Institute of Parasitology, Biology Centre CAS
Folia Parasitologica 2016, 63: 040
doi: 10.14411/fp.2016.040

Folia Parasitologica
http://folia.paru.cas.cz

Research Article
OPEN ACCESS

Pentastome assemblages of the Nile crocodile, *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae), in the Kruger National Park, South Africa

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Abstract: Thirty-two specimens of the Nile crocodile, *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae), from the Kruger National Park, South Africa, and its vicinity were examined for pentastomid parasites during 1995 to 1999 and 2010 to 2011. Pentastomid parasites occurred throughout the year and were widespread in the study area with an overall prevalence of 97% and an overall mean abundance of 23.4 (0–81). Pentastome assemblages comprised six species in three sebekid genera: *Alofia nilotici* Riley et Huchzermeyer, 1995, *A. simpsoni* Riley, 1994, *Leiperia cincinnalis* Sambon, 1922, *Sebekia cesarisi* Giglioli in Sambon, 1922, *S. minor* (Wedl, 1861) and *S. okavangoensis* Riley et Huchzermeyer, 1995. The possible influence of host age, gender and geographic location (river system) on pentastome prevalence, abundance and species richness was investigated. Generally, neither host age, gender nor locality did affect infracommunities, likely because all hosts examined were adult or subadult and displayed comparable foraging behaviour, resulting in similar exposure pathways to fish intermediate hosts. Additionally, the longevity of pentastomids would contribute to accumulative infections as hosts mature. Structuring of pentastome assemblages was observed in as far as *S. minor* was the dominant species based on overall prevalence and abundance, followed by the equally common species *S. cesarisi* and *L. cincinnalis*. With an overall prevalence ranging from 34% to 41% and relatively low abundances, *A. nilotici*, *A. simpsoni* and *S. okavangoensis* form the rarer component of pentastome communities.

Keywords: *Alofia*, *Leiperia*, *Sebekia*, Pentastomida, abundance, prevalence, species richness, crocodilians

Crocodiles are considered by many a fascinating part of the African vertebrate fauna. Recent morphological and genetic studies have shown this continent to be home to three crocodylid genera, *Crocodylus* Laurenti, *Osteolaemus* Cope and *Mecistops* Gray. *Mecistops* is represented by a single species, *M. cataphractus* (Cuvier), while the traditionally two subspecies of *Osteolaemus* are now recognised as separate species, *O. tetraspis* Cope and *O. osborni* (Schmidt), with likely a third taxonomic entity present in this genus (Brochu 2007, Eaton et al. 2009).

Crocodylus niloticus Laurenti is the only species inhabiting southern Africa, with its southernmost viable natural populations found in formally protected areas in South Africa, namely the Kruger National Park (KNP), Lake St Lucia, Pongolapoort Dam and Ndumo Game Reserve (Ross 1989, Patterson 1991, Calverley and Downs 2015). While listed on the IUCN Red List of Threatened Species as of least concern (Crocodile Specialist Group 1996a), some of the populations of Nile crocodiles in South Africa have undergone severe setbacks in recent years (Calverley and Downs 2015).

Populations of *Mecistops* and *Osteolaemus* are largely restricted to the tropical forest zone of west and central Africa, where their range overlaps with that of *C. niloticus* (see Ross 1989). *Osteolaemus* is currently listed as vulnerable (Crocodile Specialist Group 1996b), but recognition of the former subspecies as two valid species would decrease the population size of each of these considerably, and *Mecistops* has reached the status of a critically endangered species (Shirley 2014). While reports of pentastomid parasites having a detrimental effect on adult crocodiles under natural conditions are scarce, pentastomids can

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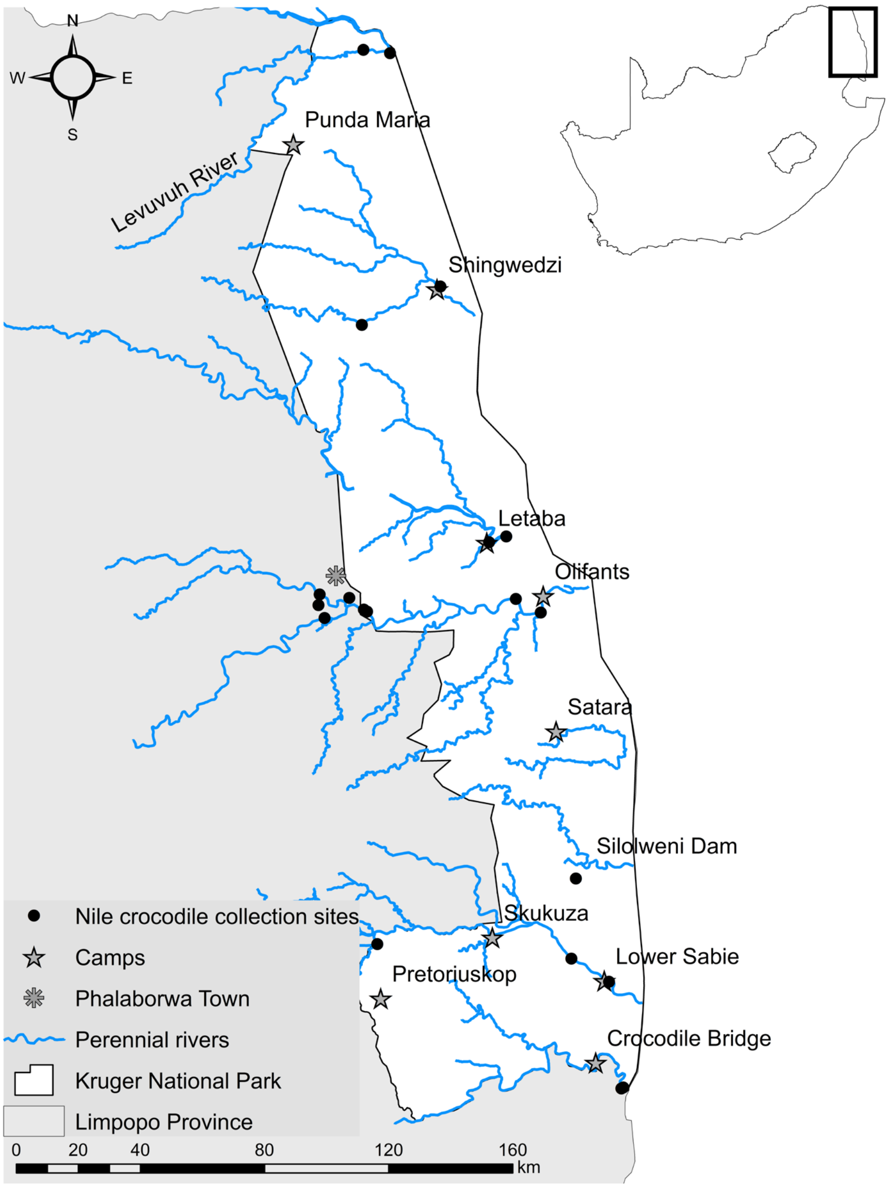

