MAMMANIDULOIDES HOKKAIDENSIS N. G., N. SP. (NEMATODA : HELIGMOSOMATIDAE) FROM VOLES IN HOKKAIDO

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Until this time, two nematode species of Heligmosomatidae (Viannaiinae) have been reported to be parasitic in the mammary gland; *Mammanidula asperocutis* SADOVSKAJA, 1952 (mammary gland of *Sorex* sp. in Primorskaya) and *Mammolongistriata mammovitae* DUBININ, 1953 (lactiferous ducts of *Clethrionomys* spp. in the Far East). In the course of examination of helminths of voles in Hokkaido, Japan, the authors obtained numerous nematode specimens from the mammary gland of female hosts and the prostate and bulbo-urethral glands in the male. The voles examined were *Apodemus speciosus ainu*, *A. argenteus hokkaidi* and *Clethrionomys rufocanus bedfordiae*. The specimens are preserved in the Department of Parasitology, Faculty of Veterinary Medicine, Hokkaido University.

DESCRIPTION OF PARASITE

Measurements are in millimeters and the average for each range is given in parentheses.

Mammaniduloides hokkaidensis n. g., n. sp.

Host: *Apodemus argenteus hokkaidi* (THOMAS)
A. speciosus ainu (THOMAS)
Clethrionomys rufocanus bedfordiae (THOMAS)

Habitat: Mammary gland in female; prostate and bulbo-urethral glands in male

Locality: Hokkaido, Japan

Description: Small nematode. Body coiled; anterior end bends slightly ventrad. Female red, male yellow to pink in life. Cuticle thin, fragile and easily desquamates. Cuticle of head, at anterior one fifth of esophagus, dilated as cephalic vesicle without regular transverse striations. Female, culticle conspicuously loose, shows preputium-like folding anteriorly and envelops cephalic vesicle. Cuticle with 26 longitudinal ridges from anterior to posterior ends without interruption. Width of ridge 0.003~0.007, ventral ridges thicker than dorsal. Each ridge engraved transversely like a rosary. No cuticle pattern except longitudinal

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ridges. Mouth reduced and cervical papillae absent.

Male Body length 7.4~9.7 (8.3), maximal width 0.258~0.292 (0.290). Dorsally, longitudinal ridges begin at 0.06~0.15 distance from head end, and end 0.11~0.13 anterior to tip of genital cone; ventrally, from 0.06~0.075 posterior to head end to about 0.2 anterior to tip of genital cone; ridges of left side end more posterior than right. Length of esophagus 0.316~0.327 (0.320); maximal width 0.042~0.054 (0.047). Nerve ring 0.152~0.204 (0.168) from head end. Prebursal papillae absent. Bursa pouched, asymmetrical and triangular; right lobe long laterally and left antero-posteriorly. Dorsal lobe differentiated indistinctly. Thickness and direction of rays asymmetrical except ventral. Size of bursa 0.350~0.450 (0.405) antero-posteriorly and 0.213~0.259 (0.240) laterally. Ventral rays arise separately from laterals; equal in length and thickness, fused proximally but apart distally. Right and left ventrals 0.068~0.076 (0.071) and 0.061~0.081 (0.072) in length respectively. Laterals asymmetrical. Right laterals fused at proximal half; medio-lateral, the longest, 0.198~0.201 (0.199), from divergence to tip 0.085~0.095 (0.089); antero- and medio-laterals straight, run antero-laterally. Right postero-lateral branches off almost rectangularly, projecting posteriad; distance between tips of postero-lateral and medio-lateral 0.095~0.110 (0.103), and that of postero-lateral and externo-dorsal 0.103~0.114 (0.106). Left postero-lateral the thickest of all, length 0.106~0.110 (0.108); arising separately from other laterals, projecting posteriad more than rectangularly. Left antero- and medio-laterals, length 0.122~0.152 (0.139), run antero-laterally, proximal one third fused. Distance between distal ends of left postero- and medio-laterals 0.190~0.290 (0.200). Externo-dorsal and dorsal arise from common base. Length of dorsal more than 0.080, of which after branching of externo-dorsal 0.065~0.072 (0.067), width 0.013~0.017 (0.015). At a distance of 0.020 from distal end, dorsal bifurcates and each branch bifurcates again; outer branch slender and curved, left one arising more distally; inner branch with minute protuberance. Left externo-dorsal cleft, length 0.071~0.083 (0.078), width 0.013~0.017 (0.015); right externo-dorsal slender, length 0.049~0.065 (0.055), width 0.005~0.007 (0.006). Left externo-dorsal runs posteriad in parallel with postero-lateral, so dorsal projecting slightly dextro-posteriad. Spicules equal and slender, yellow in color, length 0.78~0.85 (0.81), slightly expanded proximally. Distal half enclosed with thin membrane, tips fused and acute. Genital cone projecting long, about 0.050 in length, cloaca in its central portion. Gubernaculum elliptical and yellow in color, length 0.034~0.045 (0.039), width 0.011~0.015 (0.012), grooved as wrapping spicules dorsally; in ventral view, prominent on right side, but indistinct on left, overlapping with spicules.

