COEXISTENCE OF FIVE CERCOPITHIFILARIA SPECIES IN THE JAPANESE RUPICAPRINE BOVID, CAPRICORNIS CRISPUS

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Summary:

The Japanese serow, Capricornis crispus (Bovidae, Caprinae. Rupicaprini), is parasitized by five Cercopithifilaria species: C. shohoi, recently described, and reexamined in this paper, C. multicauda n. sp., C. minuta n. sp., C. tumidicervicata n. sp., and C. bulboidea n. sp. Coinfections are frequent. The location (skin or subcutaneous regions) in the host of adult worms differed between the species, as did many morphological characters of both adults and microfilariae. The location (limbs, trunk, etc.) in the host of adult worms and dermal microfilariae seemed to differ depending on the species. Male and female worms of the same species had similar head shapes, buccal capsules, and, in four species, swellings in the anterior region of the body (because of the presence of a giant ventral pseudocoelomocyte). The Cercopithifilaria spp. from C. crispus were related to the primitive forms of the genus, parasites of Bovidae and Cervidae, presently recorded in Africa and Europe. C. bulboidea was particularly close to the most primitive species, C. ruandae and C. dermicola, in Africa, with the pairs of caudal papillae numbered 8 and 9 being distant from each other, but the species also had several specialized characters. Like C. rugosicauda in a European cervid, the four other species had pairs 8 and 9 close to each other; they reflect an evolutionary trend (hypertrophy of pair 6, and reduction and posterior migration of pair 7) that suggests diversification in the host.

KEY WORDS : Filarioidea, *Cercopithifilaria*, new species, Bovidae, Caprinae, Japanese serow, specific location.

Résumé : Coexistence de cinq espèces de *Cercopithifilaria* chez le Bovidé Rupicapriné japonais, *Capricornis crispus*

Le sérow japonais, Capricornis crispus (Bovidae, Caprinae, Rupicaprini), est parasité par cinq espèces de Cercopithifilaria, C. shohoi, récemment décrite et rééxaminée ici, et quatre nouvelles espèces, C. multicauda n. sp., C. minuta n. sp., C. tumidicervicata n. sp., et C. bulboidea n. sp. Les coinfections sont fréquentes mais la localisation des adultes et des microfilaires dermiques diffère selon les espèces. Celles-ci sont distinctes les unes des autres par de nombreux caractères morphologiques; mâles et femelles d'une espèce donnée sont reconnaissables par la morphologie identique de la tête, de la capsule buccale et, quand ils sont présents, par les renflements de la région antérieure du corps (dus chacun à un pseudocoelomocyte ventral géant). Les espèces parasites de C. crispus ont des traits communs et sont proches des formes les plus primitives du genre Cercopithifilaria, qui groupent les parasites des Bovidés et Cervidés, connus d'Afrique et d'Europe. C. bulboidea est particulièrement proche des espèces les plus primitives C. ruandae et C. dermicola en Afrique, avec les paires de papilles caudales 8 et 9 éloignées l'une de l'autre; elle a cependant plusieurs caractères spécialisés. Les quatre autres espèces ont les paires 8 et 9 rapprochées; elles montrent une évolution progressive (hypertrophie de la paire 6, réduction et migration postérieure de la paire 7) qui suggère des spéciations successives chez le sérow.

MOTS CLÉS : Filarioidea, Cercopithifilaria, nouvelle espèce, Bovidae, Caprinae, sérow, distributions spécifiques.

INTRODUCTION

ercopithifilaria is a worldwide filarial genus with a very large range of mammalian hosts (Eberhard, 1980; Bain et al., 1982; Chabaud &

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Bain, 1994). Until now, it was represented in Japan by only two species, *C. japonica* (Uni, 1983) in Japanese black bears, and *C. shohoi* Uni, Suzuki & Katsumi, 1998 in a bovid Rupicaprini, *Capricornis crispus* (Temminck, 1845), the Japanese serow. The two parasites are very different, the second being particularly close to species parasitic in African bovids and European cervids (Uni *et al.*, 1998).

We show here that the species of *Cercopithifilaria* that parasitize the Japanese serow are diverse, there being as many as five species. Consistently with other members of the genus, the adult worms inhabit the skin or subcutaneous connective tissues, and microfilariae are skin-dwelling, but each species seems to dwell in different sets of locations in the host. Monoparasitism is rare in all areas of Japan; generally, two or three species are associated, and four and five species have been found together on one occasion each.

By comparative morphological analysis of the new species, and reexamination of *C. shohoi*, we have

attempted to define the relationships of the Japanese species with other members of the genus *Cercopithi-filaria*, and to determine how these five congeneric species diversified and are presently found in the same host species.

MATERIALS AND METHODS

SEROWS EXAMINED

survey for the purpose of conservation and control of Japanese serows was conducted from 1997 to 1999, and 21 serows in total from three places were examined: 17 serows on Mt. Zao (1,841 m), Yamagata Prefecture, in the northern part of Honshu; three serows in the Hida Highlands and on Mt. Ena (2,190 m) in Gifu Prefecture, in the central part of Honshu; and one serow on Mt. Sobo (1,758 m), Oita Prefecture in Kyushu, the large southern island of Japan. Except for this last animal, which was found dead, the serows were killed in accordance with the policies of the Agency of Cultural Affairs, Japan, concerning the conservation of Japanese serows.

SEARCH FOR FILARIOIDS

The entire skin and the subcutaneous connective tissues of the trunk and limbs were examined under a stereomicroscope for adult filariae. For microfilariae, skin snips were taken (except for the dead serow in Oita) from the face, ears, neck, back, limbs, olecranons, belly, nipples, and tail. Blood smears also were made from each serow and stained with Giemsa's solution but none showed any microfilariae.

MORPHOLOGICAL ANALYSIS

Adult specimens were stored in 2 % formalin in saline and cleared in lactophenol for study. Special attention was given to the body swellings, and transverse sections were made at the level of the swellings for investigation of their structure. The caudal papillae of the males were numbered as in Chabaud & Petter (1961). Giemsa-stained skin microfilariae and microfilariae extracted from the ovijector of fixed females were studied. Drawings of the parasites, histological sections, and scanning electron micrographs (SEM) of the head, cuticle, and caudal extremity were prepared as described elsewhere (Uni *et al.*, 1998); a Hitachi S-4700 scanning electron microscope was used.

