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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

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Abstract

A collection of 94 pentastomid specimens, comprising four species (three belonging to the genus *Sebekia* Sambon, 1922 and one (?)belonging to the genus *Alofia* Giglioli, 1922), were recovered from the lungs of a single Nile crocodile *Crocodylus niloticus* taken in the Okavango swamps, Botswana. On the basis of external morphology only, two specimens out of the entire assemblage were immediately distinctive, by virtue of their being comparatively long and slender. After clearing in Hoyer's medium and examination of the chitinised structures of these two specimens (hooks, fulcra, oral armature, male copulatory spicule and egg size) a new species, *Sebekia okavangoensis*, was described. It proved necessary to clear and slide-mount virtually all of the remaining specimens in the collection before any further progress towards specific diagnosis was possible. When this was done, two additional (damaged) specimens belonging to the above new species were discovered. Furthermore, it was postulated that two other female *Sebekia* specimens, described earlier under the binomial *Sebekia cesarisi* Giglioli in Sambon, 1922 by Riley, Spratt & Winch (1990), also belonged to this taxon. Seven females and one male in the present collection were assigned to *S. cesarisi*, primarily on the basis of the distinctive morphology of the oral cadre. Uniquely this cadre possessed a pair of prominent anterior flanges which line the front part of the buccal cavity. Stylised drawings of these structures are the *only* features of diagnostic significance contained within the perfunctory type description of *S. cesarisi*. The majority of the present collection (a total of 75 specimens, 72 of which were slide-mounted) comprised a single species, *S. wedli* Giglioli in Sambon, 1922, and we were able to confirm the usual variation in the key parameters of all of the chitinous structures measured: this seems to be a hallmark of pentastomid morphometrics. Nonetheless, despite this problem, all of the present *Sebekia* spp. can be discriminated by appropriate *combinations* of characters, and there is no confusion between these and the remaining eight well characterised species in the taxon (Riley *et al.*, 1990). Finally, a new species of *Alofia*, *A. nilotici*, based on two females and two males, possesses hooks that are intermediate between *Sebekia* and the other known *Alofia* spp., although the distinctive copulatory spicules leave no doubt as to its generic status. The hooks of this new species carry a dorsal patch of minute spines which suggests a rather close relationship between the two genera, as initially proposed by Heymons (1941) and later by Self & Rego (1985). Six specimens, deemed by eye to be the best preserved, were examined solely by SEM. Three were identified as *S. wedli*, one as *S. okavangoensis* n. sp., one as *S. cesarisi* and the remaining *Alofia* specimen possessed hooks without spines; we remain uncertain about the specific status of this particular specimen.

Introduction

At least five genera of pentastomids are now known to infect the respiratory tract of crocodilians, four being exclusive to these hosts, and the literature suggests that at least half of the 22 extant species of crocodilians harbour one or more species of pentastomid (Riley, 1993). Two of these genera, *Alofia* Giglioli, 1922 and *Sebekia* Sambon, 1922, have been recently reviewed and a number of new species, mainly from the marine crocodile *Crocodylus porosus*, were described. Remarkably, this host, which in terms of its ecology is particularly wide-ranging, supports three genera and a

total of five species; concurrent infections are common (Riley, Spratt & Winch, 1990; Riley, 1994).

Generally, pentastomids infecting African crocodiles are rather less well known, although the Nile crocodile *Crocodylus niloticus* is host to one very distinctive and well characterised species belonging to the genus *Leiperia* Sambon, 1922 (see Sambon, 1910, 1922; Rodhain & Vuylsteke, 1932; Fain, 1961): larval stages apparently occur in fish (Beauchamp, 1914; Southwell & Piliers, 1929), as is typical of all other crocodilian-infecting genera (Winch & Riley, 1986).

The confusion surrounding the two endemic African species belonging to the genus *Sebekia, S. wedli* Giglioli in Sambon, 1922 and *S. cesarisi* Giglioli in Sambon, 1922, has been aired by Self & Rego (1985) and Riley *et al.* (1990). In their revision of the genus, the latter authors described a total of eight adult specimens (all slide-mounted and derived from the collection at The Natural History Museum) which they believed represented these two *Sebekia* spp. The specimens they assigned to *S. wedli* definitely originated from *C. niloticus*, but the other four (provisionally ascribed to *S. cesarisi*) were recovered from an unnamed African crocodile. Riley *et al.* (1990) based their diagnoses mainly on comparisons of the buccal armature of their material with earlier drawings of these structures (p. 194 in Sambon, 1922; see also Fig. 1B,C), allegedly of the type-material. It should be noted that the types are now lost and the type descriptions are very perfunctory (Self & Rego, 1985). However, the correspondence in buccal armature was passably good: the morphology of the oral cadre can be utilised reliably in generic diagnosis in certain porocephalid genera (see Fain, 1961) and, although that of *Sebekia* spp. is more variable than most, interspecific differences do exist, and it was on this basis that Riley *et al.* (1990) concurred with the original diagnoses of Giglioli (in Sambon, 1922). It should be noted that nothing is known about the host of *S. cesarisi*, beyond that it is a crocodile.

The first record of an *Alofia* sp. from Africa was from Ghana, but again, unfortunately, the identity of the host was also unknown although it cannot be seriously doubted that this was actually a crocodile (see Riley, 1994). This observation established finally that both *Sebekia* and *Alofia* spp. are widespread in crocodiles, from South and Central America, through Africa and Asia, to Indonesia and Australia (see Fain, 1961), which, in turn, reflects the probable long host/parasite association (Riley, 1994) that might well have become established long before the isolating mechanisms associated with continental drift became significant.

In this paper a number of pentastomid species are described from a single specimen of *C. niloticus* which originated from Botswana. A total of 94 parasites were recovered from the bronchioles of the lungs and, in terms of gross morphology, all looked superficially quite similar [apart from the obvious size differences between some males and females (see Riley, 1994) and the elongate, slender form of two specimens of *S. okavangoensis* n. sp.]. It was not until most of the series was slide-mounted and cleared in Hoyer's Medium that it became apparent that *S. wedli* and *S. cesarisi* were both represented, together with the above new *Sebekia* and *Alofia* spp. The large number of specimens of *S. wedli* in particular (72 together with three specimens used for SEM studies) has permitted a more rigorous biometrical analysis; hitherto, this has not been possible with any African pentastomid species, which further emphasises the unusual degree of variability that exists in nearly all of the key diagnostic characters (a recurrent problem in pentastomid morphometrics (Riley, 1986). Despite these difficulties certain *combinations* of these key parameters readily discriminate species, and now there are no longer any doubts remaining about the specific status of these interesting endoparasites.

Materials and methods

All pentastomid material was recovered by one of us (FWH) from the bronchioles of an immature female *Crocodylus niloticus* which was captive-reared at the Okavango Swamps Crocodile Farm, Maun, Botswana. The farm is situated on the Botete River, which is inhabited by wild crocodiles and, during flooding, fish from the river penetrate into the breeding and rearing ponds. Late in 1989 the crocodile (together with a large batch of others) was purchased by Mr Calcaterra of the Crocovango Crocodile Farm, Pretoria, South Africa, where it died, soon after transport by road, from stress-related septicaemia. The crocodile had been dead for several hours prior to dissection and the cuticles of some of the worms had begun to break down and flake before the material was able to be fixed and stored in 70% alcohol. This degeneration of the cuticle effectively precluded accurate annulus counting in all specimens (apart from *S. okavangoensis* n. sp.; see below), but other important diagnostic features were unaffect-

ed. About 15% of the material was damaged in some way, so that even gross body dimensions could not be measured. Seven selected specimens, deemed by eye to have been the best preserved at the time of fixation, were processed for scanning electron microscopy on a JEOL JSM 35, after critical point drying.

For basic morphological analysis hooks, fulcra and copulatory spicules of *Sebekia* and *Alofia* spp. were measured according to the protocols outlined in Riley *et al.* (1990) (see Figs 2A–D; 4A–E; 5E–F), after specimens had been decapitated (N.B.two males only; two females were whole-mounted) with fine needles and slide-mounted in Hoyer's Medium. Detached cephalothoraces were mounted separately from the abdomen, but on the same slide, and gently flattened by coverslip pressure prior to examination. The measurement of cadre length in some specimens of *S. wedli* was difficult because the posterior limit was obscured by converging fibres of chitin in the pharynx. Such cadres could only be estimated to the nearest 10 μm under $\times 100$. Measurement of this parameter in other spp. was straightforward and precise. Measurements of the fulcra were taken from midway between the two points of articulation with the hook to the tip of the base; fulcra that were excessively bowed by muscle contraction at the time of fixation were not measured. Egg dimensions (length and maximum width) proved to be a useful additional criterion for differentiating *Sebekia* spp., and accordingly 20 of the largest, least distorted (by pressure from surrounding eggs), fully-embryonated eggs (i.e. containing fully-developed primary larvae) in the terminal part of the uterus of gravid females were measured along these two axes. Nearly all females were packed with eggs, but, on closer examination after clearing, in more than half of such females these were found to be without mature embryos and therefore were not measured. *All* slide-mounted specimens are individually numbered and these numbers are referred to wherever plates, diagrams etc. appear in the text and in tables. All specimens are now deposited in The Natural History Museum (BMNH), Cromwell Road, London SW7 5BD, under the collection No. BMNH (E) 1994.80.

