

## **CERCOPITHIFILARIA SHOHOI N. SP. (NEMATODA: FILARIOIDEA) FROM THE RELICT BOVIDAE, CAPRICORNIS CRISPUS, IN JAPAN**

UNI S.\*, SUZUKI Y.\*\* & KATSUMI A.\*\*\*

### **Summary:**

*Cercopithifilaria shohoi* n. sp. was found in the relict bovid, *Capricornis crispus*, in Japan, and is described and compared with other species in the genus. Adult male and female worms were found in subcutaneous tissues of the trunks of ♂ serows shot in Mt. Zao, Yamagata Prefecture, in the northern part of Honshu, the largest island of Japan. The one complete male found was 19.7 mm long, and the five females were 31.6-50.9 mm long. Unsheathed or sheathed microfilariae 104-122 µm long were taken from the females. One microfilaria was found in the sediment of the preservation solution of the tissues, but none were found in the blood of the infected serows, so microfilariae may be limited to the skin. Males of this species had one pair of papillae between perianal and subterminal groups of caudal papillae. In having this intermediate pair, *C. shohoi* n. sp. resembled species such as *C. faini* from an African bovid and *C. rugosicauda* from a European deer. From its morphological characteristics, *C. shohoi* n. sp. seems to be one of the more primitive species in the genus *Cercopithifilaria*.

**KEY WORDS :** Nematoda, Filarioidea, *Cercopithifilaria shohoi* n. sp., Bovidae, *Capricornis crispus*, Japan.

**Résumé :** *CERCOPITHIFILARIA SHOHOI* N. SP. (NEMATODA : FILARIOIDEA) CHEZ LE BOVIDÉ RELIQUÉ *CAPRICORNIS CRISPUS*, AU JAPON

*Cercopithifilaria shohoi* n. sp. retrouvé chez le bovidé reliqué *Capricornis crispus*, au Japon, est décrit et comparé aux autres espèces du genre. Des vers adultes mâles et femelles ont été trouvés dans les tissus sous-cutanés du tronc de ♂ serows du Japon abattus au Mont Zao, dans la Préfecture de Yamagata, dans la région nord du Honshu, la plus grande île du Japon. Le seul mâle complet qui ait été trouvé mesurait 19,7 mm de long, et les cinq femelles mesuraient de 31,6 à 50,9 mm de long. Des microfilaries dégainées ou gainées de 104 à 122 µm de long ont été relevées sur les femelles. Une microfilaire a été trouvée dans le sédiment de la solution de conservation des tissus, mais aucune n'a été trouvée dans le sang des serows infectés; ainsi, il semblerait que les microfilaries résident uniquement dans la peau. Les mâles de ce genre avaient une paire de papilles entre les groupes périanal et subterminal des papilles caudales. Du fait de cette paire intermédiaire, *C. shohoi* n. sp. ressemble à des espèces comme *C. faini* d'un bovidé africain et *C. rugosicauda* du cerf européen. D'après ses particularités morphologiques, *C. shohoi* n. sp. semble être une espèce relativement primitive du genre *Cercopithifilaria*.

**MOTS CLÉS :** Nematoda, Filarioidea, *Cercopithifilaria shohoi* n. sp., Bovidae, *Capricornis crispus*, Japon.

## INTRODUCTION

The genus *Cercopithifilaria* was named originally as a subgenus by Eberhard (1980) for a parasite of an African primate, and emended

as a genus by Bain *et al.* (1982). Bain *et al.* (1982) described many species of the genus *Cercopithifilaria* in Africa, and placed some species of *Dipetalonema* and other genera into the genus *Cercopithifilaria*. Host animals, found throughout the world, range from marsupials to primates. Chabaud & Bain (1994) suggest that the means by which the genus *Cercopithifilaria* underwent evolutionary expansion is unusual. The purpose of this report is to describe *Cercopithifilaria shohoi* n. sp. from an ungulate, the Japanese serow (*Capricornis crispus*), a relict Bovidae, Rupicaprinae, and to identify the relationship of the species to other species among the genus in terms of morphological features, geographic distribution, and evolution. This species is the second in this genus to be reported in Japan; the first was *C. japonica* (Uni, 1983) from black bears.

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

\*\* Laboratory of Veterinary Anatomy, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan.

\*\*\* Animal Husbandry Research Center, Yamagata-ken Agricultural Co-op, Yamagata 990-0894, Japan.