	C. shohoi	C. multicauda n. sp.	C. minuta n. sp.	C. tumidicervicata n. sp.	C. bulboidea n. sp.
Female	Gifu 17 (n = 4)	Gifu 27 $(n = 4)$	Yamagata 97 (n = 4)	Gifu 126 (n = 7)	Oita 31-2 (n = 8)
Body length	55 (32-55) [43]	25 (20-25) [23]	19 (19-24) [22]	32 (25-40) [33]	42 (27-42) [31]
Body width	160 (120-170) [148]	70 (60-90) [74]	100 (70-100) [79]	125 (102-145) [115]	87 (87-135) [99]
Buccal cavity, H*	8	4	5	6	6
Buccal ring, H/MED*	5/10	2/8	3/11	4/15	3/9
Nerve ring*	250 (170-250) [213]	130 (115-150) [137]	150 (150-208) [178]	220 (208-265) [244]	165 (153-204) [170]
Esophagus	920 (670-920) [760]	780 (645-800) [710]	560 (525-581) [552]	800 (745-1,173) [923]	685 (485-840) [660]
Vulva*	1,125 (710-1,125) [896]	440 (430-550) [503]	720 (570-724) [655]	760 (760-969) [881]	500 (450-612) [540]
Vagina, L/W*	125/70	150/40	75/55	120/60	105/40
Ovijector	4,700	550	1,250	3,700	2,050
Tail	225 (180-225) [201]	200 (135-200) [160]	170 (130-170) [135]	300 (177-300) [221]	170 (143-190) [151]
Microfilaria	(n = 10)	(n = 10)	(n = 10)	(n = 10)	(n = 10)
Body length	118 (105-120) [115]	167 (165-178) [170]	195 (195-211) [202]	420 (416-459) [436]	190 (190-208) [203]
Body width	5 (5-8) [6]	10 (5-10) [8]	6 (4-6) [5]	9 (8-10) [10]	6 (4-8) [6]
Male	Gifu 31 $(n = 5)$	Gifu 23 $(n = 7)$	Yamagata 108 (n = 7)	Gifu 97 (n = 6)	Oita 22-B3 (n = 7)
Body length	23 (13-32) [24]	13 (13-17) [15]	11 (11-14) [12]	18 (16-23) [20]	28 (18-28) [22]
Body width	78 (68-95) [83]	52 (52-64) [62]	61 (57-65) [60]	100 (88-105) [95]	70 (55-75) [70]
Nerve ring*	180 (160-208) [182]	145 (140-158) [150]	150 (140-182) [152]	230 (213-302) [254]	150 (140-182) [167]
Esophagus	550 (536-830) [648]	550 (550-850) [708]	555 (442-718) [565]	920 (659-945) [849]	520 (520-620) [554]
Testicle apex*	900	650	1,420	1,850	1,120
Area rugosa, L	1,125	1,250	900	1,000	1.090
Right spicule, L	75 (65-75) [69]	65 (65-83) [76]	50 (50-68) [57]	102 (83-102) [86]	55 (55-73) [65]
Left spicule, L	240 (203-243) [230]	380 (355-388) [377]	225 (225-280) [232]	380 (338-380) [362]	238 (234-250) [241]
Tail	150 (117-163) [148]	140 (93-140) [100]	115 (112-145) [114]	200 (156-200) [177]	125 (117-177) [149]
Terminal cylinder, L/W	5/8	9/5	15/6	10/10	6/6

Holotype and allotype are presented first (except for *C. shohoi*), followed by ranges and then means. n, number of parasites examined.

Table I. - Measurements of adults and microfilariae of the five species of Cercopithifilaria found in Japanese serows.

Measurements are given in Table I: the body length is expressed in millimeters, with other dimensions in micrometers; height of the buccal cavity is the distance from the oral opening to the esophagus; dimensions of the buccal capsule (or ring) are the height (H)/the maximum external diameter (MED); the distance from the apex is given for the nerve ring, the commencement of the testis, and the vulva; length (L)/width (W) is given for the vagina.

The authors' names of the new species described below are Uni and Bain.

RESULTS

haracters common to all species are presented first. Morphological findings (Figs. 1-11 and Table I) and prevalence and distribution (Table II) are given for each species, with a differential diagnosis between the Japanese species. The structure of the body swellings, present in four of these species, is described at the end of this section.

GENERAL.

Filarioidea, Onchocercidae (Leiper, 1911) Chabaud & Anderson, 1959; Onchocercinae Leiper, 1911; *Cercopithifilaria* (Eberhard, 1980) Bain, Baker & Chabaud, 1982.

Nematodes 10 to 50 mm long and 50 to 150 µm wide. No lateral alae but cuticle thickened internally, in general very slightly (Fig. 8). Transverse section of body round. Head papillae symmetrically arranged: four external-labial and four cephalic papillae; amphids with conspicuous channel. Small round oral opening; buccal capsule reduced to a small pre-esophageal cuticular ring; transverse section of the buccal cavity round anteriorly; lumen Y-shaped at its base and at the apex of esophagus; posteriorly, esophageal lumen flattened; esophagus not divided into anterior muscular and posterior glandular portions, but paired glandular pore openings in the esophageal lumen often conspicuous (three pairs along the esophagus).

In the female, vagina composed of a short wide tube perpendicular to the body wall, then a chamber lined with hypodermal cells that bulge posteriorly to form the sphincter, preceding the ovijector. Tail curved ventrally, with three terminal lappets, two lateral and one dorsal; phasmids at base of the lateral lappets.

Microfilariae with head trapezoidal in lateral view, round in ventral view; left cephalic hook; striations of the cuticle conspicuous, except at anterior end; last posterior nucleus far from tip of tail; in uteri, sheath present, loose, but fragile after fixation and often ragged. In the male, cuticle of the tail thick ventrally and forming lateral alae; posterior extremity of tail abruptly

	C. shohoi	C. multicauda n. sp.	C. minuta n. sp.	C. tumidicervicata n. sp.	C. bulboidea n. sp.	
Type locality	Mt. Zao,	Hida Highlands,	Mt. Zao,	Hida Highlands,	Mt. Sobo,	
	Yamagata	Gifu	Yamagata	Gifu	Oita	
Prevalence	9/12 (75 %)	1/3	2/12 (17 %)	2/3	1/1	
of adult worms*						
Prevalence of microfilariae**	12/17 (71 %)	1/3	4/17 (24 %)	2/3	ND***	
Total adults	38	54	24	56	56	
Other locality	Hida Highlands,		Hida Highlands,	Mt. Zao,	Hida Highlands	Mt. Zao,
(localities)	Gifu		Gifu	Yamagata	and Mt. Ena, Gifu	Yamagata
Prevalence	1/3		1/3	5/12 (42 %)	2/3	1/12
of adult worms*						
Prevalence	3/3		0	9/17 (53 %)	2/3	0
of microfilariae**						
Total adults recovered	10		3	28	21	1
Adult tissue location	Subcutaneous	Skin	Skin	Skin	Subcutaneous	
Distribution	Limbs, trunk,	Limbs	Thoracic limbs,	Abdomen,	Limbs, trunk	
of adult worms	neck		trunk	pelvic limbs, trunk, back		
Distribution	Limbs, nipples,	Face, ears	Neck, midback	Tail, posterior back,	Midback,	
of dermal microfilariae	trunk			pelvic limbs, nipples	thoracic limbs	

^{*} Serow(s) infected/serow(s) examined.

Table II. - Geographic distribution and parasitic location of the five species of Cercopitbifilaria found in Japanese serows.

^{**} In skin snips.

^{***} ND, not done. See text.

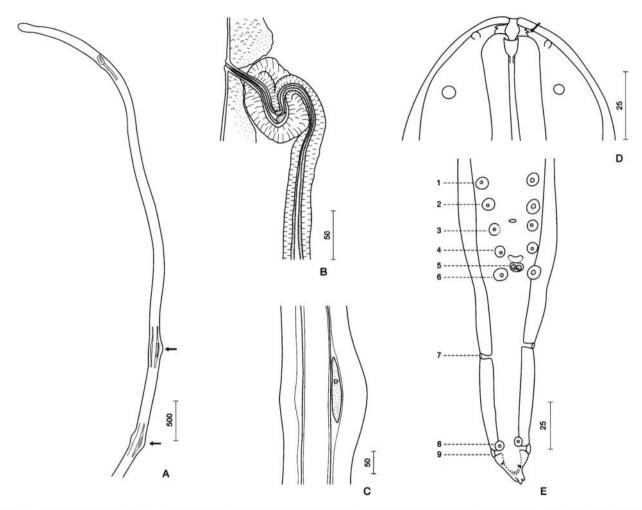


Fig. 1. – *Cercopithifilaria shoboi*. A-C, females. A. Anterior part with body swellings (arrows), lateral view. B. Vagina, lateral view. C. Body swelling with pseudocoelomocyte. D and E, males. D. Head, lateral view. Buccal capsule, external aspect (arrow). E. Posterior part with four pairs of precloacal papillae, ventral view. About papillae numbers, see text. Bars, micrometers.

attenuated ending in a soft cuticular appendage, which we refer to as the cylinder; it bears three lappets; phasmids at base of the cylinder. Set of caudal papillae slightly reduced, composed of seven to nine pairs, symmetrically arranged, pedunculated (Fig.1E): two to four pairs of papillae anterior to the cloacal aperture ("precloacal papillae") (the position of the existing papillae suggests that the most anterior pairs 1 and 2 have disappeared); pair 5 submedian, pair 6 more lateral; pairs 7, 8, and 9 in the posterior half of the tail (pair 10 always absent); in general, unpaired papilla far anterior to cloacal aperture. Area rugosa composed of transverse bands of cuticular longitudinal crests (Fig. 2O).

