LYMPHATIC DWELLING FILARIOID NEMATODES IN REINDEER *RANGIFER TARANDUS TARANDUS*, (CERVIDAE) IN FINLAND, IDENTIFIED AS *RUMENFILARIA ANDERSONI* LANKESTER & SNIDER, 1982 (NEMATODA: ONCHOCERCIDAE: SPLENDIDOFILARIINAE)

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Summary:

A filarioid nematode inhabiting the lymphatic vessels of the subserosal rumen and mesenteries associated with a high prevalence of its microfilariae in peripheral blood was observed in Finnish reindeer (*Rangifer tarandus tarandus*) in 2004 and 2006. Adult specimens were collected by dissecting lymphatic vessels from slaughtered animals, where some of the nematodes were seen through the wall of the dilated vessels as thin white winding threads obscuring the vessel. The morphology of adult worms and microfilaria is described based on light and scanning electron microscopy. These filariae belong to the subfamily Splendidofilariinae of the Onchocercidae and resemble *Rumenfilaria andersoni*, recovered from different host and localization, the ruminal veins of *Alces alces* in Canada. Comparison of paratypes of this species revealed only minor differences which were not sufficient to separate the filarioid parasitic in *R. tarandus* in Finland and we identify the nematode as *R. andersoni*. However, the findings suggest two different parasite populations. The finalizing of this taxonomic question in the future requires an integrated approach, in which the DNA-based and morphological identifications are consistent.

KEY WORDS : filarioid, lymphatic filariosis, *Rumenfilaria*, *Rangifer tarandus*, *Alces alces*.

Résumé : FILAIRES LYMPHATIQUES CHEZ LE RENNE *RANGIFER TARANDUS TARANDUS* (CERVIDAE) EN FINLANDE IDENTIFIÉES À *RUMENFILARIA ANDERSONI* LANKESTER ET SNIDER, 1982 (ONCHOCERCIDAE : SPLENDIDOFILARIINAE)

Des filaires (Nematoda) situées dans les vaisseaux lymphatiques de la séreuse du rumen et du mésentère, associées à une forte prévalence des microfilaires sanguines, ont été trouvées chez le renne finlandais, Rangifer tarandus tarandus, *en 2004 et 2006. Elles ont été récoltées en disséquant les vaisseaux lymphatiques où elles étaient visibles par transparence ou signalées par l'aspect laiteux du vaisseau oblitéré. La morphologie des adultes et des microfilaires a été étudiée aux microscopes optique et électronique à balayage. Ces filaires sont des Splendidofilariinae, Onchocercidae, et ressemblent à* Rumenfilaria andersoni, *récolté dans les veines mésentériques d'*Alces alces *au Canada. La comparaison avec les paratypes de cette espèce révèle des différences mineures qui ne sont pas suffisantes pour distinguer le parasite de* R. tarandus *en Filande et nous l'identifions à* R. andersoni. *Cependant, les observations suggèrent deux populations distinctes de parasites. Pour résoudre cette question taxonomique il sera nécessaire d'effectuer une approche intégrée, où les identifications moléculaires et morphologiques seront cohérentes.*

MOTS CLÉS : *filaire, filariose lymphatique,* Rumenfilaria, Rangifer tarandus, Alces alces.

INTRODUCTION

Reindeer (*Rangifer tarandus* L.) are circumpolar cervids that are harassed during summer by hordes of blood sucking insects, such as mosquitoes, blackflies and tabanids. The mass appearance of insects enable the occurrence of filarioid nematodes in reindeer, as they may serve as vectors and intermediate hosts for these tissue dwelling parasites. The first filarioid nematode documented and described in reindeer was *Setaria tundra* Issaitschikoff & Rajevskaya, 1928, Setariinae (in Rajevskaya, 1928), in the USSR. Species from the subfamily Onchocercinae were recorded in the USSR by Nikolaevskii (1961) and Mitskevich (1967). In northern Finland, Lisitzin (1964) found an *Onchocerca* sp. in a subcutaneous nodule in the muzzle of a reindeer. Rehbinder (1973) and Rehbinder *et al.* (1975) recorded a high frequency of subcutaneous nodules in reindeer containing an *Onchocerca* sp. which was identified by Bain *et al.* (1979) as *O. tarsicola* Bain & Schulz-Key, 1974 (Bain & Schulz-Key, 1974) later synonymized with *O. skrjabini* Ruklyadev, 1964 by Yagi, Bain & Shoho (1994). Bylund *et al.* (1981) found these worms prevalent in reindeer in Finnish Lapland. Perhaps the most detrimental filarioid infection in Northern ungulates is setariosis. *Setaria* sp. caused a peritonitis outbreak in Scandinavian reindeer in 1973, and in Finland also in 2003-2005. The causative agent was identified as *S. tundra* by its morphological (Nikander *et al.*, 2007) and molecular biological features (Laaksonen *et al.*, 2007).

