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Pentastomid parasites of the family Sebekidae Fain, 1961 in West African dwarf crocodiles *Osteolaemus tetraspis* Cope, 1851 from the Congo, with a description of *Alofia parva* n. sp.

J. RILEY[1] and F. W. HUCHZERMEYER[2]

ABSTRACT

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The lungs and viscera of 23 dwarf crocodiles (*Osteolaemus tetraspis*) obtained from markets in Brazzaville, were examined for pentastomid infection. Twenty-one animals were infected and harboured a total of 82 pentastomids, all belonging to the family Sebekidae and representing at least two genera and three species. *Sebekia okavangoensis* Riley & Huchzermeyer, was present in the body cavity and lungs; the other two species were restricted to the lungs. *Alofia parva* n. sp. (14 ♀♀; 10 ♂♂) was unusual in that the fulcrum supporting the anterior hooks carried a cowl-like extension. The remaining species, provisionally allocated to the genus *Sebekia*, could not be classified to the generic level with certainty.

Keywords: Pentastomid parasites, Sebekidae, *Sebekia okavangoensis*, *Alofia parva*, dwarf crocodiles, *Osteolaemus tetraspis*

INTRODUCTION

Three species of crocodilians are found in Africa and only one of these, the Nile crocodile, is really widespread (Neill 1971). The habitat of the West African dwarf crocodile, *Osteolaemus tetraspis* Cope, 1861, is largely, if not exclusively, rainforest and its range overlaps with that of the Nile and African sharp-nosed crocodiles (Neill 1971).

Five genera of pentastomids, four belonging to the family Sebekidae Fain, 1961 and one (*Subtriquetra* Sambon, 1922) to the family Subtriquetridae Fain, 1961, are known to infect the respiratory tract of crocodilians. The literature suggests that at least half of the 21 extant species of these hosts harbour one or more species of pentastomids (Riley 1994). In Africa, five species infect the Nile crocodile, one from each of the genera *Alofia* Giglioli, 1922 and *Leiperia* Sambon, 1922 and the remainder from the genus *Sebekia* Sambon, 1922 (Sambon 1910; 1922; Rodhain & Vuylsteke 1932; Fain 1961; Riley, Spratt & Winch 1990; Riley 1994; Riley & Huchzermeyer 1995). Nothing is known of the pentastomid fauna of the other two African crocodile species.

In this paper we describe pentastomid material collected from the lungs of 23 dwarf crocodiles slaughtered at markets in Brazzaville, Congo.

[1] Department of Biological Sciences, The University, Dundee DD1 4HN, Scotland

[2] Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

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MATERIALS AND METHODS

The lungs and viscera from African dwarf crocodiles, *Osteolaemus tetraspis* Cope, 1861, slaughtered at Mikalou and Ouenzé markets in Brazzaville, Republic

of the Congo, were purchased and examined for parasites and pathology, in cooperation with M. Agnagna of Wildlife Conservation Society (WCS) in Brazzaville, during a 2-week visit by one of us (F.W.H.). During this period, which spanned 10–24 May, 1993, viscera from 23 crocodiles were examined and 21 harboured pentastomids in the lungs: two also had adults of *Sebekia okavangoensis* Riley & Huchzermeyer (1995) in the body cavity (Table 1). Animals were numbered 1–23 in chronological order of receipt and numbers 17 and 22 were without pentastomids (Table 1).

Examination of isolated lungs, which averaged 15 cm in length, entailed making three deep longitudinal incisions with a razor blade into either the dorsal or ventral surface. They were then left for 2–5 min and any pentastomids which had migrated into the now gaping incisions, were collected. Five to ten transverse incisions were then made and the exercise repeated. Occasionally specimens were damaged by cutting, but nonetheless the method appeared to be effective in recovering intact pentastomids. Immediately after collection, pentastomids were preserved in 70% alcohol for later detailed examination.

Hooks were dissected from most of the large specimens for slide-mounting and the abdomens were preserved separately in alcohol. All other specimens were slide-mounted in Hoyer's Medium, either whole or decapitated, and the head was mounted under a second coverslip on the same slide as the abdomen. This permitted the head to be orientated so that the mouth and hooks were uppermost and subsequent gentle pressure on the coverslip caused these relatively rigid features to be flattened so that they could be measured. Mouths were observed, measured and drawn from specimens mounted ventral surface uppermost (Fig. 1). Hooks, buccal cadres and copulatory spicules were measured according to the protocols outlined in Riley *et al.* (1990) and in Fig. 1 and 2. The length and width of mature eggs (i.e. containing fully mature, hooked, primary larvae and originating from the terminal part of the uterus) were measured.

All slides were individually labelled and each bears the number of the animal from which it came (1–23) and a letter (a–n), indicating the chronological order of mounting of the specimen from individual batches of specimens (Table 1). All material is deposited in the Natural History Museum under the single accession number BMNH (E) 1995-50.

RESULTS

The prevalence of infection was 91%, but the mean intensity of infection was very low (total number of pentastomids recovered = 82; mean = 3,5; range = 1–14) (Table 1). The majority of pentastomids in the collection was small (5–7 mm) and only *Sebekia okavangoensis*, represented by just six intact specimens, could be recognized immediately by virtue of its relatively large size (14–23 mm). Relying principally upon the comparative morphology of the hooks, mouth and copulatory spicules as diagnostic criteria, these few mature individuals of *S. okavangoensis*, previously recorded from the Nile crocodile in Botswana, were easily identified. Most pentastomids were located in the lung, but two crocodiles harboured adults of *S. okavangoensis* in the body cavity (one carried a single specimen and one had three) (Table 1).

A new species of *Alofia, A. parva*, possessed a typically alofian, markedly U-shaped buccal cadre and smooth hooks (Fig. 1A, C; 2B; 3C, D). However, the anterior pair of hooks could be retracted into a spinous, cowl-like extension of the fulcrum, an important diagnostic characteristic hitherto thought to be restricted to the recently described sebekiid genus, *Selfia* (Riley 1994). *A. parva* n. sp., was well represented by adequate numbers of both sexes (9 ♂♂; 14 ♀♀).

