AFFINITIES BETWEEN *CUTIFILARIA* (NEMATODA: FILARIOIDEA), PARASITES OF DEER, AND *MANSONELLA* AS SEEN IN A NEW ONCHOCERCID, *M. (C.) PERFORATA* N. SP., FROM JAPAN

UNI S.*, BAIN O.** & TAKAOKA H.***

Summary:

A new dermal filarioid nematode, collected from *Cervus nippon nippon* (sika deer) on Kyushu Island, Japan, showed close affinities between the genera *Cutifilaria* and *Mansonella* (Onchocercidae: Onchocercinae): no buccal capsule, esophagus reduced to a thin fibrous tube, and female tail with four lappets. We propose *Cutifilaria* as a subgenus of *Mansonella*. *Cutifilaria* was distinguished from the five other subgenera, *Mansonella*, *Tetrapetalonema*, *Esslingeria*, *Sandnema*, and *Tupainema*, in having an area rugosa composed of transverse bands with tiny points, 14-16 papillae around the cloacal aperture, two prominent rhomboidal subterminal papillae, and a thick right spicule with spoon-shaped distal extremity. The host range of *Mansonella* was extended to ungulates by the addition of *Cutifilaria*, which appears to be derived from *Tupainema*, parasitic in Tupaiidae (insectivores), because of the similarity in their right spicules; *Cutifilaria* seems to have an Asiatic origin. *M. (C.) perforata* n. sp. was distinct from the sole other related species, *M. (C.) wenki*, a parasite of *Cervus elaphus* (red deer) in Europe, having a more complex right spicule with a sturdy terminal point and microfilariae with a bifid posterior end. In addition, almost all females had cuticular pores near the vulva, on the ventral line. The prevalence of microfilariae and adults of *M. (C.) perforata* in the skin of sika deer was 38 % and 21 %, respectively.

KEY WORDS : *Cervus*, cuticular pore, *Cutifilaria*, Filarioidea, Japan, *Mansonella*, microfilaria, Onchocercinae, sika deer, skin snip.

Résumé : AFFINITÉ ENTRE *CUTIFILARIA* (NEMATODA: FILARIOIDEA), PARASITE DE CERFS, ET *MANSONELLA*, RÉVÉLÉE PAR UNE NOUVELLE FILAIRE, *M. (C.) PERFORATA* N. SP., AU JAPON

Une nouvelle filaire dermique, récoltée chez *Cervus nippon nippon* au Japon, révèle les relations étroites entre *Cutifilaria* et *Mansonella* (Onchocercidae, Onchocercinae) : pas de capsule buccale, œsophage réduit à un mince tube fibreux, queue de la femelle à quatre languettes. *Cutifilaria* devient sous-genre de *Mansonella* ; il est distinct des cinq autres sous-genres, *Mansonella*, *Tetrapetalonema*, *Esslingeria*, *Sandnema* et *Tupainema* : area rugosa aux bandes transversales ponctuées, 14-16 papilles caudales groupées autour du cloaque, plus deux papilles subterminales ovales et volumineuses, spicule droit fort, à extrémité distale en cuiller. *Cutifilaria*, qui étend le spectre d'hôtes des *Mansonella* aux ongulés, semble dériver de *Tupainema*, parasite d'insectivores Tupaidés, et avoir une origine asiatique. *M. (C.) perforata* n. sp. est distinct de l'unique autre espèce, *M. (C.) wenki*, parasite de *C. elaphus* en Europe, par le spicule droit plus complexe muni d'une pointe terminale puissante, la queue de la microfilarie bifide. En outre presque toutes les femelles ont des pores cuticulaires dans la région vulvaire, sur la ligne ventrale. La prévalence de *M. (C.) perforata* est assez élevée : microfilaries dermiques et adultes détectées respectivement chez 38 % et 21 % des cerfs sika.

MOTS CLÉS : *Cervus*, pore cuticulaire, *Cutifilaria*, Filarioidea, Japon, *Mansonella*, microfilaire, Onchocercinae, cerf sika, prélèvement cutané.