Supplementary information on *C. shohoi* Uni, Suzuki & Katsumi, 1998 (Fig. 1)

Female with four conspicuous body swellings (4.9, 6.0, 17.1, and 21.8 mm from anterior end in specimen Gifu 17, Table I) and male with three or four body swel-

lings (2.5, 4.6, 5.2, and 9.0 mm from anterior end in specimen Gifu 31). Buccal capsule with the external aspect forming two or three crests (Fig. 1D, arrow). Striations of the cuticle are perpendicular to the axis of the body in the lateral field, with the terminal end of striae elevated.

In the male, unpaired papilla at level of pair 3 or between pairs 2 and 3 (Fig. 1E). Three or four pairs of precloacal papillae; papillae of pair 5 joined together or not. Cylinder present at the posterior end, as wide as it is long and with conical lappets.

Adult specimens in subcutaneous connective tissues on the muscles of the limbs, trunk, and neck. Microfilariae in the skin of the lower part of the body (limbs, nipples, and trunk). Found in Yamagata and Gifu (Table II).

CERCOPITHIFILARIA MULTICAUDA UNI & BAIN, N. SP. (Figs. 2, 8A, 9A, 10A, and 11A)

Specimens: female holotype (Gifu 27 taken from serow No. 1) and male allotype (Gifu 23 from the same

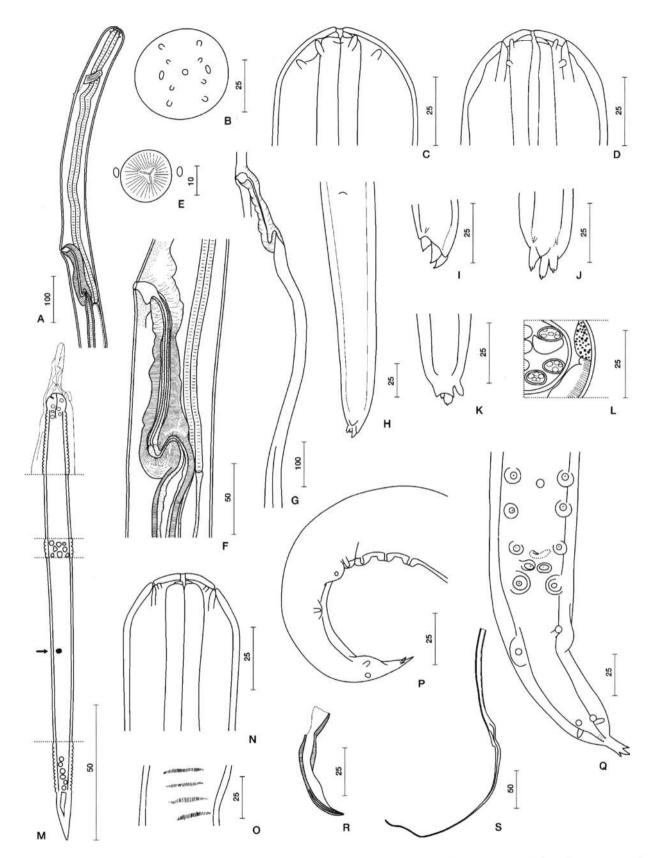


Fig. 2. – C. multicauda n. sp. A-L, females. A. Anterior part, lateral view. B. Anterior end, en face view. C. Head, lateral view. D. Head, median view. E. Esophagus and amphids, transverse section. F. vagina, lateral view. G. Ovijector. H. Tail, ventral view. I. Posterior end, lateral view. J. Posterior end, ventral view. K. Posterior end, dorsal view. L. Uterine microfilariae in midbody, rhomboid in transverse section. M. Microfilaria with sheath, median view. Arrow, rectal cell (R1). N-S, males. N. Head, median view. O. Area rugosa, ventral view. P. Posterior part, lateral view. Q. Posterior part, ventral view. R. Right spicule. S. Left spicule. Bars, micrometers.

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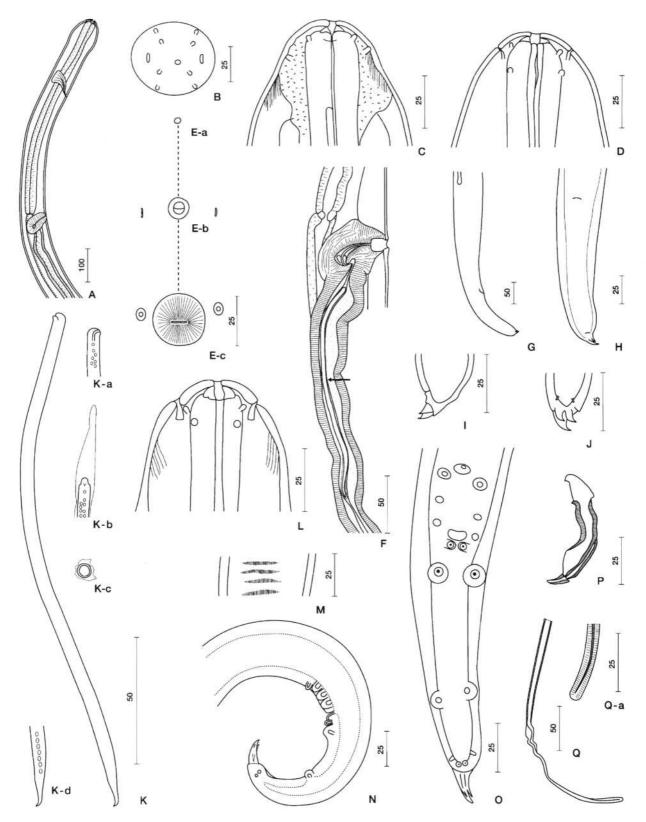


Fig. 3. – C. minuta.n. sp. A-J. females. A. Anterior part, lateral view. B. Anterior end, en face view. C. Head, lateral view. D. Head, median view. E-a. Mouth opening. E-b. Base of buccal capsule and amphids below E-a. E-c. Flat lumen of esophagus and amphids, transverse section below E-b. F. Vagina and anterior part of ovijector with microfilaria (arrow), lateral view. G. Posterior part, lateral view. H. Tail, ventral view. I. Posterior end; lateral view. J. Posterior end; ventral view. K-K-d, microfilariae. K. Entire body, median view. K-a. Anterior end, median view. K-b. Anterior end with sheath, left lateral view. K-c. Anterior part with sheath, transverse section. K-d. Posterior end, median view showing nuclei. L-Q-a, males. L. Head, median view. M. Area rugosa, ventral view. N. Posterior part, lateral view. O. Tail, ventral view. P. Right spicule. Q. Left spicule. Q-a. Left spicule, posterior end. Bars, micrometers.

