larva
Sebekia okavangoensis

3. *Crocodylus johnstoni* Krefft, 1873 (Australian freshwater crocodile)

Crocodylus johnsoni Krefft, 1873; *Tomistoma kreffti* Gray in Krefft, 1873 (*nomen nudum*); *Crocodilus* (*Philas*) *johnstoni* Gray, 1874; *Crocodylus johnstoni* Cogger, 2000

Leiperia australiensis
Sebekia johnstoni
Sebekia multiannulata

4. *Crocodylus niloticus* Laurenti, 1768 (Nile crocodile)

Crocodilus vulgaris Cuvier, 1807; *Crocodilus multiscutatus* Rüppell in Cretzschmar, 1826; *Crocodilus marginatus* Geoffroy, 1827; *Crocodilus vulgaris* Baikie, 1857; *Crocodilus vulgaris* Murray, 1863; *Crocodilus madagascariensis* Grandidier, 1872; *Crocodilus vulgaris* var. *madagascariensis* Boettger, 1877; *Crocodylus niloticus* Schmidt, 1919; *Crocodylus niloticus* Auerbach, 1987; *Crocodylus niloticus* Glaw & Vences, 1994

Subspecies *Crocodylus niloticus niloticus* Laurenti, 1768
Subspecies *Crocodylus niloticus africanus* Laurenti, 1768
Subspecies *Crocodylus niloticus chamses* Bory, 1824
Subspecies *Crocodylus niloticus cowiei* (Smith in Hewitt, 1937)
Subspecies *Crocodylus niloticus madagascariensis* Grandidier, 1872
Subspecies *Crocodylus niloticus pauciscutatus* Deraniyagala, 1948
Subspecies *Crocodylus niloticus suchus* Geoffroy, 1807

Alofia nilotici
Alofia simpsoni
Leiperia cincinnalis
Sebekia cesarisi
Sebekia okavangoensis
Sebekia wedli

5. *Crocodylus novaeguineae* Schmidt, 1928 (New Guinea crocodile)

Sebekia novaeguineae

6. *Crocodylus palustris* Lesson, 1831 (Mugger, Marsh crocodile)

Crocodylus palustris King & Burke, 1989

Subtriquetra megacephala
Subtriquetra shipleyi (“Indian crocodile”)

7. *Crocodylus porosus* Schneider, 1801 (Indo-Pacific or Saltwater crocodile)

Crocodylus natans Meyer, 1795; *Crocodilus porosus* Schneider, 1801; *Crocodilus oopholis* Schneider, 1801; *Crocodilus biporcatus* Cuvier, 1807; *Crocodilus biporcatus raninus* Müller & Schlegel, 1844; *Oopholis pondicherianus* Gray, 1862; *Croocdylus porosus australis* Deraniyagala, 1953; *Crocodylus porosus minikanna* Deraniyagala, 1953; *Crocodylus porosus* Cox, Van Dijk, Jarujin & Thirakhupt, 1998; *Crocodylus porosus* Cogger 2000

Alofia ginae (possibly *Crocodylus mindorensis,* but distributionally unlikely)
Alofia merki
Leiperia australiensis
Sebekia johnstoni
Sebekia multiannulata
Sebekia purdiae
Selfia porosus

8. *Crocodylus siamensis* Schneider, 1801 (Siamese crocodile)

Crocodilus galeatus Cuvier, 1807; *Crocodylus siamensis*, King & Burke, 1989; *Crocodylus siamensis* Cox, Van Dijk, Jarujin & Thirakhupt, 1998

Sebekia jubini, *species inquirenda*

GENUS *OSTEOLAEMUS* COPE, 1861

1. *Osteolaemus tetraspis* Cope, 1861 (Dwarf crocodile)

Crocodilus frontatus Murray, 1862; *Halcrosia afzelii* Lilljeborg, 1867; *Halcrosia nigra* Gray, 1867; *Halcrosia nigra* Gray, 1870; *Osteolaemus tetraspis* Boulanger, 1889; *Osteoblepharon osborni* Schmidt, 1919; *Osteolaemus tetraspis tetraspis* Wermuth & Mertens, 1961

Subspecies *Osteolaemus tetraspis tetraspis* Cope, 1861
Subspecies *Osteolaemus tetraspis osborni* Schmidt, 1919 (*fide* Wermuth & Mertens, 1977)

Agema silvaepalustris
Alofia parva
Sebekia okavangoensis

Family GAVIALIDAE Adams, 1854 (Gavials)

GENUS GAVIALIS OPPEL, 1811

1. *Gavialis gangeticus* (Gmelin, 1789) (Gharial)

Lacerta gangetica Gmelin, 1789; *Gavialis gangeticus* King & Burke, 1989

Subtriquetra megacephala (“Indian crocodile”)
Subtriquetra shipleyi (“Indian crocodile”)

Chelonia

Suborder Pleurodira (Side-necked turtles)

Family PELOMEDUSIDAE Cope, 1868

GENUS *PELOMEDUSA* WAGLER, 1830

1. *Pelomedusa subrufa* (Lacépède, 1788) (Cape terrapin)

Testudo subrufa Lacépède, 1788; *Testudo galeata* Schoepff, 1792; *Testudo badia* Donndorf, 1798; *Testudo rubicunda* Suckow, 1798; *Emys olivacea* Schweigger, 1812 (*non Emys olivacea* Gray, 1855); *Pentonyx capensis* Duméril & Bibron, 1835; *Pentonyx gehafie* Rüppell, 1835; *Pentonix americana* Cornalia, 1849; *Pelomedusa mozambica* Peters (*nomen nudum*) in Gray 1855 (?); *Pelomedusa mossambicensis* Peters (*nomen nudum*) in Lichtenstein 1856; *Pelomedusa nigra* Gray, 1863; *Pelomedusa gasconi* Rochebrune, 1884; *Pelomedusa galeata* Boulanger, 1889; *Pelomedusa galeata* var. *disjuncta* Vaillant & Grandidier, 1910; *Pelomedusa galeata* Schmidt, 1919; *Pelomedusa galeata orangensis* Hewitt, 1935; *Pelomedusa galeata devilliersi* Hewitt, 1935; *Pelomedusa galeata damarensis* Hewitt, 1935; *Pelomedusa subrufa wettsteini* Mertens, 1937; *Pelomedusa subrufa olivacea* Lovebridge, 1941; *Testudo emys arabica* N.-Ehrenberg in Stresemann 1954; *Pelomedusa subrufa* Auerbach, 1987; *Pelomedusa subrufa* Glaw & Vences, 1994; *Pelomedusa subrufa subrufa* Broadley & Howell, 1991.

Subspecies *Pelomedusa subrufa subrufa* (Lacépède, 1788)
Subspecies *Pelomedusa subrufa olivacea* (Schweigger, 1812)
Subspecies *Pelomedusa subrufa nigra* Gray, 1863

Pelonia africana

GENUS *PELUSIOS* WAGLER, 1830

1. *Pelusios sinuatus* (Smith, 1838) (Serrated hinged terrapin, African serrated mud turtle)

Sternotherus sinuatus Smith, 1838; *Sternotherus dentatus* Peters, 1848 (*nomen nudum*); *Sternotherus sinuatus* Gray, 1864; *Sternothaerus sinuatus* Boulanger, 1889; *Sternothaerus bottegi* Boulanger, 1895; *Pelusios sinuatus* Schmidt, 1919; *Pelusios sinuatus zuluensis* Hewitt, 1927; *Pelusios sinuatus leptus* Hewitt, 1927; *Pelusios sinuatus* Auerbach, 1987; *Pelusios sinuatus* Broadley, 1998

Pelonia africana

Family CHELIDAE Gray, 1825 (Snake-necked turtles)

GENUS *HYDROMEDUSA* WAGLER, 1830

1. *Hydromedusa tectifera* Cope, 1869 [1870] (South American snake-necked turtle, Uruguay snake-necked turtle)

Hydromedusa platanensis Gray, 1873; *Hydromedusa wagleri* Günther, 1884; *Hydromedusa tectifera* Boulanger, 1885; *Hydromedusa tectifera* Boulanger, 1886

Diesingia megastoma

GENUS *PHRYNOPS* WAGLER, 1830

1. *Phrynops geoffroanus* (Schweigger, 1812) Gorzula & Señaris, 1999 (Geoffroy's side-necked turtle)

Emys geoffroana Schweigger, 1812; *Emys geoffreana* Schweigger, 1812 (fide Boulanger 1886); *Emys geoffroyana* Gray, 1831 (nomen subst. pro *E. geoffroana* Schweigger);

Platemys geoffreana Duméril & Bibron, 1835 (nomen subst. pro *E. geoffroana*); *Platemys geoffroyana* Boulanger, 1886; *Hydraspis geoffroyana* Boulanger, 1889; *Phrynops geoffroana* Mertens *et al*., 1934; *Phrynops geoffroanus* Gorzula & Señaris, 1999

Subspecies *Phrynops geoffroanus geoffroanus* Schweigger, 1812
Emys depressa Merrem, 1820 (*non Emys depressa* Spix, 1824); *Emys viridis* Spix, 1824 (?); *Platemys waglerii* Duméril & Bibron, 1835; *Platemys neuwiedii* Duméril & Bibron, 1835 (nomen subst. pro *Emys depressa*); *Hydraspis wagleri* Boulanger, 1889; *Hydraspis boulangeri* Bohls, 1895; *Hydraspis lutzi* Ihering in Luederwaldt 1926 (?); *Phrynops geoffroana geoffroana* Müller, 1939

Subspecies *Phrynops geoffroanus tuberosus* Peters, 1870
Platemys tuberosa Peters, 1870; *Hydraspis tuberosa* Boulanger, 1889; *Phrynops tuberosa* Mertens *et al*., 1934; *Phrynops geoffroana tuberosa* Müller, 1939

Diesingia megastoma

4.3 CONCLUSION

African crocodiles harbour eight pentastome species, six of which have been recorded from the Nile crocodile, *Crocodylus niloticus*. Three species belong to the genus *Sebekia*, *Alofia is* represented by two and of *Leiperia* only one species is present. Two species, *Alofia parva* and *Agema silvaepalustris*, occur in the dwarf crocodile, *Osteolaemus tetraspis* and the slender-snouted crocodile, *Crocodylus cataphractus*, exclusively, but a single *Sebekia* species is shared with the Nile crocodile. The genus *Agema* is endemic to the African region. Infected stages of the pentastome *Subtriquetra rileyi*, thought to utilize Nile crocodiles as final hosts, have been recovered only from fishes. The largest number of pentastome species are found in the Australasian region. Of these the Indo-Pacific crocodile, *Crocodylus porosus*, harbours seven, representing the genera *Alofia, Sebekia, Leiperia* and *Selfia*, which is exclusive to the latter host. The genus *Subtriquetra* has been reported from “Indian crocodiles”, a term possibly referring to either *Crocodylus palustris* or *Gavialis gangeticus*. Ten species of pentastomes parasitising the crocodilian genera *Alligator, Caiman*, *Crocodylus* and *Melanosuchus* have been recorded from the Neotropical region including the southern states of the North American continent. The two most wide-spread pentastome genera, *Alofia* and *Sebekia* have been recorded together with representatives of the genus *Subtriquetra* and immature and larval forms of *Leiperia.*

To date the two monospecific genera, *Pelonia*, from two terrapin species, *Pelusios sinuatus* and *Pelomedusa subrufa*, in South Africa, and *Diesingia* from *Phrynops geoffroanus* and *Hydromedusa tectifera* in South America, are the only chelonian pentastomes recorded world-wide.

CHAPTER 5

THE EVOLUTION OF PRESENT-DAY CROCODILIANS AND PENTASTOMES

The following chapter is intended to provide a short summary on the phylogenesis of pentastomid parasites and that of their crocodilian hosts. While the author had no access to fossilized pentastome material during the course of this study, it was considered conducive to the interpretation of the subsequent studies on the systematics of sebekiid pentastomes to include a short chapter on the evolution of pentastomid parasites. Since the evolution of parasitic organisms can not be seen outside the context of their final hosts, a brief overview of the phylogenetic history of crocodilians, the main final hosts of the sebekiid pentastomes in this study, is provided. The evolutionary processes that forged today's crocodilians as well as their geographical distribution world-wide are certainly to be seen as one of the main factors influencing the speciation processes in their pentastome communities.

5.1 THE PHYLOGENETIC ANCESTRY OF EXTANT PENTASTOMES

The stem-group of pentastomid parasites is roughly 550 million years old and dates back to the Cambrian period of the Paleozoic era, the age of the invertebrates, characterized by marine invertebrates and algae. During the Cambrian period all invertebrate phyla became established and many classes had evolved (Parker & Haswell, 1964).

The stem-group of the Pentastomida comprises the three genera *Heymonsicambria*, *Haffnericambria* and *Boeckelericambria*, described by

Walossek & Müller (1994) based on marine fossil founds from late Cambrian limestones in Sweden. These authors provide a detailed systematic description of the larvae, and their morphology is compared extensively to hypothetical "ur-pentastomids" reconstructed by earlier authors as well as to the present-day crown-group of pentastomes. Based on the morphological characteristics, the phyletic position of the Pentastomida and their affinities to other taxa is discussed and conclusions on their adaptation to parasitism are drawn. For in depth information on the subject, the reader is referred to the work of Walossek & Müller (1994).

In the following only a few aspects pertaining to the biogeographic distribution and possible evolution of host-parasite relationships in recent pentastomes are highlighted.

According to Walossek & Müller (1994) the Palaeozoic larvae are best compared to the primary larvae of the extant Pentastomida and represent a stage of organization prior to branching into the two recent pentastome lineages of the crown-group, the Cephalobaenida and the Porocephalida. The high morphological diversity observed in the fossil larvae suggests, that the evolution of the pentastomes had begun earlier than in the late Cambrian. The external morphology of the fossil founds clearly illustrates an advanced adaptation to a parasitic way of life in a presumably protected environment, such as for example the gill chambers of a fish, and highly diverse characteristics of the head limbs indicate an adaptation of these anchoring devices to various hosts with different life habits.

The nature of the hosts of these early pentastomes remains speculative, but a co-evolution of the Pentastomida with vertebrates or chordates would not appear impossible. Walossek & Müller (1994) point out that conodonts, cephalic structures of chordates or vertebrates, were usually present in residues containing Palaeozoic pentastomes.

The change from a marine host to a terrestrial host might have occurred via the earliest tetrapods, or alternatively, represent a secondary infection of tetrapods after these had radiated on land. The above authors point out, that a secondary infection would be in keeping with the wide geographical distribution of pentastomid parasites and simultaneously explain the scattered distribution pattern in different tetrapod taxa, including amphibians, reptiles, birds and mammals.

Baer (1952) observed that about 90% of the pentastomids occur in reptilian hosts and since amphibians are final as well as intermediate hosts for some of the Raillietiellidae (Ali, Riley & Self, 1985) it is possible, that the Carboniferous amphibian stock from which the reptiles arose (Parker & Haswell, 1964) passed on their pentastomid parasites to this newly evolving class (Haugerud, 1989). The stem-reptiles, the cotylosaurs, radiated rapidly in the Mesozoic era (245-65 million years ago) and reached a high degree of diversification with numerous representatives. Of this multitude members of four orders are presently alive, the Squamata (lizards and snakes), the Rhynchocephalia, the Chelonia and the Crocodilia (Parker & Haswell, 1964). With the exception of the Rhynchocephalia, all these are known to be hosts to pentastomes rendering credit to the assumption, that pentastomes parasitised their reptilian hosts from an early evolutionary stage onwards. Since most representatives of these orders are carnivorous, there is also a possibility that pentastomes were captured by some reptilian hosts at a later stage via the food chain.

5.2 THE PHYLOGENETIC BACKGROUND OF TODAY'S CROCODILIANS

The phylogenetic ancestry of crocodilians can be followed back to roughly 265 million years ago (Alderton, 1992) at which point in geological time Pentastomida were more than 200 million years old. Crocodilians are the largest of the living reptiles and the only surviving representatives of the so-called ruling reptiles, the Archosauria, which dominated animal communities on the continents during the Mesozoic era (Neill, 1972; Ross, 1989).

It is commonly accepted, that today's crocodilians are descendants of the thecodontians, a primitive group of small, aquatic as well as terrestrial reptiles, that inhabited the super-continent Pangea in the early part of the Mesozoic era (Parker & Haswell, 1964; Alderton, 1992; Ross, 1989). During the Triassic period (230-180 million years ago) the thecodonts underwent rapid diversification giving rise to the first crocodilians (Hickman, 1966; Alderton, 1992), which in turn started to evolve during the Triassic and Jurassic period. By the end of the Jurassic period the Eusuchians, the suborder of modern crocodilians, had made their appearance and unmistakable crocodiles were common in the subsequent Cretaceous period (Parker & Haswell, 1964).

In the meantime major geographical changes had taken part and were still in process. The single massive continent Pangea, which continued during the Triassic period had begun to split during the Jurassic period (180-130 million years ago) forming the northern continent Laurasia and the southern continent Gondwana. Another several million years passed before Gondwanaland finally separated into the landmasses known today. For a long time South America was linked through a string of long-lasting islands and shallow sea to Antarctica, to Australia, to New Zealand and New Caledonia. The separation of South America from Antarctica is estimated to have occurred about 60 million years ago and about 50 million years ago Australia split from Antarctica (Stevens, 1996).

In the wake of the continental drift, individual populations, now increasingly isolated from each other, began to evolve on divergent lines (Ross, 1989).

While the crocodilian fossil record in the mid-Cretaceous period is relatively poor, findings in Late Cretaceous and Early Tertiary localities on the Laurasian continents suggest, that the basic division of the Crocodilia into the two subfamilies of the living crocodilians, alligatorines and crocodylines took place about 80 million years ago (Ross, 1989; Alderton, 1992).

The alligatorine group assumedly developed in North America and began to radiate. Eventually one of the predecessors of today's Chinese alligator *A. sinensis* crossed the land bridge in the Bering Strait spreading the genus to Asia.

Another representative of the North American alligatorine genera, *Allognathosuchus*, is believed to be the precursor of the South American genus *Eocaiman*, which in turn is assumed to be an ancestral form of the modern caimans. However, *Allognathosuchus*, was not involved in the development of today's alligators. Nevertheless this lends support to the assumption that, by evolutionary standards, the South American genus *Caiman* is more closely related to the contemporary alligators than to the genus *Crocodylus*. Ecologically and biogeographically, however, the latter genus appears to have more in common with the caimans. Especially the range of *Caiman crocodilus* overlaps largely with that of *C. acutus* in Central America but also right down into South America (Alderton, 1992).

While the crocodylines originated in Eurasia, they soon extended into Asia and Africa as well. Like their alligatorine counterparts they reached a high level of diversity in North America, but were nevertheless unsuccessful in populating the South American continent (Alderton, 1992). Today the only representatives of the genus *Crocodylus* in South America are the American crocodile, the Cuban crocodile, Morelet's crocodile and the Orinoco crocodile, which marks the southern boundary of the genus' distribution range in the New World.

Fossilized evidence suggests, that the African Nile crocodile, *C. niloticus*, arose from *C. lloydi* which was present in Egypt during the Miocene, 25-12 million years ago (Alderton, 1992). Lately genetic fingerprinting has been used to elucidate the relationships between the various species of the genus

Crocodylus. Whereas the African slender-snouted crocodile, *C. cataphractus*, appears to be standing relatively isolated, the African Nile crocodile is assumed to be closest to the ancestral form of the genus, forming a general link with the remaining members of the group (Alderton, 1992). Information on the third African crocodilian, *Osteolaemus*, is scarce and most probably this genus originates from even more primitive stock than *Crocodylus* itself.

Furthermore, there is fossil evidence, that the crocodilian populations of Africa and South America began to diverge as early as 100 million years ago, when the widening seaway between the two continents made a faunal interchange increasingly difficult. At the same time these findings from the Early Cretaceous illustrate that South America and Africa once shared closely related if not identical crocodilian species. A most spectacular example is the giant pholidosaurid *Sarcosuchus* whose skull measured over two metres (Ross, 1989; Alderton, 1992).

Genetic finger-printing has also established, that the Australian Johnston's crocodile is the closest relative of the South American Orinoco crocodile, giving credit to the assumption that some *Crocodylus* sp. inhabited Australia before Gondwanaland ever broke up, using the Antarctic land bridge. Generally speaking it has been established, that all extant species of the genus *Crocodylus* are quite closely related (Alderton, 1992).

5.3 CONCLUSION

How can the Cambrian pentastomid fossils and the phylogeny of crocodilians be related to the development and current geographic distribution of the extant genera of the Sebekidae, parasitising recent crocodilians, chelonians and monitor lizards?

Fourteen of the currently 23 living crocodilian species have been recorded as being host to one or more pentastomes (see Chapter 4), indicating a parasite-host relationship dating back to the early days of the hosts phylogenetic development. Of the five genera of the family Sebekidae parasitising crocodilians, three genera, *Leiperia*, *Alofia* and *Sebekia* can be found throughout the range of their final hosts (Riley *et al*., 1990; Riley, 1994; Riley & Huchzermeyer, 1996).

Of these three genera, the genus *Sebekia* has attained the highest species diversity as well as the widest host spectrum, having been recorded from alligators and caimans, as well as from the genera *Crocodylus* and *Osteolaemus* (Sambon, 1922; Heymons, 1941a; Overstreet *et al*., 1985; Junker *et al*., 1999; Riley & Huchzermeyer, 2000). Its wide host and geographic range combined with the fact, that each of the main geographic regions forming part of the range of crocodilians, i.e. the Ethiopian region, Australasia as well as South and North America, are characterized by their own distinct *Sebekia*-fauna suggests that the genus *Sebekia* had already emerged and was parasitising the forebears of today's crocodilians before these split into the presently known two subfamilies 80 million years ago, and possibly even before the South American and African crocodile fauna started diverging 100 million years ago. Subsequently, continued isolation and adaptation to diverging hosts have brought forth the 11 presently acknowledged species of the most successful genus of the crocodilian pentastomes.

The genus *Leiperia* has an equally wide distribution and occurs in the Australasian region as well as on the African continent (Riley & Huchzermeyer, 1996; Junker *et al*., 2000). While *L. gracilis*, recovered as larval forms from South American *C. acutus*, *C. crocodilus* and the North American *A. mississippiensis,* has to be considered as a *species inquirenda* and may well represent several different species, the morphological descriptions of some authors leave no doubt as to the presence of at least one species of *Leiperia* in the South and North American region (Riley & Huchzermeyer, 1996). Hence it is presumed that the genus *Leiperia* originated from ancestors present in primitive crocodilian stock, before the main adaptive radiations had taken place. Despite its early appearance during evolution, the genus *Leiperia* remained static and little morphological development ensued during the further evolution of its final hosts.

The genus *Alofia*, is wide spread in the Australasian and Ethiopian region, its range on the American continent, however, is limited to South America, and while reported from the two caiman species, *C. crocodilus* and *C. latirostris*, there are no records of alofian pentastomes from the four representatives of the genus *Crocodylus* in the New World or North American alligators (Fain, 1960; Self & Rego, 1985; Riley, 1994; Riley & Huchzermeyer, 1995b; Junker *et al*., 1999). If this represents a true absence rather than a lack of pertinent data, it possibly indicates that the genus *Alofia*, emerged more recently than the genera *Leiperia* and *Sebekia*, and might only have evolved after the two crocodilian lineages split up, hence its absence in *A. mississippiensis*. The fossil evidence of a close relationship between the crocodile fauna on the African continent and South American crocodilians (Alderton, 1992) might well serve to explain why the genus *Alofia* is represented on the African and South American continents, but is absent from North American alligators.

On the other hand, epidemiological data on prevalence and intensities as regards pentastome communities of the Nile crocodile obtained during the present study suggest, that the genus *Alofia* is less successful in colonizing its final host than the genus *Sebekia*. Thus it is possible, that although the genus *Alofia* occurred in Eusuchians before the development into the extant two families of crocodilians, it was not able to establish itself successfully in the Alligatoridae. If the transmissive stages of *Alofia* were not able to withstand the comparatively harsher climate in the more northerly parts of the American range of crocodilians, they might have subsequently disappeared from these parts.

The two monospecific genera of crocodilian pentastomes, *Selfia* and *Agema*, have sofar been recorded exclusively from the Australian and African continent, respectively (Riley, 1994; Riley *et al*., 1997). It is therefore assumed that the genus *Selfia* only emerged from its pentastomid ancestors after the crocodilian fauna of Australia had become isolated from the range of the remaining crocodilians. It is noteworthy, however, that, apart from the sympatric genus *Alofia*, *Selfia* shares closest morphological ties with the genus *Diesingia*, which is exclusive in South American terrapins (Riley, 1994; Junker *et al*., in press). Seeing that distinct links existed between the two continents up to about 60 million years ago, this should not be surprising, and it can thus be presumed that the genera *Alofia*, *Selfia* and *Diesingia* emerged from a common forebear with a wide geographic range. The genus *Diesingia* branched off when this common ancestor, or one of its successors, was transferred into the new chelonian host and continued its isolated adaptation.