Correspondence: S. Uni.

Tel: +81-6-645-2066 - Fax: +81-6-646-3590.

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

## MATERIALS AND METHODS

Japanese serows (*Capricornis crispus*) seem to be one of the most primitive species of bovids (Imazumi, 1966). Serows inhabit mountains higher than 1,000 m. Thirty-one Japanese serows were killed within a three-week period (February 16, February 23, and March 2) in 1997 on Mt. Zao (1,841 m) in Yamagata Prefecture in the northern part of Honshu, the main island of Japan, in accordance with the policy of the Agency of Cultural Affairs, Japan, concerning the conservation and control of Japanese serows.

Serows shot in the mountains were immediately transferred to the Animal Husbandry Research Center in Yamagata City and necropsied by members of the staff of the Japan Wildlife Research Center (Tokyo). Tissues in which parasites were seen were stored in 2 % formalin in saline, and later the worms were removed from the tissues under a dissection microscope. Blood smears were made from samples from each animal and stained with Giemsa's solution, but skin snips were not made.

One microfilaria found in the sediment of the preservation solution for tissues was stained with Giemsa's solution. Microfilariae were taken from the terminal part of the uterus of some of the preserved adult females. Part of the midbody of a female worm was embedded in 2 % agar in phosphate-buffered saline, sectioned, and stained with hematoxylin and eosin. Drawings of the worms were made with a camera lucida. Three female worms were studied by scanning electron microscopy (SEM) by methods reported previously (Uni, 1983). The ages of the serows were estimated by researchers at the Japan Wildlife Research Center, who counted the rings on the horns. Sixteen ticks were collected from the serows, and kept alive in a plastic tube containing a small piece of wet filter paper.

All parts of three Japanese serows shot between December 1983 and March 1984 in Gifu Prefecture of the central part of Honshu were preserved in 10 % formalin, and connective tissues were examined for parasites on April 9, 1997.

## DESCRIPTION

### *CERCOPITHIFILARIA SHOHOI* N. SP.

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

Small, slender nematodes inhabiting subcutaneous connective tissues. Anterior end slightly bulbous (Fig. 1); eight circumoral papillae on cephalic plate elongated along lateral axis: two laterodorsal and two lateroventral at corners of cephalic plate in outer circle, and four small papillae in inner circle. Amphidial pores at lateral side (Fig. 2). Small, refractile, pre-esophageal cuticular ring at the anterior end (Fig. 3). Esophagus short, simple, and not divided into anterior muscular and posterior glandular portions (Fig. 1). Cuticle with transverse striations. Tail of female terminated with three conical protuberances (Fig. 4). Caudal papillae of male at perianal area, area between anus and subterminal end, and subterminal area (Fig. 5). Many microfilariae from uteri were unsheathed, but some were sheathed. Parasites of Japanese serows.

### MALE

(One complete specimen and two posterior parts): Body 19.7 mm long, midbody 102-122 (mean, 115)  $\mu$ m wide. Nerve ring 104  $\mu$ m from anterior end. Esophagus 510  $\mu$ m long. Ratio of esophageal length to body length 2.6 %. Tail 166-172 (169)  $\mu$ m long, tapered, with three conical protuberances (Fig. 5).

Perianal papillar group: Single, mid-ventral, sessile papilla immediately anterior to anus; one pair of small papillae immediately posterior to anus; four pairs of pedunculate papillae lateral to anus.

