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Prevalence of Nematodes in the Asiatic Black Bear, *Ursus thibetanus*, in Central Honshu, Japan, with an Amended Description of *Cercopithifilaria japonica* [syn. *Dipetalonema* (*Chenofilaria*) *japonica*]

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Abstract

Forty-five Asiatic black bears, *Ursus thibetanus*, shot in mountaineous areas of Gifu, Fukui, and Shiga Prefectures in central Honshu, the main island of Japan, between April 1991 and March 1994 were examined for nematode parasites. Three species of nematodes were found: *Baylisascaris transfuga* (Rudolphi, 1819) was found in 16 (39%) of 41 bears; *Cercopithifilaria japonica* (Uni, 1983) n. comb. [syn. *Dipetalonema* (*Chenofilaria*) *japonica* Uni, 1983] was found in two (4%) of 45 bears, and *Dirofilaria ursi* Yamaguti, 1941 was found in 22 (49%) of 45 bears. This is the first report on the prevalence of nematodes in black bears of central Honshu, Japan. The microfilariae taken from the uterine cavity of *Dipetalonema* (*Chenofilaria*) *japonica* females were sheathed. A close morphological examination of adults and microfilariae of *D.* (*C.*) *japonica* suggests that this filaria should be placed in the genus *Cercopithifilaria*.

Key words: Nematoda; *Baylisascaris transfuga*; *Cercopithifilaria japonica*; *Dirofilaria ursi*; Asiatic black bear; Japan.

Introduction

Three species of filarial nematodes have been found in the Asiatic black bear in Japan (Yamaguti, 1941; Uni, 1983; Yokohata *et al.*, 1990). One of the species, *Dirofilaria ursi* Yamaguti, 1941, has attracted attention because it is suspected to cause subcutaneous dirofilariasis in humans (Uni *et al.*, 1980; Beaver *et al.*, 1987). However, detailed information on the prevalence of this filaria in Japanese bears has not been available. The objective of this paper is to present the prevalence of filariae and other helminths in bears in central part of Honshu, Japan. In addition, morphological and taxonomical remarks are made about *Cercopithifilaria japonica* (Uni, 1983) (syn. *Dipetalonema japonica*), one of the other filarial species known to parasitize bears.

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Materials and Methods

Forty-five Asiatic black bears were shot between April 1991 and March 1994 for research projects on the ecology and diseases of the wild animals in Gifu, Fukui, and Shiga Prefectures in central Honshu (Fig. 1). The viscera of the black bears (the tongue, pharynx, esophagus, trachea, lungs, heart, stomach, diaphragm, intestines, mesentery, kidneys, and accessory adipose tissues) were collected from 26 bears in Gifu Prefecture, 14 bears in Fukui Pref., and 5 bears in Shiga Pref., and examined under a dissecting microscope for nematodes. The filarial parasites

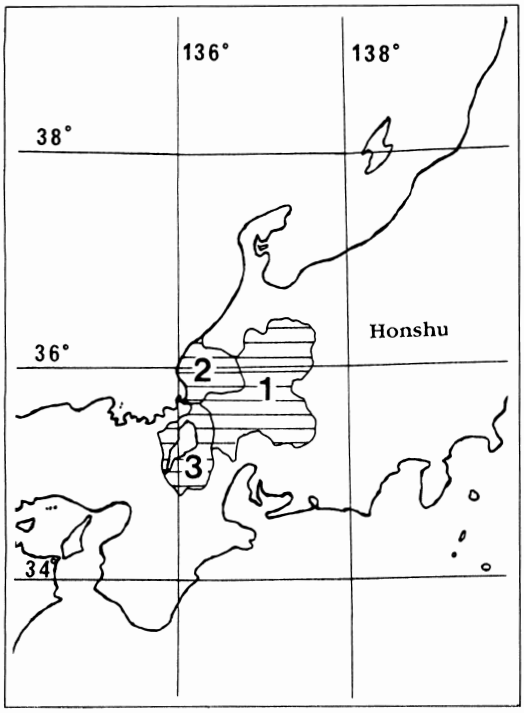


Fig. 1 Research areas for nematode parasites of Asiatic black bears in central Honshu, Japan. Gifu (1), Fukui (2), and Shiga (3) Prefectures.

obtained were fixed and stored in 2% formalin in saline (Uni, 1983), and other nematodes were fixed and stored in 10% formalin. The identifications of these parasites were made on the basis of the differentiating features described by Sprent (1968) for *Baylisascaris* spp., and by Anderson and Bain (1976) and Bain *et al.* (1982) for filarial parasites. One female of *C. japonica* was used for the *camera lucida* drawing, and an anterior fragment of another female was fixed, placed into 2% agar, embedded in paraffin, sectioned by routine histological procedures, and stained with hematoxylin and eosin.