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While blood samples were collected and studied during the recent *S. tundra* outbreak, another kind of microfilaria (mf) was observed in reindeer in January 2004. These mf were abundant, especially in reindeer originating from the southern part of Finnish reindeer herding area. Adult parasites were found in December 2006 in Kuusamo, living in the subserosal lymphatic vessels of the rumen of the reindeer.

This is the first report of a lymphatic dwelling filarioid nematode in reindeer from Finland. The general morphology is that of *Rumenfilaria andersoni* Lankester & Snider, 1982, a venuous filaria from the moose *Alces alces* in Canada (Lankester & Snider 1982). However, since the present parasites originate from another host, *Rangifer tarandus tarandus*, in another region, Finland, and in a different localization, the lymphatic vessels, the existence of two distinct species is possible. This is an important point for elucidating the origin of the infection: is it recently widespread from Canada, or a local infection which usually is at low prevalence and intensity, and unnoticed. Type specimens of *R. andersoni* were thus examined and, in the morphological analysis of both materials, a particular attention has been given to the characters which are known to evolve rapidly and are generally discriminative, such as the cuticular ornamentation of adult worms and the microfilaria (Bain & Babayan, 2003).

MATERIAL AND METHODS

Material analyzed

• Specimens from Finland

The first specimen (female) was found and collected from a slaughtered adult female reindeer in Kuusamo in the south-eastern part of the Finnish reindeer herding area in December 2006. The worm was recovered from the ruminal subserosal lymphatic vessel. More specimens were collected from reindeer in January-February 2007 from different slaughter houses locating in the southern part of the Finnish reindeer herding area. The worms were extracted by dissecting mesenteric and subserosal ruminal lymphatic vessels under a stereo microscope and fixed for morphological studies. Blood samples for mf description were collected from infected reindeer.

• Paratype specimens of *Rumenfilaria andersoni* Lankester & Snider, 1982

The comparison was made with paratypes of *Rumenfilaria andersoni*: two anterior parts (one short, one long) of fully gravid female paratypes, one long posterior part of a male paratype; kindly loaned by the United States Parasite Collection, Beltsville, numbers 077015.00, storage number MT76-B. Host: *Alces alces* (L.). Geographical region: northwestern Ontario, Canada.

Morphological study

Worms taken for scanning electron microscopy were fixed in a hot fixative containing formaldehyde (40 %, ten parts), acetic acid (one part) and water (89 parts). Three female and five male nematodes were dehydrated in increasing concentrations of ethanol and critical point dried (Bal-Tec, CPD 030). Dried samples were mounted on aluminium stubs, coated (40 sec.) with platinum (Agar Sputter Coater), and examined under a Zeiss DSM 926 scanning electron microscope (The Electron Microscopy Unit of the Institute of Biotechnology, University of Helsinki).

Mf were extracted from the uterus of female parasites or recovered from the blood samples by using modified Knott's method (Georgi, 1985), and also studied in blood smears stained with May-Grünwald-Giemsa.

For classic morphological studies, worms were fixed in 70 % ethanol, cleared in lactophenol and observed under a light microscope equipped with a drawing tube. A total of ten female and ten male parasites and several pieces of worms were examined and measured (University of Helsinki). Four complete females, three complete males and several long anterior and posterior parts of females and males, including a long posterior part of female at fourth moult, fixed in 70 % ethanol were used for the description here. They are deposited in the Museum National d'Histoire Naturelle, Paris (MNHN) collection number 249 JW (adult worms). Head papillae, the shape of mouth opening and buccal cavity were studied in apical view of a female. Using a razor blade, the head was severed under a stereomicroscope and oriented under the microscope (see for example Uni *et al.*, 2004). Particular attention was paid to cuticular ornamentations, which often provide distinctive characters.

For the study of mf, blood smears were prepared and stained. Two slides are in the MNHN collection (68 JW and 58 YU), Mf from fixed females were also studied, after extraction from the distal part of the uteri near the vagina; they were examined in lactophenol for the habitus, cephalic hook and spines, shape of posterior extremity; cephalic space, excretory and anal pores. Caudal nuclei were located in stained blood smears. The presence or absence of sheath was noted.

RESULTS

Usually the reactions on the nematode infected lymphatic vessels were visible to the naked eye, especially in older reindeer. The typical gross pathological changes associated with the infection were the dilatation of the vessels, lymphoedematous swelling of the vessel walls around the living worm, and greenish or greyish granulomatous or fibrotic reaction in the foci containing dead worms.