Results

Apart from the intact male and female specimens of *S. okavangoensis* n. sp., which were noticeably longer and more slender than any other species, there was no single aspect of gross morphology which could be used to differentiate the remaining specimens and this even applied to distinguishing *Alofia* from *Sebekia*. However, these two genera could be differentiated under $\times 40$ using a binocular dissecting microscope, because the hooks and mouth ring of *Alofia* are sufficiently large and projecting to be visible, whereas those of *Sebekia* are too small for detailed observation. Males were relatively few in number (15% of the total recovered) and normally these could be distinguished from the occasional immature female of similar small size by their white seminal vesicle and testis which remain visible through the thin cuticle even in alcohol-fixed specimens. Otherwise it proved necessary to slide-mount and clear all specimens before specific diagnosis was possible.

For the reasons discussed below, *S. okavangoensis* n. sp. is clearly quite closely related to two females (both from a Ugandan crocodile) which were collectively described, together with two males from a Ghanaian crocodile, under the binomial *S. cesarisi* by Riley *et al.* (1990). However, in the light of the present investigation it now seems likely that this latter diagnosis was incorrect. This conclusion stems from an appraisal of several specimens (seven females and one male) of a Botswanan species, which exhibit a unifying and distinctive set of characteristics (including the possession of a small spiky extension to the fulcrum), but which also had an oral cadre that was very similar to the stylised drawing of this structure allegedly from the type-series of *S. cesarisi* (p. 194 in Sambon, 1922; reproduced in Fig. 1C). Accordingly, these eight specimens are designated *S. cesarisi* in this paper, and the females of those thus described by Riley *et al.* (1990) are now considered to be closely related to, if not synonymous with, *S. okavangoensis* n. sp. The status of the two alleged '*S. cesarisi*' males (Riley *et al.*, 1990) remains unknown; they probably represent another new species, but more specimens are required before this can be confirmed.

The very large number of *S. wedli* recovered from the Botswanan crocodile reaffirms our earlier diagnosis of this species (see Riley *et al.*, 1990).

***Sebekia wedli* Giglioli in Sambon, 1922** (Figs 1A,B, 2A–D, 3; Tables I,II)

Over 75% of the collection comprised this single species (a total of 65 females and 10 males), and both sexes may be readily differentiated from the other 2 congeneric African species described below because

Table I. The principal characteristics of female *Sebekia wedli* examined in the present study

Slide number	Hook length	Blade length	Rows (spines)	Fulcrum length	Mouth (overall length)	Cadre length	Mouth width
1	76	28.5	10	165	380	230	123
2	83	33	12.5	185	375	240	130
3	83	33	12.5	184	350	237	124
4	76	35	10.5	160	360	230	130
5	85	31.5	10	158	360	220	135
6	74	28	11	178	340	185	120
7	87	33	11	178	350	240	–
8	88	36	11	192	375	230	128
9	85	31.5	11	191	370	240	126
10	76	32.5	8	202	360	238	–
11	76	35.5	11	190	335	230	–
12	80	25.5	11	163	392	235	115
13	77	31	9.5	178	340	215	128
14	75	30.5	10	144	355	230	125
15	84	30.5	13.5	180	382	247	121
16	77	30.5	12	198	377	230	127
17	78	27	11.5	155	345	230	125
18	70	29.5	10	178	320	170	105
19	70	28.5	11	165	–	220	–
20	72	26.5	11	165	367	230	110
21	–	–	12	190	393	240	113
22	84	35.5	12.5	188	362	220	123
23	74	28.5	11	170	315	195	103
24	79	31.5	12.5	184	350	225	130
25	82	29	12	190	357	238	125
26	–	–	10	187	367	220	118
27	82	33	12	174	360	240	–
28	80	27.5	11	163	342	235	117
29	82	31	12	180	350	225	110
30	80	27	12	–	365	250	120
31	89	34	12	–	360	240	–

the oral cadre is *always* open anteriorly; the intervening bridge of chitin is too delicate to be easily seen (Fig. 2A). In females for example, the lateral, anteriorly-directed prongs of the cadre are separated by a gap of 11–33 ($\bar{x}$ = 21) μm (Figs 1A, 2A) and, although the cadre of most specimens is a more or less elongate U, with sides that converge slightly anteriorly, some contraction along the long axis of the cadre is possible so that it deforms to produce a more ovoid profile.

Females (N = 62). All but 3 females (see below) were considered representative of the terminal instar and, although all of these were gravid, c. 50% were probably prepatent in that the uterus contained eggs without mature hooked larvae. The largest females measured 15–19 mm in length. The hooks of this species are comparatively small ($\bar{x}$ = 80 ± 5.6 μm), so that any variation in the orientation and presentation of hooks on slides (i.e. if they were not perfectly flattened) tended to have a disproportionate effect on the validity/consistency of the hook data. Therefore, care was taken to ensure that, as far as was possible, only well presented hooks were measured, which meant that for approximately 20% of specimens one or more hooks were discounted. Despite this attention to detail, the data are nonetheless very variable with

Table I. Continued

Slide number	Hook length	Blade length	Rows (spines)	Fulcrum length	Mouth (overall length)	Cadre length	Mouth width
32	77	30.5	9.5	185	350	220	120
33	82	28.5	12	178	342	215	125
34	84	31	12.5	185	367	217	118
35	75	28	8.5	185	315	185	100
36	74	34	10.5	195	340	215	110
37	74	28	10	155	320	215	110
38	67	31.5	9	150	350	230	126
39	74	26	11	160	320	220	120
40	84	31	10	158	345	230	121
41	86	32.5	11	180	357	221	118
42	82	29.5	10	180	375	230	110
43	79	32.5	10.5	167	365	240	105
44	79	34,5	10	185	330	230	–
45	84	38.5	10	187	347	230	108
46	72	24	10	140	336	245	128
47	70	24	9	125	327	220	121
48	85	34	11.5	195	335	215	120
49	87	34	10.5	187	347	225	–
50	76.5	30.6	12.5	165	310	205	120
51	82	28.5	11	173	365	230	118
52	80	30	10	180	360	240	–
53	88	32	12	176	355	235	130
54	86	26	11	164	370	240	123
55	87	32	10.5	187	375	235	130
56	86	35	10.5	180	345	245	125
57	81	31.5	12.5	195	350	250	120
58	82	30.5	10	169	365	235	135
59	77	28	–	170	365	245	125
60	84	33	11.5	187	340	225	125
61	87	34	11	174	350	235	135
62	80	27	10.5	173	375	240	140

mean combined hook lengths showing a range of 67–89 μm (Table I). It was also apparent from the raw data that anterior hooks are usually, but not invariably, slightly larger than their posterior counterparts ($\bar{x}$ = 81.12 ± 5.78 *vs* $\bar{x}$ = 78.2 ± 5.65) with most of this being accounted for by discrepancies in the length of the hook blade ($\bar{x}$ = 31.75 ± 3.56 *vs* $\bar{x}$ = 29.75 ± 4.5: see Fain, 1961) (NB. the hook data in Table I and Fig. 3 are the mean of the combined anterior and posterior hook measurements). In one extreme case (specimen 10), a difference of 12 μm was apparent in the overall lengths of anterior and posterior hooks. There was no obvious difference between hooks in terms of spination (estimating these is subjective anyway), but between 9–13.5 rows of spines could be discerned ($\bar{x}$ = 10.9). When data were fully collated (Table I), it was apparent that 3 females (Nos 18, 23 & 35), whilst possessing hooks that were well within the size range of the sample, had small buccal cadres (170–195 μm) compared to the remainder ($\bar{x}$ = 229 ± 14.5, range 205–265; Table I): a re-examination of these specimens revealed either no eggs (No. 35) or very few soft-shelled, unembryonated eggs (18,23) and we conclude that they alone probably represent the penultimate instar. Therefore, for some individuals, the terminal moult may entail only a relatively small increase in hook size.

