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A NEW FILARIA OF A LIZARD TRANSMITTED BY SANDFLIES

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A description is given of Madathamugadia wanjii *n. sp., a Splendidofilariinae parasite of the gecko* Ptyodactylus hasselquistii, *on the west bank of the River Jordan and of its life cycle in* Phlebotomus duboscqi.

The new species is close to M. ivaschkini *(Annaev, 1976) n. comb., of Turkmenistan, which is also transmitted by sandflies (Reznik, 1982). The genus* Madathamugadia *is now comprised of four species, two from Madagascar and two from the Mediterranean sub-region; it differs from the genus* Thamugadia *by the presence of a double row of papillae anterior to the cloaca of the male.*

The larval characters of Splendidofilariinae of lizards confirm the affinity of these parasites to the Splendidofilariinae of birds (Chandlerella and Splendidofilaria); *the first group could have arisen from the second by "captures" which could have occurred in several places.*

Key-words: taxonomy – phylogeny – Onchocercidae – reptile – vector – morphogenesis

The gecko *Ptyodactylus hasselquistii* (Donndorf, 1798), which is common on rocky banks of the River Jordan often carries microfilariae. These belong to a new filarial worm of a small, little known group, the Splendidofilariinae parasites of reptiles.

Its development takes place in sandflies, like that of a related parasite of lizards (Reznik, 1982); out of more than one hundred known life-cycles of filarial worms (Bain & Chabaud, 1986), these are the only ones in this vector.

DESCRIPTION OF THE FILARIA

Material – Material originated from two geckoes. The type material was collected in the gecko number 288 DV: 6 females, 2 males and microfilariae in blood smears. The filariae were found in the general cavity, near the kidneys and the trachea. Additional material (microfilariae in blood smears) came from the second gecko number 289 DV; the morphological study of the living microfilariae was done on this material. All specimens were deposited in the Helminth collection of the Museum national d'Histoire naturelle, Paris; female holotype and male allotype accession numbers 288 DV a and b respectively.

Morphological study – The morphology is shown on Figs 1 to 4; further details are given below.

Head: in all specimens, asymmetrical arrangement with four external labial papillae and two cephalic papillae; projecting amphids, of the same appearance as the labial papillae. Female genital apparatus: opisthodelphic; ovaries and oviducts short; uteri start at about 600 µm from the caudal extremity, then fuse at 2.350 µm behind the vulva, to form a wide thin walled impaired portion, 1.850 µm in length, followed by a muscular ovejector 500 µm long (analysis of a specimen from the type material); vulva posterior to the junction of the oesophagus and intestine in all females. Microfilaria: sheath present but fitting closely in both living microfilariae and specimens stained with Giemsa's stain; left cephalic hook short and, on the opposite side, 2 (or 3?) projecting points one behind the other.

