Helminths and pentastomes from preserved puff adder (*Bitis arietans*) specimens in reptile collections of South African museums

By

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Submitted in partial fulfilment of the requirements for the degree of Masters of Science (Tropical Animal Health) in the Department of Tropical Diseases in the Faculty of Veterinary Science, University of Pretoria

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DECLARATION

I hereby certify that this research is the result of my own investigation. Where use was made of the work of others, it has been duly acknowledged in the text. The results in this dissertation have not been submitted, in whole or in part, for a degree at any other tertiary institution.

Leandri Strydom

I hereby release this dissertation for examination in my capacity as supervisor.

E. Volker Schwan

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SUMMARY

Helminths and pentastomes from preserved puff adder (*Bitis arietans*) specimens in reptile collections of South African museums

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Apart from isolated surveys, there is a lack of information regarding the parasite fauna of serpents (snakes) in South Africa. In an attempt to contribute to the knowledge, the dissertation focusses on the identification of a total of 227 helminth and 3 pentastome specimens which were collected following dissection from thirty-nine preserved puff adders (*Bitis arietans*) from reptile collections of South African museums. The puff adder specimens originated from various localities in South Africa. Identification of helminth and pentastome specimens was based on non-molecular, conventional morphological criteria with the appropriate use of relevant taxonomic literature. Of the 227 helminths 212 (93.4 %) were nematodes and 15 (6.6 %) were metacestodes. Of the 212 nematodes, 208 (98.1 %) were

identified as ascaridoids of which 160 belonged to the genera *Hexametra* (n=122), *Ophidascaris* (n=19) and *Polydelphis* (n=19). Because of their deteriorated condition, 48 ascaridoids could not be identified to a lower taxon level. Four (1.9 %) of the 212 nematodes were identified as diaphanocephaloids of the genus *Kalicephalus*. The fifteen metacestodes were identified as plerocercoids. The pentastome specimens sampled from a single puff adder were identified as *Raillietiella boulengeri*.

The helminth and pentastome spectrum identified is in accordance with published records.

Chapter 1

INTRODUCTION

Puff adders belong to the family Viperidae (Spawls, Howell, Drewes & Ashe 2002). The puff adder is the largest viper on the African continent, with the maximum length reaching 1.8 m (Marais 2004). Together with being the largest viper in Africa, it is also the most dangerous snake in Africa, often found in open inhabited areas (Spawls *et al.* 2002). Puff adders are regarded as the most common and wide spread snake of the African continent (Broadley 1983) with their distribution including most of the Sub-Saharan Africa as well as the Arabian Peninsula (Bates, Branch, Bauer, Burger, Marais, Alexander, & De Villiers 2014). The puff adder occurs in a wide range of habitats, being absent only from very dry deserts, 2200 meters and higher above sea level and rain forest areas. Puff adders are hosts to internal parasites such as helminths and pentastomids as well as external parasites such as ticks and mites (Broadley 1983).

Few records exist on the helminths of South African reptiles (Hering-Hagenbeck & Boomker 2000), and most records are taxonomic (Hering-Hagenbeck & Boomker 2000). In South Africa surveys on helminth and other parasite infections of *Bitis arietans* have been conducted by Fantham & Porter (1949) and Hering-Hagenbeck & Boomker (2000).

The Pentastomida includes a few pentastome species which are known to parasitise puff adders. Pentastomes adults are predominantly parasites of the respiratory tract of reptiles and are regarded as minor pathogens with infections being mostly asymptomatic in wild reptile populations (Paré 2008; Pantchev & Tappe 2011). Humans may serve as accidental intermediate hosts (Pantchev & Tappe 2011) if they ingest pentastomid larvae of some species, such as *Armillifer armillatus* or *Linguatula seratta,* in raw or undercooked meat.

Plerocercoids (a type of metacestode), the infective (second) larval stage, have been found in the muscles and tissues of snakes which serve as intermediate hosts (Pantchev & Tappe 2011; Lescano & Zunt 2013), with carnivores being the definitive hosts. Similarly, to the pentastomids,

some cestodes such as several species of the genus *Spirometra*, have zoonotic potential (Lescano & Zunt 2013).

The aim of this study was to identify helminth and pentastome specimens, collected from thirty-nine preserved puff adders from reptile collections of South African museums. Identification was based on non-molecular, conventional morphological criteria by use of appropriate taxonomic literature. Due to the deteriorated condition of several specimens, identification could only be attempted to genus level.

Chapter 2

LITERATURE REVIEW

Snakes (Chordata; class Reptilia; order Squamata, suborder Serpentes) are a group of carnivorous reptiles comprising 26 families with 3508 species. Around 170 species and subspecies of snakes occur in South Africa (Marais 2004).

2.1 The family Viperidae

Puff adders are part of the family Viperidae which consists of approximately 315 species belonging to 38 genera. The family is divided into two subfamilies, the Crotalinae (Asian and New world pitvipers) and the Viperinae (Old World vipers or pitless vipers) (Bates *et al.* 2014). Only the Viperinae occur in Africa (Bates *et al.* 2014) and of these, eleven genera occur in Africa (Bates *et al.* 2014) namely, *Bitis, Causus, Atheris, Montatheris, Proatheris, Echis, Cerastes, Pseudocerastes, Daboia, Vipera and Macrovipera.* The genus *Bitis* comprises 17 species in total, with the majority of species being endemic to the African continent with the exception of *B. arietans* (Merrem) which also occurs on the Arabian Peninsula (Bates *et al.* 2014). Vipers are rather abundant in most African countries (Bates *et al.* 2014). In South Africa, there are 11 species belonging to the genus *Bitis* and two species belonging to the genus *Causus* (Bates *et al.* 2014). The genus *Bitis*, includes the puff adder and is the most widespread and diverse African snake genus, occurring in a variety of terrestrial biotopes (Lenk, Herrmann, Joger & Wink 1999). Vipers have well developed, large hinged front fangs and thus a good venom delivery system which has led to vipers being the medically most important group of snakes (Wüster, Peppin, Pook & Walker 2008).

Females of the genus *Bitis* are ovoviviparous, giving birth to 16 – 43 young per litter (Broadley 1983). Larger species such as *B. arietans* and *Bitis gabonica gabonica* (Duméril, Bibron & Duméril) typically give birth to smaller litters (Bates *et al.* 2014). The puff adder is often consumed by humans in some African countries (Bates *et al.* 2014).

The diverse *Bitis* genus currently comprises 17 species: *B. arietans*, *B. gabonica*, *Bitis rhinoceros* Peters, *Bitis nasicornis* (Shaw), Bitis albanica Hewitt, *Bitis armata* (Smith), *Bitis rubida* Branch, *Bitis caudalis* (Smith), *Bitis peringueyi* (Boulenger), *Bitis schneideri* (Boettger), *Bitis atropos* (Linnaeus), *Bitis cornuta* (Daudin), *Bitis xeropaga* Haacke, *Bitis inornata* (Smith), *Bitis worthingtoni* Parker, *Bitis parviocula* Böhme and *Bitis heraldica* (Bocage) which are endemic to Africa except for *B. arietans* which also occurs in the Arabian Peninsula (Hering-Hagenbeck & Boomker 2000; Bates et al. 2014).