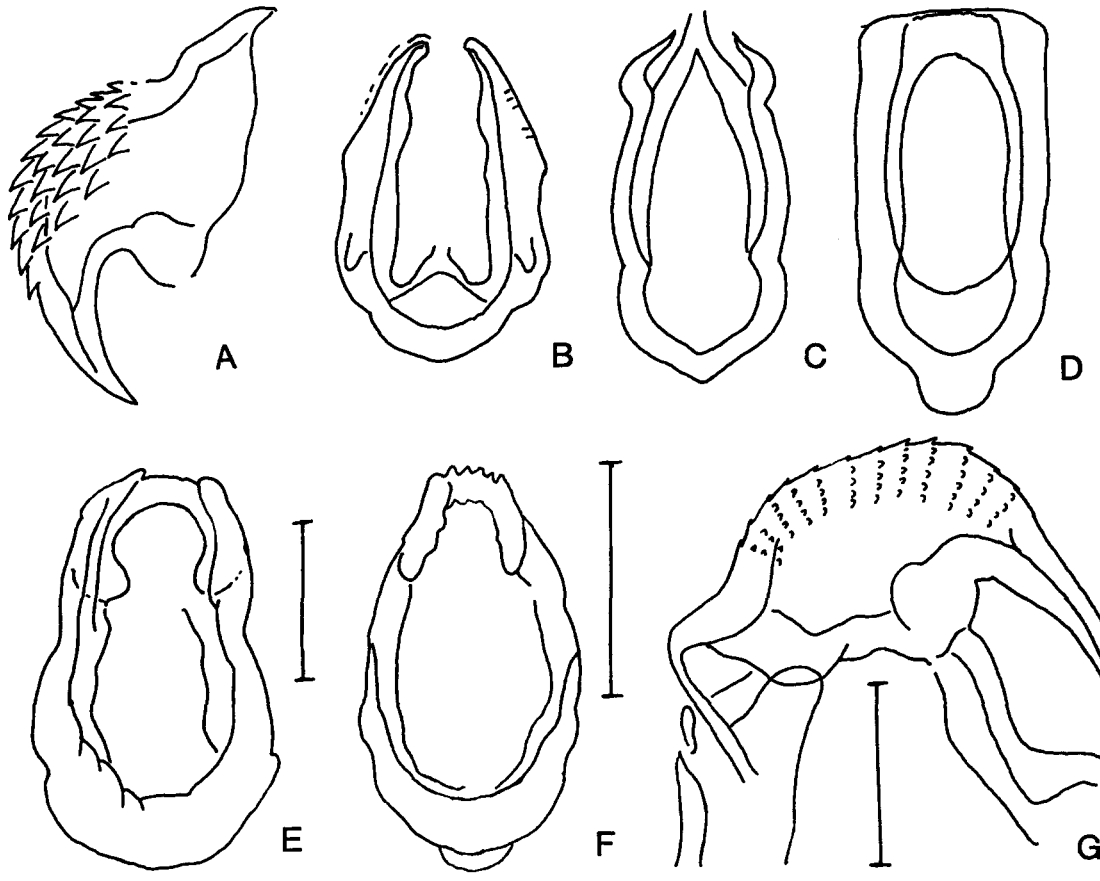

Fig. 1. A–D. The somewhat stylised diagrams, copied from Sambon (1922), of the type-material of *Sebekia* and *Alofia* spp.: (A) is the serrated hook and (B) the oral cadre of *S. wedli* (from pages 189 & 194), (C) is the cadre of *S. cesarisi* and (D) is that of *Alofia merki* (both from p. 194). No scale-bars, nor measurements of these structures were provided. For comparison (E) and (F) are the buccal cadres of female and male (specimen nos 5 & 8, respectively) of *S. cesarisi* described herein. Depending on the plane of focus, the anterior flanges assume varying degrees of prominence, and a diagram of the form shown in (C) can result. The double indentation in the lateral walls, as depicted in (C), exists in all of the present specimens, although it is not quite so obvious. The blade (G) of the posterior hook of the paratype female of *Alofia nilotici* n. sp. is offset from the long axis of the shank by about 90°. The rows of minute spines are extremely difficult to detect and their exact disposition may be slightly different from that shown. *Scale-bars*: E, F, 100 μm; G, 50 μm.

Of the remaining data, cadre dimensions (length $\bar{x}$ = 229 ± 14; width $\bar{x}$ = 121 ± 9) are again variable, as is the overall length of the buccal complex (x = 355 ± 18). Some of this variation is reconcilable because the various components of the buccal complex appear to be distorted by muscular contraction, particularly the position of the pharynx relative to the cadre, and flattening during mounting can distort it further. A graph of hook length against cadre length reveals a cluster of values which clearly differentiates mature specimens from the three individuals postulated to represent the penultimate instar (Fig. 3).

No significant differences in the length of anterior and posterior fulcra were detected ($\bar{x}$ = 176.6 ± 17 *vs* $\bar{x}$ = 176 ± 15.5), but overall length is significantly affected by the state of contraction (at the time of fixation) of the various muscle systems which attach to the fulcrum. Mature *S. wedli* eggs (N = 100) measure 84.1 ± 2.3 × 56.2 ± 2.5 μm and thus are significantly smaller than those of the other two congeneric species.

Males (N = 10). Males were up to 8.5 mm in length: a total of six of the specimens examined were within the 8–8.5 mm size range. Annuli could not be counted. As with females, the anterior hooks were very slightly larger than the posterior hooks and the former possessed longer blades (Table II). Between 8 and 12 rows of spines could be seen. The small difference

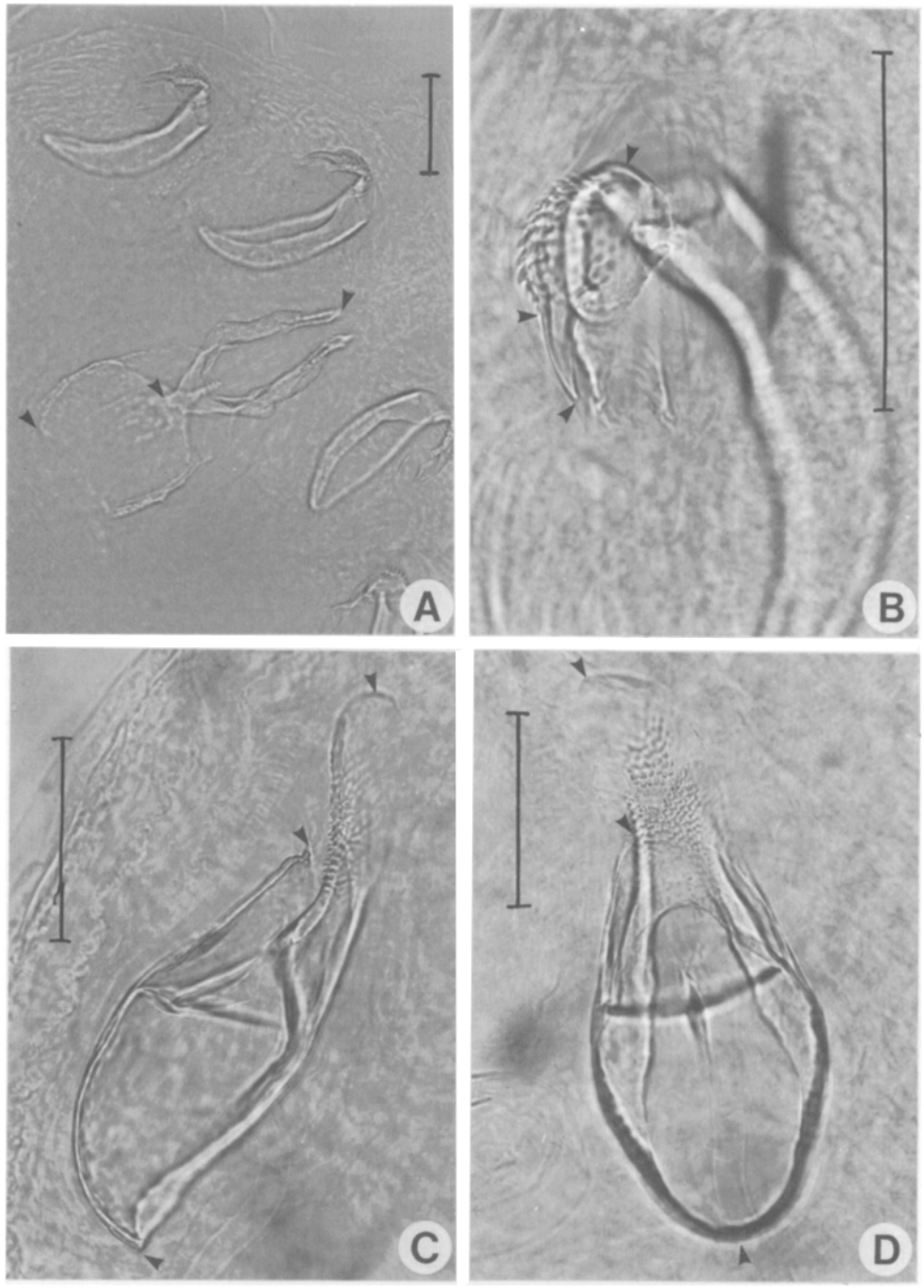

Fig. 2. Light micrographs of *Sebekia wedli*. A. Head of a female (no. 22) showing the oral cadre with the characteristic gap between the lateral prongs (cf. Fig. 1B) associated with the barrel-shaped pharynx. A deep groove, with obvious rugosities, extends from the pharynx anteriorly into the oral cavity. The outer arrows indicate overall length of the buccal complex and the middle arrow indicates the posterior limit of the cadre; this is often obscured by converging fibres of chitin in the pharynx. The four hooks are visible as are three of the supporting fulcra. B. Detail of a single posterior hook from (A) showing the dimensions measured; the large arrows indicate the overall length from the hook tip to the posterior insertion of the apodeme, whereas the central arrow indicates the posterior limit of the hook blade. C,D. The copulatory spicule (slide no. 71) from the lateral and dorsal aspects, respectively. The overall length of the cowry shell proximal region is indicated. The overall length, which includes the spatulate anterior extension is indicated by the top and bottom arrows. Rows of backward-projecting spines, which ornament the spatulate extension, gradually disappear towards the tip. *Scale-bars.* 100 μm.

