[Jpn. J. Parasitol., Vol. 44, No. 4, 298–305, August, 1995]

Redescription of *Strongyloides akbari* Mirza and Narayan, 1935 (Nematoda: Strongyloididae), an Ovoviviparous Parasite of *Suncus murinus* (Insectivora)

ISAO SHIMABUKURO[1)], HIDEO HASEGAWA[1,2)], JUN KOBAYASHI[1)], BOUASY HONGVANTHONG[3)] AND YOSHIYA SATO[1,2)]

[1)]*Department of Parasitology and* [2)]*Research Center of Comprehensive Medicine, Faculty of Medicine, University of the Ryukyus, Nishiahra, Okinawa 903-01, Japan.*
[3)]*Institute of Malariology, Parasitology and Entomology, Vientiane, Lao People's Democratic Republic.*
(Accepted August 4, 1995)

Abstract

Strongyloides akbari Mirza and Narayan, 1935 (Nematoda: Strongyloididae) was collected from a shrew, *Suncus murinus*, on Okinawa Island, Japan. This is the first record of this nematode outside of India. Redescription was made based on parasitic females, free-living males and females, first-stage rhabditoid larvae and filariform larvae. Presence of free-living generation was first proved in *S. akbari*. The parasitic female is characterized by its minute body size, non-spiraled ovaries and ovoviviparous nature. The free-living male has slightly curved spicules and the free-living female has slight postvulval reduction in body width and a vagina forming almost right angle to longitudinal body axis. The first-stage rhabditoid larva derived from parasitic female differs from the first-stage rhabditoid larva from free-living female by having a protruded ventral side of cephalic end and a larger genital primordium. The homogonic filariform larva has a larger genital primordium than the heterogonic filariform larva.

Key words: *Strongyloides akbari*; *Suncus murinus*; redescription; Okinawa; Japan.

Introduction

Strongyloides akbari Mirza and Narayan, 1935 (Nematoda: Strongyloididae) was erected on material collected from a shrew, *Suncus murinus* (referred as *Crocidula coerulea*), of India (Mirza and Narayan, 1935). However, the original description was inadequate because principal measurements were given for only one parasitic female, and the figure was very small obscuring details. No redescription has been made for this nematode. We found recently a species of the genus *Strongyloides* from *S. murinus* on Okinawa Island, Japan, and a close examination has revealed it to be *S. akbari*. In this paper, a redescription of this nematode is presented.

Correspondence: Isao Shimabukuro
島袋　勲[1]，長谷川英男[1,2]，小林　潤[1]，Bouasy Hongvanthong[3]，佐藤良也[1,2]（[1]琉球大学医学部寄生虫学教室，[2]同地域医療研究センター，[3]ラオス国マラリア寄生虫衛生昆虫研究所）

Materials and Methods

The shrews, *Suncus murinus*, were trapped at Kyozuka, Urasoe, Okinawa Island, Japan, using live traps with fish meat sausage, fried fish or fried squid as baits. They were euthanatized with ether, and then autopsied. The intestine was cut into 4 portions of equal length and examined separately. The each part of the intestine was cut open, compressed between two thick glass plates and searched for nematodes under a stereomicroscope with transillumination. In some instances, Baermann's technique was applied to recover worms. Nematodes were then collected by using a fine curved needle, and put in physiological saline. First-stage larvae were collected from the fresh feces of the infected shrews by formalin-ether concentration technique. In some instances, rhabditoid larvae were obtained by gently

compressing the eggs from the uteri of parasitic females.

In order to obtain free-living stages, Harada-Mori culture and agar-plate culture (Arakaki *et al.*, 1988) were made at 27°C with the feces and rectal contents of the shrews. First-stage rhabditoid larvae were collected by dissecting free-living female adults having motile larvae within their uteri. Collected free-living adults, rhabditoid and filariform larvae were washed in distilled water. Obtained nematodes were fixed with 70% ethanol at 70°C, cleared with glycerol alcohol solution, and mounted with 50% glycerol solution for microscopical observation. Some worms of each stage were also observed in living condition or immediately after being killed with heat. Observation was made with Nikon Optiphoto microscope equipped with Nomarski interference apparatus, and figures were made with a Nikon drawing tube. Voucher specimens were deposited in the National Science Museum, Tokyo, with the accession number NSMT As-2451.

