Jpn. J. Vet. Res., **33**, 27–43 (1985)

VARIATIONS OF SYNLOPHE OF *ORIENTOSTRONGYLUS EZOENSIS* TADA, 1975 (NEMATODA : HELIGMONELLIDAE) AMONG DIFFERENT POPULATIONS IN JAPAN

Shin-ichiro FUKUMOTO[1] and Masashi OHBAYASHI[2]

(Received for publication January 28, 1985)

The cuticular longitudinal ridges on the body surface (synlophe) of the rat nematode, *Orientostrongylus ezoensis* TADA, 1975, from different host populations in Japan were examined. The number of longitudinal ridges was found to be inconsistent among the populations, i. e., (1) in adult females and males, (2) in nematodes from wild caught hosts, *Rattus norvegicus*, collected in geographically different areas of Japan, (3) in nematodes recovered from laboratory maintained rats. The number of longitudinal ridges was differed among the various body regions and increased posteriorly. The synlophe was also studied by a scanning electron microscopy.

Keywords: *Orientostrongylus ezoensis*, *Rattus norvegicus*, synlophe, Japan

INTRODUCTION

In Japan, *O. ezoensis* TADA, 1975 was found from *Rattus norvegicus* in Hokkaido,[21] and recently was also reported in other regions of Japan.[8,23–26]

The number and distribution of longittdinal ridges (synlophe) have been used for determining species or subspecies of trichostrongylid nematodes.[2,3,5,9–17]

In this paper, the authors reexamined the specimens of *O. ezoensis* from wild hosts collected in several regions of Japan and the specimens maintained in laboratories using experimental animals. These specimens were examined especially for the differences in number and distribution of longitudinal ridges.

[1] Laboratory of Parasitology, Department of Veterinary Medicine College of Dairying, Ebetsu, 069–01, Japan

[2] Department of Parasitology, Faculty of Vaterinary Medicine, Hokkaido University, Sapporo 060, Japan.

MATERIALS AND METHODS

The following specimens of natural infections from wild caught hosts were examined (fig. 1).

a) *Orientostrongylus ezoensis* in Hokkaido: The specimens were collected from *R. norvegicus* in Sapporo, Hokkaido.

b) *O. ezoensis* in Hyogo Prefecture: The specimens were isolated from *R. norvegicus* caught on the artificial island (Port Island) of Kobe, Hyogo Prefecture (UGA et al., 1981).

c) *O. ezoensis* in Ehime Prefecture: The nematodes were isolated from the small intestine of *R. norvegicus* which was preserved in formalin and collected in Matsuyama, Ehime Prefecture.

d) *O. ezoensis* in Aichi Prefecture: The nematodes sent by Dr. K. MAKIYA, Department of Parasitology, College of Medicine, Nagoya University, which were collected from *R. norvegicus* caught in Nagoya City, Aichi Prefecture.

Samples passaged in the laboratories were as follows.

a) *O. ezoensis* in Saitama Prefecture: The nematode was passaged in Saitama Medical College using laboratory rats. The nematode was originated from *Rattus norvegicus*, collected in Saitama Prefecture.

b) *O. ezoensis* in Hokkaido: The nematode was maintained in the Department of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, and originated from *R. norvegicus*, collected in Sapporo, Hokkaido (FUKUMOTO, 1979).

The specimens were treated with lacto-phenol for microscopy. Measurements and drawings were done using a camera lucida (OLYMPUS, BH2–DA).

The synlophe was studied by examining the surface of whole mounts and preparing cross sections of fixed and cleared nematodes. Cross sections were made at three locations, i. e., (a) in the esophageal region, (b) in the midbody and (c) near the proximal extremity of spicules in males or near the vulva in females. The longitudinal ridges were examined and counted using an interference microscope (OLYMPUS, BH2–NIS).

The specimens examined by a scanning electron microscope were fixed in 5% glutaraldehyde and dehydrated through a series of increasing concentrations of ethyl alcohol. After dipping the worms in isoamyle acetate, specimens were dried by critical-point drying using CO_2 substitution (HITACHI, HCP–1). Specimens were then coated with a layer of gold in an ioncoater, (EIKO, IB–2) and studied with a scanning electron microscope (SEM, AKASHI, MSM4T) at 15KV.