Table II. The principal characteristics of male *Sebekia wedli* from *C. niloticus* examined in the present study

	Hook dimensions													
	Ant.		Post.		Rows of spines		Fulcrum length		Mouth dimensions			Copulatory spicule		
Slide No.	Length	Blade	Length	Blade	Ant.	Post.	Ant.	Post.	Overall	Cadre	Width	Overall	cs	Width
63	60*	28*	60*	26*	9*	8*	138	123	186	125	75	295	200	120
64	61*	26*	57*	26*	–	9*	140	127	216	143	76	310	200	118
65	61*	28*	59	23	9*	8*	147	140	220	138	78	338	240	120
66	61	31	61	26	10	9	145	135	217	138	79	320	218	115
67	59	31	59	26	12	11	146	138	220	143	75	340	225	120
68	61*	26*	51*	26*	–	10	134	125	217	138	74	285	200	115
69	59*	29*	–	–	9	–	132	132	211	130	74	308	225	110
70	54*	23*	61*	23*	9	9	112	200	–	–	–	295	205	105
71	61*	28*	61*	29*	8	10	127	135*	220	133	77	298	215	110
72	60	25.5	56	25.5	8	8	150	135	200	125	75	310	198	108
Mean ± SD	59.4 ± 2	28 ± 2.5	58.5 ± 3	25.5 ± 2			136 ± 10	131 ± 7	212 ± 11	136 ± 6	76 ± 2	310 ± 18	213 ± 14.6	115 ± 5

*Only one measured.

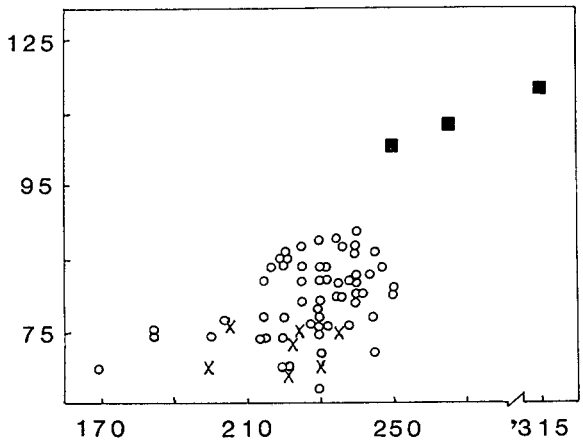

Fig. 3. A graph of mean hook length (y axis) plotted against the length of the buccal cadre (x axis). The values for *Sebekia wedli* (o) form a more or less discrete cluster, apart from the three values (bottom left), which probably represent preadult instars. *S. cesarisi* (×) resolves as a cluster which overlaps with that of *S. wedli*, whereas *S. okavangoensis* n. sp. (■) is well separated from these two. Had the sample size of *S. cesarisi* been larger, it is likely that a statistically significant difference between the overlapping cluster groups would have emerged.

between the lengths of the anterior and posterior fulcra was not significant. The buccal complex, smaller than that of the female, measures 212 ± 11 μm overall and the average length of the cadre, which, like that of the female, is always open anteriorly, is 135 ± 6 μm (Table II).

The paired copulatory spicules are distinctive, heavily sclerotised structures, the proximal portion of which is shaped like a cowry shell in lateral view with a large oval opening on the ventral surface (Fig. 2C,D). Projecting anteriorly from the cowry shell (which measures 213 ± 14.0 μm in length) is a spatulate extension which carries 12–14 rows of backward-pointing conical teeth (Fig. 2C,D). In cleared specimens the rounded tip of this extension is smooth and transparent and can only be seen using diffracted light. The amuscular paired cirrus threads, coiled within the cirrus sacs, are modified distally in that, over the terminal 0.25 mm or so, the penis first narrows to form a stout tube of thickened chitin, which abruptly changes into a section of very thinly chitinised tube ending in the tip, drawn out to form a delicate hollow needle which may be folded. Although all of the *Sebekia* males described herein are variations on this theme, the dimensions of the component parts of the structure vary markedly between species. For example, the needle-like tip in *S. wedli* is about 30 μm long, that of *S. cesarisi* is about 60 μm but very lightly chitinised, whereas that of *S. okavangoensis* n. sp. is again needle-like but 120 μm long.

***Sebekia okavangoensis* n. sp.** (Figs 3, 4A–E; Table III)

As already discussed, this species is very close to, and probably synonymous with, *females* of the species described earlier under the binomial *S. cesarisi* by Riley *et al.* (1990).

Females (N = 3). Only one female is intact, the other 2 being ruptured and with a portion of the lower abdomen missing. The holotype female (No. 2) is very long and slender (27 × 1.4 mm) with 99 annuli, much of the abdomen is of a uniform diameter and 3–4 mm of the caudal extremity is tapered gradually to a rounded point. The anterior half of each annulus carries a broad (20–75 μm wide), irregular band of chloride cells, 3–6 cells deep, the pore caps of which are visible externally as clusters of transparent papillae, measuring 2–4 μm in height.

The hook blade is long, strongly curved and sharply demarcated from a prominent, domed dorsal elevation of the hook which carries an estimated 14–17 rows of robust spines (Figs 4A,B). However the most obvious diagnostic feature is the substantial (approx. 75 × 18 μm heavily chitinised, finger-like anterior extension to all fulcra which is equipped distally with a cluster of small spines (Figs 4A,B). This extension, positioned equidistant between the fulcrum and the hook, is connected to both of these structures by thin sheets of chitin. An apodeme, connecting the posterior hook retractor muscle to the fulcrum, passes between the hook and the extension. The oral cadre is distinctly mitre-shaped and, although the lateral prongs are connected by a bridge of crenellated chitin anteriorly (Fig. 4C), slight separation of these structures is possible so that the cadre can assume a more parallel-sided configuration. The pharynx is much more lightly chitinized than in related species (Fig. 4C). Eggs, which are fully embryonated only in a single specimen (No. 3), are close to those of *S. cesarisi* and measure 99.1± 2.0 × 69.5 ± 1.85 μm.

Male. The single male is substantially longer and proportionately more slender (23 × 1.1 mm) than the males of the other species and surprisingly the dimensions of hooks, fulcra, oral cadre, etc. are virtually indistinguishable from those of the female (compare Table III with Tables II and IV). The copulatory spicules are comparatively large, measuring 625 μm in overall length, and the so-called cowry shell component of the spicule (which is not particularly obvious in this specimen) is 440 μm long and 160 μm wide (Fig. 4D,E). Virtually the whole of the rounded, spatulate anterior extension of the spicule is covered with a tightly-packed array of non-overlapping, smooth discoid scales which decrease in size towards the tip (Fig. 4D,E).

Type-host: *Crocodylus niloticus* Laurenti.
Type-locality: Okavango Swamps, Botswana.

***Sebekia cesarisi* Giglioli in Sambon, 1922** (Figs 1C,E,F, 5A–F; Table IV)

Both sexes are generally claviform, being widest in the anteriormost third of the abdomen, and the caudal extremity tapers gradually to a bluntly-rounded point; superficially this species is virtually indistinguishable from *S. wedli.*

Females (N = 7). The largest female that could be accurately measured is 16 × 1.5 mm but the three remaining intact specimens, which were folded, measure approximately 12–17 mm long. The hooks are intermediate in size between *S. wedli* and *S. okavangoensis* (Fig. 3; Table IV) and, although they carry the same spination as the former, the slightly curved blade is relatively small compared to overall hook length, and it merges smoothly with the dorsal patch of spines (Fig. 5C). Fulcra are consistently larger than those of *S. wedli*, and in most specimens a small spiky extension (c. 12 μm long) is present between each of the hooks and its fulcrum (Fig. 5C). The oral cadre is similar to that of *S. cesarisi* (compare Fig. 5A,B,D with Fig. 1C which is copied from p. 194 in Sambon, 1922) in that it is pear-shaped and closed anteriorly by a bridge of chitin (Fig. 5A). The cadre is a continuous hoop of chitin over most of its circumference, but anteriorly sclerotised triangular flanges extend dorsally from the cadre and constitute a lining in the front part of the oral cavity (Fig. 5A,B). These structures, which seem to be unique to this species, are viewed to best advantage from the lateral aspect (Fig. 5D). Egg dimensions (N = 100) are marginally greater than in other species, being 104 ± 2.7 × 68.8 ± 2.1 μm.