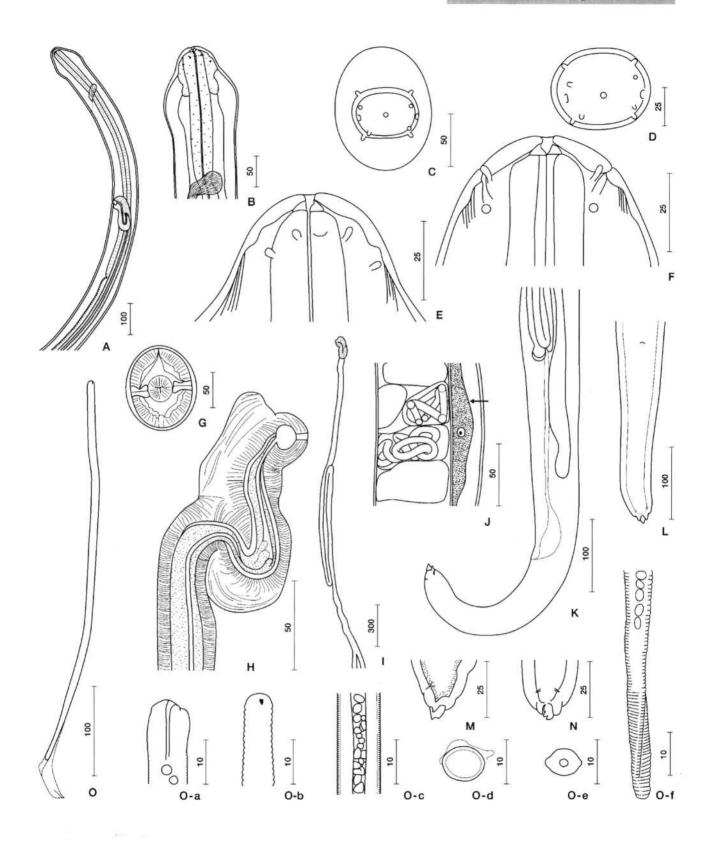


Fig. 4. – *C. tumidicervicata* n. sp. A-N, females. A. Anterior part, lateral view. B. Enlarged anterior end, lateral view. C. Anterior part with subterminal bulbous part, en face view. D. Head, en face view. E. Head, lateral view. F. Head, median view. G. Body at esophagus level, transverse section. H. Vagina, lateral view. I. Ovijector. J. Body swelling with pseudocoelomocyte (arrow). K. Posterior part, lateral view. L. Tail, ventral view. M. Posterior end, lateral view. N. Posterior end, ventral view. O-O-f, microfilariae. O. Entire body with sheath, median view. O-a. Anterior end with cephalic hook, median view. O-b. Anterior end with cephalic hook, left lateral view. O-c. Midbody, median view. O-d. Midbody, transverse section. O-e. Caudal region, transverse section. O-f. Caudal region. Bars, micrometers.

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serow), 218 HS, collection of the Muséum National d'Histoire naturelle (MNHN), Paris. 52 paratypes: 48 in the Department of Medical Zoology, Osaka City University Medical School, four in the MNHN (218 HS). Location in host: adults in skin of thoracic limbs and olecranon of the pelvic limbs; microfilariae in skin of face and ears.

Type locality: Furukawa, Hida Highlands, Gifu Prefecture, Japan.

Anterior end round. Head papillae long and conspicuous; cephalic papillae at the corners of a rectangle that is elongated dorsoventrally. Buccal capsule very

Female

Striations of cuticle 0.2 µm apart, obliquely directed in the lateral field (Fig. 10A). Vulva anterior to esophago-intestinal junction and protruding. Vagina elongated. Ovijector short. Body with four slight swellings 6.9, 7.2, 7.7, and 9.2 mm from anterior end of the paratype specimen Gifu 3. Complex caudal extremity that seemed at first sight to be composed of five or six lappets; in

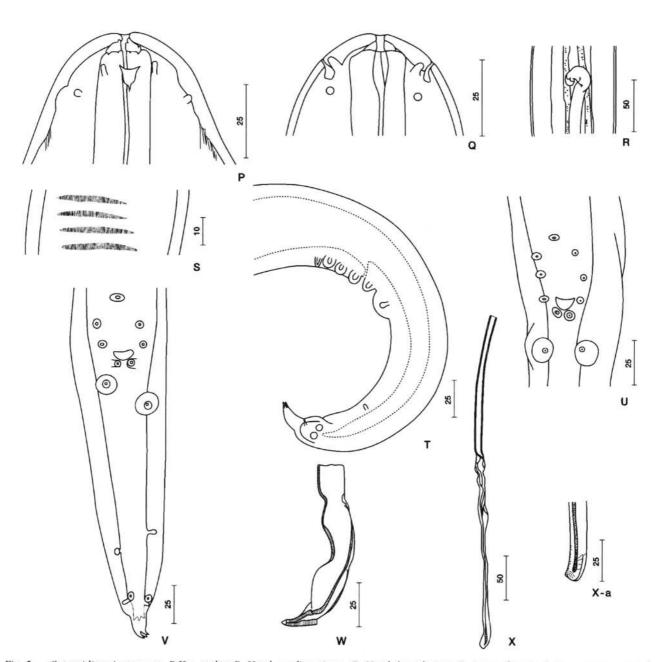


Fig. 5. – *C. tumidicervicata* n. sp. P-X-a, males. P. Head, median view. Q. Head, lateral view. R. Apex of testis. S. Area rugosa, ventral view. T. Posterior part, lateral view. U. Cloacal region with three precloacal pairs of papillae, ventral view. V. Tail, ventral view. W. Right spicule. X. Left spicule. X-a. Left spicule, posterior end. Bars, micrometers.

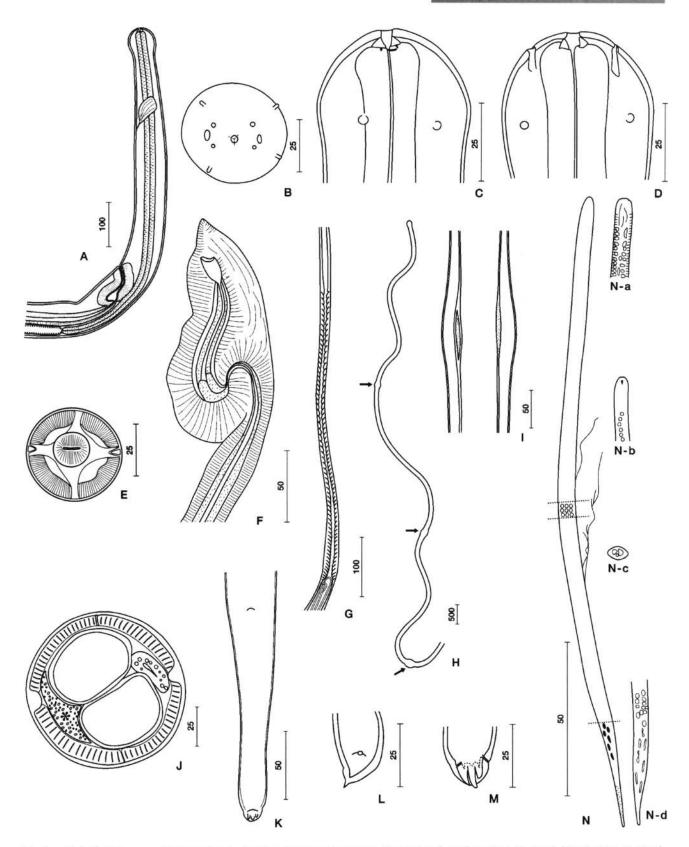


Fig. 6. – *C. bulboidea* n. sp. A-M, females. A. Anterior part, lateral view. B. Anterior end, en face view. C. Head, lateral view. D. Head, median view. E. Body at esophagus level, transverse section. F. Vagina, median view. G. Specialized part of ovijector (see text). H. Anterior part with body swellings (arrows). I. Body swelling. J. Body swelling with pseudocoelomocyte (asterisk), transverse section. K. Posterior part, ventral view. L. Posterior end, lateral view. M. Posterior end, ventral view. N-N-d, microfilariae. N. Entire body with sheath, lateral view. N-a. Anterior end, median view. N-b. Anterior end, left lateral view. N-c. Midbody, transverse section. N-d. Posterior end, median view. Bars, micrometers.