Female Body length 14.0~16.5 (15.3), maximal width 0.464~0.566 (0.503). Longitudinal ridges visible from preputium-like folding and end about 0.300 anterior to tail end dorsally and about 0.400 ventrally, in front of vulva. Esophagus generally slightly meandering, length 0.312~0.415 (0.327), width 0.057~0.066 (0.059). Nerve ring 0.167~0.204 (0.179) from the head end. Tail bends ventrally about 180°, tail end tapering bluntly. Vulva at bending of tail, opens as transverse slit, at 0.290~0.335 (0.307) from tail end. Genital organs simple, vagina short; ovejector including sphincter 0.290~0.335 (0.312) in length, runs along right side of body. Anus 0.087~0.106 (0.098) from tail end, posterior portion of alimentary canal runs along left side of body. Eggs elliptical, thin-shelled, not embryonated; 0.076~0.083 (0.0826)× 0.046~0.060 (0.0462).

Discussion: The nematode of this genus obviously belongs to the subfamily Viannaiinae of Heligmosomatidae. To compare *Mammolongistriata mammovitae* DUBININ, 1953, the parasite of the mammary gland of voles, *Clethrionomys rufocanus arsenjevi* and *C. rutilus hintoni*, with the subject species, *Mammaniduloides hokkaidensis* is similar in the longitudinal ridges of cuticle and the shape of the spicule, but differences are observed in the structures of the head and the length of the esophagus. The esophagus is very long in *Mammolongistriata*. Moreover, in *Mammolongistriata*, the shape of the bursa is trapezoidal, slightly asymmetrical, the ventral rays are conspicuously longer than the others, and the gubernaculum is fusiform. These features differ from the species presented in this paper.

On the other hand, *Mammanidula asperocutis* SADOVSKAJA, 1952, the parasite of the mammary gland of a shrew *Sorex* sp., is similar to the subject species in the character of the preputium-like folding of the cuticle in the female, the gubernaculum etc. The genus *Mammanidula*, however, has no longitudinal ridges. The shape of the bursa is similar to the subject species, but a portion of the left postero-lateral ray of the subject species is occupied by the outside branch of the bifurcated left externo-dorsal ray, and that of the postero-ventral by the antero-lateral ray, in *Mammanidula*.

Mammaniduloides n. g.

Generic diagnosis: Heligmosomatidae: Body small, coiled. Cephalic vesicle present, cervical papillae absent. Cuticle with longitudinal ridges. Male: Bursa asymmetrically triangular, dorsal lobe indistinctly differentiated; ventral rays fused at base, divergent distally; right laterals longer than left, fused at proximal half, right postero-lateral widely separated distally; left antero- and medio-lateral fused at proximal one third; left postero-lateral thick, arising separately from other laterals, projecting posteriad; dorsal and externo-dorsal arising from common base; right externo-dorsal slender, left externo-dorsal cleft; dorsal with four terminal digitations. Prebursal papillae absent. Spicules long, slender, fused distally; gubernaculum grooved. Female: Tail end folded ventrally; vulva near anus; eggs elliptical, thin-shelled.

Genotype: *Mammaniduloides hokkaidensis* n. sp.