Male. Only one male from the entire collection could be definitely ascribed to this species. It measures c. 10 mm in length and the hooks are slightly smaller than, but otherwise identical to, those of the female. Fulcrum dimensions are intermediate between those *S. wedli* and *S. cesarisi*, and the spicule is very elongate and delicate (Fig. 5E,F; Table IV). Scale-like ornamentations, which are restricted to the basal portion of the spatulate anterior extension of the spicule, when viewed from the lateral aspect appear as backwardly-projecting spines (Fig. 5F).

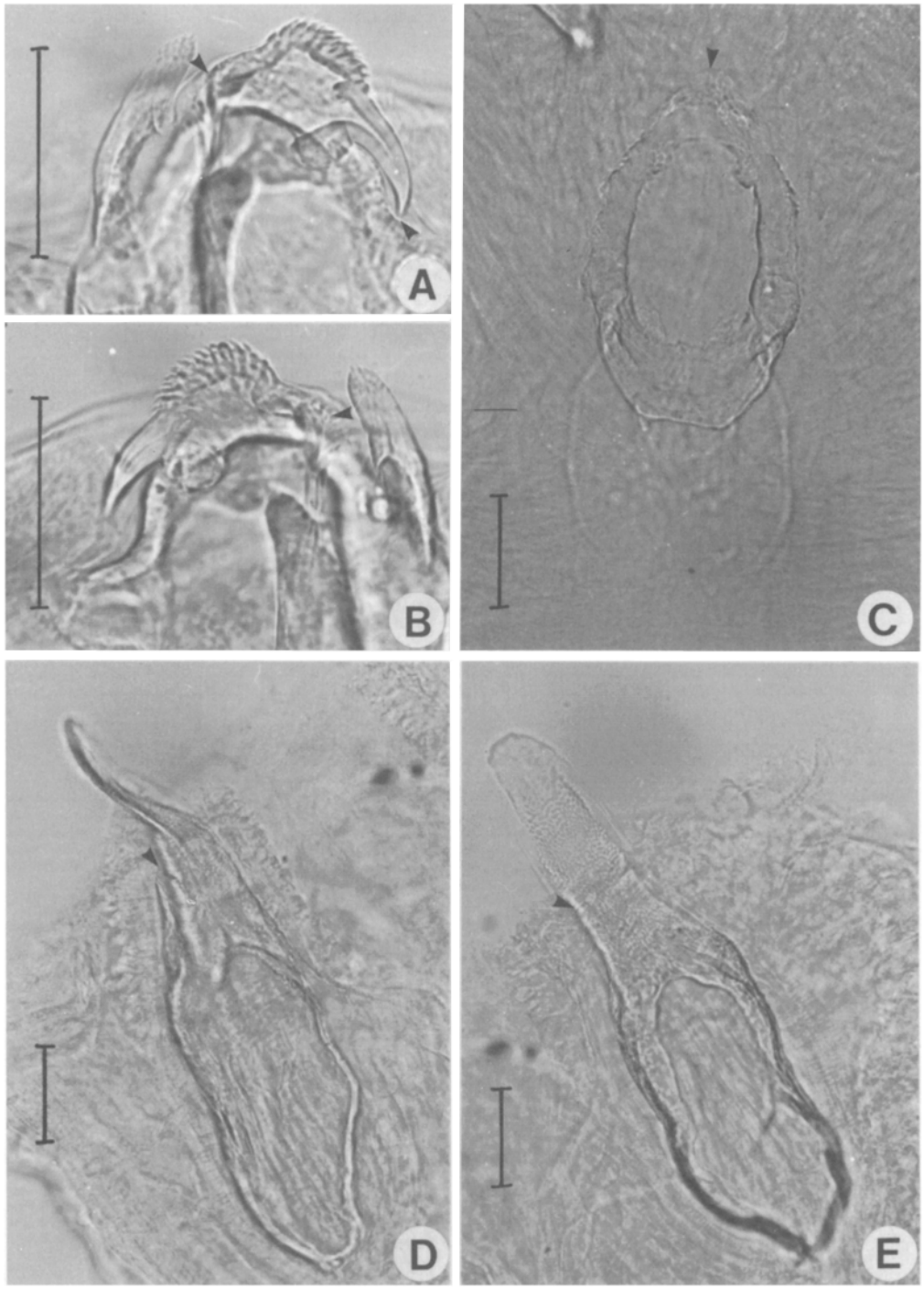

Fig. 4. Light micrographs of *Sebekia okavangoensis* n. sp. A,B. Anterior hook (A) of holotype female and the posterior hook (B) of a paratype female (Nos 2 & 3, respectively) showing the robust nature of the hooks and the prominent dome of spines on the dorsal surface. The large finger-like extension of the fulcrum, equipped with a cap of spines, is obvious. An arrow indicates the posterior insertion of the apodeme and overall length is the distance between this and the hook tip. C. The oral cadre of a paratype female (No. 1) showing its characteristic pointed apex (arrow), flattened base and the barrel-shaped, thinly chitinised pharynx. The whole assembly is more delicate and much less heavily armoured that that of related species. D,E. The copulatory spicule of the paratype male (No. 4) from the lateral (D) and dorsal C aspects showing that the cowry shell proximal component is poorly defined (an arrow indicates its anterior margin), whereas the spatulate extension is comparatively large. Much of the latter is covered by discoid scales. *Scale-bars*: 100 μm.