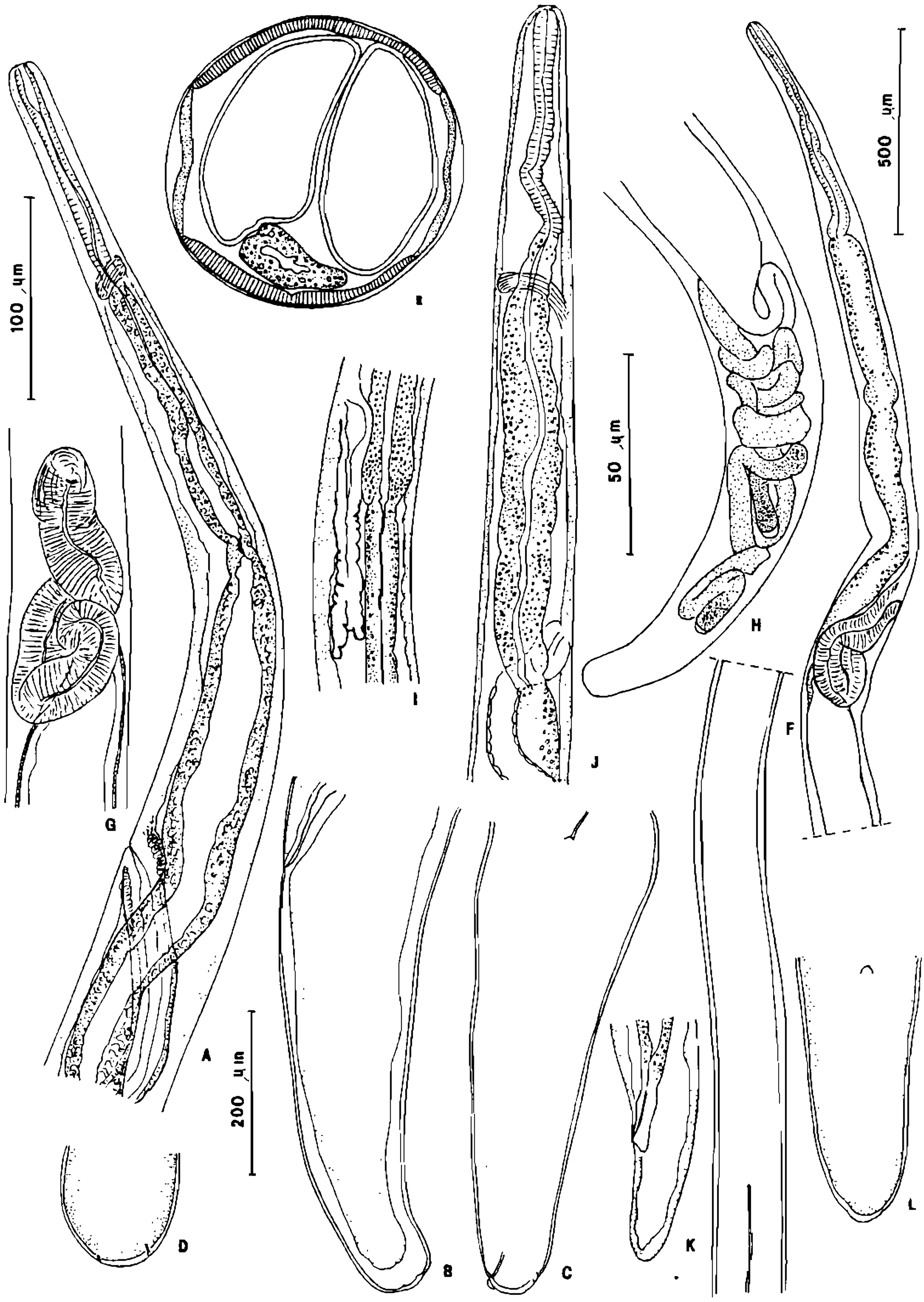

Fig. 1: *Madathamugadia wanjii* n. sp. – Female A: anterior end, lateral view. B, C: tail, lateral and ventral view. D: caudal extremity of another female, ventral view. E: transversal section at mid body. F: anterior end and ovijector (drawing in two parts). G: muscular part of ovijector and vagina, ventral view. H: posterior end with coiled ovaries and beginning of one of the uteri. – Female larvae. I: third stage, with genital primordium near the oesophago-intestinal junction. J: fourth stage, anterior end. K, L: idem, tail, lateral and ventral view. Scales: A, G, H: 200 µm; B, C, E, I, J, K: 100 µm; D, L: 50 µm; F: 500 µm