In addition, samples of the diaphragm and tongue from 17 of these bears were examined for *Trichinella spiralis* larvae by compression and digestion as follows. Small pieces of tissue totaling 1 g were compressed between two glass slides and observed under a compound microscope with magnification of ×40. Separately, 5-g samples of tissue were minced and digested with artificial gastric juice made of pepsin (Wako Pure Chemical Industries, Osaka) and HCl, and the sediment was exmained for larvae under the low-power (×4) objective of a compound microscope as described elsewhere (Ash and Orihel, 1987).

Results

Three nematode species, *Baylisascaris transfuga* (Rudolphi, 1819), *Cercopithifilaria japonica* (Uni, 1983), and *Dirofilaria ursi* Yamaguti, 1941, were found. Their prevalence and intensity are listed in Table 1. *B. transfuga* was often found in the jejunum, but sometimes found in the stomach. One gravid female *C. japonica* was collected from perirenal adipose tissue and an anterior fragment of another female was found in the connective tissue around the esophagus. *D. ursi* was found most often in the perirenal adipose tissue or connective tissue around the trachea and pharynx. *Trichinella spiralis* larvae were not detected.

The gravid female *C. japonica* (38 mm long, and 173 μm wide at midbody) had a preesophageal cuticular ring and an undivided esophagus (Fig. 2). The nerve ring was 306 μm from the anterior extremity, the esophagus was 714 μm long, and the vulva was 969 μm from the anterior extremity. In a stained cross-section of the anterior portion near the vulva (Fig. 3), the cuticle was smooth and 2.1 to 2.6 μm thick. Interior projections of the cuticle were found at both lateral chords, which were not thick but 83 to 88 μm wide. The somatic muscle cells, which were 5 to 18 μm thick, contained large contractile muscle fibers. The intestine was small, and four uterine tubes with microfilariae filled the

Table 1 Prevalence and intensity of nematode parasites in the Asiatic black bears, *Ursus thibetanus*, in Gifu, Fukui, and Shiga Prefectures in central Honshu, Japan (1991–1994)

Parasite	Prevalence*	Intensity†
Baylisascaris transfuga	39% (16/41‡)	1–35 (9)
Cercopithifilaria japonica	4 (2/45)	1
Dirofilaria ursi	49 (22/45)	1–15 (2)

* No. of bears infected/No. examined.
† No. of parasites per bear. Range, with mean in parentheses.
‡ Intestines from 4 bears were not available.

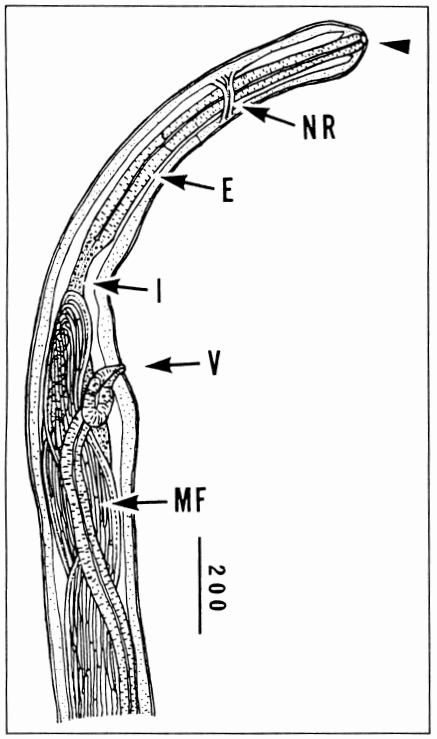


Fig. 2 *Cercopithifilaria japonica*. Anterior part of female showing a preesophageal cuticular ring (arrowhead). E, esophagus; I, intestine; MF, microfilariae; NR, nerve ring; V, vulva. Units of the scale, micrometers.