The equally isolated geographic distribution of the genus *Agema* on the African continent together with morphological evidence suggests, that the genus *Agema* evolved after its host had diverged from the remaining crocodilian stock. This assumption is supported by the fact that *Agema* is not present in the most wide-spread of the African crocodiles, *C. niloticus*. Considering the morphological similarities between *Agema* and the genus *Sebekia*, it is possible that a primitive *Sebekia*n pentastome parasitising early representatives of the genus *Crocodylus*, was isolated when *C. cataphractus* diverged from the common gene pool which, according to genetic finger-printing, happened early during the genus' diversification (Alderton, 1992), and was subsequently modified. Close ecological links could have facilitated the transfer of the new genus *Agema* from *C. cataphractus* to *O. tetraspis*. Similarly, *Agema* might have originated from primitive ancestors shared with the genus *Sebekia*, which digressed once *Osteolaemus* had branched off from the evolutionary line leading to the genus *Crocodylus*.

It is surprising that, considering the ancient relationship between pentastomes and their crocodilian hosts, only comparatively few pentastomid genera, each represented by relatively few species, have evolved, and, generally speaking, pentastomids are a static taxonomic group. It would appear that even fossil founds of pentastomes reflect this strong conservative tendency to adhere to a fairly stereotypic basic design. Storch (1984) provides evidence that even the supposed transition from external to internal parasitism did not result in major morphological changes, as can be deducted from the well developed cuticular sensory structures and glands. Wallossek & Müller (1994) assume that the early pentastomes already occupied their particular niche so successfully, that additional adaptations to parasitism were obsolete when adapting to new environments.

CHAPTER 6

NOTES ON THE SYSTEMATICS AND PHYLOGENY OF THE SEBEKIDAE

6.1 THE SYSTEMATIC RELATIONSHIPS AMONG PENTASTOMES OF THE FAMILY SEBEKIDAE

Introduction

In recent years a number of new pentastome species and genera have been described and existing genera revised within the family Sebekidae, that combine diagnostic characteristics of one or more genera included in this family (Riley, 1994; Riley & Huchzermeyer, 1995; Riley *et al*., 1997; Junker *et al*., 2000; Junker & Boomker, 2002; Junker *et al*., in press). As a result, the initially clear distinction between sebekiid taxa has become more complex.

Due to the severe reduction of external morphological features in pentastomes, brought about by their adaptation to a strictly parasitic way of life (Osche, 1963; Walossek & Müller, 1994), the identification of pentastomid parasites is based on relatively few morphological characteristics (Riley, 1986). These in turn are subject to a high level of intraspecific variation, which has often been referred to as one of the hallmarks of pentastome morphology (Riley, 1986; Riley & Huchzermeyer, 1995a). Nevertheless, the combination of various characters provides a reliable means in determining taxonomic status.

Almeida & Christoffersen (1999) subjected the Pentastomida in general to a Hennigian analysis and used this cladistic approach to phylogenetically systematize the pentastomid genera. Their study, based on 32 morphological characteristics, resulted in a new classification, which was nevertheless close to the traditional classification of pentastomes presently in use. According to the authors, the only necessary radical change, was the redistribution of genera of the family Sambonidae to other families. This, however has had a direct impact on the Sebekidae, as the family was now extended by the genus *Sambonia* Noc & Giglioli, 1922. Furthermore, Almeida & Christofferson (1999) omitted a number of genera belonging to the family Sebekidae in their analysis and new genera have been described or old genera revised since (Junker & Boomker, 2002; Junker *et al.*, in press). It thus seemed appropriate to put the wealth of morphological data on sebekiid pentastomes that were compiled during the course of this study, and the accompanying literature review, to use and to expand on the phylogenetic system of the Sebekidae as suggested by Almeida & Christofferson (1999).

Material and methods

The main source of data for the following analyses was the primary literature. However, in the case of the genera *Pelonia* and *Diesingia* and the African species of the genera *Sebekia*, *Leiperia* and *Alofia*, excepting *A. parva*, the author was able to compare and examine adult pentastomes from her own collection. The *Diesingia* material was kindly provided by Dr. J. Riley.

Illustrations accompanying the original descriptions of sebekiids were used to determine the most informative and consistent characteristics within the Sebekidae and, subsequently, these were sorted manually. Table 14 lists the characters used for the present analysis.

Table 14. List of characters of adult pentastomes utilized for the analysis of systematic relations in the family Sebekidae.

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1 Copulatory spicules: 0 = heavily chitinized, with prominent collar around anterior spatular extension; 1 = more delicate, the collar is either weakly developed or absent.

2 Oesophageal peg of oral cadre: 0 = present, 1 = absent

3 Distribution of chloride cell pores: 0 = several rows per annulus; 1 = a single row per annulus.

4 Cirrus tip in males: 0 = elaborate, shaped like a flared trumpet; 1 = reduced, simple.

5 Patches of spines on hooks: 0 = absent; 1 = present or secondarily (partially) reduced

6 Body shape: 0 = long and slender; 1 = short and massive (banana-shaped).

7 Anterior extension to fulcrum: 0 = absent; 1 = present on all fulcra or secondarily reduced in posterior and/or anterior fulcra.

8 Oral cadre: 0 = ovoid profile, 1 = U-shaped.

9 Hooks: 0 = flat-topped or slightly rounded; 1 = distinctly convex.

10 Copulatory spicules: 0 = with double hooked collar; 1 = collar with tiller-like extension.

11 Hooks: 0 = almost linear; 1 = hooks bent through right angle

12 Hooks: 0 = rows of large spines present; 1 = spines absent.

13 Fulcrum: 0 = extension present; 1 = extension absent

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0 = plesiomorphic state, 1 = apomorphic state

Results

The characters presented in Table 14 have been manually sorted into a data matrix which is given in Table 15. The ensuing cladogram for the currently know genera of the Sebekidae of reptiles is illustrated in Figure 10.

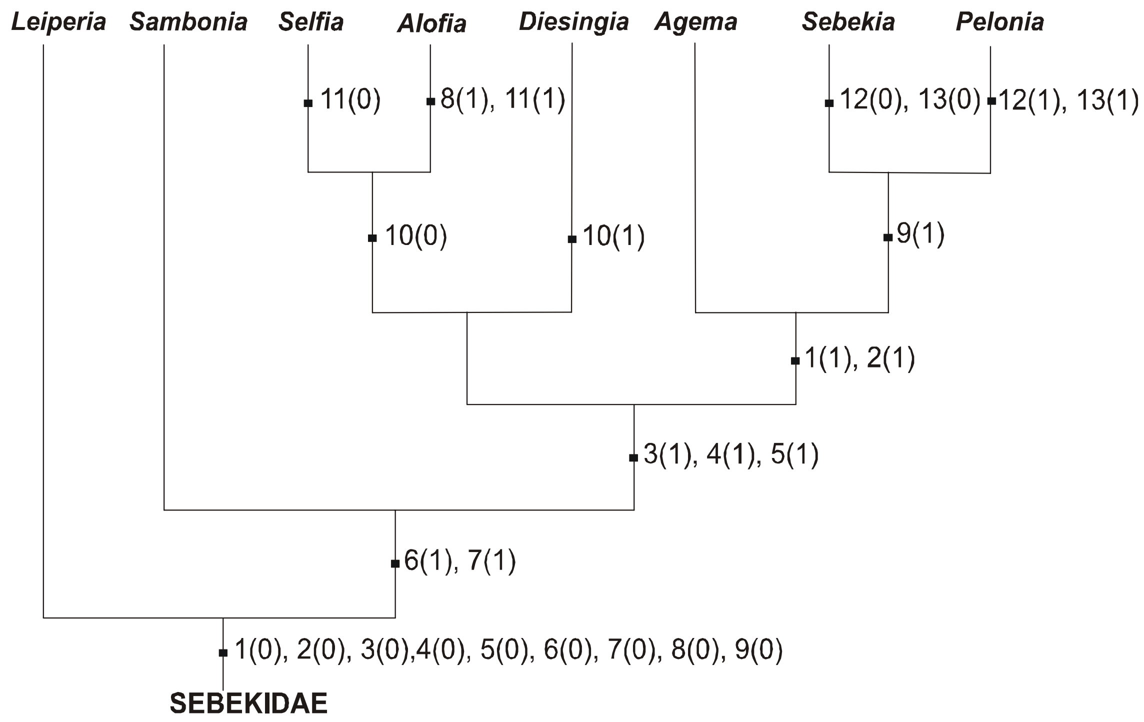

Figure 10. Cladogram for the family Sebekidae. The numbers refer to the list of characters provided in Table 14.

Table 15. Data matrix used in the cladistic analysis of the pentastome family Sebekidae.

Taxa	Characters												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Leiperia	0	0	0	0	0	0	0	0	0	-	-	-	-
Sambonia	0	0	0	0	0	1	1	0	0	-	-	-	-
Selfia	0	0	1	1	1	1	1	0	0	-	0	-	-
Alofia	0	0	1	1	1	1	1	1	0	0	1	-	-
Diesingia	0	0	1	1	1	1	1	0	0	1	-	-	-
Agema	1	1	1	1	1	1	1	0	0	-	-	-	-
Sebekia	1	1	1	1	1	1	1	0	1	-	-	0	0
Pelonia	1	1	1	1	1	1	1	0	1	-	-	1	1

0 = plesiomorphic state, 1 = apomorphic state, - = non comparable characters.

Discussion

The somewhat isolated position within the family Sebekidae allocated to the genus *Leiperia* by Almeida & Christofferson (1999) is supported. This has merits not only on morphological grounds, but is also emphasized by the different niche, namely the trachea, that this parasite occupies in the crocodilian host, and its life-cycle which is unique in the Sebekidae in that it includes an obligatory phase in the circulatory system (Riley & Huchzermeyer, 1996; Junker *et al*., 2000).

The author does not agree with Almeida & Christoffersen (1999) who accept Self & Rego's (1985) integration of the genus *Diesingia* into the genus *Sebekia* for reasons detailed in Chapter 3, and concludes that the two genera actually belong to different groups within the Sebekidae (see below).

The inclusion of the genus *Sambonia* in the family Sebekidae by Almeida & Christofferson (1999) is considered a valid move and in addition to the congruent morphological characteristics compiled by the latter authors, further similarities between *Sambonia* and other sebekiid genera exist. A comparison between the copulatory spicules of the males of *Leiperia* and those of *S. lohrmanni* shows a high degree of conformity, especially as regards the configuration of the cirrus tips. A close relation between the two genera is further emphasized by the fact, that in both genera the chloride cell pores are arranged in several rows per annulus (Fain, 1961; Riley & Huchzermeyer, 1996; Junker *et al*., 2000), whereas the remaining sebekiids usually possess a single row of chloride cells per annulus. *Sebekia okavangoensis* is an exception as it is the only other member of the family Sebekidae in which the chloride cell pores are disposed in irregular rows, three to six cells deep, on each annulus (Riley & Huchzermeyer, 1995a). A further characteristic of *S. okavangoensis*, otherwise a typical representative of the genus *Sebekia*, which sets it apart from congeneric species and at the same time forms a link to the genus *Leiperia*, is the large number of annuli and the slender and elongated, rather than squat and banana-shaped, body.

However, *S. okavangoensis* is not the only example of a sebekiid pentastome that displays morphological properties not entirely in keeping with the generic diagnosis, while in other respects it displays the typical characteristics of its genus. Usually these exceptions and atypical representatives of a certain genus offer insight on the shared, primitive characteristics of the common ancestors from which the different sebekiid genera eventually evolved.

Of the several genera of the Sebekidae, two genera, *Leiperia* and *Sambonia*, are considered morphologically close to the original design of the Sebekidae. The remaining genera of the family possess more evolved characteristics, and a simple, non-flared, cirrus tip sets them apart from *Leiperia* and *Sambonia*. Despite this shared characteristic other, divergent diagnostic characters warrant the division of the remaining genera into two groups.

The first group comprises the genera *Selfia*, *Alofia* and *Diesingia* and will in the following be referred to as the *Selfia*-group. While this group retains the primitively heavily chitinized copulatory spicules, including a well developed collar around the longer spatulate extension, as seen e.g. in the males of the genus *Leiperia*, the smooth collar has been modified into a jointed, tiller-like extension in the genus *Diesingia* (Figure 8C,D, 9E,F) and forms a double-hooked-collar in the genera *Selfia* and *Alofia* (Figure 11E,F) (Riley, 1994; Junker *et al.*, 1999; Junker *et al.* in press). Furthermore, all three genera still share the primitive peg-like extension of the oral cadre into the oesophagus (Figure 8A,B, 9A,B, 11C,D).

The second group, the *Agema*-group, includes the genera *Agema*, *Sebekia* and *Pelonia*, where the oesophageal peg has disappeared and the lateral prongs generally unite into a broad solid base posteriorly (Figure 7A,B,C, 12D,E,F). However, photographs of *S. trinitatis* and *S. mississippiensis* suggest, that an oesophageal peg might be present, but no indication of this is given in the description of either species (Overstreet *et al*., 1985; Riley *et al*., 1990). The latter supports that *Sebekia* indeed stems from ancestors in which the oral cadre extended into the oesophagus. A similar reduction that is evident in all three members of this group is the loss of the heavily chitinized support structures of the copulatory spicules. In the genus *Agema*, the collar of the spicules, while still present, is very thinly chitinized and in *Pelonia* it is virtually absent (Figure 7E,D). The genus *Sebekia*, on the other hand, accommodates species with a collar, such as *S. wedli* and *S. trinitatis* and without a collar, like *S. purdieae* and *S. johnstoni*. Intermediate forms, such as *S. multiannulata* and *S. cesarisi* illustrate a possible transformation (Figure 12H,I) (Riley *et al*., 1990; Riley *et al*., 1997; Junker & Boomker, 2002).

The shape of the copulatory spicules in the basic plan, as seen in *Leiperia* and members of the first group, is a more or less oval and compact stucture (Figure 5A). In the genera of the *Agema*-group, with the exception of the monospecific genus *Pelonia*, the shape has become more varied, resulting, for example, in a more elongated structure, which is most pronounced in *Agema silvaepalustris*, *S. cesarisi* and *S. okavangoensis* (Figure 12G,H) (Riley & Huchzermeyer, 1995a; Riley *et al*., 1997).

The features that have undergone the most drastic and possibly most frequent modifications during the evolution of the family Sebekidae are the hooks in combination with their fulcra. The morphological evidence of current species suggests that hooks, probably with minute spines were a common criterium of the sebekiid pentastomes at one stage during their evolution, and that fulcra most likely were equipped with cowl-like extensions. While none of this is in evidence in the genus *Leiperia*, the fulcra of *S. lohrmanni* possess cowl-like extensions (Fain, 1961). At present, it can not be determined, whether *Leiperia* has secondarily lost these characteristics or whether they only developed after the genus *Leiperia* had branched off from the common ancestry. Subsequently, both traits became extensively modified. In some genera spines on hooks and extensions to the fulcra became more conspicuous, in others these were reduced, but each genus usually possesses at least one species which displays one or more primitive hook characteristics. At the same time the shape and alignment of the hook, which was slightly rounded in the primitive sebekiids with a blade that was set off from the base by a slight notch also changed. It should be born in mind, that many examples exist in nature, where similar characteristics have developed entirely independent of each other, by convergent evolution with no common or immediate ancestry involved (Hickman, 1966). The author, however, agrees with Almeida & Christoffersen (1999) that the patches of spines found in *Sebekia* (Figure 12A,B,C) are homologous to the secondarily very reduced spines found in *Alofia* (Figure 11A) and, for that matter, in *Selfia*.

In the genus *Sebekia* the tendency was towards distinctly convex hooks, and the spines on the hooks became large and prominent features. The extensions of the fulcra evolved into peg-like, usually spinose, structures (Figure 12A,B,C). *Sebekia trinitatis*, however, is characterized by more or less flat-topped hooks, more reminiscent of the hooks of *Leiperia* or *Diesingia* than the dome-shaped hooks of other *Sebekia* spp. and only the anterior hooks carry minute patches of spines (Riley *et al*., 1990; Junker *et al*., 2002, in press). A spinose extension is present on the anterior fulcra, but the posterior fulcra bear smooth cowls Similarly, in *S. cesarisi* the fulcra carry spike-like anterior extensions devoid of spines, whereas fulcral extensions have been completely reduced in *S. wedli* (Figure 12B, C). Closely related to the genus *Sebekia* and most reminiscent of *S. wedli*, the only representative of the genus *Pelonia* possesses the prominent dome-shaped hooks of the former genus, but the hooks are aspinose and lack the extensions to the fulcra (Figure 7F) (Riley & Huchzermeyer, 1995a; Junker & Boomker, 2002).

The life-cycle of the genus *Agema* offers strong support for the hypothesis that spines were common hook characters in the primitive sebekiids that were secondarily reduced. During the ontogenesis of *A. silvaepalustris*, a smooth cowl is present on the fulcra of instars II and III and instar III possesses a patch of minute spines at the base of the hook blade (Riley *et al*., 1997). None of these features are retained in the adults. Nevertheless, according to the biogenetic law, stating that the ontogenesis of the individual may reflect certain phylogenetic traits (Hickmann, 1966), this development gives evidence of a spinose ancestry.

In the genus *Alofia* adaptation and speciation led to the reduction of spines and fulcral extensions and typical alofian hooks are smooth and flat-topped (Figure 11B). Riley (1994) speculates that females of *A. merki* bear a spinose extension to the fulcrum, and in *A. parva* the anterior fulcrum carries a spinose cowl. *A. nilotici* also has more primitive characteristics in that the anterior and posterior hooks of both sexes are slightly domed and carry an extensive patch of spines, resulting in a resemblance to *Sebekia* (Figure 11A) (Riley & Huchzermeyer, 1995a,b). Despite these deviations, however, all the above species of *Alofia* possess hook blades that are bent through almost a right angle, a feature which has evolved only in the genus *Alofia* (Figure 11 A,B) (Riley, 1994).

The genera *Selfia* and *Diesingia* both carry hooks that are smooth and reminiscent of the primitive hook shape of *Leiperia*, but the alignment in the genus *Selfia* is unique in that it has become almost linear. The anterior fulcra of *Selfia* are equipped with a spinose cowl, while the posterior fulcra are free of extensions. In the genus *Diesingia* on the other hand, the posterior fulcra bear smooth cowls and the anterior fulcra spinose pegs (Figure 8E,F, 9C,D) (Riley 1994; Junker *et al*., in press).

Another highly variable structure in the morphology of sebekiid pentastomes is the oral cadre. In the primitive sebekiids it is assumed to be closed, oval shaped and heavily chitinized, but subsequently has undergone modifications. The most conspicuous modification is the development of the anteriorly open, distinctly U-shaped oral cadre of the genus *Alofia*, characterized by parallel sides. Nevertheless, the oral cadre of *A. parva* with its bowed sides, and the anteriorly converging oral cadre of *A. nilotici* provide examples for a possible transformation line (Figure 11C). In the closely related genus *Selfia* the oral cadre can be either oval or more U-shaped since the anterior prongs are only loosely connected by chitinous fibres (Riley, 1994; Riley & Huchzermeyer, 1995a,b). It would, however, appear, that the reduction or the loss of chitinous fibres closing the oral cadre anteriorly, has also occurred independently within species of less closely related genera. While belonging to the second group, the oral cadres of both, *S. wedli* and *P. africana*, have an open appearance (Figure 7A,B,C, 12F), whereas the remaining members of this group have closed oral cadres. In fact, the close phylogenetic relationship between the genera *Agema* and *Sebekia* is further confirmed by the morphology of their oral cadres. In both genera the oral cadre is distinctly ovoid and a conspicuous bridge of chitin closes the cadre anteriorly (Riley *et al*., 1990; 1997).

It is obvious from the cladogram that the nature of the final host used by the presently known sebekiid genera does not influence their position within the systematic order of the Sebekidae and pentastome genera utilizing the same final host do not necessarily share a closer phylogenetic relationship. In fact, the chelonian genera *Diesingia* and *Pelonia*, have close affiliations to two different systematic groups within the Sebekidae, and more common characteristics exist between *Sambonia* utilizing monitor lizards as final hosts and the crocodilian pentastome genera *Selfia*, *Alofia*, *Agema* and *Sebekia*, than between these and *Leiperia*.

As all final hosts of the Sebekidae are amphibious reptiles, whose habitat and dietary requirements are similar, they are likely to become exposed to the infective stages of their respective parasites at one time or another.

It should be mentioned here that the intermediate host of *S. lohrmanni* is as yet unknown, similar to that of *Diesingia* and *Pelonia*, and that some authors discuss the possibility of a direct life-cycle for *S. lohrmanni* (Fain & Mortelmans, 1960). Should an intermediate host be required, however, fish would be a logical assumption. Like terrapins, Nile monitors partake in a wide variety of foods, including carrion, fish, crabs, snails, snakes, terrapins, and eggs of waterbirds and crocodiles (Patterson, 1991).

6.2 KEY TO THE GENERA OF THE PENTASTOMID FAMILY SEBEKIDAE, SAMBON 1922

Family Sebekidae

The body shape is cylindrical and lateral lines are usually distinct. Infective larvae carry double hooks. The hooks of adults are simple, prominent and their disposition is trapezoidal. The fulcrum reaches at least twice the length of the hook (Fain, 1961; Almeida & Christofferson, 1999). Adults are parasites of crocodilians, chelonians and monitor lizards.

1 Adults parasitising monitor lizards *Varanus*……………….……….***Sambonia***
Adults parasitising other final hosts……………………………….………….2

2 Adults parasitising chelonians………………………………..………………3
Adults parasitising crocodilians……..…………………………..……………….4

3 Oral cadre of adults with peg-like extension into the oesophagus and collar of copulatory spicule of males formed like a collembolan fulcrum…***Diesingia***
Oral cadre of adults without oesophageal peg and collar of copulatory spicules of males reduced...……………………………………………...***Pelonia***

4 Adults situated in the trachea, body elongated and abdomen spirally coiled………………………………………………………………….***Leiperia***
Adults situated in the lungs...……………………………………………………..5

5 Oral cadre of adults with oesophageal peg and copulatory spicules of males with prominent double-hooked collar…………………………………………6

Oesophageal peg usually absent in oral cadre, possibly present in some species. Collar of copulatory spicules of males reduced or absent, never double-hooked……………………………………………………………………..7

6 Oesophageal peg broad. Oral cadre of adults with ovoid profile, lacking parallel sides, and hooks almost linear..…………………………………..***Selfia***
Oral cadre of adults U-shaped, with parallel sides and usually open. Blade of hooks bent through almost right angle at the base……………………...***Alofia***

7 Hooks of adults smoothly curved without spines and extensions to the fulcra absent in adults. Oral cadre heavily chitinized, oval and closed anteriorly …..……………………………………………………………………..***Agema***
Hooks convex with large prominent spines and fulcra usually with anterior extension. If hooks flat-topped then anterior pair with compact group of spines in mid-region and posterior pair smooth, blades never bent at right angle. Oral cadre heavily chitinized with ovoid profile, usually closed anteriorly……………………………………………………………..***Sebekia***

6.3 SYSTEMATIC RELATIONSHIPS AMONG SEBEKIID PENTASTOMES ON THE AFRICAN CONTINENT

Of the six genera of the family Sebekidae occurring on the African continent, only the genera *Alofia* and *Sebekia* are represented by more than one species. It was attempted to establish a transformation line for the species belonging to the two latter genera. However, it is not possible to determine if any of the African *Sebekia* or *Alofia* species were the direct precursor of another *Sebekia* or *Alofia* species on this continent. It would appear that the forbearer of *S. wedli* split off early from the remaining two *Sebekia* spp. and followed its isolated speciation process, since *S. wedli* retains the original compact, oval copulatory spicules, but has lost the fulcral extensions and has an oral cadre with an open appearance (Figure 12 C,F,I). *S. okavangoensis* and *S. cesarisi*, on the other hand, possess elongated copulatory spicules, but still carry fulcral extensions and typical *Sebekia*n oral cadres (Figure 12 A,B,D,E,G,H).

It is, however, the authors opinion, substantiated by the above morphological analysis, that *P. africana* from South African terrapins is most closely related to *S. wedli* parasitising Nile crocodiles, and that *P. africana*, in fact, evolved through continued isolation and adaptation from *S. wedli* originally captured by one of the chelonian hosts.

A further example of close morphological conformity between two species of different genera exists between another species of *Sebekia* and the African genus *Agema*. The oral cadre as well as the elongated copulatory spicules of *S. cesarisi* and *A. silvaepalustris* are highly similar and the two could be easily confused were it not for differences in their hook morphology. Whether this reflects an especially close phylogenetic relationship remains speculative. In view of the fact that *Agema* occurs exclusively in *C. cataphractus* and *O. tetraspis*, while *S. cesarisi* parasitises only *C. niloticus*, the pentastome *S. okavangoensis* might be the better proposition as common ancestor, since it occurs in all three hosts.

Of the three alofian species present on the African continent, *A. parva*, parasitises *O. tetraspis* and *C. cataphractus*, but does not occur in *C. niloticus*. Combined with distinct morphological differences, this suggests, that *A. parva* split off early from the line developing towards the remaining two *Alofia* species found in the Nile crocodile only.