Fig. 1. Collection sites of Nile crocodiles in the Kruger National Park, South Africa and its vicinity. For reference of global positioning system points, see Table 1.

cause substantial necrosis and haemorrhaging in the lungs and liver of infected hosts and have the potential of causing mortalities when associated with secondary bacterial infections (Shotts et al. 1972, Hazen et al. 1978, Junker et al. 1999). Given the possible impact of these parasites on their hosts and considering that pentastomids have been reported from all three crocodilian genera in Africa, it is regrettable that data on the pentastome assemblages of African crocodiles are scant, usually limited to prevalence and intensity records from a few hosts, with a main focus on taxonomic aspects (Riley 1994, Riley and Huchzermeyer 1995a,b, Riley et al. 1997, Junker et al. 1999, 2000).

In the present paper we provide the first comprehensive study of the pentastome assemblages of a comparatively large sample of 32 Nile crocodiles from the KNP, South Africa, using prevalence, mean abundance and species richness as parasitological descriptors.

MATERIALS AND METHODS

During the period from 1995 to 1999 and from 2010 to 2011, 16 Nile crocodiles each were collected from different localities in the KNP, South Africa (Fig. 1, Table 1). These crocodiles (18 males, 14 females) were examined as part of unrelated studies on their blood chemistry and disease ecology parameters, providing the rare opportunity to collect information on their pentastomid parasites. The six rivers from which crocodiles were sampled represent the two major river systems that drain the KNP, the Limpopo System to the north of the 24°15' latitude and the Incomati System to the south of it (Pienaar 1968). From north to south, the Levuvhu (number of crocodiles = 2), Shingwedzi (n = 5), Letaba (n = 2) and Olifants (n = 10) Rivers contribute to the Limpopo System, whereas the Sabie (n = 4) and Crocodile (n = 2) Rivers form part of the Incomati System. Excepting the Shingwedzi, which is seasonal, but retains a number of permanent and semi-permanent pools along its length, these rivers

Table 1. Collection data of 32 Nile crocodiles collected in the Kruger National Park and vicinity, South Africa.

Locality	Date	Coordinates	Sex	TL (cm)	SVL (cm)	Weight (kg)	Gonads
Sabie River	22/02/1995	25°01'S; 31°15'E	M	320	-	-	-
Olifants River	20/07/1997	24°05'S; 31°45'E	M	350	-	-	-
Olifants River	23/09/1997	24°05'S; 31°45'E	F*	210	-	-	-
Olifants River	23/09/1997	24°05'S; 31°45'E	M*	260	-	-	-
Olifants River	24/09/1997	24°05'S; 31°45'E	M	350	-	-	-
Olifants River	25/09/1997	24°05'S; 31°45'E	F*	140	-	-	-
Olifants River [a]	25/09/1997	24°05'S; 31°45'E	M	420	-	-	-
Sabie River [a]	26/09/1997	24°09'S; 31°05'E	M*	240	-	-	-
Sabie River [a]	27/09/1997	24°09'S; 31°05'E	M	390	-	-	-
Shingwedzi River	10/06/1998	23°13'S; 30°12'E	F	280	-	-	-
Shingwedzi River	10/06/1998	23°13'S; 30°12'E	M*	240	-	-	-
Shingwedzi River	10/06/1998	23°13'S; 30°12'E	F	320	-	-	-
Shingwedzi River	10/06/1998	23°13'S; 30°12'E	F	270	-	-	-
Olifants River	16/03/1999	24°02'S; 31°40'E	M	380	-	-	-
PMC	06/04/1999	24°55'S; 31°10'E	M	390	-	-	-
PMC	15/06/1999	24°55'S; 31°10'E	F	260	-	-	-
Crocodile River	12/07/2010	25°27'S; 31°58'E	F	260	141	70	maturing 20 mm follicles
Crocodile River	12/07/2010	25°25'S; 31°58'E	F	292	165	102	maturing 20 mm follicles
Olifants River	13/07/2010	24°02'S; 31°13'E	M	373	202	226	mature, active testes
Olifants River	13/07/2010	24°03'S; 31°13'E	M	323	181	111	immature
Shingwedzi River	13/07/2010	23°07'S; 31°27'E	M	313	170	118	maturing, inactive testes
Levuvuh River	13/07/2010	22°43'S; 31°30'E	M	323	176	127	mature, active testes
Levuvuh River	13/07/2010	22°25'S; 31°13'E	M	385	203	268	mature, active testes
Silolweni Dam	15/07/2010	24°49'S; 31°50'E	F	304[b]	184	210	mature, active ovaries
Silolweni Dam	15/07/2010	24°49'S; 31°50'E	M*	282	147	85	immature
Letaba River	15/07/2010	23°50'S; 31°38'E	F	280	150	80	mature 40 mm follicles
Letaba River	15/07/2010	23°51'S; 31°35'E	F	280	150	83	mature 40 mm follicles
Sabie River	15/07/2010	25°07'S; 31°55'E	M	390[c]	232	338	mature testes
PMC	07/02/2011	24°02'S; 31°05'E	F	299	-	113	two 10 mm degenerating follicles
Olifants River	08/02/2011	24°04'S; 31°06'E	F	330	-	160	mature, inactive ovaries
PMC	08/02/2011	24°00'S; 31°06'E	F*	190	-	-	immature
PMC	09/02/2011	24°01'S; 31°11'E	M	346	-	170	mature, inactive testes