FIGURE 1 *Geographical origin of specimens of O. ezoensis from Rattus norvegicus*

A :	Sapporo	after TADA, 1975
B :	Saitama	sent by Dr. K. FUJIMOTO, Saitama Medical College
C :	Tokyo	after UCHIDA et al., 1984
D :	Nagoya	sent by Dr. K. MAKIYA, Nagoya University
E :	Kobe	after UGA et al., 1981 & 1983
F :	Miki	after UGA et al., 1982
G :	Matsuyama	sent by Prof. H. NISHIDA, Ehime University

RESULTS

The measurements of specimens are shown in tables 1 and 2, and the number of longitudinal ridges is shown in tables 3 and 4.

Although it was impossible to separate the specimens into different species or subspecies, variations in the number of longitudinal ridges at the midbody were observed among the groups of specimens from various localities.

The following characteristics were noted. The ridges were usually uninterrupted, and the longitudinal ridges were originated from the posterior margin of the cephalic vesicle (fig. 2). The ridges extend to the bursa in males and posterior to the anus in females (figs. 3–4). The number of transverse striations between longitudinal ridges is approximately 3 per μm (fig. 5).

The number of ridges increased porteriorly in males and females (tab. 3, figs. 6–11). The number of ridges at midbody varied from 20 to 26 in males and from 25 to 34 in females. The longitudinal ridges of females were significantly greater in number than those of males ($P<0.05$, tabs. 3 & 4). There was no significant difference in the number of longitudinal ridges between the populations collected from the wild caught *R. norvegicus* caught in Sapporo and those passaged in laboratory rats in Hokkaido.

DISCUSSION

Eight species of the genus *Orientostrongylus* DURETTE-DESSET, 1970 are known, namely, *O. tenorai* DURETTE-DESSET, 1970; *O. chinensis* (ERHARDOVA, 1959) DURETTE-DESSET, 1970; *O. brevispicularis* (SINGH, 1962) DURETTE-DESSET, 1970; *O. krishnansamyi* DURETTE-DESSET et LIAT, 1974; *O. dendrogali* DURETTE-DESSET et LIAT, 1974; *O. ezoensis* TADA, 1975; *O. ratti* OHBAYASHI et KAMIYA, 1980 and *O. siamensis* OHBAYASHI et KAMIYA, 1980 (tab. 5, fig. 12). Most of these species were reported from the small intestine of rodents mainly belonging to Murinae in Asia.

DURETTE-DESSET (1970, 1971) described that the genus *Orientostrngylus* stands at the base of the evolution branch of the line of the subfamily Nippostrongylinae from Heligmonellinae in the family Heligmonellidae, due to primitive characters in morphology, i. e., the pattern of synlophe, dorsal ray and synthetic shape of its caudal bursa. DURETTE-DESSET & LIAT (1974) confirmed the primitiveness of the genus *Orientostrongylus* by the findings from primitive hosts, i. e., *O. krishnansamyi* from Petauristinae, *Iomys horsfieldi,* and *O. dendrogali* from Tupaiidae (Primates).

In Japan, *O. ezoensis* was reported only from *Rattus norvegicus.*[21,23–26] FUKUMOTO (1979) described that *O. ezoensis* infected laboratory maintained rats and mongolian gerbils, *Meriones unguiculatus,* but not mice (BALB / cA-nu / nu, BALB / cA-nu /+ and ICR). The present authors recommend that further studies be done on the parasitic fauna of wild-rodents, especially, *Rattus* spp. and *Mus* spp., in Japan and in neibering

TABLE 1 *Measurement* of male O. ezoensis from different hosts in Japan*

HOSTS	NATURAL INFECTION			LABORATORY MAINTAINED	
	Rattus norvegicus			Laboratory rat	
Localities	Sapporo	Kobe	Matsuyama	Sapporo	Saitama
No. examined	10	4	2	25	10
Body length (mm)	2.82±0.12 (2.71−2.94)	1.48±0.28 (1.09−1.65)	(1.77 ; 1.90)	2.09±0.14 (1.85−2.33)	1.96±0.21 (1.72−2.24)
Width	95±9 (86−104)	54±8 (48−67)	(65 ; 71)	55±9 (43−76)	69±8 (57−83)
Esophagus length	318±22 (296−340)	239±16 (218−255)	(203 ; 206)	346±26 (306−391)	316±35 (261−378)
Nerve ring**	132±2 (129−133)	123±12 (111−135)	(113 ; 206)	134±25 (88−167)	126±26 (104−160)
Excretory pore**	194±8 (186−201)	199±8 (191−207)	(149 ; 158)	185±33 (141−247)	169±32 (152−217)
Spicule length	91±7 (84−98)	73±9 (59−83)	(78 ; 78)	90±12 (75−117)	63±13 (54−80)