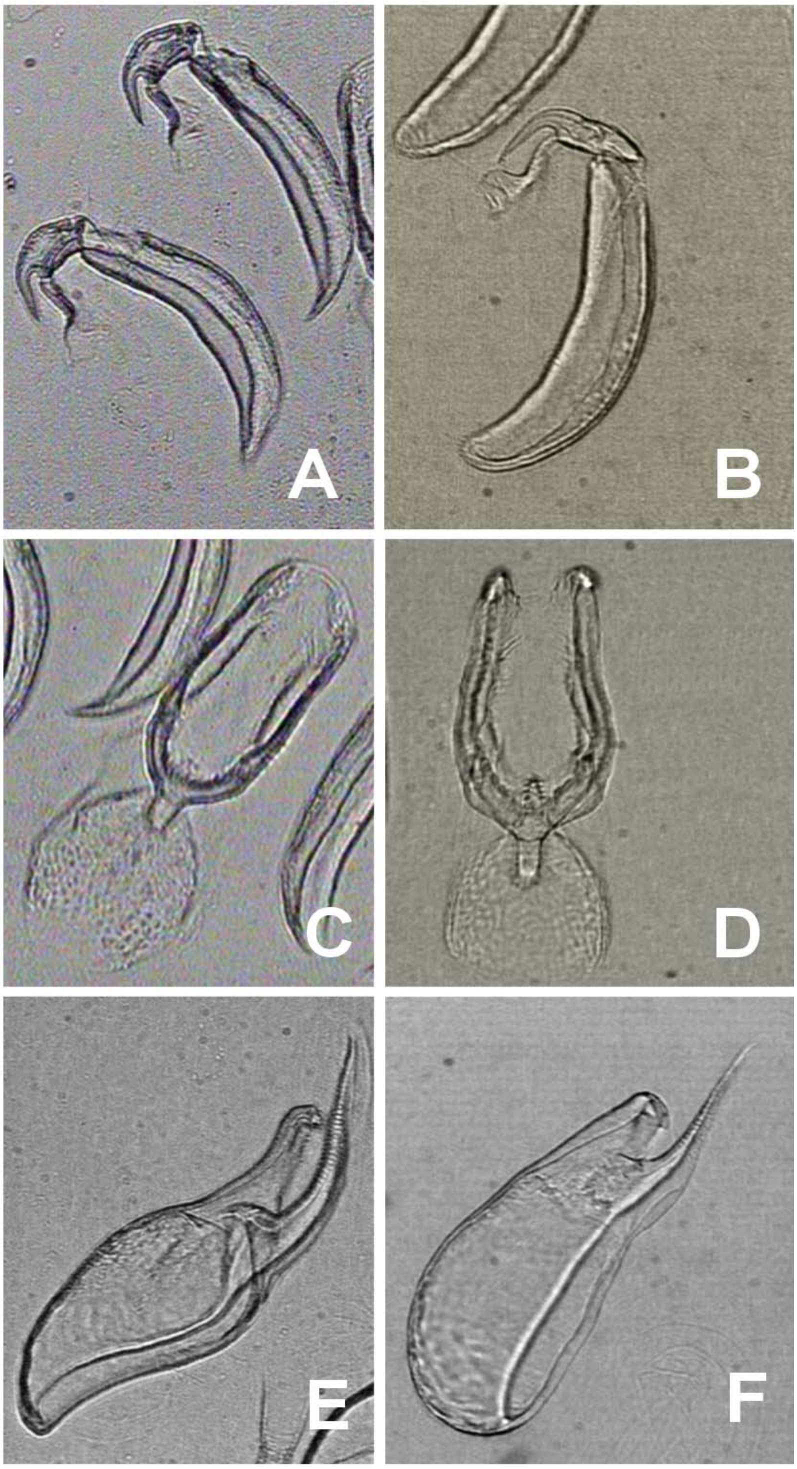

Figure 11. A,C,E. *Alofia nilotici*: A. Slightly domed anterior and posterior hook, both carrying minute spines. C. Oral cadre with peg-like extension into the oesophagus, anteriorly closed. E. Lateral view of right copulatory spicule with double hooked collar. B,D,F. *Alofia simpsoni*. B. Flat-topped posterior hook without spines. D. Anteriorly open oral cadre with oesophageal peg. F. Lateral view of right copulatory spicule with double hooked collar.

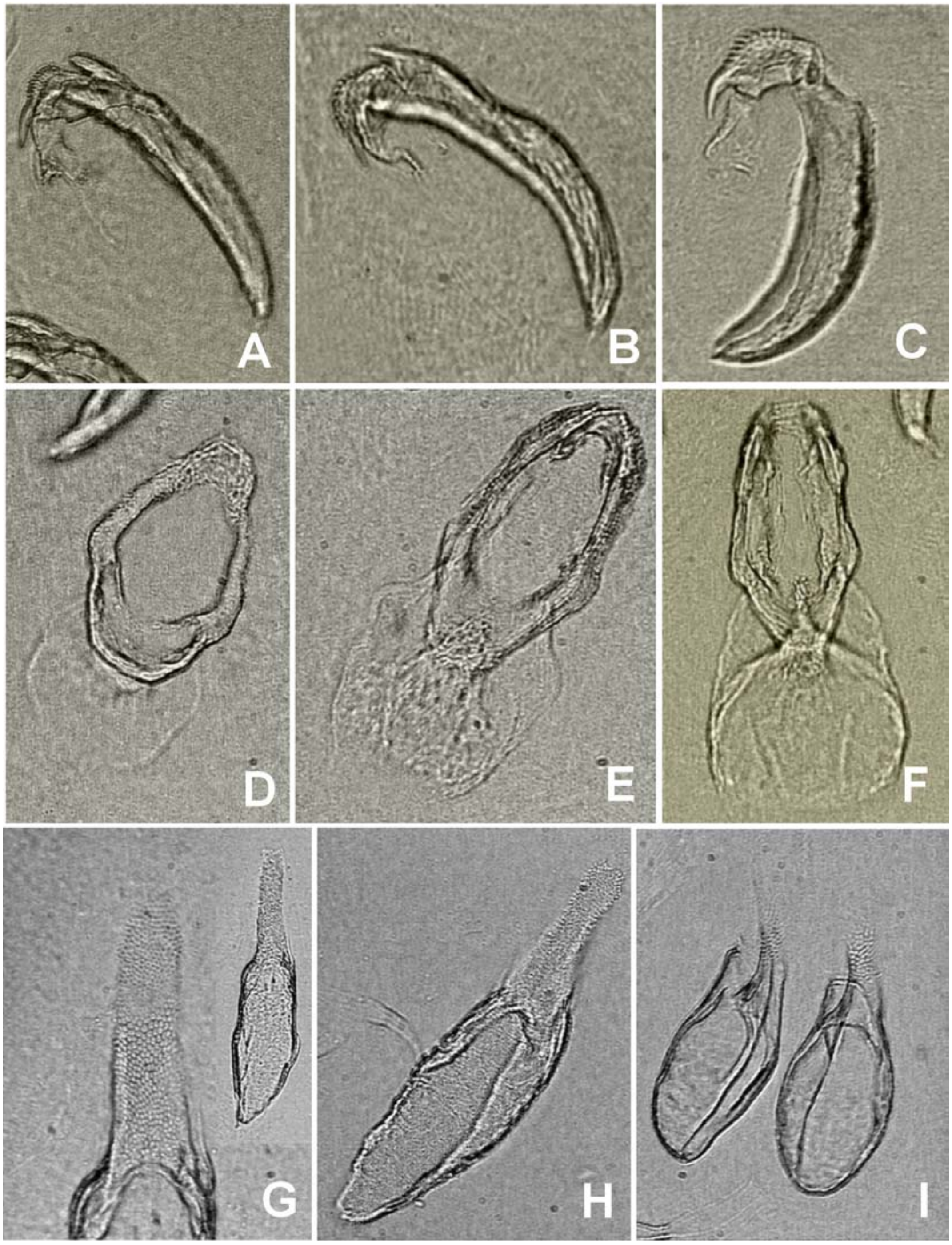

Figure 12. A,D,G. *Sebekia okavangoensis*. A. Anterior hook with prominent spines and spinous, peg-like extension to the fulcrum. D. Anteriorly closed, heavily chitinised oral cadre. G. Copulatory spicule. The cowry shell part is elongated. Note the discoid scales on the long spatulate extension. B,E,H. *Sebekia cesarisi*. B. Convex, spinous anterior hook. Fulcrum with spike-like extension. E. Oral cadre with ovoid profile. H. Ventral view of elongated copulatory spicule. The spatulate extension carries scale-like rugosities. C,F,I. *Sebekia wedli*. C. Anterior hook with spines, fulcrum without extensions. F. Oral cadre, closed anteriorly through bridge of delicate chitin. I. Lateral and ventrolateral view of the rounded copulatory spicules. Collar smooth without hooks. Spatulate extension with chitinous teeth.

Both species, *A. parva* and *A. nilotici*, display primitive characters. *Alofia parva* still possesses fulcral extensions, but already has flattenend hooks, while *A. nilotici* still has slightly dome-shaped hooks, but already lacks the fulcral extensions. Because of the shared final host, *A. simpsoni* is assumed to have a closer ancestry with *A. nilotici* than with *A parva,* although morphologically, this is not a foregone conclusion.

In evolutionary terms the more primitive species of a given taxon can often be found to occur with a higher frequency and to be more abundant than their more recent counterparts, since the latter have had less time to spread within the host population and might not yet be equally well adapted to the host environment. The dynamics of the pentastome community of *C. niloticus* supports the above scenario. *Sebekia wedli* has been identified as one of the core species in Nile crocodiles, having the highest prevalence of infection (80%), as could be expected from phylogenetically older species. The prevalence decreases towards the *Sebekia*n and alofian species with the more typical generic characteristics (71% and 50% in *S. cesarisi* and S. *okavangoensis*, respectively; 50% and 14% in *A. nilotici* and *A. simpsoni*, respectively).

CHAPTER 7

PENTASTOMID INFECTIONS IN NATURALLY INFECTED SOUTH AFRICAN NILE CROCODILES

7.1 RANGE, CONSERVATION STATUS AND BIOLOGY OF THE NILE CROCODILE

The Nile crocodile used to occur through most of the African continent, and despite recent contractions of its range, it is still wide-spread in sub-saharan Africa (Alderton, 1992). It is the only crocodile species found in southern Africa, i.e. Zambia and states to the south of it. In southern and eastern Africa the status of the Nile crocodile is considered relatively secure and abundant. However, survey data for most of west and central Africa are either very poor or altogether absent. In South Africa, Botswana, Ethiopia, Kenya, Malawi, Mozambique, Tanzania, Zambia and Zimbabwe, Madagascar and Uganda the Nile crocodile is listed on CITES Appendix II whereas throughout its remaining range it is listed on Appendix I (King & Burke, 1997).

In South Africa viable numbers of Nile crocodiles are only found in the game reserves of northern Zululand and in their major stronghold, the KNP (Patterson, 1991). In KwaZulu-Natal, Lake St Lucia is one of the most important habitats for crocodiles and supports a population of about 1200 crocodiles. The lake, however, is connected to the sea by an extensive estuary and, due to reduced water influx resulting from water removal for irrigational purposes further upstream, is becoming increasingly saline. Despite a certain tolerance of the Nile crocodile towards brackish conditions, the above changes are known to have already caused the death of crocodiles (Alderton, 1992).

Historically the severe depletion of the wild crocodile population was mainly caused by hide-hunting. The belly hide of the Nile crocodile produces a high quality leather and it is estimated that between 1950 and 1980 three million individuals were killed for their skin (Patterson, 1991; Alderton, 1992).

Modern threats to the survival of the Nile crocodile are climate changes and increasingly arid conditions, especially in the southern and northern parts of their range, as well as loss of habitat and habitat modification due to ever-expanding human settlements and increased pressure on the water resources.

On the other hand, Nile crocodiles are estimated to kill about 300 people in Africa per year, and human-crocodile conflicts have become a serious problem in some areas, especially where crocodiles and people live in close proximity. It was in order to solve this conflict, that sustainable management programs were developed and implemented.

Over the centuries the Nile crocodile has retained an important status in the cultural tradition of rural South African communities and even today it is a sought after ingredient for potions (muthi) prepared by traditional healers, so called sangomas, for a number of ailments. In the tradition of the Tsonga from southern Zululand, crocodile fat is said to protect against lightning, and a tooth or claw from a crocodile, especially a man-eating one, worn around the neck is believed to protect against crocodile attacks (Alderton, 1992). Hence, small scale poaching for crocodile organs still occurs and a crocodile might fetch up to R 1000 on the black market (Hoffmann, personal communication, 2000). However, illegal trade in general is thought to be insignificant (King & Burke, 1997).

In the 1960's the first conservation laws were enacted to protect wild crocodilians as certain species had declined drastically. This, combined with the diminished natural resources and an increasing demand for skins, resulted in a rise in prices. Prompted by these developments, conservationists as well as skin producers began to investigate the possibility of farming or ranching with crocodiles in order to produce skins on a sustained yield basis (Ross, 1989).

The first two crocodile ranches in Africa were established in Zimbabwe in 1965 and in the same year a crocodile research station was established in the Ndumu Game Reserve, in South Africa to, amongst other aims, research the captive rearing of crocodiles. Since 1990 South Africa has transferred to the CITES Appendix II ranching systems and the majority of the 80 000 skins marketed worldwide in 1993 originated from Zimbabwe and South Africa (54% and 15%, respectively) (Collins, 1995). Initially the ranching system that was commonly used, was that sustainable numbers of crocodilian stock, either eggs, hatchlings or adults, are harvested from the wild and commercially reared. Recently crocodile farms have been very successful in breeding crocodiles to the F2 generation and use these as breeding stock (Ross, 1989).

Nile crocodiles are known to utilize a large variety of habitats and may be found in large lakes, rivers, flood plains and fresh water swamps (Foggin, 1987; Ross, 1989; Patterson, 1991; Penny, 1992). They are well adapted to their aquatic way of life and when submerged little but the eyes can be detected above the surface. Being nocturnal hunters, their eyes are well adapted for night vision, they have a keen sense of smell and acute hearing. Nile crocodiles are opportunistic feeders and usually ambush their prey. Once seized the animal is dragged into the water and drowned. Crocodiles will scavenge, but they usually prefer a fresh kill (Trutnau, 1986; Alderton, 1992). It is well documented that the diet of the Nile crocodile changes as it grows in size from predominantly insects and small invertebrates by hatchlings to mainly vertebrate prey by larger crocodiles (Cott, 1961).

7.2 THE PREVALENCE AND INTENSITY OF PENTASTOMID INFECTIONS IN NILE CROCODILES

Fifteen Nile crocodiles were examined during the course of this study and with the exception of a single specimen, Crocodile 1/97, all crocodiles harboured pentastome infections, representing a prevalence of 93%. A total of 484 (565*) pentastome specimens were recovered.

Table 16. List of Nile crocodiles *Crocodylus niloticus* examined for pentastomes in this study.

Host Number	Collection date	Locality	Sex	Length (m)	Pentastome burden	Condition
A/97	20-07-97	Olifants River	M	3,5	76	bad, open fracture of right hind foot
1/97	23-09-97	Olifants River	F	2.1	0	good
2/97	23-09-97	Olifants River	M	2.6	7	good
3/97	24-09-97	Olifants River	M	3.5	4	good
4/97	25-09-97	Olifants River	F	1.4	11	good
5/97	25-09-97	Olifants River	M	4.2	43*	good
6/97	26-09-97	Sabie River	M	2.4	109*	good
7/97	27-09-97	Sabie River	M	3.9	54*	good
1/98	10-06-98	Silwervis Dam	F	2.8	52	good
2/98	10-06-98	Silwervis Dam	M	2.4	3	good
3/98	10-06-98	Silwervis Dam	F	3.2	2	good
4/98	10-06-98	Silwervis Dam	F	2.7	76	good
1/99	16-03-99	Olifants River	M	3.8	43	emaciated
2/99	06-04-99	PMC Dam	M	3.9	73	slightly thin
3/99	15-06-99	PMC Dam	F	2.6	12	good

F = female, M = male, * = pentastomes were recovered from the right lungs only and the number counted doubled

The intensity of infection varied largely, and while it was found to be as low as two and as high as 109*, the average pentastome burden per host was 40* (see Table 16).

Discussion

Pentastomid parasites form an integral part of the parasite community of Nile crocodiles in South Africa and the high prevalence of 93% obtained from *C. niloticus* in the present investigation is in compliance with records of a generally high frequency of pentastome infections in crocodilian hosts in the available literature. Characteristically the intensity of pentastome infections between individual Nile crocodiles examined during this study varied largely and covered a wide range (2 to 109).

The above corresponds with data from two South African Nile crocodiles collected by Junker *et al.* (1999) which harboured 29 and 239 pentastomid parasites, respectively, and Riley & Huchzermeyer (1995a) found 94 adult pentastomes infecting a single *C. niloticus* from Botswana.

An equally wide range of intensity (2 to 224, mean = 22,3) and a similarly high prevalence (81%) as was found in the Nile crocodiles, was reported from Swamp forest dwarf crocodiles *O. tetraspis osborni* examined in the Northern Congo Republic in 1995 (Riley & Huchzermeyer, 2000). The prevalence of pentastomid parasites in hosts of the same species examined in 1993 was 91 %. The mean intensity (3,5) was found to be unusually low and was speculated to be due to the technique used to retrieve the parasites (Riley & Huchzermeyer, 1995b). Data on the third African crocodilian species, the slender-snouted crocodile, *C. cataphractus*, are scarce, but a single individual was examined and eight pentastomes were recovered from its lungs (Riley *et al.*, 1997).

The pattern of high prevalence and a wide range of intensity of infection seen in African crocodiles is repeated in the American alligator, *Alligator mississippiensis*, for which a number of data are available. Cherry & Ager (1982) found 93% of 30 alligators from southern Florida to be infected with an average density of 10,6 (1 – 77) adult pentastomes, and seven alligators from Georgia carried a pentastome burden of about 30 to 40 each (Deakins, 1971).

Summarized, the above data show the frequency of pentastome infections to be consistently high, while the mean intensity of infection is commonly low and parasite densities of 109 and certainly 224 or 239 pentastomes per host must be considered as unusually high.

While epidemiological data may not always be available, the literature clearly shows pentastomes to be common parasites of crocodiles and alligators with a nearly worldwide distribution (compare Chapter 4). Riley (1993) speculated that at least half of the 23 extant species of crocodilians harbour one or more pentastome species. It is the authors opinion, that if more extensive studies were possible, this number would certainly be found to be considerably higher.

7.3 SPECIES COMPOSITION IN NATURALLY INFECTED NILE CROCODILES

All pentastomid parasites recovered from *C. niloticus* belonged to three genera of the family Sebekidae, namely *Alofia*, *Leiperia* and *Sebekia*. The genus *Sebekia* comprised the three species *S. cesarisi*, *S. okavangoensis* and *S. wedli* and of the genus *Alofia*, *A. nilotici* and *A. simpsoni* were present. *Leiperia cincinnalis* was the only representative of the genus. Table 17 lists the numbers of individuals belonging to the various species recovered from the different hosts according to their sex and their developmental stage. The genera *Selfia* and *Agema* were not recovered from the South African crocodiles and none of the hosts examined harboured *Subtriquetra*.

A total of 76 pentastomes were recovered from Crocodile A/97. One *L. cincinnalis* female was anchored in the tracheal wall and two infective larvae were taken from the pulmonary artery. The genus *Sebekia* was represented by three species. Thirteen *S. wedli* males and 38 patent females were found in the bronchioles and lung parenchyma, as well as six males and five patent females of *S. cesarisi*. A single *S. okavangoensis* male and patent female, and a single immature *Sebekia* sp. female were taken from the same site. In addition, an immature *S. okavangoensis* female and a mature *S. wedli* male were recovered from the pulmonary artery. Of the genus *Alofia* two species, *A. simpsoni*, of which two patent females and a single male were collected and *A. nilotici,* of which only three patent females were recovered.

Crocodile 1/97 harboured no pentastomes. A single patent *S. cesarisi* female and four males, as well as two *S. wedli* males were recovered from the lungs of Crocodile 2/97. No pentastomes were present in either the heart or the trachea.

The pentastome fauna of Crocodile 3/97 comprised two infective larvae of *S. okavangoensis* and a single infective larva and patent female of *S. wedli*. All the specimens were found in the lungs.

Crocodile 4/97 harboured 11 pentastomes in its lungs. In addition to a single *A. nilotici* male, a single male and immature female of *S. cesarisi* and five *S. wedli* males and two females were present. One of the *S. wedli* females had not reached patency yet. A single sebekiid specimen was damaged and could not be further identified.

From the right lung of Crocodile 5/97, 21 pentastomes were recovered. *S. cesarisi* was represented by three males and three patent females. A single male, immature female and infective larva of *S. okavangoensis*, respectively, were taken. While ten *S. wedli* males were collected, females of the latter species were not found. One patent *A. nilotici* female was present. Another sebekiid specimen could not be further identified. A single *L. cincinnalis* female was attached to the wall of the trachea.

Forty-three pentastomid parasites were collected from the right lung of Crocodile 6/97. The majority of these belonged to *S. wedli* of which 18 prepatent females, four males and eight infective larvae were collected. The eleven specimens of *S*. *cesarisi* comprised a single fully mature female, four prepatent females, and six males. The uteri of a single *S*. *okavangoensis* female and a single *A*. *nilotici* female contained no eggs. From the trachea seven patent *L. cincinnalis* females were recovered. Twelve infective larvae together with three immature males and a single immature female were attached to the pulmonary artery, where, together with cast off cuticles, they formed a huge cluster embedded in mucous.

The right lung of Crocodile 7/97 harboured 17 pentastomes. *S. wedli* was the dominant species with five adult males and eight patent females. A single *S*. *cesarisi* female had not reached patency yet. Two *Sebekia* sp. females and a single sebekiid specimen could not be further identified. Eight *L. cincinnalis* females were anchored in the trachea and 12 infective larvae of the same species inhabited the aorta pulmonalis.

Crocodile 1/98 harboured no *L. cincinnalis* in its trachea, however, 11 infective larvae and a total of 6 adult males and 30 pre-adult males and females were recovered from the pulmonary artery, where they formed a conspicuous cluster. A single infective larva was found in the lungs. The genus *Alofia* was not present and of each of *S*. *okavangoensis* and *S. wedli* only a single male was collected from the lungs. A single *S*. *cesarisi* female was taken from the same site, as well as a single *Sebekia* sp. female.

Only three specimens, two young females and a single male of *L. cincinnalis* were recovered from the trachea of Crocodile 2/98.

Crocodile 3/98 harboured only a single *S*. *okavangoensis* and a single *S. wedli* male in its lungs.

Table 17. List of pentastome species and numbers recovered from Nile crocodiles *Crocodylus niloticus* in South Africa.

Pentastomid parasites		Crocodile hosts													
Species	Sex	Host Number													
		A/97	2/9	3/9	4/9	5/9	6/97	7/9	1/9	2/9	3/9	4/9	1/9	2/9	3/99
Alofia nilotici	F	3	0	0	0	2*	0	0	0	0	0	0	0	0	0
(n = 13*)	PPF	0	0	0	0	0	2*	0	0	0	0	0	0	0	2
	M	0	0	0	1	0	0	0	0	0	0	0	1	2	0
Alofia simpsoni	F	2	0	0	0	0	0	0	0	0	0	0	0	0	0
(n = 6)	PPF	0	0	0	0	0	0	0	0	0	0	2	0	0	0
	M	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Leiperia	F	1	0	0	0	1	7	8	0	2	0	7	1	0	0
cincinnalis	PPF	0	0	0	0	0	1	0	2	0	0	6	0	0	0
(n = 171)	M	0	0	0	0	0	0	0	6	1	0	10	0	0	0
	PMM	0	0	0	0	0	3	0	2	0	0	5	0	0	0
	PM	0	0	0	0	0	0	0	26	0	0	11	0	0	0
	IL	2	0	0	0	0	12	12	12	0	0	33	0	0	0
Sebekia cesarisi	F	5	1	0	0	6*	2*	0	1	0	0	0	0	1	0
(n = 72*)	PPF	0	0	0	1	0	8*	2*	0	0	0	0	4	5	0
	M	6	4	0	1	6*	12*	0	0	0	0	0	3	1	3
Sebekia	F	1	0	0	0	0	0	0	0	0	0	0	0	0	0
okavangoensis	PPF	1	0	0	0	2*	2*	0	0	0	0	0	0	0	0
(n = 16*)	M	1	0	0	0	2*	0	0	1	0	1	1	0	0	0
	IL	0	0	2	0	2*	0	0	0	0	0	0	0	0	0
Sebekia wedli	F	38	0	1	1	0	0	16*	0	0	0	0	7	5	1
(n = 265*)	PPF	0	0	0	1	0	36*	0	0	0	0	0	0	16	0
	M	14	2	0	5	20*	8*	10*	1	0	1	0	27	33	5
	IL	0	0	1	0	0	16*	0	0	0	0	0	0	0	0
Sebekiid	F	0	0	0	0	0	0	4*	1	0	0	0	0	0	0
(n = 22*)	PPF	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	IL	0	0	0	0	0	0	0	0	0	0	0	0	10	1
	?	0	0	0	1	2*	0	2*	0	0	0	0	0	0	0
Total (n = 565*)		76	7	4	11	43*	109*	54*	52	3	2	76	43	73	12

F = mature female, IL = infective larva, M = mature male, PM = pre-mature developmental stages of undetermined sex, PMM = pre-mature male, PPF = pre-patent female, Sebekiid = unidentified sebekiid pentastome other than *Leiperia cincinnalis*, ? = developmental stage undetermined, * = of Crocodiles 5/97, 6/97 and 7/97 the numbers of pentastomes recovered from the right lung only have been doubled and marked by *.