[a] pentastomids collected from right lung only, counts doubled for comparison; [b] part of the tail missing, estimated length: 340 cm; [c] part of the tail missing, estimated length: 460 cm; Fulton's k – body condition factor (see Zweig 2003); F – adult female; M – adult male; PMC– sedimentation dams at the Phalaborwa Mining Complex; SVL – snout-vent length; TL – total body length; * subadult.

are perennial (Pienaar 1968). In addition, an impounded internal stream (Silolweni Dam; n = 2) and several permanent sedimentation dams of the Phalaborwa Mining Complex (PMC; n = 5) just outside the KNP, with crocodiles therein having migrated from either the Olifants or Selati Rivers, were sampled. A single additional crocodile collected from the Letaba River in 1995 harboured an unusually large number (n = 239) of pentastomids in its heart, trachea, bronchi and lungs and was therefore excluded from this study. Its pentastome assemblage and associated pathological lesions were described by Junker et al. (1999).

The first group of crocodiles were shot and immediately processed in the field, whereas the second group was shot in the field and subsequently transported to the *post mortem* facilities at Skukuza, the Park's scientific headquarters. All crocodiles were examined within 12 h of death, as some had to be stored overnight. Night temperatures did not exceed 5 °C and no significant *post mortem* migration of pentastomids was observed in the respiratory tracts or hearts of these animals. Total length (TL, from the tip of the snout to the tip of the tail), sex, overall body condition and, in the case of the second group, gonad development, weight and snout-vent length (SVL; n = 12; 141–232 cm) were determined. The overall mean TL was 303.2 cm (140–420 cm), and the overall mean body weight was 151 kg (70–338 kg).

Based on the information obtained from the 12 crocodiles for which information on TL, SVL and gonad development was available, crocodiles were classified as subadults or adults (Table 1). Crocodiles with maturing gonads were included in the group of adults for the purpose of this study. As such, the shortest female that had reached sexual maturity, as evidenced by maturing follicles of 20 mm diameter, had a TL of 260 cm (SVL = 141 cm). We therefore classified females with a TL of < 260 cm as subadult and those with a TL of ≥ 260 cm as adult females. A male of 323 cm TL (SVL = 181 cm) was found to be immature. However, another crocodile of the same TL and a shorter SVL of 176 cm possessed mature testes and a male of slightly smaller size (TL = 313; SVL = 170) possessed maturing testes. We therefore decided to take the TL of the next smaller male with immature gonads as cut-off point for sexual maturity. Thus, males of ≤ 282 cm TL were classified as subadults, and males with a TL of > 282 cm as adults.

This classification conforms with the size ranges for mature crocodiles established by Kofron (1990) for Nile crocodiles in Zimbabwe, where females attained sexual maturity after having reached a length of 262–287 cm, and males of 270–295 cm. Calverley and Downs (2015) categorised Nile crocodiles in Ndumo Game Reserve, South Africa, as subadults (150–250 cm) and adults (> 250 cm). When applying their classification to the pres-

Table 2. Prevalence and mean abundance (MA) of pentastomid parasites collected from 32 Nile crocodiles in the Kruger National Park and vicinity, South Africa.

Species[a]	Infected hosts (N)	Prevalence (%)	Total count	MA	MA range
Leiperia cincinnalis Sambon, 1922	18	56	170	5.3	0–39
Leiperia cincinnalis IL	11	34	95	3.0	0–33
Alofia nilotici Riley et Huchzermeyer, 1995	13	41	37	1.2	0–12
Alofia simpsoni Riley, 1994	11	34	42	1.3	0–11
Sebekia cesarisi Giglioli in Sambon, 1922	23	72	111	3.5	0–22
Sebekia minor (Wedl, 1861)	22	69	360	11.3	0–54
Sebekia okavangoensis Riley et Huchzermeyer, 1995	11	34	29	0.9	0–9
Sebekidae gen. sp. adults[b]	10	31	17	0.5	0–6
Sebekidae gen. sp. IL[b]	12	38	66	2.1	0–16
Pentastomida total[c]	31	97	749	23.4	0–81

[a] adult stages unless otherwise stated; [b] excepting *Leiperia cincinnalis*; [c] based on adult specimens identified to species level; IL – infective larvae.

ent data set, two males currently classified as subadults would be considered adult. Since one of these males was clearly identified as immature based on gonad development, we have retained the age classes as set out earlier.

