## A Study of the Internal Parasites of *Clethrionomys* rufocanus bedfordiae (Thomas)

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## Introduction

Clethrionomys rufocanus bedfordiae (Thomas) occurs only in Hokkaido, Japan. The parasites of C. r. bedfordiae have been studied by Chabaud et al. (1963), Ohbayashi et al. (1968) and Ishimoto (1974). In this paper, we introduce further information obtained from a recent survey.

## **Materials and Methods**

During May to October, 1982, 175 voles, *C. r. bedfordiae*, were collected at Nopporo National Forest (43°25'N, 141°25'E), Hokkaido, Japan, in order to examine their parasitic fauna.

The skulls were prepared in order to determine the species and the age of host. All guts from esophagus to anus and other organs except the heads of voles were examined parasitologically and after naked eye examination, helminths were collected under a dissecting microscope. Nematodes were fixed and preserved in 5% formalin solution and examined microscopically with lactophenol solution. Trematodes and cestodes were fixed and kept in 70% ethanol, stained with Delafield's haematoxylin or Semichon's aceto-carmine, and mounted with Canada balsam. Measuring and drawing of these worms were done with the aid of a camera lucida, OLYMPUS Model BH2-DA.

The skeletal muscles in which *Sarcocystis* were found were fixed with Bouin solution and embedded in paraffin wax. These paraffin-embedded sections were stained with haematoxylin-eosin or periodic acid Schiff staining.

## **Results and Discussion**

Twelve species of helminths and one protozoon were obtained (Table 1); Capillaria sp., Tenorastrongylus speciosus (Konno, 1958), Anoplocephaloides sp. and Catenotaenia pusilla (Goeze, 1782) were the first records of these species from C. r. bedfordiae.

A. Nematoda

1. Capillaria sp.

One female was obtained from the stomach of a vole.

Body length 15.07 mm, width 0.069 mm at the vulval region. Esophagus 3.40 mm long. Vulva at 3.530 mm from head end. Eggs barrel-shaped, 0.030-0.033 mm  $\times$  0.061-0.065 mm. Cuticle swelling and a small spine at vulval region.

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PARASITES	HABITAT		
Nematoda			
Capillaria sp.	stomach	1*	(0.5)
Tenorastrongylus speciosus	small intestine	10	(5.7)
Heligmosomum yamagutii	small intestine	132	(65.4)
Heterakis spumosa	large intestine	44	(25.1)
Syphacia montana	large intestine	27	(15.8)
Trichuris sp.	large intestine	5	(2.9)
Capillaria hepatica	liver	6	(3.6)
Mammanidula hokkaidensis	genital organs	3	(1.8)
Trematoda			
Plagiorchis muris	small intestine	10	(5.7)
Cestoda			
Anoplocephaloides sp.	small intestine	3	(1.8)
Catenotaenia pusilla	small intestine	10	(5.7)
Cysticercus sp.	liver	13	(7.6)
Protozoa			
Sarcocystis clethrionomysi	skeletal muscle	2	(1.1)

Table 1Internal parasites of Clethrionomys rufocanus bedfordiae (Thomas)from Nopporo National Forest, Hokkaido, Japan

\* Number of voles infected.

† Percentage of voles infected (%).

No species of the genus *Capillaria* have been reported from the alimentary tract of *C. r. bedfordiae* hitherto, although *C. muris sylvatici* (Diesing, 1851) (Sołtys, 1949; Kisielewska, 1970 a, b) and *C. annulosa* (Dujardin, 1845) (Tenora and Zejda, 1974; Mészáros, 1978) have been reported from the intestine of *C. glareolus*. Our specimen was similar to *C. muris sylvatici*, although positive identification was not possible because of the absence of males.