DISTRIBUTION AND OCCURRENCE

For 2 months, June 4 to August 4, 1967, the authors examined 134 voles captured at five districts Kuromatsunai, Nopporo, Bibai, Rumoi and Aizankei, in western Hokkaido, the nematode was found in 20 voles (14.9%) (figure 1, table 1).

The infection rates among host animals were 11/64 (Nos. of positive cases/cases examined) (17.2%) in *Apodemus argenteus hokkaidi*, 3/16 (18.8%) in *A. speciosus ainu* and 6/54 (11.1%) in *Clethrionomys rufocanus bedfordiae*. The infection rate by sexes of the hosts were 9/29 and 2/35 in the male and female *Apodemus argenteus hokkaidi* respectively, 1/6 and 2/10 in *A. speciosus ainu* and 4/19 and 2/35 in *Clethrionomys rufocanus bedfordiae*.

The habitat is the mammary gland (lactiferous ducts) in the female, and the prostate and bulbo-urethral glands in male host. Numbers of the nematodes per host is 2~96 (average 24), and the sex ratio of the nematodes is male 1: female 1.46.

FIGURE 1 *Map showing localities where voles were collected*

TABLE 1 *Animals examined (Number of hosts positive / examined)*

LOCALITY (DATE)	*APODEMUS ARGENTEUS HOKKAIDI*		*A. SPECIOSUS AINU*		*CLETHRIONOMYS RUFOCANUS BEDFORDIAE*	
	♂	♀	♂	♀	♂	♀
Rumoi (4/VI'67)	—	1/1	—	—	—	—
Bibai (24/VI'67)	3/4	1/2	—	—	—	—
Nopporo (6/VII'67)	5/15	0/17	0/3	1/4	—	—
Aizankei (4~10/VII'67)	0/4	0/4	1/2	1/2	1/1	0/1
Kuromatsunai (2~4/VIII'67)	1/6	0/11	0/1	0/4	3/18	2/34
TOTAL	9/29	2/35	1/6	2/10	4/19	2/35

Clethrionomys rutilus mikado (THOMAS), in addition to the voles mentioned above, inhabits Hokkaido, however this vole species was not obtained during this examination. Discovery of the nematode, however, in *C. rutilus mikado* is probably high and will be the authors' problem in the near future.

TABLE 2 *Distribution of parasites*

HOST			HABITAT	NO. OF PARASITES			LOCALITY
Species	Sex & Case No.			♂	♀	Total	
Apodemus argenteus hokkaidi	♀	1	M	38	58	96	Rumoi
	"	2	M	5	6	11	Bibai
	♂	1	B	18	39	57	"
	"	2	B	3	12	15	"
			P	2	1	3	
	"	3	P	1	2	3	"
	"	4	B	13	16	29	Nopporo
	"	5	B	3	6	9	"
	"	6	P	9	10	19	"
	"	7	P	2	3	5	"
	"	8	B	3	5	8	"
	"	9	P	2	2	4	Kuromatsunai
A. speciosus ainu	♀	1	M	34	47	81	Nopporo
	"	2	M	1	1	2	Aizankei
	♂	1	B	1	6	7	"
Clethrionomys rufocanus bedfordiae	♀	1	M	35	36	71	Kuromatsunai
	"	2	M	2	4	6	"
	♂	1	B	2	3	5	"
	"	2	B	16	18	34	"
	"	3	B	3	6	9	Aizankei
	"	4	B	2	4	6	Kuromatsunai

Remarks: M: Mammary gland
P: Prostate gland
B: Bulbo-urethral gland

PATHOLOGICAL FINDINGS

After naked-eye examination, the tissue material was fixed in formalin solution and paraffin sections were stained with hematoxylin-eosin.

Mammary gland Discovery of the nematodes was limited to the inguinal mammary glands, with the exception of one vole in which the nematodes were found in the thoracic glands. Through flaying, coiled or stretched nematodes were found on the surface. The infected mammary gland was swollen; dark gray in color and without transparency. On the cut-surface, the nematodes and

a blackish fluid were found within cystic structures of various sizes, the blackish color of the fluid changed to milky white when diluted with water. The fluid contained numerous eggs and cells or tissue elements.