* Values mean ± SD, range in parentheses Measurements in μm unless otherwise indicated
** Distance from anterior extremity

TABLE 2 *Measurement* of female O. ezoensis from different hosts in Japan*

HOSTS	NATURAL INFECTION			LABORATORY MAINTAINED	
	Rattus norvegicus			Laboratory rat	
Localities	Sapporo	Kobe	Matsuyama	Sapporo	Saitama
No. examined	10	5	5	30	10
Body length (mm)	3.91±0.64 (3.48+0.64)	2.58±0.28 (2.60−3.48)	2.35±0.25 (2.07−2.63)	2.90±0.55 (2.09−4.13)	2.96±0.28 (2.29−3.28)
Width	90±7 (84−98)	48±4 (43−52)	60±4 (54−65)	57±12 (41−75)	81±9 (65−98)
Esophagus length	308±22 (298−326)	291±27 (260−326)	260±25 (228−304)	362±51 (300−431)	301±41 (257−381)
Nerve ring**	166±24 (120−189)	161±22 (139−183)	128±15 (113−143)	140±23 (95−167)	169±32 (120−283)
Excretory pore**	206±11 (198−226)	190±7 (183−196)	159±22 (135−182)	265±38 (149−250)	195±42 (152−283)
Vulva***	130±10 (122−144)	140±16 (112−156)	126±30 (133−156)	125±18 (101−167)	150±31 (126−167)
Anus***	72±10 (62−85)	58±10 (52−74)	41±6 (33−48)	57±15 (33−69)	66±11 (48−80)

* Measurements in μm unless otherwise indicated Values mean ± SD, range in parentheses
** Distance from anterior extremity
*** Distance from posterior extremity

TABLE 3 *Number of longitudinal ridges in various body regions of O. ezoensis from different hosts in Sapporo, Japan*

Hosts	NUMBER OF LONGITUDINAL RIDGES*					
	Male			Female		
	Anterior**	Midbody	Posterior***	Anterior**	Midbody	Posterior***
Rattus norvegicus collected in Sapporo	16±2 (12−20) N=7	23±1 (21−24) N=15	26±2 (26−29) N=7	17±2 (16−19) N=5	26±1 (25−28) N=21	30±2 (27−33) N=6
Laboratory rat maintained at Hokkaido University Sapporo	16±2 (13−19) N=7	23±1 (21−24) N=25	27±1 (25−29) N=8	17±1 (15±19) N=6	26±1 (25−28) N=23	30±2 (27−33) N=10

* Mean ± SD, range in parentheses; N means number of specimens examined

** Cross section made in esophageal region

*** Cross sections made in near proximal extremity of spicules in males or anterior to vulva in females

TABLE 4 *Number of longitudinal ridges (midbody) of O. ezoensis collected from different hosts in Japan*

HOSTS	LOCALITY HOST COLLECTED OR MAINTAINED	NUMBER OF LONGITUDINAL RIDGES*	
		Male	Female
Rattus norvegicus	Sapporo, Hokkaido	23±1 (21−24) N=15	26±1 (25−28) N=21
R. norvegicus	Kobe, Hyogo Prefecture	24±1 (23−26) N=5	26±1 (25−28) N=5
R. norvegicus	Matsuyama, Ehime Prefecture	25, 25 N=2	28, 30, 32, 34 N=4
Laboratory rat	Hokkaido University, Sapporo	23±1 (21−24) N=25	26±1 (25−28) N=23
Laboratory rat	Saitama Medical College, Saitama Prefecture	22±2 (20−24) N=15	29±2 (25−32) N=13

* Mean ± SD, range in parentheses; N means number of specimens examined

areas. At present, the origin and the invasion route of the genera *Rattus* and *Mus* into the Japanese Archipelago remain unclear. The authors suggest that the origin of rats in Japan be given further reconsideration and study.

The number of longitudinal ridges is used as an important criterion for classification of species of the genus *Orientostrongylus* DURETTE-DESSET, 1970. Up to now, however, variations in the number of longitudinal ridges in the species have not been reported.

LICHTENFELS (1971) revealed considerable differences in the synlophe among various body regions of *Citellinema grisei* (Trichostongyloidea : Heligmosomidae) of *Sciurus griseus*.