2.2 The puff adder (*Bitis arietans*)

The puff adder is a highly venomous and dangerous snake and is responsible for the most serious snake bite incidents in humans in Africa (Marais 2004). The venom is cytotoxic, attacking the tissues around the bite wound as well as blood vessels, often leading to amputations of limbs in wet bites (Marais 2004). Other symptoms of puff adder bites include extreme pain in the bitten area, extreme oedema and sometimes blistering (Marais 2004). The venom is slow acting, generally taking 24 h before being fatal to humans (Marais 2004). Fatalities are less than ten percent of bite incidents, since most cases are treated with antivenom and fluids, fatalities are typically due to complications of extreme oedema and kidney failure (Marais 2004).

Puff adders of 1.8 m have been documented in Kenya, making the puff adder the largest pitless viper (Marais 2004). Puff adders have an average length of about 90 cm, a thick body, thin neck and a triangular shaped head (Broadley 1983; Marais 2004). They range in colour from bright yellow to light yellow, orange-brown, light brown or even grey with black chevron patterns on top of their body (Marais 2004). Puff adders are common across most areas of South Africa (Marais 2004). They are very adaptable and are found in a wide variety of habitats, excluding only mountain tops, very dry desert areas or very dense forest areas (Marais 2004; Bates *et al.* 2014).

Puff adders are slow moving and will hiss or blow when disturbed (Broadley 1983; Marais 2004). Puff adders usually wait motionless until a potential prey such as rodents, rabbits, birds, lizards and frogs are within striking distance (Marais 2004). The strike of a puff adder is quick

and the head and neck is often pulled back in a s-shape before striking (Marais 2004), swallowing their prey head first (Marais 2004). Puff adders will typically stay close to the ground, only rising to the top of small shrubs in order to bask in the sun (Marais 2004). They are mainly nocturnal and will readily lie on roads where they are often killed by vehicles (Marais 2004). Puff adders depend on their excellent camouflage, often rather freezing than fleeing (Marais 2004). The puff adders are ovoviviparous, giving birth to an average of 20-40 young during spring (Marais 2004). In captivity they have been known to live for 14 years or longer under optimal conditions (Broadley 1983).

2.3 Nematode studies of reptiles

It appears that ascaridoid nematodes feature strongly as parasites of snakes in general.

Dujardin (1845) was the first who embarked on the taxonomy of ascaridoids of amphibians and reptiles. Based on the presence of four uterine branches in *Ascaris anoura*, he suggested a subgenus called *Polydelphis* (Dujardin 1845). Skrjabin (1916) raised the subgenus to generic rank after observing the presence of six uterine branches.

Reptile nematodology has been a topic in South Africa for quite some time (McAllister, Bursey & Freed 2010). Studies on the helminth fauna of reptiles date back to von Linstow (1899) who reported on *Ascaris attenuata* (syn. *Polydelphis anoura*) from a puff adder originating from the Durban area (Linstow 1899). Fantham & Porter (1949) provided the first summary of endoparasites from South African snakes of the Viperidae, Boidae, Colubridae and Elapidae families. Hering-Hagenbeck & Boomker (2000) subsequently compiled a check-list of nematodes from South African snakes and lizards. McAllister *et al*. (2010) performed a study on nematodes collected from 107 reptiles of 11 families (32 species). A single puff adder was examined in which no nematodes were found (McAllister *et al.* 2010). Few records however exist on helminth parasites from the 400 reptile species in South Africa (Hering-Hagenbeck & Boomker 2000). Most studies on helminths from South African reptiles were performed many years ago, before the development of advanced molecular techniques which can be used for more accurate identification of species (Ronai 2017).

2.4. Helminths and pentastomes recorded from puff adders

Research indicates that studies on the helminths of puff adders date back to 1861 when Wedl discovered *Polydelphis quadricornis* from a puff adder in Africa. Following Wedl, studies were performed by Daubney (1923), Goodey (1924), Sandground (1933), Schuurmans Stekhoven (1937), Fantham & Porter (1949), Mozgovoi (1953), Sprent (1978) and in 2000 Hering-Hagenbeck & Boomker compiled a check-list of nematode occurring in South-African snakes.

Cestodes reported from puff adders are *Ophiotaenia marenzelleri* from two puff adders in South Africa (Fantham & Porter 1949) as well as *O. adiposa* and *O. amphiboluri* from a single puff adder from Cameroon (McAllister *et al.* 1992).

Studies on pentastomes of puff adder are few, with De Meneghi (1848) & Fantham & Porter (1949) finding *Armillifer armillatus* and Ali, Riley & Self (1982) reporting *Raillietiella boulengeri* from puff adders (Table 2.1 & Table 2.2).

2.5. Description of helminth and pentastome species reported from puff adders

2.5.1 *Ophidascaris amucronata* Schuurmans-Stekhoven, 1937

Ophidascaris is found mainly in the stomachs of snakes (Sprent 1988). They are often intertwined with the tissues of the prey which makes up the stomach content (Sprent 1988). The worms seem to attach to the stomach wall by embedding their body into the mucous membrane of the stomach, and can be found this way when the stomach is empty of prey (Sprent 1988). *Ophidascaris* was identified as a group of large ascaridoid parasites with an even body width or posteriorly wider in females, occurring in serpents (Sprent 1988). The group is characterised by helminths that contain three lips of a square shape with interlabia present (Sprent 1988). The oesophagus of *Ophidascaris* may be invaginated into the intestine to appear as caecum-like structures which are inconsistent (Sprent 1988). Two uterine branches are present in the females of *Ophidascaris* (Sprent 1988). Other characteristics of *Ophidascaris* include the presence of denticular ridges, an indented interior margin and an oral groove (Sprent 1988). In living specimens of *Ophidascaris* the intestine have typical folds of the internal surface which produces an iridescent sheen and a pattern that can be herringbone or reticulate

and in some cases papillate in appearance (Sprent 1988). The intestine varies in colour from a brown-red to a lemon-yellow colour (Sprent 1988). The vulva is located close to the middle of the body, sometimes slightly posterior to the exact middle (Sprent, 1988). There is no gubernaculums present in the genus (Sprent 1988). Papillae are present in high numbers with 30 or more precloacal papillae, 2 subventral papillae, 2 subdorsal postcloacal papillae and one double paracloacal papilla as well as a single median precloacal plaque or papilla (Sprent 1988).

Sprent (1977) reported *O. amucronata* specimens to be yellow-brown in colour, with square lips and no alae present. The caecum is also absent and the tail tapers towards the smooth rounded tip (Sprent 1988). The vulva of females is situated between a third and two thirds of the body length from the anterior end (Mozgovoi 1953). Spicules of males are almost twice as long as the ejaculatory ducts (Sprent 1977). Males are smaller than females and females have phasmids near the tip of their tail (Sprent 1988).

