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Review

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## Behaviour of filariae: morphological and anatomical signatures of their life style within the arthropod and vertebrate hosts

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### Abstract

This paper attempts to pinpoint the most original morphological anatomical features of the biology of filariae *per se* and those which are or could be important for triggering regulatory processes in the arthropod vector and uncontrolled pathogenic processes in the vertebrate hosts. The following stages are considered: the motile egg or newly-hatched larva, the microfilaria, in the lymphatic or blood vessels of its vertebrate host; the larva, its migrations and its intrasyncytial development in the hematophagous arthropod subverted as vector; its transfer to the vertebrate host, migratory properties through the lymphatic system, maturation, mating and, finally, egg laying in the tissues they reach. This synthesis is based on parasite morphological features and their functional interpretation, histological features in the different niches the filariae reach, and on quantitative analyses of filarial development at its different phases, as well as on the rare and valuable observations of living parasites *in situ*. Data have been drawn from various species of Onchocercidae from amphibians, reptiles, birds and mammals. These comparative analyses have revealed the major constraints to which the filariae, including those parasitizing humans, have been subjected during their evolution from their ancestors, the oviparous and heteroxenic spirurids. Emphasis is placed on mechanical events: resistance of the microfilariae to the currents in the blood or lymph vessels, regulatory processes induced in the vector mesenteron by the movements of the ingested microfilariae, transient disruption by the microfilarial cephalic hook of the vectors' tissues and cell membranes during microfilarial translocation, attachment of males to females during mating by means of 'non-slip' systems, etc. Like other nematodes, filariae are equipped with sensory organs and a locomotor system, composed of the muscles and of the original osmoregulatory-excretory cell. Any change in one of these elements will result in the destruction of the filaria, at some stage of its development. In the vertebrate host, the intravascular stages will no longer be able to resist being carried passively towards the organs of destruction such as the lymph nodes or the lungs.

### Background

The study of human filariae is limited for obvious ethical reasons. The proportion of inoculated larvae that develop, their route of migration, pairing of the sexes, egg laying and migration of the microfilariae, to name only a few

processes, remain unknown. However these filariae belong to a homogeneous group, the family Onchocercidae, from other members of which information can be extrapolated. Parasites of amphibians, reptiles, birds and non-human mammals are available for observation and

further experimentation [1]. The study of each species provides a fragment of information and these pieces, when assembled as in palaeontology, allow outlining the main features of the biology of filariae, which can be extended to the parasites of humans.

1 - The microfilaria: the morphological diversity of this stage reflects its different behavioural traits and niches accessible to the vectors

The filariae have developed unique and highly evolved biological features as compared to their parasitic spirurid ancestors. Spirurids have their eggs expelled from the host and transmitted by omnivorous arthropods [2]. Filariae, which are confined inside the host tissues, have adapted to transmission by haematophagous arthropods by laying motile vermiform eggs or very young larvae in diapause, the microfilariae (Fig. 1), which are able to reach the peripheral cutaneous blood or lymphatic vessels and thus become available to the arthropods.

One constraint affecting microfilariae is the restriction resulting from the narrow bore of these vessels. Most of the cells of the internal primordia of the microfilariae are reduced to a thin cytoplasmic layer fringing the nuclei. This first larval stage is still an embryo; its musculature is only half formed, with 4 submedian longitudinal files of muscle cells; a mesenchymal cell, R1 cell, is dormant and it will begin to participate in the elaboration of the definitive musculature only when the microfilariae have reached the vector (Fig. 2C). This peculiarity in ontogenesis has also been observed in ancestral groups of filariae [5,6] and in free-living rhabditids [7]; the filariae are only exploiting a general feature of other nematodes.

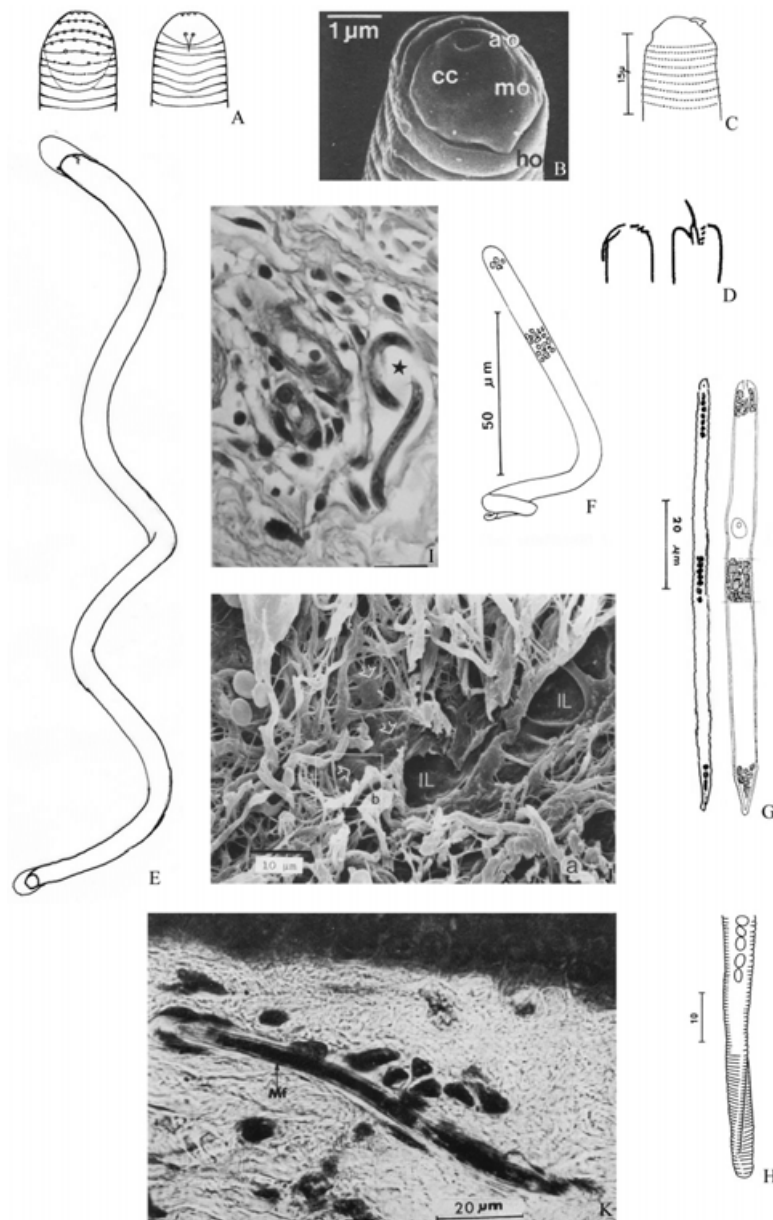
As a consequence of the slenderness of microfilariae, the female filaria may have considerable offspring, which is dependent on the vector for the completion of the larval development. Thus the tendency will be for the microfilariae to be borne earlier and earlier. This may explain some of the characters used empirically to distinguish human-dependent filarial strains: the lower number of nuclei in the microfilaria of *Wuchereria bancrofti* [3] indicates a more precocious arrest of cellular divisions; in *Brugia malayi*, the relatively high proportion of exsheathed microfilariae in blood films [4] indicates more rapid eclosion.

