

ONCHOCERCA EBERHARDI N. SP. (NEMATODA: FILARIOIDEA) FROM SIKA DEER IN JAPAN; RELATIONSHIPS BETWEEN SPECIES PARASITIC IN CERVIDS AND BOVIDS IN THE HOLARCTIC REGION

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Summary:

Onchocerca eberhardi n. sp. from the sika deer, *Cervus nippon*, in Japan is described. Adult worms lived in the carpal ligament; infection reached high levels (up to 25 female and 16 male worms in a single carpal limb). Skin dwelling microfilariae were mainly found in the ears. Prevalence of infection was 81 % at the type locality, Mt. Sobo, in Kyushu. The new material was compared to the 31 species of *Onchocerca* presently known. *Onchocerca eberhardi* n. sp. females were characterized by a long slender anterior end and a thin esophagus ≤ 1 mm long with no or only a slight glandular region. The vulva was located near the level of the mid-esophagus and the cuticle had transverse external ridges and internal striae (two striae between adjoining ridges). The most similar species were *O. stilesi* (re-examined), *O. lienalis*, and to a lesser extent *O. gutturosa*, all from bovids (cattle). Two main lineages of *Onchocerca* are recognized in cervids with either primitive or with derived characteristics (as exemplified by the new species). The species in both lineages are not restricted to cervids but are also found in bovids in the Holarctic region, suggesting that the species diversified in the two host groups simultaneously, when these host groups lived in the same geographic area.

KEY WORDS : Filarioid, *Onchocerca eberhardi* n. sp., *Cervus nippon*, sika deer, carpal ligament, Japan, *Onchocerca stilesi*, Bovidae, Holarctic region.

Résumé : *ONCHOCERCA EBERHARDI* N. SP. (FILARIOIDEA, NEMATODA) CHEZ LE CERF SIKA AU JAPON; RELATIONS ENTRE LES ONCHOCERQUES DE CERVIDAE ET DE BOVIDAE EN RÉGION HOLARCTIQUE

Description d'*Onchocerca eberhardi* n. sp., parasite de *Cervus nippon*, au Japon. Les adultes vivent dans le ligament carpal; l'infection peut être forte (un cas avec 25 femelles et 16 mâles). La localisation principale des microfilaries dermiques est le lobe des oreilles. La prévalence est de 81 % dans la localité-type, Mont Sobo, Kyushu. Le nouveau matériel est comparé aux 31 espèces décrites dans le genre. L'espèce est distincte par plusieurs caractères de la femelle, oesophage fin, ≤ 1 mm de long, avec une partie glandulaire faiblement différenciée ou non distincte, la vulve assez antérieure, la cuticule ornée de côtes et de stries (deux stries par intercôtes). Les espèces les plus proches sont *O. stilesi* (réétudié ici), *O. lienalis* et, à un moindre degré, *O. gutturosa*, toutes parasites de Bovidae (bétail). Deux principales lignées sont reconnues chez les onchocercs de Cervidae, respectivement avec des caractères primitifs ou évolués (cas de la nouvelle espèce). Dans les deux lignées, les représentants ne sont pas restreints aux Cervidae et se trouvent aussi chez des Bovidae, en région holarctique, suggérant qu'ils se sont diversifiés simultanément chez les deux groupes d'hôtes quand ceux-ci étaient dans la même région géographique.

MOTS CLÉS : Filaire, *Onchocerca eberhardi* n. sp., *Cervus nippon*, cerf sika, ligament carpal, Japon, *Onchocerca stilesi*, Bovidae, région holarctique.

INTRODUCTION

Until recently, *Onchocerca* species known in Japan were only those species from cosmopolitan domesticated animals: in horses *O. cervi-*

calis Railliet & Henry, 1910, in cattle *O. gibsoni* Cleland & Johnston, 1910, *O. gutturosa* Neumann, 1910, and *O. lienalis* Stiles, 1892 (Itagaki, 1954; Takaoka, 1999).

During the last decade, searches for filarioids in wild Japanese ungulates revealed three species of *Onchocerca*, one not endemic and two endemic (Yagi *et al.*, 1994; Uni *et al.*, 1998, 2001). *Onchocerca skrjabini* Rukhlyadev, 1964 (= *O. tarsicola* Bain & Schulz-Key, 1974) was described from the European deer (Rukhlyadev, 1964) where it is common (Schulz-Key, 1975; Baruš & Koubek, 1993). The species was also recorded in reindeer (Bylund *et al.*, 1981). In Japan the species is a parasite of a caprine bovid, the Japanese serow (Yagi *et al.*, 1994), in which it causes chronic tendinitis in the olecranon and fibrous bursa formation in the carpal and tarsal regions (Suzuki *et al.*, 1982, 1997). The findings of *O. skrjabini* from the sika deer in this study are indicated below.

Onchocerca suzukii Yagi, Bain & Shoho, 1994 is another parasite of the serow. *Onchocerca dewittei japo-*

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nica Uni, Bain & Takaoka, 2001 is a parasite of the wild boar in Kyushu, the large southern island of Japan. Unexpectedly, this species appeared to be locally an agent of zoonotic onchocerciasis (Takaoka *et al.*, 2001, 2004, and 2005), whereas in other parts of the world species of *Onchocerca* from cattle or horses are generally identified or suspected in zoonotic onchocerciasis (Engelkirk *et al.*, 1982; Orihel & Eberhard, 1998).

The new *Onchocerca* species described here is a parasite of the sika deer in Kyushu and resembles some parasites of cattle, particularly *O. stilesi* Eberhard, 1979, a rare species from the United States, which we have examined here to specify precise morphologic characteristics for diagnosis.

MATERIALS AND METHODS

The host animals examined for species of *Onchocerca* were sika deer (*Cervus nippon* Temminck) from Kyushu. Fifty-four sika deer living on Mt. Sobo, Oita Prefecture, and nine sika in Kumamoto Prefecture were killed between September 1998 and February 2006 in accordance with the conservation and control policies of the Ministry of the Environment, Japan. The head, the entire skin of the body, and the limbs were shipped refrigerated to a laboratory for filarial examination one or two days after the animals were killed.

For the detection of adult worms, subcutaneous connective tissues and the ligaments of joints of the limbs were inspected under a stereomicroscope. In addition, we used the collagenase technique (Schulz-Key, 1988) to obtain complete adult parasites in the joints of six sika deer, because the *Onchocerca* adults lived deep inside the ligaments: the carpal or tarsal joints were incubated in saline solution containing 0.3-0.5 % collagenase type I (Worthington Biochemical Corporation, New Jersey) for 8 to 24 h at 38° C.

For the detection of microfilariae, skin snips were taken from the face, ears, neck, back, limbs, and tail of each animal by the methods described by Uni *et al.* (2002). Microfilariae that came out were examined for identification and compared to microfilariae extracted from the ovejector of female worms. The latter microfilariae were stained with Giemsa's solution. In addition, blood films were made from each deer and stained with Giemsa's solution. The eyeballs of the sika deer (ID nos. S28, S30-34) were dissected under a stereomicroscope to examine the cornea and retina for lesions.

To investigate the co-existence of filarial parasites in sika deer, other species of filarioids [*Cercopithifilaria crassa* Uni & Bain, 2002, *C. longa* Uni & Bain 2002, *Mansonella (Cutifilaria) perforata* Uni, Bain & Takaoka,

2004, and *O. skrjabini*] were sought in skin snips for microfilariae and in skin and limbs by dissection under a stereomicroscope for adult worms (Uni *et al.*, 2002, 2004).