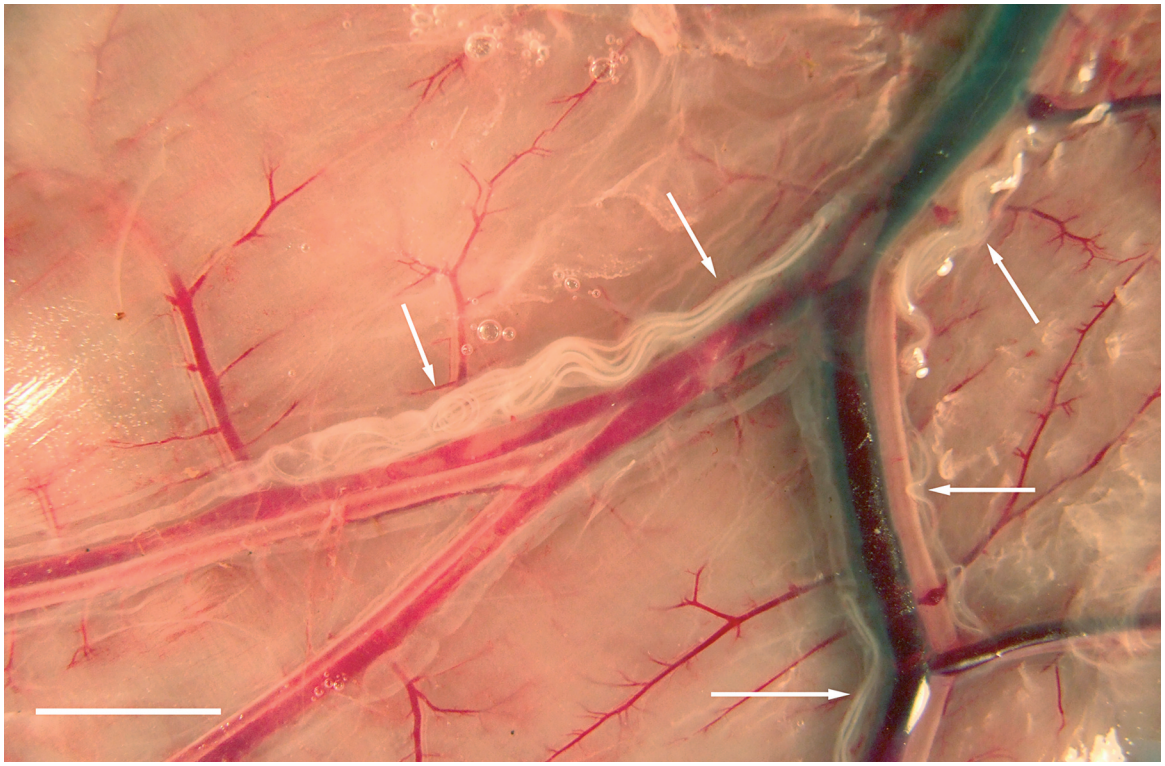

Fig. 1. – The serosal surface of the rumen of the reindeer. *Rumenfilaria andersoni* nematodes can be seen through the wall of the dilated lymphatics as thin white winding threads obscuring the vessels (arrows); scale bar = 3 mm.

Some of the nematodes were seen through the wall of the dilated vessels as thin white winding threads occluding the lumen of the vessel. The nematodes were readily visible especially in emaciated reindeer calves with scant or no fat around the lymphatic vessels on the serosa of the rumen (Fig. 1). The presence of the nematodes had often resulted in lymphangiectatic varices containing usually one longer female and 2-3 male worms.

The nematode species found in the lymphatic vessel was identified following the key of Filarioidea by Anderson & Bain (1976) as belonging to the family Onchocercidae (Leiper, 1911), subfamily Splendidofilariinae Chabaud & Choquet, 1953 with characters of the genus *Rumenfilaria* Lankester & Snider, 1982: mainly caudal papillae in two longitudinal rows consisting of preanal, adanal and postanal pairs, vulva in posterior half of oesophagus, female tail extremity rounded (Lnakester & Snider, 1982).

MORPHOLOGICAL ANALYSIS

• Specimens from *Rangifer tarandus*, in Finland (Figs 2-5)

General

Measurements of some specimens are given in Table I; maximum size was 160 mm long and 350 µm wide for female, and 50 mm/250 µm for male worms. Anterior

	Female		Male	
	Average	**Range**	**Average**	**Range**
Body length	65 mm	40-100 mm	45 mm	30-55 mm
Body width	240 µm	140-350 µm	200 µm	100-255 µm
Length of oesophagus	1,260 µm	1,100-1,530 µm	1,420 µm	1,300-1,550 µm
Distance to nerve ring	225 µm	195-250 µm	230 µm	210-250 µm
Length of tail	170 µm	140-200 µm	130 µm	100-155 µm
Distance to vulva	830 µm	750-900 µm	–	–
Length of ovejector	1,050 µm	–		
Right spicule	–	–	130 µm	110-148 µm
Left spicule	–	–	140 µm	130-159 µm

Table I. – Morphometrics of female and male worms (four complete females, three complete males and several long anterior and posterior parts of females and males) of *Rumenfilaria andersoni* from the reindeer in Finland.