Results

Fifteen of the 16 *S. murinus* examined during the period from September to December, 1994, harbored *S. akbari* (prevalence: 94%). It was found that the parasitic females were distributed in the all intestinal portions from duodenum to immediately anterior to rectum. The number of worms found was few in the first portion including the duodenum (7.3% of the total worms recovered), then increased in the second and third portions (32.1 and 31.2%, respectively), and somewhat decreased in the last section (29.4%). All of the parasitic females collected immediately after death of the hosts have eggs of various developmental stage, but intrauterine hatch was not observed. Only eggs were observed within mucous membrane around the parasitic female when the intestinal wall was observed under a stereomicroscope immediately after the death of the host. After several hours incubation by Baermann's method, intrauterine hatch was often observed. When the intestinal wall was observed after being placed in physiological saline for 1 hr., some eggs with motile larvae and hatched larvae were seen around the parasitic female.

With the Harada-Mori culture of fresh feces and rectal fecal masses, numerous homogonic filariform larvae were collected after 24 hrs of culture, and free-living males (mostly dead) were also observed in addition to the homogonic filariform larvae on the 2nd day of culture. The heterogonic filariform larvae appeared in the culture medium after 5 days of culture. Only a few free-living adult males were collected with Harada-Mori culture. On the other hand, many free-living males and females were detected by agar-plate culture for 2 days, but homogonic filariform larva was not observed. At 40th hr of culture, free-living worms were still immature, but at 45th hr most of the free-living females were already gravid and many eggs were observed around the females on the agar surface. Intrauterine hatch was also observed in the senile free-living females. Heterogonic filiarform larvae were observed on the agar surface of the 5th day of culture.

Redescription
Strongyloides akbari Mirza and Narayan, 1935
(Figs. 1–22)

Parasitic female (10 worms): Body minute, tapering anteriorly, 0.73–1.54 mm long by 30–38 μm wide at vulval region. Cuticle with extremely fine transverse striations. Lateral alae absent. Cephalic apex with hexagonal mouth surrounded by 4 cephalic papillae and 2 amphidial pores (Fig. 1). Esophagus filariform, slender, 330–436 μm long, corresponding to 26.1–47.9 (mean 33.3) % of worm length, by 18–27 μm wide at thickest portion near posterior end (Figs. 2–4). Esophago-intestinal junction dividing worm length in ratio of 1:1.1–2.8 (mean 1.8). Distance from anterior extremity to nerve ring 60–88 μm, and to excretory pore on small protrusion slightly posterior to nerve ring, 75–101 μm (Fig. 2). Vulva slightly protruded, dividing worm length in ratio of 1.6–3.2 (mean 2.4) : 1. Postvulval body 230–498 μm long, i.e. 25.2–42.7 (mean 30.1) % of worm length (Fig. 6). Uterus extending anteriorly and posteriorly (Figs. 5–7). Oviducts with narrowed distal portions, abruptly expanded and then gradually narrowed to be joined with ovaries (Figs. 4, 7). Anterior ovary flexed at level near esophago-intestinal junction, running posteriorly and ending at level anterior to vulva

(Figs. 4, 5); posterior ovary flexed at level slightly anterior to anus, running anteriorly and ending at level slightly anterior to end of anterior ovary (Figs. 5–7). Uterus containing up to 25 eggs of various developmental stages, arranged in longitudinal row (Figs. 4–7). Tail conical, with round tip, 25–37 μm long, i.e. 2.2–3.4 (mean 2.9) % of worm length (Fig. 7). Eggs ellipsoidal, with thin soft shell, 43–53 by 18–24 μm. First-stage larvae hatched within uterus in worms collected by Baermann's technique (Fig. 8).

Free-living adult

General: Minute worm tapering to both extremities (Figs. 11, 12, 14–16). Cuticle with extremely fine transverse striations. Lateral alae absent. Esophagus rhabditiform (Figs. 11, 14). Nerve ring near posterior portion of isthmus of esophagus (Figs. 11, 14). Excretory pore situated near level of esophago-intestinal junction (Figs. 11, 14).