Adult worms detected were placed in 2 % formalin in saline solution and the specimens were cleared in lactophenol for study. For histologic examination, the carpal lesions infected with the parasites were embedded with paraffin by routine methods and the sections were stained with hematoxylin and eosin. Scanning electron microscopic (SEM) micrographs of the females and males were prepared as described elsewhere (Uni *et al.*, 2001).

Measurements are given in Tables I and II: the body length of adult worms in millimeters and the other dimensions in micrometers. In females, characteristics of the external cuticular ridges and number of internal striae are described; in this study, following Railliet & Henry (1910), "striae" means the furrows or lines, as in Bain & Schulz-Key (1974), not the thick part of the cuticle demarcated between two consecutive furrows, as in Beaver *et al.* (1974, 1983). This precision is important as the number of striae between two adjoining ridges (striae/inter-ridge) is a distinctive characteristic. In the male, the caudal pairs of papillae are tentatively numbered on the basis of a set of 10 pairs, as in Chabaud & Petter (1961) for spirurids and Bain *et al.* (1976) for a primitive species of *Onchocerca*. The authorities of the new species are Uni and Bain.

Two specimens of *O. stilesi* Eberhard, 1979 from Minnesota, U.S.A., were examined: the female holotype, USNM Helminth collection No. 75108 and a male paratype, USNM Helminth collection No. 75109. The host was *Bos taurus*; the habitat, connective tissues around the stifle joint.

RESULTS

ONCHOCERCA EBERHARDI UNI & BAIN, N. SP.

(Tables I-III; Figs 1, 2, 4-16)

Filarioidea, Onchocercidae (Leiper, 1911) Chabaud & Anderson, 1959, Onchocercinae Leiper, 1911, *Onchocerca* (Diesing, 1841) Railliet & Henry, 1910.

Adult worms loosely coiled in the carpal ligament. The specimens recovered were abundant: one complete female and 139 female fragments (74 anterior, 31 midbody, and 34 posterior parts); 47 complete males and 24 male fragments (nine anterior, seven midbody, and eight posterior parts).

- Female (one complete, 10 anterior, and 10 posterior parts): very thin and straight anterior end 3 mm long. Slight cervical swelling posterior to nerve ring (Fig. 1A). Head: external labial papillae arranged in a rectangle elongated dorsoventrally; cephalic papillae arranged in

	<i>O. eberhardi</i> n. sp.	<i>O. stilesi</i> ¹	<i>O. lienalis</i> ²	<i>O. gutturosa</i> ³
Female	S59-C36 (n = 10)			
Body length	175	ND	316	ND
Body width	230 (170-230) [207]	140-220 (180)	180-220	140-225
Body width at end of esophagus	70 (51-70) [60]	55**	50	75-80
Nerve ring*	190 (170-235) [192]	120-180 (140), 180**	210	200-260
Cervical swelling	Yes (slight swelling)	Yes**	No	Yes
Vulva*	480 (425-620) [508]	360-740 (440)	543-560	480-640
Glandular esophagus	ND (610-795) [685]	370-630 (480)	525-770	Not divided
Esophagus	980 (880-1,080) [975]	540-910 (730)	865-1,195	1,000-1,210
Cuticle thickness	30 (28-32)	16-18**	12-17	25-50
Cuticle surface	Smooth	Wrinkled**	Longitudinal wrinkles	Smooth
Number of striae between ridges	2	3	2	4-7
Ridge shape	Straight	Undulated ¹ **	Undulated	Undulated
Branching of ridges at lateral field	No	Yes, (No**)	Yes	Yes
Cuticular thickening at lateral fields	No	No**	No	Yes
Tail	150 (115-150) [135]	60-120 (80)	150-228	140-170
Microfilariae	(n = 20)	(n = 5)**		
Body length	ND (200-258) [235]	177-190**	225-260	260-280
Body width	ND (4-5) [5]	4-5**	5.0-5.3	5

Holotype (S59-C36) of *O. eberhardi* n. sp. is presented first, followed by range and then mean. Unit: See text. * From anterior end. n: Number of parasites examined. ND: Not done. References: ¹Eberhard, 1979, ²Present study, ³Bain *et al.*, 1978b and ³Bain, 1975.

Table I. – Measurements of females and microfilariae of *Onchocerca eberhardi* n. sp. from sika deer and closely related species from bovids.

	<i>O. eberhardi</i> n. sp.	<i>O. stilesi</i> ¹	<i>O. lienalis</i> ¹	<i>O. gutturosa</i> ²
Male	S59-C38 (n = 10)			
Body length	22 (17-26) [21]	19-28	18.5-24.5	22.8-33.4
Body width	65 (50-68) [58]	55-80 (70)	50-80	85-90
Nerve ring*	178 (163-205) [186]	100-150 (120)	110-170	250
Glandular esophagus	493 (493-565) [541]	270-480 (380)	–	–
Esophagus	780 (690-870) [785]	470-720 (580)	590-800	880-970
Right spicule	60 (60-70) [66]	50-70 (60)	60-80	85
Left spicule	208 (185-223) [200]	150-200 (170)	190-230	270-290
Pairs of caudal papillae	8 (7-9)	8-9	7	7
Tail	60 (50-83) [63]	50-70 (60)	55-80	105-115
Host animal(s)	<i>Cervus nippon</i>	<i>Bos taurus</i>	Bovids, <i>Bos taurus</i>	<i>B. taurus</i> , <i>B. indicus</i>
Parasitic location	Carpal ligament	Connective tissues around stifle joint	Gastrosplenic ligament	Nuchal ligament
Locality	Japan	USA	USA, France	West Africa

Allotype (S59-C38) of *O. eberhardi* n. sp. is presented first, followed by range and then mean. Unit: See text. * From anterior end. n: Number of parasites examined. – Not reported. References: ¹Eberhard, 1979 and ²Bain, 1975.

Table II. – Measurements of males, hosts, parasitic locations, and localities of *Onchocerca eberhardi* n. sp. from sika deer and closely related species from bovids.

a rectangle elongated laterally (Figs 1B-D and 13, arrows). Buccal capsule absent but very thin cuticular plate present; lumen at base of buccal cavity Y-shaped in transverse section. Esophagus thin, with no distinct or with slightly granulous glandular part. Body width narrow at posterior end of esophagus (Table I). Vulva near mid-length of esophagus; its distance to head two to three times the distance from head to nerve ring (Table I; Fig. 1A and E). Main part of body curled (Fig. 4).

Cuticle: thin in anterior part with external delicate striations, not visible at low magnification (Fig. 1A). Cuticle progressively thickened and complex in structure. Cuticle 28-32 µm thick in coiled main part of body, composed of four or five layers (Figs 1F, I, and 7).

External layer with transverse salient ridges, 25-45 µm apart, straight, interrupted, not branched on lateral fields (Figs 1G, H, and 14, *); incomplete ridges very rare (Fig. 1G, *). Median layer of cuticle thick, divided with internal transverse striae; a constant two striae between adjoining ridges (Figs 1H, I, 8, and 9, arrows). Layers appeared dotted or not in transverse section, depending on whether the cuticular fibers were transverse (Fig. 1F) or longitudinal (Fig. 1I).