As we cannot directly observe the microfilariae in their natural environment, their behaviour is almost unknown. However, there are other ways of approaching this problem: analyses of the distribution of microfilariae in host skin snips, and of the ingestion by the vectors of different filarial species and of inert microspheres; and the study of

the functional anatomy of microfilariae. This phase of the filarial life cycle appears to be astonishingly complex.

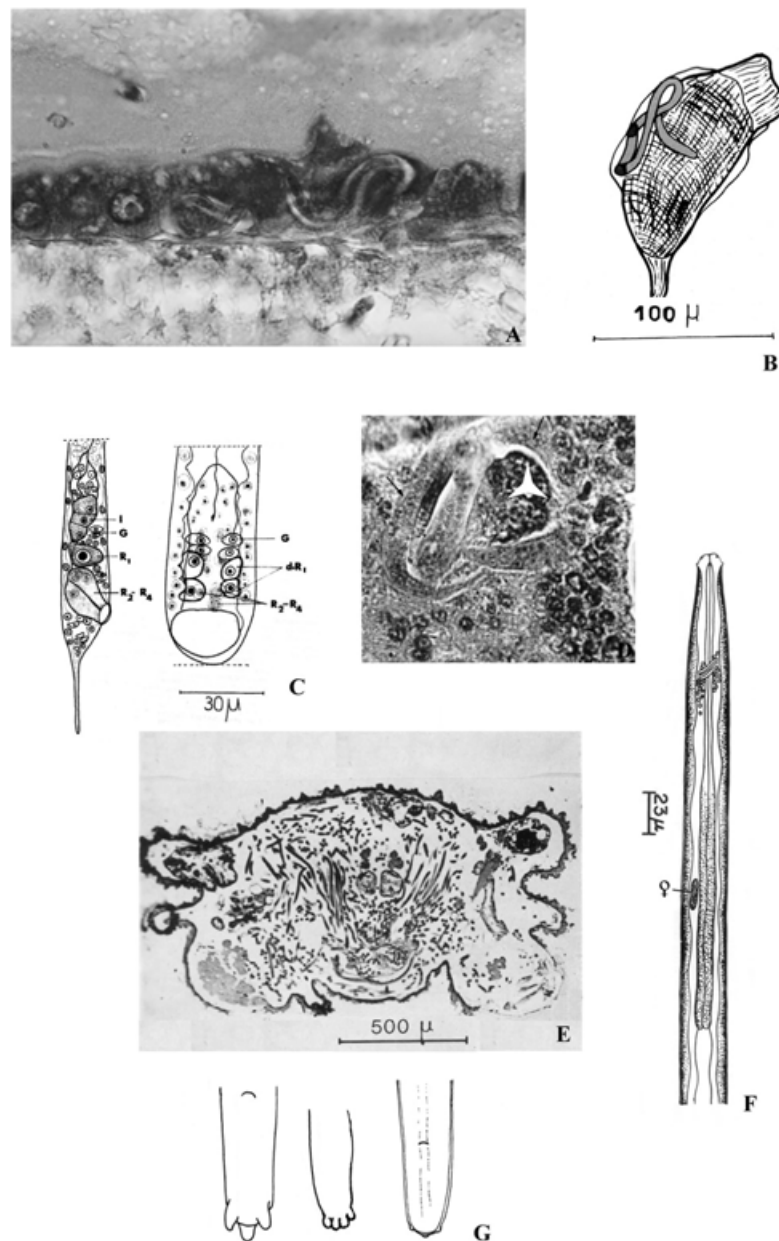
The lymphatic localization of the so-called dermal microfilariae is probably the primary condition. The filariae actually deposit their embryos in the connective tissue, drained by the initial lymphatic vessels [8] (Fig. 1I,1J). Lymph is a less aggressive medium than blood: no platelets, no complement system, incomplete coagulation system and no granulocytes; in addition, its flow is much less violent. These lymphatic microfilariae are not randomly distributed in the host, but are more often concentrated in precise regions, and this cannot be explained by the proximity of the female filariae [9-11]. The blood microfilariae also are not uniformly distributed and the patterns are characteristic of each species; this has been shown using a particularly favourable experimental model: a saimiri monkey naturally and concomitantly infected with four species of *Mansonella*; thus, the ingestion by the hematophagous vector of the different microfilarial species could be compared under identical conditions [12]. It was found that each species is differently ingested and the observations suggest that, in the peripheral vessels accessible to the mouthparts of the vectors, each tends to have a particular niche, mainly defined by the vascular diameter.