A total of 76 pentastomes, however, were collected from Crocodile 4/98. Fifty-nine of these originated from a cluster of different developmental stages of *L. cincinnalis* in the pulmonary artery. Two prepatent females were recovered from the lungs, while 11 females, seven of which were patent, were attached to the trachea. Two prepatent *A. simpsoni* females were taken from the lungs, one of which was in copula with the single male. A single *S. okavangoensis* male was found in the body cavity.

The majority of the 43 pentastomes recovered from Crocodile 1/99 belonged to *S. wedli* which made up 79% of the infection. Seven patent *S. wedli* females and 27 males were collected from the lungs. A single *A. nilotici* male together with three *S. cesarisi* males as well as four prepatent *S. cesarisi* females were present at the same site. A single female *L. cincinnalis* was taken from the trachea.

Crocodile 2/99 harboured a total of 73 sebekiids in its lungs, but no pentastomes were recovered from the heart or trachea. The majority of specimens belonged to *S. wedli*. Of the latter species 33 were males and 21 females. Five of the females were gravid, the remainder had not reached patency yet. A single *S. cesarisi* male was found together with six females. Eggs were present in only one of the females. The genus *Alofia* was represented by two *A. nilotici* males. Ten infective sebekiid larvae other than *L. cincinnalis* were not identified to the species level.

While no pentastomid parasites were collected from the heart and trachea of Crocodile 3/99, 12 specimens were taken from its lungs. Five *S. wedli* males and a single patent female were present, whereas of *S. cesarisi* only three males could be found. The uteri of two *A. nilotici* females contained few eggs, without fully developed primary larvae. The species of a single infective sebekiid larva other than *Leiperia* was not determined.

All but one of the fourteen Nile crocodiles harbouring pentastomid parasites carried multiple infections (93%) and Crocodile A/97 was host to all six pentastome species recovered during this survey. However, the number of species collected per host ranged from one to six, with an average species richness of 3.3 ± 1.4 (see Table 18).

Table 18. The number of species and individuals recovered from infected Nile crocodiles, *Crocodylus niloticus*, in South Africa.

Pentastome species	Crocodile host number													
	A/97	2/97	3/97	4/97	5/97	6/97	7/97	1/98	2/98	3/98	4/98	1/99	2/99	3/99
A. nilotici	3	0	0	1	2*	2*	0	0	0	0	0	1	2	2
A. simpsoni	3	0	0	0	0	0	0	0	0	0	3	0	0	0
L. cincinnalis	3	0	0	0	1	23	20	48	3	0	72	1	0	0
S. cesarisi	11	5	0	2	12*	22*	2*	1	0	0	0	7	7	3
S. okavangoensis	3	0	2	0	6*	2*	0	1	0	1	1	0	0	0
S. wedli	52	2	2	7	20*	60*	26*	1	0	1	0	34	54	6
No. of different species	6	2	2	3	5	5	3	4	1	2	3	4	3	3

* = numbers, obtained from right lung only, were doubled for comparison

As is clear from Tables 18 and 19, *S. wedli* is by far the most commonly encountered of the various species of pentastomes recovered from South African Nile crocodiles. In fact, it was present in 12 of the 14 infected hosts and in eight of these the majority of pentastomes collected belonged to the former species. With a maximum of 60 individuals collected from a single host it was not only the species with the highest mean intensity of infection, but also with the second highest maximum intensity. The latter was only exceeded by *L. cincinnalis* of which 72 specimens infected Crocodile 4/98. In Crocodile 2/98 *L. cincinnalis* was the only species present. While *L. cincinnalis* came out third regarding prevalence, its mean intensity was only marginally lower than that of *S. wedli*.

Sebekia cesarisi occurred in the second highest percentage of hosts, but the mean number of individuals per host was distinctly below that of *S. wedli* and *L. cincinnalis*. *Sebekia okavangoensis* was part of the pentastome fauna of half the infected hosts, however, together with that of *A. nilotici* and *A. simpsoni*, its mean intensity was the lowest established during this survey. *Alofia simpsoni* was certainly the species least frequently encountered.

Table 19. The prevalence and intensity of different pentastome species from infected Nile crocodiles in South Africa.

Pentastome species	Prevalence (%)	Mean intensity	Range
A. nilotici	50	2 ± 0.7*	1 – 3*
A. simpsoni	14	3	3
L. cincinnalis	57	21 ± 26.1	1 - 72
S. cesarisi	71	7 ± 6.4*	1 – 22*
S. okavangoensis	50	2 ± 1.8*	1 – 6*
S. wedli	86	22 ± 22.8*	1 – 60*

* = numbers, obtained from right lung only, were doubled for comparison

Discussion

The pentastome fauna of South African *C. niloticus* is characterized by a high species diversity and all but one crocodiles examined in this study had multiple infections. In fact, 71% of the infected hosts harboured more than two with up to six different species, which sets crocodilian pentastomes apart from those parasitising other hosts, such as e.g. snakes, where infections with more than one species are rare (Fain, 1961).

Further reports from southern Africa confirm the species richness of the pentastome community of Nile crocodiles. Junker *et al.* (1999) recovered five and six species, respectively, from two Nile crocodiles from South Africa and the collection of pentastomid species from a single Nile crocodile from

Botswana comprised four species belonging to two genera (Riley & Huchzermeyer, 1995a).

Similar records were published on crocodilians from other continents and Riley (1994) collected *Selfia porosus* and *Alofia merki* concurrently from the Indopacific crocodile *C. porosus*. The same crocodilian is known to be the host of seven species of pentastomes representing four genera (Riley *et al.*, 1990; Riley & Huchzermeyer, 1996).

The pentastome species collected from Nile crocodiles in the present investigation were identical to those reported by Junker *et al*. (1999) and it is thus concluded that the three genera *Sebekia*, *Alofia* and *Leiperia* represented by the species *S. wedli*, *S. cesarisi, S*. *okavangoensis*, *A. nilotici*, *A. simpsoni* and *L. cincinnalis* form the main body of the pentastome fauna of *C*. *niloticus* in South Africa.

Four of the above species of pentastomes were also recovered from a Nile crocodile from the Okavango Delta in Botswana (Riley & Huchzermeyer, 1995a). The taxonomic status of a fifth species, *Alofia* sp., was left undetermined by the authors. The single female specimen carried smooth hooks, and a single row of chloride cells was disposed on the anterior border of each annulus. Descriptions of *A. simpsoni* (Riley, 1994; Junker, 1996; Junker *et al*., 1999) strongly suggest, that the single unidentified *Alofia* specimen was indeed *A. simpsoni*, giving evidence that the range of the species recovered from final hosts in the present study extends further into southern Africa.

The initial publications on the pentastomes of Nile crocodiles originated from Central Africa. *Sebekia wedli* was reported from a Nile crocodile from the then Belgian Congo (Devos, 1939) and Rodhain & Vuylsteke (1932) reported *L. cincinnalis* from *C*. *niloticus* from the same country. *Alofia simpsoni* was initially described from an unknown host in Ghana (Riley, 1994) and *S*. *okavangoensis* was present in a crocodile from Uganda (Riley & Huchzermeyer, 1995a).

While type-hosts and type-localities have not always been included in earlier publications, the above list suggests, that *L. cincinnalis*, *Sebekia* spp., and most likely *A. simpsoni* and *A. nilotici* parasitise Nile crocodiles throughout their entire range on the African continent.

In their book on patterns and processes in parasite communities, Esch, Bush & Aho (1990) discuss the role of core species and satellite species as part of a component community concept. Contrary to the core species which occur with relatively high frequencies and densities in their respective hosts, the satellite species are found less frequently and are also relatively less numerous (Holmes & Price, 1986). Bush & Holmes (1986*a*, b) described species that were intermediate in frequency and abundance as secondary species. In the following an attempt is made to structure the pentastome community of Nile crocodiles using the above terminology.

Clearly, *S. wedli* is the pentastome exhibiting the highest prevalence in Nile crocodiles examined during this study and, combined with the highest mean intensity of infection, is the most conspicuous core species of the pentastome community in *C. niloticus*. Findings from additional Nile crocodiles support this classification. Riley & Huchzermeyer (1995a) report that 80% of the pentastomid population of a single Nile crocodile was made up by *S. wedli*, and over 50% of the pentastomes retrieved from a Nile crocodile by Junker *et al*. (1999) were identified as *S. wedli*. Based on the results of the present investigation, two more core species, *S. cesarisi* and *L. cincinnalis*, were identified. The mean intensity of infection seen in *S. cesarisi* is not as high as that found in *S. wedli* and *L. cincinnalis*, but the high prevalence justifies its classification as a core species. While *L. cincinnalis* was seen with less frequency than the two *Sebekia* species, it usually occurred in high numbers. Reports from Junker *et al*. (1999) that *L. cincinnalis* was found in each of two Nile crocodiles examined and that a total of 72 *L. cincinnalis* were recovered from one of the hosts, confirm *L. cincinnalis* as a core species.

The prevalence and mean intensity of *S. okavangoensis* and *A. nilotici* in the present survey were virtually identical and they were therefore allocated the same status of importance. Due to their abundance being relatively low, but their prevalence being intermediate they are considered secondary species. *Alofia simpsoni* was recovered from two of the 14 infected hosts only, and occurred in low densities. Hence, *A. simpsoni* is considered a satellite species in the pentastomid component community of Nile crocdiles.

Interestingly, *A. simpsoni* was recovered in large numbers from Crocodile B/95 (Junker *et al*., 1999) highlighting the possible influence of single accidental events on the interpretation of results when dealing with a small data base.

Too few crocodilian hosts were made available to us to facilitate a complete analysis of the community dynamics of pentastomes of Nile crocodiles, but the current data do not suggest that the absence or presence of one of the pentastome species influences the frequency or density of another. The latter might be surprising as these pentastomes are specialists, occurring exclusively in crocodiles. Furthermore, they belong to the same feeding guild, i.e. most adult pentastomes feed on blood which is sucked from ruptured capillaries by an oral papilla coupled to a pharyngeal pump (Doucet, 1965; Riley, 1973, 1986). Another common resource shared by the crocodilian pentastomes is the microhabitat within the final host. With the exception of *L. cincinnalis*, which colonizes a separate niche in the trachea and bronchi of the final host, the two remaining genera, *Alofia* and *Sebekia*, both inhabit mainly the bronchioles of the lungs.

However, given the generally low mean intensity of pentastome infections in Nile crocodiles in relation to the size of the crocodiles' lungs, neither the availability of food nor the number of attachment sites would appear to be a sufficiently limited resource to cause competition among pentastomes. Based on the concept of Holmes & Price (1986), the author concludes that pentastomes form isolationist communities in their crocodilian hosts. According to Holmes & Price (1986), the latter contain "species with low colonizing ability, interspecific interaction is weak even amongst guild members and vacant niches exist".

Moreover, the millions-of-years old association between pentastomes and their crocodilian hosts (see Chapter 5) and the apparent absence of competition between the different crocodilian pentastome species supports the cospeciation model of Mitter & Brooks (1983) which suggests that the "phylogenies of host and parasite communities have co-evolved for so long that competition is no longer a powerful organizing force and that, consequently, the infracommunities are no longer interactive".

7.4 PENTASTOME INFECTIONS AT DIFFERENT STUDY SITES

Pentastomid parasites were present at all four study sites examined. In Table 20 the prevalence and mean intensity of infection as regards the various species at the different study sites, as well as the general prevalence and mean intensity of all the pentastomes present at a certain study site, are given.

All six pentastome species recovered from South African crocodiles occurred in hosts from the Olifants River. From the Sabie River *A. simpsoni* was lacking and *A. nilotici* from the Silwervis Dam. Three species, namely *A. simpsoni*, *L. cincinnalis* and *S. okavangoensis*, were not found in the PMC Dam, the locality with the lowest species diversity.

Two species, *S. wedli* and *S. cesarisi,* were encountered at all four the localities examined. At three of these, the Olifants River, the Sabie River, and the PMC Dam, *S. wedli* had or shared the highest prevalence and was the species with the highest abundance. *Sebekia cesarisi* had an equally high or slightly lower prevalence and in two localities came second as regards mean intensity of infection.

Table 20. The prevalence an intensity of pentastomes found in infected Nile crocodiles from the different localities examined in this study.

Parasite	Olifants River (n = 6)		Sabie River (n = 2)		Silwervis Dam (n = 4)		PMC Dam (n = 2)	
	Prevalence (%)	Mean Intensity (Range)	Prevalence (%)	Mean Intensity (Range)	Prevalence (%)	Mean Intensity (Range)	Prevalence (%)	Mean Intensity (Range)
A. nilotici	50	1.8* ±1.0 (1-3)	50	2*	0	0	100	2.0±0
A. simpsoni	17	3	0	0	25	3	0	0
L. cincinnalis	50	1.7±1.2 (1-3)	100	21.5±2.1 (20-23)	75	41.0±35.0 (3-72)	0	0
S. cesarisi	83	7.4*±4.2 (2-1*)	100	12.0*±14.1 (2*-22*)	25	1	100	5.0±2.8 (3-7)
S. okavangoensis	50	3.7*±2.1 (2-6*)	50	2*	75	1.0±0	0	0
S. wedli	100	19.5*±20.2 (2-52)	100	43.0*±24.0 (26*-60*)	50	1.0±0	100	30.0±33.9 (6-54)
Pentastomida	100	30.0*±28.0 1-52	100	78.5*±43.1 (2*-60*)	100	33.0±36.7 (1-72)	100	37.0±36.8 (2-54)

* = Numbers, obtained from right lung only, were doubled for comparison

Leiperia cincinnalis was one of the most frequent species in two of the three localities it occurred in. In the same localities it was also the species with the highest and second highest density, respectively.

Apart from the Olifants River, the Silwervis Dam was one of the study sites, where *A. simpsoni* was present and here it was in fact the pentastome with the second highest mean intensity of infection, only preceded by *L. cincinnalis*.

The frequency of *A. nilotici* was amongst the highest and second highest at the PMC Dam and in the Sabie River, respectively, while the number of individuals recovered per host remained low in all the localities.

Discussion

A closer look at the prevalence and mean intensity of the various pentastome species at the different localities confirms the role of *S. wedli* as the main pentastome of South African crocodiles. With the exception of the crocodiles taken from the Silwervis Dam, its mean intensity, and even more so its prevalence, is constantly high throughout the KNP.

The remainder of the pentastome species collected at the different localities equally reflect the general trend in the species distribution. However, one should not loose sight of the fact, that only limited numbers of crocodiles were available and trying to make general deductions from so few hosts per locality is difficult.

As would be expected to be the case in a recently created artificial biotope, the species diversity in the PMC Dam was low and at this stage the pentastome fauna is largely determined by accidental pentastomes, i.e. those that were already present in the crocodilian hosts before these left their original habitat and began populating the PMC Dam. Since the access of crocodiles from the Olifants River to the PMC Dam is not limited, the local pentastome diversity is likely to increase as more hosts with their respective pentastome populations become established. At the same time, a larger host reservoir would ensure that the host population remains above the threshold density required for the effective transmission of the parasites between hosts and thus to ensure the survival of pentastome species at a given locality (Kermack & McKendrick, 1927).

While a superficial look at the results of the Silwervis Dam crocodiles suggests a locally high abundance of pentastomes, the high mean intensity of infection regarding *L. cincinnalis* is due to a large number of pre-adults taken from clusters in the pulmonary artery of two of the four hosts. Of the remaining species only few pentastomes were present (Table 17).

Apart from the slight differences stated above, the distribution of the various pentastome species as well as their respective abundance appears to be homogenous throughout the KNP and adjacent areas, confirming the assumption of Junker *et al*. (1999) that pentastome infections are common and wide spread among the crocodile population of the KNP.

7.5 PENTASTOME INFECTIONS IN CROCODILE HOSTS OF DIFFERENT SEX AND SIZE

Of the 15 Nile crocodiles examined in this study six were females and nine were males. Crocodiles up to 3.2 m in length were of either sex, although females clearly outnumbered their male counterparts (3 males and 6 females). Crocodilies that had reached a length of 3.5 m or more were exclusively male. In Table 21 the hosts according to their length and parasite burden in an ascending order irrespective of their sex, are listed.

Table 21. Pentastome infections in South African Nile crocodiles of different sex sorted according to the length of the host and pentastome burden in ascending order.

Host Number	Sex	Length (m)	Pentastome burden
4/97	F	1.4	11
1/97	F	2.1	0
2/98	M	2.4	3
6/97	M	2.4	109*
2/97	M	2.6	7
3/99	F	2.6	12
4/98	F	2.7	76
1/98	F	2.8	52
3/98	F	3.2	2
3/97	M	3.5	4
A/97	M	3.5	76
1/99	M	3.8	43
7/97	M	3.9	54*
2/99	M	3.9	73
5/97	M	4.2	43*

F = female, M = male, * = numbers, obtained from right lung only, were doubled for comparison

Pentastomes were recovered from male and female final hosts and while all male crocodiles harboured pentastomes, a single female was found to be uninfected resulting in a slightly lower prevalence of infection in the female hosts. Data representing the distribution of pentastomes in crocodiles of different gender are compiled in Table 22, indicating a slightly higher intensity of infection in male crocodiles.

Table 22. The prevalence and intensity of pentastome infections in male and female Nile crocodiles, *Crocodylus niloticus*, in South Africa.

Crocodile hosts		Pentastomes		
Sex	Average length (m)	Prevalence (%)	Mean intensity	Range
Female (n = 6)	2,5	83	31	2 - 76
Male (n = 9)	3,3	100	46*	3 - 109*

* = numbers, obtained from right lung only, were doubled for comparison

As mentioned above, the average size of male crocodiles was distinctly larger than that of the females and in order to compensate for the suspected influence of host size on the intensity of pentastome infections, crocodiles were subsequently grouped into different size classes. The results included in Table 23 represent the mean intensity of infection irrespective of the sex of the various hosts.

Table 23. The prevalence and intensity of pentastome infections in different size Nile crocodiles, *Crocodylus niloticus*, in South Africa.

Crocodile hosts		Pentastomes		
Size class	Length (m)	Prevalence (%)	Mean intensity	Range
1	< 2 (n = 1)	100	NA	11
2	2 –2.4 (n = 3)	67	NA	3; 109*
3	2.5 – 3.4 (n = 5)	100	30	2 - 76
4	≥ 3.5 (n = 6)	100	49*	4 - 76

NA = not applicable; * = numbers, obtained from right lung only, were doubled for comparison

Little difference was seen in the prevalence of pentastome infections between the four size classes of Nile crocodiles and the decrease in prevalence in the second size class was due to a single uninfected host. The mean intensities of infection in the different size classes varied. For the crocodiles less than 2 m in length only the data of a single host are available and little comparison is possible. For the same reason the author has not included a mean intensity for size class 2, as a pentastome burden of 109* is untypically heavy and would have largely influenced the figures. Nevertheless, the last two size classes exhibit a clear tendency. In size class 3 the mean intensity of infection is 30, and 40% of the hosts carry a pentastome load that is above the general average of 40. In the largest size class this figure increases to 83% and the mean intensity of infection is as high as 49. Exclusion of the intensity of infection of host 3/97, which is distinctly out of line, increases the mean to 58. In fact, 63% of all the crocodiles with an above average intensity of infection belong to size class 4.

Discussion

Nile crocodiles under 3.5 m in length examined in this study were represented by male and female animals, but crocodiles larger than 3.5 m were exclusively male. This is in compliance with published reports that the initial rapid growth rate of juvenile American alligators slows down after about four years and that from the third year onwards a difference in size between the two sexes becomes noticeable. This development is assumed to be under hormonal control, and females will maintain a slower growth rate and will stay smaller than their male counterparts throughout their lives (Alderton, 1992). The latter confirms well to the smaller average size of the females examined during this investigation versus the larger average size of the male Nile crocodiles.

It is obvious from the results, that the differences in the mean intensity of pentastome infections in male and female crocodilian hosts (46* and 31, respectively) were not truly gender-related, but caused by the difference in average size between the two sexes.

Even the smallest Nile crocodile made available for this investigation harboured pentastomid parasites, indicating that Nile crocodiles come into contact with infective pentastome larvae from an early age onwards and are exposed to the infective agents throughout their life. However, while the prevalence of infection remains constantly high irrespective of the size, i.e. age, of the crocodilian host, the pentastome burden increases with size. This can readily be explained by the shift in the crocodilian's diet during its ontogenesis which results in a higher probability of contact with potential intermediate hosts.

Studies on the stomach contents of Nile crocodiles reveal that their food habits alter significantly as the individuals grow older and therefore larger. Hatchlings up to 50 cm in length primarily rely on insects as a source of food, followed by frogs and spiders. The main dietary item of crocodiles between 2,5 and 3,0 m are fish, found in over 60% of the examined stomachs, while snails, mammals, birds, reptiles and shellfish are taken to a lesser extent. Large crocodiles from 4,5 m onwards mainly prey on mammals and reptiles, but fish were still recovered from the stomachs of close to 40% of the crocodiles (Ross, 1989; Alderton, 1992). Add to this that growing crocodiles prey on increasingly large fish which in turn have an increased likelihood to be infected, and that a large crocodile would have to consume larger numbers of fish to meet its dietary requirements, the possibility of exposure to infective pentastome larvae further increases with the size of the final host.

Observations of Winch & Riley (1986) that 12 juvenile South American *C. sclerops* (< 1 m) were uninfected, whereas 75% of the larger caimans harboured *S. oxycephala*, support this scenario.

Furthermore, pentastomes are long-lived parasites, the patent period of *Porocephalus crotali*, another member of the Porocephalida, was reported to be at least 6 years (Riley, 1981). Thus an individual hosts can accumulate pentastome burdens over a number of years.

7.6 SEX RATIO OF THE PENTASTOMES RECOVERED

The total number of male and female pentastomes collected from infected hosts during the present investigation reveals the sex ratio to be evenly distributed (48% and 52%, respectively).

Table 24 illustrates the sex ratio of different pentastome species. In *A. simpsoni* and *A. nilotici*, which together with *S. okavangoensis*, occurred in the lowest densities, female pentastomes are dominant over the males, but the genus *Sebekia* shows no difference regarding the number of males and females recovered.

Table 24. Sex ratio within alofian and *Sebekia*n pentastome species collected from Nile crocodiles, *Crocodylus niloticus*, in South Africa.

Pentastome species	Males		Females	
	Sex ratio (%)	Number of individuals	Sex ratio (%)	Number of individuals
Alofia nilotici	31*	4	69*	9*
Alofia simpsoni	33	2	67	4
Sebekia cesarisi	49*	35*	51*	36*
Sebekia okavangoensis	50*	6*	50*	6*
Sebekia wedli	51*	126*	49*	122*

* = numbers, obtained from right lungs of Crocodiles 5 - 7/97 only, were doubled for comparison

For *Leiperia*, however, a more complex picture emerges and the sex ratio of *L. cincinnalis* at the different predilection sites differs. From aortal clusters a total of 72 instars, past the developmental stage of the infective larva, were isolated of which nine were young females, and 26 young or mature males. The sex of a group of 37 pre-adults was not determined, but might have contained more females than males, as the developing copulatory spicles of the males begin to show early during ontogenesis. Contrary to this, of the 32 specimens of *L. cincinnalis* that were collected from the trachea of the final hosts, only a single individual was male, while the remaining 31 (97%) were females.

Discussion

The number of male and female *Sebekia* encountered at their predilection sites in the individual crocodilian hosts was randomly distributed and no specific pattern discernible. In some of the hosts females outnumbered the males of the species, while in other crocodiles more males were found. The numbers were balanced frequently and the total number of male and female *Sebekia* recovered from all the infected hosts shows an even distribution of the sexes. This was not the case in the genus *Alofia*. While comparatively few pentastomes belonging to the latter genus were present in the hosts examined during this survey, the majority of hosts infected with *A. nilotici* and/or *A. simpsoni* harboured more females than males.

Data gained by Junker *et al*. (1999) from two Nile crocodiles suggested a shift of the sex ratio in mature sebekiid infections in favour of the females. The latter authors reported that females accounted for 79% of the 61 *A. simpsoni* collected from a single host. The same phenomenon was observed in the genera *Waddycephalus* and *Kiricephalus*, that are parasites of snakes, and is assumed to be due to the comparatively shorter lifespan of pentastome males (Riley & Self, 1980; 1981b). The comprehensive set of data acquired during the present study supports the latter theory for the crocodilian genus *Alofia*, but not for the genus *Sebekia*. The most striking example for a shift in the balance of male and female pentastomes during their life history is provided by *L. cincinnalis*. Young males and females seem to be evenly distributed in the circulatory system, but males are seldom present in the trachea.

7.7 SEASONALITY OF PENTASTOME INFECTIONS

Nile crocodiles were caught during spring, autumn and winter months, respectively (see Table 16), and the relative distribution of the different developmental stages, mature pentastomes, immature pentastomes and infective larvae, was established. The results are summarized in Table 25.