Another species, provisionally ascribed to the genus *Sebekia* and represented in the final instar by just two gravid females, also had a novel combination of characteristics that were intermediate between *Alofia*, *Selfia* and *Sebekia*, although the shape of the buccal cadre (Fig. 1D, E, F; 3F, G, H) effectively ruled out the first of these from serious consideration. However, in the absence of males we remain uncertain as to its taxonomic status, although it is clearly a new species. Paradoxically, a substantial population (22 individuals) of preadult pentastomids, all at approximately the same stage of development, and possessing simple hooks—the rear parts of which were protected by a small, smooth, cowl-like extension to the fulcrum (Fig. 2C; 3G)—were provisionally diagnosed as juveniles of this species, mainly on the basis of hook and buccal-cadre morphology (Fig. 1E; 2C; 3G). Furthermore, we postulated that another batch of 21 "double-hooked" nymphs [each possessed a long, spiky extension to the fulcrum overlaying each hook, giving it a doubled appearance (Fig. 2D; 3H)], represented an even earlier instar of this same species, again principally because of similarities in the buccal cadre (Fig. 1F; 3H). All nymphs of the family Sebekidae possess such hooks (Southwell & Piliers 1929; Rodhain Vuylsteke 1932; Winch & Riley 1986; Riley *et al.* 1990).

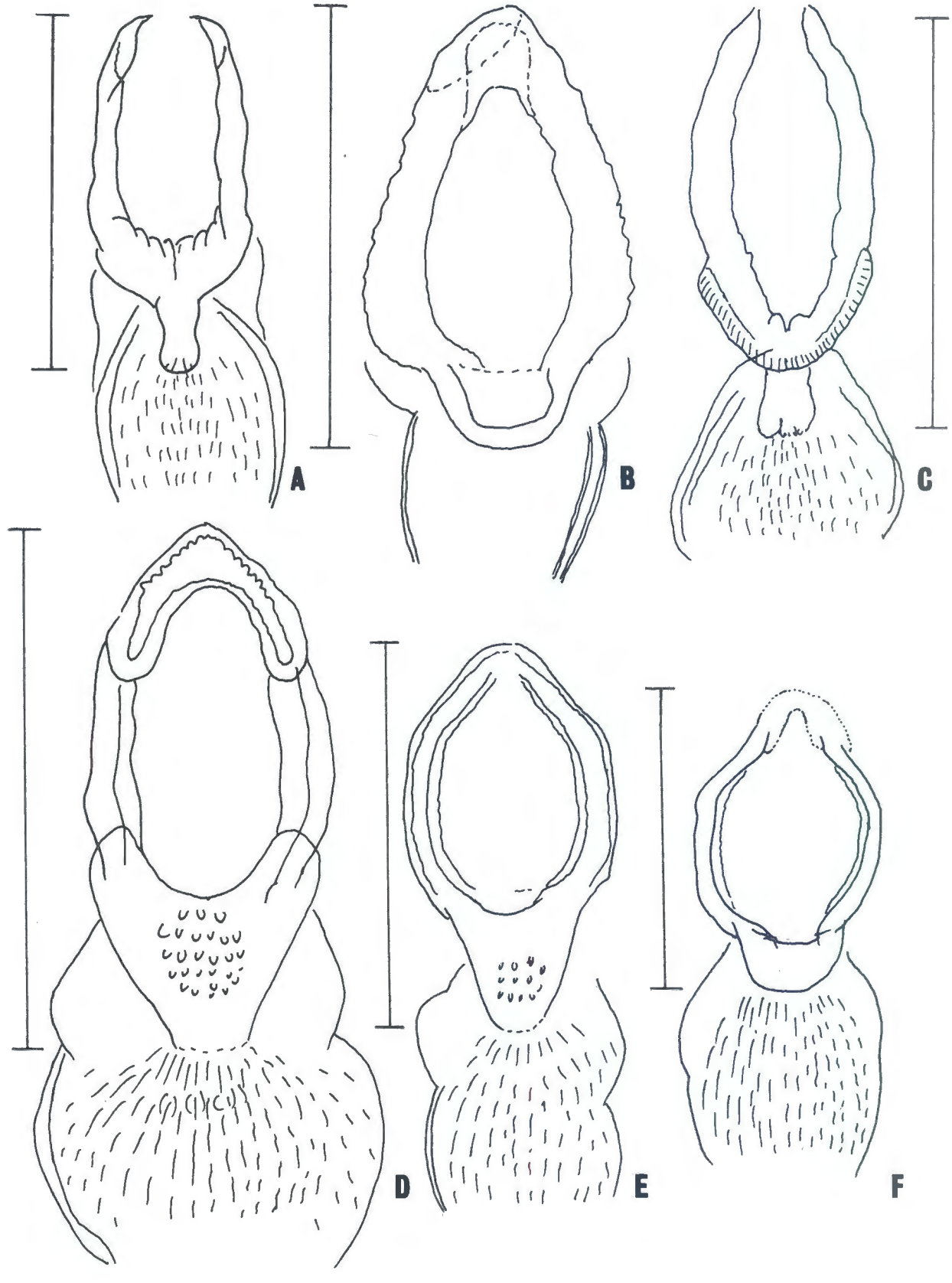
Sebekia okavangoensis Riley & Huchzermeyer (1995)

A total of seven fully adult specimens of this species was recovered [slide 2a was a female abdomen only; 2 ♀♀ from 10; 1 ♀ from 16; 1 ♂ and 2 ♀♀ from 23 (Table 1)] and both sexes were immediately distinguished by virtue of their being comparatively large, elongated and slender, with a gently tapered caudal

TABLE 1 Data on the prevalence and intensity of infection of three species of pentastomids from *Osteolaemus tetraspis* in May 1993

Crocodile examined		Length (m)	Sex	Specimen, no. examined, slide identification letter					Total	Comments
				Alofia parva	*Sebekia okavangoensis*	*Sebekia* sp.				
No.	Date					Adult	Preadult	Nymph		
1	13/5	–#	♀	2a, g(1d)	–	2c, f	–	2b, e	7 + 1h*	1d = immature female, 1h* = gravid female abdomen (not *S. okavangoensis)*
2	15/5	1,25	♂	2b, e	1a	–	–	2c, d	5	*S. okavangoensis* in body cavity (abdomen only)
3		1,13	♀	2d, e	–	–	–	4a, c, g, f	6	–
4		1,11	♀	1a	–	–	1b	–	2	–
5		1,47	♂	–	–	–	1a	2b, c	3	–
6		0,90	♀	–	–	–	1a	–	1	–
7	16/5	–	♀	–	–	–	1a	–	1 + 3*	Fragments of three pentastomids, not identified
8		1,13	♂	–	–	–	–	–	2*	Fragments of three pentastomids, not identified
9		–	♀	–	–	–	1a	1b	2	–
10		1,40	♂	1c	2a, d	–	–	1b	4	–
11	18/5	1,20	♂	–	–	–	–	1a	1	–
12		1,37	♂	3b, c, g (1f)	–	–	–	2d, e	6	1f = immature
13		1,25	♂	–	–	–	–	1a	1	–
14		1,15	♂	–	–	–	–	1a	1	–
15		1,46	♂	4a, e, g, l	–	–	7b, c, f, h, j, k, m	3d, i, n	14	–
16	19/5	1,33	♂	–	1a	–	5b, c, d, e, f	–	6	–
17		–	♀	–	–	–	–	–	0	–
18		–	♀	1a	–	–	–	–	1	–
19		–	♂	3, b, c, d	–	–	3a, e, f	–	6	–
20	21/5	–	♀	1a	–	–	–	–	1	–
21		–	♂	2b, c	–	–	–	1a	3	–
22		–	♂	–	–	–	–	–	0	–
23		–	♀	–	3a, b, c	–	1a	–	4	All *S. okavangoensis* in cavity
Total				24	7	2	21	21	75 + 6*	