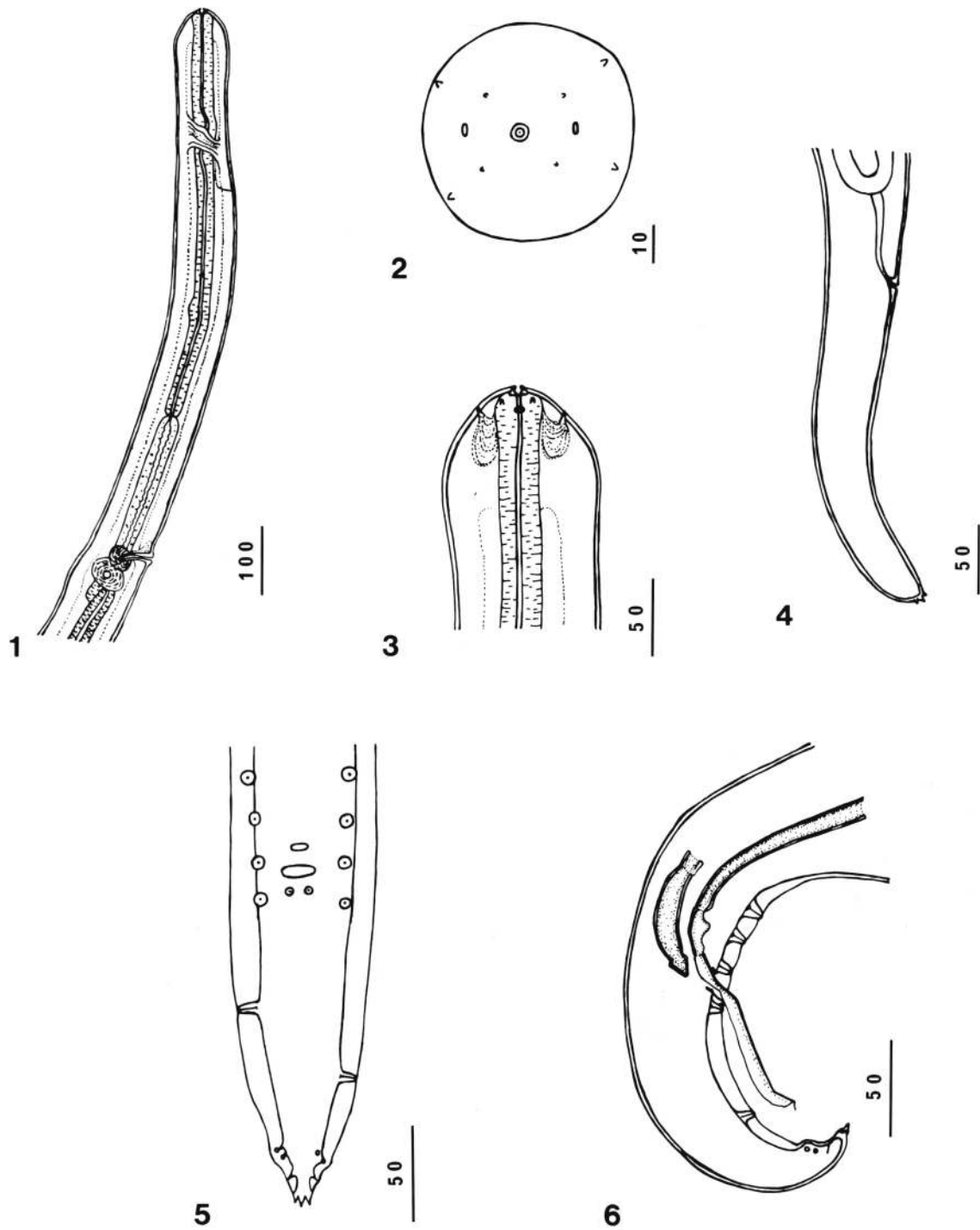
Intermediate group: One pair of pedunculate papillae located subventrally approximately halfway between anus and posterior end.

Subterminal group: Two pairs of semipedunculate papillae and one pair of phasmidial pores present just anterior to posterior end.

Caudal end terminated in a cuticular cone and two cuticular petaloid appendages. Narrow caudal alae present. Spicules were dissimilar in structure and of unequal lengths (Fig. 6); left spicule 257-286 (269)  $\mu$ m long, divided into shaft and lamina; right spicule with bulbous end 68-70 (69)  $\mu$ m long; spicule ratio 3.8-4.1 (3.9). Gubernaculum absent. Area rugosa present at coiled caudal region of the males. Raised transverse bands numbering 117-124 (121) each with longitudinal striations found on the ventral surface, extending from the anterior border 1,404-1,560 (1,482)  $\mu$ m from the anus to the posterior border 156-338 (247)  $\mu$ m from the anus.

### FEMALE

(Five complete specimens, two anterior parts, and three posterior parts): Body 31.6-50.9 (41.4) mm long, midbody 112-158 (141)  $\mu$ m wide. Microfilariae were in the uteri at the midbody level (Fig. 7). The small round mouth was surrounded by elevated cuticle when viewed by SEM (Figs. 8 and 9). Nerve ring 192-245



Figs. 1-6. — *Cercopitthifilaria shoboi* n. sp. 1. Female anterior end; lateral view. 2. En face view of head of female. 3. Enlarged anterior end; lateral view. 4. Female posterior end; lateral view. 5. Male posterior end; ventral view. 6. Male posterior end; lateral view; Scale bar, micrometers.