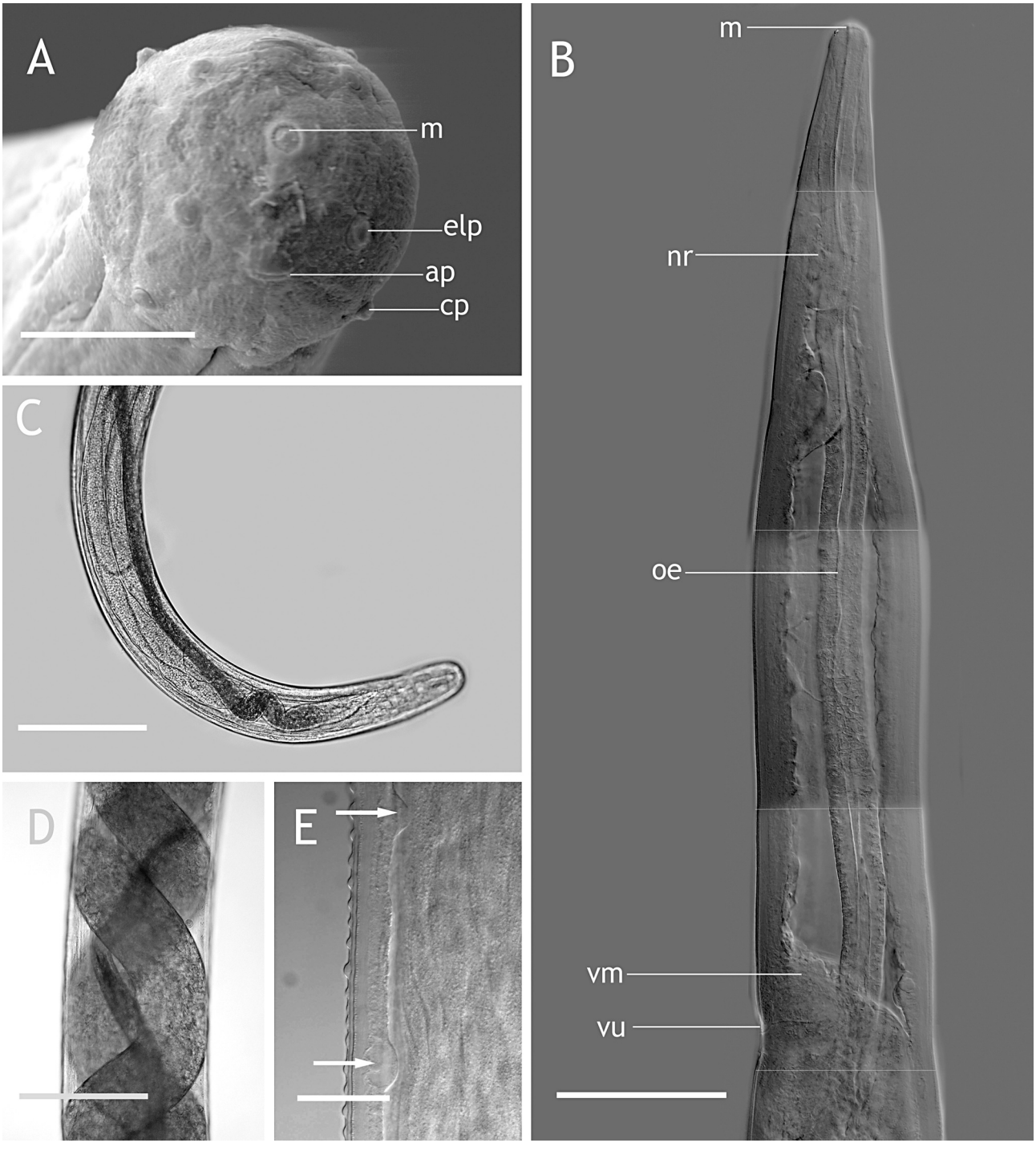

Fig. 2. – A. The anterior end of *R. andersoni* as seen in SEM; m = mouth, elp = externo-labial papillae, ap = amphidial pore, cp = cephalic papillae; scale bar = 20 µm. B. The anterior part of female; five micrographs combined together; the points of junction indicated; m = mouth, nr = nerve ring, oe = oesophagus, vm = vaginal musculature, vu = vulva; scale bar = 200 µm. C. The posterior part of the female; scale bar = 400 µm. D. Uterus; scale bar = 200 µm. E. Cuticular ornamentation and blisterlike structures (arrows) between epicuticle and internal structures; scale bar = 50 µm.