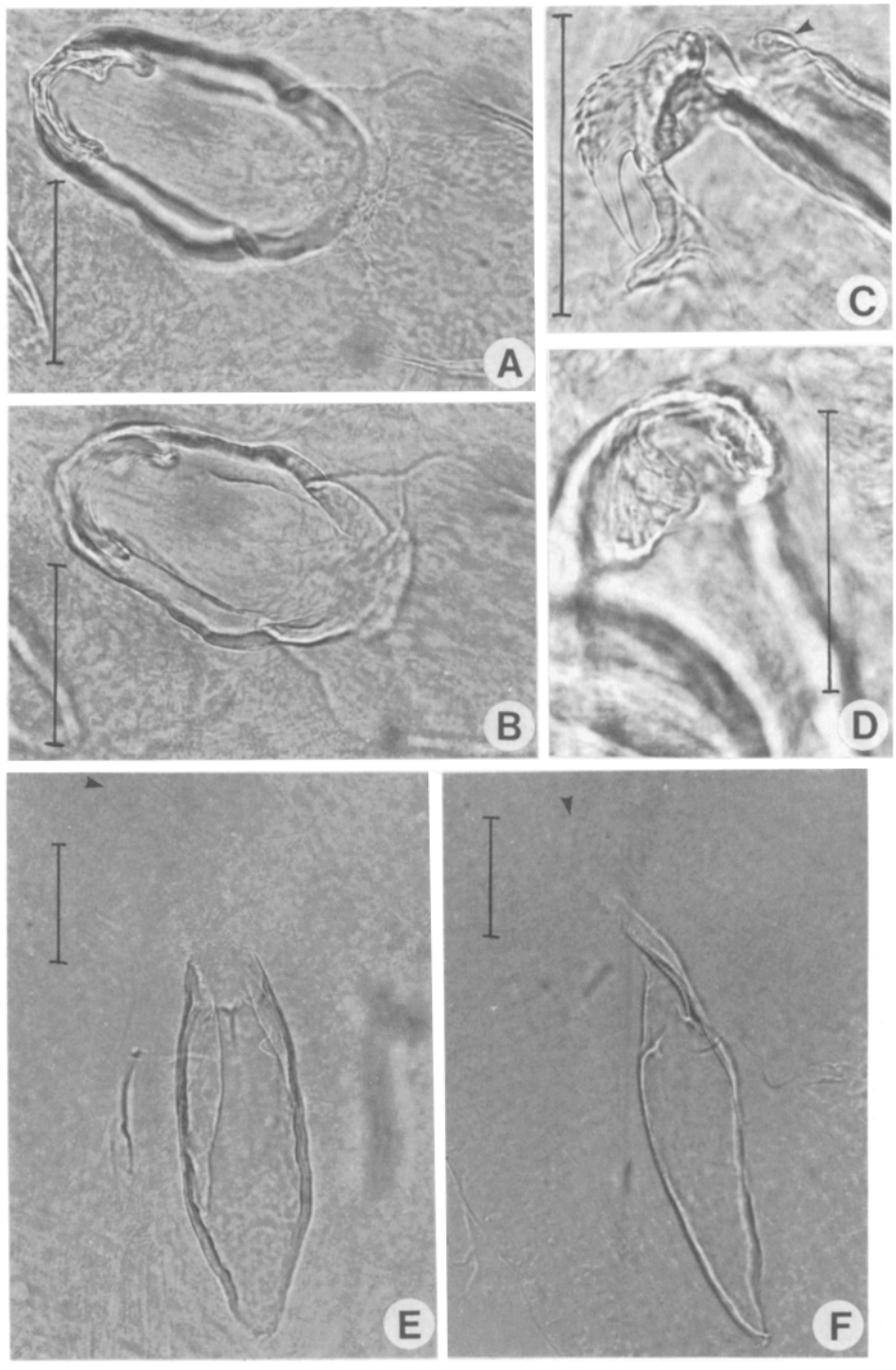

Fig. 5. Light micrographs of *Sebekia cesarisi*. A,B. Two micrographs of the oral cadre of a female (slide No. 4) in different planes of focus which reveal the anterior sclerotised extensions lining the front part of the oral cavity. Although the overall shape resembles that of *S. okvangoensis* n. sp. (see Fig. 4C), the cadre is composed of thicker chitin and the posterior border is rounded rather than flattened. C. The anterior hook of a female (No. 5) showing the small peg-like extension to the fulcrum (arrowed) that characterises all of the hooks of both sexes. In most other respects these hooks are difficult to differentiate from those of *S. wedli*. D. The dorsally-projecting, triangular flanges lining the front of the oral cavity of a female (No. 7) viewed obliquely from the side. The shadow in the bottom left corner is part of a fulcrum overlying the cadre. E,F, The copulatory spicules of the single male showing their distinctive features. The cowry shell is delicate and markedly pyriform. (E) is viewed dorsally and (F) laterally. Scale-like ornamentation is limited to the basal third of the spatulate extension with the result that its transparent tip is difficult to observe (arrows indicate its position). *Scale-bars*: 100 μm

Table III. The principal characteristics of female (1–3) and male (4) *Sebekia okavangoensis* n. sp. from *C. niloticus* examined in the present study

	Hook dimensions										
	Ant.		Post.		Rows of spines		Fulcrum length		Mouth dimensions		
Slide No.	Length	Blade	Length	Blade	Ant.	Post.	Ant.	Post.	Overall	Cadre	Width
1	102	50	104	50	16	15	280	290	340	250	170
2	112*	48	112	47	16*	17	283	295	440	315	190
3	106*	51*	108*	51*	14*	15*	285	290*	–	265	180
Mean ± SD	106 ± 4	49.5 ± 1	108 ± 3	49 ± 2	–	–	283 ± 2	292 ± 2	–	284 ± 21	180 ± 8
4	107	54	107*	59*	15	16*	250	245	380	260	180

*Only one measured.

***Alofia nilotici* n. sp.** (Fig. 6A–D; Table V)

This species represented by 2 males and 2 females may be immediately recognised as belonging to the genus *Alofia* by the possession of a U-shaped buccal cadre, hooks with blades that are fine, canaliculated and bent almost through a right angle (NB. in females only; see Fig. 6A,B), and the flask-shaped, hook-necked copulatory spicule (Fig. 6C; and see Riley, 1994). Uniquely, the anterior and posterior hooks of both sexes carry an extensive dorsal patch of minute spines (Fig. 1G).

Females (N = 2). The lengths of the paratypes are 16 and 17 mm. Spines on the dorsal edge of the hooks of the paratypes (Slides 1 & 2) are arranged in approximately 13 discrete rows; these are quite widely separated anteriorly (by about 7–8 μm) but more crowded together posteriorly. About 12 spines can be counted (with great difficulty) in each row (Fig. 1G). Anterior and posterior fulcra are of equal length. The lateral prongs of the large U-shaped mouth converge anteriorly leaving a gap of c. 50 μm, and are inwardly bowed in the mid-section, giving an overall hour-glass appearance (Fig. 6A,B). The small peg-like projection from the barrel-shaped pharynx extends into the cadre and carries about 9 rows of prominent spines (with up to 5 spines in each row) in a groove. Eggs (N = 20) measure 84 ± 2.2 × 60 ± 1.1 μm.

Males (N = 2). The small holotype and paratype males, which measure 9–10 mm in length, are distinctly claviform and taper gradually over the posterior half of the body to a conical tail. Hooks similar in overall length to those of the female but only because the blade and shank are less sharply bent; the hooks are in fact smaller. Oral cadre U-shaped but slightly different from that of females, as the lateral prongs bow out, giving an overall oval appearance. The cadre is open at the front in the paratype male but delicately bridged by chitin in the holotype male. Copulatory spicules are typical of the genus *Alofia* in being flask-shaped, with an extensive elliptical opening on one side of the cowry shell base; the elongate neck of the flask is a longitudinally grooved projection which terminates in a double-hooked lip. Opposite this is a longer, finer spatulate extension covered by rows of non-overlapping scales which gradually peter out towards the tip (Fig. 6C). The distal portion of the penis is massive relative to the spicule. Following the narrow, heavily chitinised tubular section (present in *Sebekia* spp.), a delicate, thinly-chitinised tube, 70-100 μm in diameter by 1.6 mm long terminates in a folded, transversely striated region (the striations are hoops of chitin), the end of which is pulled out to form a very finely tapered hollow needle of chitin measuring c. 240 μm in length (Fig. 6D).

Type-host: *Crocodylus niloticus* Laurenti.
Type-locality: Okavango swamps, Botswana.

SEM observations

The initial selection of the six specimens (5 females and one male) for SEM examination was made purely on the grounds of external appearance and state of preservation; fortuitously all three *Sebekia* species were represented. Originally we thought that all 4 species were present, but closer examination of the single *Alofia* specimen revealed that unaccountably its hooks were devoid of spines. The 2 remaining specimens had flak-