The nasopharynx, trachea, lungs, heart and aorta of each crocodile were examined for parasites as described by Junker et al. (2000). For one crocodile from the Olifants River and two crocodiles from the Sabie River, only the right lungs were available for parasite collection; counts were doubled for comparison. Pentastomes were fixed in hot 70% ethanol, stored in cold 70% ethanol and subsequently mounted and cleared in Hoyer's medium for identification under a compound microscope. Voucher material of larval and adult pentastomes was deposited in the National Collection of Animal Helminths, Agricultural Research Council-Onderstepoort Veterinary Institute, Onderstepoort, South Africa (accession numbers S/2015/15-20). For the purpose of this study, we did not distinguish between the infective larvae of the genera *Alofia* Giglioli in Sambon, 1922 and *Sebekia* Sambon, 1922, but recorded them as sebekid infective larvae. They were easily differentiated from the infective larvae of *Leiperia* Sambon, 1922.

The terms prevalence, (mean-) abundance and species richness are used as per the definitions of Bush et al. (1997). The classification of pentastomid parasites follows Poore (2012).

Statistical procedures

For data analysis, crocodiles were assigned to different abiotic and biotic population subgroups: season (spring: September–November, summer: December–February, autumn: March–May and winter: June–August); river system (Incomati System and Limpopo System); sex (male and female) and age (subadult and adult). Because of the low number of crocodiles obtained from them, the PMC and Silolweni Dam were not included in statistical analyses pertaining to differences between systems. Similarly, only descriptive statistics are presented with regards to crocodiles collected during the various seasons. Species richness and overall/total counts were determined based on adult pentastomids identified to species level.

We tested for the effect of crocodile sex, age and river system in which a crocodile was collected on the number of (a) adult pentastomids identified to species level, (b) infective larvae of *Leiperia cincinnalis* Sambon, 1922, (c) infective larvae of the Sebekidae, excluding those of *L. cincinnalis*, i.e. *Alofia* spp. plus *Sebekia* spp., and (d) number of pentastomid species (based on adult pentastomids only and excluding unidentified sebekids) harboured by an individual crocodile. Tests were performed using linear mixed-effects models with a river from which a crocodile was collected as a random factor (to control for multiple crocodile collections from the same locality) using package 'nlme'(Pinheiro et al. 2016) implemented in the R 3.3.0 statistical environment (R Core Team 2016). The frequency distribution of dependent variables did not significantly deviate from normality after log-transformation (Kolmogorov-Smirnov tests, d = 0.14–0.25, $p > 0.20$ for all).

In addition, we tested whether the sex ratio of each pentastome species within an individual crocodile differed significantly from the null expectation of unity using χ^2 tests.

Test results were considered statistically significant if they differed at the 5% level, i.e. $p < 0.05$.

RESULTS

Excepting a single subadult female from the Olifants River, all crocodiles harboured pentastomid parasites (total prevalence 97%), with an overall mean abundance of 23.4 pentastomids per individual (0–81) (Table 2). A total of 749 adult pentastomes were collected and identified to species level, representing six species comprising three genera of the family Sebekidae: *A. nilotici* Riley et Huchzermeyer, 1995, *Alofia simpsoni* Riley, 1994, *Leiperia cincinnalis*, *Sebekia cesarisi* Giglioli in Sambon, 1922, *S. minor* (Wedl, 1861) and *S. okavangoensis* Riley et Huchzermeyer, 1995. Overall mean species richness was 3.1 (0–6) and infections with more than one pentastome species were common. In fact, 65% (20/31) of the infected hosts harboured three or more species. Single infections were found in 16% (5/31) of the infected crocodiles, whereas 7% (2/31) carried the full species complement.

Although the nasopharyngeal region of each crocodile was examined, members of the Subtriquetridae were not found. Representatives of *Alofia* and *Sebekia* were collected from the bronchioles and lung parenchyma, whereas mature adult *L. cincinnalis* were found in the trachea and bronchi, with infective larvae and immature adult males and females being located in the heart and pulmonary artery.

We did not find any significant effect of either explanatory variable, be it biotic (host sex and age) or abiotic (river system), on either dependent variable (Table 3). In general,

Table 3. Summary of linear mixed-effects models of the effect of a crocodile sex, age and river system in which it was collected on the number of adult pentastomid individuals (AP) and species (SR), infective larvae of *Leiperia cincinnalis* (ILLC) and infective larvae of *Alofia*/*Sebekia* (ILAS). Reference levels for independent variables were female for sex, adult for age and Limpopo for river system.

Dependent variable	Explanatory variable	Coefficient estimate ± SE	*t*	*p*
AP	Sex	-0.10 ± 0.22	-0.46	0.65
	Age	-0.12 ± 0.29	-0.42	0.68
	River system	-0.14 ± 0.25	-0.55	0.59
ILLC	Sex	-0.53 ± 0.26	-1.98	0.18
	Age	0.54 ± 0.36	1.49	0.27
	River system	0.04 ± 0.40	0.10	0.92
ILAS	Sex	0.29 ± 0.33	1.63	0.24
	Age	0.34 ± 0.47	0.71	0.55
	River system	-0.02 ± 0.35	-0.07	0.95
SR	Sex	-0.03 ± 0.07	-0.40	0.69
	Age	-0.10 ± 0.09	-1.12	0.28
	River system	-0.06 ± 0.09	-0.62	0.56

crocodiles harboured similar numbers of adult pentastomids, infective larvae of *L. cincinnalis* and infective larvae of *Alofia*/*Sebekia* as well as pentastomid species independently of sex, age or a river system which they inhabited.