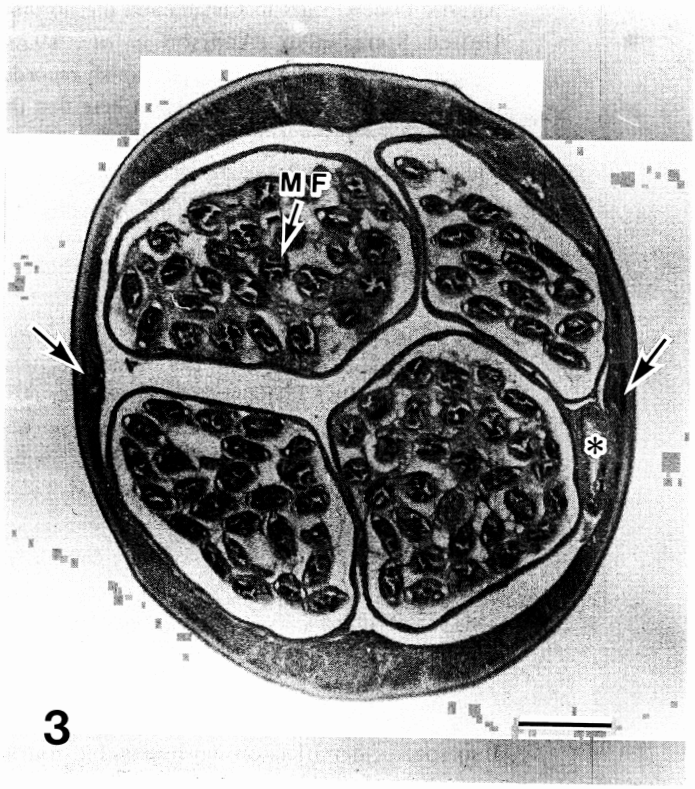
pseudocoelom. Microfilariae taken from the uterine cavity near the vulva were sheathed, 364 to 416 μm long, and 13 to 16 μm wide (Fig. 4). Refractile granules were found in the anterior or posterior portion (or both) of the sheath (Fig. 5). The microfilariae had a tail with the end flattened dorsoventrally (Figs. 6 and 7).

Discussion

Dirofilaria ursi was the most prevalent parasite in the black bears in central Honshu. This nematode is common also in American black bears in New Brunswick, Canada, where the prevalence is 33.7% (Duffy *et al.*, 1994). Because *Baylisascaris transfuga* has been found in an Asiatic black bear in Akita, northern Honshu (Uni *et al.*, 1981), it seems to be widespread in the bears inhabiting Honshu. This parasite infects bears in Canada and the northern United States also (Addison *et al.*, 1978). *Cercopithifilaria japonica* was previously recorded in Akita (Uni, 1983), and we report here that this parasite is widespread in central Honshu, as well. The prevalence of this filaria was only 4%. However, the prevalence might have been found to be higher if whole carcasses could have been examined, because the parasite inhabits subcutaneous tissue as well as the adipose tissue around the visceral organs of bears (Uni, 1984). *Mansonella* (*Mansonella*) *akitensis* (Uni, 1983), another filarial species identified in black bears of Akita (Uni, 1983), was not found in this study; the parasite may have a limited geographical distribution, or more likely, the organs and tissues being provided separately made it difficult to detect.

The incidence of cases of zoonotic filarial infections in humans is increasing, and species of the genus *Dipetalonema* as well as the genus *Dirofilaria* may be causative agents in Japan (Ando *et al.*, 1985; Yazaki *et al.*, 1993). The filarial species in bears could cause human infections in rural areas of Japan. If so, species identification may be possible from the worm being found in histological sections. *D. ursi* has a cuticle with longitudinal ridges on the outer surface (Uni *et al.*, 1980). *C. japonica* has a smooth cuticle with small interior projections at the lateral chords, not described before. *M.* (*M.*) *akitensis* has scanty musculature and lateral chords without interior cuticular projections (Uni, 1983).