LICHTENFELS (1974) also reported the consistent number and distribution of longitudinal ridges of *Nippostrongylus brasiliensis* (TRAVASSOS, 1914) (Heligmonellidae : Nippostrongylinae), a parasite of rats, among several populations. In his study, no differences in synlophe were found among five populations : (a) each raised in laboratory rats, hamsters and mice for a long period ; (b) raised in different hosts ; (c) immature and stunted nematodes ; (d) different sizes or ages of nematodes ; (e) wild caught hosts from widely separated geographic areas.

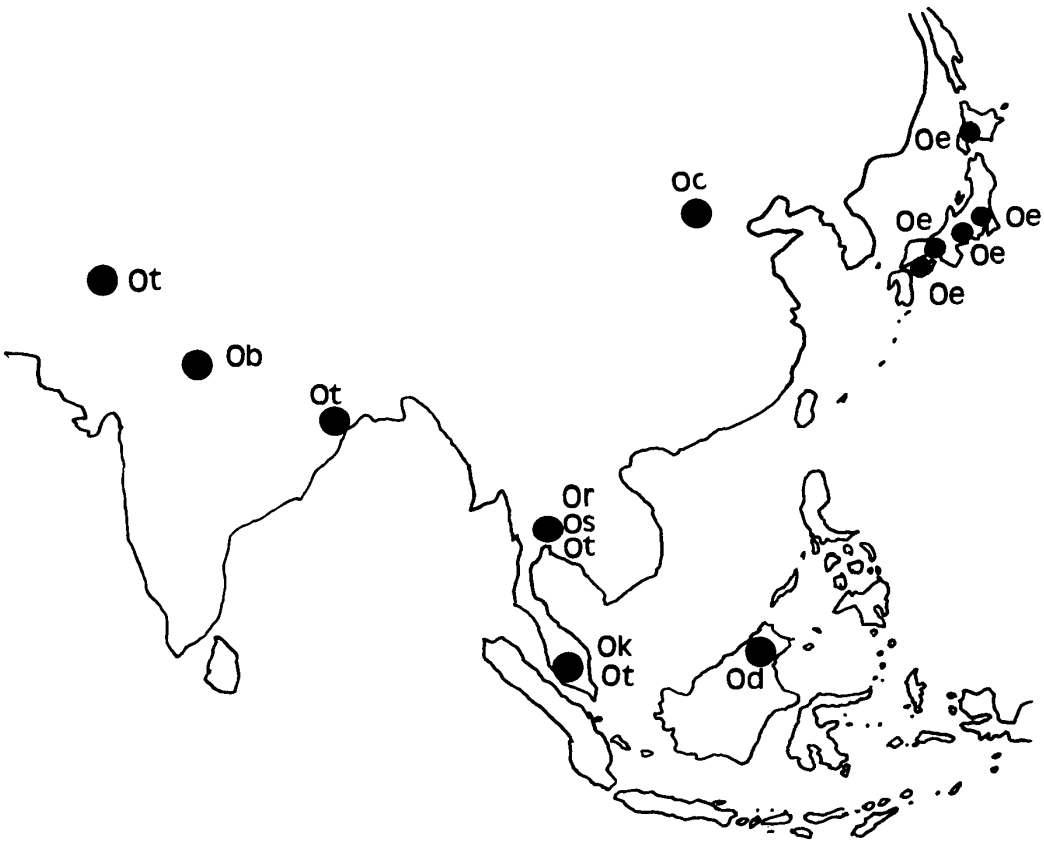
LICHTENFELS (1977, 1983), and LICHTENFELS & PILITT (1983 a, b) described that the differences in synlophe of the genera *Cooperia, Nematodirella* and *Nematodirus* (Trichostrongyloidea : Trichostrongylidae) are very useful for identifying closely related species. In these genera, the synlophe provides a character for identifying

TABLE 5 *Comparison of eight species of the genus Orientostongylus*

SPECIES & AUTHORS	HOST	LOCALITY	NUMBER OF LONGITUDINAL RIDGES (MIDBODY)	
			male	female
O. tenorai DURETTE-DESSET, 1970	*Mus musculus* *Bandicota bengalensis*	Afghanistan India	15	16
OHBAYASHI & KAMIYA, 1980	*Bandicota* spp.	Thailand	15	15,17
OW YANG et al., 1983	*Rattus* spp.	Malaysia	ND*	ND
O. chinensis DURETE-DESSET, 1970	*Cricetulus barabensis*	China	20	18
O. brevispiculais SINGH, 1962	*Rattus norvegicus*	India	12	ND
O. dendrogali DURETTE-DESSET & LIAT, 1974	*Dendrogale melanula*	Malaysia	14	ND
O. krishnansamyi DURETTE-DESSET & LIAT, 1974	*Iomys horsefieldi*	Malaysia	16	ND
OW YANG et al., 1983	*Rattus* spp.	Malaysia	ND	ND
O. ezoensis TADA, 1975	*Rattus norvegicus*	Japan	23	25
Present authors, 1985	*Rattus norvegicus*	Japan	20–26	25–34
O. ratti OHBAYASHI & KAMIYA, 1980	*Rattus* spp.	Thailand	19	19
O. siamensis OHBAYASHI & KAMIYA, 1980	*Rattus surifer*	Thailand	21	22