Like all nematodes, the microfilariae detect their position owing to two amphids (Fig. 1B), receptors of physico-chemical stimuli [13]. Their powerful musculature and their internal osmotic pressure maintained by the excretory cell, allow them to stay in their niche against the lymph or blood flow. They are generally round in transverse section and, as can be seen in a drop of blood on a slide, they undulate laterally, like the free-living nematodes; but in the highly evolved worms with dorso-ventrally flattened microfilariae (Fig. 1G), they likely undulate like dolphins. Some microfilariae have developed means of maintaining the stability of their position in order to reduce to a minimum the expenditure of energy; in the large (350/10  $\mu\text{m}$ ) microfilaria of *Mansonella (Tetrapetalonema) colombiensis* of the saimiri, the body is barely mobile whereas the tail moves rapidly around its axis like a propeller; in a *Cercopithifilaria* species from a Japanese caprin, the cuticle has spiral ridges in the caudal region (Fig. 1H), which facilitate the flow of the surrounding fluid; even the whole body of some microfilarial species has acquired a spirally coiled shape, thus multiplying points of contact with the vessel walls. It is noticeable that this last morphological development appeared in widely disparate groups, a filaria of frogs and a species of *Onchocerca* (Fig. 1E,1F), which indicates that these features could be critical for the sustained maintenance of the transmissible developmental stages. Any alteration of that capacity has important consequences



### Figure 1

Shapes and dwellings of microfilariae. (A) Head of microfilaria *Chabfilaria jonathani* Bain, Purnomo & Dedet, 1983, a parasite of edentate, left lateral view with hook and right lateral view with transversal rows of cuticular points, respectively. (B) *Onchocerca volvulus*, head, apical view with the hook half inserted in the left amphidial pocket (in 19). (C) *Diplotriaena bargusina* Skrjabin, 1917, an oviparous filaria from a bird showing the primitive cephalic anatomy of the stage I in spirurids, ventral view, with numerous rows of spines (in 64). (D) *Acanthocheilonema viteae*: when the hook is raised, the vestigial cephalic spines placed on the right side clutch its basis (in 65). (E) *Waltonella* sp., from an Asiatic frog, with helical body (original). (F) *Onchocerca suzuki* Yagi, Bain & Shoho, 1994 from a wild caprine, with helical posterior region (in 66). (G) *Cercopithifilaria kenyensis* Eberhard, 1980, a parasite of an African monkey, with a dorsoventrally flattened body, lateral view (left) and median view (right) (in 67). (H) In *Cercopithifilaria tumidicervicata*, the thick lateral alae of the cuticle of the caudal extremity are helically switched (in 68). (I) *O. volvulus* inside a subcutaneous lymphatic vessel (in 69). (J) Initial lymphatics (IL, transversal section) are identified among the connective fibres of a subcutaneous tissue (SEM micrograph, in 8; arrows indicate transitional zones in which the endothelial lining becomes discontinuous; sparse endothelial cells are present). (K) *Mansonella (Tetrapetalonema) colombiensis* Esslinger, 1982 in a subcutaneous vessel of a saimiri: this microfilaria stays in vessels of ear lobe and tail extremity and is extremely rare in blood recovered from the vessels of the flank (in 12). Scales: the width of the mentioned microfilariae varies from 4 to 8  $\mu\text{m}$  according to the species; the head of *Diplotriaena* is 15  $\mu\text{m}$  wide.



**Figure 2**

The filaria in the vector. (A) *Breinlia (B.) booliati* Singh & Ho, 1973, a parasite of *Rattus* in Malaysia: microfilaria in several contiguous digestive cells of a mosquito, two hours after the blood meal (original). (B) *Acanthocheilonema viteae*: microfilaria coiled up in a muscular fibre, a few hours after the blood meal of the tick on the infected rodent (in 24). (C) *Setaria labiatopapillosa* from cattle, organogenesis of the first larval stage: on left, a 30 hours p.i. larva: the two R1 daughter cells are placed ventrally to the digestive primordia, itself composed by the 5 intestinal cells (3 on the right side, 2 on the left side), the 3 rectal cells and, anterior, the oesophagus; the 4 genital cells are just anterior to R1 cell; on right, a 72 hours p.i. larva: the 8 daughter cells of R1 arranged into 4 longitudinal submedian files (only two files are seen in this ventral view); the anal plug and the 3 rectal glands, R2, R3, R4, are transformed into an organ of absorption (in 70). (D) *Monanema martini* Bain, Petit & Bartlett, 1986, a parasite of an African murid: first stage larva in the hypodermis of a tick; with nuclei of the neoformed syncytium grouped (white star) close the bent larval back (in 71). (E) *Acanthocheilonema viteae*: migration of infective larvae towards the rostrum (below) 5 mn after the tick was attached to the rodent skin (transversal section of a tick: in the middle, the chelicers; below the basis of the rostrum and the Y shaped pharynx) (in 24). (F) *Brugia timori*: anterior region of a female larva with genital primordium at level of the glandular oesophagus (in 72). (G) Tails of infective larvae: on left, *Loa loa* with well developed lappets; on right, *O. volvulus* with reduced lappets; in middle, *Mansonella (Esslingeria) streptocerca* with 4 lappets, the median being divided (in 30).