Histologically, the lactiferous ducts and ductules were dilated and became cystic, the nematodes and eggs were enclosed. The interstitial connective tissue increased with scattered atrophied alveoli. Sometimes the alveoli disappeared, however a few alveoli manifested dilatation. In the alimentary tract of the nematode, small numbers of cell elements and large amounts of acidophil fluid substances were seen. The epithelium of cystic structures showed signs of metaplasia to transitional epithelium, and at times hyperkeratosis. In cystic structure of moderate size, stratification of the epithelium was markedly polylayered, and its inner margin was serrated showing lacuna-like figures. Desquamation of the epithelium was also observable.

Bulbo-urethral gland Normally, this organ was observed after removal of the surrounding tissue. In infected cases, however, the discovery was easy, because the infected gland was enlarged, became cystic and protruded on the surface. The duct was dilated in cases of light initial infection, but became thin and elongated in advanced cases. The ratio of the numbers of nematodes from the left and right sides was 1 : 1.4. The gland was dark gray in color on the surface, contained a fluid substance of blackish-gray color with the nematodes and eggs the same as the mammary gland. Some of the cysts walls were uniformly thin, and others irregular.

Histologically, the glandular tissue was reduced. The cyst wall was fibrous, and the inner surface was lined by several layers of low epithelial cells, in some cases, slender internal projections could be seen.

Prostate gland No remarkable macroscopical changes were observed in cases of moderate infection. In heavy infections, masses of large and small cysts of dark gray color were confirmed. Histologically, an increase in the interstitial connective tissue was evident in many cases, and many histiocytic cells with acidophile cytoplasm appeared. Dilatation of gland tubules was not remarkable in general with the exception of severe cases. Metaplasia and desquamation of the epithelium also observed. Eggs were found in the cyst cavity, and the nematodes were observed in the larger ones.

In the genera *Mammanidula* and *Mammolongistriata*, parasitism of the mammary glands or lactiferous duct was reported, however in the reproductive organs of male animals were not described. In the subject species, differences were found in the habitat between sexes of the host. In male host animals, careful inspection is necessary for the discovery of the parasite. Clarification of the life cycle of this parasite will be examined in the near future.

SUMMARY

Examination of voles, *Apodemus argenteus hokkaidi*, *A. speciosus ainu* and *Clethrionomys rufocanus bedfordiae* in various districts of Hokkaido, revealed a nematode of new genus and species, *Mammaniduloides hokkaidensis*. This species was found in the prostate and bulbo-urethral glands of male hosts and in the mammary gland in female hosts. The canalicular system of the infected glands was dilated and became cystic.

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EXPLANATION OF PLATES

PLATE I Camera lucida drawings

Fig. 1 Anterior end of male; lateral view

Fig. 2 Anterior end of female; lateral view

Fig. 3 Posterior end of female; lateral view

Fig. 4 Posterior end of male; ventral view

Fig. 5 Posterior end of male; ventral view

Fig. 6 Dorsal and externo-dorsal rays; ventral view


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0.2
0.2
0.1
0.2
0.05
①
②
③
④
⑤
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PLATE II

Fig. 7 Anterior end of male; lateral view × 160

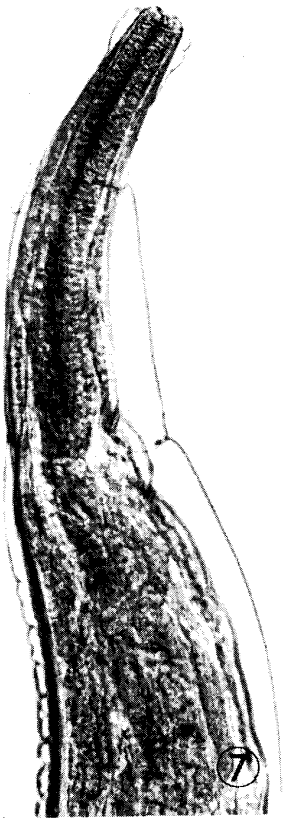
Fig. 8 Longitudinal ridges of cuticle ×320