2.5.2 *Ophidascaris radiosa* (Schneider, 1866)

Ophidascaris radiosa have square to longer than wide lips (Sprent 1988). The interlabia are prominent and about half the length of the lips with rounded edges (Sprent 1988). The lips are concave at the anterior edge which gives the appearance of a scoop to the lips (Sprent 1988). A postlabial groove is present while a ventriculus and caecum are both absent (Sprent 1988). The denticular ridges consists of very small denticles and the isthmus may vary in width (Sprent 1988). The lips have rounded posterior angles and no labial pillars posteriorly (Sprent 1988). Lateral alae may be present or absent in the anterior region (Sprent 1988). The oesophagus contains no demarcated ventriculus or bulb (Sprent 1988). The oesophagus is relatively short, being 2.5 – 5.9 % of the body length (Sprent 1988). The duct of the oesophageal gland opens behind the lips while the ducts of the subventral glands open at the posterior end of the oesophagus (Sprent 1988). The nucleus of the dorsal oesophageal gland is located in the dorsal sector (Sprent 1988). The nuclei of the subventral glands are located within the ventral region of the subventral sectors (Sprent 1988). The excretory system is bilateral with the excretory pore not far behind the nerve ring (Sprent 1988). The excretory nucleus can be found within the ommissure or at the junction of the ommissure with the left lateral filament (Sprent 1988). The

vulva is not prominent, but can be located between 59 – 75 % of the body length (from anterior end of the specimen) (Sprent 1988). The vagina extends approximately the same length as the undivided uterus in a posterior direction (Sprent 1988). The uterine coils are all located behind the vulva (Sprent 1988). The eggs are finely pitted; oval shaped, and vary in size from 0.065 to 0.085 mm in length and 0.052 to 0.077 mm in width (Sprent 1988). The level of the cervical papillae is either with the excretory pore or slightly behind the excretory pore (Sprent 1988). All reproductive organs are situated behind the vulva (Sprent 1988). The tail curves towards the ventral direction (Sprent 1988). Precloacal papillae vary from 32 to 37 in number. A digitiform mucron can be seen (Sprent 1988). In males the mucron is prominent and the spicules are short (2.2 to 2.8 % of body length) and of equal length, alate and with a rounded tip (Sprent 1988). The ejaculatory duct is about 57 to 73 % of spicule length (Sprent 1988).

2.5.3 *Polydelphis anoura* Dujardin, 1845

The genus *Polydelphis* has trapezoidal lips which are longer than they are wide, denticular ridges and the postlabial grooves and interlabia are absent (Mozgovoi 1953; Sprent 1978). In females, the vulva is located in the anterior third of the body and four uterine branches are present (Mozgovoi 1953; Sprent 1970, 1978). The vulva is situated between 18 and 38% of its body length (Sprent 1978). Males have alate spicules without a gubernaculum (Sprent 1978). The spicules are between six (6) and 12% of its body length and are longer than the ejaculatory ducts (Sprent 1978). While the tails of large female specimens may have no mucron, the tails of males have a mucron present (Sprent 1978). The oesophagus is between five (5) and 13 % of its body length from the anterior end (Sprent 1978). Between 30 and 50 precloacal papillae are present as well as two paracloacal papillae, two subventral papillae and two subdorsal papillae (Sprent 1978). Lateral phasmids are present near the tip of the tail (Sprent 1978). Infected snakes are usually asymptomatic (Sprent 1978).

Sprent (1978) reported *P. anoura* only from various species of pythons, none from the viper snake family. Jones (1979) also reported *P. anoura* from various python species in Australia and Papua New Guinea.

Sprent (1977) found *P. anoura* in the small intestine of pythons and noted that the parasites were found with their heads embedded into the mucous membrane of the upper part of the small intestine. The effects of the worms on snakes are are largely unknown and appear to be non-significant (Sprent 1978). *Polydelphis anoura* is synonymous with *Ascaris attenuata* Molin, 1858, *Ascaris rubicunda* Schneider, 1866*, Ascaris oculata* Linstow, 1899*, Polydelphis bicornuta* Robinson, 1934, *Polydelphis mucronata* Panagia, 1933 and *Ascaris anoura* Dujardin, 1845 (Sprent 1978).

Polydelphis anoura is a medium to large sized helminth with both sides tapering towards the anterior and posterior ends (Sprent 1978). The lips are trapezoid-shaped and the length of the lips is approximately equal to the width (Sprent 1978). A notch can be found at the posterior corners of the lips (Sprent 1978). The teeth (denticular ridges) extend around the entire margin of the lip and the anterior prolongations of the labial pulp are in the shape of a foot (Sprent 1978). The posterior end of the oesophagus has a bulge allowing space for the nuclei of the oesophageal glands (Sprent 1978). The shape of the nuclei differs between the different glands, with the nuclei of the subventral glands being spherical in shape and the nucleus of the dorsal gland being oval shaped and larger than the nuclei of the subventral glands (Sprent 1978). The nucleus of the dorsal gland does not extend into the subventral sectors (Sprent 1978). In some mature specimens of *P. anoura* a small intestinal caecum can be seen while in most immature specimens the caecum is clearly visible (Sprent 1978). The wall of the intestine of mature helminths has a fish-bone pattern (Sprent 1978). The vulva is located between 18 and 39 % of the body length from the anterior margin and the vulva is without any noticeable lips (Sprent 1978). In younger and smaller females, the vulva is located more posterior (Sprent 1978). The vagina of *P. anoura* is rather long in length and sinuous in shape (Sprent 1978). A vagina of 5.4 mm was measured in a female which was 134 mm in length (Sprent 1978). The vagina is about half the length of the undivided part of the uterus (Sprent 1978). The tail is short and conical in shape with a conical mucron at the terminal portion (Sprent 1978). In large females, the mucron is sometimes absent (Sprent 1978). Phasmids are located at approximately 25% of the distance from the tail towards the anus (Sprent 1978). The eggs are finely pitted and oval shaped. The dimensions of the eggs are approximately 0.060 to 0.071mm in width and 0.081 to

0.090mm in length (Sprent 1978). The tail of male helminths is rounded and a mucron is present near or at the terminal end (Sprent 1978). The spicules of males are almost twice the length of the ejaculatory duct and between six (6) and 12 percent of the body length (Sprent 1978). Spicules have two alae (Sprent 1978). Many precloacal papillae are present in numbers ranging from 30 to 50, double papillae are located paracloacal and postcloacal papillae are two subventrally situated and two subdorsally situated papillae (Sprent 1978). Lateral phasmids are located near the tip of the tail (Sprent 1978). A median plaque is present anterior to the cloaca (Sprent 1978).

2.5.4 *Hexametra quadricornis* (Wedl, 1861)

Sprent (1978) maintains that there is little difference between *Hexametra boddaertii* and *H. quadricornis* and that they may be the same species.

Dietary preferences of snakes have an influence on the infection potential of *H. quadricornis* since the third stage larvae has to develop in a mammalian intermediate host such as rodents (Sprent 1978). Third stage larvae only seem to develop in some rodent species (Sprent 1978).