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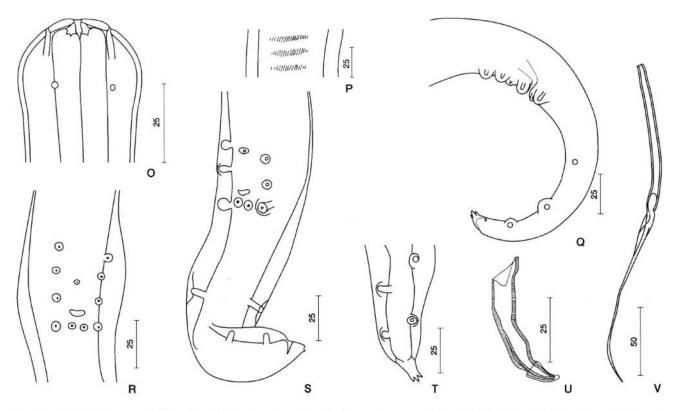


Fig. 7. – C. bulboidea n. sp. O-V, males. O. Head, median view. P. Area rugosa, ventral view. Q. Posterior part, lateral view. R. Cloacal region with three pairs of precloacal papillae, ventral view. S. Tail. T. Posterior end, ventral view. U. Right spicule. V. Left spicule. Bars, micrometers.

fact, there are three lappets as in other species, long and digitiform, the median one being the longest, and all with an acute apex; at their base, a conical bulging ventral crest and a bulging dorsal crest divided into two short conical points (Fig. 2I-K, Fig. 11A).

Microfilaria: body short, flattened dorsoventrally, transverse section rhomboidal (Fig. 2L). Short cephalic space. Caudal end lanceolated; last nucleus 8 µm from extremity. In uteri, sheath ragged and rarely present; present on all skin microfilariae.

Male

Apex of testis slightly behind the end of esophagus. Area rugosa: 86 or so transverse bands 3 μm high and distance between adjoining bands 7 μm. Caudal papillae: three large precloacal pairs, pair 6 just posterior to, and larger than, pair 5; pair 7 at mid-length of tail and large; pairs 8 and 9 close together, the former ventral, the latter laterodorsal (Fig. 2Q). Cylinder twice as long as wide, slightly constricted in posterior half, with pointed lappets, the median one longer. Spicules: right spicule with little or no dorsal heel; left spicule long with lamina twice the length of the handle (130 μm long), slender distal extremity.

Differential diagnosis

C. multicauda n. sp. is distinguished from C. shohoi by many characters (Table I): the shorter and more

slender body in both sexes but larger spicules, the right lacking a heel, the cephalic papillae on a dorsoventrally elongated rectangle, the very reduced buccal capsule, the longer microfilaria and the peculiar shape of its tail, the vagina elongated, the very slight body swellings, and the shape of the cylinder and caudal lappets.

CERCOPITHIFILARIA MINUTA UNI & BAIN, N. SP. (Figs. 3, 8B, 9B, 10B, and 11B)

Specimens: female holotype (Yamagata 97 from serow No. 2), male allotype (Yamagata 108 from the same serow), 220 HS, MNHN, Paris. Five paratypes: one in the Department of Medical Zoology, Osaka City University Medical School, four in the MNHN (220 HS). Location in host: adults in skin of trunk and thoracic limbs; microfilariae in skin of neck and midback.

Type locality: Mt. Zao, Yamagata Prefecture, Japan. Other localities: Gifu. Three specimens.

No body swellings. Anterior end with small subterminal bulb, rhomboidal in transverse section, with greater dorsoventral diameter (Fig. 9B); head muscle with a vacuolated structure in some specimens. Labial papillae arranged in a square; cephalic papillae arranged in a dorsoventrally elongated rectangle (Fig. 3B). Buccal cavity cylindrical; buccal capsule moderately wide.

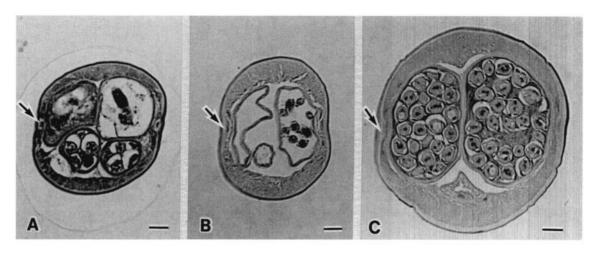


Fig. 8. – Stained transverse sections of female midbody. Lateral internal thickening of the cuticle (arrow). A. *C. multicauda* n. sp. B. *C. minuta* n. sp. C. *C. tumidicervicata* n. sp. Bars, 10 µm.

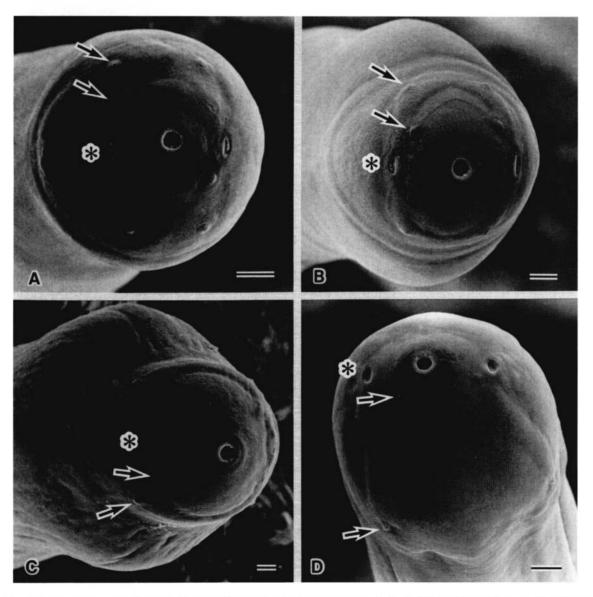


Fig. 9. – SEM of anterior end of females. A. C. multicauda n. sp. B. C. minuta n. sp. C. C. tumidicervicata n. sp. D. C. bulboidea n. sp. Papillae (arrows) and amphid (*). Bars, 5 µm.

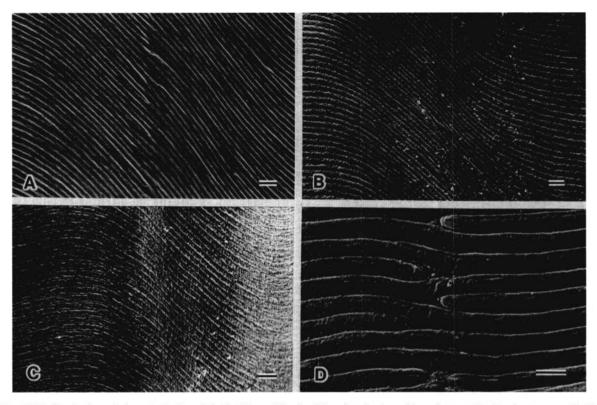


Fig. 10. – SEM of cuticular striations in the lateral field of the midbody of females. A. C. multicauda n. sp. B. C. minuta n. sp. C. C. tumi-dicervicata n. sp. D. C. bulboidea n. sp. Bars, 1 µm.

Female

Cuticular striations 0.2 µm apart, obliquely directed in the lateral field (Fig. 10B). Vulva at level of esophago-intestinal junction; vagina spherical; ovijector short and thick. Muscle layer of the midbody thick (Fig. 8B). Caudal lappets directed ventrally, conical, the median one longer, each with an acute end; at their base, a ventral and a dorsal crest (Fig. 3I-J).