INTRODUCTION

Mansonella Faust, 1929 is a filarioid genus of the subfamily Onchocercinae well known because three species are human parasites, and many species parasitize other primates (Eberhard

& Orihel, 1984; Petit *et al.*, 1985; Bain *et al.*, 1986, 1995). In addition, a few insectivores (Mullin & Orihel, 1972), carnivores (Price, 1962; Uni, 1983), and rodents (Price, 1962; Eberhard & Orihel, 1984) are hosts of *Mansonella* species: seven of the total of 28 species. However, ungulates were not thought previously to be involved in the history of the host animals of this filarioid genus (Bain, 2002).

This study of new material recovered from a cervid in Japan indicates that the genus *Cutifilaria* Bain & Schulz-Key, 1974, which was previously known to have only a single species, *C. wenki* Bain & Schulz-Key, 1974, found from *Cervus elaphus* in Europe, has many of the characteristics of the genus *Mansonella*, and we propose that the *Cutifilaria* forms a subgenus of *Mansonella*. Our finding extends the host range of *Mansonella* to cervids.

* Department of Medical Zoology, Osaka City University Medical School, Osaka 545-8585, Japan.

** Parasitologie comparée et Modèles expérimentaux, associée à l'INSERM (U567), Muséum National d'Histoire Naturelle et École Pratique des Hautes Études, 75231 Paris Cedex 05, France.

*** Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita 879-5593, Japan.

Correspondence: S. Uni, Department of Medical Zoology, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan. Tel.: +81-6-6645-3760 – Fax: +81-6-6645-3762.

E-mail: uni@med.osaka-cu.ac.jp

MATERIALS AND METHODS

Japanese material: 58 *Cervus nippon nippon* Temminck (sika deer), from Kyushu Island, Japan, were examined: 49 deer on Mt. Sobo (1,756 m) and elsewhere in Oita Prefecture, and nine deer in Kumamoto Prefecture. The deer were killed between September 1998 and February 2003 in accordance with the policies of the Ministry of the Environment, Japan, concerning their conservation and control. The entire skin with subcutaneous tissues and four limbs were shipped refrigerated to a laboratory for examination one or two days after the animals were killed on the mountains. In examination for microfilariae, skin snips (2 cm²) were taken from the ears, back, thoracic and pelvic limbs, abdomen, and tail, and the kind of microfilariae, position on the body, and number of microfilariae found were recorded as described elsewhere (Uni *et al.*, 2002). Blood films were made from each deer and stained with Giemsa's solution, but microfilariae were not found. The skin of various parts of the body and limbs was examined for adult filarioids under a stereomicroscope; the entire skin and all four limbs of two deer (S20 and S38) were examined to find where adult worms were distributed in the body. The skin was cut into large pieces (260-330 cm²), and the number of worms detected under a stereomicroscope in each piece was recorded. The adult worms found were placed in 2 % formalin in saline and the specimens were cleared in lactophenol for study. Microfilariae taken from the ovejector of living females were stained with Giemsa's solution as mentioned elsewhere (Uni *et al.*, 2002). Some specimens of adult worms and microfilariae were seen by differential interference contrast microscopy. Drawings of the parasites, histologic sections, and scanning electron micrographs (SEM) were prepared as described elsewhere (Uni *et al.*, 2001). Supplementary material: we examined two females and one male of *Cutifilaria wenki* Bain & Schulz-Key, 1974 recovered from *Dama dama* (fallow deer) in Germany and identified in 1975; collection number, 122 JR, Muséum National d'Histoire Naturelle (MNHN), Paris. In addition, for examination of microfilariae extracted from the uteri of fixed females of both Japanese and European material, we used a simple technique: a drop of diluted vital stain, Meldola's Blue, was added. By this method, nuclei of the microfilariae were rapidly stained and could be examined within a few hours. Measurements are given in Table I: the body length of adult worms is in millimeters and other dimensions are in micrometers. The distance from the apex is given for the nerve ring, vulva, and commencement of the testis. The length of the area rugosa (from the anterior and posterior extremities to the tip of the tail) was measured.