Similarly, irrespective of the biotic or abiotic factors considered, prevalence and mean abundance of overall pentastomid infection did not vary substantially within subgroups (Table 4).

On pentastomid species level, overall prevalence ranged from 34% to 72% (Table 2), and was highest in *S. cesarisi*, followed by *S. minor* and *L. cincinnalis*. Led by *S. minor*, these were also the three species with the highest total count and mean abundance. Infective larvae of *L. cincinnalis* and those of the remaining Sebekidae did have a similar overall prevalence (34% and 38%, respectively) and mean abundance (3.0 and 2.1, respectively).

Table 5 summarises the prevalence and mean abundance of adults of the six pentastome species as well as the two groups of infective larvae within the various population subgroups. A similar trend as for the overall adult pentastome population can be observed for adults of a given species which appear largely unaffected by biotic or abiotic factors.

No seasonal tendencies could be observed with regard to prevalence, abundance or species richness in overall pentastomid infections, nor with regard to prevalence and abundance in adults of individual pentastome species or the two groups of infective larvae.

Lastly we had a look at the distribution of male and female pentastomes within a given species. At a first glance all species except one demonstrated female bias in the sex ratio (Fig. 2), but statistically this was confirmed for *L. cincinnalis* ($\chi^2 = 29.4$, $p < 0.03$) and *S. minor* ($\chi^2 = 52.3$, $p < 0.001$) only, whereas sex ratio in infrapopulations of the remaining species did not differ from unity ($\chi^2 = 5.9–11.1$, $p > 0.52$ for all).

Table 4. Prevalence, species richness and means of overall pentastomid infection in Nile crocodiles in the Kruger National Park and its vicinity, South Africa.

Population	Subgroup	N	Prev (%)	MA (range)	MSR (range)
Sex	Males	18/18	100	26.0 (1–81)	3.2 (1–6)
	Females	13/14	93	20.1 (0–59)	2.9 (2–5)
Age	Adults	25/25	100	25.5 (1–73)	3.3 (1–6)
	Subadults	6/7	86	15.9 (0–81)	2.5 (1–5)
System	Incomati	6/6	100	28.0 (8–81)	3.8 (2–5)
	Limpopo	18/19	95	25.0 (0–73)	3.2 (1–6)
Season	Spring	6/7	86	24.9 (0–81)	2.7 (1–5)
	Summer	5/5	100	9.8 (1–16)	2.8 (1–5)
	Autumn	2/2	100	53.0 (43–63)	3.5 (3–4)
	Winter	18/18	100	23.3 (1–73)	3.2 (1–6)

N – number of infected hosts *vs* number of hosts examined; Prev – prevalence; MA – mean abundance; MSR – mean species richness.

DISCUSSION

In the present study we investigated the influence of biotic (host age and sex) as well as abiotic factors (river system) on the pentastome assemblages of Nile crocodiles. Pentastomids were found to be widespread parasites in Nile crocodiles in the KNP and its vicinity, with a high overall prevalence and mean abundance recorded from both river systems draining the park (Table 4). These results suggest a widespread and even distribution of pentastomes in the primary source of infection to the crocodiles, i.e. fish intermediate hosts, throughout the study area. Information on which of the numerous fish species inhabiting the waters of the KNP are susceptible to pentastomid infection is scant (see below). However, two of the known intermediate hosts of pentastomids parasitising Nile crocodiles, Mozambique tilapia, *Oreochromis mossambicus* Peters, and redbreast tilapia, *Tilapia rendalli* Boulanger (Cichlidae) (Junker et al. 1998), are widely distributed within the KNP and have been recorded from all its perennial rivers, the Levuvhu, Letaba, Olifants, Sabie and Crocodile Rivers, as well as from two seasonal rivers, the Shingwedzi and Nwanedzi Rivers (Roux et al. 2008).

Infections persisted throughout the year and typically (65% of infected hosts) comprised a minimum of three of the six species present in *Crocodylus niloticus*. Both *Sebekia cesarisi* and *S. minor*, and to a lesser extent *Leiperia cincinnalis*, were distinctly more prevalent than *Alofia nilotici*, *A. simpsoni* and *S. okavangoensis*, the latter two of which shared the lowest prevalence (Table 2). Similarly, mean abundance was considerably higher in *S. minor* than in all other species, followed by that of *L. cincinnalis* and *S. cesarisi*. Hence, based on prevalence and abundance, *S. minor* clearly emerges as the dominant species in the examined pentastome assemblages, followed by *L. cincinnalis* and *S. cesarisi*. *Sebekia okavangoensis* and both *Alofia* spp. were characterised by an intermediate prevalence of more than 10% but less than 50% and an overall mean abundance of 1.3 and less, and formed the rarer component of pentastome communities. The observed differences in prevalence and abundance between the various pentastome

Table 5. Infection parameters of pentastomid species collected from Nile crocodiles in the Kruger National Park, South Africa.