Hexametra quadricornis is a white, evenly wide, medium to large sized helminth (Sprent 1978). The lips have no interlabia, and the interlabial space is concave-shaped (Sprent 1978). The denticular ridges consists of sharp, elongate teeth which extends along the around anterior angles to the level of the double papillae (Sprent 1978). On the middle area of each lip, are double papillae (Sprent 1978). The cuticular bars are V-shaped in specimens originating from the African continent (Sprent 1978). The length of the oesophagus is approximately four (4) – eight (8) percent of their entire body length and has a bulb-shaped terminal portion which contains the nuclei of the oesophageal glands (Mozgovoi 1953; Sprent 1978). In the dorsal oesophageal gland, the nucleus is located on the right side of the dorsal part of the gland; the nucleus occasionally invades the subventral sector (Sprent 1978). The opening of the duct of the dorsal gland of the oesophagus is located between the dorsal lip and the nerve ring, while the opening of the subventral glands are located anterior to the nuclei of the oesophageal glands (Sprent 1978). *Hexametra boddaerti* and *H. quadricornis* appear to be synonymous as they have identical excretory systems and excretory pores (Sprent 1978). The excretory pores

are located close behind the nerve ring and in some specimens, a short caecum may be present (Sprent 1978).

In female specimens, the vulva can be identified near the middle of the body or slightly posterior between 47 and 60 % from the front (Sprent 1978). The undivided uterus is about twice the length of the vagina (similar to *O. radiosa)* and is approximately 2.4 mm (Sprent 1978). The uterus divides into six uterine horns which is characteristic for the genus *Hexametra* (Mozgovoi 1953; Sprent 1978). The dimensions of the eggs are given as 0.065 to 0.103 mm long and 0.059 to 0.082 mm wide (Sprent 1978). Female specimens have a short tail with a digitiform mucron and phasmids on both sides of the mucron close to the terminal end of the mucron (Sprent 1978).

Males have similar mucrons on their tails and the tail is rounded and short (Sprent 1978). Two or sometimes, three, subventral and precloacal papillae can be seen and one or two subdorsal papillae (Sprent 1978). Paracloacal papillae may be single or double (Sprent 1978). Lateral phasmids are present close to the tail's tip (Sprent 1978). Pre-cloacal papillae can range from fifty to a hundred (Sprent 1978). Spicules are 40 to 90 % of the ejaculatory duct length and have rounded tips and two alae (Sprent 1978).

2.5.5 *Rhabdias fuscovenosa* (Railliet, 1899)

Species of the genus *Rhabdias* are either parthenogenic or hermaphroditic and typically inhabit the lungs of reptiles or amphibians (Goodey 1924). The bodylength was reported to vary between three (3) and six (6) mm (Goodey 1924). The lips are all formed from six papillae (Goodey 1924). The vulva is situated just anterior to the middle of the adult worm (Goodey 1924). Adults have a receptacle in which they store spermatozoa (Goodey 1924). *Rhabdias fuscovenosa* is the only species reported of the genus Rhabdiasidea species that was reported from *B. arietans* (Goodey 1924).

2.5.6 *Kalicephalus* spp.

The genus *Kalicephalus* is a strongylid (order Strongylida) belonging to the Diaphanocephaloidea, family Diaphanocephalidae. Paratenic hosts appear to be involved in the

life cycle of the diaphanocephalids (McAllister *et al.* 2010). The predilection sites of *Kalicephalus* species are the oesophagus, stomach and intestine of their hosts (Yamaguti 1961; Kuzmin, Kinsella, Tkach & Bush 2013; Kavitha, Latha, Bino Sundar, Jayathangaraj, Senthil Kumar, Sridhar & Abdul Basith 2014). Junker, Lane, Dlamini, Kotze & Boomker (2009) report of finding *K. colubri colubri* in several snake species of different families, namely the puff adder, red-lipped herald snake (*Crotaphopeltis hotamboeia*), brown house snake (*Lamprophis fuliginosus)*, Snouted cobra (*Naja annulifera*), Mozambique spitting cobra (*Naja mossambica*), Short-snouted grass snake (*Psammophis brevirostris brevirostris*) and the Mole snake (*Pseudaspis cana*). Although infections are largely asymptomatic, clinical signs reported from infected snakes include hyperaemia and inflammation (Junker *et al.* 2009).

Yamaguti (1961) provides some information regarding species characteristics of Diaphanocephalidae and more specifically, of *Kalicephalus* species occurring in reptiles. The diaphanocephalids have characteristic features which include a bivalvular buccal capsule which is laterally compressed (Yamaguti 1961; Kavitha *et al.* 2014). The oesophagus is highly muscular with a triradiate lumen visible as well as a thick chitinous lining (Yamaguti 1961; Kavitha *et al.* 2014). Male specimens have contiguous ventral bursal rays and the spicules are of equal length (Yamaguti 1961; Kavitha *et al.* 2014). Female specimens have two sets of reproductive organs with the vulva located close to the posterior terminal end (Yamaguti 1961). Female specimens have grouped uterine branches (Yamaguti 1961). Grouped in the family Diaphanocephalidae are the genera *Diaphanocephalus* and *Kalicephalus* (Yamaguti 1961). *Diaphanocephalus* species have two chitinous ledges in the lateral walls of their capsule while *Kalicephalus* species have only one chitinous ledge in the lateral walls of their capsule (Yamaguti 1961). *Diaphanocephalus* species also have chitinous processes which are fairly delicate, projecting into the capsule from the ventral cuticular pad while *Kalicephalus* species have no chitinous processes which projects into their capsule (Yamaguti 1961). As yet, there are no records of *Diaphanocephalus* species from puff adders.

Yamaguti (1961) holds that the genus named *Occipitodontus* is synonymous to *Kalicephalus*. *Kalicephalus* species recorded from puff adders include *K. obliquus* (Fantham & Porter 1949), *K. viperae obliquus* and *Kalicephalus colubri colubri* (Hering-Hagenbeck & Boomker 2000).

Kalicephalus species may or may not have a simple leaf-crown (Yamaguti 1961). The buccal valves of *Kalicephalus* species are supported by a singular internal transverse chitinoid ridge (Yamaguti 1961). The rigde is V-shaped both dorsally and ventrally (Yamaguti 1961). A typical feature of *Kalicephalus* species which distinguishes it from *Diaphanocephalus* species is that *Kalicephalus* species have no chitinous processes that project from the ventral cuticular pad into the buccal cavity (Yamaguti 1961). Three small teeth may be present in the oesophagus (Yamaguti 1961). Males do not have a dorsal hump which is situated anterior to the trilobed bursa (Yamaguti 1961). The dorsal lobe projects past the lateral lobes (Yamaguti 1961). The dorsal ray bifurcates distally to three digits or varying length (Yamaguti 1961). The spicules of *Kalicephalus* are equal in length and there is a telamon and a gubernaculum (Yamaguti 1961). Females have a long and conical-shaped posterior extremity (Yamaguti 1961). The vulva of the female is situated at the caudal end of the body (Yamaguti 1961). The uterine branches of females can be either opposed or parallel and females are oviparous (Yamaguti 1961).

