

**OSWALDOFILARIA KANBAYA n. sp., A NEW FILARIOID WORM
(NEMATODA ; FILARIOIDEA)**

from the saltwater crocodile *Crocodilus porosus* from Australia

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SUMMARY. A new species of filarial worm, *Oswaldofilaria kanbaya* is described from *Crocodilus porosus* from Australia. This is the fourth species of the genus inhabiting a crocodilian host. Morphologically these four species are found to be very similar in some primitive features, although they are dispersed over the three southern continents.

***Oswaldofilaria kanbaya* n. sp., filaire nouvelle (Nematoda ; Filarioidea) de *Crocodilus porosus* en Australie.**

RÉSUMÉ. Description d'une nouvelle Filaire, *Oswaldofilaria kanbaya* n. sp., chez *Crocodilus porosus* en Australie. Quatre espèces du genre sont maintenant connues chez les Crocodiles. Bien qu'elles soient réparties dans les 3 continents austraux, elles sont morphologiquement proches par un ensemble de caractères primitifs.

Introduction

The three filarial parasites of crocodilians so far known all belong to the genus *Oswaldofilaria* Travassos 1933, sensu Bain (1974). Two of them were found in the Neotropical Region (Brazil), i.e. *O. bacillaris* (Molin, 1858) in *Caiman crocodilus* and in *Melanosuchus niger* (= *Caiman sclerops* and *Champsia nigra* respectively as referred to by Travassos, 1933) and *O. medemi* Marinkelle 1981 in *Paleosuchus trigonatus* from Colombia. The third species, *O. versterae* Bain, Kouyaté and Baker, 1982, occurs in *Crocodilus niloticus* from the Ethiopian Region (« South Rhodesia »).

A new *Oswaldofilaria* species is described below ; it is of particular interest as it also occurs in a crocodilian host, but was collected from the Australian Region.

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Material examined

All specimens and bloodsamples were collected by Elisabeth Hagon, University of Sydney, Australia, and kindly submitted to the author. 5 male and 10 female worm specimens were removed from the connective tissues and serous membranes of the body cavity of one individual *Crocodilus porosus*, found infected near Maningrida, Northern Territories; Australia.

The worms were killed in 4 % hot formalin solution. Microfilariae were observed in dry and stained bloodfilms and in fresh bloodsmears prepared with vital stains (New Blue R).

Description

Oswaldofilaria kanbaya

Measurements in *table I*.

Cuticle with clearly evident transverse striation over whole length of body except at the anterior and posterior ends. Body cylindrical in anterior part, tapering in posterior fifth of length to fine rounded tip of tail. Head end inflated with cuticle surrounding mouth protruding from inflated part, with evenly rounded apex (*fig. 1 A*; *2 A*). Four externolateral labial papillae distributed in rectangle (*fig. 2 D*; *3*), other papillae inconspicuous, amphid in groove (*fig. 4*), circular mouth with opening of 8 μm in diameter in center of head end (*fig. 5*). Buccal capsule narrow at entrance, widening towards base to maximal diameter of 20 μm . Walls of buccal capsule separable into three parts, comprising a) flat cuticularised ring around mouth opening with tapering outer margins curved posteriad; b) walls of buccal capsule funnel-shaped with wider diameter posteriad; c) flat cuticularised ring covering anterior tip of oesophagus. Oesophagus measuring three millimeters or more in both sexes, with short anterior muscular part (about 15 % of whole oesophagus) and posterior glandular part. Nerve ring loosely structured around posterior part of muscular oesophagus (*fig. 1 A*; *2 B*). No deirids seen. Oesophageal lumen enlarged at oesophago-intestinal junction, bordered by folded cuticular lining of oesophageal valve (*fig. 1 B*; *2 C*). Diameter of intestine at junction much smaller than diameter of glandular oesophagus. Intestine containing clearly distinguishable, partially or undigested red and white blood cells and few microfilariae as solid particles in specimens of both sexes (*fig. 2 E*). Phasmids forming prominent cone in subterminal lateral position of tail in males and females (*fig. 8*; *9*).