Male (9 worms): Length 498–645 μm and width at midbody 25–30 μm. Total esophagus 70–96 μm long, corresponding to 13.3–18.4% of worm length; pharynx 6–8 μm long; corpus 35–44 μm long by 10–14 μm wide; isthmus 5–6 μm wide; bulb 13–19 μm long by 11–16 μm wide. Nerve ring and excretory pore 59–77 μm and 75–101 μm, respectively, from anterior extremity. Anterior part of testis not recurved, ending at 106–165 μm from anterior extremity (Fig. 11). Posterior body bent ventrally (Fig. 12). Caudal papilla 1 only slightly posterior to preanal organ; papillae 3 and 4 at same level, and distance between papillae 2 and 3 slightly shorter than that between papillae 2 and 4 but apparently longer than that between papillae 3 and 4; papilla 6 at level slightly posterior to papilla 5. Tail conical, 48–61 μm long, corresponding to 8.7–10.2% of worm length (Fig. 13). Spicules slightly curved ventrally, 30–36 μm long (Fig. 13). Gubernaculum 19–23 μm long (Fig. 13).

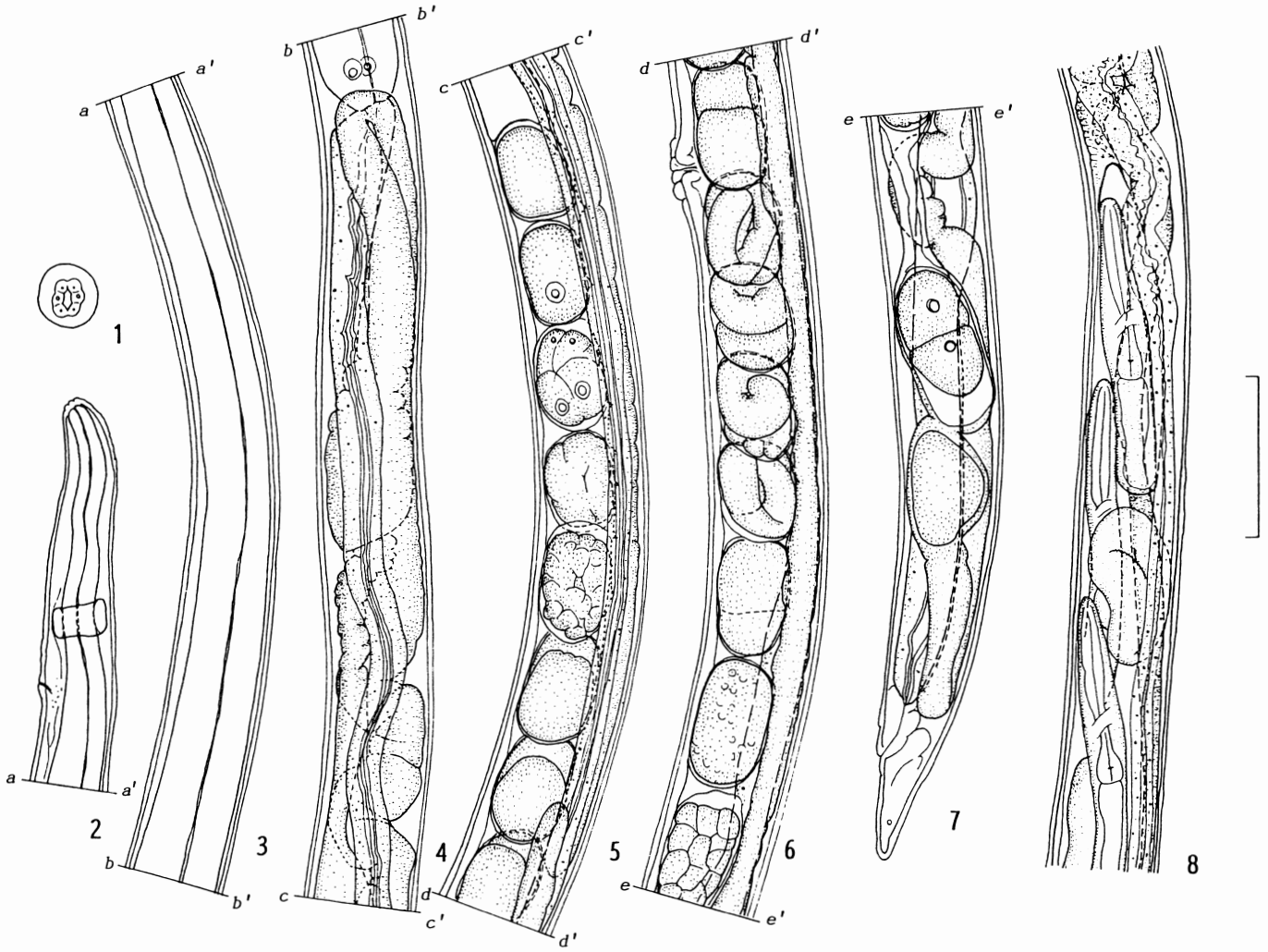
Female (10 worms): Length 655–795 μm and width at vulva 35–42 μm. Total esophagus 89–118 μm long, corresponding to 13.5–15.0% of worm length; pharynx 8–11 μm long; corpus 43–51 μm long by 13–16 μm wide; isthmus 6–8 μm wide; bulb 16–21 μm long by 15–18 μm wide. Distance from anterior extremity to nerve ring, excretory pore and gonad 72–91, 86–107 and 126–160 μm, respectively (Fig. 14). Vulva at 330–410 μm (47.6–52.2% of worm length) from anterior extremity (Figs. 14, 15). Vagina forming angle of 90–105° from longitudinal axis of worm (Fig. 15). Body width reduced 4–9% posterior to vulva (Fig. 15). Uterus containing 2 to 7 eggs arranged in longitudinal row (Fig. 15). Anterior ovary flexed posteriorly at level posterior to esophago-intestinal junction and posterior ovary flexed anteriorly at level anterior to anus, both ending near vulval level (Figs. 14–16). Tail long conical, 54–69 μm (7.9–10.0% of worm length) long (Fig. 16). Uterine eggs oval, thin-shelled, containing embryos of various developmental stages, 42–58 by 26–39 μm (Fig. 15).