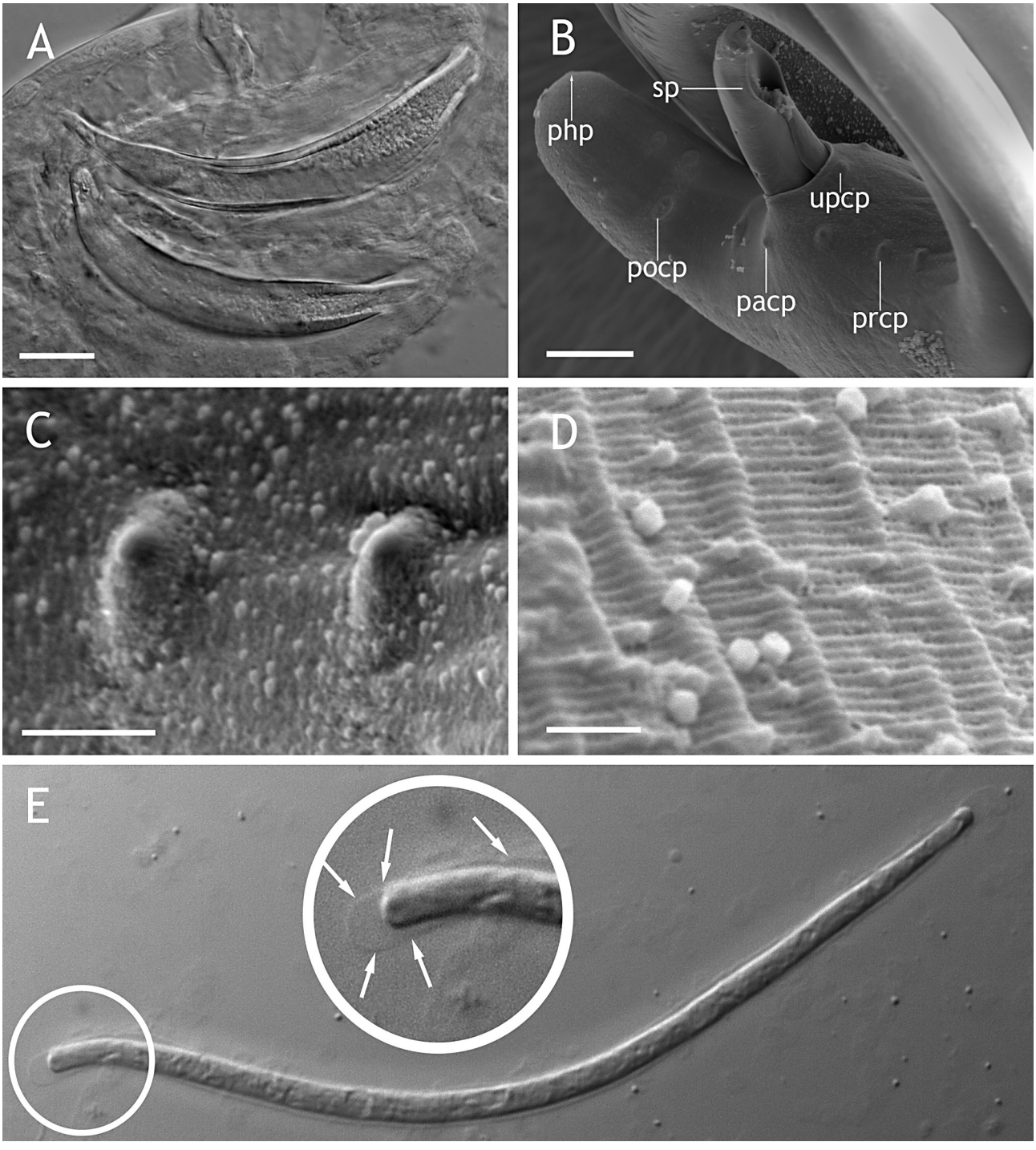

Fig. 3. – A. Spicules; scale bar = 20 µm. B. The posterior end of the male as seen in SEM; sp = spicule, prcp = precloacal papilla, upcp = unpaired precloacal papilla, papc = paracloacal papilla, pocp = post-cloacal papilla, php = phasmidial pore; scale bar = 20 µm. C. *Area rugosa* as seen in SEM, showing cuticular bosses and two precloacal papillae; scale bar = 5 µm. D. Cuticular surface in SEM showing transverse ridges and delicate longitudinal striation with tiny cuticular bosses; scale bar = 1 µm. E. Microfilaria, 5 µm wide, in the peripheral blood of the reindeer; the arrows indicate the sheath in close-up micrograph.