Transverse section of body round; cuticle not thickened laterally (Figs 1F and 7). Body musculature well-developed, lateral chords narrow (Fig. 1H, dotted area). Ridges less distant in posterior end, internal striae not visible (Fig. 1J). Tail cuticle with transverse external

Sika ID no.	<i>O. eberhardi</i> n. sp.									Co-existence with other filarioids			
	Face	Ears	Neck	Thoracic limbs	Midback	Abdomen	Pelvic limbs	Tail	<i>C. crassa</i>	<i>C. longa</i>	<i>M. (C.) perforata</i>	<i>O. skrjabini</i>	
S11	+	++	+	-	-	ND	-	-	+	+	+	-	
S14	-	+	-	ND	-	-	-	-	-	+	+	-	
S15	+	+	+	-	-	-	-	-	+	+	++	++	
S18	+	++	+	+	-	-	-	-	-	-	-	+	
S19	+	+	-	-	-	-	-	-	-	+	+++	+++	
S20	ND	ND	+	-	-	-	-	-	+	+	+++	+++	
S21	ND	+	+	-	-	-	-	-	-	-	-	-	
S23	-	+	+	-	-	+	-	-	+	+	+	+	
S24	-	-	-	+	-	-	-	-	+	+	-	-	
S25	+	+	+	-	-	-	-	-	+	+	+	+	
S28	ND	+++	ND	-	-	-	-	-	-	+	-	-	
S30	ND	+	ND	+	-	-	-	-	-	+	-	+	
S31	ND	+	ND	-	-	-	-	-	-	-	+	+++	
S32	ND	++	ND	-	-	-	-	-	-	+	-	-	
S33	ND	+++	ND	-	-	-	-	-	-	+	-	-	
S34	ND	+	ND	-	-	-	-	-	-	-	+	+++	
Total	5	14	7	3	0	1	0	0	6	12	9	9	
Detection rate	(5/8)	(14/15)	(7/10)	(3/15)	0	(1/15)	0	0	(6/16)	(12/16)	(9/16)	(9/16)	

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

Table III. – Distribution of microfilariae of *Onchocerca eberhardi* n. sp. in skin snips of sika deer with such microfilariae in Oita, Kyushu, and their co-existence with other kinds of microfilariae of filarioids.

striations and no ridges; sometimes, a few tiny bosses on cuticle (Fig. 1K, L). Posterior end twisted (Fig. 1J). Tail straight; posterior end smooth but with internal terminal point and two subterminal elongated phasmids (Fig. 1K, L).

- Microfilaria (n = 20): microfilariae from ovejector and skin of sika deer unsheathed; body dorsoventally flattened (median view, 4-5 µm wide; lateral view, 3.75 µm wide; non-fixed microfilariae as indicated in Table I), body widening slightly at anterior extremity; tiny cephalic hook present; tail extremity attenuated to smooth point (Figs 1M and 11). Uterine microfilariae stained with Giemsa's solution (n = 10): body length 210-238 (mean, 223) µm and width 4-5 (4) µm; nerve ring 55-63 (58) µm from anterior end: 26 % of body length; anal pore 192-213 (199) µm from anterior end: 89 % of body length (Fig. 12).

- Male (n = 10): slight cervical swelling at anterior end (Fig. 2A). Striations on cuticle of main part of body interrupted in lateral fields (Figs 2D and 15, *). Exceptionally, three body swellings present (two specimens: 4, 8, and 14 mm from the anterior end in a male worm, S47-5, 19 mm long; 5, 12, and 19 mm from the anterior end in another male worm, S47-6, 22 mm long). *Area rugosa* in posterior end absent. Right spicule with knobbed posterior end; sub-terminal dorsal heel (Fig. 2F). Left spicule with handle and lamina; attenuated long distal extremity (Fig. 2G). Nine pairs of caudal papillae arranged in two groups: *i*) one unpaired precloacal papilla near the cloacal aperture; four paracloacal sublateral pairs (probably pairs 2, 3, 4, and 6) and one

subventral postcloacal pair (pair 5); *ii*) four pairs aligned in posterior third of tail: small pair 7, large pair 8, pointed pair 9 with phasmids at base, and terminal pair 10. In several specimens, the cloacal group contained only three pairs, whereas the posterior group remained constant (Table II; Fig. 2I, J).

PREVALENCE AND DISTRIBUTION OF ADULT WORMS AND MICROFILARIAE OF *O. EBERHARDI* N. SP. IN SIKA DEER AND THEIR CO-EXISTENCE WITH OTHER FILARIOIDS

Adult worms were found in the limbs of sika deer from Oita (17/21 or 81 %) by dissection or treatment by collagenase (six of 17 deer with such parasites were treated by collagenase). In one (S59) of two deer treated by collagenase, 25 anterior parts of adult females (with one complete female) and 16 complete males of *O. eberhardi* n. sp. were found in a carpal ligament; none were found in the tarsal ligament treated by collagenase or anywhere on the entire skin inspected by a stereomicroscope. In another sika (S64), 10 anterior parts of females and six complete males were found in the carpal ligament examined and only one female fragment was taken from the tarsal ligament. Thus, rather than the tarsal ligament, almost all the adult parasites had wound themselves within the carpal ligament (Table II). Inflammation with congestion was found in the carpal ligament parasitized by many worms (Figs 5, 6 and 10).

Microfilariae of *O. eberhardi* n. sp. were found in skin snips of 44 of 54 (81 %) sika deer from Oita. Skin