* ND : not described.

FIGURE 12 *Geographical distribution of eight species of the genus Orientostrogylus*



Ob : *O. brevispicularis* (SINGH, 1962) DURETTE-DESSET, 1970
Oc : *O. chinensis* (ERHARDOVA, 1959) DURETTE-DESSET, 1970
Od : *O. dendrogali* DURETTE-DESSET et LIAT, 1974
Oe : *O. ezoensis* TADA, 1975
Ok : *O. krishnansamyi* DURETTE-DESSET et LIAT, 1974
Or : *O. ratti* OHBAYASHI et KAMIYA, 1980
Os : *O. siamensis* OHBAYASHI et KAMIYA, 1980
Ot : *O. tenorai* DURETTE-DESSET, 1970

species by female specimens.

MEASURES & ANDERSON (1983 a, b, c, 1984) identified *Obeliscoides cuniculi* (GRAYBILL, 1923) GRALBILL, 1924 into two subspecies, *O. cuniculi cuniculi* (GRAYBILL, 1923) and *O. cuniculi multistriatus* MEASURES et ANDERSON, 1983 using characteristics of the synlophe, incidence among wild hosts and hybridization experiments with two subspecies.

In the case of *O. ezoensis*, the number of ridges differs among various body regions, among populations from geographically different hosts, and between sexes. The variations in number did not change between the wild-caught host population and the laboratory maintained population using rats.

The ridges at the midbody of *O. ezoensis* are greatest in number among the eight species of the genus *Orientostrongylus* in Asia (tab. 5, fig. 12). The ridges seem to be greater in number in the north-eastern species than in the south-western species. It was considered that the number of ridges might have increased during the geographical spreading of their hosts, mainly *Rattus* spp..

ACKNOWLEDGEMENTS

The authors express their gratitude to Drs. K. FUJIMOTO, Saitama Medical College, K. MAKIYA, Nagoya University, S. UGA, Kobe University and Prof. H. NISHIDA, Ehime University, for sending us the nematode specimens. We would especially like to extend our appreciation to Drs. K. YAGI, Hokkaido Institute of Public Health, and Y. OKU and M. KAMIYA, Hokkaido University, for their constant advice, enthusiasm and kind assistance.

REFERENCES

1) DURETTE-DESSET, M. -C. (1970): Caractères primitif de certains Nematodes Heligmosomes de Murides et Cricétidés Orientaux d' *Orientostrongylus* n. gen. *Ann. Parasitol. Hum. Comp.*, **45**, 829–837
2) DURETTE-DESSET, M. -C. (1971): Essai de classification des Nématodes Héligmosomes Correlátion avec la paléobiogeographie des hotes *Mem. Mus. Nat. Hist. Nat., A. Zool.*, **49**, 1–126
3) DURETTE-DESSET, M. -C. (1983): Keys to genera of the superfamily Trichostrongyloidea In: CIH Keys to the Nematode Parasites of Vertebrates, No. 10 Eds. ANDERSON, R. C. & CHABAUD, A. G., 1–83 Farnham Royal, Bucks, England: Commonwealth Agricultural Bureaux
4) DURETTE-DESSET, M. -C. & LIAT, L. B., (1974): Description de deux nouvelles espèces du genre *Orientostrongylus* DURETTE-DESSET, 1970 (Nématode, Héligmosome) chez un Tupaiiné et un Petauristiné de Malaisie *Ann. Parasitol. Hum. Comp.*, **49**, 669–675
5) DURETTE-DESSET, M. -C. & CHABAUD, A. G. (1977): Essai de classification des Nématodes Trichostrongyloidea *Ibid.*, **52**, 539–558