[14]. In onchocerciasis, the pathogenesis seems to result from the accidental extravasation of the microfilariae into the peri-vascular connective tissue. Driving the microfilariae out of their niche seems to be a key factor of drug activity [14]. DEC for example induces a simultaneous extravasation of lymphatic microfilariae, causing a multitude of inflammatory reactions in the subcutaneous and epidermal tissues that will eliminate the microfilariae but also have disastrous pathological consequences (Mazzotti reaction). Ivermectin, which is thought to act as an inhibitor of neuro-muscular transmission, very rapidly induces a release of *Onchocerca volvulus* microfilariae from their niche by centripetal migration. They are found firstly in the deepest layers of the subcutaneous tissue [15], and then in the draining lymph nodes, where they are destroyed [16]. As a result of their passive intravascular migration to the regional lymph nodes, no new lesion is induced in the subcutaneous tissue with this drug [14].

In this context, the periodicity of microfilariae represents an astonishing specialization, because it implies repeated migrations in the blood. Direct observation and recording of the non-periodic microfilariae of *Litomosoides sigmodontis* in the mesenteric vessels of a jird (see Additional file 1) revealed that microfilariae are violently tumbled over by the moving mass of red blood cells that surrounds them. Hawking, who studied in depth the periodic filariae of humans and animals, concluded that the microfilariae actively maintain their position in the lung vessels during the phase of low microfilaraemia. Lung capillaries have a specialized structure, with internal supporting columns [17], and it is possible that these help the microfilariae to anchor themselves. According to Hawking [18] the stimuli which regulate this behaviour of the microfilariae are the partial pressures of oxygen and carbon dioxide, which is a plausible assumption given the presence of amphids. However, restriction to the lungs, or at least its synchronicity, is not obligatory since *Wuchereria bancrofti* has strains both with and without periodicity. The behaviours and niches of microfilariae are rapidly selected under the pressure of the vectors' habits.

The behaviours and shapes being linked, microfilariae are a relevant source of taxonomic features. However, as expected, convergences are frequent, so it is generally impossible to identify the genus on the basis of the microfilarial morphology alone when studying new material. The cephalic hook of the microfilaria seems to have generic value (Fig. 1A,1D), but its very minute size does not facilitate the use of this character.

2 - The larval stages in the vector: the movements of the ingested microfilariae induce important regulatory processes of translocation through the vector's stomach wall

The life cycle in the vector (Fig. 2) follows a constant pattern: migration of microfilariae through the digestive cells (Fig. 2A), and through the basal lamina, which may offer particular resistance (hematophagous Diptera); arrival in the hemocoel, penetration into a cell, and migration into several contiguous cells of the same tissue: these cells are immediately differentiated into a syncytium (unless the tissular structure is already syncytial, like a muscle). The cephalic hook of the microfilaria, situated on the left side and resting in one of two amphidial pockets [19] (Fig. 1B), plays an important role during this phase. Observation of a microfilaria of *Acanthocheilonema viteae*, which had just entered a muscle fibre of an argasid tick, showed that the hook caught and stretched the cytoplasmic membrane (which, interestingly, was not ruptured thereafter) while the very motile microfilaria coiled up in the peripheral undifferentiated sarcoplasm (Fig. 2B). During their development in the syncytium, the larvae are often arched, with the ventral face towards the exterior, and the anus and future rectal plug buried in the cytoplasm; the syncytial nuclei are grouped close to the dorsal face (Fig. 2D). Stage 1, from the microfilaria to the first moult, is unmotile, relatively long-lasting and corresponds to the end of organogenesis: connection of the diverse digestive primordia and multiplication of the mesenchymal R1 cell (Fig. 1C). Growth occurs mainly during stage 2. After the second moult, the larva is transformed in a motile resistant form (cytoplasmic reduction), the infective larva, which is freed into the hemocoel and will escape from the vector by rupture of the latter's mouthparts.

The vector's reactivity is initiated principally at the onset of the filaria cycle, in the mesenteron [20-22]. When the parasite load is too high, the stomach wall can be largely abraded by the numerous microfilariae migrating from a digestive cell to another; however the vector is prevented from haemorrhage by the basal lamina, which remains intact; the vector ultimately dies because it can no longer digest its blood meal. Penetration through the stomach wall may also be regulated, reducing drastically the proportion of microfilariae that escape into the hemocoel [limitation phenomenon, [20]]. The stomach wall of hematophagous arthropods reacts to the physical stimuli caused by the microfilariae moving on the clot surface with remarkably diverse mechanisms, due to the zoological diversity of the vectors. For example, in some *Simulium* species from savannah, limitation is linked to more rapid and more extensive secretion of the peritrophic matrix: in non or low infected flies the peritrophic membrane is very thin, whereas it thickens immediately after feeding on heavily infected carriers. The percentage of ingested microfilariae that reach the hemocoel can decline from 33 % to 0.4 % [21]. In *Aedes aegypti*, limitation is due to a kind of "acquired" reactivity of the digestive epithelium: whereas the first microfilariae cross the stomach wall

freely, later each digestive cell attacked by a microfilaria dies; the microfilaria is prevented from migrating further, probably due to the destruction of the cell's internal structures [22].