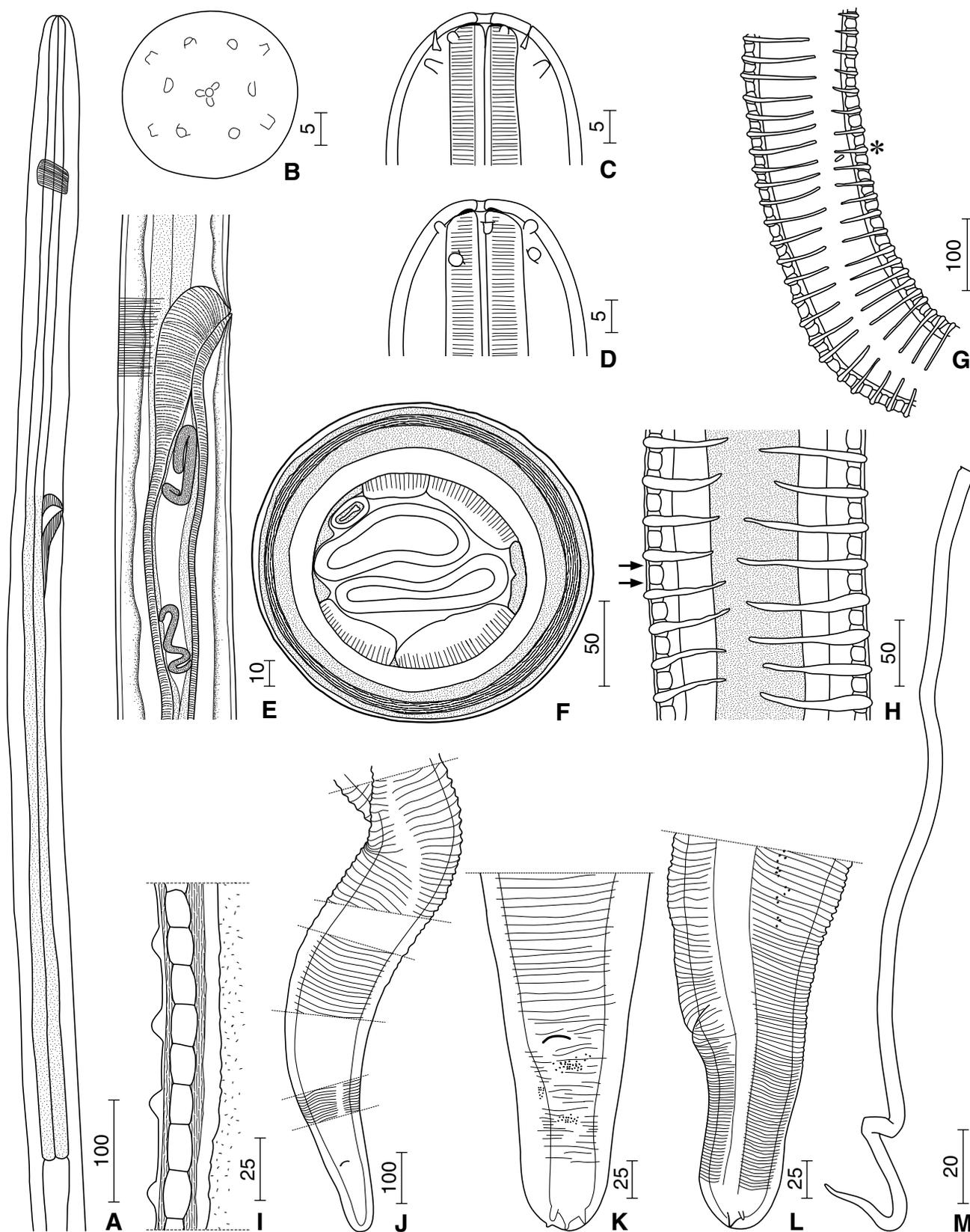


Fig. 1. – *Onchocerca eberhardi* n. sp., females. A. Anterior end, right lateral view. B. Head, apical view. C. Head, median view. D. Head, lateral view. E. Region of vagina, right lateral view. F. Cross-section of body in the coiled main part. G. Cuticular ornamentation of body, sublateral view. Incomplete ridge (*). H. Ridges and striae (arrows), lateral view (dotted area: lateral chord). I. Detail of cuticle, longitudinal view. J. Posterior region, twisted. K. Tail, ventral view. L. Tail, lateral view. M. Microfilaria from ovejector. Bars, micrometers.

microfilariae were in the ears (41/50 or 82 %), neck, and face; a few were found in the skin of thoracic limbs (10/52 or 19 %), back (6/52 or 12 %), or abdomen (3/51 or 6 %); no skin microfilariae were in pelvic limbs (51 deer examined) or tail (46 deer examined). Distribution of microfilariae from 16 sika deer is listed in Table III. In addition, skin microfilariae of *O. eberhardi* n. sp. were found in four of the nine sika deer from Kumamoto Prefecture, Kyushu. No microfilariae were

found in the blood films and no lesions were found in the eyeballs of the six sika deer examined. Adult worms and microfilariae of *O. eberhardi* n. sp. were found co-occurring with one, two, or three species of *C. crassa*, *C. longa*, *M. (C.) perforata*, and *O. skrjabini* in sika deer (Table III). Four sika deer (S15, 20, 23, and 25) were infected with all five filarial species including *O. eberhardi* n. sp. Parasitic locations of the adult worms of these filarioids were different: *C. crassa*

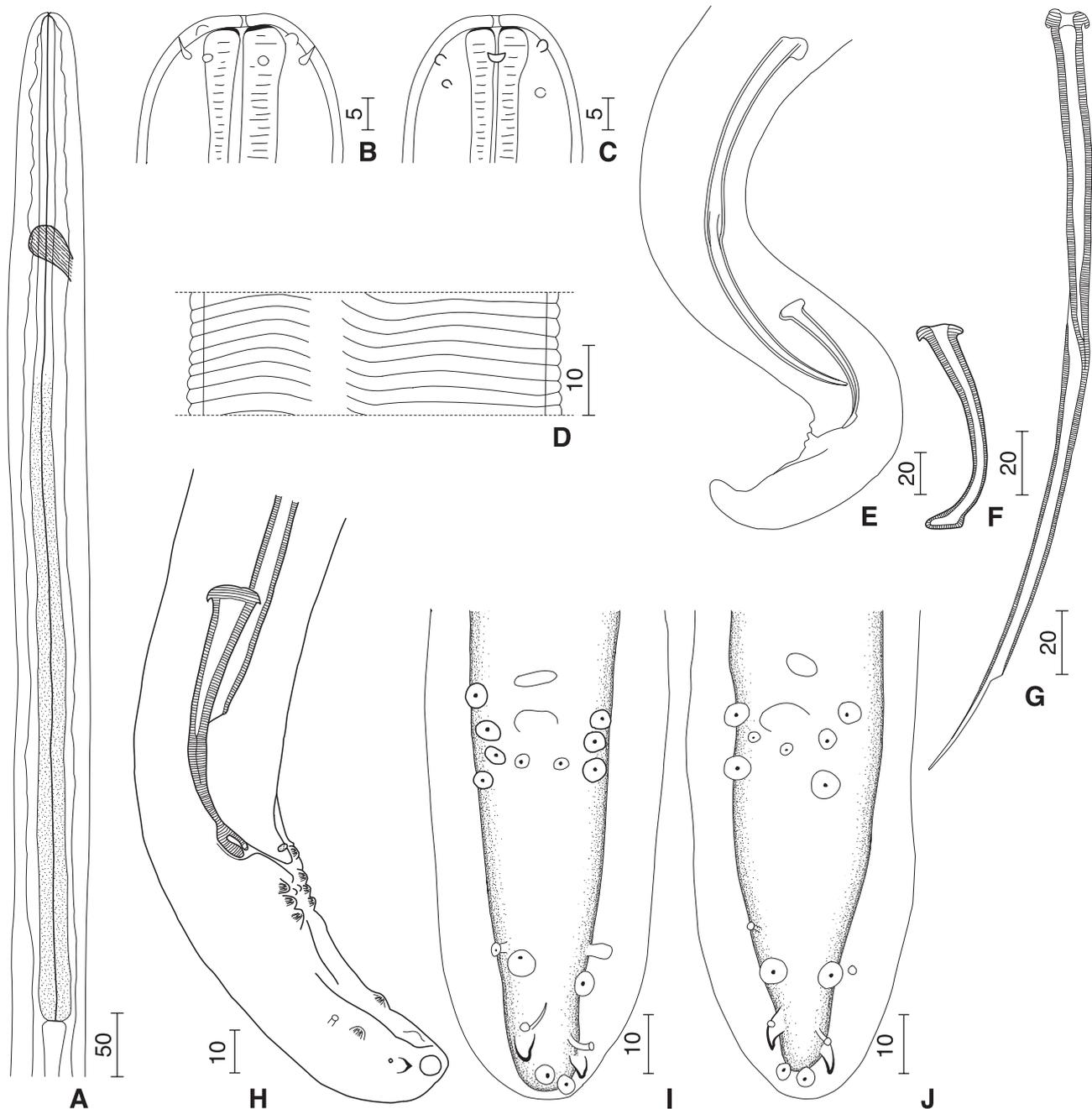


Fig. 2. – *Onchocerca eberhardi* n. sp., males. A. Anterior end, right lateral view. B. Head, median view. C. Head, lateral view. D. Cuticular ornamentation at midbody, lateral view. E. Posterior region, lateral view. F. Right spicule, lateral view. G. Left spicule, lateral view. H. Tail, right lateral view. I. Tail, ventral view. J. Tail of another male, ventral view. Bars, micrometers.