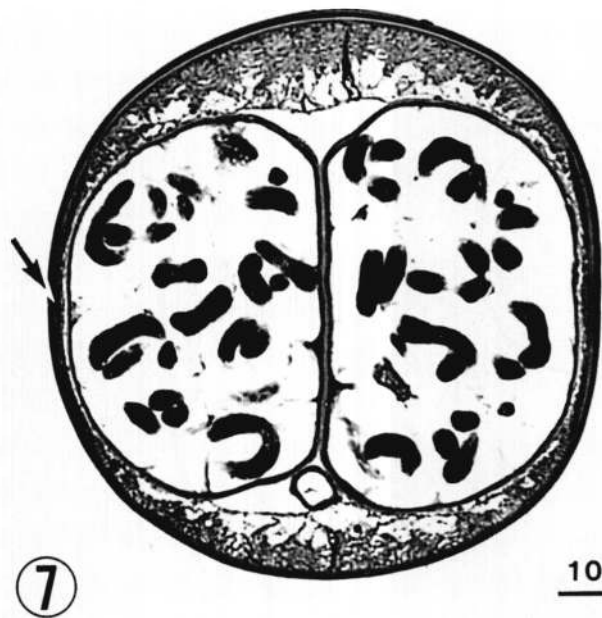


Fig. 7. — *Cercopithifilaria shoboi* n. sp.; stained cross-section of mid-body of female. Lateral internal thickening of the cuticle (arrow). Scale bar, micrometers.

(220)  $\mu\text{m}$  from anterior end (Fig. 1). Esophagus 632-847 (708)  $\mu\text{m}$  long. Ratio of esophageal length to body length 1.7-2.3% (1.7%). Vulva 643-999 (830)  $\mu\text{m}$  from anterior end, at a postesophageal position. Cuticle with fine transverse striations at intervals of 0.4-1.3  $\mu\text{m}$  on part of the midbody (Fig. 10). Tail 203-294 (245)  $\mu\text{m}$  long, tapered toward tip, curved ventrad, with three conical protuberances (Fig. 11).

#### MICROFILARIAE

(15 specimens from terminal part of uteri of females): 104-122 (109)  $\mu\text{m}$  long by 5-10 (7)  $\mu\text{m}$  wide. Delicate sheath was found on some microfilariae (Fig. 12), but many microfilariae at the terminal part of the uterus were unsheathed (Fig. 13). Anterior end bluntly rounded, and refractile apparatus at head of the cephalic space. The posterior part after the phasmids tapered sharply (Fig. 13, arrow). The microfilariae were flattened dorsoventrally.

One microfilaria taken from the sediment of the preservation solution matched the microfilariae taken from female specimens of *C. shoboi* n. sp. (Fig. 14). Microfilariae were not found in the blood smears of the infected serows or 20 histological sections of connective tissues. Four large ticks (*Haemaphysalis* sp.) were selected and dissected three weeks later, but larvae of filarial parasites were not found.

This description was based on the specimens from Yamagata Prefecture. The parasites were found in six serows all 0.5-9.5 years old, although the serows examined were up to 20 years of age.

Specimen from Gifu Prefecture: One female specimen was found in the connective tissue of a hind leg of a Japanese serow. Body 49.0 mm long, midbody 143  $\mu\text{m}$  wide. Nerve ring 247  $\mu\text{m}$  from anterior end. Esophagus 969  $\mu\text{m}$  long. Ratio of esophageal length to body length 2.0%. Vulva 1,255  $\mu\text{m}$  from anterior end. Tail 234  $\mu\text{m}$  long, with three conical protuberances. Microfilariae (five specimens from terminal part of uterus): 109-117 (114)  $\mu\text{m}$  long, and 5-8 (7)  $\mu\text{m}$  wide. They were unsheathed.

Morphological characteristics, including pre-esophageal cuticular ring and undivided esophagus of the female, and the measurements of this female and the microfilariae corresponded with those of the specimens from Yamagata Prefecture described above.

Type host: *Capricornis crispus* (Temminck, 1845).

Location in host: Subcutaneous connective tissues of trunk.

Type locality: Mt. Zao, Yamagata Prefecture, Japan.