RESULTS

MANSONELLA (CUTIFILARIA) PERFORATA N. SP.
(Tables I and II; Figs 1-11 except for Fig. 2F-J)

Head round, mouth extremely small (Fig. 1A-C), the four labial papillae in a rectangle elongated somewhat dorsoventrally and the four cephalic papillae in a dorsoventrally elongated rectangle (Figs 1C and 3), amphids slightly anterior to the labial papillae, no buccal capsule; anterior unspecialized part of digestive tract fused with the surrounding body muscles (Fig. 1D-F; arrow in E), with lumen slightly dilated, followed by a fibrous thread-like esophagus (Fig. 1B, arrow); rarely, posterior part of esophagus not fibrous, and wider than anterior part, atrophied esophagointestinal junction, especially in females (Fig. 1A, arrowhead, and G), and intestine with small lumen and thick wall containing conspicuous granules (Figs 1L, 4, and 6, abbreviation, I), colored in differential interference contrast microscopy. No body swellings in either sex. Measurements of females, microfilariae, and males in Table I.

Female

Vulva an inconspicuous slit and always posterior to esophagus (Fig. 1A, asterisk). The vagina spherical or

	<i>M. (C.) perforata</i> n. sp.	<i>M. (C.) wenki</i> *	
Female	S38-B5 (n = 10)		
Body length	96 (56-96) [77]	64	102
Body width	150 (120-170) [152]	220	215
Nerve ring**	360 (330-390) [343]	–	325
Esophagus	900 (670-900) [825]	880	625
Vulva**	1,350 (850-1,460) [1,145]	1,000	1,200
Ovejector	(2,270; 2,300)***	–	2,270
Tail	225 (165-300) [212]	240	205
Microfilaria	n = 10		
Body length	198 (183-208) [197]	170-180	
Body width	5 (3-5) [4]		3
Male	S38-51 (n = 10)		
Body length	22 (22-30) [26]	24.5	
Body width	80 (65-90) [80]	115	
Nerve ring**	270 (230-300) [260]	270	
Esophagus	610 (556-870) [659]	560	
Testicle apex**	590 (580-590) [586]	–	
Area rugosa	875 (726-1,335) [1,077]	1,230	
Right spicule	250 (200-255) [234]	320	
Left spicule	850 (675-940) [810]	1,000	
Tail	100 (65-100) [81]	120	

Holotype and allotype of *M. (C.) perforata* n. sp. are presented first, followed by range and then means. n, number of parasites examined. Units: see text.

* From Bain & Schulz-Key (1974).

** See text.

*** Two females examined.

— Not reported.

Table I. — Measurements of adults and microfilariae of *Mansonella (Cutifilaria) perforata* n. sp. and *M. (C.) wenki*.

Fig. 1. – *Mansonella (Cutifilaria) perforata* n. sp. A-L, anterior part of females. A. Anterior part, lateral view. Esophagointestinal junction (arrowhead), vulva (*), and cuticular pores (arrows). B. Enlarged anterior part, lateral view. Fibrous thread-like esophagus (arrow). C. Head, en face view. D. Transverse section of head, anterior part of digestive tract fused with the surrounding body muscles. E. Head, lateral view. Body muscles (arrow). F. Head, median view. G. Esophagointestinal junction. H. Vagina with the initial cuticular part of vulva. I. Ovary. J. Transverse section at the level of the vagina. K. Vulva (*) and cuticular pores (arrows), ventral view. L. Intestine. The thick wall contains granules. Bars, micrometers.

elongated and the outside of the vagina fused with body muscles; its lumen with a proximal part lined with cuticle very conspicuous at low magnification (vagina vera, Figs 1H and 4); this part with two bends close to the body surface; posterior part of the vagina lined with epithelium and straight (vagina uterina, Fig. 4, arrowhead). Cuticular pores aligned on the ventral line (Figs 1A, K, and 5, arrows), generally posterior to vulva; rarely, one or two pre-vulvar pores; total number from one to eight (mean, four); two of the 25 females examined were without these pores; no relationship between the number of pores and the body length of females. The pores were on elevated parts of the body (Fig. 5). Cuticle with striations perpendicular to the long axis of the body in the lateral field visible by SEM (not shown), striations 0.3 μm apart, and in transverse sections, cuticle somewhat thick in the lateral fields of the anterior part of the body (Figs 4 and 6, arrows). Anus a small transverse slit (Fig. 2B); from this level, the tail curved slightly (Fig. 2A) but generally straight; caudal extremity with two rounded lateral lappets and a round, divided terminal lappet (Fig. 2C-D).