MALE : Posterior end coiled ventrally (*fig. 1 C*). Spicules unequal and dissimilar, left spicule being almost five times longer than right spicule. Both spicules positioned along midline of body, right spicule appearing at cloaca posterior to left spicule. Left spicule curved ventrally in longitudinal axis, slender, with head poorly developed, anterior shaft cylindrical and as long as posterior blade. Blade with core

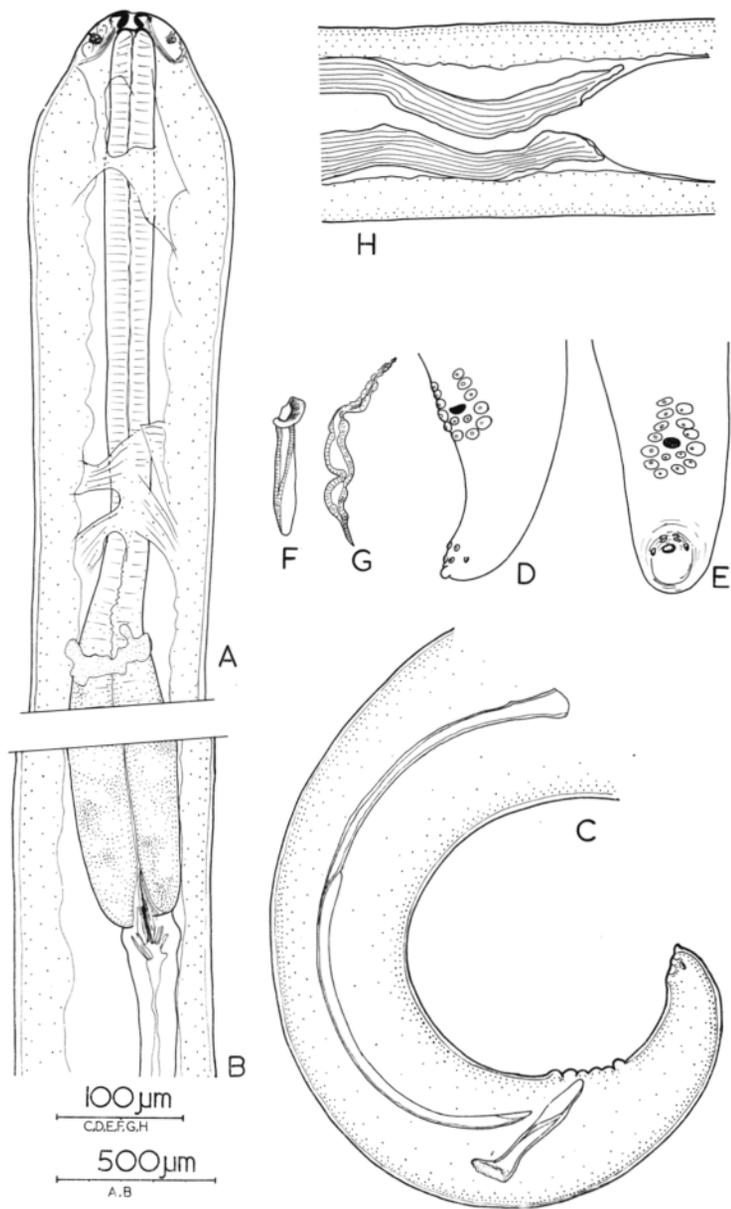


FIG. 1. — *Oswaldofilaria kanbaya*, male.

- A : Anterior end with muscular oesophagus. Note articulated buccal capsule and swelling of subapical part of body of worm.
 B : Oesophago-intestinal junction.
 C : Tail, with spicules *in situ*.
 D : Tail, latero-ventral view.
 E : Tail, ventral view.
 F : Right spicule, drawing with transmitted light from right side.
 G : Right spicule, aberrant form found in one specimen.
 H : Posterior part of ejaculatory duct, 6.3 mm distant from tip of tail, showing isthmus and anterior end of muscular layer with parallel striation.