The adaptations of the parasite to the vector assessed by an improved success of transmission are rarely visible, but two examples can be cited. A filaria of a Malaysian rodent, which develops in the adipose tissue of a mosquito, has acquired a remarkable high degree of independence of the vector's trophic habits: contrary to what happened in another filaria-mosquito pair, the infected syncytium is no longer stimulated by a blood meal, which normally induces the synthesis of provitellogen [23]. *A. viteae* shows another adaptation: the infective larvae occupy empty spaces throughout the whole body, but half a minute after the tick has attached itself to the skin of its rodent host, they migrate to the mouthparts (Fig. 2E), thus facilitating their transmission [24].

Although physiological functions – such as enzyme secretion – are lost by the filariae, species of a given genus, and sometimes the same species, may be transmitted by vectors as different as ceratopogonids and simuliids (*Onchocerca* species for example), alike a spirurid which can develop in orthopters as well as in coleopters. The vector, interesting and important as it may be [25,26], does not seem to have a significant evolutionary impact.

There is, however, one exception, the genus *Cercopithifilaria*, the whole evolution of which seems to have been determined by its vectors. Its distribution is worldwide and its host range is "incoherent" [27,28]: cercopithecids, bovids and cervids, several groups of rodents, carnivora, and marsupials. The vectors are rhipicephalid ticks; their lifespan is long, their capacity for travelling great, and their trophic cycles necessitate several different hosts. All these features probably favoured captures, i.e. the passage of a parasite from one host group to another, zoologically distant, one [29].

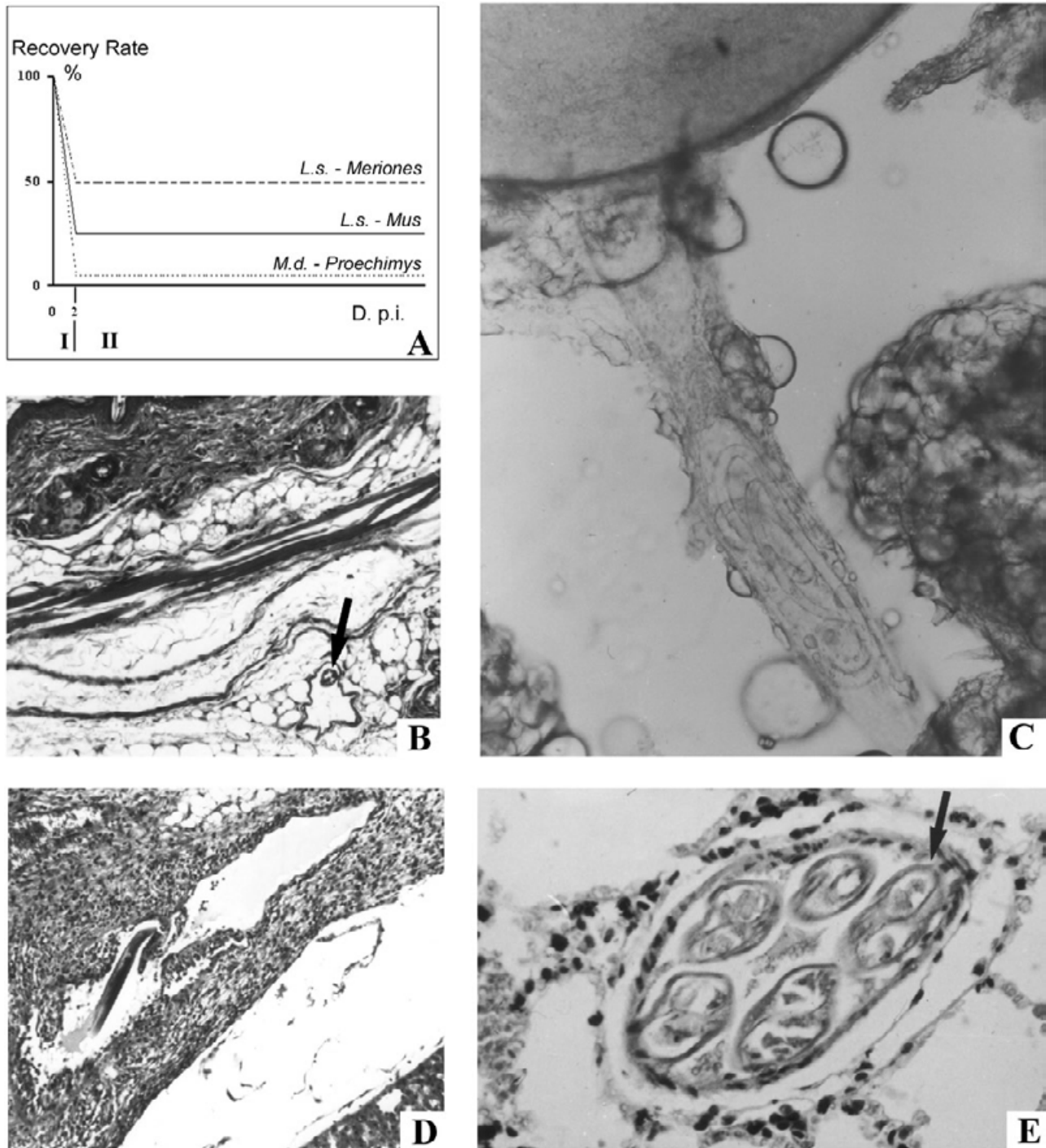
As a result of their intracellular habitat in the vector, the morphology of filariae is homogeneous during the larval development. The infective stages, specialized for penetrating the skin of the host, are all thin with a large glandular part of oesophagus and, in general, an attenuated head (Fig. 2F). However they have some morphological traits with phyletic value, such as the caudal extremity (Fig. 2G), and they are identifiable at the generic level [30].