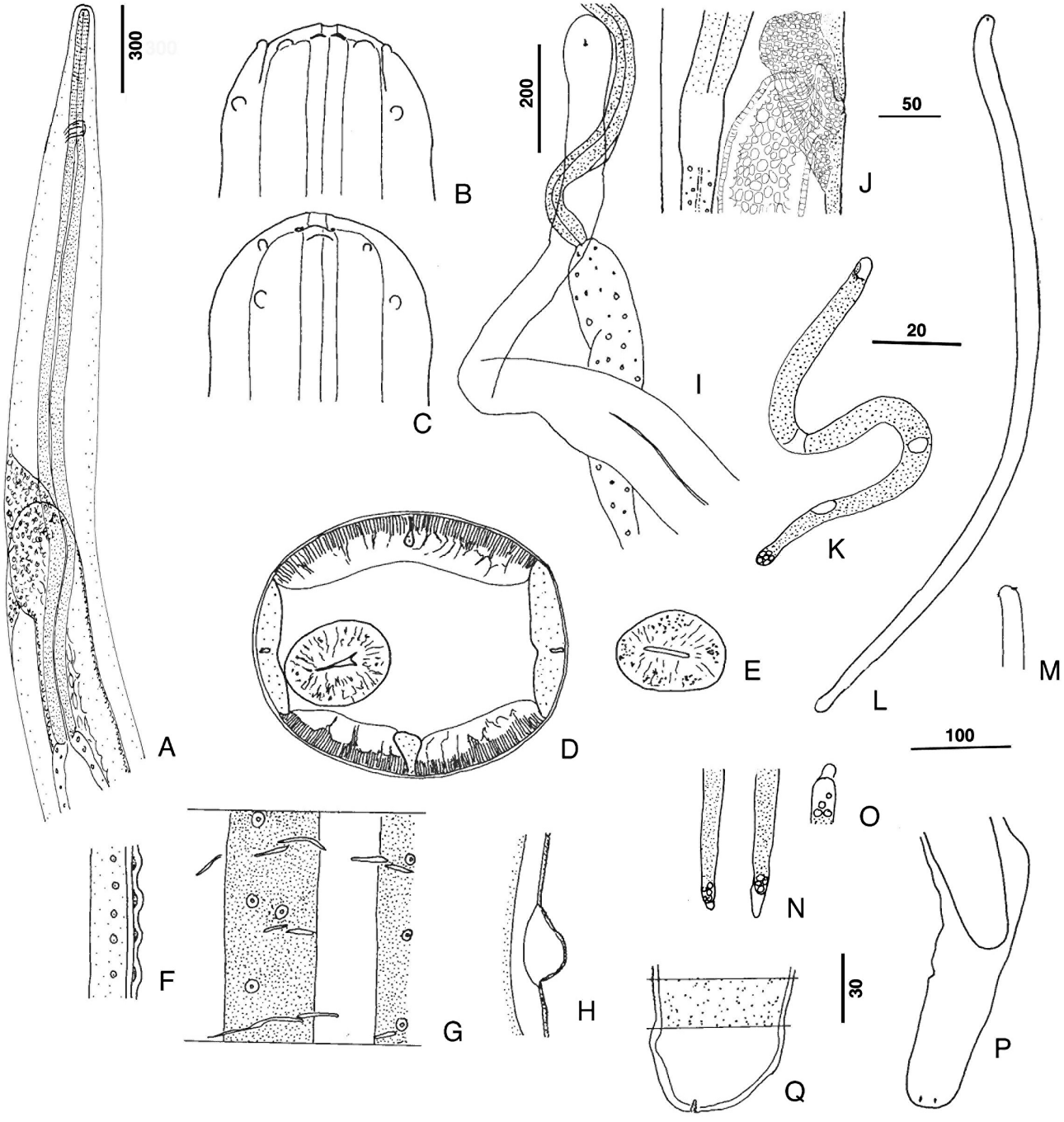

Fig. 4. – *Rumenfilaria andersoni*, female. A-M. From *Rangifer tarandus*. A. Anterior region, left lateral view. B & C. Head, dorso-ventral and lateral view. C. Anterior part of body, musculo-glandular oesophagus. D. Transverse section of body at musculo-glandular level. E. Transverse section of oesophagus, just posterior to D. F. Cuticular body crests above lateral chord, dorso-ventral view. G. Surface view of the transverse crests, lateral view. H. Structure of a crest., longitudinal section. I. Oesophageal-intestinal junction, vulva, vagina, and ovejector, dissected out. J. Other female, vagina and distal part of ovejector, at level of oesophageal-intestinal junction, righ lateral view. K. Microfilaria from a stained blood smear. L. Microfilaria dissected out from the ovejector. M. Anterior extremity with hook and spines, ventral view. N-O. From *Alces alces,* paratypes. N. Two posterior extremities with sheath. O. Anterior extremity with sheath. P & Q. From *R. tarandus*, female at fourth moult. P. Tail and exuvium. Q. Exuvium with bosses and phasmids. Scales in µm: A: 300; B, C, H, K, L, M, N, O: 20; D, E, G, Q: 30; F, P: 100; I: 200; J: 50.