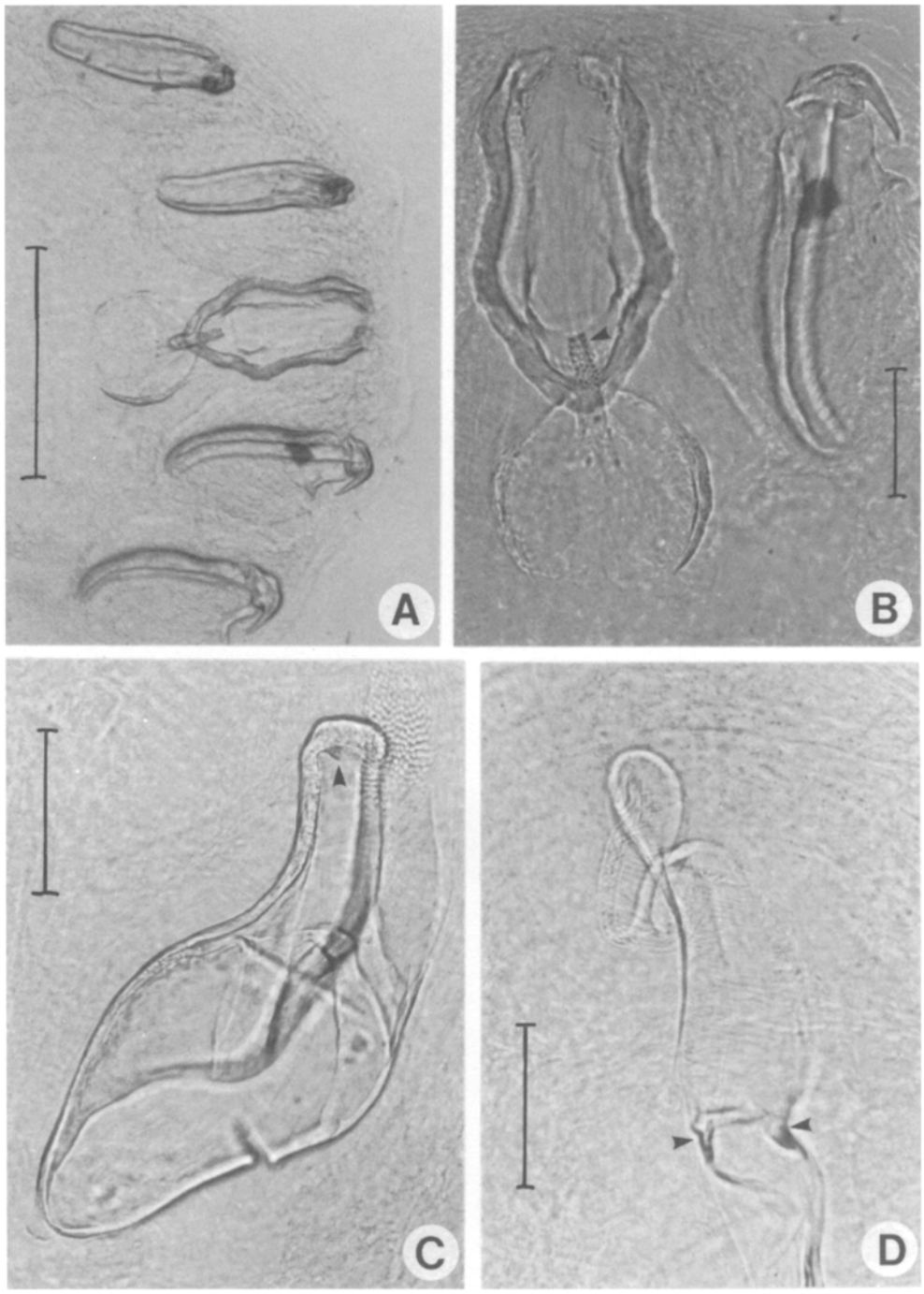
A
B
C
D

Fig. 6. Light micrographs of *Alofia nilotici* n. sp. A. The head region of a paratype female (slide No. 1) showing the large U-shaped cadre flanked by two hook pairs which are associated with long fulcra which are slightly curved proximally. Only two hooks could be measured. B. Detail of the mouth and anterior hook of (A) showing the characteristic slender bent blade of the hook and the buccal complex. The bowed sides of the cadre is typical of females whereas in males the prongs are bowed in the opposite direction giving a more ovoid profile. Note the anterior grooved extension of the pharynx into the cadre; the groove appears to be lined with spines (arrow). C. The flask-shaped copulatory spicule of the holotype male (slide No. 3) from the lateral aspect showing the large opening in the cowry shell component and the long grooved neck which terminates in a double-hooked lip (one hook is arrowed). Opposite the neck is a spatulate extension with scale-like rugosities which peter out towards the tip (the latter is not shown on this micrograph); this structure resembles the hypostome of a tick. D. The cirrus tip of the holotype male. The terminal portion of the cirrus is a wide diameter chitinous tube which narrows distally to a thickened collar of chitin (arrows). Beyond the collar the tube is twisted and or folded, and ornamented with hoops of chitin before terminating in a finely tapered hollow needle measuring 250 μm in length. *Scale-bars*: A, 400 μm; B–D, 100 μm.

Table IV. The principal characteristics of female (1–7) and male (8) *Sebekia cesarisi* from *C. niloticus* examined in the present study

	Hook dimensions										
	Ant.		Post.		Rows of spines		Fulcrum length		Mouth dimensions		
Slide No.	Length	Blade	Length	Blade	Ant.	Post.	Ant.	Post.	Overall	Cadre	Width
1	76.5*	28*	74*	23*	10*	12*	207	202	365	235	150
2	76.5*	26*	76.5*	26*	11*	9*	190	183	340	225	132
3	–	28	74	26*	–	9.5	187	175	350	223	118
4	73	23	66	29*	11	11	165	160	320	200	115
5	79*	29	74	29	10*	10.5	185	165	330	205	135
6	68	29	73	27	10.5	10*	185	183	340	230	–
7	71*	30*	68*	25*	10*	8*	200	145*	350	220	123
Mean ± SD	74 ± 4	27.5 ± 2.4	72 ± 3.7	26.5 ± 2	–	–	188 ± 13	173 ± 18	342 ± 15	219 ± 13	128 ± 13
8	67.5	31	647*	25.5*	8?	12*	174	174	245	178	92

* Only one measured

ing cuticles and were of limited use. Only novel observations are briefly reported here.

Sebekia wedli (N = 3)

A maximum of 10 rows of spines could be counted on any one hook using SEM (Fig. 7A), and each row carried up to 10 spines (Fig. 7B). Each hook is flanked by a domed lateral sensory papillae, and above the anterior hook pair, just inside of the frontal papilla, is the emergent duct of the frontal gland (Fig. 7A,B). Three spiky sensory sensilla are positioned above this duct (Fig. 7C).

Sebekia okavangoensis n. sp. (N = 1)

SEM observations of the single male revealed that chloride cell pore caps tend to increase in density towards the mid-ventral line (Fig. 7D); the genital pore, although partly obscured by adherent material, appears to lack flanking sensory papillae (Fig. 7F). Lateral sensory papillae flank each hook (Fig. 7D) although these have a much lower profile than those of *S. wedli*. At least 3 sensilla occur on the frontal papillae, and 2(?) more are located within the efferent duct of the frontal gland. The dome of spines on the hooks is prominent and those present on the extension to the fulcrum are significantly smaller (Fig. 7E).

Sebekia cesarisi (N = 1)

Very similar to *S. wedli* in all major respects, [including arcs of 3(?) chloride cell pore caps between each hook and the mouth (Fig. 8A)]. The small (20 μm long) spiky extension to the fulcrum, visible only on one (posterior) hook, may be sensory (Fig. 8A).

Alofia sp. (N = 1)

Three of the 4 hooks of this single female were visible but there were no evidence of spination on any hook at any magnification (Fig. 8B). Because of this, we remain uncertain as to the specific status of this particular specimen. Lateral lines were well developed and a single row of chloride cell pore caps demarcate the leading edge of each abdominal annulus (Fig. 8A). The caudal extremity is bluntly rounded and the vagina and anus occupy a common groove.

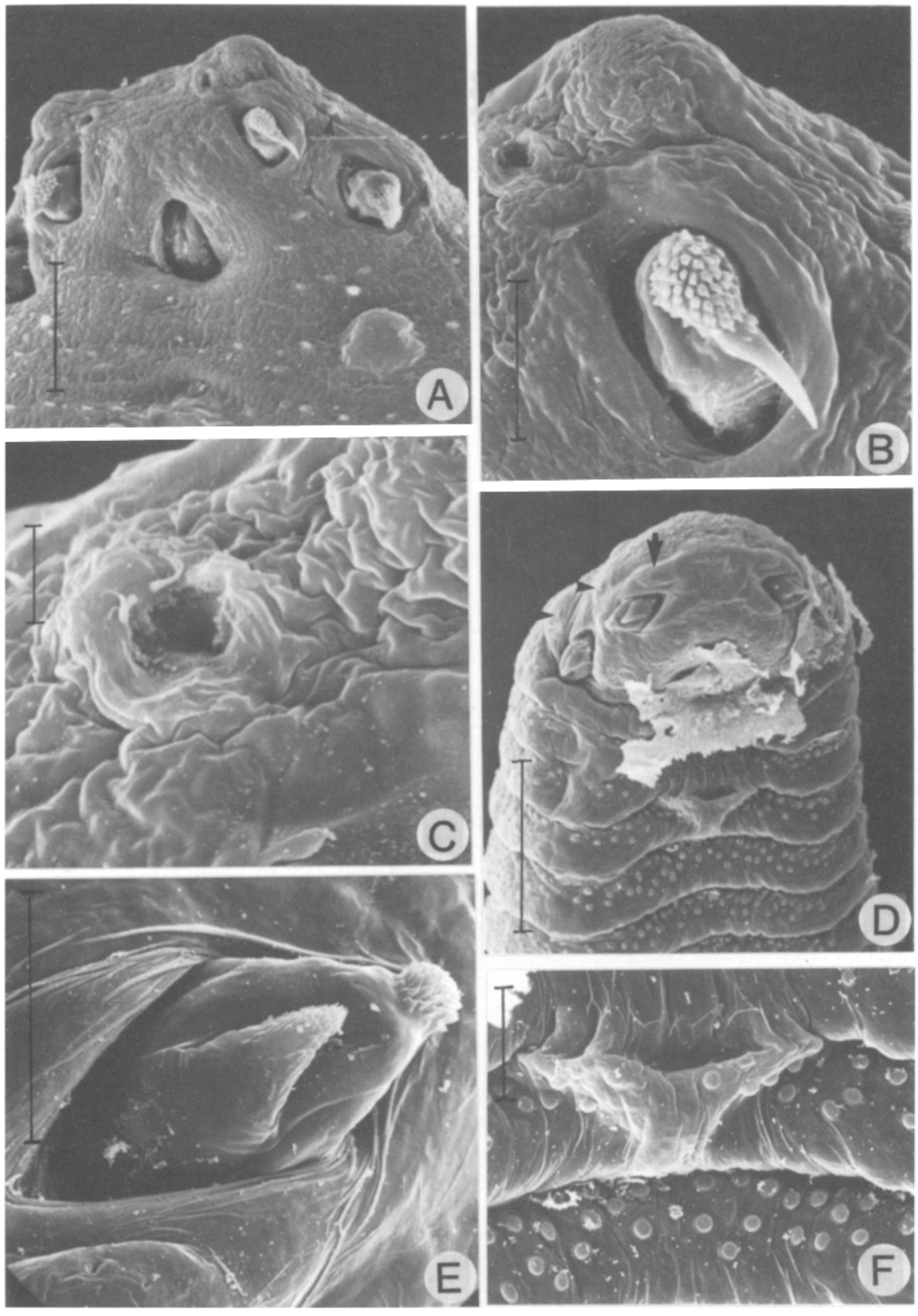
A
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Fig. 7. SEM micrographs of *Sebekia* spp. A–C. A single specimen of *S. wedli*. A. The head from the ventral aspect showing the mouth which narrows anteriorly, and the hooks with their adornment of spines. The emergent ducts of the frontal gland are visible above the anterior hooks and inside of the large frontal papillae. Lateral papillae flanking the hooks are arrowed. B. Detail of the frontal papilla, frontal gland duct and an anterior hook – 10 rows of spines may be counted on the latter. C. Detail of the frontal gland duct showing the three spiky sensillae which are positioned above it. D–F. A single male of *S. okavangoensis* n. sp. D. The head region and abdomen. The frontal papillae (large arrow) and the lateral papillae (small arrow) are indicated, and the broad bands of chloride cells occupying the anterior half of each annulus increase in number towards the mid-ventral line. E. Detail of an anterior hook from above showing the prominent patch of spines on the dorsal margin of the hook together with the substantial extension to the fulcrum which itself carries a cluster of smaller spines. F. Detail of the genital pore. The chloride cell pore caps are obvious but there are no flanking sensory papillae. *Scale-bars:* A,E,F, 100 μm; B, 50 μm; C, 10 μm; D, 500 μm.