First-stage rhabditoid larva

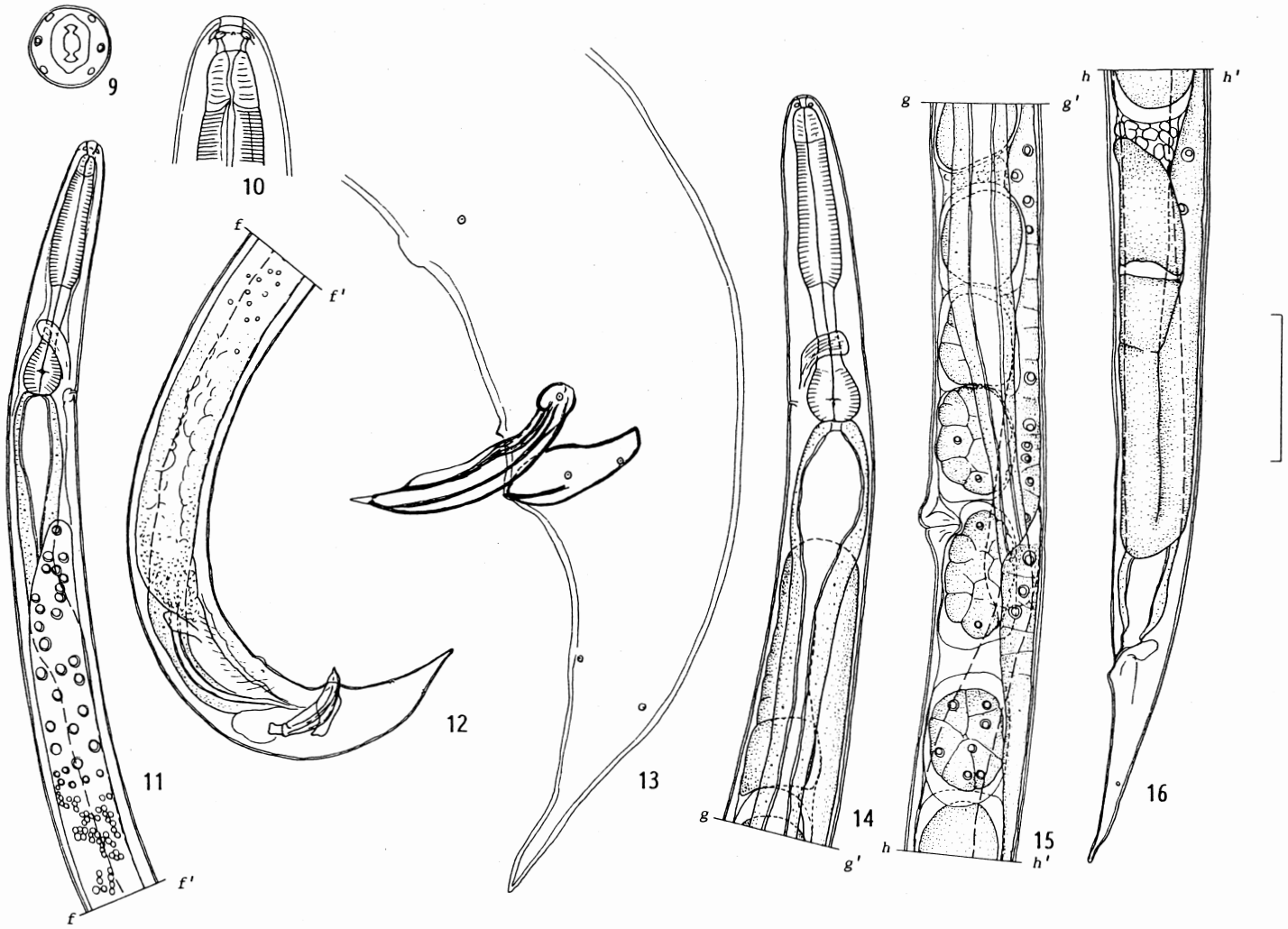
General: Body minute, tapering in posterior extremities (Figs. 17, 18). Cuticle with extremely fine transverse striations. Lateral alae absent. Buccal cavity with clear wall, slightly shorter than pharynx; esophagus rhabditiform (Figs. 17, 18). Nerve ring in anterior portion of isthmus of esophagus (Figs. 17, 18). Excretory pore situated anterior to esophago-intestinal junction (Figs. 17, 18). Genital primordium ellipsoidal, located at ventral side of midlevel of intestine (Figs. 17, 18). Tail long conical (Figs. 17, 18).

Larva derived from parasitic female (10 worms): Length 225–290 μm and maximum width 14–16 μm. Ventral side of cephalic extremity protruded more than dorsal side (Fig. 17). Total length of esophagus 68–78 μm, corpus including pharynx 41–48 μm long by 6–8 μm wide, isthmus 3–5 μm wide, esophageal bulb 13–16 μm long by 7–10 μm wide. Distance from anterior extremity to nerve ring 49–60 μm, excretory pore 69–78 μm and gonadal primordium 115–139 μm. Gonadal primordium large, 26–35 μm long. Tail 34–40 μm long.

Larva derived from free-living female (2 worms): Length 253–301 μm and maximum width 16–17 μm. Ventral side of cephalic extremity not protruded (Fig. 18). Total length of esophagus 79–86 μm, corpus including pharynx 42–48 μm long by 9–10 μm wide, isthmus 5 μm wide, esophageal bulb 16 μm long by 12 μm wide. Distance from cephalic extremity to nerve ring 65–66 μm, excretory pore 79–86 μm and gonadal primordium 136–160 μm. Gonadal primordium small, 13 μm long. Tail 39–43



Figs. 1–7 Parasitic female of *Strongyloides akbari* collected from *Suncus murinus* immediately after autopsy. 1. Cephalic end, apical view. 2–7. Whole body, left lateral view. Fig. 8. Anterior portion of anterior uterus of parasitic female of *Strongyloides akbari* collected by Baermann's method, left lateral view. Scale bar: 20 μm for Fig. 1; 50 μm for others.