2. Tenorastrongylus speciosus (Konno, 1958)

This species was obtained from the small intestine of 10 voles. From 1 to 27 worms were collected from each host. Body coiled and reddish when alive. Synlophe originated from posterior end of cephalic vesicle, and composed of 28–32 ridges at mid-body. The number of ridges gradual-

## **Explanation of Figures**

- Figs. 1-12 Heligmosomum yamagutii Chabaud, Rausch and Desset, 1963.
- Fig. 1 Synlophe of anterior extremity of female, left-subventral view.
- Fig. 2 Synlophe of anterior extremity of female, right-subdorsal view.
- Fig. 3 Synlophe of posterior extremity of female, left-subdorsal view.
- Fig. 4 Synlophe of posterior extremity of female, right-subventral view.
- Fig. 5 Cross section of anterior extremity of female; D; dorsal, V; ventral, L; left-lateral, R; right-lateral.
- Fig. 6 Cross section of mid-body of female.
- Fig. 7 Cross section of posterior extremity of female.
- Fig. 8 Anterior extremity of male, left-lateral view.
- Fig. 9 Posterior extremity of male, sublateral view.
- Fig. 10 Posterior extremity of male, ventral view.
- Fig. 11 Dorsal ray.
- Fig. 12 Posterior extremity of female.



ly decreased in the anterior region.

Male: 5 specimens. Body length 2.35-3.11 mm, width 0.065-0.077 mm. Cephalic vesicle 0.028-0.036 mm×0.049-0.055 mm. Nerve ring at 0.163-0.169 mm and excretory pore at 0.233-0.326 mm from head end. Esophagus 0.31-0.37 mm long. Prebursal papillae absent. Spicules equal, 0.33–0.42 mm. Gubernaculum filiform. boat-shaped.  $0.009-0.014 \text{ mm} \times 0.035-0.054$ mm. Genital cone well-developed. Bursa symmetrical. Dorsal ray well-developed and bifurcated distally.

Female: 6 specimens. Body length 0.01–0.89 mm, width 0.076-0.118 mm. Cephalic vesicle 0.029–0.038 mm×0.029–0.055 mm. Nerve ring at 0.117–0.235 mm and at 0.256–0.347 mm from head end. Esophagus 0.33–0.36 mm long. Vulva at 0.114–0.161 mm and anus at 0.026–0.042 mm from tail end. Eggs oval, 0.030–0.038 mm×0.049–0.063 mm.

T. speciosus has previously been recorded from Apodemus spp. in Japan (Konno, 1958; Chabaud et al., 1963; Ishimoto, 1974; Yagi and Kamiya, 1981), but this is the first record of T. speciosus from C. r. bedfordiae.

3. Heligmosomum yamagutii Chabaud, Rausch and Desset, 1963

This species was found in 132 voles.

From 1 to 32 worms were collected from the small intestine of each host. Body slender and reddish when alive. Cephalic vesicle symmetrical, with transverse striations.

The cuticle was provided with oblique synlophe arising from the right lateral line and running obliquely and anteriorly to the left lateral line across either the dorsal or the ventral part. At the cross section of mid-body, the number of ridges were 10–14 in ventrally and 7–9 in dorsally. Towards the anterior or posterior ends, the number of ridges decreased gradually.

Male: 5 specimens. Body length 9.84-15.58 mm, width 0.200-0.233 mm. Cephalic vesicle 0.070-0.102 mm×0.102-0.138 mm. Nerve ring at 0.214-0.298 mm and excretory pore at 0.233-0.326 mm from head end. Esophagus 0.69-0.75 mm long. A pair of prebursal papillae was present at the base of the ventro-ventral rays. Spicules equal, filiform, 0.76–1.01 mm long. Gubernaculum absent. Bursa symmetrical; ventroventral ray curved inwards; latero-ventral thick and slightly curved inwards; anterolateral thickest among laterals; mediolateral longest and parallel with anteropostero-lateral shortest lateral: among laterals and slightly curved inwards; extero-dorsal slender and arising from base

Figs. 13-19 Tenorastrongylus speciosus (Konno, 1958).