TABLE I. — Measurements of *Oswaldofilaria kanbaya*.

	MALE SPECIMENS			FEMALE SPECIMENS		
	Holo- type	5 specimens, range	Average	Allo- type	10 speci- mens, range	Average
Body length	18	16-18	17	31	26-36	32
Oesophagus length	3.20	3.0-3.5	3.2	3.5	3.5-4.5	4.2
Oesophagus length as % of body length	18 %	18-22	19 %	11 %	11-15 %	13 %
length of glandular oesophagus	2.8	2.4-3.0	2.76	3	3.0-4.0	3.6
Depth of buccal capsule	0.015	0.015	0.015	0.02	0.02	0.02
Tail length	0.18	0.17-0.18	0.17	0.32	0.28-0.32	0.31
Vulva, from ant. end	—	—	—	9.2	8.2-9.9	9.2
Vulva, as % of body length	—	—	—	30 %	25-35 %	29 %
Left spicule	0.55	0.48-0.55	0.51	—	—	—
Right spicule	0.11	0.11-0.12	0.11	—	—	—
Length of right spicule as % of left spicule	20 %	20-25 %	22 %	—	—	—
Body width :						
at post. end of oes.	0.16	0.15-0.16	0.15	0.25	0.22-0.28	0.25
at midbody/vulva	0.15	0.15-0.16	0.15	0.25	0.23-0.26	0.25
at cloaca	0.09	0.08-0.09	0.09	0.08	0.07-0.09	0.08

flattened and margins laterally expanded and bent over ventral side to form tube leaving anterior opening at junction with shaft and posterior opening near tip were margins decrease in size. Tip of left spicule solid, pointed, formed by tapering core. Right spicule straight with head conspicuous with antero-ventral margin elongated and directed towards anterior of body of worm (*fig. 1 F*); adjacent shaft cylindrical, flattening posteriorly to form channel with convex side facing towards left spicule. Tip formed by rounded, but sharp-edged, flattened margin. Right spicule of one specimen apparently deformed (*fig. 1 G*). Cloacal opening small and oval (long axis measuring 10 μm (*fig. 7*)). Tail papillae distributed in two distinct, symmetrical groups as follows: In pericloacal group, 14-15 papillae arranged in pairs along midventral line of body with three precloacal pairs in linear alignment and four postcloacal pairs (*fig. 1 D, E*). In subterminal group between phasmidial cones two pairs of smaller papillae. Tail tip with salient knob. Posterior part of ejaculatory duct covered by thick outer muscular layer with clearly defined parallel striations (*fig. 1 H*), not extending beyond distinct isthmus, which in one specimen was situated 6.3 mm from tail tip.

FEMALE: Vulva at a point 31 % (25 %-35 %) of body length from anterior end (*fig. 2 G; 6*). Cuticle around vulva slightly raised. Vagina complex (*fig. 2 F*), comprising atrium with internal walls smooth and wide (about 20 μm) lumen, surrounded by thick muscle layer, and thin canal (50 μm long) opening asymmetrically into base of atrium and leading through concentric muscular tissue to vaginal chamber. Chamber with internal walls sinuous and with fine cuticular cross striation.