Figs. 9–16 Free-living adults of *Strongyloides akbari* collected by agar-plate culture. 9, 10. Cephalic extremity of female, apical (9) and left lateral (10) views. 11, 12. Male whole body, right lateral view. 13. Posterior portion of male, left lateral view. 14–16. Female whole body, left lateral view. Scale bar: 20 μm for Figs. 9, 10 and 13; 50 μm for others.

μm long.

Filariform larva

General: Body slender, tapering to both extremities (Figs. 19–22). Cuticle with extremely fine transverse striations. Double lateral alae commencing at level of nerve ring and extending to tail end (Figs. 19–22). Esophagus long cylindrical, gradually thickened posteriorly (Figs. 19, 21). Genital primordium situated ventral side of and at anterior to midlevel of intestine (Figs. 20, 22). Tail long conical, with notched tip (Figs. 20, 22). Phasmids situated near midtail (Figs. 20, 22).

Homogonic larva (10 worms): Length 370–410 μm and maximum width in midbody 13–16 μm. Esophagus 145–170 μm long by 6–10 μm wide near posterior end. Nerve ring, excretory pore and genital primordium 54–63 μm, 63–71 μm and 209–238 μm, respectively, from anterior extremity. Genital primordium elongated, 21–36 μm long (Fig. 20). Anus and phasmids 33–41 μm and 24–28 μm, respectively, from posterior extremity.

Heterogonic larva (10 worms): Length 334–407 μm and maximum width in midbody 11–16 μm.