Species		*Leiperia cincinnalis* IL			*Leiperia cincinnalis*			*Alofia nilotici*			*Alofia simpsoni*		
Population	Subgroup	N	Prev (%)	MA (range)	N	Prev (%)	MA (range)	N	Prev (%)	MA (range)	N	Prev (%)	MA (range)
Sex	Males	5/18	28	1.7 (0–12)	11/18	61	2.1 (0–11)	9/18	50	1.5 (0–12)	6/18	33	1.1 (0–5)
	Females	6/14	43	4.6 (0–33)	7/14	50	9.4 (0–39)	4/14	29	0.7 (0–5)	5/14	36	1.6 (0–11)
Age	Adults	9/25	36	3.2 (0–33)	14/25	56	6.0 (0–39)	11/25	44	1.4 (0–12)	11/25	44	1.7 (0–11)
	Subadults	2/7	29	2.0 (0–12)	4/7	57	2.9 (0–11)	2/7	29	0.4 (0–2)	0/7	0	0.0
System	Incomati	4/6	67	4.7 (0–12)	6/6	100	6.0 (2–11)	4/6	67	1.5 (0–5)	1/6	17	0.8 (5)
	Limpopo	6/19	32	3.4 (0–33)	10/19	53	6.7 (0–39)	7/19	37	1.3 (0–12)	9/19	47	1.4 (0–4)
Season	Spring	2/7	29	3.4 (0–12)	3/7	43	2.9 (0–11)	3/7	43	0.7 (0–2)	0/7	0	0.0
	Summer	2/5	40	1.0 (0–3)	2/5	40	1.4 (0–4)	1/5	20	0.2 (0–1)	1/5	20	0.4 (2)
	Autumn	0/2	0	0.0	1/2	50	0.5 (0–1)	2/2	100	1.5 (1–2)	0/2	0	0.0
	Winter	7/18	39	3.7 (0–33)	12/18	67	7.9 (0–39)	7/18	39	1.6 (0–12)	10/18	56	2.2 (0–11)
Species		*Sebekia cesarisi*			*Sebekia minor*			*Sebekia okavangoensis*			Sebekidae gen. sp. IL*		
Population	Subgroup	N	Prev (%)	MA (range)	N	Prev (%)	MA (range)	N	Prev (%)	MA (range)	N	Prev (%)	MA (range)
Sex	Males	13/18	72	4.2 (0–22)	12/18	67	15.9 (0–54)	6/18	33	1.2 (0–9)	6/18	33	2.8 (0–16)
	Females	10/14	71	2.5 (0–8)	10/14	71	5.3 (0–25)	5/14	36	0.6 (0–4)	6/14	43	1.1 (0–10)
Age	Adults	19/25	76	3.2 (0–12)	18/25	72	12.2 (0–54)	10/25	40	1.1 (0–9)	10/25	40	2.0 (0–11)
	Subadults	4/7	57	4.4 (0–22)	4/7	57	7.9 (0–44)	1/7	14	0.3 (2)	2/7	29	2.4 (0–16)
System	Incomati	5/6	83	5.3 (0–22)	4/6	67	12.3 (0–44)	3/6	50	2.0 (0–9)	3/6	50	4.7 (0–16)
	Limpopo	13/19	68	3.4 (0–12)	14/19	74	11.5 (0–52)	7/19	37	0.7 (0–4)	5/19	26	1.3 (0–10)
Season	Spring	5/7	71	6.1 (0–22)	6/7	86	14.3 (0–44)	2/7	29	0.9 (0–4)	3/7	43	3.0 (0–16)
	Summer	4/5	80	2.2 (0–7)	4/5	80	3.0 (0–7)	2/5	40	2.6 (0–9)	3/5	60	2.6 (0–11)
	Autumn	2/2	100	7.0 (7)	2/2	100	44.0 34–54)	0/2	0	0.0	1/2	50	5.0 (10)
	Winter	12/18	67	2.4 (0–11)	10/18	56	8.7 (0–52)	7/18	39	0.6 (0–3)	5/18	28	1.2 (0–10)

IL – infective larvae; MA – mean abundance; N – number of infected hosts versus number of hosts examined; Prev – prevalence; * excluding *Leiperia cincinnalis*.

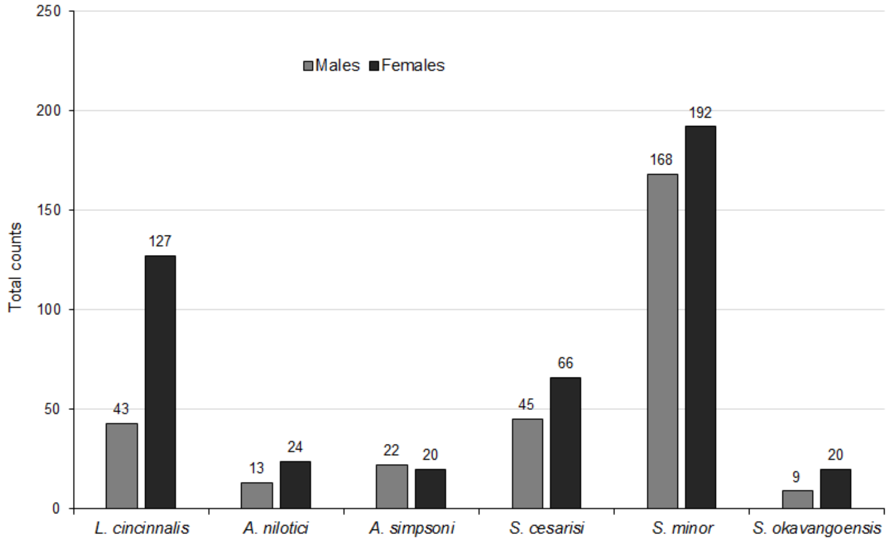

Fig. 2. Comparison of total counts of males and females within species of the pentastomid genera *Leiperia* Sambon, 1922, *Alofia* Giglioli in Sambon, 1922 and *Sebekia* Sambon, 1922 in Nile crocodiles in the Kruger National Park and vicinity, South Africa.

species might be related to variations in the life histories of these trophically transmitted parasites, e.g. their ability to utilise various fish species as intermediate hosts, and, in turn, the importance of these fish in the crocodiles' diet as preferential or occasional food items.