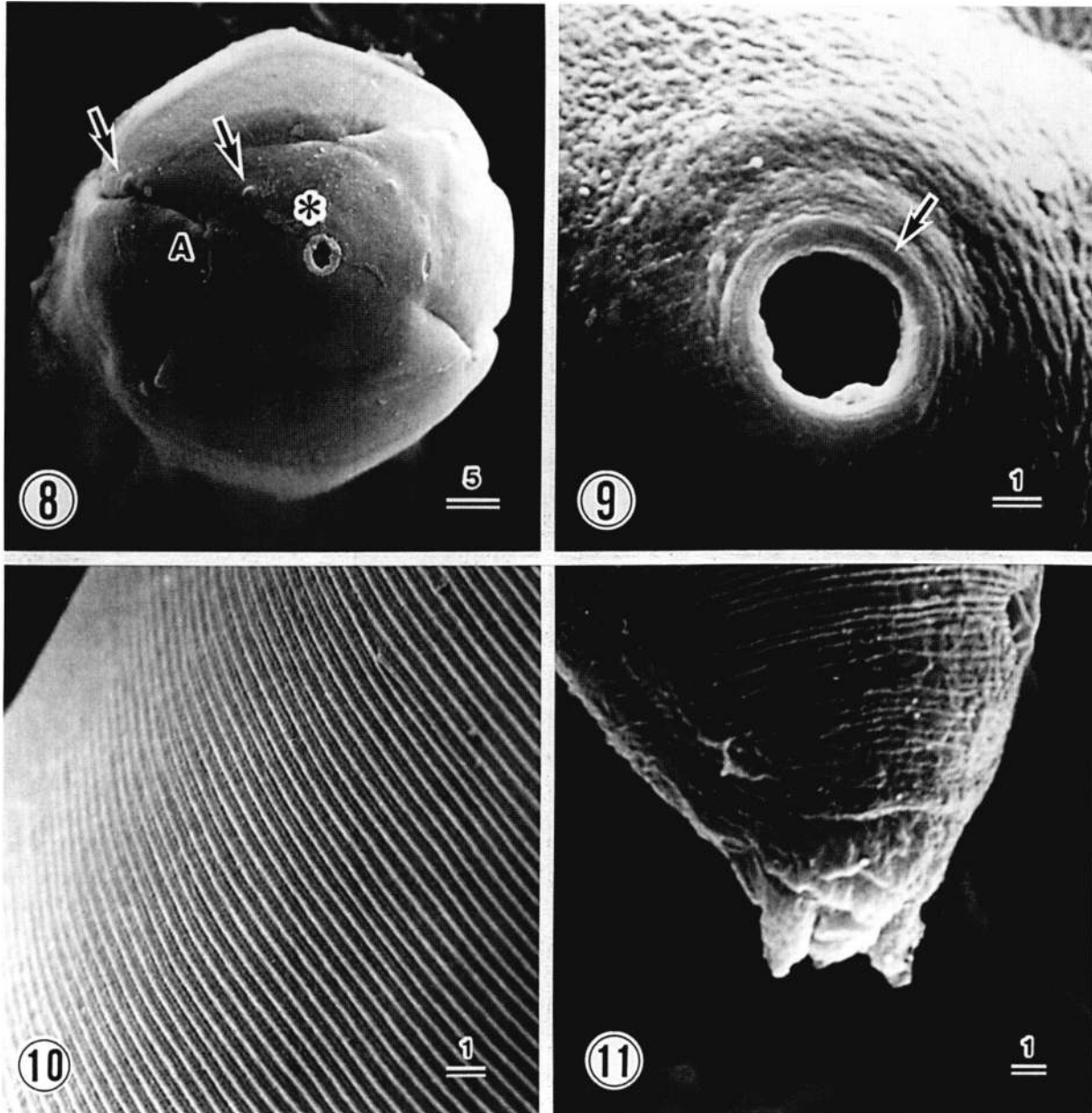
Type specimens: Holotype (male), Museum National d'Histoire Naturelle, Paris, No. 318 SE; allotype (female) at the same place. Other specimens have been deposited in the Department of Medical Zoology, Osaka City University Medical School.

Etiology: In honor of Dr. Chuzaburo Shoho, Japanese parasitologist.