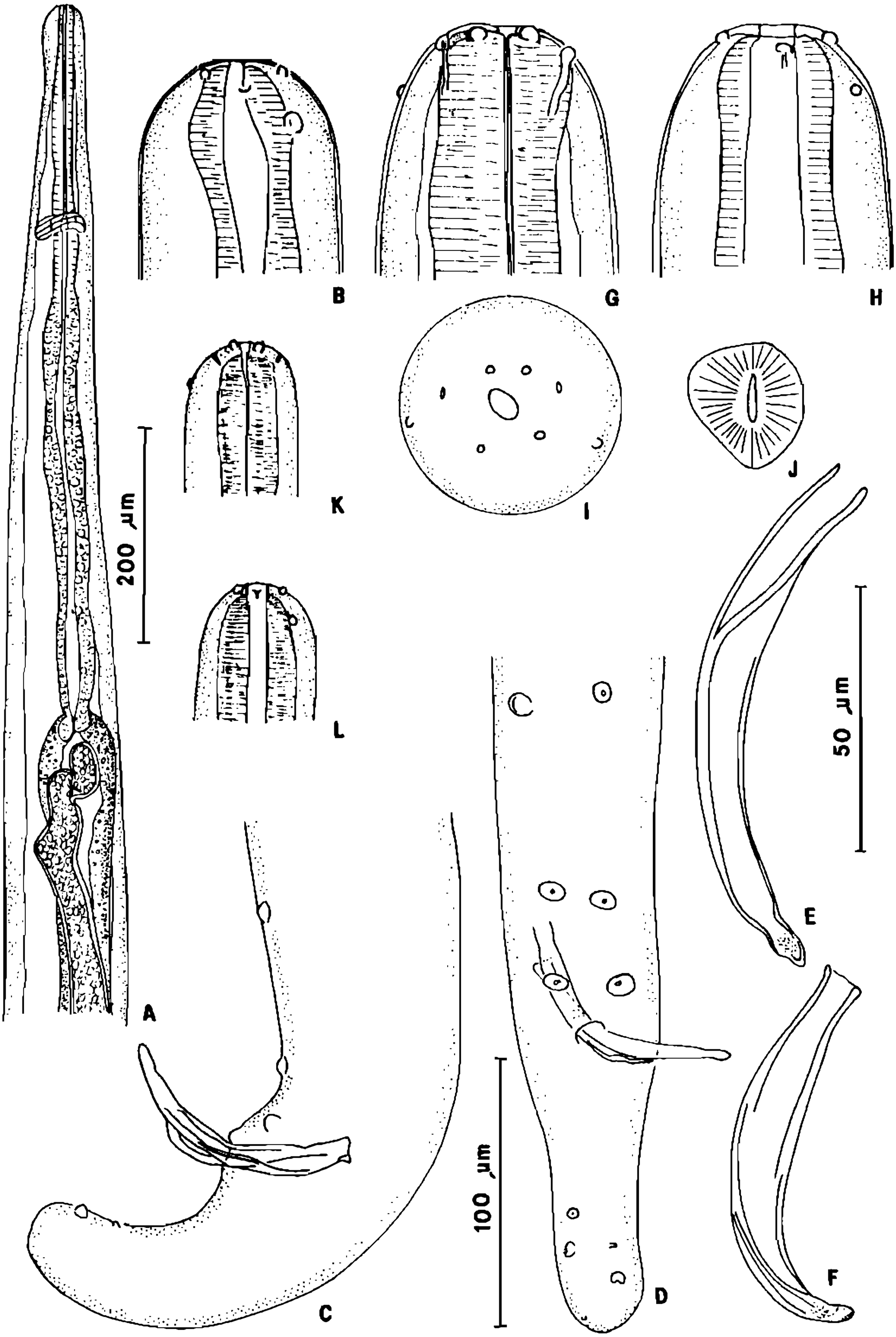

Fig. 2: *Madathamugadia wanjii* n. sp. - Male A anterior end. B: head, lateral view. C, D: tail, left lateral and ventral view. E, F: left and right spicules, right lateral view. G – I: head of female, respectively median, lateral and on face view. J: same specimen, transversal section of oesophagus. K, L: head of a fourth stage larva, median and lateral view. Scales: A: 200 µm; B, E, F, G, H, I, J, K, L: 50 µm; C, D: 100 µm.