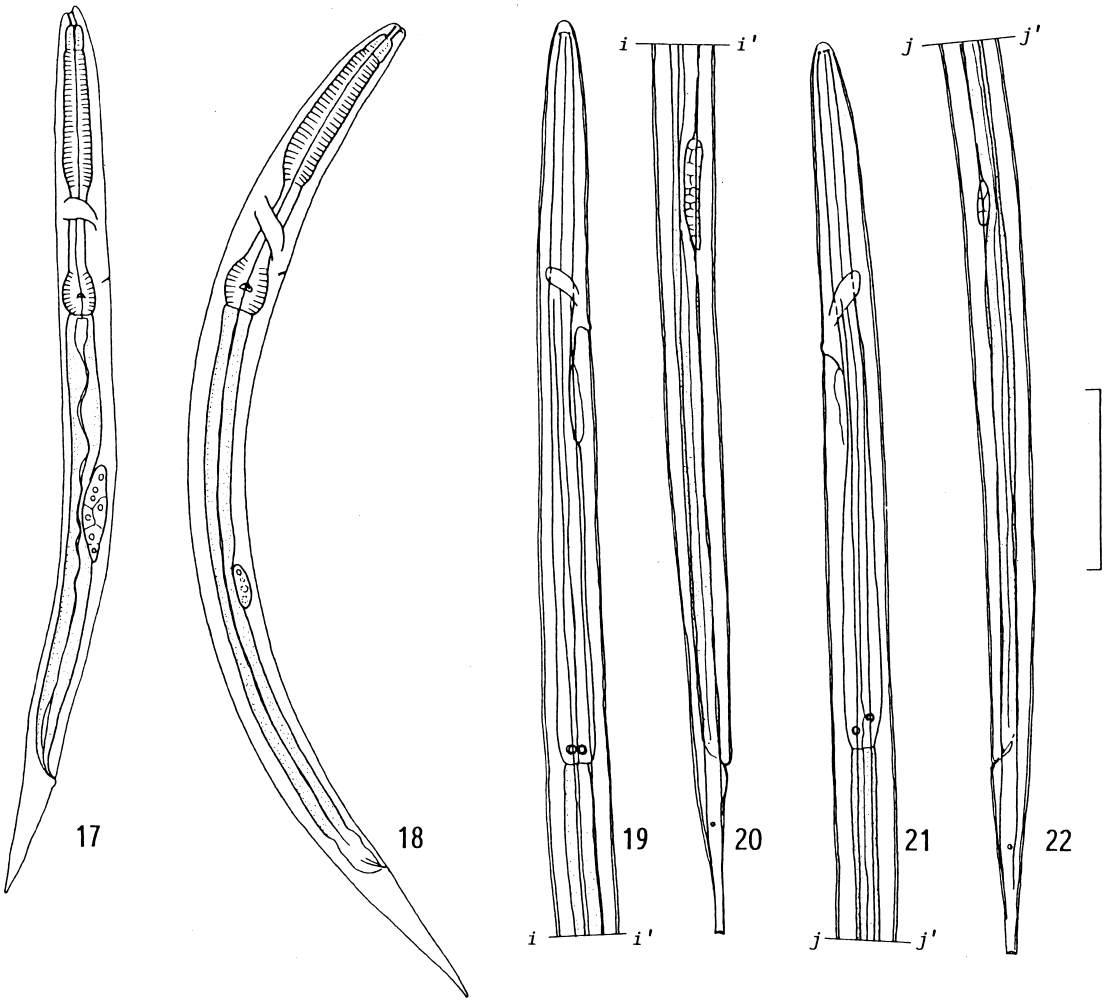


Fig. 17 First-stage rhabditoid larva derived from parasitic female, right lateral view. Fig. 18. First-stage rhabditoid larva derived from free-living female, right lateral view. Figs. 19, 20. Homogonic filariform larva, right lateral view. Figs. 21, 22. Heterogonic filariform larva, left lateral view. Scale bar: 50 μm.

Esophagus 136–170 μm long by 7–9 μm wide near posterior end. Nerve ring, excretory pore and genital primordium 54–65 μm, 60–69 μm and 196–245 μm, respectively, from anterior extremity. Genital primordium ellipsoidal, 8–11 μm long (Fig. 22). Anus and phasmids 33–41 μm and 23–28 μm, respectively, from posterior extremity.

Discussion

The morphology of the present parasitic females agrees with that of *S. akbari* (Mirza and Narayan, 1935). The type material has a longer esophagus (0.65 mm in the worm with length of 1.544mm), but its ratio to the worm length (42%) falls within the range for the present material (Mirza and Narayan, 1935). In the figure of the parasitic female in the original description, the esophagus occupies only about 20% of worm length, but it might be a mistake on drawing. The vulva in the type material divided the worm length in ratio of 1.7 : 1, also within the range for the present worms (Mirza and Narayan, 1935). Although the ratio of the tail to the worm length (2.0%) in the parasitic females in the original description was slightly smaller than the present material (Mirza and Narayan, 1935), it is presumed to be an intraspecific variation. Thus, the present material is identified as *S. akbari*.

Only a few records have been made for *S. akbari* after the description by Mirza and Narayan (1935). Srivastava (1964) collected one parasitic female, of which uterus contained no egg, from a honey budger, *Mellivora capensis* (syn. *Mellivora indicus*), and identified it as *S. akbari*. However, this