2.5.7 *Raillietiella boulengeri* (Vaney & Sambon, 1910)

According to Ali et al. (1982) the number of annuli on pentastomes is used as a key to determine the species and it remains difficult to determine the exact numbers of annuli on pentastome species since the anterior annuli are often difficult to differentiate. The hooks are also an important morphological feature for species identification and should preferably be measured from completely mature female specimens in order to standardise the measurement and ensure the accuracy of comparisons between the sizes of the hooks of different specimens.

The length of three specimens varied from 18 to 33 mm (Ali *et al.* 1982). The number of annuli was estimated to be between 32 and 34 (Ali *et al.* 1982). The spicules of male *R. boulengeri* easily distinguishes it from other Raillietiellids occurring in snakes (*Raillietiella orientalis & Raillietiella agcoi)* (Ali *et al.* 1982). The shaft of the spicules of males is strongly curved and the

base of the spicule is significantly smaller than the other Raillietiellids occurring in snakes (Ali *et al.* 1982).

The broad conical area of the anterior aspect contains the mouthparts as well as the hook pairs (Ali *et al.* 1982). This area is referred to as the cephalothorax (Ali *et al.* 1982). The cephalothorax is separated from the elongated, slender abdominal region by a neck-like constriction (Ali *et al.* 1982). The mouth opens ventrally and is located subterminally (Ali *et al.* 1982). A pair of spikes called apical papillae is located on either side of the mouth (Ali *et al.* 1982). The hooks are usually embedded and retracted into podial lobes (Ali *et al.* 1982). Smaller, parapodial lobes are located on either side of the podial lobes (Ali *et al.* 1982). The posterior hooks are larger than the anterior hook pair and the tips of these hooks are sharp (Ali *et al.* 1982). The abdomen widens to about 3.5 mm at 20 to 25 % of the total body length from the anterior end, and hereafter tapers towards the tail (Ali *et al.* 1982). Males are significantly smaller than females with a maximum length reaching 17 mm and maximum width about two millimeter in diameter (Ali *et al.* 1982). The cephalothorax of males is similar to females and the abdomen of males is also pyriform in shape as in females (Ali *et al.* 1982).

2.6. Significance of helminths and pentastomes as pathogens of snakes

Snakes can serve as intermediate hosts when they consume other infected snakes (Ali *et al.* 1982). The significance of auto-reinfection in the epidemiology of *Raillietiella* spp. in general remains unclear but is usually associated with snakes in captivity (Ali *et al.* 1982). Pentastomes can be highly pathogenic in snakes kept in captivity due to stress factors attributable to excessive handling and improper husbandry (Ali *et al.* 1982). Junker *et al.* (2009) noted that the severity of pathogenic effects of helminths in snakes differs significantly between free-ranging and captive ones, with captive snakes mostly having heavier infections and more severe clinical signs. Junker *et al. (*2009) highlights the significance of concomitant infections which can potentiate the pathogenic effects of helminths.

2.7. Significance of helminths and pentastomes as zoonoses

Reptiles are becoming more common as pets in many countries (Pantchev & Tappe 2011; Rataj, Lindtner-Knific, Vlahović, Mavri & Dovč 2011). Reptiles in captivity often come into contact with their own excrement as a result of space confinement and lack of hygiene (Rataj *et al.* 2011). This predisposes these reptiles to reinfection. Helminths of reptiles are one of the common problems of pet reptiles with which veterinarians are confronted (Pantchev & Tappe 2011). Some of the helminths and pentastomes such as *Spirometra* spp. and *Armillifer armillatus,* found in snakes have zoonotic implications (Pantchev & Tappe 2011; Rataj *et al.* 2011). Parasitic zoonoses of reptile origin are poorly studied (Pantchev & Tappe 2011). Raw or undercooked snake meat is one of the principal sources of infection for humans (Meyers & Neafie 2011; Pantchev & Tappe 2011).

Table 2.1 Helminths and pentastomes recorded from puff adders

Parasite	Locality	Reference/Study
Helminths		
Polydelphis quadricornis	Africa	Wedl (1861); Fantham & Porter (1949)
Kalicephalus obliquus	Africa	Daubney (1923)
Rhabdias fuscovenosa	South Africa	Goodey (1924)
Ophidascaris amucronata	Africa	Schuurmans Stekhoven (1937); Hering-Hagenbeck & Boomker (2000); Mozgovoi (1953)
Thubunea grayicola	Ukerewe Island, Tanganyika	Sandground (1933)
Rhabdias fuscovenosa	South Africa	Fantham & Porter (1949); Hering-Hagenbeck & Boomker (2000)
Ophidascaris intorta (= *radiosa*)	South Africa	Fantham & Porter (1949); Sprent (1988); Mozgovoi (1953); Hering-Hagenbeck & Boomker (2000)
Ophiotaenia marenzelleri	South Africa	Fantham & Porter (1949)
Ophiotaenia adiposa	Cameroon	McAllister (1992)
Ophiotaenia amphiboluri	Cameroon	McAllister (1992)
Hexametra quadricornis	South Africa	Mozgovoi (1953); Sprent (1978); Hering-Hagenbeck & Boomker (2000)
Kalicephalus colubri colubri,	South Africa	Hering-Hagenbeck & Boomker (2000)
Kalicephalus viperae obliquus,	South Africa	Hering-Hagenbeck & Boomker (2000)
Physalopteroides grayicola	South Africa	Hering-Hagenbeck & Boomker (2000)
Polydelphis anoura	South Africa	Hering-Hagenbeck & Boomker (2000)
Pentastomes		
Armillifer armillatus	Zambia	Fantham & Porter (1949)
Raillietiella boulengeri	Africa	Ali *et al*. (1982).

Table 2.2 Classification of nematode, cestode and pentastome species recorded from puff adders

Phylum	Class	Order	Family	Species and Authority
Nematoda	Secernentea (Phasmidia)	Rhabditida	Rhabdiasidae	*Rhabdias fuscovenosa* (Railliet, 1899)
		Strongylida	Diaphanocephalidae	*Kalicephalus obliquus* (Daubney, 1923)
				Kalicephalus viperae (Rudolphi, 1819)
				Kalicephalus vipera obliquus (Daubney, 1923)
				Kalicephalus colubri colubri
		Ascaridida	Ascarididae	*Polydelphis anoura* Dujardin, 1845
				Polydelphis quadricornis (Wedl, 1862)
				Hexametra quadricornis (Wedl, 1861)
				Ophidascaris amucronata Schuurmans-Stekhoven, 1937
				Opidascaris intorta Schneider, 1866
		Spirurida	Physalopteridae	*Physalopteroides grayicola* (Sandground, 1933)
Platyhelminthes	Cestoda	Proteocephalidea	Proteocephalidae	*Ophiotaenia marenzelleri* (Fantham & Porter 1949)
				Ophiotaenia adiposa (McAllister, 1992)
				Ophiotaenia amphiboluri (McAllister, 1992)
Pentastomida			Porocephalidae	*Armillifer armillatus* (Wyman, 1848)
				Raillietiella boulengeri (Vaney & Sambon, 1910)

Chapter 3

MATERIALS AND METHODS

A total of 227 helminths and three pentastomes, collected from thirty-nine puff adders, were received for identification to the lowest taxonomical level possible.