Microfilaria

Body thin and unsheathed; the tail curved, nuclei extending to tip of tail (Figs 2E-b-c and 8), and posterior end bifid with lobes in median view by SEM (Fig. 7, arrow). Striations of the posterior part 0.2 μm apart seen by SEM (Fig. 7). Measurements of the microfilariae without staining, taken from the ovejector of fixed females, are in Table I. Of the microfilariae ($n = 12$) stained with Giemsa's solution (Fig. 8), body length 173-195 (mean, 181) μm , width 3-4 (3) μm , cephalic space 2-3 (3) μm , nerve ring 40-50 (45) μm from anterior end: 25 % of body length, excretory cell 53-63 (59) μm from anterior end: 33 % of body length, rectal cells 115-143 (129) μm from anterior end: 71 % of body length, and anal pore 155-172 (162) μm from anterior end: 90 % of body length. A microfilaria taken in the preparation of a skin snip had a curved tail (Fig. 9, asterisk). (For comparison with *M. (C.) perforata* females and microfilariae, some drawings of *C. wenki* females and microfilaria are placed in Figure 2 and the supplementary information is indicated at the end of this results section).

Male

Apex of testis attached to the posterior end of esophagus (Fig. 10A and D, asterisks). Area rugosa composed of 113-240 (mean, 169) transverse bands with tiny points (46-220 points per band) (Fig. 10E; arrow in F), each band 4 μm high and 8 μm apart, and extended from 858-1,440 (mean, 1,189) to 100-132 (mean, 111) μm from the tip of tail (Fig. 10H-I, asterisks). Caudal papillae arranged in two groups (Fig. 10F-G): *i*) of 14 to 16 papillae around the cloacal aperture,

the majority anterior to the cloaca, with two pairs on the median line, four or five subventral pairs supported by narrow alae, a single papilla visible just anterior to cloaca in some specimens, and one larger pair close to the posterior aspect of the cloacal aperture; *ii*) on posterior third of tail (Fig. 10G, arrow), a pair of large, prominent rhomboidal papillae, delineated by a thin cuticular groove. Spicules somewhat large (Fig. 10I, arrowhead and arrow): the right spicule thick with a strongly cuticularized and complex distal part that was roughly spoon-shaped, with its right aspect developed and triangular, a posterior groove with subdorsal hook and terminal thick point (Fig. 10J-J-a). The posterior part of the left spicule generally outside the cloaca (Fig. 10H). Long, thin, and divided left spicule with blade much shorter than the handle (Fig. 10K-K-b). Caudal extremity (Fig. 10L-M): a transparent cuticularized and flattened cone with complex divided internal structure; two median thread-like points, and two lateral points shorter and thicker or else thread-like.

Regarding the prevalence and distribution of microfilariae and adult worms of *M. (C.) perforata* in the skin of the deer, microfilariae of the species were found in 22 (38 %) of 58 sika deer from which skin snips were taken (Table II). As shown in the table, microfilariae