- Fig. 13 Synlophe of anterior extremity of male, left-lateral view.
- Fig. 14 Synlophe of mid-body of male.
- Fig. 15 Synlophe of posterior extremity of male, right-subventral view.
- Fig. 16 Anterior extremity of male.
- Fig. 17 Posterior extremity of male.
- Fig. 18 Dorsal ray.
- Fig. 19 Posterior extremity of female.
- Figs. 20-21 Heterakis spumosa Schneider, 1866.
- Fig. 20 Anterior extremity of male, left-lateral view.
- Fig. 21 Posterior extermity of male, right-subventral view.
- Figs. 22-24 Syphacia montana Yamaguti, 1943.
- Fig. 22 Anterior extremity of female, right-lateral view.
- Fig. 23 Posterior extremity of male, right-lateral view.
- Fig. 24 Egg.



of laterals; dorsal 0.042–0.058 mm long, reduced asymmetrical and bifurcating twice distally.

Female: 5 specimens. Body length 18.64-22.83 mm, width 0.186-0.251 mm. Cephalic vesicle  $0.084-0.098 \text{ mm} \times 0.112-0.135 \text{ mm}$ . Nerve ring at 0.242-0.372 mm and excretory pore at 0.372-0.586 mm from head end. Esophagus 0.68-0.77 mm long. Vulva at 0.456-0.558 mm and anus at 0.186-0.251 mm from tail end. Tail ending in a cone with a fine spine at its extremity. Eggs oval,  $0.051-0.062 \text{ mm} \times 0.075-0.089 \text{ mm}$ .

H. yamagutii was also reported from C. r. bedfordiae collected at the same place by Chabaud et al. (1963) and Ishimoto (1974). Surkov (1972) and Surkov and Nadtochi (1971) also described H. yamagutii from C. r. rufocanus in Sakhalin Island, USSR. Previous authors, however, have not described the morphology of the dorsal ray and synlophe, hence they are described here in detail.

4. Heterakis spumosa Schneider, 1866

Forty-four voles were infected with this nematode. From 1 to 13 worms were collected from the large intestine of each host. Body large and white, lateral alae welldeveloped.

Male: 4 specimens. Body length 10.65– 11.08 mm, width 0.38–0.42 mm. Nerve ring 0.28–0.42 mm and excretory pore 0.44– 0.90 mm from head end. Esophagus 0.86– 0.95 mm long. Spicules equal, 0.33–0.45 mm long. Preanal sucker 0.084–0.099 mm in diameter, 0.60–0.72 mm from tip of tail. Ten pairs of caudal papillae present; 2 pairs beside preanal sucker, 5 pairs near cloaca and 3 pairs on slender portion of tail.

Female: 4 specimens. Body length 14.24–15.35 mm, width 0.39–0.43 mm. Nerve ring 0.09–0.35 mm and excretory pore 0.44–1.17 mm from head end. Esophagus 0.93–1.01 mm long. Vulva with prominent cuticular swelling, 7.48–8.62 mm from tail end. Eggs 0.044–0.054 mm×0.070-0.082 mm.

5. Syphacia montana Yamaguti, 1943

Twenty-seven voles were infected with this nematode. From 1 to 420 were collected from the large intestine of each host.

Male: 4 specimens. Body length 1.12– 1.47 mm, width 0.107–0.163 mm. Nerve ring 0.100–0.134 mm and excretory pore 0.296–0.350 mm from head end. Esophagus 0.233–0.256 mm long. Tail 0.154–0.177 mm long. Spicule 0.070–0.092 mm and gubernaculum 0.040–0.047 mm long.

Female: 4 specimens. Body length 4.03–5.64 mm, width 0.233–0.302 mm. Nerve ring 0.163–0.205 mm and excretory pore 0.372–0.586 mm from head end. Esophagus 0.372–0.437 mm long. Vulva with prominent cuticular swelling, 0.558–0.809 mm from head end. Eggs fusiform, 0.033–0.047 mm×0.100–0.121 mm.

The specimens collected during this study corresponded well with the original description of *S. montana* by Yamaguti (1943), Chabaud *et al.* (1963) and Ishimoto (1974) with prominent cuticular swelling

- Fig. 26 Synlophe of mid-body of female.
- Fig. 27 Synlophe of posterior extremity of female.
- Fig. 28 Anterior extremity of male.
- Fig. 29 Posterior extremity of male, ventral view.
- Fig. 30 Dorsal ray.
- Fig. 31 Posterior extremity of female.