3.1 Puff adders and recovery of helminth and pentastome specimens

Samples of helminths and pentastomes were received by the Helminthology Laboratory of the Department of Veterinary Tropical Diseases for identification. Samples were collected from thirty-nine preserved puff adders archived in various museum collections. Twenty-nine samples originated from puff adders of the Port Elizabeth Museum/Bayworld, six samples originated from the Ditsong National Museum of Natural History (formerly Transvaal Museum) in Pretoria and three samples originated from the National Museum in Bloemfontein. One sample was from an unknown source (Table 3.1).

The helminth and pentastome specimens were, according to the information received preserved in either ethanol or propanol and their condition varied considerably. The preservation fluid in many container bottles had a slightly brown colour. Specimens in several sample containers were highly distorted and discoloured. Upon receipt, all samples were re-preserved in freshly prepared 70 % ethanol glycerol fixative.

3.2 Identification of helminth and pentastome specimens

Specimens were identified to the lowest taxonomical level possible based on morphological and morphometrical published criteria (Goodey 1924; Mozgovoi 1953; Yamaguti 1961; Sprent 1970, 1977, 1978, 1988; Anderson, Chabaud & Wilmott 1989; Paré 2008; Kelehear, Spratt, Dubey, Brown & Shine 2011; Christoffersen & De Assis 2015).

Microscopic examination was performed after specimens were cleared in lactophenol and stained with Horen's trichrome (Horen 1957). Some specimens were hand-sectioned following the technique described by IIP (1996) ((IIP) International Institute of Parasitology 1996).

Microscopic examination was conducted with an Olympus BX 50 compound microscope and images taken with an attached CC12 digital camera. Some measurements were taken using the software programme, AnalySIS v 3.2 (Soft Imaging System, Germany). Other measurements and visualisation of specimens were performed with an Olympus SZX9 stereomicroscope. Measurements of body length and egg size were compared with findings of previous studies.

Scanning electron microscopy (SEM) was performed on three selected *H. quadricornis* specimens. The helminth specimens were chemically fixed in 2.5 % glutaraldehyde and subsequently in 1 % osmium tetroxide, dehydrated in an ethanol series, dried with hexamethyldisilazane, then mounted on carbon tape and finally coated with a layer of carbon before being examined with the ultrahigh resolution Field emission SEM (JEOL 6000F) scanning electron microscope (Kuo 2007).

Table 3.1: Origin of the 39 puff adders from which helminth and pentastome specimens were collected

Geographical location	Number of puff adders
Eastern Cape province	21
Western Cape province	1
Northern Cape province	1
Mpumalanga	1
Limpopo province	4
Kwa-Zulu Natal	7
North West province	1
Mozambique	1
Zambia	1
Unknown location	1

Chapter 4

RESULTS

A total of 230 parasite specimens (227 helminths and three pentastomes) were examined and identified to the lowest possible taxon. Fifteen of the 227 helminths were metacestodes and the remaining 212 were nematodes (Table 4.1).

4.1 Cestodes

Fifteen metacestodes were identified as plerocercoids and were sampled from three puff adders. Two of these originated from the Eastern Cape Province and one from Kwa-Zulu Natal (Figure 4.6).

4.2 Nematodes

Of the 212 nematodes, 208 specimens were identified as ascaridoids (Nematoda: Ascaridida: Ascaridoidea) four specimens were identified as diaphanocephaloids (Nematoda: Strongylida: Diaphanocephaloidea). Of the ascaridoids, 48 specimens were in a condition that did not permit definitive identification.

The ascaridoid helminths (n=160) were identified as *P. anoura* (Ascaridida: Ascaridoidea: Ascarididae) (n=19), *H. quadricornis* (Ascaridida: Ascaridoidea: Ascarididae) (n=122) (Figures 4.1 to 4.5) and *O. radiosa* (Ascaridida: Ascaridoidea: Ascarididae) (n=19).

The *P. anoura* specimens were recovered from five puff adders. Three of these originated from Eastern Cape province, one from Mpumalanga and one from Kwa-Zulu Natal. The *H. quadricornis* specimens were recovered from 14 puff adders. Eight of the 14 puff adders originated from Eastern Cape province, four from Kwa-Zulu Natal, one from Limpopo province and one from Mpumalanga. The *O. radiosa* specimens were recovered from five puff adders. Two of the puff adders originated from Mpumalanga, one from Western Cape province, one from Kwa-Zulu Natal and one from the Eastern Cape Province.

The diaphanocephaloids (n=4) were identified as *K. colubri colubri* (Strongylida: Diaphanocephaloidea: Diaphanocephalidae). The four *K. colubri colubri* specimens originated from two puff adders, one from the Eastern Cape Province and one from Kwa-Zulu Natal.

4.3 Pentastomes

The three pentastome specimens were identified as *R. boulengeri* (Pentastomida: Cephalobaenida) (Figure 4.7). The specimens were recovered from a single puff adder originating from North West province.

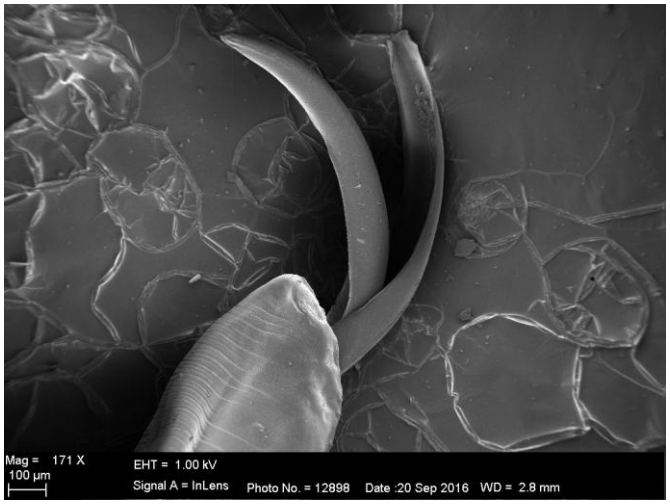

Figure 4.1: Scanning electron micrograph of the spicules of a *Hexametra quadricornis* specimen

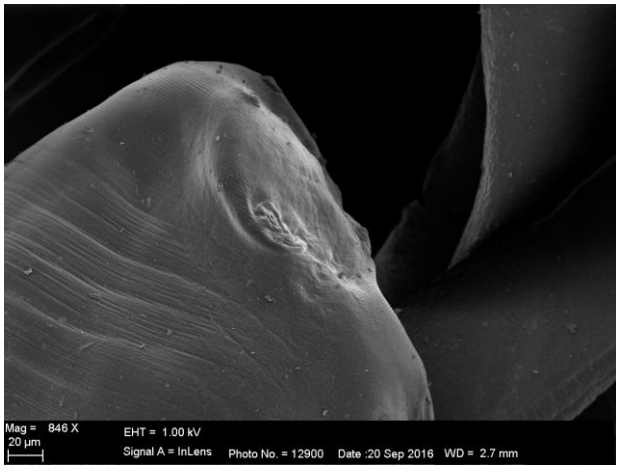

Figure 4.2: Scanning electron micrograph of the tip of the tail of a male *Hexametra quadricornis* specimen