Deer ID no.	Thoracic		Midback	Abdomen	Pelvic	
	Ears	limbs			limbs	Tail
10	-	-	+++	ND	-	-
15	-	-	++	+	-	-
16	ND	-	+	+	-	ND
17	ND	-	+	-	-	-
19	-	-	++	-	-	-
20	ND	-	+	+++	-	-
23	-	-	+	+	-	+
25	-	-	+	-	-	-
29	-	-	+++	-	-	-
31	-	-	-	+	-	-
34	-	-	+	+	-	-
38	-	-	+++	+++	-	+
39	-	-	+	-	-	+
41	+	-	+++	++	-	+
44	-	-	+	-	-	ND
45	-	-	++	+++	-	ND
46	-	-	+	+	-	ND
47	-	+++	-	-	-	ND
49	-	-	+	-	-	ND
K-6	+	-	-	-	-	ND
50	-	-	+	-	-	-
51	-	-	+++	+	-	-
Total	19	22	22	21	22	15
Detection rate	(2/19)	(1/22)	(19/22)	(11/21)	0	(4/15)

Number of microfilariae: (-) not found; (+) 1-9; (++) 10-19; (+++) 20 or more.

ND: not done.

Table II. - Distribution of microfilariae of *M. (C.) perforata* n. sp. in skin snips of sika deer with such microfilariae.

Fig. 2. – *M. (C.) perforata* n. sp. A-D, posterior part of females. E, E-a-c, microfilariae. A. Posterior part, lateral view. B. Posterior end, lateral view (*). C. Caudal lappets, lateral view. D. Caudal lappets, ventral view. E. Microfilaria. E-a. Anterior end, median view. E-b. Posterior end, lateral view. E-c. Posterior end, ventral view. *C. wenki*. F-I, females. J, microfilaria. F. Anterior part, lateral view. G. Head, with beginning of lateral chord (arrow), lateral view. H. Head, median view. I. Vagina. J. Posterior end of microfilaria, ventral view. Bars, micrometers.

Fig. 3. – SEM of anterior end of *M. (C.) perforata* n. sp., partly median view. Mouth (arrowhead), amphids (*), and papillae (arrows). Bar, micrometers.

Fig. 4. – Transverse section of *M. (C.) perforata* n. sp. at the level of the vulva. Vulva (*), vagina uterina (arrowhead), thick cuticle at lateral field (arrow), and intestine (I). Bar, micrometers.

Fig. 5. – Vulva (*) and cuticular pores (arrows) of a female *M. (C.) perforata* n. sp. seen by differential interference contrast microscopy, lateral view. Bar, micrometers.

Fig. 6. – Transverse section of anterior part of female *M. (C.) perforata* n. sp., microfilariae in uteri, thick cuticle at lateral fields (arrows), and intestine (I). Bar, micrometers.

Fig. 7. – SEM of the posterior end of a microfilaria of *M. (C.) perforata* n. sp., bifid end (arrow) in ventral view. Bar, micrometers.

Fig. 8. – Microfilaria taken from ovejector of a female *M. (C.) perforata* n. sp., stained with Giemsa's solution. Nerve ring (arrow), anus (arrowhead), and curved posterior part (*). Bar, micrometers.

Fig. 9. – Microfilaria of *M. (C.) perforata* n. sp. taken in the preparation of a skin snip from the midback of sika deer, differential interference contrast image. Crook-shaped tail (*). Bar, micrometers.

Fig. 10. – *M. (C.) perforata* n. sp. A-M, males. A. Anterior part, apex of testis (*) and esophagointestinal junction (arrowhead). B. Head, lateral view. C. Head, median view. D. Apex of testis (*) and esophagointestinal junction (arrowhead). E. Area rugosa, transverse bands composed of tiny points. F. Posterior part, lateral view. Area rugosa (arrow). Posterior end of right spicule (*). G. Posterior part, ventral view. A pair of large papillae (arrow). H. Posterior part, area rugosa (*), right spicule, and left spicule (the posterior part outside the cloaca). I. Posterior part, left spicule (arrow) next to right spicule (arrowhead) at the cloaca. J. Right spicule, lateral view. J-a. Right spicule, posterior part. K. Left spicule, handle-lamina junction (arrow). K-a. Enlarged handle-lamina junction of left spicule. K-b. Posterior end of left spicule. L. Caudal end, lateral view. M. Caudal end, ventral view. Bars, micrometers.