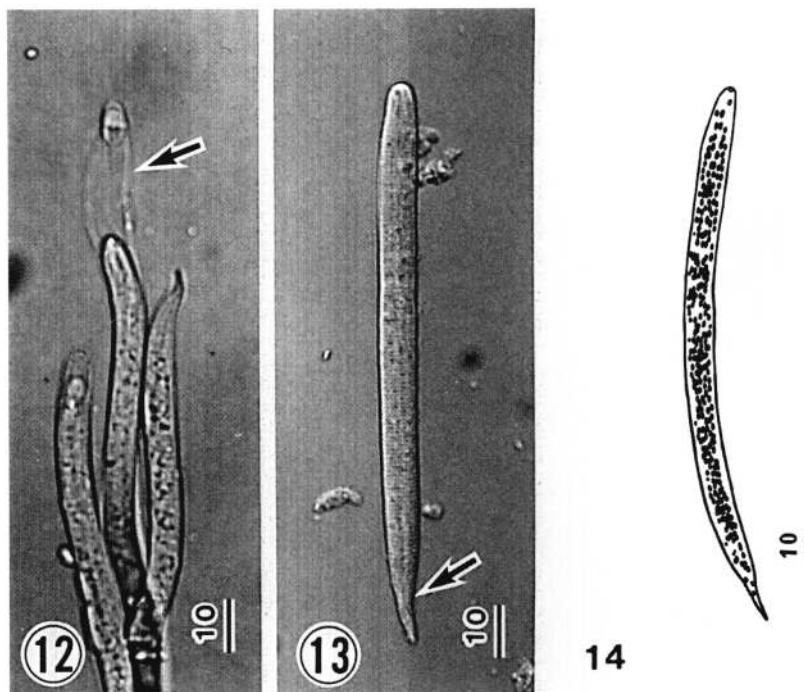
## DISCUSSION

*Cercopithifilaria shoboi* n. sp. was found in Japanese serows (*Capricornis crispus*) in Yamagata and Gifu Prefectures in the northern and central parts of Honshu. The many caudal papillae of the males and the somewhat long esophagus of the females, which are characteristics key to the classification of the species, seemed to be primitive for species in this genus.

According to Bain *et al.* (1982) and Chabaud & Bain (1994), 22 species have been placed in this genus: (a) 12 species from Africa; *C. kenyensis* Eberhard, 1980, *C. degraaffi* Bain *et al.*, 1982, *C. narokensis* Bain *et al.*, 1988, *C. eberhardi* Bain *et al.*, 1988, and *C. verveti* Bain *et al.*, 1989 from primate species, *C. ruandae* (Fain & Herin, 1955), *C. dermicola* (Fain, 1977), *C. faini* (Chabaud *et al.*, 1978), and *C. cephalophi* Bain *et al.*, 1982 from ungulates, *C. gabonensis* Bain *et al.*, 1982 and *C. roussilboni* Bain *et al.*, 1986 from a rodent, and *C. corneti* Bain *et al.*, 1987 from a carnivore; (b) five species from North and South America; *C. leporinus* Bartlett, 1983 from a hare, *C. didelphis* (Esslinger & Smith, 1979) from marsupials, *C. grassii* (Noé, 1907) (cited in Bain *et al.*, 1982; Almeida & Vicente, 1982) and *C. baina* Almeida & Vicente, 1984 from a carnivore, and *C. venezuelensis* (Eberhard



Figs. 8-11. — SEM of *Cercopithifilaria shohoi* n. sp. 8. En face view of head of female. The mouth is surrounded by an elevated cuticular ring (\*). Papillae (arrows), A; amphid. 9. Enlarged mouth opening with cuticular ring (arrow) of another female specimen. 10. Transverse striations on cuticle of midbody of female. 11. Posterior end of female with three conical protuberances. Scale bar, micrometers.



Figs. 12-14. — Microfilariae of *Cercopithifilaria shoboi* n. sp. 12. Delicate sheath of anterior part of microfilaria taken from female (arrow). 13. Unsheathed microfilaria taken from female, with sharply tapered tail end (arrow). 14. Microfilaria stained with Giemsa's solution. Scale bar, micrometers.

*et al.*, 1993) from armadillos (Edentata), (c) two species from Europe; *C. grassii* from a carnivore and *C. rugosicauda* (Böhm & Supperer, 1953) from an ungulate, (d) two species from Asia; *C. laemmleri* (Dasgupta *et al.*, 1978) from a rodent, and *C. japonica* (Uni, 1983) from a carnivore, and (e) two species from Australia; *C. johnstoni* (Mackerras, 1954) from marsupials and rodents and *C. pearsoni* (Spratt & Varughese, 1975) from a marsupial. *C. baina* was named only on the basis of morphological differences of the microfilariae from those of *C. grassii*, and the drawing was not published in the original paper (Almeida & Vicente, 1984). *C. venezuelensis* was reported as a new genus, *Strianema* (Eberhard *et al.*, 1993), but it was later placed in the genus *Cercopithifilaria* (Chabaud & Bain, 1994).