The classification of the genus *Dipetalonema*, which had contained many species with different morphology, vectors, and host animals, was extensively revised by Chabaud and Bain (1976). They listed six subgenera within the genus *Dipetalonema*: *Dipetalonema, Orihelia, Loxodontofilaria, Molinema, Acanthocheilonema*, and *Chenofilaria*, and Eberhard (1980, 1982) added two subgenera, *Cercopithifilaria* and *Dasypafilaria*. Thereafter, Bain *et al.* (1982) raised seven of these eight subgenera to the generic level, but placed *Chenofilaria* in synonym with *Acanthocheilonema*. Some species with a preesophageal cuticular ring and an undivided esophagus, considered to be specialized *Acanthocheilonema*, have been placed in the genus *Cercopithifilaria*. *Dipetalonema japonica* was placed first in the subgenus *Chenofilaria* (Uni, 1983).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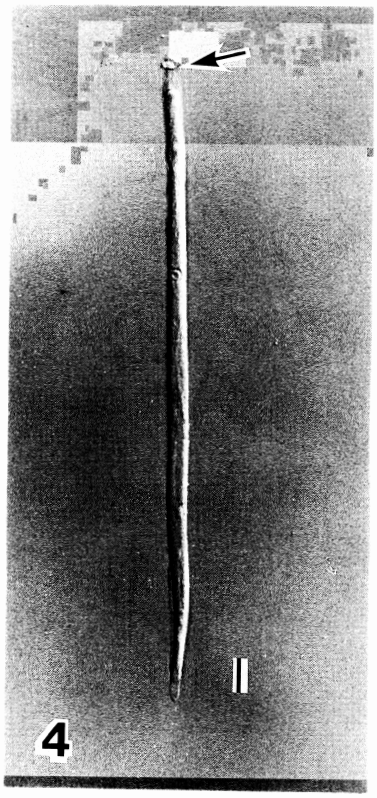
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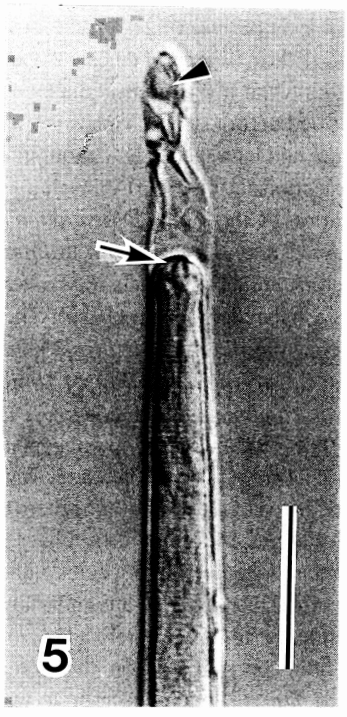
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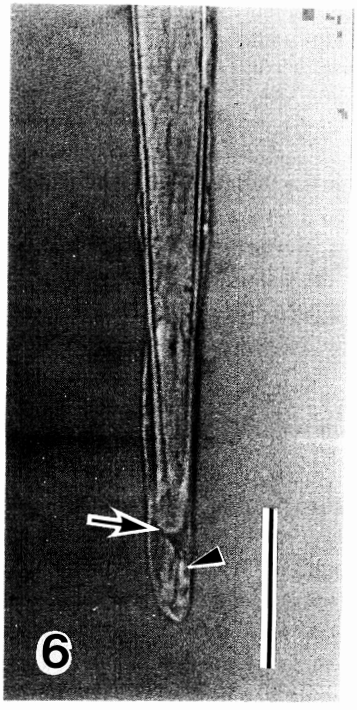
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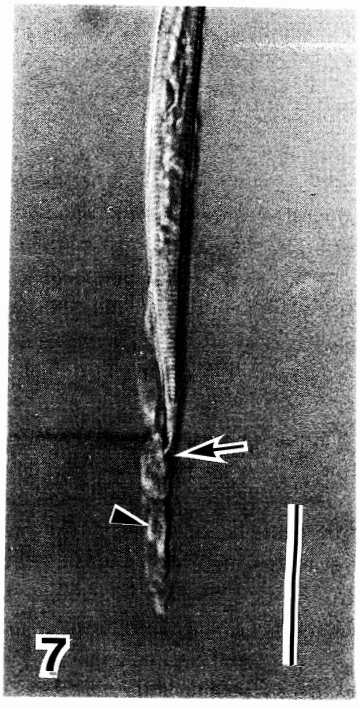
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A preesophageal cuticular ring and an undivided esophagus were found in the female specimens examined in this study, as before.

Bain *et al.* (1986, 1988) obtained microfilariae from females in the genus *Cercopithifilaria* and found that they were usually sheathed and had dorso-ventral flattening; refractile granules in the sheath were found in some species. In previous examinations (Uni, 1983, 1984), a sheath was not found on *D. japonica* microfilariae taken from ticks (Ixodidae) or from a solution used to preserve bear skin, but microfilariae taken from females in this study had sheaths. These morphological features of adult specimens and microfilariae correspond with such features of species in the genus *Cercopithifilaria*, so we suggest that *D. japonica* be transferred to this genus.

Trichinella spiralis larvae were not found in this survey, although this parasite in bear meat is the only known causative agent for human trichinellosis in Japan. This parasite has been found only in two black bears from Iwasaki village, Aomori, and a raccoon dog from Yamagata in northern Honshu (Yamaguchi, 1989). Its prevalence and distribution are of interest because bear meat is eaten by hunters and tourists.

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Figs. 3–7 Photomicrographs of *Cercopithifilaria japonica* female worm and microfilariae. Bars = 25 μm.
Fig. 3 Transverse section of anterior part near vulva of female worm. Arrows: interior cuticular projections. * intestine; MF, microfilaria. ×480.
Fig. 4 Sheathed microfilaria taken from the uterine cavity. Arrow: sheath ×200.
Fig. 5 Anterior part of a sheathed microfilaria. Arrow: a pair of refractile bodies at the mouth of the microfilaria. Arrowhead: refractile granule in the sheath ×850.
Fig. 6 Dorso-ventral side of posterior part of a microfilaria. Arrow: blunt end of the tail inside the sheath. Arrowhead: refractile granule ×850.
Fig. 7 Lateral side of posterior part of a microfilaria. Arrow: sharp end of the tail inside the sheath. Arrowhead: refractile granule ×850.

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