Fig. 11. – Distribution and number of adult worms of *M. (C.) perforata* n. sp. found in the entire skin of body of two sika deer, S20 and S38 (head and limbs are not described; see text).

of the species were found in the skin of the midback of 19 of 22 deer and in the skin of the abdomen; very few were found in the skin of ears or limbs. In all, 107 adult filarioids (89 females and 18 males) of the species were found in seven (21 %) of the 34 deer dissected. Adult worms of the species were looked for in the entire skin of two deer (S20 and S38) in a study of the distribution of adult worms in sika deer. Forty-one worms (33 females and eight males) were found in the entire skin (3,700 cm²) of deer S20 and 48 worms (41 females and seven males) were found in the entire skin (3,900 cm²) of another deer, S38, in the locations shown in Figure 11. Many worms were found in the skin of the posterior back of both deer (maximum density, four worms per 100 cm² of skin from the posterior back of deer S38); none was found in the skin of the head and limbs.

Taxonomic summary

Type host: *Cervus nippon nippon* Temminck (sika deer).

Location in host: adult worms in the skin, mainly of the posterior back; microfilariae in the skin, mainly of the midback and abdomen.

Type locality: Mt. Sobu, Ogata-cho, Oita Prefecture, Kyushu, Japan.

Collection dates: type specimens in 14 December 2001; other specimens between September 1998 and February 2003.

Specimens deposited: female holotype (S38-B5) and male allotype (S38-51), collection number, 421 HS, MNHN, Paris. Paratype: seven in MNHN and 39 in the Department of Medical Zoology, Osaka City University Medical School. Other specimens: nine in MNHN and 50 in the Department of Medical Zoology, Osaka City University Medical School.

Etymology: we named this species *M. (C.) perforata* n. sp., because of the cuticular pores present near the vulva in almost all females.

Supplementary information on *C. wenki* Bain & Schulz-Key, 1974 (Fig. 2F-J): the identification of this material as *C. wenki* was confirmed and further details were elucidated about the anterior part of the females and the

microfilaria for comparison with *M. (C.) perforata* females and microfilariae: the body width of the anterior part (Fig. 2F) was larger than that of *M. (C.) perforata*; the position of the head papillae (Fig. 2G-H) was slightly different from that of *M. (C.) perforata*; the esophagus larger (Fig. 2F); and the vulva was larger and more developed (Fig. 2I). The microfilaria had a blunt tail end (Fig. 2J).

DISCUSSION

M. (C.) perforata n. sp. taken from sika deer in Kyushu, Japan, resembled the specimens of *C. wenki* from red deer in Germany (Bain & Schulz-Key, 1974) and fallow deer in the same country, as described in this study: no body swellings, general structure of vagina (doubly bent vagina vera, followed by a straight vagina uterina), and microfilariae with nucleated tail tip. However, we distinguished the Japanese species from *C. wenki* by the characteristics of the less developed vagina, microfilariae with a bifid tail end, and the more complex posterior part of the right spicule. Features of the posterior end of microfilariae are useful in characterization of species of *Mansonella* (Orihel, 1984; Bain *et al.*, 1995). In addition, the measurements of Japanese specimens seem to be slightly different from those of *C. wenki*; smaller body width of adult worms, longer body of the microfilariae, shorter spicules, and shorter tail of the males (Table I). The cuticular pores found near the vulva in almost all female worms are the first to be reported among filarioids, to the best of our knowledge. The pores probably are related to specialized mating behavior of this species (Bain *et al.*, in preparation). That many microfilariae of *M. (C.) perforata* were found in the skin of the midback and abdomen of sika deer suggested a specific parasitic location for the filarioids in the host animals. Many of the adults of the species inhabited the skin of the posterior part of the hosts. Many microfilariae of *M. (C.) perforata* were found near the adult worms, as suggested by Schulz-Key (1975) for *C. wenki* from red deer. That the distribution of microfilariae and adult worms in the host animals is

specific has been suggested for *Cercopithecophilaria* species from Japanese serows and sika deer (Uni *et al.*, 2001, 2002).