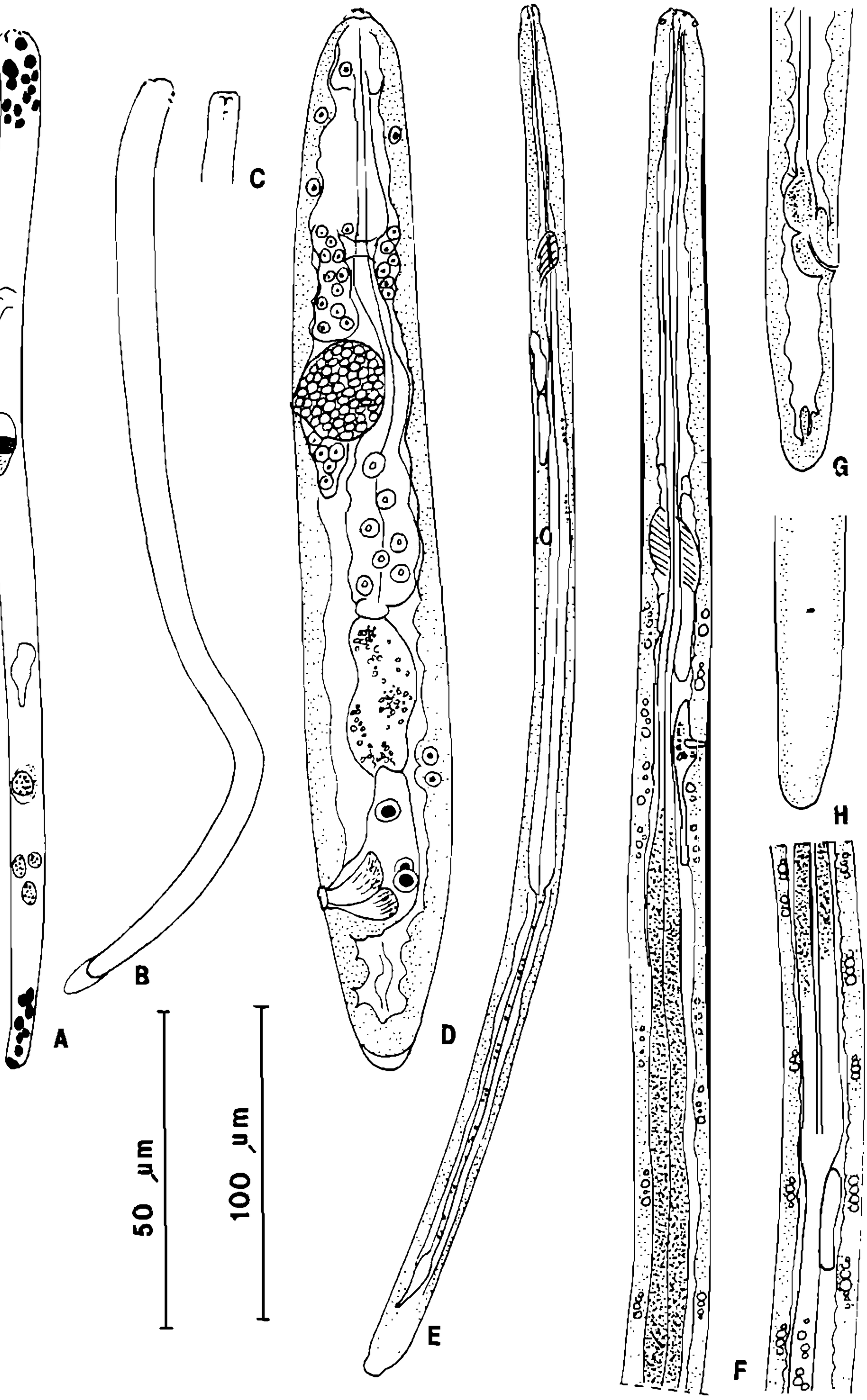

Fig. 3: *Madathamugadia wanjii* n. sp. – Microfilaria A: Giemsa stained, B, C: from ovijector of a fixed female (holotype), hook in lateral (B) or face view (C). D: young second stage, 5 days after the infective blood meal. Infective larva. E: general aspect. F: anterior end of a male larva (in two parts). G, H: tail, lateral and ventral view. Scales: E: 100 µm; others: 50 µm.