The intermediate host preference of sebekids has been found to vary. Junker (2002) recorded a higher prevalence of *L. cincinnalis* in *O. mossambicus* when compared to *T. rendalli* (maximum of 61% and 20%, respectively, in fish ≥ 250 mm) in the KNP. Conversely, sebekids other than *L. cincinnalis* were more prevalent in *T. rendalli* than in *O. mossambicus* (maximum of 60% and 2%, respectively, in fish ≥ 250 mm). In addition, all sebekids showed a tendency of increasing prevalence and intensity in the larger hosts of a given fish species (Junker 2002). Information on intermediate hosts used by each of the pentastome species is scant. *Sebekia minor* (syn. *S. wedli* Giglioli in Sambon, 1922) had a prevalence of 40.5% in *T. rendalli*, but only of 2.5% in *O. mossambicus* in the KNP (Junker et al. 1998). An additional host reported for this pentastome in South

Africa is the bulldog, *Marcusenius macrolepidotus* Peters (Mormyridae) (see Luus-Powell et al. 2008). Further intermediate host records for Sebekidae in South Africa are: *L. cincinnalis* from Lowveld largemouth, *Serranochromis meridianus* Jubb (Cichlidae), in the Incomati System; *S. okavangoensis* from sharptooth catfish, *Clarias gariepinus* Burchell (Clariidae), in the Incomati System; and *Alofia* sp. from *O. mossambicus* in the Olifants System (Junker 2002, Luus-Powell et al. 2008). Given the large diversity of fish in aquatic systems in South Africa, little is known about their capacity as hosts for pentastomid parasites.

Host age did not have a significant effect on the total number of either larval or adult pentastomes infecting crocodiles (Table 3), with a possible exception on species level, as *A. simpsoni* was not found in any of the seven subadults examined (Table 5). This is contrary to studies in the American alligator, *Alligator mississippiensis* (Daudin) (Alligatoridae), where prevalence and intensity of infection with *S. mississippiensis* Overstreet, Self et Vliet, 1985 was found to increase with size and its related age classes (Moreland et al. 1989, Tellez et al. 2014). Tellez et al. (2014) attributed their observations to a shift in the dietary preferences of alligators from a predominance of invertebrate prey in juveniles to mainly fish in subadults and adults, as well as to the ability of larger/older animals to capture larger intermediate fish or paratenic hosts, which can harbour heavier parasite burdens than smaller hosts.

A similar ontogenic diet shift has been demonstrated in *C. niloticus* (see Hutton 1987, Wallace and Leslie 2008). In Nile crocodiles from the Okavango Delta, Botswana, invertebrates made up 46% of the diet of yearlings (17.0–38.9 cm SVL) with fish only contributing 12%, whereas fish constituted 68% of the diet of subadults (66.4–115.8 cm SVL) (Wallace and Leslie 2008). In Nile crocodiles in Zimbabwe, the diet of juveniles changed from mainly insects to fish and birds at a size of 60 cm SVL (Hutton 1987). In the present study, the smallest crocodile, a subadult female, had reached a TL of 140 cm, suggesting that adult as well as subadult animals had reached a size where fish form an integral part of their diet.

The lack of an effect of host age on pentastome prevalence or burden in the current study likely reflects the homogeneity of the age/size composition of the hosts examined, the resulting similarities in their diet and, consequently, exposure to infective stages of pentastomid parasites. In addition, pentastomids are long-lived arthropods, with patency reported to last as long as six years in a member of the Porocephalida (Riley 1981). Hence, infracommunities present in the crocodiles at any given moment are the accumulative result of subsequent infections over an extended period of time and not necessarily of a single infection. The observed absence of *A. simpsoni* in the seven subadult crocodiles possibly reflects the low colonisation ability of this species when compared to the more common species in the pentastome assemblages of Nile crocodiles.

Similarly, host sex had no significant effect on either larval or adult overall pentastome counts (Table 3), but it might be noteworthy that female crocodiles carried more than four times higher burdens of *L. cincinnalis* than males. Differences in habitat preference or prey selection between the two sexes might lead to variance in the exposure of male and female crocodiles to different intermediate hosts and/or varying numbers thereof, especially if the intermediate hosts themselves have spatially heterogeneous distributions. In American alligators, males and females were found to utilise different niches within the same habitat with a resulting difference in prey selection (Tellez et al. 2014). Although *O. mossambicus* (syn. *T. mossambica* Peters) and *T. rendalli* (syn. *T. melanopleura swierstrae* Gilchrist et Thompson), for example, have largely similar habitat preferences and both are common prey of *C. niloticus* (see Branch 1994), *T. rendalli* was recorded more frequently from perennial rivers themselves, whereas *O. mossambicus* was found to be more common in pools of seasonal rivers and dams (Pienaar 1968).