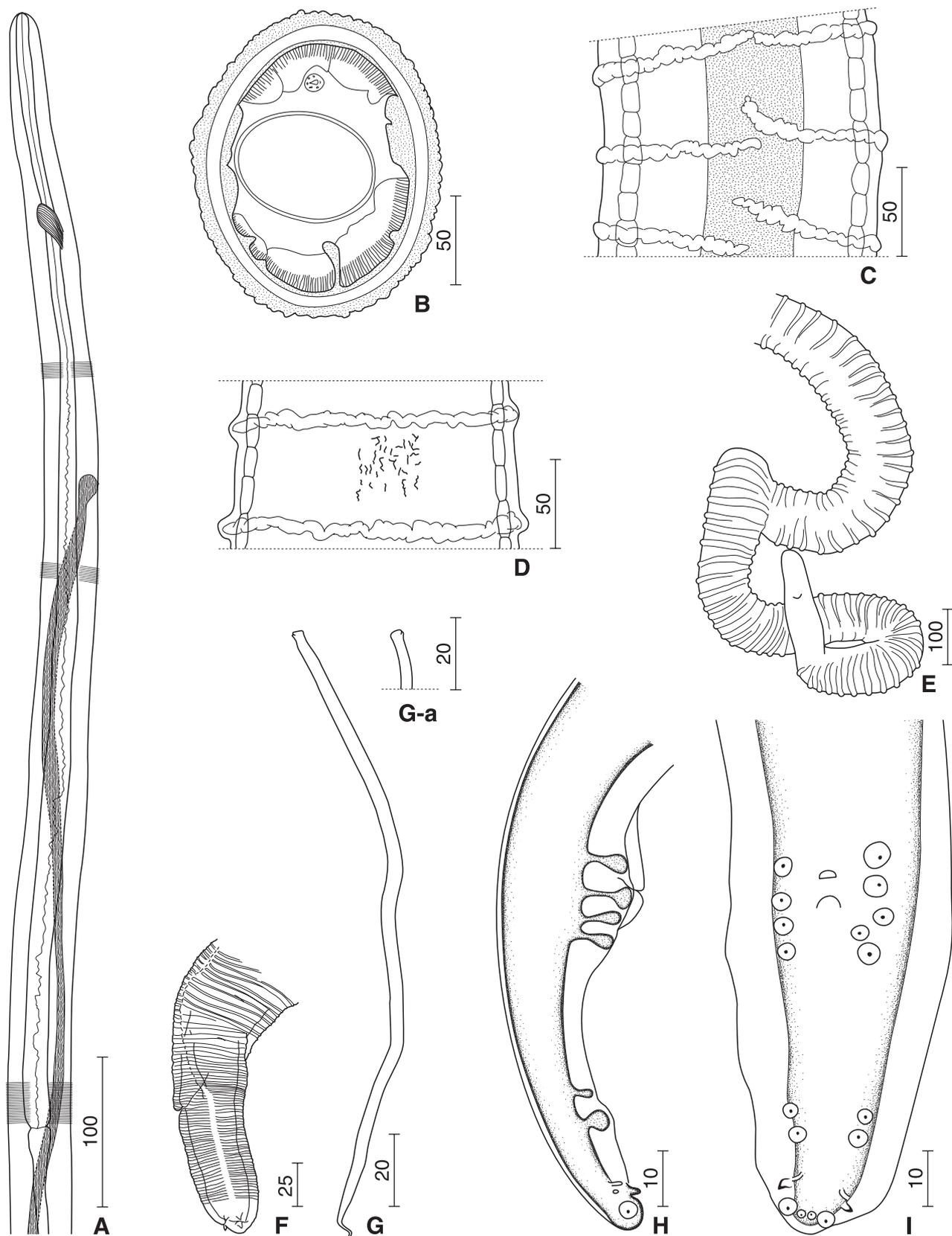


Fig. 3. – *Onchocerca stilesi* Eberhard, 1979. A-F. Female holotype. A. Anterior end, right lateral view. B. Cross-section at midbody. C. Cuticular ornamentation at midbody, lateral view (dotted area: lateral chord). D. Cuticular ornamentation, median view. E. Posterior region, twisted. F. Tail, left lateral view. G. Microfilaria in ovejector, median view. G-a. Anterior end of microfilaria, median view. H and I. Male paratype. H. Tail, right lateral view. I. Tail, ventral view. Bars, micrometers.