( ) = Immature, but probably an adult instar
* = Unidentified fragment
\# = Indication that the length of the crocodile was measured only when the collectors were present at the time of slaughter. In other cases the internal organs were purchased after the animal had been dismembered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extremity terminating in a blunt point. Annuli could be counted with reasonable accuracy in wet-mounted specimens because a broad, elevated band of chloride-cell-pore caps projected from the anterior half of each annulus (Riley & Huchzermeyer 1995). Hooks were equally distinctive in that the hook blade was long and strongly curved and sharply demarcated from a prominent domed elevation which bore an estimated 14–17 rows of robust spines (Fig. 2A; 3A).

FEMALE

[N = 5 intact females, together with part of the abdomen of another (diagnosis was confirmed by use of the criterion of egg dimensions—see below)]

The characteristics of this species are presented in Table 2. Females attained a length of 23 mm with 93/94 annuli, and although this was less than the type female (27 mm and 99 annuli, respectively), there was no statistically significant difference in hook and fulcrum dimensions between the present specimens and the type series [hook-length mean = 105,5 ± 4,5, blade 48,5 ± 2,8 *contra* mean = 107 ± 3,0, 49 ± 2,5; fulcrum 284 ± 24 *contra* 288 ± 2, respectively (Table 2), Riley & Huchzermeyer 1995]. Mouth dimensions could be measured in only one of the present specimens (Fig. 2B) and, although this precluded statistical comparison with the type series, these dimensions were virtually identical to the largest of the type females (Riley & Huchzermeyer 1995).

The largest fully embryonated eggs in the terminal region of the uterus measured 102 ± 2 x 69 ± 1,8 µm. Since both slide-mounted specimens were ruptured, it was not easy to identify the *terminal* portion of the uterus precisely.

MALE (N = 1)

The single male, though shorter than the single type male (14 *contra* 23 mm) and with smaller hooks, possessed fulcra and spicules that were virtually identical (Fig. 3A) (Riley & Huchzermeyer 1995). Spicules, particularly, were very distinctive and could not easily be confused with those of any other *Sebekia* spp. (Fig. 3B) (Riley *et al.* 1990). In the present specimen, the average dimensions of the two spicules were: 698 µm overall length, the cowry-shell component was 450 µm long and its width was 118 µm (Fig. 3B).

Alofia parva n. sp.

DESCRIPTION

Diagnosis is based upon a holotype, eight paratype sexually mature males and one immature male, together with 14 paratype females, all but one of which were gravid (Table 1). The hook and mouth dimensions of the single prepatent female and the immature male were within the size range of that of mature specimens and both were therefore considered representative of the ultimate instar.

MALE (N = 9 mature and 1 immature)

Very small, 4,5 (4,3–5,0) mm long and 0,5–0,7 mm wide. Head strongly united with the abdomen and

FIG. 1 Scale drawings of the oral cadres of the three species described in this study compiled through several depths of focus: in each the scale bar indicates the length of the cadre; the overall length is from the anterior tip of the cadre to the posterior limit of the pharynx; the maximum cadre width was also measured

A. *Alofia parva* n. sp., holotype male (slide 1g), showing the stirrup-shaped cadre with its peg-like extension into the pharynx
Scale bar = 178 µm

B. *Sebekia okavangoensis*. Female (slide 10a) showing the pear-shaped cadre, the sides and front of which are lightly armoured, with the delicate pharynx
Scale bar = 320 µm

C. *Alofia parva* n. sp., a paratype female (slide 18a) showing a cadre with bowed sides with a striated flange rimming the structure near to the pharynx. This feature appears to be limited to the females of this species
Scale bar = 230 µm

D, E, F. Cadres of the three instars postulated to belong to a single species, tentatively identified as a *Sebekia* sp.

D. From an adult female (slide 1f) where a hollow U of chitin defines the anterior of the cadre and a solid crescent of chitin is found posteriorly. The latter is equipped with fenestrations which extend onto the pharynx
Scale bar = 250 µm

E. The cadre of an immature female (slide 16e) is a less armoured version of D and, although the double sides of the cadre are evident, the ornamentations anteriorly and posteriorly are scarcely formed. A few fenestrations are present
Scale bar = 210 µm

F. The cadre of a double-hooked nymph (slide 9b) which has the beginnings of a double-sided cadre and crescents front and back; no fenestrations are present in this instar
Scale bar = 140 µm