6) ERHARDOVA, B. (1959): *Oswaldonema rysavyi* n. sp. und *Viannella chinensis* n. sp. (Nematoda : Heligmosomidae) bei chinesischen Nagern *Chesk. Parasitol.*, **6**, 93–96
7) FUKUMOTO, S. -I. (1979): Life history of *Orientostrongylus ezoensis* Tada, 1975 (Nematoda : Heligmonellidae) *Jpn. J. Parasitol.*, **28**, 465–471 (in Japanese with English summary)
8) FUKUMOTO, S. -I., YAGI, K., & OHBAYASHI, M. (1981) : Genus *Orientostrongylus* in Japan *Jpn. J. Parasitol.*, **30**, Suppl., 31 (in Japanese)
9) LICHTENFELS, J. R. (1971) : *Citellinema grisei* sp. n. (Nematoda : Trichostrongylidae) from the western gray squirrel, *Sciurus griseus Proc. Helminthol. Soc. Wash.*, **38**, 257–261
10) LICHTENFELS, J. R. (1974) : Number and distribution of ridges in the cuticle of *Nippostrongylus brasiliensis* (Travassos, 1914) (Nematoda : Heligmosomatoidea) *J. Parasitol.*, **60**, 285–288
11) LICHTENFELS, J. R. (1983) : Synlophe and species determination of Trichostrongyloidea In : Concepts in Nematode Systematics Eds. STONE, A. R. & PLATT, H. M., 273–291, London : Academic Press
12) LICHTENFELS, J. R. & PILITT, P. A. (1983a) : Cuticular ridge patterns of *Nematodirella* (Nematoda : Trichostrongyloidea) of North American ruminants, with a key to species *Syst. Parasitol.*, **5**, 271–285
13) LICHTENFELS, J. R. & PILITT, (1983b) : Cuticular ridge patterns of *Namatodirus* (Nematoda : Trichostrongyloidea) parasitic in domestic ruminants of North America, with a key to species *Proc. Helminthol. Soc. Wash.*, **50**, 261–274
14) MEASURES, L. N. & ANDERSON, R. C. (1983a) : New subspecies of the stomach worm, *Obeliscoides cuniculi* (Graybill) of lagomorphs *Ibid.*, **50**, 1–14
15) MEASURES, L. N. & ANDERSON, R. C. (1983b) : Characteristics of infections of the stomach worm, *Obeliscoides cuniculi* (Graybill) in lagomorphs and woodchucks in Canada *J. Wildl. Dis.*, **19**, 219–224
16) MEASURES, L. N. & ANDERSON, R. C. (1983c) : Development of the stomach worm, *Obeliscoides cuniculi* (Graybill), in lagomorphs, woodchucks and small rodents *Ibid.*, **19**, 225–233
17) MEASURES, L. N. & ANDERSON, R. C. (1984) : Hybridization of *Obeliscoides cuniculi cuniculi* (Graybill, 1923) Graybill, 1924 and *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983 *Proc. Helminthol. Soc. Wash.*, **51**, 179–186
18) OHBAYASHI, M. & KAMIYA, M. (1980) : Studies on the parasite fauna of Thailand II Three nematode species of the genus *Orientostrongylus* DURETTE-DESSET, 1970 *Jpn. J. Vet. Res.*, **28**, 7–11
19) OW YANG, C. K., DURETTE-DESSET, M. -C. & OHBAYASHI, M. (1983) : Sur les Nématodes de Rongeurs de Malaisie II Les Trichostrongyloidea *Ann. Parasitol. Hum. Comp.*, **58**, 467–492
20) SINGH, K. S. (1962) : Parasitological survey of Kumaun region Part XI Four nematodes from the rat, *Rattus norvegicus Indian J. Helminthol.*, **14**, 98–111.
21) TADA, Y. (1975) : *Orientostrongylus ezoensis* n. sp. (Nematoda : Heligmosomidae) from the brown rat, *Rattus norvegicus* BERKENHOUT *Jpn. J. Vet. Res.*, **23**, 41–44

22) TENORA, F. (1969): Parasitic nematodes of certain rodents from Afghanistan *Vest. Cesk. Spol. Zool.*, **33**, 174–192

23) UCHIDA, A., ARAKAWA, O., MURATA, Y & UDAGAWA, T. (1983): Helminthological surveys of brown rats on a reclaimed land in Tokyo Harbor *Jpn. J. Parasitol.*, **33**, 317–321 (in Japanese with English summary)

24) UGA, S., MATSUMURA, T. & EMOTO, M. (1981): A survey of the parasitic helminths of wild rats on the artificial island (Port Island) of Kobe City: Especially concerning the invasion of *Angiostrongylus cantonensis Ibid.*, **30**, 387–390 (in Japanese with English summary)