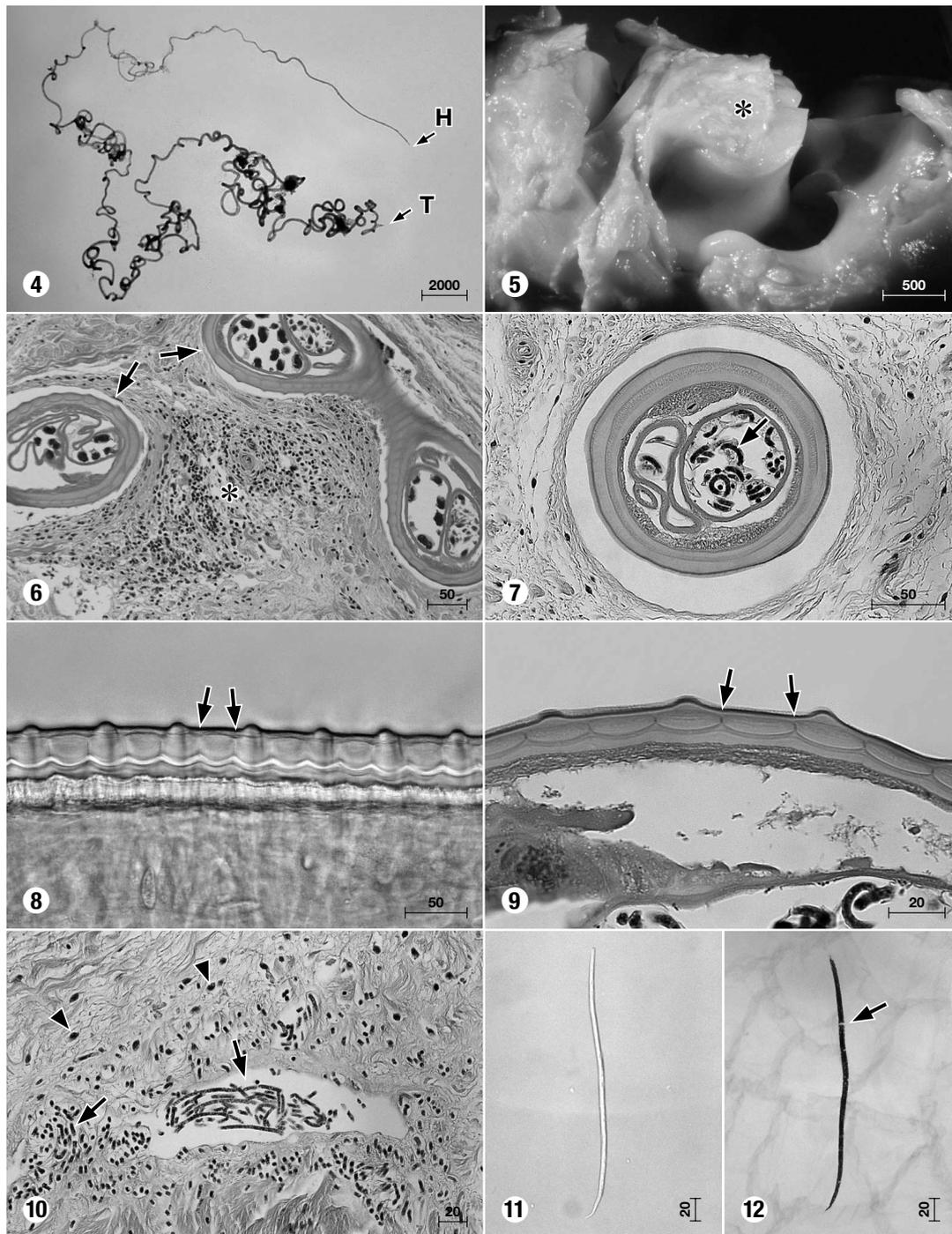


Fig. 4. – A complete female of *Onchocerca eberhardi* n. sp. Head (H) and tail (T). Bar, micrometers.

Fig. 5. – Carpal ligament (*) of sika deer, parasitized by adult worms of *Onchocerca eberhardi* n. sp. after collagenase treatment. Bar, micrometers.

Fig. 6. – Parasitic lesion of the carpal ligament. Inflammation seen near females (arrows) of *Onchocerca eberhardi* n. sp. Inflammatory cells (*). Bar, micrometers.

Fig. 7. – Transverse section of a female *Onchocerca eberhardi* n. sp. in the carpal ligament. Microfilaria (arrow). Bar, micrometers.

Fig. 8. – Cuticle of a female *Onchocerca eberhardi* n. sp. seen by differential interference contrast microscopy. Striae (arrows). Bar, micrometers.

Fig. 9. – Longitudinal histologic section of the cuticle of a female *Onchocerca eberhardi* n. sp. Striae (arrows). Bar, micrometers.

Fig. 10. – Microfilariae (arrows) in the tissue of the carpal ligament parasitized by females of *Onchocerca eberhardi* n. sp. Large inflammatory cells (arrowheads). Bar, micrometers.

Fig. 11. – Microfilaria taken from ovejector of a female *Onchocerca eberhardi* n. sp. Bar, micrometers.

Fig. 12. – Microfilaria taken from ovejector of a female *Onchocerca eberhardi* n. sp., stained with Giemsa's solution. Nerve ring (arrow). Bar, micrometers.

in the dermis of the anterior back, *C. longa* in the subcutaneous connective tissues of the limbs, and *M. (C.) perforata* in the dermis of the posterior back.

Microfilariae of *O. skrjabini* were found in the skin of sika deer (8/22 or 36 %), mainly of the ears and neck. The adult worms of the species were found in the subcutaneous connective tissues around the carpal and tarsal regions of sika deer. One complete female worm (S20-1; 60.5 cm long and 300 µm wide) was found in a sika deer (S20) on Mt. Sobo, Taketa City, Oita Prefecture, Kyushu. The findings constitute a new host record for *O. skrjabini* in the sika deer.

TAXONOMIC SUMMARY

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

Location in host: adult worms in the carpal ligament. Microfilariae in the skin, mainly of the ears.

Type locality: Mt. Sobo, Kiyokawa-cho, Oita Prefecture, Kyushu, Japan.

Collection dates: type specimens on 15 February 2004; other specimens between 26 September 1998 and 13 February 2006.

Specimens deposited: female holotype (S59-C36), collection number 140 JW, the Museum National d'Histoire Naturelle (MNHN), Paris; male allotype (S59-C38), 227 JW, MNHN. Paratype: three males (S59-C28, S59-C29, and S59-C37), 228 JW, 229 JW, and 230 JW, MNHN, respectively; three males (S59-C1, S59-C3, and S59-9) in the Department of Medical Zoology, Osaka City University Medical School (OCUMS). Other specimens: the anterior part of one female (S34-21) and one male (S34-

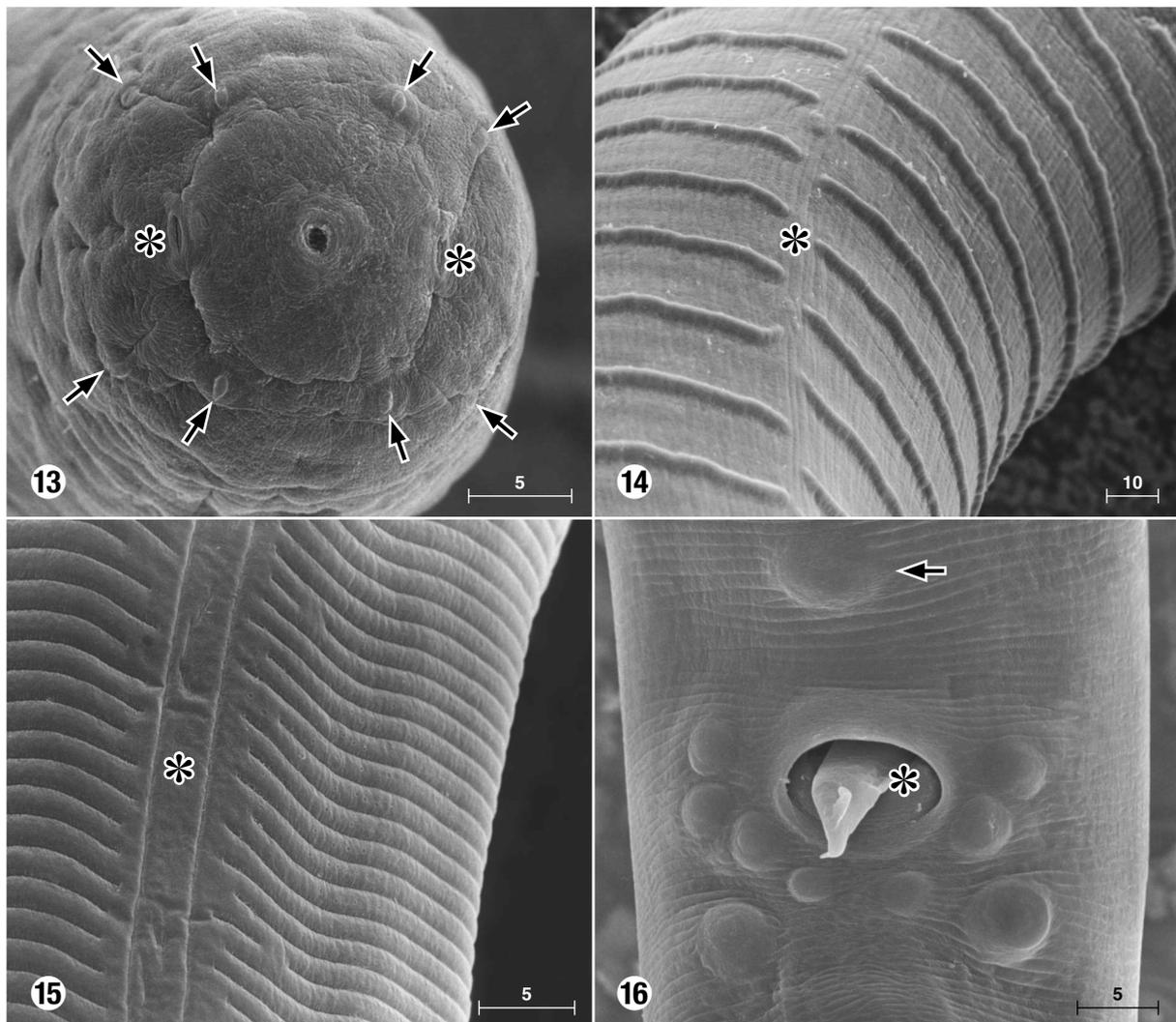


Fig. 13. – SEM of anterior end of a female *Onchocerca eberhardi* n. sp., amphids (*) and papillae (arrows). Bar, micrometers.