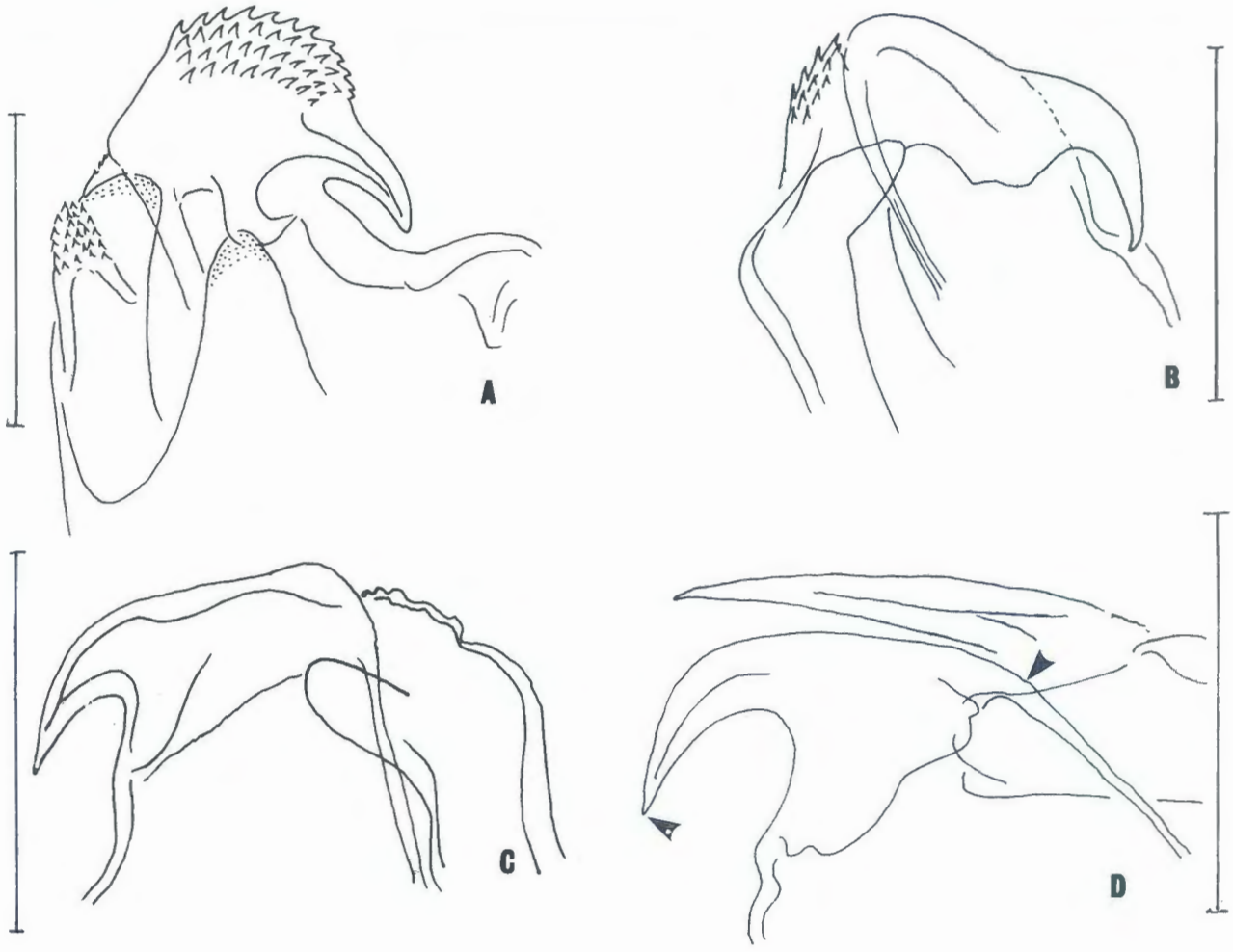


FIG. 2 Scale drawings of the hooks of the three species described, compiled through varying depths of focus

A. The short, squat posterior hook of a male *S. okavangoensis* (slide 23a) showing the domed dorsal surface with its patch of prominent spines, and the spiny, finger-like extension to the fulcrum which is also covered in spines. Fulcra are shaded
Scale bar = 86 µm

B. An anterior hook of a paratype female (slide 10c; Fig. 3D) of *Alofia parva* n. sp. showing the cowl bearing spines on its dorsal surface and the smooth, flat-topped hook with a blade that is bent through a right angle. The bend at the tip is typical of most *Alofia* spp.; male hooks have an identical morphology, but are smaller
Scale bar = 79 µm

C. An anterior hook of the preadult female (slide 1h) of a putative *Sebekia* sp. A very thinly chitinized cowl extends from the fulcrum, the hook is flat-topped and the barb is smoothly curved. In both mature females the hooks are poorly aligned, but essentially identical
Scale bar = 64 µm

D. The posterior hook of a double-hooked nymph (slide 5b) showing the spine overlying the smooth hook; the arrows indicate the overall-length measurement from the hook tip to the origin of the posterior apodeme
Scale bar = 84 µm

possessing prominent frontal papillae. Abdomen distinctly claviform, attaining its largest diameter over the anterior one third and gradually tapering posteriorly to a rounded point. Despite the fact that in this species each annulus was demarcated anteriorly by a prominent single row of chloride cells projecting 5–10 µm above the surface of the cuticle, annuli could be counted only in the single immature specimen (specimen 12f); the estimated annulus number was 59. Hooks were typical of *Alofia* spp., in that the long, slender blade was slightly bent at the tip, whereas at the base it was bent almost through a right angle

(Fig. 2B; 3C). However, the whole blade was aligned along the long axis of the delicate and elongated shank so that the entire structure appeared more or less linear. Holotype hooks measured 61 µm in overall length and the distance of the hook tip to the notch on the dorsal margin was 36 µm: paratypes mean = 67,3 ± 4,2 and 37,4 ± 3,6, respectively. Fulcra appeared long and slender (156 ± 13,3 µm) when viewed from the lateral aspect, and those attached to the anterior hooks carried a spinose, cowl-like anterior extension (Fig. 2B).

In a few specimens, where the long axis of the hook was oriented at right angles to the plane of the slide, five or six rows of spines could be discerned on the cowl, with up to ten spines in each row: posterior hooks were smooth and there was no corresponding extension to the fulcrum (Fig. 3C). The heavily chitinized mouth was U-shaped with a prominent peg-like extension projecting into the pharynx so that, when flattened under gentle coverslip pressure, the whole assembly resembled a stirrup. The anterior prongs sometimes converged, but the tips were always separated by a gap of at least 15–25 µm (Fig. 1A; 3C). Prominent flanges, which may serve for the attachment of muscles, extended dorsally from the free ends of these prongs. It should be noted that the oral cadre, in particular, is a three-dimensional structure, bowed in the dorsoventral plane, which cannot be measured reliably without some flattening, but it is easily distorted by excessive pressure.

The paired copulatory spicules were club-shaped and the edges of the open "cowry-shell" base united anteriorly to form a spatulate extension, the base of which was ornamented by small, ridged, scale-like rugosities (Fig. 3E). These were difficult to count, but up to ten rows may be present. The closed side of the cowry shell terminated anteriorly in a double-hooked collar. Overall length was 257 ± 11 µm, the length of the cowry shell was 213 ± 13 and its width 92 ± 5. The cirrus tip, which was very thinly chitinized and obscured in most specimens, could occasionally be seen as a delicate hollow needle of chitin folded over to form a minute hook.