25) UGA, S., TAKAHASHI, J., MATSUYAMA, T. & FUJIWARA, S. (1982): A parasitological survey of wild rats in Hyogo Prefecture Endo- and ectoparasites of wild rats collected in Miki City *Jpn. J. Public Health*, **29**, 419–423 (in Japanese with English summary)

26) UGA, S., MATSUMURA, T., ARAKI, K., GONDO, M., MURATA, K. & KAGEI, N. (1983): A survey of the parasitic helminths of wild rats at a zoo in Hyogo Prefecture *Jpn. J. Parasitol.*, **32**, 597–600 (in Japanese with English summary)

EXPLANATION OF PLATES

PLATE I

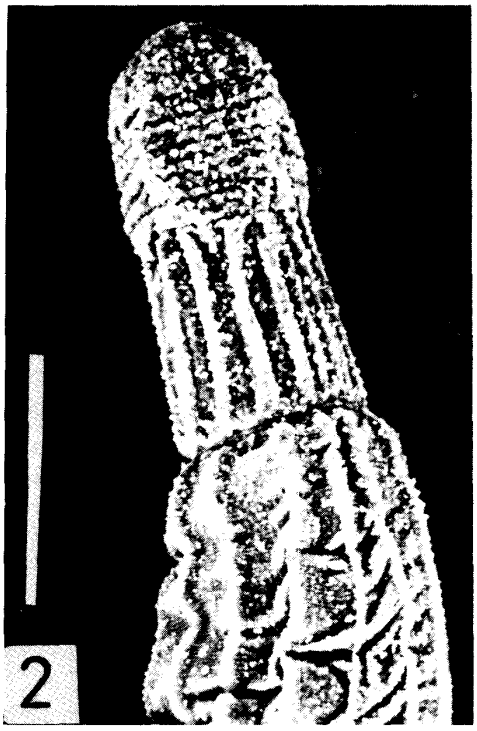
Figs. 2–5 Scanning electron micrographs of adult *O. ezoensis* from *Rattus norvegicus* in Hokkaido

Fig. 2 Cephalic region of female Note the cross striations at anterior part of cephalic vesicle and the longitudinal cuticular ridges extending to the posterior margin of cephalic vesicle Scale 50 μm

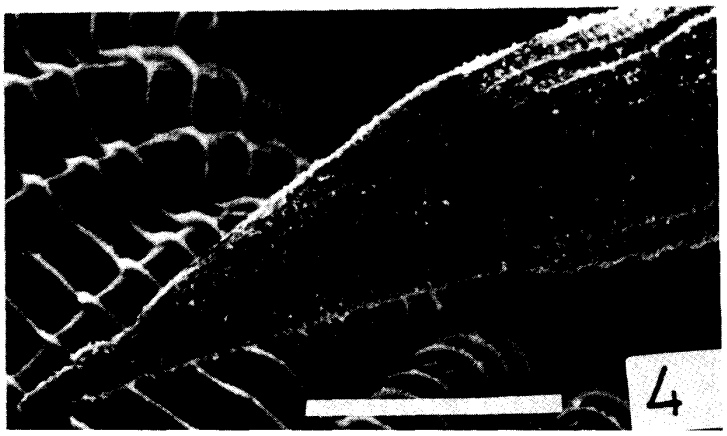
Fig. 3 Bursa of male Note the longitudinal ridges extending to the bursa Scale 30μm

Fig. 4 Posterior end of female Note the longitudinal ridges extending posterior to the anus Scale 50μm

Fig. 5 Fine striations on body surface (midbody) Scale 1μm


2


3


4

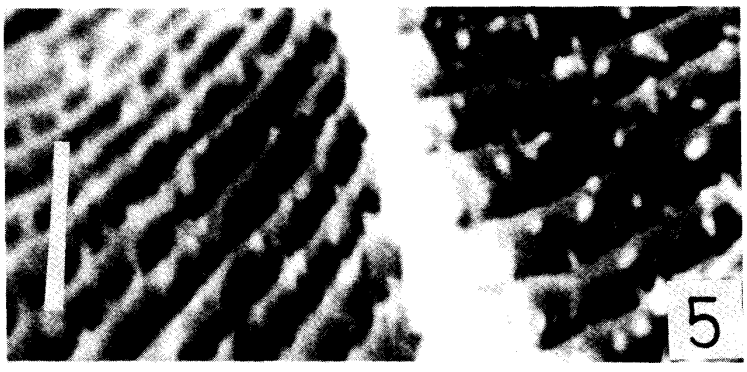

5

PLATE II

Figs. 6–11 Cross sections of males and females of *O. ezoensis* in various body regions