Little seasonal variation in the occurrence of mature and immature pentastomes as well as infective larvae can be found if the numbers of the various developmental stages of all the sebekiid species recovered from *C. niloticus* are included in the calculations.

Table 25. Percentage of different developmental stages of pentastomes of all species recovered from Nile crocodiles *Crocodylus niloticus* in South Africa. Sebekiids of undetermined developmental stage (? in Table 17) were not included.

Stage of development	Spring Aug. – Oct.	Autumn Feb.– Apr.	Winter May – Jul.
Mature	50,2 % *	69,8 %	52 %
Immature	29,6 % *	21,6 %	26 %
Infective larva	20,2 % *	8,6 %	22 %

* = numbers, obtained from right lungs of Crocodiles 5 - 7/97 only, were doubled for comparison

A closer look at Table 17, however, reveals that in Crocodiles 6/97, 7/97, 1/98 and 4/98 these figures are largely influenced by the high numbers of infective larvae and immature stages of *L. cincinnalis* that are typically associated with clusters in the pulmonary artery. In Table 26 *L. cincinnalis* was therefore excluded from the count.

The numbers of immature pentastomes and infective larvae recovered from Nile crocodiles that were shot during the spring months were slightly higher than those of autumn crocodiles, whereas in regards of mature specimens these figures were reversed.

Table 26. Percentage of different developmental stages of pentastomes of all species, excepting *Leiperia cincinnalis*, recovered from Nile crocodiles *Crocodylus niloticus* in South Africa. Sebekiids of undetermined developmental stage (? in Table 17) were not included.

Stage of development	Spring Aug. – Oct.	Autumn Feb.– Apr.	Winter May – Jul.
Mature	53, 63 %*	69,6 %	93 %
Immature	34,64 %*	21,7 %	6 %
Infective larva	11,73 %*	8,7 %	1 %

* - Numbers, obtained from right lungs of Crocodiles 5 - 7/97 only, were doubled for comparison

By far the majority of pentastomes recovered from crocodilian hosts in winter were mature specimens, while the number of immatures and even more so that of infective larvae was extremely low.

During all seasons, spring, autumn and winter, however, the number of mature pentastomes clearly exceeded that of immatures and infective larvae.

Discussion

Nile crocodiles acquire pentastome infections throughout the year, but the data in Table 25 suggest that South African Nile crocodiles are least likely to become infected with pentastomid parasites during winter, while the likelihood of infection increases during the warmer months of the year. The numbers of infective pentastome larvae, other than *L. cincinnalis*, recovered from the different Nile crocodiles were generally low, yet they further decreased during autumn and reached an absolute minimum in hosts collected in winter. This phenomenon coincides with the period of least activity on the part of the final host which is brought about by the drop in temperature as recorded in Tables 1-4 (see Chapter 2).

Incidentally, the winter months from May to June onwards are also the season during which the likewise poikilothermic fish intermediate hosts prefer to lay resting close to the bottom of lakes and rivers. Most fish remain inactive until the water temperatures begin to rise again during August/September (Boomker, personal communication, 2002), further decreasing the chances of a crocodile ingesting an infected intermediate host. The combined effects of the seasonal behavioral changes of the final as well as the intermediate hosts result in a percentage of infective larvae as low as 1% of the total number of developmental stages recovered.

When evaluating the data from the final hosts collected during the winter months, the special circumstances under which the Nile crocodiles from the Silwervis Dam were obtained must be taken into consideration. A drought period in the Tsende Sandveld region had caused the water levels in the Shingwedzi River to drop to such an extent that the Silwervis Dam had become isolated from its main feeder. At the time of capture, 26 Nile crocodiles had been confined to a body of water not more than 20 m wide and 15 m long for an extended period of time. While this emphasizes that crocodiles will tolerate closer than usual contact to their own kind and live peacefully under overcrowded conditions when water holes are drying up (Alderton, 1992), it leaves little doubt as to the fate of the fish that were equally trapped in the Silwervis Dam.

In order to rule out that the results were influenced or, in effect, caused by a prolonged unavailability of intermediate hosts, the calculations for the winter months were repeated excepting the Silwervis Dam group from the counts. The results were virtually identical with a 1% increase in the number of mature specimens and a 1% decrease in the number of immature pentastomes.

In early spring the rise in air and water temperature triggers the Nile crocodiles to resume their feeding activity (Ross, 1989) which, aided by the increased activity levels in the intermediate hosts, leads to a higher exposure of the final hosts to pentastome infections, as evidenced by the increased percentage of infective larvae recovered.

In the subtropical range of the Nile crocodile the laying season begins in late September and lasts through the middle of December (Ross, 1989) which may well explain the fact that five out of the nine crocodiles obtained in spring at the end of September 1997 were males, since female Nile crocodiles are known to guard their nests and spent only short periods drinking, and apparently fast during the 3 months incubation period (Ross, 1989). Consequently, the percentage of infective larvae recovered from females during or shortly after the incubation period should be lower than that found in male crocodiles unless females increase their food intake just before the laying season to compensate for the long fast. However, documentation pertaining to this behavior is not available, and as not all females start laying at the same time, the effects would not necessarily be distinct. The only infected female obtained in September 1997, Crocodile 4/97, was sexually immature and the absence of infective larvae in this specific crocodile cannot be attributed to breeding behaviour. Nile crocodiles generally reproduce for the first time when they are between 12 and 15 years old, by which time they measure over 2 m (Ross, 1989; Alderton, 1992).

At first sight it might appear odd that the number of immatures collected from spring crocodiles should be as high as 35%. Immature pentastomes develop for a period of about three to four months before reaching adulthood (Junker *et al.*, 1998b), thus, the immature developmental stages recovered in spring partly represent infective larvae ingested in late autumn, although the majority of immature stages recovered in late September certainly originate from nymphs ingested in early spring. While life-cycle data concerning crocodilian pentastomes are extremely scarce, Buckle, Riley & Hill (1997) have investigated the *in vitro* development of infective larvae of the pentastomid *P. crotali* from the Western diamondback rattlesnake, *Crotalus atrox*. According to these authors, the infected larvae spend between 13 and 15 days in culture prior to moulting into instar VIII. Two more moults separated immature male *P. crotali* from the adult male, a development which was completed in about 66 days and which took roughly 130 days in female *P. crotali* including an additional moult. It is with good reason that Buckle *et al*. (1997) point out the shortcomings of comparing results obtained *in vitro* to *in vivo* data, but these findings emphasize that the chances of recovering immature instars from the final host are much better than finding the infective larvae.

Since crocodiles were not available during the summer months, no comparative data for this season exist. One would have expected to find continuously high numbers of infective larvae and a percentage of immature and mature pentastomes ranging between the percentages established for the spring and autumn hosts. Junker *et al*. (1998b) have established that the development from the infective larva to the patent female in *S. wedli* takes about 7 months and it is therefore to be expected that some of the mature pentastomes recovered from crocodiles shot in winter were actually acquired during the summer months.

The results obtained in autumn show an increase in the number of mature pentastomes with a concomitant decrease in the counts of immature instars and infective larvae, when compared to the spring data. While this creates a smooth transition to the winter figures, it should be taken into account, that only two crocodiles were available, one of which, Crocodile 1/99, was severely emaciated. The advanced depletion of its fat stores suggests an extended fast or severely reduced food intake of several months. Large crocodilians are known to be able to fast for up to two years in the absence of suitable prey, relying solely on their fat reserves (Ross, 1989). Despite the presence of mature males of *S. cesarisi*, none of the *S. cesarisi* females was patent, which suggests, that these pentastomes had been ingested less than six to eight months ago (Riley, 1986; Junker *et al*., 1998b). The exclusion of the data of Crocodile 1/99 of the autumn counts increases the percentage of immature parasites to 29%.

The steady increase in the percentage of fully mature pentastomes accompanied by declining numbers of immature instars from spring to winter reflects the continuous development of pentastomid parasites ingested in previous seasons.

Junker (1996) examined two Nile crocodiles from the KNP, obtained in February and June 1995, respectively. The results conform well to the seasonal distribution pattern of pentastomid developmental stages discussed above. The host shot in early autumn harboured a pentastome population of mature sebekiids (52%, excepting *L. cincinnalis*) and infective larvae (48%, excepting *L. cincinnalis*). Immature pentastomes were absent, suggesting that the two developmental stages found, represented pentastomes acquired during a minimum of two incidents of infection, spaced considerable time apart. Had the older infection occurred as recently as during the previous winter, some prepatent females should have been present. The percentage of infective larvae recovered from the crocodile taken in the autumn of 1995 was distinctly higher than that obtained from crocodiles during the present investigation, emphasizing, that conclusions from a single host, should be treated with reserve.

The discrepancy between the adult pentastome population and the number of infective larvae (97% and 3%, respectively, excepting *L. cincinnalis*) in the winter crocodile obtained by Junker (1996), confirms the results of the present investigation, but it should be taken into consideration that the crocodile was extremely emaciated and moribund. As in crocodile 1/99, an influence on the composition of the pentastome community would be expected.

Throughout the different seasons, as well as in the majority of the final crocodile hosts examined in this study (86%), the number of adult pentastomes exceeded that of immature pentastomes and infective larvae. This is to be seen as a result of the relative longevity of adult pentastomes. Data from captive final hosts suggest that many pentastomid parasites live up to several years with egg production continuing for as long as 6 to 10 years (Riley, 1983, 1986; Buckle *et al*., 1997). Therefore, mature pentastomes derived from a specific host at a given time may originate from a number of different transmissions.

An interesting aberration from this pattern has been described for *A. silvaepalustris* from *O. tetraspis osborni* by Riley & Huchzermeyer (2000). The latter authors found the number of developing stages to far exceed that of adults indicating that the latter probably persist for less than one year. It was thus concluded, that there is an annual cycle of transmission in *A. silvaepalustris* (Riley & Huchzermeyer, 2000).

Some authors (Boyce, 1985; Overstreet *et al*., 1985) discussed the probability of a seasonal cycle in the occurrence of *S. mississippiensis* nymphs and adults in the lungs of the American alligator. This theory is well supported by the above data on Nile crocodiles. Moreover, the range of the American alligator extends northwards into the temperate zone, where winter generally lasts longer and is characterized by lower temperatures. Thus, the effects of seasonal behavioural changes in the final hosts on their pentastomid parasites should be even more pronounced than in their subtropical counterparts.

7.8 HOST SPECIFITY AND A COMPARISON OF THE PENTASTOME FAUNA OF *CROCODYLUS NILOTICUS*, *CROCODYLUS CATAPHRACTUS* AND *OSTEOLAEMUS TETRASPIS*

Generally speaking host specifity is more pronounced in adult stages of parasites than in their larval stages (Nicoli & Nicoli, 1966) and the same is true for pentastomid parasites. The adults of *Sebekia*, *Alofia, Leiperia, Agema* and *Selfia* are found in crocodilian hosts exclusively, with a possible exception of a single species of *Sebekia* reaching maturity in terrapins (Riley, 1994; Riley *et al*., 1997). A look at the distribution of crocodilian pentastome species on the African continent suggests that the pentastome fauna of *C. niloticus*, the most widespread of the African crocodiles, is distinct from that of *C. cataphractus* and *O. tetraspis*. While the distribution of *C. niloticus* ranges from tropical to southern Africa, the West African slender-snouted crocodile and the West African dwarf crocodile are limited to the equatorial rain forest zone of west and central Africa (Ross, 1998).

A new monotypic pentastomid genus *Agema* Riley, Hill & Huchzermeyer, 1997 was described from *C. cataphractus* and *O. tetraspis*, but to this date *A. silvaepalustris* has not been found in Nile crocodiles. The same is true for *A. parva* Riley & Huchzermeyer, 1995 a species collected from *O. tetraspis* and which is also present in *C. cataphractus* (Riley *et al*., 1997).

Riley & Huchzermeyer (1995b) dissected 23 *O. tetraspis*, originating not only from the Northern Congo but possibly from the neighbouring Zaire as well. Twenty-one of these harboured pentastomes and *A. parva*, *A. silvaepalustris* and *S. okavangoensis* were present. The same species were recovered from a single juvenile *C. cataphractus* and the lungs and viscera of 15 *O. tetraspis* (Riley *et al*., 1997). With the exception of *S. okavangoensis* none of the pentastomes commonly infecting *C. niloticus* were reported.

Nevertheless, the description of *A. simpsoni* (Riley, 1994) was based on two slide-mounted female specimens derived from an unknown host in Ghana. All three African species of crocodilians have been recorded from Ghana (Neill, 1972) and the former author speculates that any of the three could be the final host of *A. simpsoni*. Fain (1961) refers to a report of a single infective larva of *L. cincinnalis* from *C. cataphractus*, but the author believes this to be the infective larva of *S. okavangoensis*, as the latter pentastome also possesses a long and slender body with up to 99 annuli (Riley & Huchzermeyer, 1995a).

Considering the number of hosts examined by Riley & Huchzermeyer (1995b) and Riley *et al*. (1997), other sebekiids would most likely have been collected, had they actually been present. It is therefore reasonable to assume that the pentastome fauna of *C. niloticus* differs distinctly from that of *C. cataphractus* and *O. tetraspis*. The latter two in turn share the same pentastomid parasites.

Ecological differences might well be the cause of the absence of *Agema* and *A. parva* from the Nile crocodiles and the absence of the majority of *Sebekia* spp., *Alofia* spp. and *L. cincinnalis* from the remaining two crocodiles. Although the distribution ranges of *C. niloticus* and the two remaining African crocodilians overlap in certain regions, the different habitat requirements of the three crocodilian species result in more or less strict geographical isolation, limiting a free exchange between the various pentastome populations.

Osteolaemus tetraspis and *C. cataphractus* are both deep forest species (Waitkuwait, 1989, 1990; Kofron, 1992), with *O. tetraspis* preferring swamps bordering rivers, or small periodically flooded forest pools, and the latter inhabiting more permanent bodies of open, flowing water, such as lagoons, lakes or rivers. Depending on the flood levels of rivers, their limited range overlaps to a large extent and this close geographical and ecological proximity of the two crocodiles could well account for their shared pentastome fauna. At the same time, the habitat preference of *C. cataphractus* forms a possible link to the pentastome community of *C. niloticus*.

Riley *et al*. (1997) discuss the possibility of fish intermediate hosts transferring pentastomid parasites from *C. cataphractus* to *O. tetraspis* and vice versa. Depending on the flood levels these fish have access to both habitats thus being exposed to pentastome eggs deposited by either of the two crocodile hosts.

Under the assumption that the genus *Agema*, as well as the species *A. parva*, initially evolved in dwarf crocodiles and was subsequently captured by *C. cataphractus* the above way of transmisssion might explain the presence of *Agema* in *C. cataphractus* and its absence in *C. niloticus*.

Similarly, there is a possibility of *C. cataphractus* having acquired *S. okavangoensis* when ingesting fish infected with eggs of the latter parasite deposited by the original final host, *C. niloticus*. Since both species prefer open water they would prey on the same intermediate hosts in certain areas of their range. There is a strong likelihood that, having captured *S. okavangoensis*, *C. cataphractus* would indirectly transfer the parasite to *O. tetraspis*.

Clearly, the above theory does not satisfactorily explain why only *S. okavangoensis* was transferred from the Nile crocodile to the remaining crocodiles and not *S. wedli* or *S. cesarisi* as well.

This is even more remarkable as *S. okavangoensis* was the least common of its congeneric species retrieved in the present study which is consistent with findings of Riley & Huchzermeyer (1995a). Out of a total of 94 pentastomes taken from the lungs of *C. niloticus* only five were *S. okavangoensis*. By the same token, out of a total of 357 pentastomes recovered from 16 Dwarf crocodiles *S. okavangoensis* was represented by only six specimens (Riley & Huchzermeyer, 2000).

Regrettably, all recent material, i.e. material acquired after the reassessment of the genera *Sebekia* and *Alofia* (Self & Rego, 1985; Riley *et al.*, 1990; Riley, 1994; Riley & Huchzermeyer, 1995a), from *C. niloticus* has been derived from Nile crocodiles in southern Africa. It would certainly be interesting to compare the pentastome populations of Nile crocodiles from central and West Africa versus that of their southern counterparts. While *Agema* and *A. parva* are absent in Nile crocodiles in Botswana and South Africa, they might actually parasitise *C. niloticus* in that part of its range that overlaps with that of the two equatorial species.

CHAPTER 8

PENTASTOMID INFECTIONS IN NATURALLY INFECTED SOUTH AFRICAN TERRAPINS

8.1 BIOLOGY, HABITAT AND RANGE OF THE HOSTS

Southern Africa offers habitat to 23 different species of chelonians, including marine turtles, land-dwelling tortoises and freshwater terrapins. The latter all belong to the suborder Pleurodira, which fold in their necks side-ways (side-necked turtles). Alternatively, the Cryptodira (hidden-necked turtles) withdraw their head straight back into the shell (Patterson, 1991). Like today's crocodiles, chelonians have evolved from common cotylosaurian ancestors as early as about 135 million years ago and since then the unique protective "shell" formed by the carapace and plastron, has remained largely unmodified (Patterson, 1991).

The genus *Pelusios* is the largest representative of the side-necked turtles on the African continent and is wide-spread throughout southern Africa, where it is represented by four species (Patterson, 1991). Its generic name is a Greek reference to its habitat meaning earth or mud, hence they are often referred to as the side-necked mud turtles (Kirkpatrick, 1995). Its South African common name, serrated hinged terrapin, is very descriptive, since the plastron of *Pelusios* is hinged anteriorly and can close upwards. Thus, the head can be sealed effectively when threatened (Patterson, 1991). *Pelusios sinuatus* is the most wide-spread of the hinged terrapins in southern Africa, inhabiting large rivers and pans in the north-east of southern Africa. It is also the largest representative of the genus and adults can reach a length of about 30 cm (Patterson, 1991).

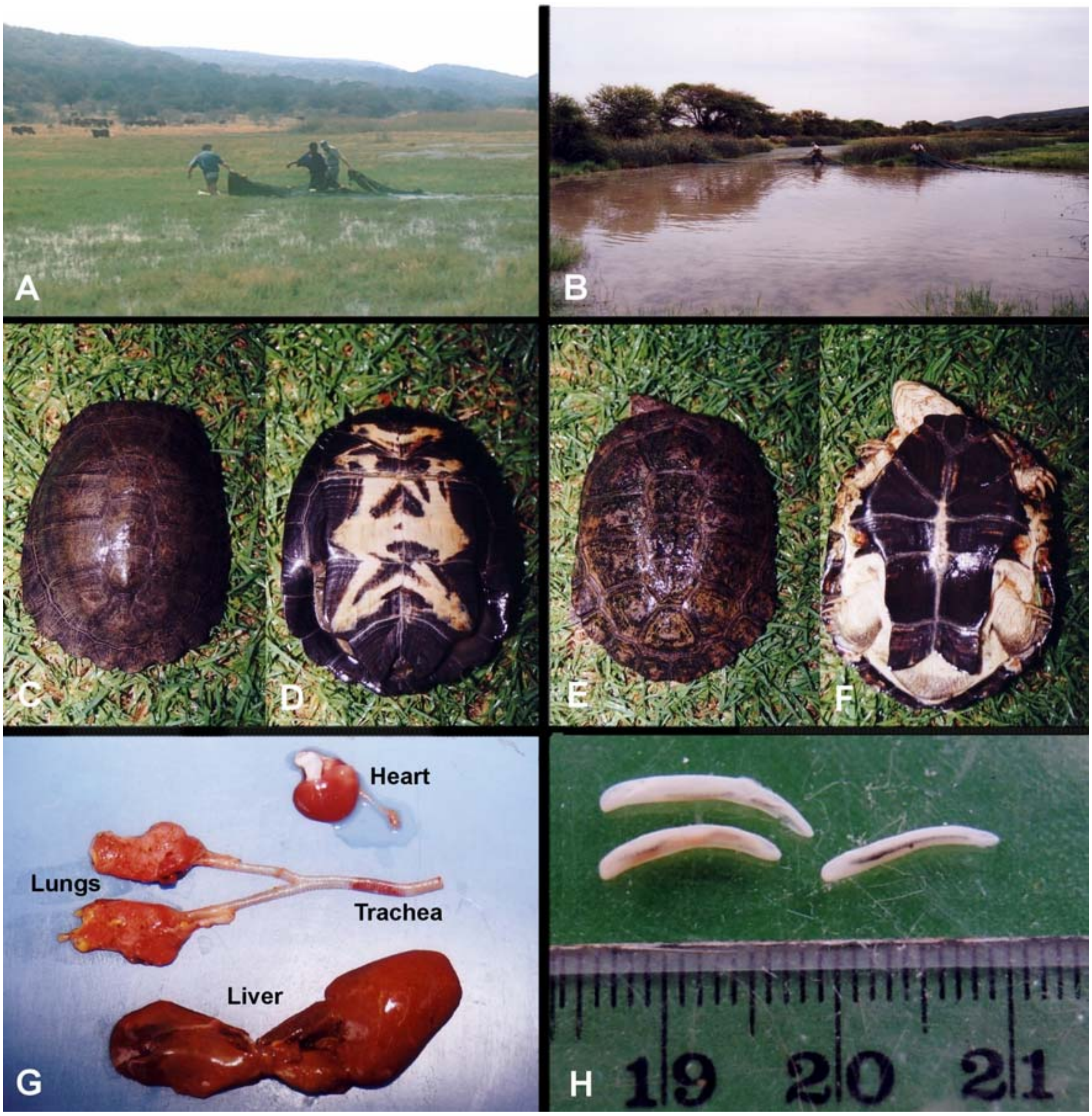

Figure 13. A. Netting terrapins at a marshy pond in the Arabie Dam vicinity. B. A different aspect of the same localitiy as in A. C, D. The carapace and plastron of *Pelusios sinuatus*. E,F. The carapace and plastron of *Pelomedusa subrufa*. G. Organs of *P. subrufa* examined for pentastome collection. H. *Pelonia africana* males.

Another pleurodiran terrapin with a wide range throughout southern Africa is the Cape terrapin *Pelomedusa subrufa* which occurs in seasonal pans, marshy areas and slow moving waters. They leave drying pans and dig into the soil to await the rains. *Pelomedusa subrufa* lacks a hinged plastron and merely retracts its head under the overhang of the carapace. Cape terrapins grow to a length of about 25 cm. Four dermal stink glands at the top of the legs exude an offensive odour when the animal feels threatened (Patterson, 1991).

Both terrapins are omnivorous and include a wide variety of foods, such as snails, small crabs, tadpoles, frogs, fish, small birds, carrion and various water plants in their diet (Patterson, 1991).

8.2 PREVALENCE AND INTENSITY OF INFECTIONS

A new species of pentastomid parasites, *Pelonia africana*, was described from both species of South African terrapins, *P. sinuatus* (n = 12) and *P. subrufa* (n = 8), examined in the present study. The species description is included in Chapter 3. Data pertaining to the hosts and their respective pentastome burdens are presented in Table 27. The nasopharynx and trachea of all hosts examined were free of pentastomid parasites and no other pentastome species were present.

Five of the 17 (29%) randomly collected hosts (terrapins WIII, Psub2 and Psub3 were included after they had been found to be infected) harboured pentastomes in their lungs. The prevalence of *P. africana* was found to be higher in *P. sinuatus* and four of the 11 randomly collected hosts supported the parasite (36%). Only one of six randomly netted *P. subrufa* hosted *P. africana*, resulting in a prevalence of 17%. The prevalence of pentastome infections was slightly higher in male serrated hinged terrapins than in females (40% versus 33%).

Table 27. List of terrapins, *Pelusios sinuatus* and *Pelomedusa subrufa*, from the Arabie Dam examined for pentastomes in the present study.

Host number	Collection date	Sex	Length (cm)	Pentastome burden	Condition
WIII[a]	?-2000	?	?	1, immature F	?
Psin1	09-03-00	M	21	0	good
Psin2	09-03-00	F	25	1, immature F	good
Psin3	09-03-00	F	24	0	good
Psin4	04-09-00	F	15	0	good
Psin5	04-09-00	M	19	0	good
Psin6	14-02-02	F	20	0	good
Psin7	14-02-02	F	20	1, mature M	good
Psin8	14-02-02	M	18	1, mature M	good
Psin9	14-02-02	M	17	1, mature M	good
Psin10	14-02-02	F	17	0	good
Psin11	14-02-02	M	16	0	good
Psub1	04-09-00	M	19	0	good
Psub2[a]	?-2000	?	?	1, mature F	?
Psub3[a]	?-2000	?	?	1, mature M	?
Psub4	14-02-02	M	22	2, mature M	good
Psub5	14-02-02	M	22	0	good
Psub6	14-02-02	F	17	0	good
Psub7	14-02-02	I	13	0	good
Psub8	14-02-02	I	12	0	good

F = female, I = immature, M = male, ? = unknown, [a] = hosts were not collected randomly

The intensity of infection was found to be extremely low in both terrapin species, and 88% of all the infected hosts included in this study harboured a single specimen of *P. africana* only. The maximum number of pentastomes per host, recovered from a single *P. subrufa*, was two.

No pentastomid parasites were collected from chelonian hosts under 17 cm in length. When the remainder of the hosts were divided in two size classes, ranging from 17 cm to 20 cm and from 21 cm to 25 cm, no significant increase in the prevalence of pentastome infections could be seen in the group containing the larger hosts (38 and 40%, respectively).