Figure 4.3: Scanning electron micrograph of a precloacal papilla of a male *Hexametra quadricornis* specimen

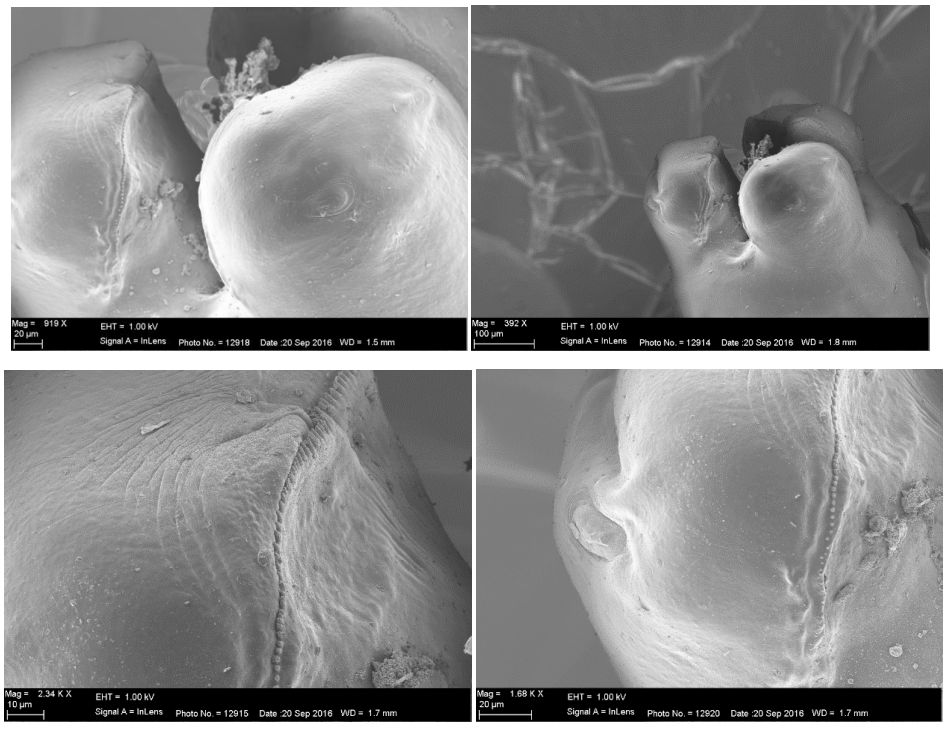

Figure 4.4: Scanning electron micrograph of the lips and denticular ridges of a *Hexametra quadricornis* specimen

Figure 4.5: Lateral aspect of the head of a *Hexametra* specimen showing the absence of interlabia (200X)

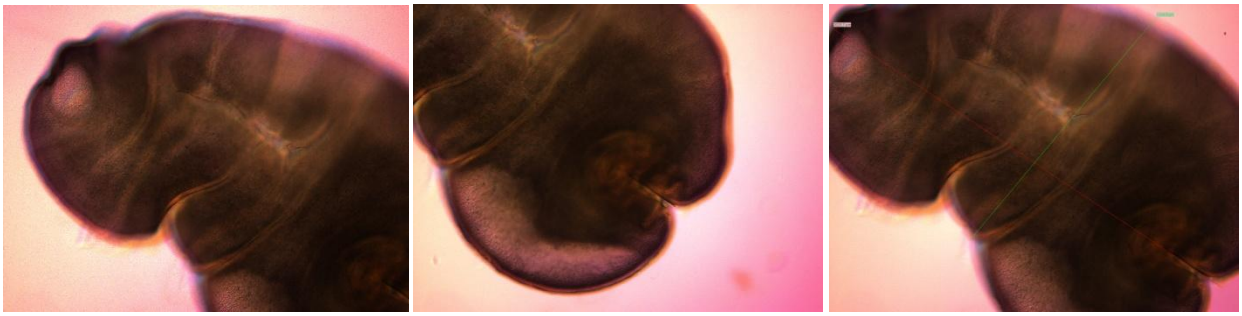

Figure 4.6: Plerocercoid specimen (40X)

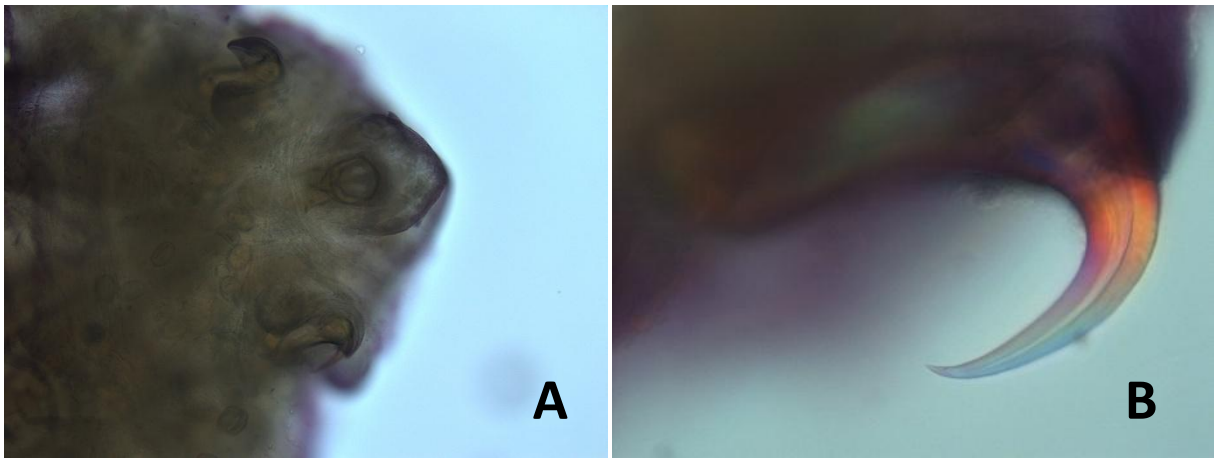

Figure 4.7: Anterior cephalothorax with first pair of hooks (A) (100X) and hook (B) (400X) of a *Raillietiella boulengeri* specimen

Table 4.1: Overview of helminths and pentastomes recovered from 39 puff adders collected at various South African (SA) provinces as well as Mozambique and Zambia indicating the genera and number (n) of specimens recovered

Province / (Country)	**Puff adder ID**	**Genera (n)**
Eastern Cape Province (SA)	BA45	Ascaridoid (multiple immatures) *Ophidascaris* (11)
	PEM11385	*Hexametra* (12)
	PEM11387	No parasites found
	PEM11738	*Polydelphis* (1)
	PEM13672	Ascaridoid (2)
	PEM13720	*Polydelphis* (13)
	PEM13832	Plerocercoid (5)
	PEM17112	Ascaridoid (6)
	PEM17113	Ascaridoid (2)
	PEM17114	*Hexametra* (23)
	PEM17115	No parasites found
	PEM17117	*Hexametra* (16)
	PEM17118	*Hexametra* (10)
	PEM17120	*Hexametra* (12) Ascaridoid (1)
	PEM17121	*Hexametra* (6)
	PEM20266	Ascaridoid (2) *Hexametra* (6) *Polydelphis* (3)
	PEM2499	Plerocercoid (4)
	PEM8257	*Kalicephalus* (3)
	PEM912	Ascaridoid (1)
	PEM9831	*Hexametra* (1)