FEMALE (N = 14)

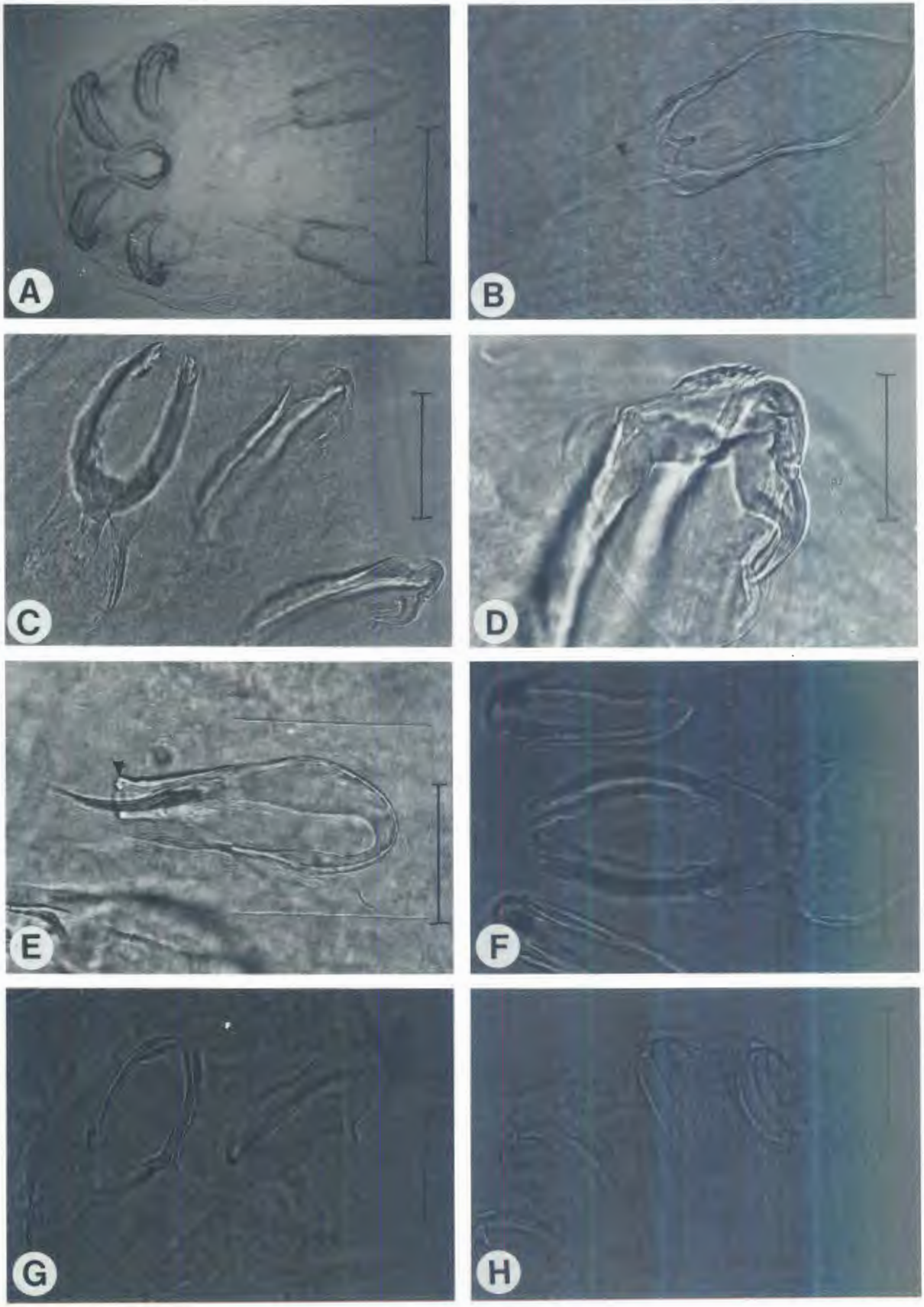
Females possess a stouter and more massive body than males, attaining a maximum length of 7,5 mm ($\bar{x}$ = 6,2). The head (diameter at base = approximately 0,5 mm) merges smoothly with the abdomen which is swollen anteriorly and attains a maximum diameter of about 1,1 mm. The posterior half of the body is of uniform diameter and the caudal extremity is very bluntly rounded, although in most specimens it is not swollen into a bulb. Annulus number, which could be estimated in only one specimen (12g), was 65. Hooks and fulcra slightly larger than those of the male, but otherwise identical to them, and the spinous cowl associated only with the anterior hooks (Fig. 3D). Similarly, the mouth was bigger, as was the gap between the anterior prongs (20–30 µm) (Fig. 1C). The posterior margin of the cadre was flared by a U-shaped rim of chitin furnished with delicate radiating striations (Fig. 1C), a feature which was not apparent in males (Fig. 1A; 3C). Undistorted eggs, located in the terminal coils of the vagina and containing fully developed larvae, measured 93 ± 3,5 x 66,5 ± 2,3 µm.

Sebekia sp.?

The remaining new species was left unnamed because in the adult form it was represented by only two mature individuals, both gravid females (one was punctured, with the result that most of the uterus was missing). The singular combination of hook and oral-cadre characteristics possessed by this species, effectively meant that, in the absence of mature males, it was not possible to ascribe this species with certainty to any of the existing sebekiid genera. Additionally, 21 preadult specimens of indeterminable sex—all with very thinly chitinized, delicate hooks and buccal cadres, together with 21 nymphs, each carrying accessory spines over each hook, giving them a doubled appearance—were, for the reasons already expounded, also thought to belong to this species.

FEMALE (N = 2)

The intact, slide-mounted female (1f) was distinctly claviform, measuring 12 mm in length and 1,6 mm at maximum width which occurred in the anterior quarter of the abdomen. Before the head was removed, 56 annuli could be estimated on a wet-mounted specimen. The remainder of the abdomen tapered gradually and the caudal extremity tapered more abruptly to form a bluntly rounded tip (NB: The other specimen was punctured); chloride-cell-pore caps were not visible in cleared specimens. Hooks, which were small, delicate and smooth, most resembled those of *Alofia* spp., except that the blade was not quite so angular and its base was not bent through a right angle, but was more smoothly curved. Unfortunately, in both specimens hooks were not orientated optimally and hook dimensions could only be estimated at 60 µm in length and the blade was about 38 µm from its tip to the notch on the dorsal margin. Fulcra associated with both hooks were equipped with a very thin, cowl-like extension into which hooks could retract; this was more robust and prominent on anterior fulcra and devoid of ornamentation. The oral cadre was distinctive and it was this character which was thought to preclude a close relationship with *Alofia* spp., and suggested that these females, together with the two groups of immature specimens described below, were part of a developmental series. The cadre was ovoid with bowed "double" sides. Focusing through the cadre showed that, anteriorly, a hollow, inverted "U" of chitin formed a bridge while, posteriorly, a similarly shaped, but solid, more crescentic segment of chitin constituted the front part of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the pharynx: this was equipped with rows of irregular fenestrations which continued deep into the pharynx (Fig. 1D; 3F). Fully embryonated eggs in the terminal uterus measured, on average, 92,3 ± 3,5 x 66,3 ± 2,3 µm.