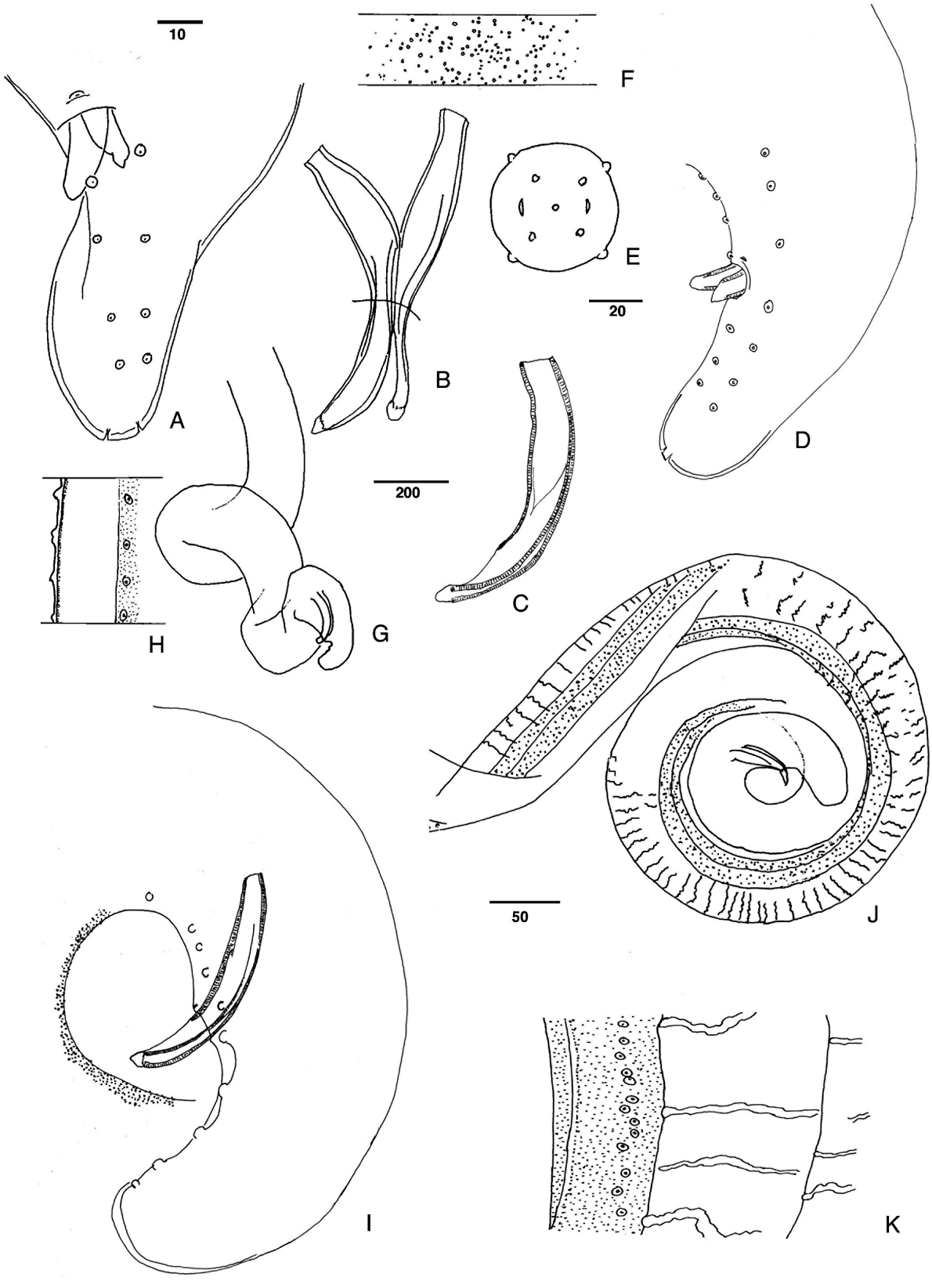

Fig. 5. – *Rumenfilaria andersoni*, male. A-H. From *Rangifer tarandus*. A. Postcloacal papillae of a male with four pairs, ventral view. B. Spicules, ventral view. C. Right spicule, left lateral view. D. Caudal papillae, subventral view. E. Head, en face view. F. *Area rugosa*, ventral view. G. Coiled posterior extremity. H. Cuticular crests (groves), longitudinal lateral view. I-K. From *Alces alces*, paratypes. I. Caudal region showing papillae, *area rugosa* and right spicule, left lateral view. J. Coiled posterior region with transverse crests, mainly dorsal. K. A subdorsal quadrant with transverse crests, and half a lateral chord. Scales in µm: A: 10; B, D, I, K: 50; E: 20; G, J: 200.

extremity attenuated, head round (Fig. 2A, B and 4A, B, C). Eight head papillae: four externo-labial and four cephalic papillae arranged in two rectangles slightly stretched dorso-ventrally in female (Fig. 4B, C), in two squares in male (Fig. 2A and 5E); an elevation posterior to each amphidial pore in female. Mouth minute, round (Fig. 2A and Fig. 5E). Buccal cavity small, limited posteriorly by a minute ring 2 µm long, often reduced to a bright lamella (Fig. 4B, C). Tail with round smooth extremity (Fig. 2C) and conspicuous phasmids.