All nine specimens of *P. africana* recovered from the lungs of the various hosts were adults and the male pentastomes outnumbered the females. All the six male specimens were considered mature and of the three females a single specimen had reached patency.

Discussion

A review of the literature on pentastome infections in terrapins reveals a lack of data on the prevalence and intensities of infections. Generally speaking, few publications on chelonian pentastomes are available, and authors often confined themselves to the mere description of the pentastomes without providing any epidemiological data.

Compared to the prevalence of sebekiid pentastomes in Nile crocodiles from South Africa, the prevalence of *Pelonia* in South African terrapins is low, despite its belonging to the same pentastome family. The latter may be explained through different feeding habits of crocodiles and terrapins. Carrion is a major constituent of both the Cape and serrated hinged terrapins' diet (Patterson, 1991). From a certain size onwards, fish are one of the main dietary items of Nile crocodiles. Thus, the probability of transmission via the food-chain is lower in terrapins than in Nile crocodiles. Furthermore, terrapins of a maximum body length of about 25 to 30 cm (Patterson, 1991) prey on smaller fish than a crocodile of up to 4 m. During the present study it was established, however, that the incidence of pentastome infection increases in fish from about 17 cm and longer. Consequently, the chances of exposure to viable infective pentastome larvae are lower in terrapins than in crocodiles.

Nevertheless, it is the author's opinion that the decreased likelihood of exposure of terrapins to transmissive stages of pentastomes when compared to crocodiles is somewhat counterbalanced by the gregarious behaviour of the chelonian hosts. It is not uncommon to find large numbers of terrapins basking on the edges of small pans or lakes. In turn, their local abundance increases the probability of host numbers remaining above the density which is critical for the parasites' successful transmission (Kermack & McKendrick, 1927). Similarly, large numbers of terrapins feeding on the same source at the same time decrease the chance of food spoiling. Thus, the chance of one of them becoming infected with viable infective larvae is enhanced.

Clearly the difference in host size has an effect on the pentastome burden that can be sustained by the two groups of hosts, too. The lungs of a big crocodile easily weigh several kilograms, while the lungs of even a fully-grown Serrated hinged terrapin would weigh only a few grams (personal observation, 2002). The body size of *P. africana* correlates well with that of crocodilian pentastomes such as *Alofia* spp. or *Sebekia* spp., and is not reduced in order to adapt to the smaller size of its host's lungs. Hence, it is reasonable to expect lower parasite intensities in the terrapins.

The low mean intensity of *P. africana* in the South African terrapins compares well to the two male specimens of *Diesingia* recovered from, most probably, a single South American terrapin (Heymons, 1941a). In the South African genus *Pelonia* as well as in the South American *Diesingia*, male specimens seem to outnumber females, but too few data exist to determine whether this is accidental or represents a true tendency.

To date the true intermediate host for *P. africana* is not known, although fish most probably fulfil this role, since they are intermediate hosts for the remaining sebekiids. The only fish present in the marshy pan the infected terrapins were obtained from were catfish, *Clarias gariepinus*, but these harboured no pentastomes (see Chapter 10). Therefore, it is reasonable to assume, that the close-by Arabie Dam, habitat to a wide spectrum of fish species and terrapins, is the actual reservoir from which the pentastomes originate. Due to lack of intermediate hosts in the marshy pan the life-cycle can not be successfully completed and thus a viable population of pentastomid parasites can not be sustained. Hence, the presumption is that infected terrapins recovered from the marshy pan acquired their complement of pentastomes before leaving the Arabie Dam itself and migrating into the pan.

While the genera of some of the pentastomid families seem to be restricted to a specific systematic group of final hosts, adult specimens of the families Porocephalidae as well as Armilliferidae appear to occur in snakes only (Riley & Self, 1979, 1980, 1981a; Fain, 1961; Heymons, 1935), other families offer examples of a wider host spectrum. The family Cephalobaenidae, for instance, combines genera parasitising snakes, lizards, amphisbaenians and amphibians (Von Haffner & Rack, 1971; Ali *et al*., 1985). Thus, it is not exceptional that some members of the family Sebekidae use crocodilians as final hosts, while others are found in chelonians.

Within the various pentastome genera of the order Porocephalida themselves, however, there seems to be considerably less leeway as regards host specifity, and the available literature suggests that host specifity within a certain genus is indeed strict (Heymons, 1935; Fain & Mortelmans, 1960; Fain, 1961; Riley & Self, 1979, 1980, 1981a,b; Riley *et al*.,1990; Riley, 1994).

There has been speculation, whether *S. mississippiensis* from the North American alligator could reach maturity in turtles as well (Dukes *et al*., 1971). Nymphs of this pentastome have been reported from the turtles *Chrysemys scripta elegans, Sternotherus odoratus* and *Trionyx spiniferus* from Louisiana (Overstreet *et al*., 1985). Dukes *et al*. (1971) experimentally infected the snapping turtle, *Chelydra serpentina*, with nymphs of *S. mississippiensis* collected from naturally infected largemouth bass and recovered nymphs from the lungs of the experimental host one month later. Boyce (1985) succeeded in experimentally infecting captive raised Florida cooters, *Pseudemys nelsoni*, and one Florida softshell, *Trionyx ferox*, with infective larvae of the same pentastomid. After two weeks, and in the case of the Florida cooters, after four months, nymphs were recovered from various organs, including the lungs.

While the above data show that infective larvae of *S. mississippiensis* are able to survive in a number of terrapins, the latter does not necessarily imply that the terrapins act as true intermediate hosts for this pentastome. They might, in fact, serve as paratenic hosts only. The latter appears reasonable to assume, especially in view of the fact, that four months after the infection of the Florida cooters the pentastome nymphs had not undergone any further development in the terrapin host. Junker *et al.* (1998b) reported that 95 days after the experimental infection of *C. niloticus* with infective larvae of another crocodilian pentastome, *S. wedli*, mature males and immature females were present in the lungs. In the genus *Porocephalus* the same development has been completed within 75 to 86 days (Riley, 1981). Since the development in pentastomid parasites is generally speaking very stereotypic (Riley, 1986), there is no cause to expect that the development of two members of the same genus should take place at a distinctly different pace.

On the other hand, terrapins would easily fit the role of paratenic host for *S. mississippiensis*, since fish form part of their catholic diet, and they in turn are readily taken by alligators and crocodiles (Patterson, 1991; Alderton, 1999). The data gathered from South African crocodiles and terrapins suggest that the pentastome communities of the two final hosts are separate and no overlap exists, despite the close proximity of terrapins and crocodiles in many of the localities. Considering the species richness of crocodilian pentastomes in South Africa, one would suspect that terrapins are equally parasitised by more than just a single pentastome species.

What is true for so many of the pentastomid genera, is certainly reflected by the ones utilizing chelonian hosts. Little is known about their life-cycles and there is much scope for further studies.

CHAPTER 9

EXPERIMENTAL INFECTIONS OF FINAL AND INTERMEDIATE HOST

9.1 EXPERIMENTALLY INFECTED CROCODILES

Introduction

To date few attempts have been made to elucidate the life cycle of crocodilian pentastomes by experimentally infecting final hosts. This is partly due to the conservation status of many of the crocodilians, making it difficult to obtain permission to use these reptiles as experimental animals. Furthermore, it is not always easy to provide the crocodilians with enclosures that reflect the environmental conditions of their natural habitat sufficiently well to allow normal behavioural patterns. Winch & Riley (1986b) observed that the inability of captive *C. sclerops* to return to the water at night, as it would under natural conditions, had a negative impact on the experimental life-cycle studies of *S. subtriquetra*, and that the prolonged passage of larvae through the gut due to chronic constipation caused by the caiman's forced inactivity resulted in larval death. Thus the enclosure for the Nile crocodiles used for the experimental infections during the present study was designed to provide the hosts with sufficient space to move around and to allow them access to water in a small pond. Keeping experimental *C. niloticus* under similar conditions, Junker *et al.* (1998b) successfully investigated the life-cycle of *S. wedli*.

The aim of the current experimental infections of Nile crocodiles with L. cincinnalis was to confirm the assumption, based on data from natural infections, that the development of L. cincinnalis includes an obligatory phase in the cardiovascular system of the host and to gain insight on the number of moults as well as migration routes of the various instars. In the case of S. *rileyi* it was furthermore intended to describe the adults of this parasites hitherto only known as infective larva (Junker *et al.*, 1998a) and to confirm the Nile crocodile as its final host.

Material and methods

These have been discussed in detail in Chapter 2.

Results

Clinical signs of infection were not detected in any of the experimentally infected crocodiles and no lesions attributable to pentastome adhesions or tissue migration were noted at necropsy.

The stomach contents of Crocodile W4 three days after infection contained no pentastomid residues.

While S. *rileyi* was not present in any of the seven infected crocodiles, three of them harboured L. cincinnalis. All instars of L. cincinnalis, excepting the specimen recovered from Crocodile W5, were at the same developmental stage as at the time of infection, i.e., in all specimens the strongly recurved hooks were overlain by a less-curved spikey extension and the annuli carried rows of spines characteristic for the infective larva.

At day 98 and 138 after infection three infective larvae of L. cincinnalis, specimens W1/1, 2 and 3, were found in the right ventricle of the heart of Crocodile W1. One of these, specimen W1/1, was still alive at recovery.

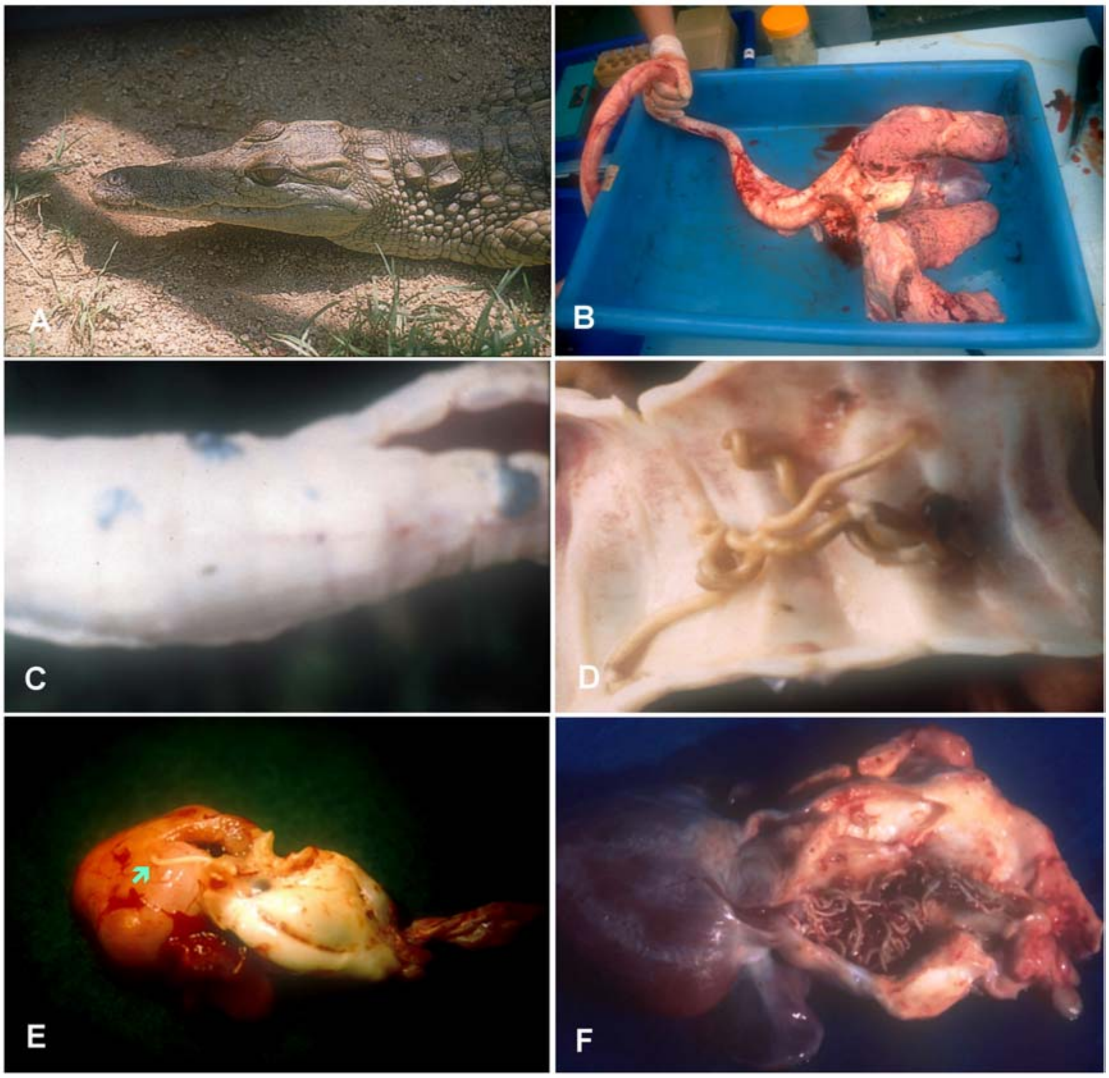

Figure 14. A. One of the experimental Nile crocodiles. B. The trachea, lungs and heart of a naturally infected crocodile. C. A section of the trachea showing the dark brown nodules often associated with *Leiperia cincinnalis* attachments sites. D. Trachea split open, revealing three *L. cincinnalis* females. Note the spirally coiled abdomen. The anterior third is burrowed in the tracheal wall. E. The heart of an experimentally infected crocodile. The arrow marks an infective larva of *L. cincinnalis* leaving the organ during *post mortem* migration. The heart of a naturally infected crocodile. The opened pulmonary artery harbours a cluster of immature stages and infective larva of *L. cincinnalis*.

Crocodile W4 harboured two viable infective larvae 3 and 38 days after infection. While specimen W4/1 was recovered from the bronchioles, specimen W4/2 had attached itself to the wall of the pulmonary artery.

A single specimen of L. cincinnalis, W5/1, was collected from the abdominal cavity of Crocodile W5 102 and 67 days after infection. Its head was lost during recovery. The abdomen, however, still moved at the time of collection, and the absence of abdominal spines clearly indicates this to be a later instar than the infective larva.

Of specimen W1/1 only a single hook could be measured. The latter was 246 µm long and carried a spike measuring 276 µm in length. The right and left anterior hook of specimen W4/1 measured 281 µm and 265 µm, respectively, with accessory hooks that were 258 µm and 235 µm long. The posterior hooks reached a length of 287 µm and 278 µm, respectively, with a spike-length of 308 µm and 278 µm. While the anterior fulcra measured 607 µm and 626 µm in length, both posterior fulcra were 598 µm long. The oral cadre was 292 µm long, with an overall length of 324 µm and a width of 117 µm.

Discussion

Pathologic changes were not evident in the crocodiles experimentally infected with *L. cincinnalis* in this study. The infection neither resulted in behavioural changes nor was the physical condition of any of the infected hosts adversely affected. Crocodiles W1, 4 and 5 tolerated the pentastomid parasites well. This is contrary to reports of four-week old alligator hatchlings from an alligator farm in Florida dying of sebekiosis (Boyce, Cardheilac, Lane, Buergelt & King, 1984). At two weeks of age these hatchlings began showing signs of anorexia, weight loss and respiratory distress. The symptoms worsened progressively and eventually led to the alligators' death. Upon necropsy *S. mississippiensis* (mean intensity = 7) was recovered from all seven hatchlings. Mosquito fish, *Gambusia* *affinis*, that formed part of the daily diet and had a high prevalence of *S. mississippiensis* were established as the source of infection. The same authors then proceeded to force-feed 15 *S. mississippiensis* to each of two uninfected alligator hatchlings, weighing 80 g at the time of infection. After a week these started showing the same symptoms as their naturally infected counterparts. Necropsy 14 days after infection revealed 5 and 8 larvae of *S. mississippiensis* in the lungs.

No histopathology was done on the experimental hosts in the present study, but histopathological changes commonly associated with pentastome infections are pulmonary haemorrhage and oedema, multifocal filling of bronchi with red blood cells and necrosis. The damage migrating and/or feeding pentastomid parasites cause to the lung epithelium or other tissues is often associated with fungal infiltration and gives way to secondary bacterial infections, as has been recorded for alligators and Nile crocodiles (Deakins, 1971; Shotts, Gaines, Martin & Prestwood, 1972; Hazen, Aho, Murphy, Esch & Schmidt, 1978; Boyce *et al*., 1984; Boyce, Kazacos, Kazacos & Engelhardt, 1987; Ladds & Sims, 1990; Junker *et al*., 1999).

The severity of the pathological picture varies with the suitability of the host, and is usually more pronounced in atypical or aberrant hosts (Boyce & Kazacos, 1991). Studies on the migration of larval pentastomids indicate that tissue damage and lesions caused by the larvae in their natural intermediate and final hosts are minimal (Self & Kuntz, 1967). This was also observed in impala, *Aepyceros melampus*, and kudu, *Tragelaphus strepsiceros*, that were naturally infected with *Linguatula* nymphs and where tunnels without any appreciable tissue reaction or haemorrhage were seen in the livers (I.G. Horak & J. Boomker, 2002, pers. comm.).

The number of pentastomes together with the size, age and general condition of the host are further determinants as to the host response to pentastome infections.

When interpreting the results of Boyce *et al*. (1984) it should be taken into consideration, that at the time of hatching the average American alligator measures hardly more than 22 cm in length from the tip of its snout to the tip of its tail, and while they nearly triple their size during their first year of growth (Alderton, 1992), at four weeks of age the hatchlings referred to above would have been tiny compared to the size of alligators that would prey on fish and thus become naturally infected (see above). Therefore, a parasite load of seven pentastomes in the lungs of these hatchlings should have certainly had a severe impact. By comparison, the Nile crocodiles infected with *L. cincinnalis* were considerably older (2 to 3 years) at the time of infection, with a body-length varying from 80 to 100 cm, and a minimum weight of 15 kg. In addition, fewer pentastomes were recovered from the Nile crocodiles at necropsy than from the alligators, the latter having been exposed to unusually large numbers of pentastomes. In comparison, the course of infection in the Nile crocodiles was close to natural infections, where free-ranging individuals are exposed to smaller numbers of pentastomes over a longer time (Moreland, Forrester & Delaney, 1989).

The numbers of infective larvae of *L. cincinnalis* available for the experimental infections of *C. niloticus* were not as high as one would have liked and the recovery rate remained below the expectations. Seven crocodiles were fed *L. cincinnalis*, but in only three of the hosts did some of the infective larvae survive and continue to develop. Junker *et al*. (1998b) obtained higher recovery rates (35 and 40%, respectively, in two out of four infected hosts) when infecting *C. niloticus* with only encysted larvae of *S. wedli*.

It is assumed that part of the problem encountered in the present study stems from the fact that the infective larvae of *L. cincinnalis* began to actively exit their cysts almost immediately upon recovery. The same phenomenon has been recorded for *S. oxycephala* upon removal from the body cavity of mosquito fish (Boyce *et al*., 1984). However, *S. wedli* remained in their cysts when collected from the swim bladders of *O. mossambicus* and *T. rendalli*. While the cyst surrounding the infective larvae is of host origin (Winch & Riley, 1986a) and is a result of the host's response to contain the parasite (Riley, 1986), it also provides a protective casing for the infective larva inside. Without this fibrous capsule the exposure to environmental changes increases. Consequently, unfavorable storage conditions prior to using *L. cincinnalis* specimens in the experimental infections would have had a greater impact. Nevertheless, Junker (1996) stored a single specimen of *L. cincinnalis* that had been dissected out of its cyst for about 2 weeks in phosphate buffered saline at 4 – 8 ºC, and, warmed to room temperature, the infective larva was found to be alive. It's capacity to infect a final host was, however, not been established. In addition, the unencysted infective larvae of *L. cincinnalis* were directly exposed to the extremley acid environment of the crocodilian stomach, the pH of which can be as low as 2 (Alderton, 1992). The latter, however, does not seem to have incapacitated the equally excysted infective larvae of *S. mississippiensis* used by Boyce *et al*. (1984).

Sofar it has not been established with certainty whether infective pentastome larvae start their migration by progressing through the stomach wall or through the intestinal wall of the final host under natural conditions.

The author assumes that the specimens of *L. cincinnalis* recovered from Crocodiles W1, 4 and 5 are some of the encysted *L. cincinnalis* used during the experimental infection. However, too few data are available and *L. cincinnalis* did not develop in Crocodile W2, despite it also being infected with three encysted *L. cincinnalis*. Some of the excysted infective larvae of *L. cincinnalis* were still moving at the time of infection, but it is likely that prolonged storage has reduced the viability of especially the excysted infective material. While differences in the physiological adaptivity of the two pentastome genera *Leiperia* and *Sebekia* might have played a role in the outcome of the experimental infections, they do after all occupy different niches in their final hosts, we believe that the shorter storage period was vital to the success of the alligator infections and the infection of *C. niloticus* with *S. wedli*.

Buckle *et al*. (1997) have developed an *in vitro* method of successfully growing *Porocephalus crotali* from the infective instar to the adult stage. According to the authors, this is relatively easily achieved under laboratory conditions, but it is unfortunately not as practicable under field conditions. It would nevertheless be interesting to see if the method could be adjusted to keep infective larvae collected for experimental infections alive and infective for longer periods than is currently the case. Despite the usage of antibiotics in the culture medium the authors report constant problems with bacterial contamination of their cultures. This is assumed to also have negatively effected the experimental material used in the present investigation.

With the exception of specimens W1/2 and W1/3 (see below), all *L. cincinnalis* were alive at the time of recovery from the experimentally infected crocodiles, thus confirming the results to be representative of an ongoing developmental processes.

The distribution of the *L. cincinnalis* larvae recovered from the experimental hosts follows the distributional patterns seen in natural infections, where the same developmental stages were located mainly in the pulmonary artery, chambers of the heart as well as in the bronchioles (Rodhain & Vuylsteke, 1932; Junker *et al*., 2000). Our data suggest that a cueing system directs the migratory route of *L. cincinnalis* in *C. niloticus* to its various attachment sites during development, although at this stage we can only speculate on the nature of this direction finder. Boyce (1985) speculated on a 'pathfinder' for *S. mississippiensis* in natural alligator hosts and the latter was supported by the aberrant location of the same parasite in rodent experimental hosts.

The hook measurements together with the dimensions of the oral cadre of the infective larvae recovered from the experimental crocodiles in the present study, did not differ significantly from morphometric data of infective larvae of *L. cincinnalis* from fish intermediate hosts nor from final hosts recorded by other authors (Riley & Huchzermeyer, 1996; Junker *et al*., 1998a; Junker *et al*., 2000).

Indications are that the experimental infective larvae that established themselves in the crocodiles followed the regular pattern of development as discussed by Junker *et al*. (2000), and that infective larvae upon entry into the cardiovascular system of the final host do not grow in size by subsequent moults. Instead, they undergo a single moult transforming them into adults.

Data obtained from the experimental infection of Crocodile W4, sacrificed when the infection was 3 and 38 days old, suggests that once the infective larvae have entered the alimentary canal of the final host the migration to the pulmonary artery is accomplished in a relatively short period, no longer than about 38 days although it could be considerably shorter, about 3 days. Two interpretations of the results are possible. Firstly, both infective larvae recovered from Crocodile W4 could have been ingested during the first experimental infection. This would imply that the migration requires about 38 days. Since larvae do not necessarily migrate equally fast, specimen W4/1 had not progressed past the bronchioles when the host was killed. Alternatively, it is possible that specimen W4/2 recovered from the pulmonary artery resulted from the second infection, suggesting that the infective larvae of *L. cincinnalis* gain access to the bronchioles of the crocodilian hosts in as little as three days.

Access from the bronchioles to the pulmonary artery is straight forward and the obligatory phase in the vascular system is an extended developmental period taking up to two months, possibly longer. These experimental findings confirm speculations of Rodhain & Vuylsteke (1932) who observed tufts of different developmental stages of *L. cincinnalis* attached to thickened parts of the aortal endothelium of a young Nile crocodile from the Congo. The extent of the host's tissue reaction, where the larval hooks were embedded, caused the latter authors to believe that the attachment had lasted several weeks.

From Crocodile W5 two adult *L. cincinnalis* were recovered, one 67 days after the second and the other 102 days after the first experimental infection while in Crocodile W1 the earlier infection was 98 days old and the development of *L.*

cincinnalis had not yet progressed past the infective stage. These experimental data suggest that the transition from the infective larva to the adult stage occurs about 100 days after infection of the final host. The author assumes that the specimens recovered from Crocodile W1 originate from the second infection and that the first infection was either unsuccessful or that the developing pentastomes became aberrant while migrating to the final attachment site of the adults in the trachea, and/or were killed by an immune reaction of the host. Field studies indicate that the latter might happen in natural infections. Natural infections are often characterized by a high number of infective larvae and subsequent developmental stages of both sexes forming clusters in the pulmonary artery, but comparatively low numbers of adults, especially males, are found in the trachea (Junker *et al*., 2000). The paucity of males might also be due to their shorter life-span, as seems to be the case in some pentastomes (Riley & Self, 1980, 1981). In the case of the females failure to find a suitable attachment site could result in their being coughed out in the attempt. Similarly, males migrating in search of receptive females might irritate the trachea and trigger an expulsion reflex, such as coughing or excessive mucous production which would suffocate the parasites.