However, little is known about foraging strategies, territorial behaviour or habitat utilisation in Nile crocodiles in the KNP and much of our current knowledge on the distribution and movements of these ancient reptiles is based on studies undertaken on populations of Nile crocodiles inhabiting large lakes elsewhere in (South-) Africa (Modah 1967, Hutton 1989, Calverley and Downs 2015). Information on home range size differs somewhat with the geographic origin of crocodile populations. Home range increased with size in both male and female Nile crocodiles in the Ndumo Game Reserve, South Africa, with adult crocodiles (> 2.5 m TL) occupying larger home ranges than subadults as well as using these more expansively (Calverley and Downs 2015). Contrary to this, in Kenya and Zimbabwe, large subadult females were found to travel widely throughout the available habitat without distinct home ranges, large breeding females occupied small home ranges in the vicinity of prime nest sites and large males usually had distinct home ranges (Modah 1967, Hutton 1989). If male and female Nile crocodiles in the KNP express similar behavioural differences, this might expose them to a different segment of the pentastome population. However, in this context, it is also interesting to remember that overall pentastome counts, larval or adult, were not influenced by locality, i.e. river system.

Seasonal differences have been reported in the feeding behaviour of Nile crocodiles suggesting that in subadult and adult crocodiles feeding activity is reduced during the lower temperatures prevailing in winter (Kofron 1989, Wallace and Leslie 2008). It would have been interesting to see if such altered feeding behaviour and implied changes in the exposure to infected intermediate hosts would influence pentastome abundance or whether such effects would be counterbalanced by the accumulative nature of pentastome infections. Unfortunatley, too few crocodiles were collected during the various seasons to allow meaningful interpretation of the data.

Female bias in sex ratios of polygamous endoparasites is not a scarce phenomenon (Poulin 2007), and members of the Sebekidae in the present study pose no exception. As with many of the nematodes, Riley (1972) found the sex ratio in mature infections of *Reighardia sternae* (Diesing, 1863) (Reighardiidae) to shift in favour of females, and females of the pentastomid genera *Kiricephalus* Sambon, 1922 (Porocephalidae) and *Waddycephalus* Sambon, 1922 (Sambonidae) survived longer after infection than their male counterparts (Riley and Self 1980, 1981). Poulin (1997) considered differential mortality, with higher longevity seen in females, a key factor of varying sex ratios in helminth infections.

With currently six species of pentastomid parasites recorded from it, *C. niloticus* harbours one of the most species-rich pentastomid communities among crocodilians. In fact, its pentastome diversity is only exceeded by that of the saltwater crocodile, *Crocodylus porosus* Schneider (Crocodylidae), from which ten nominal pentastomid species have been recorded to date (Junker and Boomker 2006, Poore 2012, Christofferson and De Assis 2013).

Interestingly, despite a considerable overlap in the distribution range of the three African crocodylid genera (Uetz and Hošek 2015), *S. okavangoensis*, one of the less common species in Nile crocodiles, is so far the only pentastome species shared by all. In addition, *L. cincinnalis* was collected from both *C. niloticus* and *Mecistops cataphractus* (see Fain 1961, Junker and Boomker 2006). *Mecistops cataphractus* and *Osteolaemus osborni* [syn. *O. tetraspis* and *O. tetraspis osborni* (Wermuth, 1953)] on the other hand, both harbour *A. parva* Riley et Huchzermeyer, 1995 and *Agema silvaepalustris* Riley, Hill et Huchzermeyer, 1997 (see Riley and Huchzermeyer 1995b, Riley et al. 1997, Riley and Huchzermeyer 2000), species not hitherto recorded from the Nile crocodile. It is noteworthy that Riley and Huchzermeyer (1995b) as well as Riley et al. (1997) refer to *O. tetraspis* obtained from Ouenze and Impfondo markets in the Republic of the Congo; in a later publication, Riley and Huchzermeyer (2000), animals from the same locality are assigned to the subspecies *O. tetraspis osborni*.

Members of a second family of pentastomids, the Subtriquetridae, belong to a single genus and are known to parasitise the nasopharyngeal region of crocodilian hosts (Riley 1986). To date, no adult subtriquetrids have been found in any of the African crocodiles, despite the presence of infective larvae of *Subtriquetra rileyi* Junker, Boomker et Booyse, 1998 in cichlids in the KNP (Junker et al. 1998). The question arises if other aquatic organisms, such as terrapins, might serve as definitive hosts for this pentastomid.

As the majority of pentastomid records from African crocodiles are based on incidental findings from single or few individuals, with only *O. osborni* in central Africa (Republic of the Congo) (Riley and Huchzermeyer 1995b, Riley et al. 1997) and *C. niloticus* in southern Africa (South Africa; the present study) having been studied in larger numbers, the apparent differences in their pentastome assemblages might as yet reflect a lack of data. Reports of unidentified pentastomid specimens, preliminarily assigned to *Sebekia* sp., from dwarf crocodiles in the Republic of the Congo as well as in Nigeria (Riley and Huchzermeyer 1995b, Enabulele et al. 2013), suggest that the extent of crocodilian pentastome diversity in Africa is not fully explored yet.

Acknowledgements. The authors wish to acknowledge the late Dr Fritz Huchzermeyer, South Africa, for his contribution to this crocodile survey and for bringing the parasitologists aboard. Over the years, his contribution to our knowledge on crocodiles, their diseases and parasites has been invaluable. The South African National Parks Board and the Management of the Phalaborwa Mining Complex are thanked for making the crocodiles available. Ms Chantel de Beer, ARC-Onderstepoort Veterinary Institute, kindly compiled the map indicating the collection sites. This is publication no. 914 of the Mitrani Department of Desert Ecology.

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Received 5 August 2016 Accepted 10 October 2016 Published online 15 November 2016

Cite this article as: Junker K., Calitz F., Govender D., Krasnov B.R., Boomker J. 2016: Pentastome assemblages of the Nile crocodile, *Crocodylus niloticus* Laurenti (Reptilia: Crocodylidae), in the Kruger National Park, South Africa. Folia Parasitol. 63: 040.