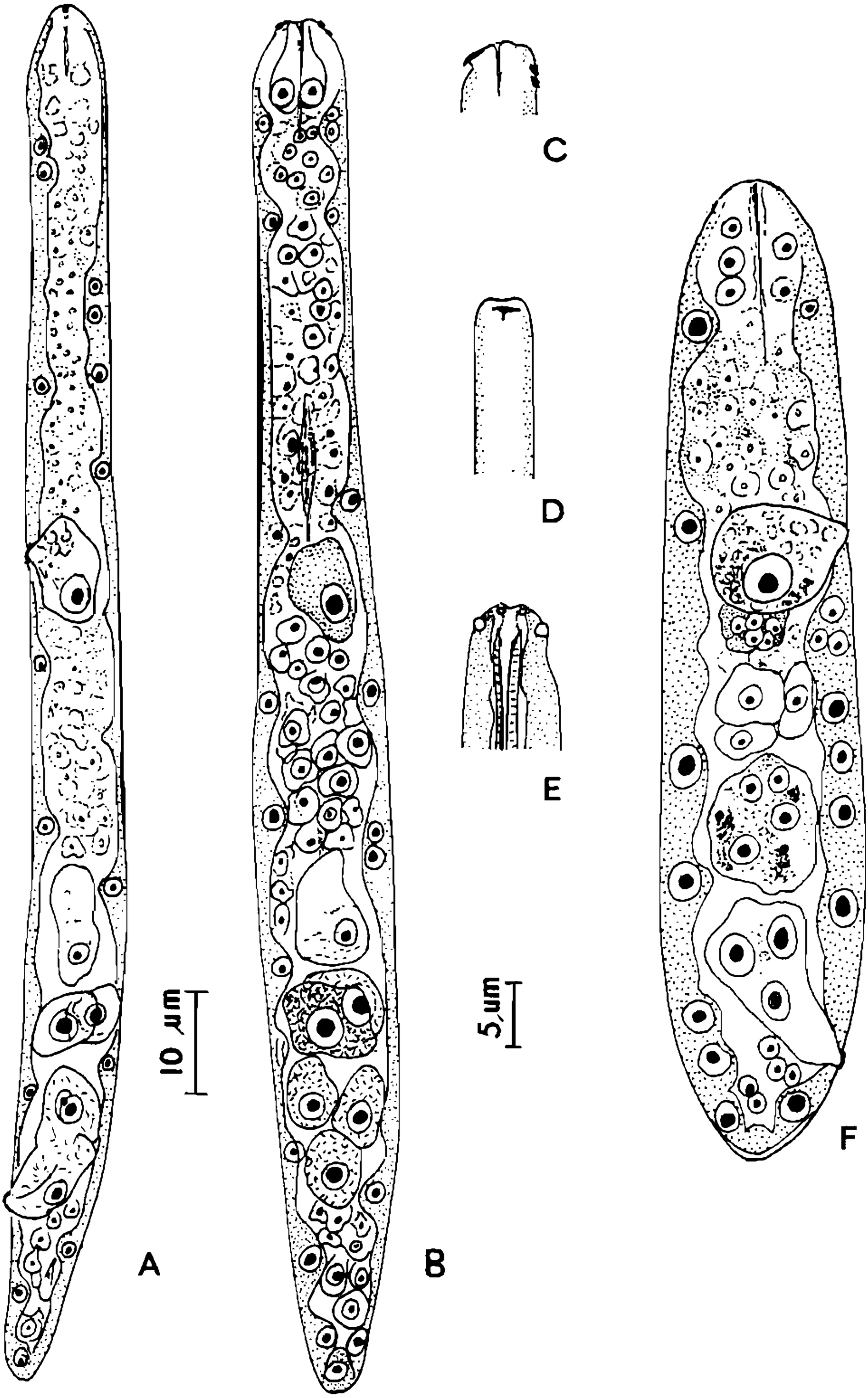

Fig. 4: *Madathamugadia wanjii* n. sp. – A-B: first stage, 28-36 h after the blood meal (R1 x 2), lateral and dorsal view. C, D: head of microfilaria, dorsal and left lateral view. E: head of infective larva, lateral view. F: first stage, 72-80 h after the blood meal, lateral view (molting is starting at posterior end). Scales: A, B, F: 10 µm; C, D, E: 5 µm.

Measurements – Female holotype and, in parentheses, a paratype: body length 17 (21) mm, width 200 (170) µm; nerve ring, 300 (300) µm from apex; oesophagus length 700 (650) µm; vulva, 1.160 (1050) µm from apex; tail length 235 (325) µm; length of microfilariae extracted from the ovijector of the holotype: 152-170 µm long and 5-6 µm of maximum width at cephalic region.

Male allotype, and, in parentheses, paratype: body length 9,6 (9,6) mm, width 110 (120) µm; nerve ring, 240 (225) µm from apex; oesophagus length 630 (700) µm; tail length 100 (120) µm; respective length of left and right spicules 113 (125) and 85 (97) µm.

DISCUSSION

On the characters of the anterior position of the vulva, the tail without lateral alae, the non-terminal anus and the subequal spicules, the new species falls into subfamily Splendidofilariinae Chabaud & Choquet, 1953.

According to Anderson & Bain (1976), four genera are parasites of reptiles: one, *Cardianema* Alicata, 1933, in a tortoise, has long spicules and a complex membranous blade, and three, in lizards, which have contrasting characters. Our specimens belong to the second group which is composed of:

– *Pseudothamugadia* Lopez-Neyra, 1956 with a short tail, identical left and right spicules and a few papillae around the cloaca; the single species, *P. physignathi* (Johnston, 1912), redescribed by Johnston & Mawson in 1943, is a parasite of agamids in Australia.