Microfilaria: transverse section round. Tail with anucleated part 13 µm long; extremity bent; in a stained microfilaria, cephalic end with an isolated pair of nuclei; nerve ring 45 µm (18 %) from anterior end. Uterine microfilariae with ragged sheath; sheath present in skin forms.

Male

Apex of testis far behind esophagointestinal junction. Area rugosa (Fig. 3M): about 120 bands, 3 µm high, distance between adjoining bands 5 µm. Caudal papillae (Fig. 3O): three precloacal pairs, more or less regularly arranged, pair 6 larger than all others, distant from pair 5; pair 7 at two-thirds of the tail length, pairs 8 and 9 subterminal, the first laterodorsal, the second ventral. Cylinder twice as long as wide, conical; lappets sharp, the median one larger. Spicules short: the right with conspicuous dorsal heel, the left with handle (130 µm

long) slightly shorter than the lamina; extremity of lamina thick and round.

Differential diagnosis

The adult worm is small (Table I), like *C. multicauda*, but the species is distinguished by the shape of the head, the longer microfilaria with a different tail extremity, the right spicule with heel and left spicule with thick distal extremity, and the female caudal extremity. The adult worm is smaller than *C. shohoi* and is also distinguished by the microfilariae being twice as long as those of *C. shohoi*, position of cephalic papillae (Uni *et al.*, 1998), and position and size of pairs 6 and 7 of caudal papillae.

CERCOPITHIFILARIA TUMIDICERVICATA
UNI & BAIN, N. SP.
(Figs. 4, 5, 8C, 9C, 10C, and 11C)

Specimens: female holotype (Gifu 126 from serow No. 1), male allotype (Gifu 97 from the same serow), 219 HS, MNHN, Paris. 45 paratypes: 43 specimens in the Department of Medical Zoology, Osaka City University Medical School, two in the MNHN (219 HS).

Location in host: adults in skin of abdomen, pelvic limbs, trunk, and back; microfilariae in the skin of the posterior part of the body (tail, posterior back, pelvic limbs, and nipples).

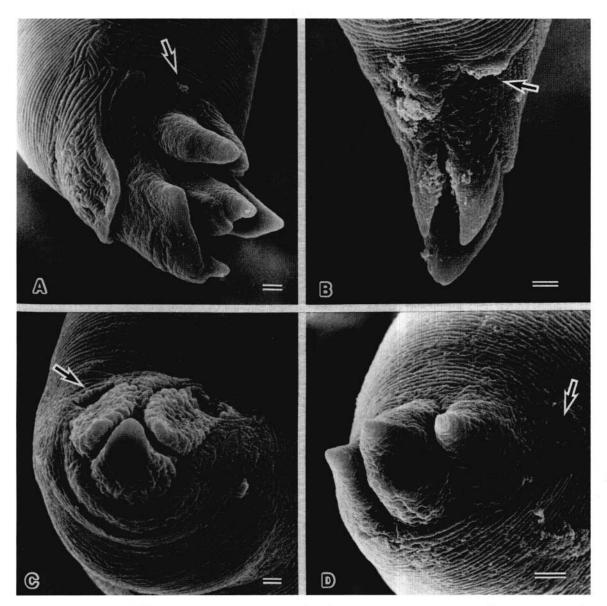


Fig. 11. – SEM of posterior end of females. A. C. multicauda n. sp., lateral view. B. C. minuta n. sp., ventral view. C. C. tumidicervicata n. sp., apical view. D. C. bulboidea n. sp., laterodorsal view. Phasmid (arrow). Bars, 1 µm.

Type locality: Furukawa, Hida Highlands, Gifu Prefecture, Japan.

Other localities: Yamagata. 28 specimens; one female in the MNHN (253 HS).

Anterior end with a subterminal cervical bulb, which is rhomboidal in transverse section with a greater dorsoventral diameter (Fig. 4A-C, Fig. 9C). Body swellings present (Fig. 4J). Cuticle of head thick and papillae with peduncles; labial and cephalic papillae at the corners of two laterally elongated rectangles (Fig. 4D-F).

Female

Three body swellings (8.2, 12.2, and 20.0 mm from anterior end in the holotype specimen), often slight. Striations of the cuticle $0.2~\mu m$ apart, obliquely directed

in the lateral field (Fig. 10C). Head muscles and anterior part of esophagus with refringent granulations in some specimens. Vulva generally anterior to esophagointestinal junction; vagina moderately elongated; ovijector long. Muscle layer thick at transverse section of the midbody (Fig. 8C). Caudal lappets almost as wide as long, the lateral ones blunt, the dorsal one larger and with a pointed ventral extremity; around the base of the lappets, ventral and dorsal crests (Fig. 4M-N). Microfilaria: body very long and thick (Table I). Transverse section oval, cuticular lateral alae in the posterior region; caudal region straight but outer surface spirally twisted, thick blunt end (Fig. 4O-f). Long cephalic space; narrow central column of nuclei; anucleated caudal extremity 40 µm long; in a stained microfilaria,

nerve ring 76 μ m (19 %) and excretory pore 106 μ m (26 %) from anterior end. Each microfilaria with a sheath in the uterus; sheath absent from skin forms.

Male

One body swelling (5.4 mm from anterior end in the allotype specimen). Buccal capsule sometimes with tiny posterior crests anchored in the esophagus (Fig. 5P). Apex of testis at anterior level of intestine, its distance from the head approximately twice the length of the esophagus (Fig. 5R). Area rugosa: about 160 transverse bands, each 3 µm high, distance between adjoining bands 6 µm. Caudal papillae (Fig. 5V): two or three precloacal pairs; pair 6 far behind pair 5, and the largest of all pairs; pair 7 small and in the posterior third of the tail; pairs 8 and 9 close together, the former ventral, the latter laterodorsal. Caudal cylinder as wide as it is long; caudal lappets short and conical. Spicules large: the right with terminal cap, forming a conspicuous subterminal dorsal heel; the left with lamina slightly longer than handle (170 µm long), and distal part of the lamina with slightly striated alae, extremity round.

Differential diagnosis

The large size of the microfilaria distinguishes this species from the four other species. The adults greatly resemble those of *C. minuta* with the subterminal bulb, the thick muscle layer of the midbody, the arrangement of caudal papillae, and the shape of the left spicule; however, *C. tumidicervicata* n. sp. is distinguished by the greater size of adults and spicules, the cephalic papillae forming a laterally elongated rectangle, the longer ovijector, the presence of body swellings, the shape of the caudal lappets in both sexes, and pair 7 of caudal papillae being smaller and more posterior.

Adults of *C. tumidicervicata* n. sp. also resemble *C. shohoi* in the body size, the size and arrangement of caudal papillae, and the position of head papillae (Uni *et al.*, 1998). *C. tumidicervicata* has a left spicule as long as that of *C. multicauda* n. sp., but the body is larger (Table I); the other differential characters are those listed for *C. shohoi*.

CERCOPITHIFILARIA BULBOIDEA UNI & BAIN, N. SP. (Figs. 6, 7, 9D, 10D, and 11D)

Specimens: female holotype (Oita 31-2 from serow No. 1 in Oita), male allotype (Oita 22-B3 from the same serow), 217 HS, MNHN, Paris. 54 paratypes: 50 in the Department of Medical Zoology, Osaka City University Medical School, four in the MNHN (217 HS).

Location in host: adults in subcutaneous connective tissues on muscles of limbs and trunk; microfilariae in skin of the midback region and thoracic limbs in two serows from Gifu.

Type locality: Koubaru on Mt. Sobo, Taketa City, Oita Prefecture, Kyushu, Japan.

Other localities: Gifu and Yamagata. 22 specimens; one female in the MNHN (252 HS).