PREADULTS (N = 22)

The postulated preadults of the above species, present in low numbers in nine hosts (Table 1), were sexually immature but preadult, in that their hooks were single, smooth and thinly chitinized. However, one specimen (no. 1h) was diagnosed as an immature female because sperm was visible in the spermatheca. The hooks of this specimen measured 64 µm in length and the notch on the dorsal surface was poorly defined (Fig. 3G). These hooks were intermediate in their state of development between the mature females and the remaining 20 preadults (Table 2). No members of this cohort had hooks with the doubled appearance typical of the earlier instars of nymphal sebekiids (see below and Discussion). Accurate measurement of hooks, fulcra and mouths was difficult because these features, with the single exception of the postulated immature female specimen (slide 1h), were exceedingly thinly chitinized and frequently deformed by slide-mounting. Most fulcra were excessively bowed (by muscle contraction?) and were not measured; only those that were relatively straight are included in Table 2. The cowl-like extension to the fulcrum folded to form a peak in some specimens (e.g. slides 15b and f) although the significance of this is unclear. It was as though all of these specimens had just emerged from ecdysis, although statistically this was highly improbable.

NYMPHAL SP. (N = 21)

The remaining population, found in low numbers in 13 hosts (Table 1), was represented only by earlier nymphal forms which, as far as could be determined, were all at approximately the same stage of development. Nymphs were distinctly claviform, being widest in the anterior one third and, posteriorly, tapering smoothly to a bluntly pointed tail. There was no significant development of sexual organs, nor could the male sexual opening be reliably identified, with the result that the sexes could not be differentiated.

FIG. 3 Micrographs of distinctive features of the three species of pentastomids described in this paper

A, B. The entire cephalothorax with detail of a copulatory spicule of the single male of *S. okavangoensis* (slide 23a)

A. The highly characteristic shape of the oral cadre mirrors that of the female (cf. Fig. 1B), and the domes on the dorsal surfaces of the hooks carry patches of spines
Scale bar = 500 µm

B. The copulatory spicule, entirely lacking the hooked collar typical of *Alofia* spp. (see Fig. 3E) is large and the anterior spatulate projection is covered by scale-like rugosities. The overall length and "cowry shell" length are indicated by arrows
Scale bar = 200 µm

C, D, E. *Alofia parva*

C. The cephalothorax of the holotype male (slide 1g) showing the disposition of the buccal complex relative to the hooks—note that the buccal cadre has bowed sides and is open anteriorly; the peg-like extension into the pharynx is plainly visible. The whole buccal complex is bowed in the dorso-ventral plane so that in this particular ventral view, the cadre is superficial. Note, however, that its anterior flanges and peg extension are out of focus
Scale bar = 100 µm

D. Detail of an anterior hook of the paratype female (slide 10c) showing the spines on the cowl extension to the fulcrum
Scale bar = 50 µm

E. A composite photograph of the copulatory spicule (slide 15l) taken in two planes of focus. The "cowry shell" component terminates anteriorly in a double-hooked collar (arrowed), and the other anterior projection carries rows of backwardly directed spines and resembles the hypostome of a tick
Scale bar = 100 µm

F, G, H. *Sebekia* sp.?

F. Detail of the mouth (drawn in Fig. 1D) and flanking anterior hooks of a mature female. Hooks were not flattened during slide mounting and therefore could not be measured. The two crescents of chitin on opposite sides of the cadre are obvious
Scale bar = 100 µm

G. Detail of the preadult female (drawn in Fig. 1E) to show the delicate mouth compared to the adult stage, and detail of a hook
Scale bar = 100 µm

H. The cephalothorax of a double-hooked nymph (slide 9b) to show the lightly chitinized mouth flanked by the relatively large hooks, each bearing an accessory spike extension of the fulcrum
Scale bar = 200 µm

TABLE 2 The principal characteristics of the pentastomids (family Sebekidae) from *Osteolaemus tetraspis* examined in this study. (Measurements in µm unless stated otherwise)

Species	Sex	No.	Length (mm)	Width (mm)	No. of annuli	Hook dimensions		Fulcrum length	Mouth dimensions		
						Overall length	Blade length		Overall length	Cadre length	Cadre width
Sebekia okavangoensis	♀	6	18–23	1,3	93/4	105 ± 24	48,5 ± 24	284 ± 24	450	320	210
	♂	1	14	1,1	87(?)	86	40	240	–	240	175
Sebekia spp.	♀	2	10	2,2	56	60	38	160 ± 3	345 355	250 250	117 115
"Preadult"	♀	1	6,4	0,95	–	64	33	150	–	210	120
Smooth-hooked nymphs	?	20	4,1–6,8 (5,5)	0,6–1,1 (0,9)	–	47 ± 4	–	140*–170	256 ± 20	174 ± 15	94 ± 11
Double-hooked nymphs	?	21	3,5–7,3 (5,5)	0,6–1,0 (0,8)	56–61	81,5 ± 6,3	–	165 ± 22	202 ± 17	133 ± 11	88 ± 10
Alofia parva n.sp.	♀	14	5,3–7,5 (6,4)	0,8–1,3 (1,0)	65(?)	79 ± 4,2	41 ± 3,5	180 ± 16,5	307 ± 18	240 ± 13,5	97,5 ± 6,0
	♂	10	4,3–5,0 (4,5)	0,5–0,7 (0,55)	59(?)		37,5 ± 3,6	156 ± 13,5	234 ± 13	185 ± 12	74 ± 4,3

* Probable underestimate—many were bowed

All possessed comparatively large hooks (length 81,5 ± 6,3 µm) overlain by a substantial spiky extension (length 86,0 ± 5,0 µm) to the fulcrum which gave the hooks a doubled appearance (Fig. 2D; 3H). The blade was very large compared with the shank and there was no notch on the dorsal margin (Fig. 2D). From observations of a number of specimens, with hooks in a variety of positions with respect to the fulcrum, it was apparent that the hooks were guarded by this extension, but that they could move independently of it: the distance from the hook tip to the rear of the extension was 101 ± 5,3 µm. Annuli were easily counted in intact specimens because the posterior margin of each annulus was fringed by a prominent row of backwardly directed spines. A single row of non-projecting chloride cells girdled the mid-annular region. The distinctive buccal cadre was totally unlike that of *Alofia* spp., in that it was never U-shaped with parallel sides (Fig. 1F; 3H). Rather, its round or ovoid profile was interrupted anteriorly by a delicate U-shaped bridge of chitin which united the open ends of the ring. Posteriorly, a broad crescent- or U-shaped segment of chitin extended from the ring into the pharynx. Mouthrings of this general type are typical of certain species belonging the genus *Sebekia* (Riley *et al.* 1990).