The field data indicates that the parasites pass the host's lungs twice during their development. Not only were infective larvae found in the bronchioles, but young females also (Junker *et. al*., 2000). To date the exact route followed by *L. cincinnalis* in the crocodiles is not yet known, but the presence of infective larvae in the bronchioles, as seen in Crocodile W4, confirms the assumption of Junker *et al*. (2000) that prior to the invasion of the pulmonary artery, *L. cincinnalis* finds its way into the lungs, which offer a large target area for the parasite.

Once in the lungs the pulmonary veins would appear to be the most likely access route to the cardiovascular system, following the flow of oxygenated blood into the left artrium. However, *L. cincinnalis* infective larvae were instead recovered from the right ventricle and the pulmonary artery of experimentally as well as naturally infected hosts during this study. The latter implies that, upon entry into the blood stream, the infective larvae have to migrate against the bloodflow. The powerful double hooks characteristic for these developmental stages might well present an answer to this challenge, providing a means for the pentastome to anchor itself in the epithelium. In this context one should consider that, contrary to all other reptilians, the crocodilian heart is distinctly divided into four chambers, efficiently dividing oxygenated blood from deoxygenated blood. The foramen of Panizza, situated between the right and left aortic branch allows a certain degree of mixing. When the crocodile breathes, the blood pressure generated in the right ventricle exceeds that in the left ventricle. Consequently, all the blood from the right ventricle flows into the pulmonary artery, while blood from the left ventricle flows into the right and, through the foramen of Panizza, also into the left aortic branch. When submerged, the crocodile is able to regulate its blood flow directing oxygenated blood to the brain and heart, while less vital organs receive deoxygenated blood. In order to achieve this blood shift, blood flow to the pulmonary arteries is reduced and the resulting increase in pressure in the right ventricle eventually exceeds that of the left ventricle. Part of the blood from the right ventricle now flows into the left aortic branch (Ross, 1989; Eckert, 1993). As a result of these special adjustments in the circulatory system of crocodilians, pentastomes are at most times exposed to less pressure in the right ventricle and the pulmonary artery than they would experience in the left ventricle and the aortic arches, without suffering an oxygen shortage. For young adults traveling from their gathering sites in the pulmonary artery through the lungs to the trachea were they eventually reside, the direction of the blood-flow in the pulmonary artery would actually be beneficial.

It is assumed that the single young adult *L. cincinnalis* recovered from the abdominal cavity of Crocodile W5 migrated there when leaving its attachment site after the death of the host or was accidentally displaced during the necropsy.

Rodhain & Vuylsteke (1932) discussed the possibility of an obligatory developmental phase in the circulatory system but thought it exceptional rather than the rule. That it is indeed a specific pattern of development within the genus of *Leiperia* has since been confirmed in *L. australiensis* and by this study. We recovered infective larvae, as well as young females, from the lungs. Thus the lungs are a route of migration for the infective larvae on their way to the cardio-vascular system and for the adults on their way to their attachment sites in the trachea. Considering the large numbers of infective larvae and young adults often encountered in the pulmonary artery, the question arises why comparatively few adults are found in the trachea. This is especially true for the males, which occur in substantial numbers in the cardio-vascular system, but hardly ever in the trachea or lung. Possible explanations are a relatively shorter life-span of male versus female pentastomes (Riley, 1986; Junker *et al.*, 1998b), aberrant migration of the pre-adults, an immune reaction from the host or failure to attach to the trachea and subsequent expulsion as mentioned previously. However, the reasons will remain speculative until more extensive studies can be undertaken.

A male and a female *L. cincinnalis*, recovered from one of the naturally infected crocodiles, were attached next to each other in the trachea and probably had been *in copula* but were separated during recovery. We conclude that *L. cincinnalis* copulate in the trachea and not in the circulatory system. Riley & Huchzermeyer (1996) had already stated their doubts as to the likelihood of such a difficult procedure taking place in the narrow surroundings of the pulmonary artery.

Males with single hooks and completely developed copulatory spicules still encased in the cuticle of the infective larva have been in the pulmonary artery and this supports the speculation of Riley & Huchzermeyer (1996) that only one moult is necessary for the infective larva to develop into a fully adult male. However, another fully mature male surrounded by both the cuticula of the infective larva and the cuticula of a preceding single-hooked stage indicates that the males of *L. cincinnalis* undergo at least one additional moult after having reached sexual maturity. Unlike moulting in females, moulting in the males does not seem to be related to any further growth. The measurements of specimen No. 4.18/98, in addition to the male recovered from the trachea, was well within the range of the measurements of the other males (Table 4). Once again, these data emphasise the variability of structures encountered when dealing with pentastomes, as already stated by Riley & Huchzermeyer (1995).

It was not possible to successfully establish *S. rileyi* in the experimentally infected Nile crocodiles. This, in combination with the absence of adult *S. rileyi* in the nasopharyngeal area of naturally infected Nile crocodiles, suggests that *C. niloticus* might not be the true final host of *S. rileyi*, contrary to the assumption of Junker *et al*. (1998a). Terrapins would be another likely final host for *S. rileyi*, but neither terrapins, occurring in large numbers in the Phabeni Dam from which the infected fish originated, nor Nile crocodiles from this specific habitat were made available for research. Members of the family Ardeidae (herons, egrets and bitterns) of which 19 species occur in southern Africa and most of which can be found in the KNP, as well as other piscivorous birds such as darters, *Anhinga melanogaster*, or cormorants, *Phalacrocorax carbo* and *Phalacrocorax africanus*, are further possible hosts for this pentastomid parasite. However, to date no data are available and further field studies would be necessary to elucidate the matter. Sofar, pentastomid infections involving avian hosts have only been reported from marine birds. *Reighardia sternae* has a direct life-cycle in the herring gull, *Larus argentatus*, and guillemots, *Uria* spp., and *Reighardia lomviae* is known to occur in puffins, *Fratercula arctica*, (Dyck, 1975; Banaja, James & Riley, 1975).

9.2 EXPERIMENTALLY INFECTED FISH

With the exception of the genera *Sambonia*, *Diesingia* and *Pelonia*, for which the intermediate host still requires final confirmation, fish are the common intermediate hosts of pentastomes belonging to the family Sebekidae. Eggs dispersed in the faeces of the crocodilian final hosts are the infective agent for intermediate hosts, and transmission occurs via the food chain.

Different groups of pentastomes have developed different means to ensure that only fully embryonated eggs are released into the environment. In some cephalobaenids egg-laying only commences once all the eggs contain fully developed primary larvae. In the raillietiellids females become patent before all eggs are fully mature, but a vaginal "sieve" allows only fully developed eggs to be deposited. In the porocephalids eggs mature on their way to the terminal part of the uterus and the vagina, and only fully mature eggs are shed (Riley, 1986). Riley (1983) observes that the number of eggs produced by the pentastome females increases with body size. In *P. crotali* females reach a body length of up to 70 mm and deposit between 520 and 2300 eggs a day over a period of at least six years (Riley, 1981).

The high fecundity of most pentastome females is thought to compensate for the high losses that occur when eggs deposited in the water are not ingested by fish, or other causes of high egg mortalities. This is also followed by many helminth endoparasites (Riley, 1986).

Materials and mehods

From the terminal part of the uteri of seven patent females recovered from Crocodile 1/99 an egg-solution of the concentration of 5207 eggs/ml was obtained. Of this solution 104 eggs were counted randomly and their developmental status established. Only 11 eggs (10.5%) contained fully developed primary larvae, of which 25% displayed movement, indicating viability. When heated to 30ºC a single primary larva hatched.

Two groups of seven platys each (P1 – P14/99) were infected by placing them in a 2 l beaker containing 1 l water to which 12 ml of the egg suspension had been added. The fish were left in the beakers overnight.

Furthermore, eggs were recovered from three *S. wedli* with uteri partially filled with eggs and three platys were infected with this egg suspension (P15 – 17/99). Platy P15/99 was necropsied 1 d p.i. and P16/99 and 17/99, 22 d p.i..

Results

No pentastomes were recovered from intermediate hosts P15/99, P16/99 and P17/99.

At two days p.i. two fish were examined for developing larvae and six pentastome eggs were recovered from the intestine of one of the intermediate hosts (P1/99). These eggs were immature and did not contain fully developed primary larvae. The second host (P2/99) harboured neither pentastome larvae nor eggs. Two fish each were examined 5, 9, 11 and 22 d p.i. and three fish were necropsied 44 d p.i.. All intermediate hosts were found to be negative.

Discussion

The presence of eggs in the intestine of one of the hosts is a good indication, that the platys ingested some of the eggs added to the water and lends credit to the assumption that predatory fish can become infected mistaking pentastome eggs for invertebrate prey (see Chapter 10). It nevertheless appears that the egg-suspension used for the experimental infection of the platys contained too few fully developed eggs to successfully establish pentastomes, and it is assumed that undeveloped eggs that passed through the intestine were shed in the faeces.

The above emphasizes the necessity of ingesting or administering fully developed eggs if the intermediate host is to become infected. Pentastome eggs possess smooth egg shells and are devoid of anchoring devices. The passage through the alimentary canal can therefore not be protracted and does not offer sufficient time for immature eggs to reach hatchability. Two possible exceptions should be pointed out in this context. The egg shells of the genera *Sambonia* and *Parasambonia* Stunkard & Gandal, 1986 are equipped with small spines. However, according to observations of Heymons & Vitzthum (1936) on *Sambonia lohrmanni*, these spines only occur during embryogenesis and Riley & Self (1982) report mature eggs of *Parasambonia minor* to possess a spiny egg-shell, whereas undeveloped eggs are smooth. It is therefore more likely that the gut passage of fully embryonated eggs is prolonged once the outer mucous capsule is removed and the spines become exposed, allowing more time for the primary larva to hatch, than that the eggs are attached to the intestinal wall to allow for further development. To date the real function of these spines has not yet been elucidated and remains speculation.

CHAPTER 10

PENTASTOMID INFECTIONS IN NATURALLY INFECTED SOUTH AFRICAN FISH

10.1 THE INTERMEDIATE HOSTS, BIOLOGY AND HABITAT

A total of 465 fish were collected from the Phabeni Dam, the main collection site of fish in the KNP. Of these, 260 were Mozambique *Tilapia*, *Oreochromis mossambicus* (120 – 290 mm), and 202 were redbreast *Tilapia*, *Tilapia rendalli swierstrai* (75 – 270 mm). Two sharptooth catfish, *Clarias gariepinus* (350 and 410 mm), and a single threespot barb, *Barbus trimaculatus* (85 mm), were obtained at the same site.

Few fish were caught in Lake Panic, KNP and a total of 18 *O. mossambicus* (165 – 290 mm), a single *T. rendalli* (200 mm) and a single Lowveld largemouth, *Serranochromis meridianus* (165 mm), originating from this locality were examined.

Outside the KNP a total of 58 *O. mossambicus* (210 - 420 mm), 20 *T. rendalli* (150 - 260 mm), 14 largescale yellowfish, *Barbus marequensis* (210 - 310 mm), three papermouth, *Barbus mattozi* (300 – 320 mm), 15 rednose labeo, *Labeo rosae* (230 – 420 mm), and four silver catfish, *Schilbe intermedius* (300 – 320 mm), were collected from the Arabie Dam. An additional 10 *C. gariepinus* (400 – 490 mm) were collected from a small marshy area close-by.

In the following a short summary on the main intermediate hosts examined in the present study, *O. mossambicus* and *T. rendalli* is given. All information on the distribution, biology and ecology of these cichlids has been gathered from Skelton (1993). The tilapiines are a major branch of African cichlid fishes and can roughly be divided into two branches, substrate spawning species (*Tilapia*) and mouthbrooders (*Oreochromis*).

The redbreast *Tilapia* is common in the Cunene, Okavango and Zambesi River systems in southern Africa and also occurs in Zaire as well as in estuaries in Mozambique. In South Africa it occurs throughout KwaZulu/Natal and is present in Mpumalanga and the Northern Province. Its preferred habitat is quiet, well-vegetated waters along river littorals or swamps, floodplains and backwaters. Waterplants and algae form the major part of its diet but aquatic invertebrates, and occasionally small fish, are also included. *Tilapia* spp. tend to feed on coarser foods and are usually smaller than *Oreochromis* spp. which explains the difficulties in obtaining *T. rendalli* larger than 250 mm for this investigation. Males and females excavate nests in shallow water after clearing the vegetation.

Oreochromis mossambicus occurs in the east coastal rivers from the lower Zambesi system south to the Bushman system in the Eastern Cape Province. It has also been translocated to South African inland regions and to the south-west and west coastal rivers including the lower Orange River and some rivers in Namibia, and has been introduced worldwide into tropical and warm temperate regions. Excepting fast-flowing waters, the Mozambique *Tilapia* can be found in a variety of habitats but does exceptionally well in standing waters. Like *T. rendalli* it is tolerant of a wide range of temperatures and relatively high salinity. While larger individuals may feed on insects and other invertebrates, *O. mossambicus* mainly feeds on algae and detritus. Males create a nest on sandy substrate and the females mouthbrood the eggs, larvae and small fry.

Both cichlids are popular angling species and widely used in aquaculture, and commercial and subsistence fisheries.

10.2 The prevalence and mean intensities of pentastomid infections in naturally infected fish

All fish collected from the Arabie Dam were negative as regards pentastomid infections and neither the two catfish nor the single *B. trimaculatus* caught in the Phabeni Dam harboured pentastomes. The single *S. meridianus* from Lake Panic, however, hosted three infective larvae of *L. cincinnalis*. The remainder of the infective larvae collected during this study originated from the two cichlid species, and a total of 718 pentastomid larvae were recovered. In the following, fish from Lake Panic and the Phabeni Dam are grouped together.

Of *S. rileyi* 377 larvae were collected, all of which moved freely on the inner wall of the swim bladder of the hosts. The majority of the 97 sebekiid nymphs, excepting *L. cincinnalis*, recovered from the swim-bladder were encysted and *L. cincinnalis* was taken from the mesenterium surrounding the intestine. All 244 infective larvae of *L. cincinnalis* were found encysted. Contrary to alofian or *Sebekia*n infective larvae, which remained in their cysts, *L. cincinnalis* began to actively leave its cyst immediately upon recovery from the host tissue.

Not discriminating between different pentastome species, the general prevalence of pentastomes in *O. mossambicus* was 74% and double infections with *S. rileyi* and *L. cincinnalis* occurred in 32% of the infected fish. Only a single *O. mossambicus* (260 mm long) harboured *S. rileyi* and *L. cincinnalis* together with a single encysted sebekiid larva.

The general prevalence of pentastome infections was markedly lower in *T. rendalli* and reached 50%. Double infections occurred in 20% of the infected fish and a single *T. rendalli* (190 mm) harboured *Subtriquetra*, *L. cincinnalis* and other sebekiids.

While the number of double infections remained virtually the same in *O. mossambicus* smaller or larger than 200 mm, the number of double infections increased by 10% to 26% in *T. rendalli* from 200 mm and longer.

Table 28. Prevalence and mean intensity of pentastomid infections in different size classes of *Oreochromis mossambicus* from the KNP.

Size class (mm)	*Leiperia cincinnalis*		*Subtriquetra rileyi*		Sebekiid	
	Prevalence (%)	Mean intensity (Range)	Prevalence (%)	Mean intensity (Range)	Prevalence (%)	Mean intensity (Range)
100-149 (n = 17)	12	1 (1,1)	18	2.3 (1 - 5)	0	0
150-199 (n = 87)	36	1.3 (1 - 5)	45	1.2 (1 - 2)	2	2 (1, 3)
200-249 (n = 113)	44	1.6 (1 - 6)	58	1.7 (1 - 6)	0	0
250- (n = 61)	61	2 (1 - 10)	69	2.5 (1 - 11)	2	1(1)

The prevalence and mean intensity of pentastomid infections in *O. mossambicus* grouped according to the different pentastome species and different size classes of fish are presented in Table 28.

Table 29. Prevalence and mean intensity of pentastomid infections in male and female *Oreochromis mossambicus* of comparable size classes caught in the KNP.

Size class (mm)	Sex	Prevalence; Mean intensity (Range)	
		Leiperia cincinnalis	*Subtriquetra rileyi*
150 - 199	M (n = 22)	32%; 1.7 (1 – 3)	41%; 1.2 (1 – 2)
	F (n = 21)	38%; 1.8 (1 – 3)	57%; 1.1 (1 – 2)
200 – 249	M (n = 62)	45%; 1.4 (1 – 4)	63%; 1.7 (1 – 6)
	F (n = 47)	40%; 1.9 (1 – 5)	55%; 1.8 (1 – 5)
250 -	M (n = 53)	64%; 2.2 (1 – 10)	68%; 2.6 (1 – 11)
	F (n = 7)	43%; 2.6 (1,1,6)	86%; 1.8 (1 – 3)

F = female, M = male

The prevalence and mean intensity of pentastome infections in male versus female *O. mossambicus* of the same size are given in Table 29. As the number of sebekiids other than *L. cincinnalis* recovered from *O. mossambicus* was negligible and only two immatures and a single male *O. mossambicus* harboured these pentastomes, data on the prevalence and mean intensity of infections of *O. mossambicus* with sebekiids were omitted from this table.

Table 30. Prevalence and mean intensity of pentastomid infections in different size classes of *Tilapia rendalli* from the KNP.

Size class (mm)		*Leiperia cincinnalis*		*Subtriquetra rileyi*		*Sebekiid*	
		Prevalence (%)	Mean intensity (Range)	Prevalence (%)	Mean intensity (Range)	Prevalence (%)	Mean intensity (Range)
< 100	(n = 8)	0	0	0	0	13	2 (2)
100-149	(n = 47)	2	1 (1)	2	1 (1)	17	1.4 (1 – 3)
150-199	(n = 96)	5	3.8 (1 - 12)	27	1.4 (1 – 3)	31	1.4 (1 – 5))
200-249	(n = 47)	0	0	62	2 (1 – 4)	28	1.7 (1 – 7)
250-	(n = 5)	20	1 (1)	60	2.3 (1 – 3)	60	5 (1 - 11)

Table 31. Prevalence of pentastomid infections in male and female *Tilapia rendalli* of comparable size classes caught in the KNP.

Size class (mm)	Sex		Prevalence (%)		
			Leiperia cincinnalis	*Subtriquetra rileyi*	Sebekiid
150 - 199	M	(n = 39)	8	31	28
	F	(n = 16)	0	38	25
200 – 249	M	(n = 32)	0	63	31
	F	(n = 6)	0	67	33
250 -	M	(n = 4)	25	75	50
	F	(n = 0)	NA	NA	NA

F = female, M = male, NA = not applicable

Table 30 summarizes the main data for *T. rendalli* and Tables 31 and 32 the information on the prevalence and mean intensity of pentastomid infections in male and female *T. rendalli* of comparable size.

Table 32. Mean intensity of pentastomid infections in male and female *Tilapia rendalli* of comparable size classes caught in the KNP.

Size class (mm)	Sex	Mean intensity (Range)		
		Leiperia cincinnalis	*Subtriquetra rileyi*	Sebekiid
150 - 199	M (n = 39)	4.7 (1 - 12)	1.2 (1 - 2)	1.7 (1 - 5)
	F (n = 16)	0	1.5 (1 - 2)	1.3 (1 – 2)
200 – 249	M (n = 32)	0	2.2 (1 - 6)	1.8 (1 - 7)
	F (n = 6)	0	2.3 (1 – 4)	1.5 (1 – 2)
250 -	M (n = 4)	1 (1)	2.3 (1 - 3)	2 (1,3)
	F (n = 0)	NA	NA	NA

F = female, M = male, NA = not applicable

Table 33. The prevalence of pentastome infections in *Oreochromis mossambicus* from the KNP during summer and winter.

Size class (mm)	Season		Prevalence (%)		
			Leiperia cincinnalis	*Subtriquetra*	Sebekiid
100 - 149	Summer	(n = 16)	13	25	0
	Winter	(n = 0)	NA	NA	NA
150 -199	Summer	(n = 80)	33	45	3
	Winter	(n = 8)	75	50	0
200 - 249	Summer	(n = 88)	41	59	0
	Winter	(n = 25)	56	56	0
250 -	Summer	(n = 43)	58	67	2
	Winter	(n = 18)	66	72	0

NA = not applicable

In order to establish whether seasonal changes had an influence on the prevalence of pentastome infections, *O. mossambicus* and *T. rendalli* caught in winter (June 1999) were separated from the summer catches (January and March 1999) and the prevalence of pentastome infections in the above size classes determined independently. The results are presented in Tables 33 and 34.

Table 34. The prevalence of pentastome infections in *Tilapia rendalli* from the KNP during summer and winter.

Size class (mm)	Season		Prevalence (%)		
			Leiperia cincinnalis	*Subtriquetra*	Sebekiid
< 100	Summer	(n = 8)	0	0	13
	Winter	(n = 0)	NA	NA	NA
100 - 149	Summer	(n = 42)	0	2	14
	Winter	(n = 5)	20	0	40
150 -199	Summer	(n = 72)	7	25	25
	Winter	(n = 24)	0	29	50
200 - 249	Summer	(n = 39)	0	59	31
	Winter	(n = 7)	0	85	14
250 -	Summer	(n = 1)	0	0	100
	Winter	(n = 4)	25	75	50

NA = not applicable

Discussion

The pentastome communities of *T. rendalli* and *O. mossambicus* differed distinctly as regards the Sebekidae. *Subtriquetra* on the other hand was the pentastome most commonly encountered in both intermediate host species and little difference could be found between the prevalence and mean intensity of these infections in *T. rendalli* and *O. mossambicus* from 200 mm onwards.

However, the numbers of *T. rendalli* harbouring *S. rileyi* increased appreciably in fish of 150 mm long and more, while *O. mossambicus* of between 100 and 149 mm already had an infection rate of 18%. In the next size class, 150 –199 mm, the prevalence of *S. rileyi* is distinctly higher in *O. mossambicus* than in *T. rendalli*.

The results of the present investigation reflect a distinct host preference of *L. cincinnalis* for *O. mossambicus*. In fact, the prevalence and mean intensity of *L. cincinnalis* in the various size classes of *O. mossambicus* corresponds very well with the data gained for *S. rileyi* and remain only slightly below the levels obtained by the latter parasite. Hence, both are considered core species of the pentastome community of *O. mossambicus.* The remaining sebekiid parasites, *Alofia* and *Sebekia*, found encysted in the swim bladder, play a minor role in the component pentastome community of *O. mossambicus* as they never reached a prevalence higher than 2%.

Tilapia rendalli on the other hand, was found to be the main host for sebekiids, excepting *L. cincinnalis*, which was present in few hosts only and reached a prevalence of 20% in hosts larger than 250 mm, but remained at a low prevalence of maximally 2% - 5% in the remaining size classes. In the smaller size classes the prevalence of sebekiids exceeded that of the subtriquetrids and reached the same level in fish between 150 and 199 mm in length. In fish larger than 199 mm *Subtriquetra* had a distinctly higher prevalence.

Generally speaking, the intensity of pentastome infections in both cichlids was very low, and the majority of intermediate hosts harboured only a single or two pentastomid parasites. However, the maximum number of infective larvae of *L. cincinnalis* per host was 12 and that of *Subtriquetra* and other sebekiids 11.

Few publications contain information on the prevalence and intensity of pentastome infections in fish, but Boyce (1985) included data on the larvae of *S. mississippiensis* collected from mosquito fish. The mosquito fish were collected from a manmade lake in Florida, and although the size of the lake is unknown, the reference to its being heavily populated by adult alligators suggests that its general conditions may have been similar to those of the Phabeni Dam in the KNP. The results as regards host densities and other habitat parameters might thus be comparable. However, the difference in size between the African cichlids and *G. affinis*, which reaches a maximum length of only about 6 cm, is considerable.

Boyce (1985) on three occasions during 1983 and 1984 found the prevalence of *S. mississippiensis* in mosquito fish to range from 60% to 86%, with a mean intensity ranging between 4.5 and 9.7 (Range: 1 – 79). A total of 185 fish were examined. These figures clearly exceed the maximum prevalence as well as intensity obtained for *L. cincinnalis* and for the remaining sebekiids in any of the two cichlid species from the KNP. The author believes that a possible explanation for this can be found in the different feeding habits of the mosquito fish. *Gambusia affinis*, its dorsally directed mouth indicative of a surface feeder, preys on small live organisms including mosquito larvae and fish larvae, taken at the surface or when suspended on different levels in the water (Skelton, 1993). In fact, a healthy individual can consume its own weight in mosquito larvae every day (Axelrod *et al*., 1981). It is probable that floating pentastome eggs, when stirred up from the bottom sediment by water movement, fall into the prey items of *G. affinis* and are therefore actively pursued and ingested. This would certainly result in a higher intake of pentastome eggs than would be the case with the chance ingestion while grubbing by predominantly herbivourous or detrivorous bottom feeders.