Fig. 9 Anterior end of female; lateral view × 160

Fig. 10 Posterior end of male; dorsal view × 160

Fig. 11 Posterior end of female; lateral view × 160

Fig. 12 Eggs × 320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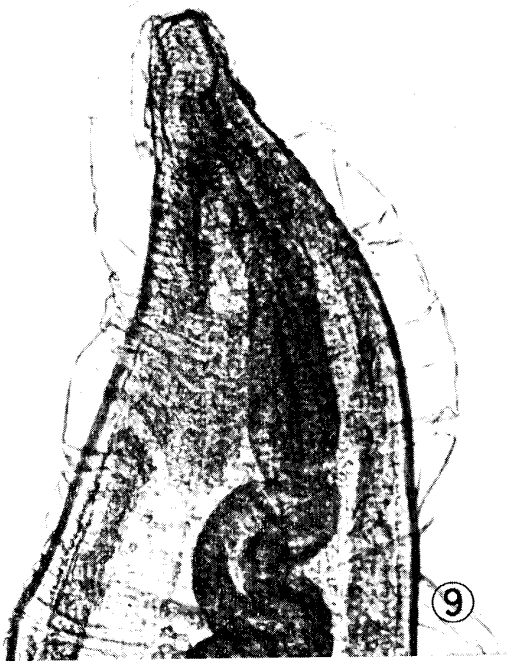
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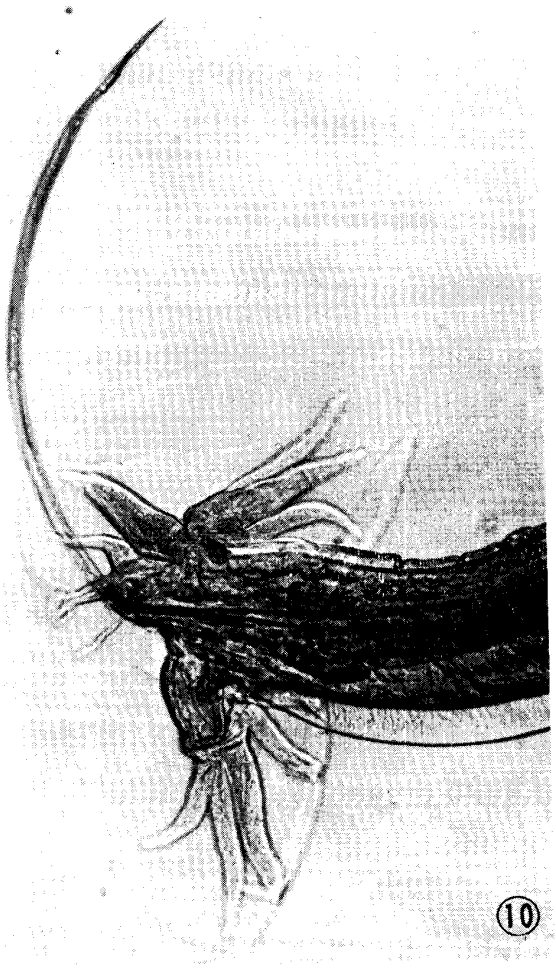
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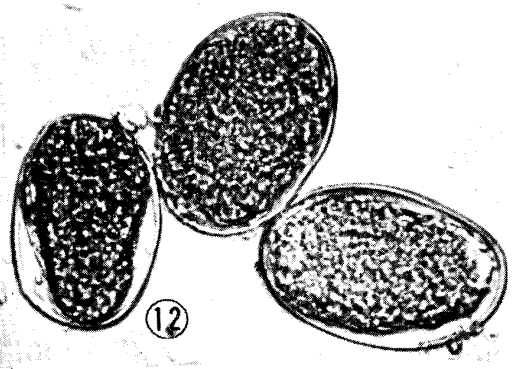
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PLATE III