– *Madathamugadia* Chabaud, Anderson & Brygoo, 1959, also with a short tail but distinct from *Pseudothamugadia* by the subequal spicules of different form and papillae in a double set anterior to the cloaca. This genus is represented by two Madagascan species described by Chabaud, et al. (1959); they are *M. zonosauri* and *M. hopluri,* parasites of, respectively, gerrhosaurids and iguanids. They are close to one another with a divided oesophagus, the vulva anterior to the oesophagus and notably small (50 µm) microfilariae.

– *Thamugadia* Seurat, 1917 (= *Brygoofilaria* Sulahian & Schacher, 1968) with a long tail and identical spicules. Although four species are now placed in this genus, only three correspond to its definition. These are the type species, *T. hyalina* Seurat, 1917, a parasite of geckoes in North Africa; *T. agamae* (Sulahian & Schacher, 1968), a parasite of agamids in Lebanon; and *T. skrjabini* Annaev & Sonin, 1973, a parasite of lacertids and agamids in Turkmenistan. The morphology of these species is very variable with or without a divided oesophagus, a vulva anterior or posterior to the oesophagus and, in the male, either no caudal papillae (as the type species) or with a double row posterior to the cloaca (the other two species).

The fourth species, *T. ivaschkini* Annaev, 1976, a parasite of geckonids in Turkmenistan, differs from the other three by the subequal left and right spicules of different shape, the small number of postcloacal papillae, and the double row of precloacal papillae. These characters are present in the genus *Madathamugadia.* This led us to put Annaev's species in this genus in spite of the characters of the two Madagascan species: short microfilariae, vulva anterior to the oesophagus with a tail barely longer than the width.

Our specimens are very close to *M. ivaschkini* (Annaev, 1976) n. comb., but differ by the morphology of microfilariae: rounded tail with tightly packed nuclei in our specimens compared to the pointed tail with the two terminal nuclei far from each other in *M. ivaschkini,* from the description of Reznik (1982).

The filaria of *Ptyodactylus hasselquistii* is therefore a new species, *Madathamugadia wanjii* n. sp., dedicated to our colleague Samuel Wanji.

Development of the filaria in *Phlebotomus duboscqi*

In the laboratory, the life cycle of the worm was obtained in colonized *Phlebotomus dubosqci* Neveu-Lemaire, 1906, which originated from Keur Moussa, Senegal, in 1987. Since that date, the colony was maintained at Imperial College, Ascot, by the methods described by Killick-Kendrick & Killick-Kendrick (1987). The sandflies, sent to Paris as late-stage larvae or pupae, were used minimum 8 days after, then put with an infected gecko which was immobilized in wire mesh. The sandflies often engorged on the tip of the nose. They were maintained at 28-29 °C and dis-

sected at varying times in 20% foetal calf serum in R. P. M. I. medium; the filarial larvae stayed in perfect condition throughout the time of the morphological examinations made on fresh preparations.

Two trials made with 20 insects showed that a gecko with 600 microfilariae per 10 mm^3 of blood (taken from the ocular sinus) gave a mean of 6 (0-22) ingested microfilariae and 1,9 infective larvae.

Morphogenesis – Morphogenesis is shown on Figs 3 and 4; only the stage 1 and infective larva were analyzed, because the infected insects were also used to inoculate new lizards.

The development occurred in the flight muscles. Molt 1 began at the posterior end of the larva 3 days after the insect blood meal; larvae were infective in 7-8 days; some of them were found in the head, but most of them were in the thorax and abdomen; although the larvae seemed in perfect condition, they were not very motile: only some slow oscillations of the anterior end were seen.

Measurements – Fresh blood microfilaria: body length 165-200 µm, width 6-6,5 µm; for a microfilaria 176 µm long, excretory pore and R1 cell respectively at 68 µm and 128 µm from anterior end; anal pore at 20 µm from caudal extremity.

Stage 1: – larva 24-36 h after the insect blood meal (R1 cell divided once): body length 148 µm, width 15 µm; excretory pore and R1 cell respectively at 60 µm and 108 µm from anterior end; tail length 20 µm; – 60-72 h old larva: body length 108 µm, width 22 µm; excretory and anal pores respectively at 40 µm and 95 µm from anterior end; intestinal length 15 µm.

Molt 1: body length 140 µm, width 20 µm; nervous ring and excretory pore respectively at 33 and 50 µm from anterior end; length of oesophagus, intestine and rectum 80 µm, 25 µm and 30 µm respectively; tail length 18 µm.