Table V. The principal characteristics of female (1,2) and male (3,4) *Alofia nilotici* n. sp. examined in the present study

	Hook dimensions		Fulcrum length		Mouth dimensions			Copulatory spicule	
Slide no.	Ant.	Post.	Ant.	Post.	Overall length	Cadre length	Cadre width	Overall length	Cowry shell length
1	107	117	290	295	480	330	180	–	–
2	122	112	230	220	–	300	170	–	
3	104	108	224	216	350	235	130	585	445
4	101	104.5	243	242	365	230	125	520	400

Discussion

All of the chitinous structures measured in this study (of *S. wedli* in particular) display continuous variation and this, coupled with the highly disparate sample sizes of the four species under consideration, serves to illustrate the difficulties inherent in the specific diagnosis of pentastomids. At least three sources of this variation can be identified: that which is genetically based (about which we know nothing), that which results from environmental effects during ontogeny, and that which results from specimen preparation. Despite the fact that a single host was involved, the second is likely to be significant. For example, cohorts of infective nymphs of the various species (encysted in fishes), would probably have gained access to the host over a protracted period. Thereby, individual nymphs will have been exposed to (say) a developing acquired immunity at different phases and for different periods of time, and this could introduce a diversity of growth conditions. Although care was taken to minimise the effect of specimen preparation, clearly it can never be eliminated entirely because hooks, fulcra and buccal complexes are manifestly three-dimensional structures which are difficult to flatten without distortion. Nonetheless, whilst no single character can consistently discriminate *all* congeneric (*Sebekia*) species (see Riley *et al.*, 1990), unless differences are marked and the sample size is large, appropriate combinations of characters can.

The diagnosis of *S. wedli* is quite straightforward, despite the perfunctory nature of the type description (see Self & Rego, 1985). The buccal cadre of the type-series clearly opens anteriorly [i.e. the lateral prongs are united by a very delicate bridge of chitin which may be scarcely visible in cleared specimens (Fig. 1B)], a trait shared by *S. trinitatis* Riley, Spratt & Winch, 1990 and *S. mississippiensis* Overstreet, Self & Vliet, 1985: in all other species the prongs are, to varying degrees, much more strongly united (Riley *et al.*, 1990). According to Fain (1961), both sets of hooks of a single female specimen (taken from *C. niloticus* on the river Luapula, a tributary of the Congo in Zaire) measured 80μm (overall length) and the blade of the anterior set was 5–10 μm longer than that of the posterior pair. The four female specimens examined by Riley *et al.* (1990) had hooks in the range 81–90 μm, fulcra 202–213 μm and an oral cadre of 195–220 μm (all overall length). This accords well with the present data and, on the basis of this limited data set, it seems that any geographical variation that might exist in this species, does not extend to the independent variables that we have measured. Thus *S. wedli* appears to be widely distributed through tropical central Africa

S. cesarisi remains particularly problematical because relatively few specimens were available for examination, and the only useful clue to its identity was the stylised drawing of the oral cadre of the type-material (in Sambon, 1922; Fig. 1C). Cadres are three-dimensional structures, and by combining fea-

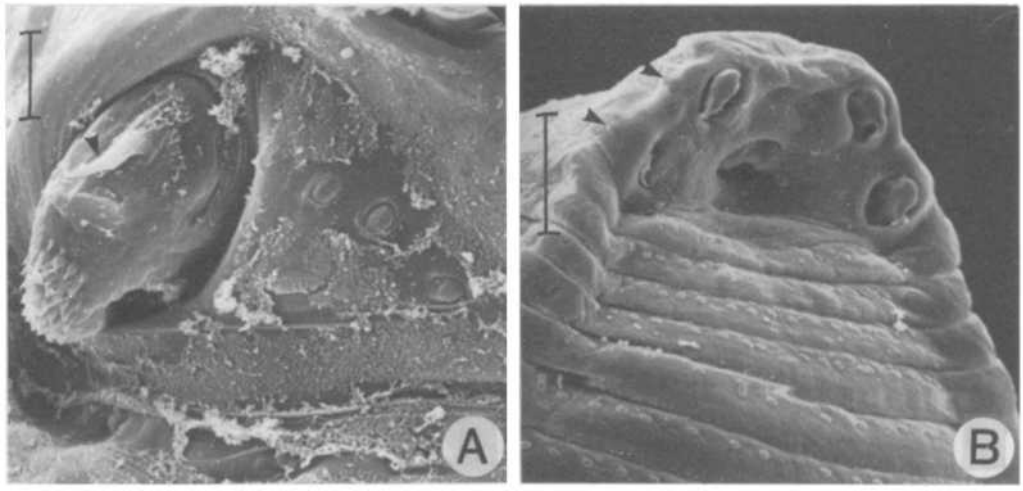

Fig. 8. A. Detail of a posterior hook of *Sebekia cesarisi* showing the sensory (?) spike between the hook and the fulcrum (arrowed) and the three chloride cell pore caps (?) which extend from it in an arc towards the mouth. B. The head region of a female *Alofia* sp. from the ventral aspect. The lateral line is prominent in this species and chloride cell pore caps are arranged in a line along the anterior edge of each annulus. The barb of one hook is disposed to show the characteristic bend and lateral papillae flanking the hook are arrowed. No spination was apparent on any hook. *Scale-bars:* A, 20 μm; B, 200 μm.

tures observed in several planes of focus, a cadre such as that shown in Fig. 5A,B can be constructed to resemble that of *S. cesarisi* (Fig. 1C). Incidentally, Terzei, the illustrator in much of Sambon's (1922) taxonomic work, exhibited a persistent tendency to oversimplify diagrams. Nonetheless, the double indentation in the lateral arms of the cadre (Fig. 1C), apparent in all of the present specimens, leads us to conclude that we have (finally) identified *S. cesarisi*. The spiny extension to the fulcrum, present on the one or both fulcra of the eight *Sebekia* spp. that have been examined in detail (Riley *et al.*, 1990), is completely absent only in *S. wedli*. In *S. cesarisi* it is much reduced, aspinose and generally difficult to observe (Figs 5C, 8A), which would explain why it was not mentioned in the type-description. This further vindicates our conclusion that we have now correctly diagnosed *S. cesarisi*.

We postulate that *S. okavangoensis* n. sp., established from a type/series of just four specimens, should correctly include the two females described earlier by Riley *et al.* (1990) under the binomial *S. cesarisi* [BM(NH) Reg. Nos 1937.10.6.15 & 1957.12.20.16]: it is equally apparent that the males described at that time were incorrectly ascribed to this species. These may represent yet another species. At least seven other *Sebekia* species possess a dorsal patch of spines on both the hooks and an extension to the fulcrum, but the extent, number and size of the spines in *S. okavangoensis* n. sp. readily differentiate this species (see Riley *et al.*, 1990)

Comparative egg dimensions also proved to be a useful adjunct to specific diagnosis, but it is worth emphasising that all specimens were identically fixed in 70% alcohol, and only embryonated eggs, with hooked, fully-developed larvae, were selected for measurement. Providing that these two criteria are met, and the sample size is large enough, egg size could provide a useful adjunct to diagnosis.

This is the first time that cirrus dimensions of congeneric pentastomid species have been described, and, although the data are sparse, evidence of reproductive isolation is emerging. Riley (1986, 1992) has postulated that during copulation the cirrus tip (i.e. the hollow needle of chitin in the species considered herein) lodges tightly within the spermathecal duct of the female. This snugness of fit ensures that during sperm transfer, sperm contacts only the lining of the male reproductive tract before reaching the lumen of the spermatheca. This has important consequences for the prolonged storage of sperm.

Heymons (1941) noted that the type-descriptions of virtually all *Sebekia* and *Alofia* species were based on relatively few specimens. After measuring many more representatives of both genera, from the same area and host, Heymons concluded that most of the diagnostic characters, including body shape, buccal complex and hooks, were rather variable in shape and

size. This opinion was broadly endorsed by Self & Rego (1985) in their review, but it now seems likely that Heymons (1941) was confusing several species (Riley *et al.*, 1990; Riley, 1994). The present discovery of a new *Alofia* with spiny hooks does suggest a genuinely close relationship between these two genera.

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