Bulbous head followed by a neck (Fig. 6A; Fig. 9D). Body swellings present, each with a ventral giant granulated pseudocoelomocyte (Fig. 6I-J). Labial papillae very small and seen only in apical view. Labial and cephalic papillae at the corners of two laterally elongated rectangles, with the cephalic papillae posterior to the maximum diameter of the bulb (Fig. 6C-D, Fig. 9D). Buccal capsule small; longitudinal section trapezoidal, the external aspect forming two crests (Fig. 6C). Conspicuous internal lateral thickenings of the cuticle in the anterior part (Fig. 6E).

Female

Three body swellings (3.4, 8.2, and 13.0 mm from anterior end of the holotype specimen). A cuticular circular elevation around the mouth opening is seen in two specimens by SEM. Striations of cuticle 0.5 µm apart; they are not oblique in the lateral field and are often interrupted (Fig. 10D). Vulva anterior to esophagointestinal junction; vagina moderately elongated; ovijector very long, with characteristic specialized structure from 1,000 µm behind the vagina to the division of the uteri (Fig. 6G): lumen lined with large hypodermal cells directed backwards, probably to control microfilarial migration to vagina. Tail slightly constricted in the second third; three lappets, longer than wide, the median one longer, all inserted ventrally.

Microfilaria: transverse section rhomboidal (Fig. 6N-c) and body flattened dorsoventrally; on lateral view, one file of nuclei; on median view, two to three files of nuclei. Anucleated caudal end 15 µm long. Ragged sheath present; sheath absent from skin microfilariae.

Male

Body usually with four swellings (2.7, 3.5, 5.2, and 8.8 mm from anterior end of the allotype specimen), sometimes only two (2.4 and 8.7 mm from head). Distance from apex of testis equal to twice the length of the esophagus. Area rugosa: some 70 bands 4 µm high, distance between adjoining bands 7 µm. Caudal papillae (Fig. 7R-T): two or three pairs of precloacal papillae, pairs 5 and 6 on almost the same transverse line, pair 7 at the midlength of the tail, pairs 8 and 9 distant (20-26 µm). Cylinder slightly conical, three short lappets of similar length. Spicules: the right with dorsal heel; the left with handle 115 µm long, lamina of similar length, slender extremity.

Differential diagnosis

C. bulboidea n. sp. is distinguished from the four species described above by special features: pair 8 of

caudal papillae being far anterior to pair 9, pairs 5 and 6 at the same transverse level, the ovijector with a specialized epithelium in the posterior part, the lateral thickenings of the cuticle being more developed and salient, and striations of the cuticle not being obliquely directed in the lateral field and often interrupted. It also has more posterior cephalic papillae, and females have a bulging tail. The microfilariae are of the same size as those of *C. minuta* n. sp. but are flattened dorsoventrally.

STRUCTURE OF BODY SWELLINGS

A body swelling often appears if the vagina is voluminous; this is not a real body swelling. When present, such swellings are scattered along the first few postvulvar centimeters. Body swellings are more conspicuous ventrally and arise because of the presence of a giant ventral pseudocoelomocyte, the cytoplasm of which may contain dark pigment (*C. tumidicervicata*, Fig. 4J) or granules (*C. bulboidea*, Fig. 6J). Thus, contrary to an earlier report (Bain & Chabaud, 1988), this specialized apparatus for facilitating the copulation of the male and the female has the same structure as in the genus *Mansonella* Faust, 1929, and in *Onchocerca* species parasitic in suids (Bain *et al.*, 1993). The structures in the three genera suggest convergence.

DISCUSSION

apricornis is a member of a subfamily, Caprinae (Grubb, 1993; Hassanin et al., 1998) and the genus has been placed in the tribe Rupicaprini together with a few other genera of animals, for their adaptation to high mountains (Geist, 1987). No species of *Cercopithifilaria* have been found in the Caprinae, except for *Capricornis cripsus* in Japan, but the detection of small filarioids in the skin is unlikely unless they are specifically looked for.

RELATIONSHIPS OF *CERCOPITHIFILARIA* SPP. FROM *C. CRISPUS* WITH OTHER MEMBERS OF THE GENUS

The four new species described and *C. shohoi* (see Uni et al., 1998) have several characters that show their close relationships with *Cercopithifilaria* species parasitic in bovids and cervids, until now known only in Africa and Europe. The specialized caudal cuticular appendage in the male, here called the cylinder, the large size and regular arrangement of the caudal papillae, and their number being close to the basic 10 pairs (Chabaud & Petter, 1961) with pair 7 present, and eight head papillae symmetrically arranged are the fundamental characters also found in *C. ruandae* (Fain &

Herin, 1955), *C. dermicola* (Fain, 1977), *C. faini* (Chabaud, Landau & Petit, 1978), *C. cephalophi* Bain, Baker & Chabaud, 1982, known by the female only, and *C. rugosicauda* (Böhm & Supperer, 1953).

All of these species differ largely from the parasites from primates (Eberhard, 1980; Bain *et al.*, 1982, 1988, and 1989), lagomorphs (Bartlett, 1983), rodents (Spratt & Varughese, 1975; Bain *et al.*, 1982; Bain *et al.*, 1986), carnivores (Noè, 1911; Uni, 1983 and 1984; Almeida & Vicente, 1984; Bain *et al.*, 1987), and marsupials (Spratt & Varughese, 1975; Esslinger & Smith, 1979; Bain *et al.*, 1982). In species from these host groups, differences are the fewer head and caudal papillae, with pair 7 conserved in only a few species (*C. leporinus* Bartlett, 1983; *C. roussilhoni* Bain, Petit & Chabaud, 1986), the loss of symmetry in head and caudal papillae, migration of precloacal papillae to the cloacal opening, and reduction of the size of caudal papillae; the cuticular cylinder is not present.

Ruminants diversified during the Miocene (Lavocat, 1967), the Caprinae appearing in the Upper Miocene, according to several authors (reviewed in Hassanin *et al.*, 1998). However, whatever their origins were and geographical distributions are, ruminants seem to be parasitized by *Cercopithifilaria* spp. with a common ancestor, and these species appear to be the most primitive ones in the genus. This filarial genus probably appeared at the time when bovid and cervid expansion began, during the early Miocene.

COEXISTENCE OF *CERCOPITHIFILARIA* SPP. IN *C. CRISPUS*

The coexistence of congeneric species of endoparasites in a host occurs more frequently than was formerly thought (Durette-Desset, 1970; Schulz-Key, 1975; Grulet & Landau, 1982; Landau & Chabaud, 1994). Each example raises the question of differentiation and maintenance of closely related species in one host. Two hypotheses may explain this phenomenon. *i*) The congeneric diversity could result from species acquired, at the time of their expansion, from allied hosts, and the parasitic fauna found now reflects this ancient evolutionary stage (Inglis, 1971). *ii*) The congeneric species could have been derived from a single primitive species that diversified in the host (Chabaud & Durette-Desset, 1978).

In *C. crispus*, two morphological types of *Cercopithi-filaria* species seem to be present. *C. bulboidea* n. sp. is close to the two most primitive African species parasitic in cattle, *C. ruandae* and *C. dermicola*; the three species have the caudal pairs 7, 8, and 9 of papillae almost equally separated, and of similar size (Fig. 7S). However, the Japanese species has several specialized characteristics in the reduced number of precloacal papillae, close apposition of papillae pairs

5 and 6, lateral thickenings and cuticular striae, and the structure of the ovijector.

The four other species form another group (caudal pairs 8 and 9 close together near the tail extremity) that seems to have diversified in the Japanese serow. This diversification is shown by a particular evolutionary trend: the hypertrophy of pair 6 of the papillae, and the reduction and posterior migration of pair 7 (Fig. 5V). In this group, *C. shohoi*, with a complete set of precloacal papillae, appears to be the most primitive form, from which have been derived *C. multicauda* n. sp., *C. minuta* n. sp., an intermediary stage, and then *C. tumidicervicata* n. sp., in which this trend is more pronounced.