Figs. 25-31 Mammanidula hokkaidensis (Ohbayashi, Orihara and Fujimaki, 1968).

Fig. 25 Synlophe of anterior extremity of female, subventral view.



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- Fig. 32 Plagiorchis muris Tanabe, 1922, ventral view. Figs. 33-34 Anoplocephaloides sp.
- Fig. 33 Scolex.
- Fig. 34 Mature segment.
- Figs. 35-37 Catenotaenia pusilla (Goeze, 1782).
- Fig. 35 Scolex.
- Fig. 36 Mature segment.
- Fig. 37 Gravid segment.

(36)



Fig. 38-40 Capillaria sp. (scale 0.03 mm).

- Fig. 38 Anterior extremity of female.
- Fig. 40 Posterior extremity of female. Fig. 41 Fig. 42 Scolex of Anoplocephaloides sp. (scale 0.6 mm).
- Figs. 43-45 C. pusilla.
- Fig. 43 Scolex (scale 0.5 mm).
- Fig. 45 Gravid segment (scale 0.8 mm).

Fig. 39 Fig. 41 Vulval region, right-lateral view. Dorsal ray of *H. Yamagutii* (scale 0.02 mm).

Fig. 44 Mature segment (scale 0.3 mm). Fig. 46 Sarcocystis clethrionomysi Ohbayashi and Kitamura, 1959 (scale 0.4 mm).

of vulval region and host specificity.

6. Trichuris sp.

Only 5 females were obtained from the large intestine of 5 voles.

Female: 2 specimens. Anterior body filiform and posterior thick. Body length 21.20–28.59 mm, width 0.47–0.55 mm. Esophagus 13.54–17.71 mm long. Vulva, 14.00– 17.92 mm from head end. Eggs barrelshaped, 0.033–0.039 mm×0.068–0.076 mm.

These specimens were similar to *Trichuris muris* (Schrank, 1788), although we were not able to identify them specifically because of the absence of males. Ishimoto (1974) also reported *Trichuris* sp. from *C. r. bedfordiae* at the same locality, but also failed to obtain males.

7. Capillaria hepatica (Bancroft, 1893)

This nematode was found in the liver of voles. These worms were usually degenerated in the host tissue, and only eggs were detected, therefore, it was impossible to obtain intact worms.

Female: Body filiform, width 0.054 mm in the vulval region. Vulva with a prominent cuticular projection.

This nematode is well known as a cosmopolitan species of rodents and has been reported from *Clethrionomys* spp. (Chabaud *et al.*, 1963; Lubinsky *et al.*, 1971; Ishimoto, 1974; Romashov, 1979).

8. Mammanidula hokkaidensis (Ohbayashi, Orihara and Fujimaki, 1968)

Three voles were infected with this nematode. From 1 to 10 worms were collected from mammary glands of female hosts and the prostate and bulbo-urethral glands of male.

Body coiled in 1 or 3 spirals. Synlophe originated from cephalic vesicle and ran longitudinally. The synlophe composed of 26–30 ridges at mid-body. The number of ridges decreased gradually in the anterior and posterior region.

Male: 4 specimens. Body yellow to pink in color when alive. Body length 8.43–8.67 mm, width 0.10–0.14 mm. Cephalic vesicle,  $0.05-0.07 \text{ mm} \times 0.06-0.07 \text{ mm}$ . Nerve ring 0.16-0.18 mm and excretory pore 0.37 mm from head end. Esophagus 0.37-0.40 mm long. Spicules equal and filiform, 0.91-1.00 mm long. Gubernaculum present. Prebursal papillae absent. Bursa asymmetrical and triangular, right lobe larger than left; lateral rays asymmetrical, right laterals longer than left; dorsal well-developed and bifurcating twice distally.

Female: 6 specimens. Body reddish when alive. Body length 13.98-15.15 mm, width 0.16-0.27 mm. Cephalic vesicle, 0.06-0.09 mm $\times 0.07$  mm. Nerve ring 0.28-0.33 mm and excretory pore 0.47-0.54 mm from head end. Esophagus 0.38-0.47 mm long. Vulva 0.31-0.35 mm and anus 0.11-0.13 mm from tail end. Eggs oval, 0.047-0.058 mm $\times 0.077-0.091$  mm.