*C. shoboi* n. sp. can be distinguished from these species from these characteristics: the length and features of spicules, numbers and arrangement of caudal papillae of males, lengths of microfilariae and adults, features of the posterior ends of females and males, and the relation of the position of the vulva to the esophageal end. By light microscopy, longitudinal striations were seen under or on the cuticle, but by SEM, the cuticle was striated only transversely. The cuticle with fine transverse striations of *C. shoboi* n. sp. seemed to be slightly different from that with thicker striations of *C. venezuelensis* (Eberhard *et al.*, 1993), but *C. shoboi* n. sp. differed more clearly in having both a pre-esophageal cuticular ring and an area rugosa, and in the microfilariae not having a filamen-

tous tail. From the morphological characteristics of a pre-esophageal cuticular ring and undivided esophagus, the species described in our report belongs in the genus *Cercopithifilaria*. *C. japonica* in black bears (*Ursus thibetanus*) in Japan has been described (Uni, 1983, 1984, Uni *et al.*, 1995), and can be distinguished from *C. shoboi* n. sp. by its large microfilariae (about four times longer), the dilatation of the anterior part of the female, and the few papillae of the male.

Of the five species found in ungulates, *C. shoboi* n. sp. may be closely related to *C. faini* (Chabaud *et al.*, 1978), to judge from the arrangement of caudal papillae and the bulbous end of the right spicule (both in males), but can be distinguished by its shorter microfilariae and by the three conical projections of the tail end of the male. The resemblance of the two parasites may reflect the historical relationship between Asian and African bovinds. *C. shoboi* n. sp. differs slightly from *C. ruandae* (Fain & Herin, 1955) in its short microfilariae and the small subterminal papillae of the male. *C. shoboi* n. sp. may be similar to *C. rugosicauda* (Böhm & Supperer, 1953) in the arrangement of caudal papillae of the male, but differs in its short spicules, short microfilariae, less dilatated anterior part of the female, and postesophageal position of the vulva. *C. shoboi* n. sp. differs from *C. cephalophi* Bain *et al.*, 1982 in the shorter esophagus of the females, the postesophageal position of the vulva, and the shorter microfilariae. *C. shoboi* n. sp. differs from *C. dermicola* in its longer females and shorter microfilariae (Fain, 1977).

One pair of papillae was seen between the perianal and subterminal groups of papillae in the males of *C. shoboi* n. sp. Among species of the genus *Cercopithifilaria*, such papillae were described in the species from ungulates, a hare, a rodent, and armadillos: *C. rugosicauda* (Böhm & Supperer, 1953), *C. ruandae* (Fain & Herin, 1955), and *C. faini* (Chabaud *et al.*, 1978); *C. leporinus* in Canada (Bartlett, 1983); *C. roussilboni* from a porcupine (Bain *et al.*, 1986); and *C. venezuelensis* (Eberhard *et al.*, 1993). *C. shoboi* n. sp. from an ungulate seems to belong on this list. Intermediate papillae have not been found in species from primates, carnivores, a rodent, and marsupials: *C. kenyensis*, *C. eberhardi*, and *C. narokensis* from African primates (Eberhard, 1980; Bain *et al.*, 1988); *C. grassii*, *C. japonica*, and *C. corneti* from carnivores (Almeida & Vicente, 1982; Uni, 1984; Bain *et al.*, 1987); *C. gabonensis* from a rodent (Bain *et al.*, 1982); and *C. pearsoni* and *C. didelphis* from marsupials (Spratt & Varughese, 1975; Esslinger & Smith, 1979). *C. johnstoni* belongs in this group if the specimen shown in Spratt & Varughese (1975) is referred to, but papillae near the subterminal group were present as well in the specimens shown in Mackerras (1954) and Spratt & Varughese (1975).