3 - The final larval stages and adults in the vertebrate host: once they have escaped from the immune effectors at point of entry, they must resist liquid fluxes.

The details of many fewer life cycles are known in the vertebrate host than in the vector: 18 compared to a hundred [31]. These species belong mainly to the Onchocercinae and Dirofilarinae: their hosts are mammals or, rarely, birds or reptiles. The laboratory filarial models using cats [32] and, especially, rodents [33-39] are those that have provided most data. Comparative analyses have revealed a fundamental uniformity, which remains generally unrecognized. Thus it is justified to extrapolate these experimental results to the human parasites.

In primary infection, the inflammatory process, which is probably exacerbated by the vector's bite, results in the immediate destruction of a large number of larvae at the site of penetration. Some of them, a proportion characteristic of the filaria-host pair, however, reach the peripheral lymphatic vessels (Fig. 3A,3B,3C). Subsequent mortality is insignificant during a period that is also characteristic of each pair and may be as long as 8 months [38]. This suggests that the process of moulting does not particularly favour the release of immunogenic material, contrary to a fairly common assertion [40].

The lymphatic system offers shelter to the larvae at periods when their developmental stages are the most vulnerable. It allows them to escape different molecules, leukocytes, present and/or recruitable in the skin. It then provides easy routes of migration within the connective tissues, which rapidly react as assessed by leukocyte infiltrates. The inflammatory process follows the migration of the larvae (Fig. 3D) but the lymphatic wall generally prevents the penetration of granulocytes into the vessel lumen; even if thrombosis occurs, the larva can continue to migrate to its next sites. A lymphatic phase is thus not an exclusive property of the so-called lymphatic filariae; Wenk [41] revealed it with *L. sigmodontis* and, previously, Menon et al. [42] had done so with a lizard filaria. This behaviour seems to be a common trait during the first phase of the life of filariae in the vertebrate host [43,44]. From this perspective, it becomes possible to interpret certain biological enigmas. (i) The nodule of *O. volvulus* may originate from a lymphatic vessel, in which a thrombosis developed as a result of a dysregulated inflammatory process, within which the filaria secondarily adapted in order to survive. (ii) The cardio-vascular localization of *Dirofilaria immitis* and its migrations: the filariae initially remain in the connective and aponeurotic tissues, alike *D. repens*; they are then found in the abdominal cavity for a long period [45]; finally, they arrive in the heart when they are as long as 6 cm. These successive localizations are identical to those of a filaria of the genus *Monanema* that, as its natural host is a small rodent, provides a model easier to study [38] (Fig. 3E). This model has shown that the filarial worms recovered at necropsy from the peritoneal cavity in fact inhabit the lymphatics. The lymphatic



**Figure 3**

The filaria in the vertebrate host. (A) Kinetics of the filarial recovery rate (recovered filariae  $\times$  100 / inoculated larvae) in three filaria-host pairs: *Litomosoides sigmodontis* (L. s.) in *Meriones unguiculatus*, *L. sigmodontis* in laboratory mice, *Molinema dessetae* (M. d.) in its natural host, *Proechimys oris*. (B) An infective larva (arrow) in a lymphatic vessel of the subcutaneous tissue (*B. malayirodenti*) (in 43). (C) Two infective larvae migrating in a mesenteric lymphatic vessel to a lymph node (*Monanema martini*-rodent; larvae of this species are coiled up when migrating) (in 38). (D) Lymphatic vessel with a larva and surrounded by an inflammatory reaction (inflammatory cells have infiltrated the valves of the vessel but not its lumen) (in 43). (E) Adult filaria in a pulmonary blood vessel (*M. martini* rodent; in 38). Scales: the diameters of the infective larvae are 12–18  $\mu$ m, in fixed tissues; that of *M. martini* adult male worm is 30  $\mu$ m.