Fig. 14. – SEM of transverse ridges on the midbody of a female *Onchocerca eberhardi* n. sp. Lateral field (*). Bar, micrometers.

Fig. 15. – SEM of midbody of a male *Onchocerca eberhardi* n. sp. Lateral field (*). Bar, micrometers.

Fig. 16. – SEM of caudal papillae near cloaca of a male *Onchocerca eberhardi* n. sp. Precloacal papilla (arrow) and cloaca (*). Bar, micrometers.

10), 139 JW, MNHN; two anterior parts of females with microfilariae (S29-8 and S59-C44), one posterior part of young female (S29-32), and two males (S29-26 and S29-30), 127 JW, MNHN; 130 fragment females, 37 complete males, and 20 fragment males in OCUMS.

Etymology: we named this species *O. eberhardi* n. sp. in honor of Dr. Mark L. Eberhard, U.S.A., for his contribution to the knowledge of filarioids.

ONCHOCERCA STILESI EBERHARD, 1979

(Tables I, II; Fig. 3)

- Female (holotype): Very thin anterior end 5 mm long (Table I; Fig. 3A). Esophagus slender without distinct glandular part. Vulva in anterior half of esophagus; its distance to head equal to twice distance from nerve ring to head. Cuticle thin in anterior part of body, with conspicuous transverse external striae. Body musculature well developed, lateral chords narrow (Fig. 3B). Body round in transverse section, cuticle not thickened laterally. In main part of body (Fig. 3C, D), two cuticle layers, 25-30 μm thick: external layer with transverse ridges, undulated, jointed or overlapped on lateral fields, surface cuticle wrinkled; internal layer divided with transverse striae; a constant three striae between adjoining ridges. Posterior end coiled (Fig. 3E). Ridges present in preanal region but closer, 5-7 μm apart; tail with external striations, but no ridges (Fig. 3F). Tail straight; extremity round with lateral point near each phasmid.

- Microfilariae (from ovejector of female holotype): Body 177-190 μm long, 4-5 μm wide; slight constriction behind head; tiny cephalic hook; tail extremity attenuated (Fig. 3G, G-a).

- Male (paratype): Esophagus with glandular part. Caudal papillae in two groups: *i*) paracloacal group with one unpaired papilla and four to five aligned sublateral pairs (two anterior and three posterior to cloacal aperture); *ii*) four pairs in posterior third of tail with pair 7 small, pair 8 large, pair 9 pointed with phasmid at base, and pair 10 terminal. Tail tip with two small ventral transparent papilla-like formations (Fig. 3I). Right spicule with knobbed extremity and subterminal dorsal heel; left spicule with attenuated long distal extremity, as previously described (Eberhard, 1979).

TAXONOMIC DISCUSSION

For the diagnosis of the material from the sika deer we used the morphologic characteristics selected in the dichotomous key proposed by Bain (1981) for 24 species of *Onchocerca*, after revision and description of many species were made by several authors (Bain, 1975, 1981; Bain & Schulz-Key, 1974, 1976; Bain

et al., 1978a, b, 1980; Bain & Beveridge, 1979a, b; Eberhard, 1979; Denké & Bain, 1981). At that time, 26 species of *Onchocerca* were known, but two of these species were not included in the key because they were considered insufficiently defined: *O. stilesi* from cattle, for which an important distinctive characteristic, the microfilaria, was not described (Eberhard, 1979) and *O. lupi* Rodonaja, 1967 from a wolf (Rodonaja, 1967) which at the time was thought to be either a transfuge parasite from an ungulate, or an error of collection labelling.

In the present study, the short microfilaria of *O. stilesi* confirmed the validity of this species (Table I). The identity of *O. lupi* has been established by morphologic analysis of new materials collected from dogs in Eastern Europe (Demiaszkiewicz & Matsaberidze, 1991; Demiaszkiewicz *et al.*, 1991; Egyed *et al.*, 2001; Sréter *et al.*, 2002) and by molecular analysis of the filarial worms and their *Wolbachia* symbionts (Egyed *et al.*, 2002a, b). In addition to these two species, the present taxonomic discussion includes the five species described after 1981: *O. alcis* Bain & Rehlinger, 1986 from cervids (Bain & Rehlinger, 1986), two species *O. denkei* Bain, Vassiliades & Delbove, 1982 and *O. suzukii* from bovids (Bain *et al.*, 1982; Yagi *et al.*, 1994), and two species *O. ramachandrini* Bain, Wahl & Renz, 1993 and *O. dewittei japonica* from suids (Bain *et al.*, 1993; Uni *et al.*, 2001), totaling 31 *Onchocerca* species (with one subspecies).

Onchocerca eberhardi n. sp. stands in contrast to those six species with an esophagus longer than 2,000 μm and with thicker posterior glandular part, in female and male, and vulva not far from head (twice the distance from the head to nerve ring). These species are *O. railletii* Bain, Muller, Khamis, Guilhon & Schillhorn Van Veen, 1976, *O. reticulata* Diesing, 1841, and *O. cervicalis* Railliet & Henry, 1910 from equids; *O. flexuosa* (Wedl, 1856) from cervids; *O. armillata* Railliet & Henry, 1909 from bovids. *Onchocerca fasciata* Railliet & Henry, 1910 from camelids, [which in the key (Bain, 1981) is shown as apart from these species because the vulva is slightly less anterior], has similar esophageal morphology. In addition, females of these species have a thick anterior end (about 200 μm wide at the posterior end of the esophagus).

Among the remaining 25 species with an esophagus shorter than 2,000 μm in both sexes, *O. eberhardi* n. sp. stands in contrast to those of the nodular lineage with muscles atrophied and hypodermis hypertrophied in the female (Bain & Beveridge, 1979a; Bain, 2002). The seven species are *O. volvulus* (Leuckart, 1893) from humans; *O. gibsoni*, *O. cebei* Galliard, 1937, *O. ochengi* Bwagamoi, 1969, *O. dukei* Bain, Bussieras & Amégée, 1974, *O. schulzkeyi* Denké & Bain, 1981, and *O. hamoni* Denké & Bain, 1981, all from bovids.

Among the following six species, some do not have a complex female cuticle with ridges and striae, contrary

to *O. eberhardi* n. sp. *i*) Transverse ridges are absent in *O. suzukii* from bovids and *O. bohmi* (Supperer, 1953) from equids; in addition, the former has a distinctly divided esophagus, 1,660-1,910 μm long in females, and large spicules (Yagi *et al.*, 1994); the latter has a microfilaria with a long caudal filament (Supperer, 1953). In *O. cervipedis* Wehr & Dickmans, 1935 from cervids, the ridges are flat and not distant, so that they resemble the primitive common external striation of nematodes; the anterior end of the female is thick (220-270 μm wide at the posterior end of the esophagus). *ii*) The internal striae are absent in *O. dewittei dewittei* Bain, Ramachandran, Petter & Mak, 1977, *O. ramachandrini*, and *O. dewittei japonica* from suids (Takaoka *et al.*, 2004); in addition, body swellings are present in females of these three species (Uni *et al.*, 2001). The anterior region of the female of *O. ramachandrini* is not very attenuated (≥ 90 μm wide at posterior end of esophagus).