DISCUSSION

Crocodile lungs are well developed, consisting of a system of tubes ending in sacs, and these organs are tough and fibrous. The technique used to recover intact parasites from lungs depended upon the parasites migrating from increasingly anoxic lung tissue towards cut surfaces where they could be collected. What proportion of the pentastomid population present in lungs was retrieved by this technique, remains uncertain and, as a result, the accuracy of the data presented in Table 1 concerning prevalence and intensity, which was very low, is questionable.

Wild-caught African dwarf crocodiles are regularly brought to markets in Brazzaville, where they are slaughtered for their meat (Behra 1990). According to Agnagna (personal communication, Brazzaville 1993) animals are captured all year round, and kept alive in small villages before being traded during the high-water season when river boats can get far upstream. In the present study it was estimated that at least one month had elapsed between capture and slaughter. Unfortunately it was not possible to ascertain the origin of the animals, although the probability was that each batch of crocodiles coming to market on a particular day, came from a particular locality (see dates of purchase in Table 1). Since pentastomids are relatively long-lived [many months to several years (Riley 1986)], the presence of two populations of immature forms, each composed predominantly of a single instar putatively representing a single species, strongly suggests that in this particular case, there is a seasonal influence on transmission and/or development.

All four genera comprising the family Sebekidae are restricted to crocodilians, the only known exception being *Sebekia mississippiensis* Overstreet, Self & Vliet, 1985, which can also attain maturity in the lungs of a piscivorous turtle (Dukes, Shealy & Rogers 1971). Sebekiids, in common with all other pentastomids, have traditionally been assorted into taxa on the basis of relatively few anatomical characteristics (Riley 1986). The diagnostic characteristics in question vary discretely—there is no problem in identifying species—but the present work has highlighted the fact that the genera are indeed arbitrarily constructed. Self & Rego (1985), who reviewed the family and focused particularly on the genera *Alofia* and *Sebekia,* concurred with Heymons's (1941) earlier assessment in concluding that these genera were so closely related they could be synonymous. Self & Rego (1985) could find only two features that reliably separated these genera, namely the hooks—those in *Sebekia* being claw-like whereas those in *Alofia* are fang-like—and the ring-shaped oral cadre in *Sebekia* contrasted with the U-shaped one of *Alofia*. They further noted that even though the oral cadre was referred to as ring-shaped in *Sebekia* spp., the sclerotized lateral prongs did not always meet anteriorly and the ring could be united by fibres (see the cadre of *S. oxycephala* in their Fig. 1C).

While recent reviews have reaffirmed many of the distinguishing characteristics of these two genera, they have also revealed certain ambiguities. For example, Riley *et al.* (1990), in their review of the genus *Sebekia*, described a total of nine species, five of which were new, and noted that the buccal cadre, though highly variable in profile, being oval to elongate, generally lacked the long, parallel sides, and never carried the peg-like extension into the pharynx, typical of all *Alofia* spp. Rather, the cadre is united anteriorly by fibres, except in the single example of *S. wedli* where the anteriorly directed prongs are always separated by a gap of 11–33 µm although there is no peg-like extension into the pharynx (Sambon 1922; Riley *et al.* 1990; Riley & Huchzermeyer 1995). Both sets of hooks are small and curved with small blades and, in at least nine of the *Sebekia* spp. described to date, a patch of spines is present on the domed dorsal surface; all fulcra carry a spinous, finger-like extension. Again one species, *S. trinitatis* (Riley, Spratt & Winch 1990), is atypical in this regard, in that the fulcrum extension, which is limited to the anterior pair of hooks, is more reminiscent of the cowl-like extension of *Selfia* (Riley 1994). The distinction between cowls and fingers was not fully appreciated at the time that *S. trinitatis* was described, because cowls, when viewed from the lateral aspect, appear as fingers (see Fig. 1C in Riley *et al.* 1990). Similarly, the anterior hook in this species, though bearing a dorsal patch of spines, has a long, flat-topped shank and is therefore more *Alofia*-like. Although the gross morphology of the copulatory spicule of all *Sebekia* spp. resembles that of *Alofia*, the anterior "collar" is usually ill-defined, thinly chitinized and never double-hooked. Therefore, with the notable exceptions of *S. wedli* (mouth) and *S. trinitatis* (hooks/fulcra), the genus *Sebekia* presents a reasonably homogeneous assemblage.

By contrast, the hook shank in most *Alofia* spp. is elongated and flat-topped, and the blade is comparatively large and finely pointed with a base that is bent almost through a right angle. However, the hooks of *A. nilotici* [Riley & Huchzermeyer (1995)] are more compact and possess a more domed dorsal surface. Another feature of this species, and of *A. merki* (Riley 1994), are the patches of minute spines associated with the dorsal surface of either the anterior set of hooks, or both. However, these are almost insignificant when compared with the robust spines of typical *Sebekia* spp. (Riley *et al.* 1990).

In all *Alofia* spp. fulcra lack extensions, the oral cadre resembles a stirrup or tuning fork in having long, parallel sides and a peg-like projection extending back into the pharynx, and copulatory spicules carrying a heavily chitinized, double-hooked collar (Riley 1994). Equally important, female *Alofia* spp. have an obviously rounded and bulbous caudal extremity (Giglioli 1922; Sambon 1922; Riley 1994; Riley & Huchzermeyer 1995), a feature which appears to have no counterpart in other sebekiid genera.