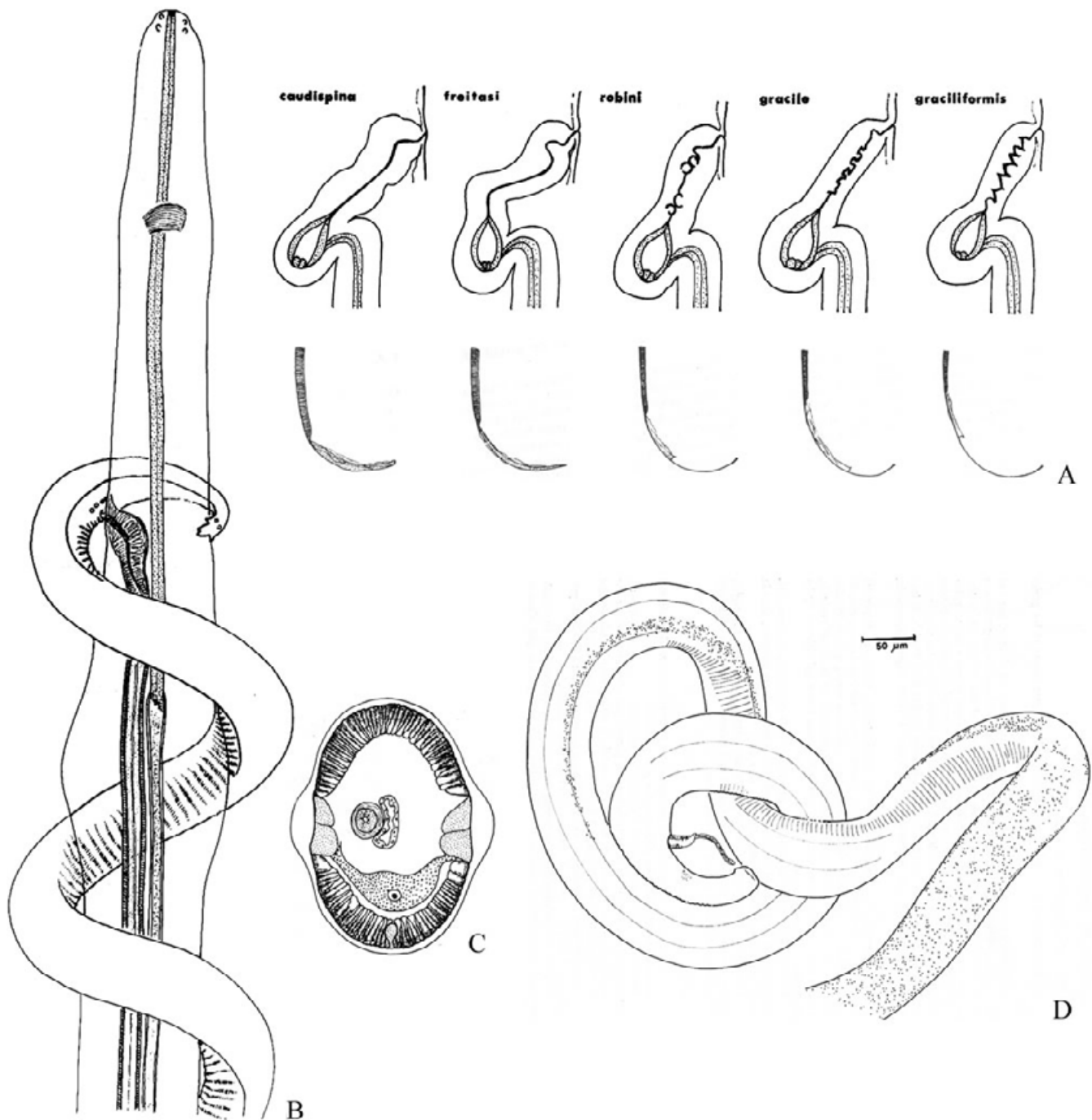
system is connected anatomically to the cardio-pulmonary circulation, so that the lymph, collected by the thoracic duct, mingles with the blood just before reaching the heart, carrying with it, for example, developing *D. immitis* worms. It is no longer necessary to envisage a dangerous and improbable perforation of the myocardium by the larvae, or a migration via the blood. In addition, micrographs published by Kume and Itagaki [46] show that *D. immitis* larvae in the vena cava are not in its lumen but in the wall, and are surrounded by a structure that suggests a lymphatic vessel. Lymphatic vessels have a remarkable capacity for dilatation, because of the interlocked structure of their cells, and they can contain large worms (38). The speciality of *D. immitis* is that it has adapted to living in blood. (iii) The filarial pulmonary nodules: the migrating larvae or the adult filariae must actively resist the centripetal lymphatic flow in order to move forward or even to stay in one place. Those of *M. martini* normally migrate against the lymph stream from the abdominal lymph nodes to the lymphatic vessels of the mesentery and then to those of the large intestine; once there, they resist expulsion by means of contractions of their body (which are adapted to the peristaltic movements of the host-organ, features which can account for the mild lesions with this filarial species). Similarly, an adult female of *B. malayi*, inside a very dilated lymphatic vessel of which the transparent wall had been hardened by fibrosis (this is a signature of the inflammatory process), was observed to undulate constantly in a plane parallel to the flow. Likewise *W. bancrofti* was found dancing in a lymphatic vessel [47] thereby maintaining its position. However either by chance or, more especially, following drug delivery as seen with *M. martini*, weakened worms may be carried along passively with the lymph and thus reach, via the right heart, the pulmonary arteries (Fig. 3E) where they are destroyed. This would explain the presence of nodules of *W. bancrofti* and *B. malayi* in the lungs of humans [48].

On its arrival in the host, the larva immediately begins its development: the head loses its attenuated shape and chemical changes occur at the surface of the cuticle [49]. The third larval stage hardly grows and seems to have little trophic requirement, so that this stage may develop *in vitro* apparently without its duration being modified either in this artificial environment or in a surrogate host. Two moulting strategies have been demonstrated [50]. Moulting no. 3 occurs generally between 7 and 10 days post inoculation, as it does in *Wuchereria*, *Brugia*, *Acanthocheilonema*, *Litomosoides*, *Monanema* and *Molinema* among the Onchocercinae, and *Loa loa* in the Dirofilarinae. In contrast, in *Onchocerca* and in *Dirofilaria*, respectively typical genera of these two subfamilies, the third moulting occurs much earlier, 2–3 days p.i. [51,45,52]. This biological character, as well as molecular markers of both

these filariae [53] and their endosymbiotic bacteria *Wolbachia* [54], suggest a close relationship between *Dirofilaria* and *Onchocerca* and on the contrary places *Loa* closer to the other Onchocercinae. The morphology of the infective stages also fits better with these new hypotheses.