The main finding from the Japanese material, confirmed by reexamination of *C. wenki*, was that *Cutifilaria* has many of the characteristics of *Mansonella* as defined by Chabaud & Bain (1976), Anderson & Bain, (1976), Eberhard & Orihel (1984), and Bain (2002): no buccal capsule, and esophagus reduced to a thin fibrous tube, its anterior part fused with the body muscle; a caudal extremity with four lappets in the female, and in the male, a caudal extremity more or less completely fused and forming a cone. Such an esophageal structure has been observed earlier in only a few specimens of the type material of *C. wenki*, so the link with *Mansonella* was not recognized previously. Furthermore, in some specimens of the Japanese material, the esophagus was not striated longitudinally, reminiscent of that reported in 1974 for *C. wenki*. The close relationship of *Cutifilaria* and *Mansonella* was indicated also by a characteristic to which attention was drawn some years ago (Petit *et al.*, 1985; Bain *et al.*, 1986): the apex of the testis is attached to the digestive tract in the esophagointestinal region. Eberhard & Orihel (1984) defined five subgenera in the genus *Mansonella* Faust, 1929: *Mansonella* Faust, 1929, *Tetrapetalonema* Faust, 1935, *Esslingeria* Chabaud & Bain, 1976, *Sandnema* Eberhard & Orihel, 1984, and *Tupainema* Eberhard & Orihel, 1984. *Cutifilaria* is distinct from these subgenera in the following characteristics: the area rugosa of transverse bands with tiny points (instead of longitudinal ridges), the right spicule with a stout spoon-like posterior part, caudal papillae not greatly reduced in number (maximum, nine pairs) but grouped close and anterior to the cloacal aperture, roughly in a semicircle; and a single subventral pair in the posterior third of the tail, prominent and rhomboidal in face view. There were no body swellings in the *Cutifilaria* species. The adult worms and microfilariae of the *Cutifilaria* species inhabited the skin. We therefore propose that *Cutifilaria* be included as a subgenus of the genus *Mansonella*.

The history of *Mansonella* probably began with the emergence of tupaied insectivores during the Eocene, and expanded with the primates (Bain, 2002). The small *Cutifilaria* lineage, *M. (C.) wenki* and *M. (C.) perforata* in the Palearctic region, must be much more recent, contemporaneous with cervids, during the Miocene, about 15 M years ago (Thomas, 1992). *Mansonella (Cutifilaria)* could be derived from *Tupainema*, restricted to the Asiatic region, as this primitive form of *Mansonella* has an analogous right spicule, with a spoon-like simple posterior part, and a left spicule already elongated. The cervids are woodland dwellers like tupaiids, unlike the almost contemporaneous bovids, which are devoid of such filariae. The habitat of cer-

vids might have been the 'trump card' accounting for the capture of *Mansonella* species, an event that probably occurred in the Asiatic region.

ACKNOWLEDGEMENTS

We thank Dr. Y. Takao, Kurume University, Kurume, and Mr. K. Kamata, Kumamoto, for providing sika deer, Ms. C. Katayama, Mr. H. Mori, and Mr. H. Tamiya of Osaka City University Medical School for help in examination of the parasites, and Mr. T. Kenkou of the Central Laboratory of the same medical school for the histologic preparation and Mr. H. Nakagawa of the same laboratory for SEM examination of the parasites. We thank Dr. J. Baker and Ms. C. Latta for reading the manuscript. This study was supported in part by grants 10306020 and 12877043 from the Ministry of Education, Science, Sports, and Culture, Japan. The authors dedicate this paper to the memory of Professor Y. Suzuki, Laboratory of Veterinary Anatomy, Gifu University, Gifu, Japan, who discovered *Onchocerca* species in Japanese serows and contributed greatly to the study of parasitic diseases of wild animals in Japan.