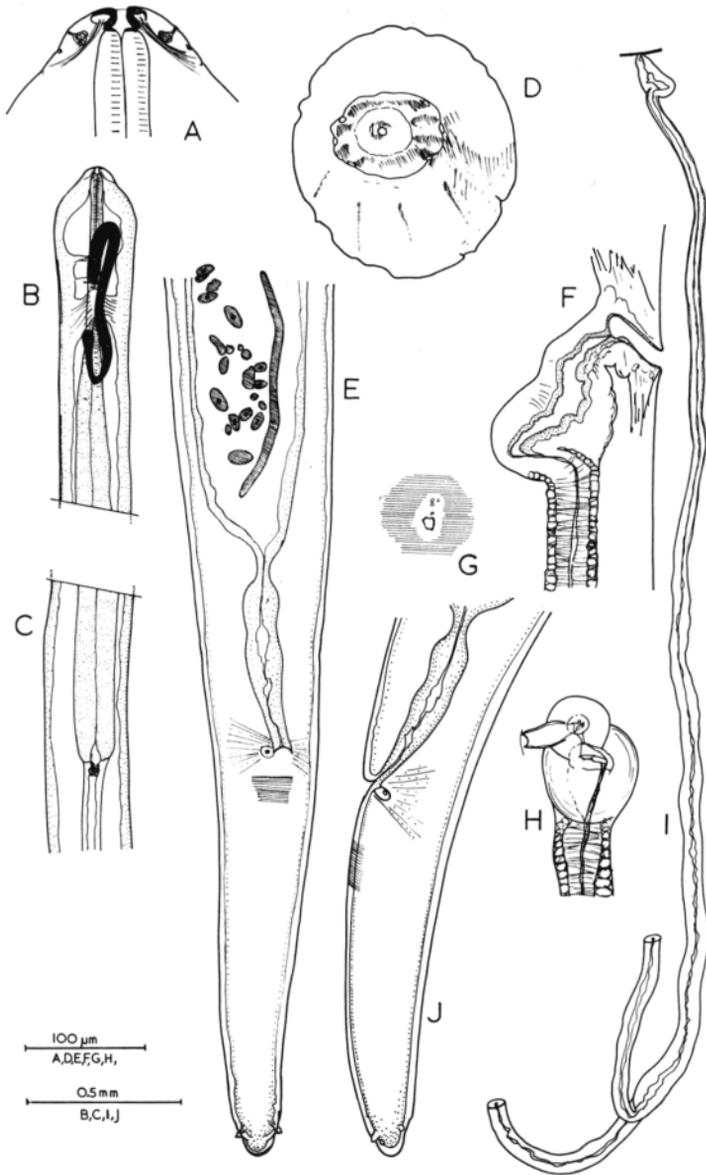


FIG. 2. — *Oswaldofilaria kanbaya*, female.

- A : Anterior end with articulated buccal capsule and amphids (dorsal aspect).
 B : Anterior end with muscular oesophagus and start of female genital duct, i.e. ovary and part of oviduct (in black).
 C : Oesophago-intestinal junction.
 D : *En face* view.
 E : Tail, ventral view. Note microfilariae inside intestine and rectal sphincter. Cuticular striation shown posterior to anus.
 F : Vagina, lateral view, with dilated atrium.
 G : Vulva, ventral view, with surrounding cuticular striation.
 H : Vagina, ventral view. Note oblique position of atrium.
 I : Female genital duct with vagina, unpaired uterus and bifurcation of uterus.
 J : Tail, lateral view.

Deep end of vaginal chamber with a lumen appearing oval in cross section in four specimens examined. At base of chamber small canal opening in lateral position, connecting in an S-shape vaginal chamber with unpaired section of uterus. Vagina and uterus at first running anteriorly than flexing posteriorly. In one specimen, one ovary seen at level of nerve ring (*fig. 2 B*), the other ovary at a point 13.9 mm (or 42 % of body length) from anterior end. Intestine separated from rectum by narrow constriction. Tail slightly bent towards dorsal side. Tail rapidly tapering on dorsal side at level of conically protruding phasmidial cones, leaving small evenly rounded tip covered by double-layered cuticle (*fig. 2 E, J*). In one specimen, ventral side of tail end with knob-like elevation as described for male specimens.