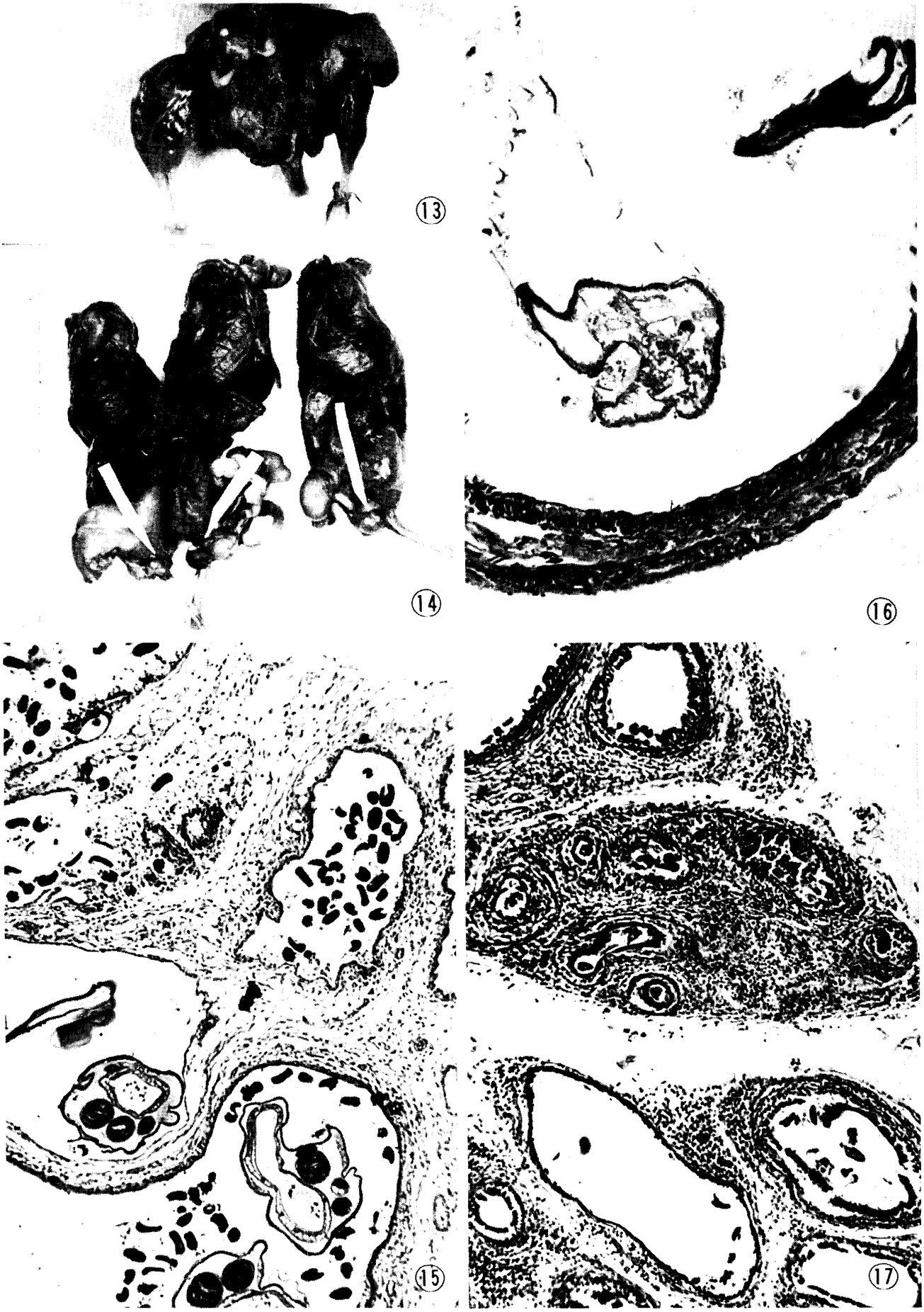
Fig. 13 Inguinal mammary glands of female *Apodemus argenteus hokkaidi* showing parasites. Flayed material × 1.2

Fig. 14 Cystically enlarged bulbo-urethral glands (arrow) of male *A. argenteus hokkaidi*. Flayed material × 0.8

Fig. 15 Mammary gland of *A. argenteus hokkaidi* showing an increase of interstitial tissue and dilated lactiferous ducts with parasites and eggs × 80

Fig. 16 Bulbo-urethral gland of *A. argenteus hokkaidi* showing cystic dilatation and a parasite in situ × 180

Fig. 17 Prostate gland of *A. argenteus hokkaidi* showing and increase of interstitial tissue, dilatation of gland tubules etc. × 80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