Figs. 6–8 Males

Fig. 6 At esophageal region

Fig. 7 Midbody

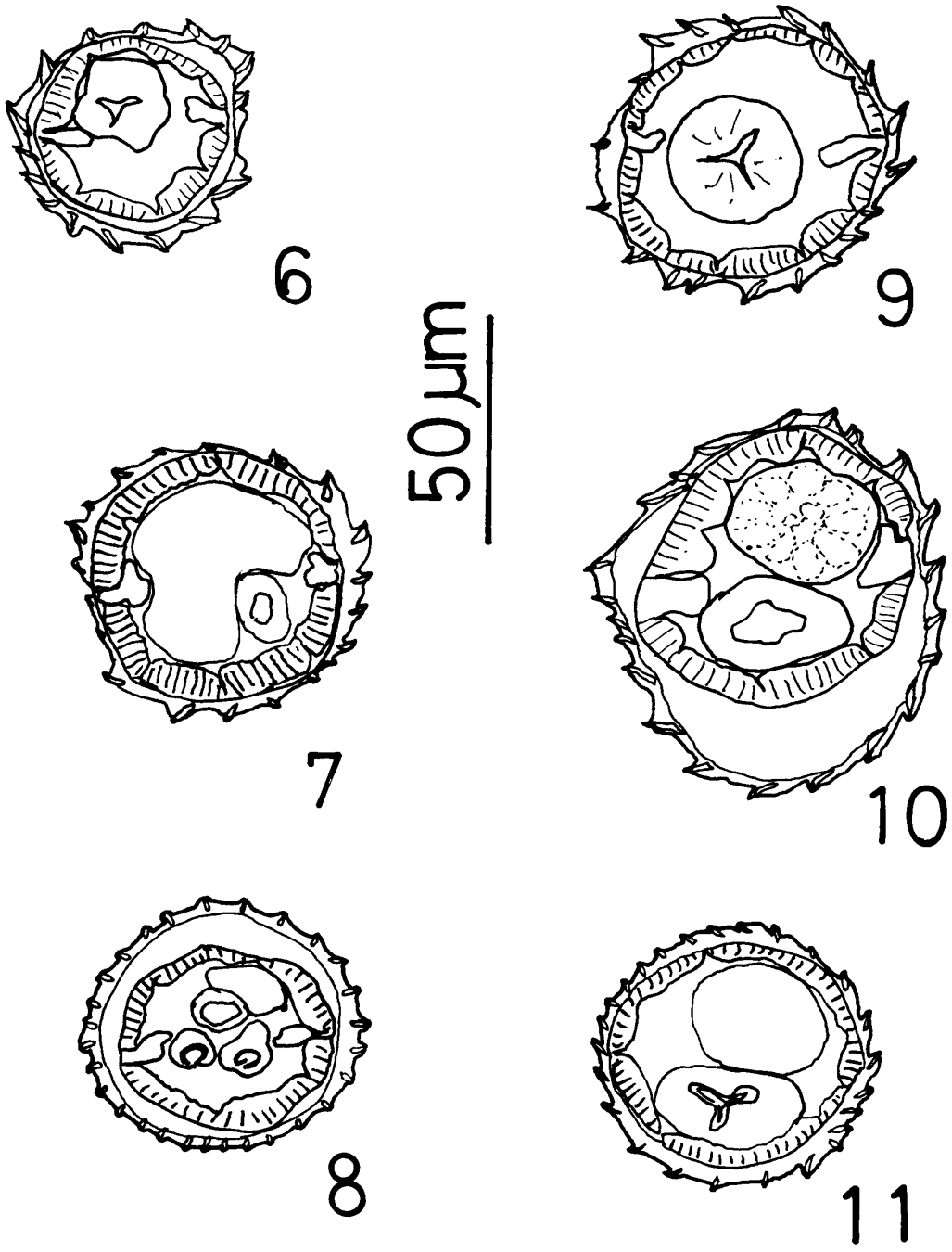
Fig. 8 Near the proximal extremity of spicules

Figs. 9–11 Females

Fig. 9 At esophageal region

Fig. 10 Midbody

Fig. 11 Near vulva


6
9
50μm
7
10
8
11