MICROFILARIA: Sheath little longer than microfilaria, width of sheath about equal to width of microfilaria, sheath did not contain refractile granules (*fig. 10 A-D*). Average length of microfilariae 170 μm , average width 6.5 to 7 μm , measured in anterior half of body; posterior half of body tapering to blunt tip of tail. Dimensions of vital-stained material were about 20 % bigger, possibly due to swelling during 2 1/2 days' transportation in unrefrigerated, heparinised blood sample. Cuticle transversally striated, 14.5 striae per 10 μm on posterior half of body. Excretory cell and pore easily visible in vital-stained microfilariae (*fig. 10 D, E*) at a distance equal to 31.5 % (average of 5 specimens measured) of body length from anterior end. Pharyngeal thread visible in its anterior and posterior course (*fig. 10 D*). "Innenkörper" about 18 μm long, extending between the points 46 % and 35 % of body length from posterior end. R_1 nucleus larger than R_2 , R_3 and R_4 nuclei, with large protoplasm; R_1 nucleus situated at a point 30 % of body length from posterior end, R_2 , R_3 and R_4 positioned between the points 18 % and 25 % of body length from posterior end. Position of posterior three R-cells varying as shown in *figures 10 D, F, G*. Anus marked by small protrusion of primordium of anal plug. Tail 26 μm long, corresponding to 15 % of body length. Cell nuclei of body wall staining clearly and drawn in *figure 10 D* as optical longitudinal section. Some small cell nuclei visible in anterior oesophageal region. Giemsa-stained microfilaria showing nuclear column almost reaching head-end with few loosely spaced nuclei. Gaps in nuclear column occurring at positions of excretory cell, "Innenkörper", cell of nucleus R_1 , and at anal region. Gap of "Innenkörper" very conspicuous.

Hagon (personal communication) commented that she found the circulating microfilariae to be aperiodic (the number of microfilariae in bloodsamples from the "caudal sinus" was determined every 4 hours for 24 hours); the bloodsamples contained 69-88 microfilariae/ μl .

Fig. 3. En face view. L = externo-lateral labial papillae; A = amphidial opening; C = possible cephalic papillae. *Fig. 4.* Head end, lateral view, lettered as above. Note groove posterior to amphidial opening. *Fig. 5.* Mouth opening, with triradiate oesophagus at base of buccal capsule. *Fig. 6.* Vulva, with vagina leading to the right side. *Fig. 7.* Ventral view of male tail. *Fig. 8.* Tail end of male, lateral view from left, with protruding tail tip (T) and cone-like phasmid (P). *Fig. 9.* Tail end of male, ventral view, with four subterminal papillae arranged in square.