Infective larvae: body length 435-500 µm, width 14-15 µm; measurements of two larvae, a male and, in parentheses, a female: body length 500 (475) µm, width 15 (14) µm; nerve ring and excretory pore respectively at 80 (80) µm and 110 (115) µm; buccal cavity 3,5 (3,5) µm high; oesophagus 295 (300) µm long and glandular part 130 (160) µm long; intestine and rectum long respectively 130 (125) and 25 (23) µm; tail length 40 (37) µm; genital primordium at 300 (185) µm from anterior end.

Discussion – The cycle of *M. wanjii* is very similar to that of *M. ivaschkini*, obtained by Reznik (1982): rapid development in the flight muscles of sandflies, notable shortening and thickening of the microfilariae during stage 1 (which may be almond-shaped, depending on species) and a short infective stage (< 500 µm).

These highly evolved cycles resemble those of certain Splendidofilariinae of birds (Anderson, 1956; Hibler, 1963; Bartlett & Anderson, 1980) as well as those of Lemdaninae of lizards and birds (Dutton, 1905; Bain, 1969). They underline the affinities of these groups.

Development in the gecko

A specimen of *P. hasselquistii* was given 25 and 20 infective larvae, with an interval of 9 days, inoculated subcutaneously into the interior skin of the thighs. The animal was autopsied 13 days after the first inoculation. Four larvae were recovered; one, the largest, was in the subcutaneous abdominal tissue and the other 3 were between the muscles of the pelvic region and the thighs. The shape of the larvae was in an arc: three of them were studied (Fig. 1 I, J, K; Fig. 2 K, L).

A stage 3 male: morphology close to that of the infective stage but body thicker and without cephalic constriction; body length 550 µm, width 25 µm; genital primordium oval, not developed, 20 µm long and placed just posteriorly to the beginning of the intestine.

A female larva 1650 µm long, 50 µm wide which is probably a young stage 4; vaginal primordium placed slightly anterior to the oesophago-intestinal junction.

A stage 4 female 2000 µm long and 50 µm large; buccal cavity 4 µm high, flattened laterally; four prominent externo-labial papillae and one cephalic papilla; oesophagus 360 µm long with thick glandular part 220 µm long; tail 70 µm long; vulvar primordium slightly anterior to the oesophago-intestinal junction; ovejector and genital cords differenciated.

Discussion – During the morphogenesis of *M. wanjii* in the vertebrate host, notable features are the poor development of the anterior region of the filaria, the slight elongation of the oesophagus and the regression of the cephalic papillae.

The reduced oesophagus and the small, straight vagina, characteristic of many Splendidofilariinae, appear, therefore, to be primitive characters which result from the persistence of larval characters.

CONCLUSION

Only 8 species of Splendidofilariinae of lizards are known: one Australian (genus *Pseudothamugadia*), two Madagascan (genus *Madathamugadia*) and five in the Mediterranean subregion (2 *Madathamugadia* and 3 *Thamugadia*). These genera are not restricted to a single family of hosts: 3 are parasites of agamids, 2 of geckonids, and a single species can parasitize several families, lacertids and agamids, for example.

The phylogenetic interpretation is tentative because the grouping of the species is based on only a few characters provided by a meagre morphology. There are two possible hypotheses.

1) An origin in Gondwana in reptiles, with a migration from the East coast of Africa and an explosion in the Mediterranean subregion.

However the very localized geographical distribution and the characters of the host spectrum seem to indicate a recent evolution and are not in favour of this hypothesis.

2) The "capture" by lizards from the Splendidofilariinae of birds, having the same larval characteristics, such as *Splendidofilaria* and *Chandlerella* (eg. Bain et al., 1981). This may have happened separately in Australia, Madagascar (that is to say on islands where the phenomenon of "capture" is frequent) and a third time in the Mediterranean subregion. It may be that the two species *M. zonosauri* and *M. hopluri,* which both have short microfilariae, do not belong to the same group as the two apparently close species, *M. ivaschkini* and *M. wanjii,* which have microfilariae which are long.

In the Mediterranean subregion, there are two more hypotheses: (1) The Mediterranean *Madathamugadia* could have given rise to *Thamugadia* with a slightly more evolved morphology (disappearance of the papillae anterior to the cloaca). (2) The Mediterranean *Madathamugadia* and the *Thamugadia* arose separately from two groups of Splendidofilariinae of birds, distinguished by the position of caudal papillae.

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