This nematode was identified as M. hokkaidensis by its specific habitat in each host and by its morphology.

B. Trematoda

9. Plagiorchis muris Tanabe, 1922

Ten voles were infected with this trematode. From 1 to 16 specimens were collected from the small intestine of each host.

Three specimens. Body length 1.065-1.079 mm, width 0.460-0.499 mm, minute spines on body. Oral sucker, 0.130-0.140 mm in diameter. Acetabulum at anterior one third of body, 0.126-0.149 mm in diameter. Cirrus pouch clubform, dorsodextral to acetabulum. Ovary elliptical, 0.135-0.143 mm in diameter, situated postero-dextral to cirrus pouch. Vitelline gland well-developed, extending to lateral part. Uterus originated from hindbody, winding up the part between the testes and reaching the posterior part of the acetabulum. Testes lobed, almost oval and equal in size; anterior testis 0.209-0.260 mm and posterior one 0.219-0.242 mm in diameter; situated obliquely. Eggs oval, 0.016-0.021 mm×0.028-0.033 mm.

## C. Cestoda

Two species of mature cestodes were obtained during this study and they represented the first records of this group from the alimentary tract of *C. r. bedfor-diae*.

## 10. Anoplocephaloides sp.

Anoplocephaloides sp. was obtained from the small intestine of 3 voles.

specimen. Body wedge-shaped, One length 10.09 mm, maximum width at posterior end of mature segments 2.645 mm. Strobila thick, gradually widening toward the posterior end with 35 segments. Length/width ratio in mature segments 1:5.6—1:7.1. Scolex well-developed, 0.977 mm in diameter. Four suckers unarmed and equal in size, 0.363-0.386 mm in diameter. Neck 0.809 mm wide. Genital pores unilateral, situated in anterior part of the margin. Cirrus pouch strongly developed, pyriform, 0.102–0.124 mm wide, 0.195– 0.233 mm long. Ovary irregular, 0.167-0.214 mm wide. Vitelline gland elliptical and small, 0.065-0.074 mm wide. Testes about 60-70 in number, ranged widely in mature segments. Eggs spherical, 0.033-0.038 mm in diameter, with pyriform apparatus.

The morphology of the present specimen corresponded to the description of the genus Anoplocephaloides Baer, 1923. Rausch (1976) reported A. baeri from Apodemus argenteus (Temminck) collected at Mt. Moiwa-yama, near Sapporo, Hokkaido. The present specimen was similar to A. baeri. Precise classification, however, was not possible because the specimens were few in number and damaged.

11. Catenotaenia pusilla (Goeze, 1782)

Ten voles were infected with this cestode. One or 2 specimens were collected from the small intestine of each host.

Three specimens. Body length 23.0– 29.0 mm, maximum width 0.90–1.04 mm. Scolex small, 0.586–0.796 mm long, 0.246– 0.312 mm wide. Rostellum absent. Four suckers unarmed, 0.112-0.167 mm in diameter. Neck slender, 0.205-0.233 mm wide. Strobila, with 44-47 segments, gradually increasing in width toward the posterior end. Length/width ratio in mature segments 1:0.96—1:1.32, in gravid segments 1:2.29-1:3.29. Mature segments trapezoidal. Genital pores irregularly alternating, situated near the middle of margin. Cirrus pouch small, 0.177-0.195 mm long, 0.042-0.047 mm wide. Ovary strongly lobed, ranged extensively in the anterior field, 0.209-0.256 mm×0.381-0.446 mm. Vitelline gland also lobed, situated posterior to ovary, 0.153-0.186 mm in diameter. Testes posterior to ovary, 39-57 in number in each segment. 0.019-0.056 mm in diameter. Uterus well-developed in each gravid segment, taenioid with median stem and lateral branches. Eggs ellipsoid, 0.012- $0.016 \text{ mm} \times 0.024 - 0.040 \text{ mm}$ , with pyriform apparatus.

The same species has also been reported from C. r. rufocanus in Sakhalin Island, USSR, by Surkov (1972).