might be misidentification because the tail (70 μm long in a female with worm length of 1.53 mm long) was more than twice the length in the original description (31 μm in a female with worm length of 1.544 mm long; Mirza and Narayan, 1935). Recently Hemkar and Renapurkar (1991) recorded *Strongyloides muris* from *Suncus murinus*. However, *S. muris* seems to be an invalid taxon because no nematode has been described under this name. Moreover, their host identification was quite doubtful because they claimed that they detected some rodent nematodes, i.e. *Hymenolepis nana, Hymenolepis diminuta, Pterygodermatites tani* (as *Rictularia tani*) and *Trichuris muris* from the insectivore (Hemker and Renapurkar, 1991). Thus, it remains unclear whether their *Strongyloides* was *S. akbari* or not. Anyway, the present report is the first one of *S. akbari* outside of India.

In the original description of *S. akbari*, it was stated that the eggs hatch within the uterus of parasitic female (Mirza and Narayan, 1935). In the present study, all parasitic females recovered from the intestinal wall immediately after the death of the host had unhatched eggs while the worms collected by Baermann's technique had hatched larvae within their uteri. It is considered therefore that the parasitic female lays eggs normally, and the eggs hatch shortly after oviposition. If the parasitic female is transferred in an unnatural condition such as physiological saline, the oviposition may be inhibited but the uterine eggs may continue to develop and finally hatch within the uterus. Similar phenomenon was observed in *Strongyloides felis* Chandler, 1926, a cat parasite. Chandler (1925) stated that the eggs hatch within the uterus of parasitic female, but recently Speare and Tinsley (1986) observed that the parasitic female lays eggs in tunnels within the mucous membrane of the crypt.