Species with papillae intermediate in position in the males had females that seemed to have slightly higher ratios of esophageal length to body length than those of species without such papillae, when calculated from measurements given in the original texts. Species with papillae in this position had the following ratios: *C. venezuelensis*, 3.3 % (Eberhard *et al.*, 1993); *C. leporinus*, 2.4-2.6 % (Bartlett, 1983); *C. ruandae*, 2.4 % (Fain & Herin, 1955); *C. rugosicauda*, 2.2-2.4 % (Böhm & Supperer, 1953); *C. faini*, 1.5 % (Chabaud *et al.*, 1978); and *C. roussilboni*, 1.1 % (Bain *et al.*, 1986). *C. shoboi* n. sp. had the ratio of 1.7-2.3 %. Species without papillae in this position had the following ratios: *C. corneti*, 2.4 % (Bain *et al.*, 1987); *C. japonica*, 2.0 % (Uni, 1983), and 2.3 % (Uni, 1984); *C. eberhardi*, 1.9 % (Bain *et al.*, 1988); *C. didelphis*, 1.4 % (Esslinger and Smith, 1979); *C. kenyensis*, 1.4 % (Eberhard, 1980); *C. narokensis*, 1.3 % (Bain *et al.*, 1988); *C. gabonensis*, 0.8 % (Bain *et al.*, 1982); *C. johnstoni*, 0.6-0.8 %, and *C. pearsoni*, 0.4 % (Spratt & Varughese, 1975). Variation was found, but in general, species with such papillae had high ratios (2.0-3.3 %); many species without such papillae had lower ratios (0.4-1.9 %). A higher ratio means that the esophagus of that species was less atrophied than one with a lower ratio. The esophagus of *C. shoboi* n. sp. seemed to be only slightly atrophied for a species in this genus.

With regard to the evolution of *Onchocerca* species from Japanese serows (Yagi *et al.*, 1994), *O. suzukii* Yagi *et al.*, 1994, with its stout esophagus, is included in the primitive group found in tropical domestic

cattle, Palearctic Cervidae, Nearctic Cervidae, and Antilocapridae. *O. skrjabini* Rukhlyadev, 1964 has an atrophied esophagus, and is among the more evolved members of the genus. Of the various specimens of *O. skrjabini*, Japanese specimens reported by Yagi *et al.* (1994) and Suzuki *et al.* (1997) have primitive characteristics: longer esophagus, more anterior vulva, and less atrophied caudal papillae than European specimens from red deer, *Cervus elaphus* (Bain & Schulz-Key, 1974).

If structures of *Onchocerca* species being complex means that the species are primitive (Yagi *et al.*, 1994), the same pattern may apply to other genera, including *Cercopithifilaria*. Eberhard *et al.* (1993) have pointed out that several species assigned to the genus do not share the morphological features described for *C. kenyensis*. We suggest that the species of the genus *Cercopithifilaria* be divided into two groups; primitive members with intermediate caudal papillae and less atrophied esophagus and evolved members without such papillae and with an atrophied esophagus. By this definition, *C. shoboi* n. sp. is another primitive member of the genus. That *C. shoboi* n. sp. was found in a modern (bovid) host rather than in hosts such as porcupines or marsupials supports the hypothesis of Chabaud (1981) and Bain *et al.* (1986, 1988) that the evolution of hosts and nematode parasites is not in parallel in this genus. Larvae of *C. shoboi* n. sp. were not found in ticks taken from Japanese serows in this study, but investigation of the vector of this species may confirm the suggestion of Chabaud & Bain (1994) that the means of evolutionary expansion of the genus *Cercopithifilaria* is unusual in depending not on the definitive host (as is usual for nematode parasites of vertebrates) but rather on the vector.

#### ADDENDUM

While this manuscript was at the publisher's, a survey of serow parasites was done (on 22 February 1998). Microfilariae of *C. shoboi* n. sp. were found alive in skin snips of three of the seven serows examined. The microfilariae were unsheathed, 107-112  $\mu\text{m}$  long, and 5-8  $\mu\text{m}$  wide. No microfilariae were found in blood smears of the animals with microfilariae in the skin.

#### ACKNOWLEDGEMENTS

We thank Yamagata City for official permission to study serows, and Drs. A. Kimura, T. Nakashima, and S. Hirata of the Japan Wildlife Research Center, Tokyo, for their cooperation. We thank Dr. C. Shoho for his encouragement during

this study, and Ms. C. Latta for reading the manuscript. Dr. O. Bain, Museum National d'Histoire Naturelle, Paris, kindly arranged for the accessioning of the type specimens.

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Reçu le 10 septembre 1997  
 Accepté le 15 décembre 1997