Five species have transverse ridges and striae like *O. eberhardi* n. sp. In four of these species the vulva is more posterior, near the posterior end of the esophagus (three to six times the distance from nerve ring to head) and the internal cuticle presents three to four striae between adjacent ridges. These species are parasitic in cervids: *O. skrjabini*, *O. garmsi* Bain & Schulz-Key, 1976, *O. jakutensis* (Gubanov, 1964) (= *O. tubingensis* Bain & Schulz-Key, 1974; Sonin, 1975), and *O. alcis*. In addition, the microfilaria is large in *O. skrjabini* (400-410 μm long, 11-12 μm wide; Bain & Schulz-Key, 1974) and *O. garmsi* (287-330 μm long, 7.4-8.4 μm wide; Bain & Schulz-Key, 1976). The esophagus is short, ≤ 850 μm , with a thick glandular part in *O. jakutensis* and *O. alcis* (Bain & Schulz-Key, 1976; Bain & Rehlinger, 1986). *Onchocerca lupi* in carnivores has two striae between adjoining ridges; at a length of 80-105 μm , its microfilaria is short and 5-7 μm relatively thick (Demiaszkiewicz & Matarberidze, 1991; Egyed *et al.*, 2001; Sréter *et al.*, 2002).

Four species (*O. beaveri* Bain, Chabaud & Landau, 1978a, *O. lerouxi* Bain, Chabaud & Landau, 1978a, and *O. sonini* Bain, Chabaud & Landau, 1978a from bovids in a Central African forest and *O. denkei* from a West African bovid) are similar to *O. eberhardi* n. sp. in the position of the vulva (two to three times the distance from the head to nerve ring). However, the esophagus in females has a distinct glandular part in *O. beaveri*, *O. lerouxi*, and *O. sonini*; in addition, the esophagus in all three of these species is longer (1,310-1,960 μm in females and 980-1,460 μm in males; Bain *et al.*, 1978a) than in *O. eberhardi* n. sp. *Onchocerca denkei* has a short esophagus, 1,020 μm long, in the female (Bain *et al.*, 1982), close to the maximum in *O. eberhardi* n. sp. However, *O. denkei* has reduced muscles, a thick hypodermis, four striae between ridges, and a large body diameter at the posterior end of the esophagus, which is twice as thick as in our specimens.

The last three species (*O. gutturosa*, *O. lienalis*, and *O. stilesi*) resemble *O. eberhardi* n. sp., because the females have a long thin anterior body (≤ 80 μm wide at the posterior end of esophagus), and an esophagus about 1,000 μm long, with a slightly differentiated glandular part or none at all (Table I). The species are all parasitic in bovids (cattle). *Onchocerca gutturosa* has four to seven striae per inter-ridge, sinuous ridges branched in lateral fields, asymmetric female body, pronounced reduction of caudal papillae, and longer left spicule in the male (Bain, 1975; Bain *et al.*, 1978b). *Onchocerca lienalis* and *O. stilesi* are 50-55 μm wide in body width at the posterior end of esophagus like the majority of our specimens (Table I). *Onchocerca lienalis* has two striae per inter-ridge, like our specimens, but ridges are present only in the posterior end, and the female has longitudinal wrinkles on the body (Bain *et al.*, 1978b). *Onchocerca stilesi* has three striae per inter-ridge; the ridges are sinuous and joined on the lateral field or overlapped (Fig. 3C); the microfilaria is shorter than that of *O. eberhardi* n. sp. (177-190 μm versus 200-258 μm). The measurements of *O. eberhardi* n. sp. seem to be different from those of *O. stilesi*: the more posterior position of the nerve ring, longer esophagus, and longer tail in both sexes and longer left spicule in male worms. Also, *Onchocerca eberhardi* n. sp. is distinct from these three bovid-host species in its location, the carpal ligament (Tables I, II).

CONCLUSION

Previously it was emphasized that the species of *Onchocerca* from cervids were polyphyletic with two main lineages identified: one of primitive species, *O. flexuosa* and *O. cervipedis*; the other of derived species, *O. skrjabini*, *O. jakutensis*, *O. garmsi*, and *O. alcis* (Bain, 1981; Yagi *et al.* 1994). A bovid parasite, *O. suzukii* from the Japanese serow, was found to resemble the primitive cervid parasite, *O. flexuosa*, suggesting a common ancestor (Yagi *et al.*, 1994).

The new species, *O. eberhardi*, appears to be an evolved species with the complex cuticle, reduction of glandular esophagus, and transformation of papillae pair 9 into a cuticular point. But this species differs from the other evolved parasites of cervids in a rather important characteristic, the more anterior position of the vulva. This difference brings *O. eberhardi* n. sp. closer to a group of species parasitic in bovids: *O. stilesi*, *O. lienalis*, and *O. gutturosa*.

The lineages of *Onchocerca* in cervids are probably the results of successive waves of diversification, which may have occurred simultaneously in bovids. However, these relationships were restricted to the areas where the cervids lived, almost all of which lie in the Holarctic

region. In tropical areas, other distinct lineages of *Onchocerca* may have expanded in bovids.

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- 9 - Immunology
- 10 - Parasites of fishes and other ectothermic vertebrates
- 11 - Population genetic structure and molecular epidemiology
- 12 - Innovative epidemiology
- 13 - Innovative strategies in diagnosis
- 14 - Ethics, teaching and epistemology
- 15 - Innovative prevention strategies

Satellite Symposia and Round Tables

Round tables and workshops will be organized upon request to the organizers. Satellite symposia will be organized by industrial firms.

Venue of the Multicolloquium

EMOP X will be organized at the “Cité Universitaire de Paris”. This campus is located in the middle of a nice park, nearby downtown Paris. The opening ceremony will be held in the “Grand amphithéâtre” of the historical Sorbonne.

EFP Young Scientist Award (YSA)

The EFP will offer Young Scientist Awards to stimulate the participation of young parasitologists and thus to contribute to an increasing development of parasitology in Europe. Awards will be in two areas: “Research in basic parasitology” and “Research in applied parasitology”. Up to three prizes may be awarded in each field. Details instructions on : www.emop10.eu

Scholarships for Parasitologists

Following the tradition, the EFP will offer a number of scholarships for young scientists to attend EMOP X in Paris. The French Society of Parasitology will also provide support for scientists not belonging to the EU and reduced registration fees will be available for students.

Accommodation

Low-cost accommodation will be available on the campus but conventional accommodation will be also organized in near-by hotels.

Important dates

Deadline for abstracts : 2 april 2008
Deadline for early registration : 2 may 2008

EMOP web site : www.emop10.eu

Registration, submission of abstracts, YSA, scholarships, accommodation and definitive program will be available through these regularly updated web pages.

Conference secretariat

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EMOP X

Honorary President

Jean-Antoine Rioux

European Federation of Parasitology

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International Scientific Committee

Eduardo Dei-Cas (Coordinator) and the chairs of the different symposia