The monotypic genus *Selfia* (Riley 1994) is probably most closely related to *Alofia*, because spicules are virtually identical. However, its minute, smooth hooks are "linear", in that the *Alofia*-like blade is not presented at right angles to the shank but is rotated dorsally to become aligned more along the long axis of the shank; this is both flat-topped and creased transversely. Furthermore, the anterior hooks can be retracted into a large, spinous, cowl-like extension to the fulcrum, the oral cadre is closed by fibres and a hoop of chitin anteriorly, and the backward extension into the pharynx is broad and spatulate (Riley 1994). Finally, the caudal extremity of the female is tapered to a rounded point and is therefore closer to that of *Sebekia* spp. than that of *Alofia* (Riley 1994).

In this context it becomes apparent that two of the three species described here are not easily accommodated—even within this classification which is based on relatively few generic characters. The spinous, cowl-like extension of the fulcrum of *A. parva* n. sp. is unique amongst *Alofia* spp., it being reminiscent of both *Selfia porosus* and *Sebekia trinitatis.* Furthermore, the "linear" hooks combine features intermediate between *Selfia* and *Alofia*. However, despite the fact that the sides of the oral cadre are not parallel but converge in most specimens, they are nonetheless never united anteriorly and the backward extension into the pharynx is a peg. This, combined with the bulbous tail of females, and the dou-

ble-hooked copulatory spicules, suggests that, on balance, we are dealing with an *Alofia* sp.

The remaining anomalous species, provisionally allotted to the genus *Sebekia*, is more problematical and it also calls into question the criteria once thought to be central to generic diagnosis within the family Sebekidae. We accept that extant pentastomids are likely to possess a mosaic of ancestral and derived characters, despite the recent discovery of highly conserved fossilized forebears from the late Cambrian (Walossek & Muller 1994). However, since we do not yet understand pentastomid systematics, or indeed the functional significance of many of the features described here, the criteria traditionally used to define taxa are unquestionably quite arbitrary. In any event, the true status of the postulated *Sebekia* species cannot be resolved until the male is described. In the interim, all that can be stated is that the mouth and hooks are very different from those of *Alofia* spp. and that the caudal extremity is not bluntly rounded. The absence of spination on hooks, coupled with the lack of an extension to the fulcrum, portends the possible erection of another genus.

The alternative view, and one espoused particularly by Self & Rego (1985) (although at that time these authors could not be aware of the "intermediate" forms mentioned here, namely that all pentastomids infecting crocodilians should be regarded properly as a single genus), has increasing appeal. Taxonomic techniques based on molecules may ultimately shed more light on this dilemma.

Finally, the presence of *S. okavangoensis* in the body cavity indicates that some *post-mortem* migration had occurred, and that this phenomenon may be restricted to just one of three recorded species.

REFERENCES

BEHRA, O. 1990. Sex ratio of dwarf crocodiles (*Osteolaemus tetraspis* Cope, 1861) exploited for food in Congo. *Proceedings of the 10th working meeting of the Crocodile Specialist Group, IUCN,* Gainsville 23–27 April 1990, 1:3–5.

DUKES, G. H., SHEALY, R. M. & ROGERS, W.A. 1971. *Sebekia* oxycephala (Pentastomida) in largemouth bass from Lake St. John, Concordia Parish, Louisiana. *Journal of Parasitology,* 57: 1028.

FAIN, A. 1961. Les pentastomides de l'Afrique centrale. *Annales du Musée Royal de l'Afrique Centrale, Series 8,* 92:1–115.

GIGLIOLI, G.S. 1922. The new genus *Alofia* of the family Linguatulidae. An anatomical account of *A. ginae. Journal of Tropical Medicine and Hygiene,* 30:371–377.

HEYMONS, R. 1941. Beiträge zur Systematik der Pentastomiden VI. Die Arten der Gattung *Alofia* im Vergleich mit *Sebekia* I. Übersicht über Arten. *Zeitschrift für Parasitenkunde,* 12:419–423.

NEILL, W.T. 1971. The last of the ruling reptiles: alligators, crocodiles and their kin. New York and London: Columbia University Press: 293–322.

RILEY, J. 1986. The biology of pentastomids. *Advances in Parasitology,* 25:46–128.

RILEY, J. 1994. A revision of the genus *Alofia* Giglioli, 1922 and a description of a new monotypic genus *Selfia*: two genera of pentastomid parasites (Porocephalidae: Sebekidae) inhabiting the bronchioles of the marine crocodile (*Crocodylus porosus*) and other crocodilians. *Systematic Parasitology,* 29:23–41.

RILEY, J., SPRATT, D.M. & WINCH, J.M. 1990. A revision of the genus *Sebekia* Sambon, 1922 (Pentastomida) from crocodilians with descriptions of five new species. *Systematic Parasitology,* 16:1–25.

RILEY, J. & HUCHZERMEYER, F. W. 1995. Descriptions of four species of pentastomid parasites belonging to the genera *Alofia* Giglioli, 1922 and *Sebekia* Sambon, 1922, from a single Nile crocodile (*Crocodylus niloticus*) from Botswana. *Systematic Parasitology,* 31:221–238.

RODHAIN, J. & VUYLSTEKE, C. 1932. Contribution à l'étude des porocéphales des crocodiles africaines. *Revue de Zoologie et de Botanique Africaines,* 23:1–11.

SAMBON, L.W. 1910. Preliminary notes on three new species of tongue worms (Linguatulidae). In the collection of the "Museum d'Histoire Naturelle", Paris. *Transactions of the Society of Tropical Medicine and Hygiene,* 3:134–154.

SAMBON, L.W. 1922. A synopsis of the family Linguatulidae. *Journal of Tropical Medicine and Hygiene,* 25:188–206; 391–428.

SELF, J.T. & REGO, A.A. 1985. Reassessment and revisions of certain genera and species of the family Sebekidae (Pentastomida) including description of *Sebekia microhamus* n. sp. *Systematic Parasitology,* 7:33–41.

SOUTHWELL, T. & PILIERS, A.W.N. 1929. A note on a nymphal linguatulid—*Leiperia cincinnalis* Sambon—from the musculature of the fish *Tilapia nilotica. Annals of Tropical Medicine and Parasitology,* 23:130.

WINCH, J.M. & RILEY, J. 1986. The development of *Sebekia oxycephala* (Pentastomida) from a South American crocodilian (*Caiman sclerops*) in experimentally infected fish. *Zeitschrift für Parasitenkunde,* 72:251–264.

WALOSSEK, D. & MULLER, K. J. 1994. Pentastomid parasites from the Lower Palaeozoic of Sweden. *Transactions of the Royal Society of Edinburgh: Earth Sciences,* 85:1–37.