REFERENCES

- ANDERSON R.C. & BAIN O. Keys to genera of the order Spirurida. Part 3. Diplotriaenoidea, Aprocotoidea and Filarioidea. In: CIH Keys to the Nematode Parasites of Vertebrates. Anderson R.C., Chabaud A.G. & Willmott S. (eds). Commonwealth Agricultural Bureaux, Farnham Royal, England, 1976, No. 3, 59-116.
- BAIN O. Evolutionary relationships among filarial nematodes. In: The Filaria. Klei T.R. & Rajan T.V. (eds). World Class Parasites. Kluwer Academic Publishers, Boston, 2002, 5, 21-29.
- BAIN O., MOISSON P., HUERRE M., LANDSOU-D-SOUKATE J. & TUTIN C. Filariae from a wild gorilla in Gabon with description of a new species of *Mansonella*. *Parasite*, 1995, 2, 315-322.
- BAIN O., PETIT G. & ROSALES-LOESENER L. Filaires de singes sud-américains. *Bulletin du Muséum national d'Histoire naturelle*, Paris, 4^{ème} série, 8, 1986, Section A, 513-542.
- BAIN O. & SCHULZ-KEY H. Une filaire intradermique chez le cerf européen : *Cutifilaria wenki* n. gen., n. sp. (Onchocercinae). *Tropenmedizin und Parasitologie*, 1974, 25, 450-453.
- CHABAUD A.G. & BAIN O. La lignée *Dipetalonema* : nouvel essai de classification. *Annales de Parasitologie Humaine et Comparée*, 1976, 51, 365-397.
- EBERHARD M.L. & ORIHIEL T.C. The genus *Mansonella* (Syn. *Tetrapetalonema*): a new classification. *Annales de Parasitologie Humaine et Comparée*, 1984, 59, 483-496.

- MULLIN S.W. & ORIHIEL T.C. *Tetrapetalonema dunni* sp. n. (Nematoda: Filarioidea) from Malaysian tree shrews. *Journal of Parasitology*, 1972, 58, 1047-1051.
- ORIHIEL T.C. The tail of the *Mansonella streptocerca* microfilaria. *American Journal of Tropical Medicine and Hygiene*, 1984, 33, 1278.
- PETIT G., BAIN O. & ROUSSILHON C. Deux nouvelles filaires chez un singe, *Saimiri sciurus*, au Guyana. *Annales de Parasitologie Humaine et Comparée*, 1985, 60, 65-81.
- PRICE D.L. Description of *Dipetalonema interstitium* n. sp. from the grey squirrel and *Dipetalonema llewellyni* n. sp. from the raccoon. *Proceedings of the Helminthological Society of Washington*, 1962, 29, 77-82.
- SCHULZ-KEY H. Untersuchungen über die Filarien der Cerviden in Süddeutschland. 2. Die Filarien des Rothirsches (*Cervus elaphus*). *Tropenmedizin und Parasitologie*, 1975, 26, 348-358.
- THOMAS H. Crise climatique et événements géodynamiques. Leur rôle dans l'évolution des primates anthropoïdes. Bibliothèque d'Orientation Menta, Paris, 1992, 92 p.
- UNI S. Filarial parasites from the black bear of Japan. *Annales de Parasitologie Humaine et Comparée*, 1983, 58, 71-84.
- UNI S., SUZUKI Y., BABA M., MITANI N., TAKAOKA H., KATSUMI A. & BAIN O. Coexistence of five *Cercopithifilaria* species in the Japanese rupicaprine bovid, *Capricornis crispus*. *Parasite*, 2001, 8, 197-213.
- UNI S., BAIN O., TAKAOKA H., KATSUMI A., FUJITA H. & SUZUKI Y. Diversification of *Cercopithifilaria* species (Nematoda: Filarioidea) in Japanese wild ruminants with description of two new species. *Parasite*, 2002, 9, 293-304.

Reçu le 29 août 2003

Accepté le 3 mars 2004

ERRATUM

Figure 5 page 26 of the article by Lhermitte-Vallarino N. & Bain O.: "Morphological and biological study of *Rhabdias* spp. (Nematoda) from African chameleons with description of a new species" was badly reproduced in PARASITE issue of March 2004.

The publisher makes his apologies to the authors and readers of PARASITE.

Please note reprints with accurate figure are available from Dr Odile Bain: bain@mnhn.fr