	PEM4066	No parasites found
Kwa-Zulu Natal Province (SA)	NMB10879	*Hexametra* (1)
	PEM5270	Plerocercoid (6)
	PEM6063	*Polydelphis* (1) Ascaridoid (1) *Hexametra* (7)
	TM44026	*Hexametra* (16)
	TM46810	Ascaridoid (1)
	TM47963	*Hexametra* (9) *Kalicephalus* (1) *Ophidascaris* (1)
	TM62743	Ascaridoid (5)
Limpopo Province (SA)	PEM9023	Ascaridoid (6)
	TM75177	*Hexametra* (2)
	NMB5994	Ascaridoid (4)
	PEM9879	*Ophidascaris* (2)
Location unknown	PEM65200	Ascaridoid (2)
Mozambique	PEM15501	Ascaridoid (1)
Mpumalanga Province (SA)	PEM9880	*Hexametra* (1) *Polydelphis* (1) *Ophidascaris* (3) Ascaridoid (5)
Northern Cape Province (SA)	NMB10853	Ascaridoid (6)
North-West Province (SA)	TM85331	*Raillietiella* (3)
Western Cape Province (SA)	PEM6552	*Ophidascaris* (2)
Zambia	PEM6218	Ascaridoid (3)

Chapter 5

DISCUSSION & CONCLUSIONS

The following nematode species have been reported from puff adders in Africa (*B. arietans*): *H. quadricornis, K. colubri colubri*, *K. viperae obliquus*, *O. intorta*, *O. amucronata*, *P. grayicola*, *P. anoura*, *R. fuscovenosa* (Hering-Hagenbeck & Boomker 2000). The helminths and pentastomes identified, all originate from the puff adders within a country or continent which the species have previously been identified from (Table 2.1 & Table 4.1). Cestodes reported from puff adders include, *Ophiotaenia marenzelleri* from two puff adders in South Africa (Fantham & Porter 1949) as well as *O. adiposa* and *O. amphiboluri* from a puff adder from Cameroon (McAllister et al. 1992).

Two-hundred and twenty-seven (227) helminths were identified as *Polydelphis* (19), *Ophidascaris* (19), *Hexametra* (122), *Kalicephalus* (4) and cestode (15) specimens. Three (3) pentastomes were recovered from a single puff adder and were identified as *R. boulengeri*. Measurements of ascarids and pentastomes closely correlated with those of previous studies.

The helminths and pentastomes were collected from puff adders which were preserved many years ago, dating back as far as 1959 and the most recent puff adders preserved in 2006. As a result of age and unknown preservation conditions of the puff adders, many of the helminths had extensive damage. Several helminth specimens were not intact or only parts of helminths were available for identification.

No conclusion on infection intensities could be drawn as it is not evident that the puff adders were systematically sampled, i.e. if all specimens were collected.

Most of the taxonomic literature on helminths applicable to the topic dates back to before 2000. More recent studies, performed after the 20th century, are available on pentastomes. Most notable are changes concerning the nomenclature, classification and systematics of the helminths recorded from puff adders and other serpents and reptiles in general. Many species described and recorded in the past are now known to be synonymous (Sprent 1978) such as

Polydelphis anoura being synonymous to *Polydelphis attenuatta*, *P. bicornuta*, *P. oculate* and *P. amucronata*. McAllister *et al.* (2010) also noted the previously described *Ascaris attenuata* as being synonymous with *Polydelphis anoura.*

Usually, only a few pentastome specimens from serpents are examined in studies pertaining to serpents and misidentifications of species are common due to different fixation methods, intraspecific variation and the state of the preserved specimens (Kelehear *et al.* 2011). The differences and identifications of pentastomes to genus level is usually clear, however, the taxonomic status of numerous species remains controversial (Kelehear *et al.* 2011).

Studies on helminths in snakes are generally scant, therefore providing only limited information to establish the true difference between interspecific morphological variation and intraspecific morphological variation (Sprent 1978). The genus *Ophidascaris* comprises many species with multiple varying features, indicating that it is possible that this genus may comprise fewer valid species than originally thought (Sprent 1978). Also, in other studies, *Hexametra* species were incorrectly identified as *Ophidascaris* species (Sprent 1978).

Similarly, Fantham & Porter (1949) reported *Polydelphis quadricornis,* now known as *Hexametra quadricornis,* from two puff adders and a gaboon viper (Hering-Hagenbeck & Boomker 2000). Fantham & Porter (1949) also reported *Ophidascaris intorta* from a puff adder. According to Sprent (1988), *Ophidascaris radiosa* is synonymous to *Ophidascaris intorta.* However, Hering-Hagenbeck & Boomker (2000) and Mozgovoi (1953) consider *Ophidascaris intorta* and *Ophidascaris radiosa* as different species.

Kalicephalus obliquus was reported from puff adders first by Fantham & Porter (1949). Apart from *K. obliquus,* Hering-Hagenbeck & Boomker (2000) also listed *K. colubri colubri* from puff adders. McAllister *et al*. (2010) suggested that *K. obliquus* is synonymous to *K. viperae.*

Conclusions

This study contributes to the literature available on the helminths and pentastomes of puff adders in South Africa, Mozambique and Zambia.

It can be concluded that there is a dearth of information on the helminth and pentastome composition in serpents in general. This is confounded by the fact that the taxonomic status of several known helminth species is controversial. Further studies should also be aimed at the pathogenetic effects of helminths in snakes in their natural environment as well as their infection cycles, as these are unknown fields. Examination of helminth and pentastome specimens by scanning electron microscopy should be considered in future studies since it allows the visualisation of more delicate morphological features. Molecular analyses will also provide clarification on the taxonomical status of species, since molecular studies have not been performed on the helminths and pentastomes of puff adders according to published research. If possible, samples should be collected by the researchers themselves from fresh or recently preserved snakes to ensure a better state of the specimens collected. Specimens should be preserved in appropriate media to enable various analyses such as molecular analyses. Larger collections of specimens and a better geographic spread will allow more insights into the prevalence and spread of these parasites.

Chapter 7

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APPENDIX 1 ANIMAL ETHICS APPROVAL

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Identification of helminths and pentastomes sampled from preserved puff adders *(Bitis arietans)* specimens from the reptile collections of South African museums**
PROJECT NUMBER	**V005-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Ms. L Strydom**

STUDENT NUMBER (where applicable)	**U_12054683**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SAMPLES	**Helminths**	**Pentastomes**
NUMBER OF ANIMALS	**To be reported**	**To be reported**
Approval period to use animals for research/testing purposes		**February 2017-February 2018**
SUPERVISOR	**Dr. EV Schwan**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 27 February 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

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