While the natural distribution of *G. affinis* is the Gulf of Mexico, into which drain many of the rivers of North America, mosquito fish have been widely introduced into tropical and subtropical areas to assist in mosquito control. In South Africa mosquito fish were introduced before 1936 by aquarists, but later bred and distributed by the Cape Inland Fisheries Department. Isolated populations have been reported from the south-western and southern Cape as well as from

Mpumalanga and the Northern Provinces. Subsequently, *G. affinis* has been identified as an aggressive intruder, that can have a detrimental effect on indigenous fish populations by preying on their larvae (Skelton, 1993). It would be interesting to see if *G. affinis* has in the meantime become an intermediate host for African pentastomes in areas where their habitat overlaps with that of the Nile crocodile or, for that matter, with that of terrapins.

Serranochromis meridianus belongs to a large group of predatory cichlids and preys mainly on small fish and other invertebrates (Skelton, 1993). Thus, small *S. meridianus* probably become infected when mistaking floating pentastome eggs for prey, and, being mouth brooders, males might become infected while digging the nest and females when collecting the brood from the nest substrate.

A clear tendency of an increasing mean intensity as the hosts become larger was seen in all three groups of pentastomid parasites and both intermediate hosts from the KNP. The same is true for the prevalence of infection. It is assumed that this is partly due to the fact that larger fish are older, and thus had a higher previous exposure to sebekiid eggs or, in the case of *Subtriquetra*, free-living primary larvae. If pentastome eggs can accidentally be ingested during nest preparation or, in the case of mouth brooders, when collecting the brood, fish that have reached the size at which they mature would be more likely to become infected.

Junker (1996) reported the prevalence of *S. wedli* in *T. rendalli* to be 41% with a mean intensity of 1.8, and the prevalence and mean intensity of *S. rileyi* was 2% and 1.0 respectively. In *O. mossambicus* the prevalence of *S. wedli* was 3% and that of *S. rileyi* 8%, with mean intensities of 3.7 and 1.1, respectively. In the present study the more general classification "sebekiids" was used instead of *S. wedli*, since encysted or free living sebekiid larvae were not identified to species level. The average length of *T. rendalli* examined by Junker (1996) was 146 mm and that of *O. mossambicus* 186 mm. The prevalence of *S. rileyi* in *T. rendalli* as recorded by Junker (1996) corresponds well with that found in *T. rendalli* of a comparable size class (100 – 149) during the present study. The prevalence of sebekiids in the same size class, however, was lower in the current investigation. This is noteworthy as the prevalence of pentastome infections was shown to increase with the size of the intermediate host and the average size of fish collected during the present investigation was larger. The majority of fish examined during the present and previous studies (Junker, 1996) were caught in the summer months. Hence, seasonal changes cannot account for the differences in the data. Furthermore, Tables 32 and 33 clearly indicate, that there is little seasonal variation between the prevalence of pentastome infections in both fish species. The differences between summer and winter figures are interpreted to be due to the smaller number of fish available for the winter calculations.

The overall prevalence of *S. rileyi* in the Mozambique *Tilapia* caught during the present investigation, irrespective of their size, was 5% and it is only slightly below the figures obtained by Junker (1996). Compared to the high prevalence found in some of the size classes of *O. mossambicus*, this highlights the influence of the body size of the intermediate hosts on the prevalence of subtriquetrid and sebekiid pentastome infections. The overall prevalence of sebekiid pentastomes (1%) was found to be even lower than that determined by Junker (1996).

No difference as regards the prevalence of pentastome infection is apparent in *O. mossambicus* and *T. rendalli* of different sex when identical size classes are compared. As no dietary differences between the sexes have been reported and since both males and females are involved in preparing the nest site (Skelton, 1993), resulting in an equal probability of exposure to pentastome eggs, this is not surprising.

As regards the ability of crocodilian pentastomes to colonize their intermediate host, it appears that the assumedly free-living primary larvae of *S. rileyi* have a slight advantage over the Sebekidae, relying on an entirely passive mode of transmission. This may be less obvious in terms of prevalence or mean intensity of infection in a given host, but is reflected in a wider host spectrum, as *S. rileyi* successfully infects *T. rendalli* as well as *O. mossambicus*. Although data on *S. rileyi* are not available, Winch & Riley (1986b) established that the eggs of adult *S. subtriquetra* infecting *C. sclerops* hatch within the nasal passages and that primary larvae, once they are in the water, adopt a characteristic stationary fishing posture on the lake bottom. While the larvae of *Subtriquetra* rely on accidental contact with a passing fish, the orientation of the hooked limbs and tail is such, that a fish brushing against a larva while feeding on the bottom is most likely to be 'hooked'. Larvae remain infective for 4 - 5 days and limited movement, supposedly to reach a more favorable "fishing" site, is possible.

In view of the fact that *L. cincinnalis* and the remaining sebekiids occupy separate and unrelated niches in the intermediate host and thus do not directly compete for the same resources, it is surprising that they do not utilize the same cichlids. Interestingly, subtriquetrids and sebekiids have been found concurrently in *T. rendalli*, despite their identical site preferences, and the presence of one of the parasites has no perceptible effect on the prevalence nor intensity of the other. Schad (1966, cited by Esch *et al*., 1990) suggested that 'a non-reciprocal cross-immunity evoked by one (parasite) species may function to limit the infrapopulation of a competing species' and would thus lead to selective site segregation. This concept may serve to interpret why *L. cincinnalis* occurs in the mesenteries, while the predilection site of the other sebekiids is the swim bladder. Similarly, a difference in the oxygen requirements of these pentastomes might result in their choosing different sites within the host. However, it does not fully explain the more or less strict absence of sebekiids from *O. mossambicus* and that of *L. cincinnalis* from *T. rendalli*. It is possible that the two cichlids have independently developed an immunity against the respective pentastomes. It should be kept in mind, that the segregation between *L. cincinnalis* and the other sebekiids extends throughout their life-cycle, right into their final hosts, where they occupy different niches. Therefore, an immuno-incompatibility of the two groups of Sebekidae seems to be a likely evolutionary force to have moulded today's differences. *Subtriquetra*, being a member of another family of pentastomes, might genetically differ sufficiently to not be influenced by the host's immune response evoked by the respective Sebekidae. Winch & Riley (1986b) postulated that the absence of *S. Subtriquetra* from bottom-dwelling *Tilapia* spp. was due to an immune response against developing larvae on the part of the host.

Certain anatomical adaptations of the genus *Oreochromis* to a predominantly vegetarian life, including a long intestine (Skelton, 1993), might make it a more suitable host for *L. cincinnalis*, offering more sites for the parasite to become encysted in. Whether the fine teeth disposed in several rows on the jaws or the spacing of pharyngeal teeth, or a high number of gill rakers present in *O. mossambicus* would be able to inhibit the passage of sebekiid eggs other than those of *L. cincinnalis*, or if such eggs would be more likely to be damaged, possibly because of a more delicate egg shell, will have to remain speculation until more extensive studies can be conducted.

The high prevalence of *L. cincinnalis* in one of the intermediate host species, is in keeping with its high prevalence in the crocodilian final hosts from the KNP. In view of the fact that the group "sebekiids" includes the various species of the genera *Sebekia* and *Alofia*, and that *S. wedli* and *S. cesarisi* are core species of the pentastome communities of Nile crocodiles, the prevalence of this group in fish from the Phabeni Dam and Lake Panic is considered low. In order to make up for the low prevalence and mean intensity of these sebekiids in the intermediate hosts, their ability to survive and to continue their development in the final host once the intermediate host has been ingested by a crocodile must be extraordinary.

Furthermore, it is possible that bottom-feeding fish other than *T. rendalli* and *O. mossambicus* enlarge the reservoir of intermediate hosts utilized by these pentastomes. While no pentastomes were recovered from fish netted in the Arabie Dam, encysted pentastome larvae that were collected from the swim bladder of *Marcusenius macrolepidotus* at the same locality during a later, unrelated study and were submitted to the author for identification proved to be *S. wedli* (personal observation, 2002). *Marcusenius* feeds on a wide range of invertebrates, that are taken of plant stems or from the bottom of rivers and flood plains where it prefers marginal habitats (Skelton, 1993).

A number of fish have been recorded as intermediate hosts of *L. cincinnalis* (Fain, 1961), and during the present investigation a new intermediate host, *S. meridianus*, was established. Thus, it is unlikely, that the genera *Alofia* and *Sebekia* should be limited to one or three intermediate host species only.

Catfish have been examined for pentastome infections on previous occasions (Junker, 1996) as well as during the present study. However, to date no pentastomid parasites could be isolated from these fish. This is surprising, as catfish form an integral part of the diet of crocodiles and would thus be an excellent intermediate host. In addition, catfish are completely omnivorous and they prey, scavenge and grub on a wide variety of foods (Skelton, 1993). Their preference for slow flowing or standing waters should increase the probability of exposure to transmissive stages of pentastomes. It may well be that the absence of pentastomes in catfish is a pentastome population limitation device. If catfish, being one of the main prey items for Nile crocodiles, were heavily infected with pentastomid parasites, crocodiles might easily acquire extraordinarily high pentastome burdens, which in turn could affect them severely, as reported by Junker *et al*. (1999).

None of the the fish obtained from the Arabie Dam harboured pentastomes, and even the fish caught in the Arabie Dam Bay, a small area of the Dam favoured by a population of about 40 Nile crocodiles (Hoffmann, personal communication, 2000) were negative. Permission to examine Nile crocodiles from the Arbie Dam was refused, but the presence of *P. africana* in the terrapins originating from the Dam strongly suggests the presence of pentastomes in the fish intermediate hosts, and *S. wedli* collected from *M. macrolepidotis* (see above) confirms this assumption. In view of the method of transmission one can certainly speculate on the value of *S. intermedius*, *B. mattozi* or *B. marequensis* as intermediate host. *Schilbe intermedius* prefers slow flowing waters and feeds from mid- or surface waters, *B. marequensis* also prefers slow flowing waters and feeds primarily on algae and aquatic insect larvae, while *B. mattozi* is an active predator in large pools of cooler, fast flowing perennial rivers, initially feeding on small planctonic crustaceans and later (over 175 mm) mainly on small fish. It would have been interesting to see if the ontogenetic shift in the diet influences the probability of pentastome infections. However, as even the two proven intermediate hosts, *O. mossambicus* and *T. rendalli,* harboured no pentastomes, one can only speculate.

A possible explanation for the absence of pentastomes in the latter two cichlids is the surface area of the Arabie Dam, which, in comparison, is certainly more than 20 times as large as the Phabeni Dam. Even with a crocodile population of about 120 individuals, the final host density would be considerably lower in the Arabie Dam than in the smaller Phabeni Dam. It must also be borne in mind, that the Arabie Dam was only built in 1986, and, considering the low host density, it is likely that it would take considerable time for any parasite to become established in appreciable numbers throughout the system.

Considering that pentastomes were recovered from chelonian hosts originating from the Arabie Dam, it follows that final hosts are more suitable survey animals when trying to assess whether pentastomid parasites are present in a given ecological system or not.

It is noteworthy that adults of *S. rileyi*, found regularly as infective stages in both cichlid species from 150 mm and longer, and which was usually present in slightly higher numbers than the sebekiids in these intermediate hosts, was not recovered from the two crocodiles from the Phabeni Dam examined by Junker *et al.* (1999). Furthermore, it was absent from all the hosts necropsied during the present study. These new findings suggest a strong probability that, contrary to the assumption of Junker *et al*. (1998a), Nile crocodiles are not the final hosts for *S. rileyi*. Terrapins, which are abundant in the Phabeni Dam, or the equally abundant piscivorous birds such as cormorants, egrets, herons and fish eagles might function as the final hosts (Junker, unpublished observations, 1999).

The genera *Leiperia* and *Sebekia* have been recorded from humans (Meirena, Solano & Venegas, 1989) and since *O. mossambicus* and *T. rendalli* are a common source of protein in resource-poor areas of South Africa, this would appear to offer an easy route of transmission. The author, however, believes that the relatively high prevalence and intensity of infection in fish as seen in the Phabeni Dam is not representative of the situation in other localities and the results from the Arabie Dam support this. Furthermore, infective larvae were collected from the swim bladder or the mesenteries only, and not from the muscular tissues as has been recorded for *S. mississippiensis* in naturally infected fish (Overstreet *et al*., 1985; Boyce *et al*., 1987). Thus, removing the viscera of the fish, and boiling or grilling the fish at high temperatures prior to consumption, effectively eliminates the possibility of infection.

10.3 HISTOPATHOLOGY OF THE SWIM BLADDER OF NATURALLY INFECTED FISH

The presence of *S. rileyi*, and to a smaller extent that of *Alofia* and *Sebekia* prior to encystment, in the swim bladders of *Oreochromis* and *Tilapia* is often revealed by the typical suck-marks left by feeding *Subtriquetra* in the wall of the swim bladder. Depending on the number of parasites, the inner surface of the opened swim bladder displays a leopard-skin pattern of tiny discoid scars associated with a reddish-brown discoloration. These suck-marks cover the surface of the swim bladder at random and are not restricted to specific areas, such as the rete mirabilis.

The typical color of the scars is due to haematin, which is an end-product of haemoglobin digestion and is a metabolic product of pentastomid parasites (Riley, 1986). On the other hand it also accumulates during erythrophagocytosis, when blood exuding from ruptured capillaries is degraded by means of cellular processes on the part of the intermediate host.

No gross lesions other than the small roundish suck-marks on the insides of the swim bladders were seen. Some of the swim bladders of *O. mossambicus* were distincty mottled, the mottling being the result of the pigment melanin, that was arranged in an irregular pattern across the organ. In one or two cases the wall of the swim bladder was slightly thickened but because of simultaneous infection with trematodes, it can not be categorically stated that *Subtriquetra* was responsible for the thickening.

The normal swim bladder of both the cichlid species consists of a basement membrane of simple squamous epithelium, a muscle layer consisting of smooth muscles, a dense layer of fibrous connective tissue with longitudinal, circular and oblique fibres arranged in layers. A highly vascular gas gland is present in the swim bladder wall and the vasculature is made up of parallel venous and arterial rete mirabile arranged in a pallisade formation (Figure 15). Macrophages containing the pigment melanin were present in scant to reasonable quantities in some of the swim bladders of *O. mossambicus* only and should be considered as a normal occurrence.

In the uninfected hosts and in some of the hosts infected with one or two *Subtriquetra* specimens, there were no abnormalities in the structure of the swim bladder. However, as the number of parasites increased so did the structure alter. In mild infections only a slight influx of globular macrophages were observed. In severe infections the basement membrane appeared

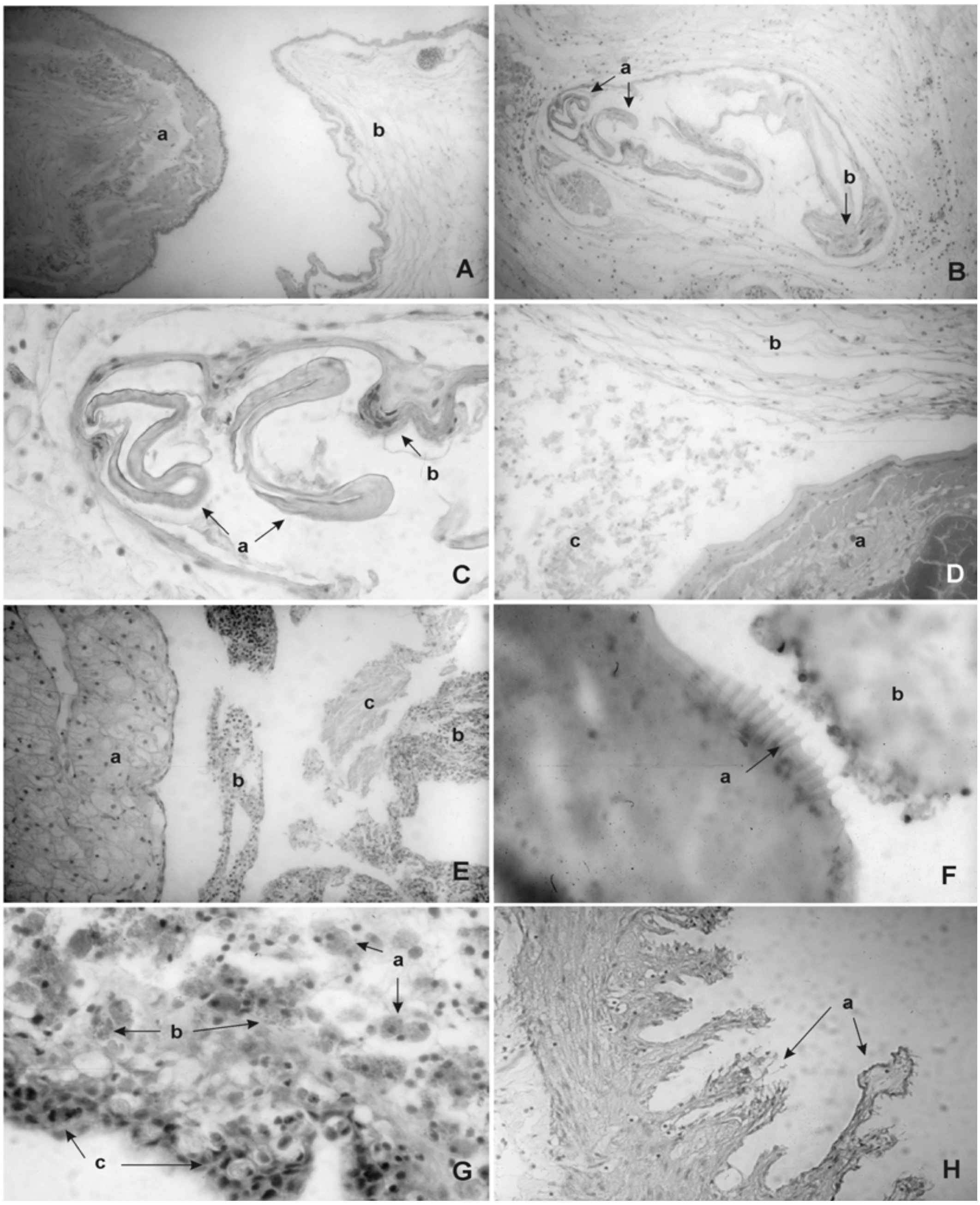

Figure15. A. Normal swim bladder showing part of the gas gland (a) and the wall with a normal basement membrane (b). B. Cast cuticles of *Subtriquetra rileyi* (a) with slight lymphocyte proliferation in the basement membrane (b). C. Higher magnification of B. D. Section through a parasite (a) with a damaged swim bladder wall (b) and cell debris in the lumen (c). E. The swim bladder wall appears normal and the debris in the lumen consists of some pycnotic lymphocytes and a few erythrocytes (b) and haematin (c). F. Section through an annulus showing the spines (a) and the adjacent damaged swim bladder wall. G. The cell reaction in the swim bladder wall consists of heterophils with large granules (a). Some degranulating heterophils occur (b) and the basement membrane is thickened by the accumulation of lymphocytes (c). H. Damage to the swim bladder wall eventually results in villous fibrosis (a). Magnification: A, B x 100; C, D, E, H x 200; F, G x 400. All stained with haematoxylin-eosin.

thickened and cellular debris occurred within the lumen of the swim bladder. The debris consisted of erythrocytes, heterophils, macrophages and some lymphocytes embedded in an amorphous matrix that often contained haematin (Figure 15). All cell types showed karyorrhexis and picnotic nuclei, which are indicative of necrosis.

Small haemorrhages underneath the basement membrane were seen in the parasitised swim bladders. These were conceivably caused by the mouthparts of the pentastomes while sucking blood, or by the hooks or the body spines and probably by all these structures. Even so, the histopathological picture of the swim bladders is one of mild changes that would not influence its normal functioning.

The continued development of *S. rileyi* in the swim bladder of its intermediate hosts involves a number of moults and the cuticles cast during ecdysis become enclosed by a layer of connective host tissue. The connective tissue is generally loose and an influx of lymphocytes indicates that the cellular immune response is combined with a humoral component.

Discussion

The fact that infections with *S. rileyi* do not produce any severe histopathological changes in the swim bladders of infected *O. mossambicus* and *T. rendalli* is indicative of a longstanding host-parasite relationship. It reflects the author's findings as regards naturally infected crocodiles, which under natural conditions tolerate pentastome infections well. It confirms the records of Junker *et al.* (1998a) that, macroscopically, infections with pentastomid parasites have no negative effect on the natural intermediate hosts. Similarly, Boyce (1985) reported that *G. affinis* tolerated even heavy infections with up to 79 infective larvae of *S. mississippiensis* and that only a mild inflammatoty response was elicited in these fish. To the contrary, Boyce *et* *al*. (1984) found that even small numbers of infected larvae of *S. mississippiensis* caused distinct lesions and pronounced inflammation in *X. helleri.* Junker *et al*. (1998b) found that *S. wedli* caused the death of experimentally infected guppies, *Poecilia reticulata.* It is noteworthy that in this case the infective larvae had not encysted in the swim bladder, the predilection site in naturally infected hosts, but were found moving freely in the abdominal cavity. The authors ascribed this to the unfavorable size ratio between the parasites and the swim bladders of their experimental host.

Nimeth, Zwerger, Würtz, Salvenmoser & Pelster (2000) investigated the histopathological changes in the swim bladder of the European eel, *Anguilla anguilla*, infected with the nematode *Anguillicola crassus* at the glass-eel stage. Some of their findings included the proliferation of connective tissue and the lamina propria resulting in a severe reduction of the lumen of infected swim-bladders. Exudate containing parts of nematodes, nematode eggs and decaying swim bladder tissue accumulated in the lumen. The oxygen and carbondioxide content of infected swim bladders was found to be significantly reduced (Würtz, Taraschewski & Pelster, 1996). While some of these pathological changes are similar to the histopathological alterations seen in the *O. mossambicus* and *T. rendalli* examined in this study, it is clear that the glass eels were considerably more severely affected by *A. crassus* than the cichlids were by *S. rileyi*. In this context it is of interest that the European eel is not the natural host of *A. crassus*, but it is a parasite of Japanese eels, *Anguilla japonica*, where infection rates are usually low with little harmful effect upon the host (Moravec & Taraschewski, 1988). Therefore, *A. crassus* further illustrates the different responses elicited by parasites in their natural versus accidental or recently conquered hosts.

It is thus concluded, that the non-pathogenicity of *S. rileyi* and sebekiids in their natural intermediate hosts reflects the extraordinary degree of adaptation that the pentastomid parasites and their hosts have reached during millions of years of co-evolution.

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SUPPLEMENT

CURRICULUM VITAE

Personal data:

Name:	Kerstin Junker
Date of birth:	October 3rd, 1968, Gütersloh, Nordrhein Westfalen
Parents:	Dipl.-Ing. Gerhard Junker Maria Elisabeth Junker, born Flaskamp
Nationality:	German
Marital status:	Single

Schools and Universities:

1998-2002	Scientific research for the degree Dr. rer. nat. (PhD), Karlsruhe, conducted in cooperation with the Department of Veterinary Tropical Diseases, University of Pretoria, South Africa, with the working theme: "A study on the Pentastomida parasitising crocodilian and chelonian final hosts, with special emphasis on the South African pentastome fauna."
1996	Masters Degree in Biology (Dipl.-Biol.)
1994-1995	MSc examinations (major subjects: Zoology and Biological engineering; minor subjects: Microbiology and Organic chemistry) Masters thesis (Diplomarbeit, 12 months) on the subject "Studies on the biology of crocodilian pentastomes in South Africa" at the Medical University of Southern Africa, South Africa and the University of Karlsruhe (TH)
1992-1993	Continuation of the studies in Biology at the University of Karlsruhe

1991	Bachelor's Degree (Vordiplom) Subjects: Zoology, Botany, Chemistry and Mathematics
1989-1991	Continuation of the studies in Biology at the University of Karlsruhe
1988	Admission to study Biology at the University of Karlsruhe (TH), Germany
1988	Matric (Abitur)
1979-1988	Secondary education at the Copernicus Gymnasium, Philippsburg
1975-1979	Primary education at the Hieronymus Nopp Grundschule, Philippsburg

Practical experience:

1997	Gained practical experience in the husbandry of wild animals in captivity at the National Zoological Gardens in Pretoria, South Africa, assisting and standing in for the curators (eight months). Sections worked in: primates, large and small carnivores, exotic birds
1989-1996	Temporary work during holidays at the water laboratory of the Kernkraftwerk Philippsburg GmbH, conducting chemical analyses, including atomic apsorption spectrometry
1992	Three month volunteer work for the Bureau of Land Management, Oregon, assisting the Wildlife Biologist with Spotted Owl and Marbled Murrelett surveys, collecting and analysing habitat data and nest site monitoring
1991	Three month volunteer work for the Bureau of Land Management, Oregon, assisting the Fishery Biologist with fish population sampling, stream habitat inventory, analysis of population and inventory data and installation of instream log and boulder structures

1988	Three month volunteer work for the Bureau of Land Management, Oregon, as assistant Park Ranger at Fishermen's Bend recreation site (maintenance, public relations, development of nature trails)

Scholarships:

1998-2000	Scholarship for the post-graduate studies by the Landesgraduierten Förderung, Baden-Württemberg
1998-1999	and the Deutscher Akademischer Austauschdienst

Hobbies:

- Hiking and skiing
- Gardening and photography
- Aviculture: breeding of parrots, finches, doves, and game birds

Pretoria, 28. September 2002 Kerstin Junker

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