Female
Transverse section of body flattened dorso-ventrally; cuticle thin; no lateral alae; lateral chord well developed, half chord with two rows of nuclei; about 4-6 muscle cells/quadrant (Fig. 4D, G). Oesophagus with short muscular portion ending at level of nerve ring (Fig. 2B, 4A) and long glandular portion; lumen of oesophagus Y-shaped in muscular anterior part, dorso-ventrally flattened in glandular part with short Y-shaped transition at end of muscular part (Fig. 4D, E). Cuticular ornamentation present, mainly in posterior part of worms; formed by delicate transverse crests (Fig. 2E, 4F, G, H), discontinuous; in longitudinal section, each crest like a blister between epicuticle and internal layer, about 8 µm long and 5 µm high. Vulva a longitudinal slit in posterior region of glandular oesophagus. Vagina very short, surrounded by massive musculature (Fig. 2B) composed of many small fibres; no vaginal chamber and cellular sphincter, but thin flattened tube composed of transverse part, bend, longitudinal part, bend, and transverse part giving access to ovejector (Fig. 4J). Ovejector 1 mm long (Fig. 4I), thick since the beginning (80 µm), lined with an outer thin layer of longitudinal muscles and a three-fold thicker internal epithelial layer of swollen cells; cells arranged in salient rows posteriorly, separated by furrows in which microfilariae lie.

Microfilariae
Blood and uterine mf with visible sheath or not (Fig. 3E, 4K, L, M). Anterior end shortly attenuated; small cephalic hook and, opposite, a group of three spines; posterior quarter of body attenuated; extremity round with subterminal constriction. Stained mf from blood (Fig. 4K): 137 µm long, 7 µm wide, cephalic space 5 µm long, excretory pore, internal body and anal pore 50 µm, 90 µm and 115 µm from head; tail nucleated till the tip. Two entire uterine mfs: length 156 and 158 µm, width 3.5 and 4 µm. Blood mfs (Knott technique; n = 100 mfs, from several reindeer): length 160.2 (157.5-164.3) µm, width 5.3 (5.25-5.4) µm.

Male
Posterior part coiled and with cuticular ornamentation (Fig. 3B, D and Fig. 5I): ventrally *area rugosa* 1,600 µm long, beginning anterior to cloacal aperture and composed of bosses (Fig. 3C and 5F); dorsally and laterally, composed of delicate transverse crests (Fig. 3D and 5H), discontinuous, undulating. Caudal papillae: unpaired precloacal papilla (identified on some specimens) and seven or eight pairs in total: three pairs precloacal; one pair paracloacal; three or four pairs post-cloacal, symmetrically arranged (Fig. 3B and 5A, D) or not. Phasmidial pore funnel-shaped. Spicules subequal; right spicule with ventral extremity bevelled (Fig. 3A, B and 5B, C).

Female at fourth molt (Fig. 4P, Q)
A posterior part of worm 27 mm long; exuvium with conspicuous cuticular phasmidial channels. Cuticular ornamentation of exuvium composed of bosses.

• Paratypes of *Rumenfilaria andersoni* Lankester & Snider, 1982 from *Alces alces*

The paratype specimens examined are consistent with the original description by Lankester & Snider (1982). In particular, mf within a sheath, as observed on a dozen mf which escaped from sectioned part of worm (Fig. 4N, O). Female vagina short, simple, wrapped in massive musculature, ovejector thick with large swollen epithelial cells. Cuticular ornamentations, previously undescribed, present in both sexes. In females, these are faint transverse crests observed in mid-region of body (posterior part of body absent). In male, these are located in posterior region, composed of a ventral *area rugosa* of bosses, delicate transverse crests dorsally 35 to 70 µm apart (Fig. 5I-K). Ten pairs of caudal papillae in one paratype: four precloacal pairs, one paracloacal pair, five postcloacal pairs. Unable to analyze in detail oesophagus and its lumen.

DISCUSSION

Taxonomic discussion. The filarioid nematodes from *Rangifer t. tarandus* are morphologically similar to those of *Rumenfilaria andersoni* recovered from *Alces alces* in Canada, except for some details. In the mf, the subterminal constriction seems more pronounced and mf are often sheathless, whereas the sheath is present in all specimens described by Lankester & Snyder (1982) and the paratypes examined here from *A. alces*. However, this is not a specific character because it was observed with the human filarioid *Brugia malayi* that the proportions of mf hatching out of the egg shell (sheath) differed according to strains (Mak, 1987 *in* Bain & Babayan, 2003). Also the number of caudal papillae must be considered: 8-9 and even ten (this study) pairs, including five postcloacal pairs, in parasites from *A. alces*; 7-8 pairs in specimens from *R. t. tarandus*, including four postcloacal pairs. Since we consider here the morphospecies, these differences are insufficient to separate the

filarioid parasitic in *R. tarandus* in Finland from the fialrioid from *A. alces* in Canada and we identify them as *Rumenfilaria andersoni*. However, this is suggestive of two different parasite populations, finalization of this taxonomic question requiring an integrated approach in future, in which the DNA-based and morphological identifications are consistent.

This study raises several questions that offer challenging opportunities for further research in rumenfilariosis in reindeer, such as the life cycle of the parasite, its clinical significance, and the pathology associated with the infection. It may even serve as a possibility for developing an animal model of human filariosis, considering the high prevalence of infection among reindeer and the observed progressive process of the disease through the obstruction, lymphaedema, lymphangitis, to the chronic changes such as granulomatous inflammation and fibrosis of the lymphatic vessels.

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