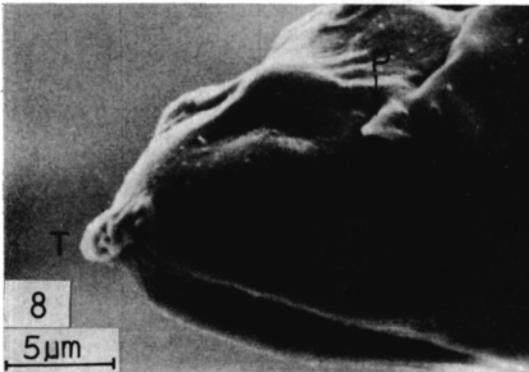
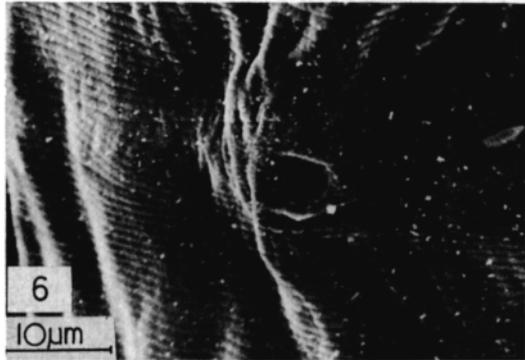
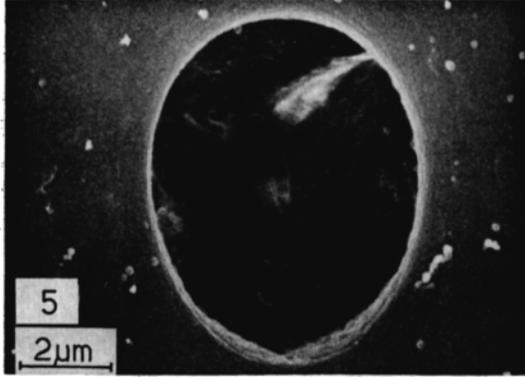
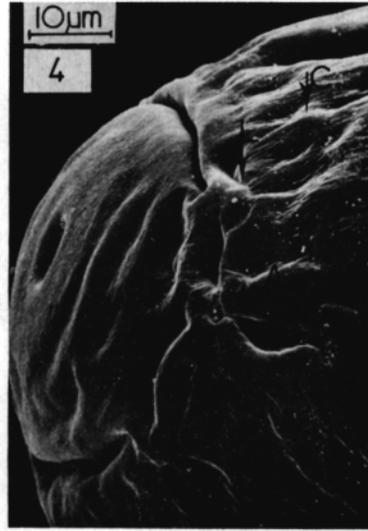
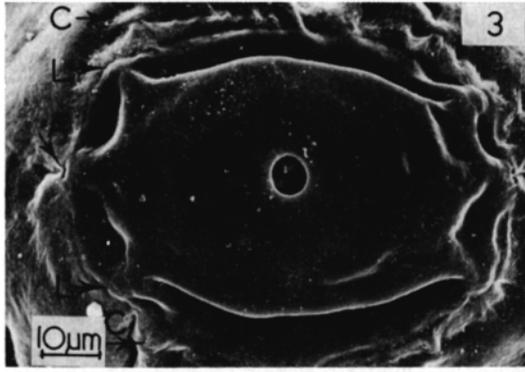


FIG. 3-9. — *Oswaldofilaria kanbaya*.

Type host : *Crocodilus porosus*
 Other hosts : none known
 Type locality : Maningrida, Northern Territories, Australia
 Other localities : none known
 Site in host : Connective tissue layers and serous membranes of body cavity
 (following Mrs. E. Hagon, pers. comm.). Microfilariae in blood.
 Etymology : Kanbaya is the name for *Crocodilus porosus* in the language of the
 aboriginal people (Kunibidji) of the area of the type locality of the
 species.

Type material lodged in the Queensland Museum under the following numbers :

Holotype and Allotype	GL4711
Paratype males	GL4712
Paratype females	GL4713
Bloodfilms, 4 slides	GL4714 to 4717

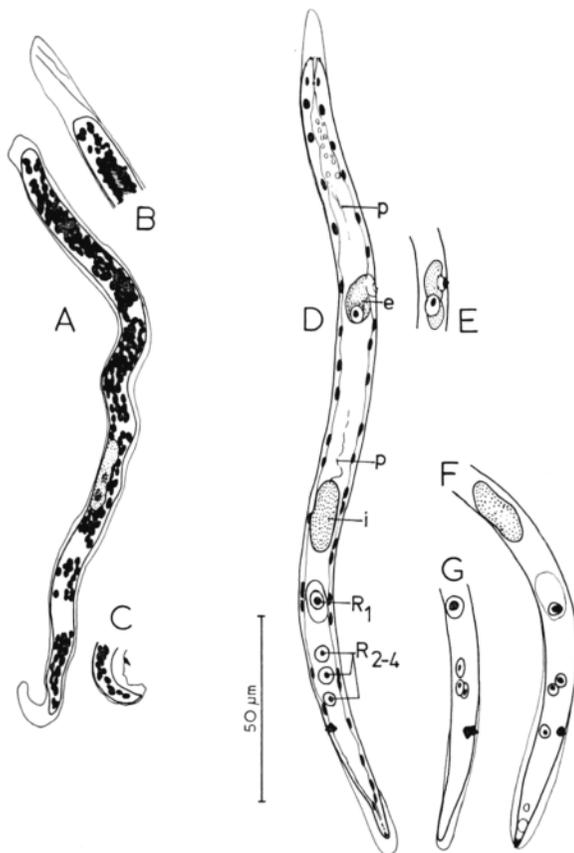


FIG. 10. — Microfilariae of *Oswaldofilaria kanbaya*.