Mirza and Narayan (1935) stated that the rhabditoid larvae became filariform larvae directly without heterogonic development. However, the present study revealed the existence of free-living generation in *S. akbari*. The culture method employed by Mirza and Narayan (1935) was not specified for *S. akbari*, but seemed to be the same method with that these authors used for *S. eryxi* Mirza and Narayan, 1935, in which the feces were mixed with sterile sand (Mirza and Narayan, 1935). Probably, the nutrient agar provided more suitable condition than the sand culture for development of free-living generation of *S. akbari*.

It is of interest that the shape of the cephalic extremity differed between the first-stage rhabditoid larvae derived from parasitic female and free-living female. To our knowledge, such difference between the larvae has not been described. Premvati (1958) and Little (1966) observed no difference between the first-stage larvae developed from eggs of parasitic and free-living females in *S. fuelleborni* and *S. stercoralis*. Presumably, the protruded ventral side of the cephalic extremity may act like the boring tooth of larval anisakid nematodes, and may be thus

advantageous in migration from the intestinal mucosa to lumen. The difference in the size of the genital primordium between homogonic and heterogonic filariform larva seems to be maintained from the first rhabditoid stage.

As shown above, the eggs hatch within the host intestinal mucosa shortly after oviposition, and rhabditoid larvae are passed with host feces. Moreover, a preliminary experiment showed that the inoculated filariform larvae of *S. akbari* migrated to the lungs (Shimabukuro *et al.*, in preparation). Because these characteristics resemble those of *Strongyloides stercoralis* of humans, *S. akbari* may suite as a candidate for laboratory model of human strongyloidiasis. *Suncus murinus*, the natural host of this nematode, has attracted attentions recently as an experimental animal because it has basic physiological mechanisms of mammals including primates. Several strains of *S. murinus* have been already established for experimental study. Thus, further experimental study on infection dynamics of *S. akbari* in *S. murinus* is necessary to prove the usefulness of this nematode-host system as a model for human strongyloidiasis.

Acknowledgments

This study was supported partly by a specified grant from the University of the Ryukyus, 1994.

References

1) Arakaki, T., Hasegawa, H., Asato, R., Ikeshiro, T., Kinjo, F., Saito, A. and Iwanaga, M. (1988): A new method to detect *Strongyloides stercoralis* from human stool. Jpn. J. Trop. Med. Hyg., 16, 11–17.
2) Chandler, A. C. (1925): The species of *Strongyloides* (Nematoda). Parasitology, 17, 426–433.
3) Hemker, R. R. and Renapurkar, D. M. (1991): *Pseudophysaloptera indiana* and *Pseudophysaloptera formosana formosana* from *Suncus caeruleus* in Bombay. Ind. J. Helminthol., 43, 35–40.
4) Little, M. D. (1966): Comparative morphology of six species of *Strongyloides* (Nematoda) and redefinition of the genus. J. Parasitol., 52, 69–84.
5) Mirza, M. B. and Narayan, S. S. (1935): *Strongyloides akbari* n. sp. – A new nematode parasite from *Crocidura coerulea*, with a note on some species of the genus *Strongyloides*. Proc. Ind. Acad. Sci., 2, 503–507.
6) Premvati (1958): Studies on *Strongyloides* of primates. I. Morphology and life history of *Strongyloides fuelleborni* von Linstow, 1905. Can. J. Zool., 36, 65–77.
7) Speare, R. and Tinsley, D. J. (1986): *Strongyloides felis*: an "old" worm rediscovered in Australian cat. Aust. Vet. Prac., 16, 10–18.
8) Srivastava, S. C. (1964): A new host record for *Strongyloides akbari* and *Artyfechinostomum surfratyfex*. Ind. J. Helminthol., 16, 24–26.