At the end of stage 3, the female genital primordium has hardly developed: only its junction with the hypodermis is formed, at the site of the future vulva; this particular phase is well identified, as in *Caenorhabditis elegans*. The two genital cords lengthen and differentiate during the stage 4. In the male, the spicular primordia are formed from the dorsal wall of the rectum as soon as the end of the third stage is reached and, because of their presence, the body is inflated anterior to the tail. Thus the sex may be easily identified. Moulting no. 4 occurs between about 20 and 50 days p.i., generally after a significant phase of growth, but there may be exceptions: the young adult of *Loa loa* is only 4.5 mm long, barely twice the length of the infective larva [50]. The spicules have attained their definitive shape and size at the end of the fourth moulting, and the sensory caudal papillae are already visible beneath the exuvium. The culmination of adult development is the production of microfilariae. This necessitates repeated mating. However, nematodes are covered by a smooth cuticle that does not facilitate copulation; oxyurids, strongylids etc. have developed various kinds of suckers or bursae that the male uses to grasp the female. Filariae, submitted to similar constraints, have developed original but inconspicuous systems, compatible with their life within tissues. These are the cuticular rugosities situated on the ventral aspect of the posterior region of the male (*area rugosa*), such as bosses and transverse bands of longitudinal short crests which form a «non-slip» surface (Fig. 4B,4D). Their arrangement is specific and permits differentiation of strains by means of minute variations that are significant on the scale of the parasite; thus it is possible to distinguish geographic forms of *B. malayi*, those from China and Korea, and those from India [55]. The body swellings situated in the anterior part of the worm in some filarial species (Fig. 4B), which have long remained enigmatic, also have a role in facilitating mating [56]. This very specialized character has appeared in distinct lineages, in *Mansonella*, and *Cercopithifilaria* as well as in the species of *Onchocerca* from suids [57]. The dorso-ventral flattening, observed in some of these species between the body swellings, also helps to facilitate the restraining of the female by the male.

The mechanisms involved in egg laying are largely unknown; they have to satisfy two constraints: the need to meet a vector and the need not to exacerbate the host immune response. In *L. sigmodontis*, it has been possible to observe microfilariae expelled in small groups of 5–6, previously accumulated in the vagina. But the



**Figure 4**

(A) Evolution of the vagina and the left spicule in the five species of *Dipetalonema* spp. sensu stricto, known from platyrrhine monkeys: the proximal lumen of the vagina is a straight tube in the primitive species *D. caudispina*; it will become coiled to control the delivery of microfilariae, the maximum of complexity being reached in *D. graciliformis*. In parallel, the distal lamina of the left spicule evolves from a thick short part to a long and thin one, capable of penetrating deep into the complex vagina (in 57). (B) Hypothetic representation of the mating in filariae: the male grasps with its posterior region the female at level of the vulva, which is situated in the anterior part; *area rugosa* on the ventral aspect of the male and a body swelling of the female are presented (in 56). (C) A body swelling of *Mansonella (Tetrapetalonema) mariae* Petit, Bain & Roussillon, 1985 due to the presence of a giant pseudocoelomocyte (transverse section) (in 56). (D) Caudal region of *Brugia malayi*, Indian strain characterized with the numerous cuticular bosses, beginning on the right side (in 55).

morphological diversity of the vagina suggests the existence of different modes of egg laying to achieve these ends. The vagina may be fairly curved, with a simple shape (*Onchocerca* for example) or it may present a succession of chambers and sphincters of great complexity (Fig. 4A), to which the spicules of the male have to adapt [58].

At the beginning of the patent phase of infection, the microfilarial density is inversely correlated with the number of adult filariae [11], because the size and fertility of female worms are reduced as the parasite load increases [ongoing work]. Later, the quantitative relationships become extremely complex because they are dependent to a large extent on the immune response acquired during infection. This response has been proved to be able to reduce the survival of microfilariae in several experimental infections, and even to totally eliminate microfilaraemia although uterine microfilariae are still abundant in the female worms [59-61]. The adaptations to ensure completion of the life cycle may vary. In *L. sigmodontis*, the microfilarial densities are exceptionally high, reaching 1500 per  $\mu\text{l}$  of blood (about 0.06% of the bodyweight of the rodent); the vector, the macronyssid mite *Ornithonyssus bacoti*, ingests only a small amount of blood due to its small size and, in addition, 80% of the microfilariae arrive dead in the digestive caeca because they are torn by the mite's pharyngeal teeth [62]. In another example, a filaria parasitic in birds, the adult worms die after one season of egg laying and the maintenance of the species is ensured by the microfilariae which are transmitted to the nestlings by the mallophagean vector [63].

## Conclusion

The above observations indicate the importance of physical factors in the relations between the filariae and their two successive hosts, haematophagous arthropods and terrestrial vertebrates. Like any other nematode, the filaria is active thanks to its locomotor system, muscles and 'endoskeleton', the high osmotic pressure maintained by the excretory cell. Any drug that affects this system should be very efficient.

The filaria uses this locomotor system either to change its location, due to the necessity of mating for example or, more fundamentally, to avoid moving. It is obvious that parasites inhabiting the digestive tract must resist expulsion by actively maintaining themselves in one place. Because of this constraint, they have developed various mechanisms, with different origins, to achieve this single end. These mechanisms include the suckers of cestodes and trematodes, the longitudinal crests of the trichostongylids that are coiled around the intestinal villi, and the cuticular cephalic flanges that ensure the anchoring of some spirurids. A number of this last group have also chosen to invade the host's tissues.

Filariae adopted a similar method to that of their spirurid ancestors. The resulting protection is still not perfect because the circulation of fluids can drive them to organs in which they will be destroyed, such as the lungs and liver. To resist this they have been forced to develop their own, more subtle, methods of active mechanical resistance.

## Additional material

### Additional File 1

*Mesenteric blood vessels of a Meriones unguiculatus with 1300 Litosomoides sigmodontis microfilariae / 1 blood  $\mu\text{l}$ . Each microfilaria is visualised in the blood stream by a pale, sometimes squirming patch amongst the red blood cells (a live L. sigmodontis microfilaria is 80–100  $\mu\text{m}$  long and 4  $\mu\text{m}$  wide).*

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