A-C : (Giemsa) showing differences in arrangement of nuclei in anterior (B) and posterior (C) extremity in comparison to A.
 D-G : (vital stain) with subcuticular nuclei lining the body wall and with excretory cell (e), pharyngeal thread (p), Innenkörper (i) and R-cells (R_{1-4}) in the body cavity.
 E Variation in the arrangement of the components of the excretory cell.
 F et G Variations in the position of R-cells.

DIAGNOSIS

With characters of *Oswaldofilaria* Travassos 1933, sensu Bain (1974). *O. kanbaya* differs from all other Australian species, namely *O. chlamydosauri* (Breinl 1913), *O. innisfailensis* (Mackerras 1962), *O. pflugfelderi* (Frank 1964) and *O. samfordensis* Manzanell 1982, by the inflated head end, the very unequal length of the two spicules, the structure of the vagina with an atrium and the S-shaped duct connecting the vaginal chamber with the uterus.

From the species described from crocodylian hosts, namely *O. bacillaris* (Molin 1858), *O. medemi* Marinkelle 1981 and *O. versterae* Bain, Kouyaté and Baker 1982, the species *O. kanbaya* differs in the length of the oesophagus, the denticulated tip of the tail in the male, the size of the spicules and the length of the microfilariae.

Amongst the remaining Neotropical species, only *O. petersi* Bain and Sulahian 1974 and *O. spinosa* Bain and Sulahian 1974 have a similarly unequal length of the spicules and similarities in the structure of the vulva ; both these species differ from *O. kanbaya* in number and arrangement of the pericloacal papillae, the length of the microfilariae, the length of the oesophagus and the structure of the buccal capsule.

Discussion

A comparison of the morphology of the four *Oswaldofilaria* species inhabiting crocodylians reveals an interesting uniformity in features, which are considered as primitive (Bain, Kouyaté and Baker 1982) in this genus :

- There are ten (or less) pairs of papillae on the male tail.
- The cloacal papillae are arranged in a strongly bisymmetrical pattern.
- The oesophagus is longer than 3 mm and 4 mm in the male and in the female respectively, i.e. the oesophagus of the species inhabiting crocodylians is longer than the oesophagus of any other species of the genus, in the male and in the female*.
- The spicules are very unequal, with the right spicule being much less than half the length of the left spicule.

It is noteworthy that this combination of primitive characteristics is not found in any species of the genus parasitic in lacertilians. The four *Oswaldofilaria* spp. of crocodylians appear therefore as a primitive group within the genus.

In spite of these primitive features and of the phylogenetic age of their host group, it is not clear, whether these parasites of crocodylians may be regarded as direct ancestors of the species of the genus occurring in lacertilians (Bain, Kouyaté and Baker 1982). However, *O. kanbaya* is readily distinguished from the other four Australian species, which exhibit closer affinities to Neotropical *Oswaldofilaria*

* On the fig. 2 of male *O. brevicaudata*, in Bain, 1974, the oesophagus length is 2,4 mm (and not 4,7 mm as written) which is in the range of the length indicated by previous authors (i.e. 1,5 mm to 3,2 mm ; Diaz-Ungria, 1979 ; Sonin, 1968 ; Caballero, 1948).

species (Manzanell, 1982). This suggests that *O. kanbaya* may not be the direct ancestor of the Australian species, but may have arrived in the Australian region independently. The wide geographical distribution of *Crocodilus porosus* (as well as of *C. niloticus*, the host of *O. versterae*) (Wermuth and Mertens, 1961) favours the geographical dispersal of its parasites and this may be important in explaining the close relationship between all *Oswaldofilaria* species parasitic in crocodylians.

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