Between these four species and *C. bulboidea*, the morphological gap is probably only an apparent one because in the evolution of *Cercopithifilaria* spp. in African and European ruminants, pair 8 has migrated toward pair 9 (*C. faini* and *C. rugosicauda*). Therefore, it is likely that *C. bulboidea* is a primitive stage of the *Cercopithifilaria* species that have diversified in the Japanese serow.

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REFERENCES

- Almeida G.L.G. de & Vicente J.J. Cercopithifilaria bainae sp. n. parasita de Canis familiaris (L.) (Nematoda, Filarioidea). Atas Sociedade de Biologia do Rio de Janeiro, 1984, 24, 18.
- BAIN O., BAKER M. & CHABAUD A.G. Nouvelles données sur la lignée *Dipetalonema* (Filarioidea, Nematoda). *Annales* de Parasitologie Humaine et Comparée, 1982, 57, 593-620.
- BAIN O. & CHABAUD A.G. Un appareil favorisant l'accouplement des filaires : les renflements de la région antérieure

- du corps. Annales de Parasitologie Humaine et Comparée, 1988. 63, 376-379.
- Bain O., Chabaud A.G. & Georges A.J. Nouvelle filaire du genre *Cercopithifilaria*, parasite d'un carnivore africain. *Parassitologia*, 1987, 29, 63-69.
- BAIN O., PETIT G. & CHABAUD A.G. Une nouvelle filaire, Cercopithifilaria roussilhoni n. sp., parasite de l'Athérure au Gabon, transmise par tiques: hypothèse sur l'évolution du genre. Annales de Parasitologie Humaine et Comparée, 1986, 61, 81-93.
- BAIN O., WAHL G. & RENZ A. Onchocerca ramachandrini n. sp. from the warthog in Cameroon. Annales de Parasitologie Humaine et Comparée, 1993, 68, 139-143.
- BAIN O., WAMAE C.N. & REID G.D.F. Diversité des filaires du genre Cercopithifilaria chez les babouins, au Kenya. Annales de Parasitologie Humaine et Comparée, 1988, 63, 224-239.
- BAIN O., WAMAE C.N. & REID G.D.F. Description de Cercopithifilaria verveti n. sp., filaire sous-cutanée d'un cercopithèque au Kenya. Annales de Parasitologie Humaine et Comparée, 1989, 64, 42-45.
- Bartlett C.M. Cercopithifilaria leporinus n. sp. (Nematoda: Filarioidea) from the snowshoe hare (Lepus americanus Erxleben)(Lagomorpha) in Canada. Annales de Parasitologie Humaine et Comparée, 1983, 58, 275-283.
- BÖHM L.K. & SUPPERER R. Beobachtungen über eine neue Filarie (Nematoda), Wehrdikmansia rugosicauda Böhm & Supperer 1953, aus dem subkutanen Bindegewebe des Rehes. Sitzungsberichte, Abt. 1, Biologie, Mineralogie, Erdkunde und verwandte Wissenschaften, Osterreichische Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Klasse, 1953, 162, 95-103.
- CHABAUD A.G. & BAIN O. The evolutionary expansion of the Spirurida. *International Journal for Parasitology*, 1994, 24, 1179-1201.
- CHABAUD A.G. & DURETTE-DESSET M.C. Parasitisme par plusieurs espèces congénériques. Bulletin de la Société Zoologique de France, 1978, 103, 459-464.
- Chabaud A.G., Landau I. & Petit G. Deux filaires de Céphalophes au Gabon. *Annales de Parasitologie Humaine et Comparée*, 1978, *53*, 285-290.
- CHABAUD A.G. & PETTER A.J. Remarques sur l'évolution des papilles cloacales chez les nématodes phasmidiens parasites de vertébrés. *Parassitologia*, 1961, 3, 51-70.
- Durette-Desset M.C. Les systèmes d'arêtes cuticulaires chez les Nématodes Héligmosomes. IV. Description de six espèces nouvelles, parasites de Sciuridés africains : redescription d'*Heligmonina magna* Baylis, 1928 et d'*Heligmonella streptocerca* Baylis, 1928. *Cahiers de la Maboké*, 1970, 8, 41-62.
- EBERHARD M.L. Dipetalonema (Cercopithifilaria) kenyensis subgen. et sp. n. (Nematoda: Filarioidea) from African baboons, Papio anubis. Journal of Parasitology, 1980, 66, 551-554.
- ESSLINGER J.H. & SMITH J.L. *Dipetalonema (Acanthocheilonema) didelphis* sp. n. (Nematoda: Filarioidea) from opossums, with a redescription of *D. (A.) pricei* (Vaz and Pereira, 1934). *Journal of Parasitology*, 1979, 65, 928-933.

- FAIN A. Parasitisme intradermique par les nématodes chez les bovins au Rwanda. Description de deux nouvelles espèces. Annales de la Société belge de Médicine tropicale, 1977, 57, 113-120.
- FAIN A. & HERIN V. Filarioses des bovidés au Ruanda-Urundi. III. Étude parasitologique. Annales de la Société belge de Médicine tropicale, 1955, 35, 535-554.
- GEIST V. On the evolution of the Caprinae, in: The Biology and Management of Capricornis and Related Mountain Antelopes. Soma H. (ed), Croom Helm, London, 1987, 3-40.
- GRUBB P. Family Bovidae, in: Mammal Species of the World. A taxonomic and geographic reference. Wilson D.E. & Reeder D.M. (eds), Smithsonian Institution Press, Washington, 1993, 393-414.
- GRULET O., LANDAU I. & BACCAM D. Les Isospora du moineau domestique: multiplicité des espèces. Annales de Parasitologie Humaine et Comparée, 1982, 57, 209-238.
- HASSANIN A., PASQUET E. & VIGNE J.-D. Molecular systematics of the subfamily Caprinae (Artiodactyla, Bovidae) as determined from cytochrome b sequences. Journal of Mammalian Evolution, 1998, 5, 217-236.
- INGLIS W.G. Speciation in parasitic nematodes, in: Advances in Parasitology. Dawes B. (ed), Academic Press, London, 1971, 9, 185-223.
- LANDAU I. & CHABAUD A.G. Plasmodium species infecting Thamnomys rutilans: a zoological study, in: Advances in Parasitology. Baker J.R. & Muller R. (eds), Academic Press, London, 1994, 33, 49-90.
- LAVOCAT R. *Histoire des Mammifères*. Edition du Seuil, Paris, 1967, 190 pp.
- Noè G. Contribuzione alla sistematica ed alla anatomia del genera Filaria. 1. La Filaria grassii (Noè, 1907). Ricerche del Laboratorio di Anatomia Normale della Regia Università di Roma, (1910) 1911, 235-252.
- 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.
- SPRATT D.M. & VARUGHESE G. A taxonomic revision of filarioid nematodes from Australian marsupials. Australian Journal of Zoology, 1975, Suppl. ser., 35, 1-99.
- UNI S. Filarial parasites from the black bear of Japan. Annales de Parasitologie Humaine et Comparée, 1983, 58, 71-84.
- UNI S. Note on *Dipetalonema (Chenofilaria) japonica* Uni, 1983 from Japanese black bear. Supplementary description. *Annales de Parasitologie Humaine et Comparée*, 1984, 59, 531-534.
- UNI S., SUZUKI Y. & KATSUMI A. Cercopithifilaria shohoi n. sp. (Nematoda: Filarioidea) from the relict Bovidae, Capricornis crispus, in Japan. Parasite, 1998, 5, 119-126.

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