12. Cysticercus sp.

Thirteen voles were infected with the larvae of a cestode. From 1 to 8 specimens were collected from the liver of each host. No taxonomical identification was possible because they were very immature, hence they are not described here.

D. Protozoa

13. Sarcocystis clethrionomysi Ohbayashi and Kitamura, 1959

Cysts of this protozoan parasite were found in the skeletal muscle of 2 voles.

Cyst, Miescher's tube, fusiform, 0.117– 0.186 mm×0.280–1.018 mm. Cyst membrane 5–13  $\mu$ m in thickness, homogeneous without any striations by haematoxylineosin stain. Cyst divided into chambers of 14–35  $\mu$ m×19–78  $\mu$ m in size. Numerous bradyzoites were within chamber. Chambers in central portion of cyst contain less bradyzoites than marginal portion, positive by periodic acid Schiff stain. Bradyzoites elongated oval,  $1.67-1.86 \ \mu m \times 4.65-6.51 \ \mu m$ . Nucleus of bradyzoites situated eccentricly,  $0.74-1.40 \ \mu m$  in diameter.

S. clethrionomysi has been also reported from C. r. bedfordiae collected in Hokkaide by Ohbayashi and Kitamura (1959).

## Summary

During May to October, 1982, the internal parasite fauna of 175 voles, Clethrionomys rufocanus bedfordiae (Thomas), col-Nopporo National lected in Forest (43°25'N, 141°25'E) in Hokkaido, Japan, was examined. Twelve species of helminths and one protozoan species were recognized. These are as follows: Capillaria sp., Tenorastrongylus speciosus (Konno, 1958); Heligmosomum yamagutii Chabaud, Rausch and Desset, 1963; Heterakis spumosa Schneider, 1866; Syphacia montana Yamaguti, 1943; Trichuris sp.; Capillaria hepatica (Bancroft, 1893); Mammanidula hokkaidensis (Ohbayashi, Orihara and Fujimaki, 1968); Plagiorchis muris Tanabe, 1922; Anoplo*cephaloides* sp.; Catenotaenia pusilla (Goeze, 1782); Cysticercus sp. and Sarcocystis clethrionomysi Ohbayashi and Kitamura, 1959.

Of them, T. speciosus, Anoplocephaloides sp. and C. pusilla were newly recorded from C. r. bedfordiae, and Capillaria sp. from the stomach of C. r. bedfordiae. The morphology of the dorsal ray and synlophe of H. yamagutii are described in detail.

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# エゾヤチネズミ Clethrionomys rufocanus bedfordiae (Thomas) の内部寄生虫相について

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1982年5月から10月にかけて,北海道江別市野幌自 然公園(北緯43度25分,東経141度25分)内で捕獲した エゾヤチネズミ Clethrionomys rufocanus bedfordiae (Thomas) 175 頭について,内部寄生虫相の検索を行 なった.得られた内部寄生虫は,線虫類8種,吸虫類 1種,条虫類3種,原虫類1種の計13種であった.そ れらの種名は以下に示すとおりである.

Capillaria sp.; Tenorastrongylus speciosus (Konno, 1958); Heligmosomum yamagutii Chabaud, Rausch and Desset, 1963; Heterakis spumosa Schneider, 1866; Syphacia montana Yamaguti, 1943; Trichuris sp.; Capillaria hepatica (Bancroft, 1893); Mammanidula hokkaidensis (Ohbayashi, Orihara and Fujimaki, 1968); Plagiorchis muris Tanabe, 1922, Anoplocephaloides sp.; Catenotaenia pusilla (Goeze, 1782); Cysticercus sp. 及び Sarcocystis clethrionomysi Ohbayashi and Kitamura, 1959.

以上の内, T. speciosus, Anoplocephaloides sp. 及 び, C. pusilla のエゾヤチネズミ C.r. bedfordiae に おける寄生例は初報告である.また,胃に寄生の Capillaria sp. も, C.r. bedfordiae では,はじめてであ る.

更に, *H. yamagutii* の背肋と, synlophe の形態に ついて詳細に観察した結果, 原記載と異なっていたの で報告した.