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Anatomy, histology and maturing of the metacercaria of Lacunovermis macomae (Trematoda: Gymnophallidae) from brackish-water Macoma balthica (southwestern Finland, Baltic Sea)

Marketta Pekkarinen

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The trematode from *Macoma balthica* (L.) here studied has the characteristics typical to the genus *Lacunovermis*, the ventral pit and wide genital pore some distance anterior to the ventral sucker. Other characteristics are quite similar to those described earlier for *Lacunovermis macomae* (Lebour).

The position of the ovarium was nearly in the proportion of 50:50 on the right side or on the left side of the worm. Tegumentary spines were broad on the face and wedge-shaped in profile; they were in fact "multiple" spines, usually with 2–4 individual spines side by side. The subtegumentary cells, the outer tegument and filamentous surface coat were alcianophilic, thus largely composed of acid mucosubstances. Large lipid droplets were present in the cells of the digestive caeca.

In brackish-water cultures in vitro at 37°C sperms began to form within 12 h, and they filled the seminal vesicle in 24 h. Later they were seen further up the genital tract, finally in the fertilisation space near the ovarium. The first capsulated eggs formed in 48 h. More viable and a greater number of eggs (13–36 in number) were obtained when metacercariae were incubated subcutaneously in mice for 55–75 h. In the adults dissected from the mice the tegument was less alcianophilic, the size of the digestive caeca was reduced and their lipids had decreased compared to metacercariae. In some individuals lipids had increased in other tissues. Alcianophilic cells had appeared in the Mehlis' gland.

Smaller metacercariae with enormous digestive caeca, with small suckers and with "double" spines on the thin tegument, were also often present in the extrapallial space of *M. balthica*. They may be the younger stages of *L. macomae*, or they may be metacercariae of a different species.

Marketta Pekkarinen, Division of Physiology, Department of Zoology, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki 10, Finland.

1. Introduction

The taxonomy and identification of larval stages of gymnophallid species have long been unsettled (c.f. James 1964, Loos-Frank 1971a, Ching 1972, 1973a). Many species need to be re-examined and re-described to clarify the often confusing situation respecting gymnophallid trematodes (Ching 1972). Further confusion is created by the existence of sympatric sibling species which have the same morphological and anatomical features but which can be distinguished on the basis of biological and ecological factors (Bartoli 1981).

The history of the nomenclature of *Lacunovermis macomae* is as follows: Lebour (1908) described a gymnophallid cercaria "Cercaria macomae", which lived between the mantle and shell of *Macoma balthica* (L.) in England. Jameson & Nicoll (1913) described an adult form *Gymnophallus macroporus* in scoter ducks (*Melanitta nigra*). Further, Markowski (1936) in Poland detected in *M. balthica* a metacercaria which he named "Metacercaria mutabilis". *Gymnophallus macroporus* was placed by James (1964) into the genus *Gymnophalloides*. Loos-Frank (1970), who found the metacercaria on the German coast of the North

Sea, discovered that "Cercaria macomae" and "Metacercaria mutabilis" were both metacercariae of *Gymnophallus macroporus*. She placed the trematode into the genus *Lacunovermis*, which was established by Ching (1965), and hence the name became *Lacunovermis macomae* (Lebour).

In the Baltic brackish water metacercariae of *L. macomae* have been described as occurring in *M. balthica* in the southern Baltic near Poland (Markowski 1936) and Germany (Reimer 1962). Also, its adults have been found in the long-tailed duck (*Clangula hyemalis*) along the Polish Baltic coast (Sulgostowska & Grytner-Ziecina 1974) and along the German coast (Reimer 1962). In Germany it was found also in the velvet scoter (*Melanitta fusca*) and common scoter (*M. nigra*) (Reimer 1962). The metacercaria found in *Macoma balthica* here in southwestern Finland (Pekkarinen 1983, 1984) has the typical characteristics of the genus *Lacunovermis*: the ventral pit and a wide genital pore (Ching 1973a). The dimensions and other anatomical characteristics of the metacercaria are given here and they are compared with earlier descriptions on the metacercaria of *L. macomae* from different geographical locations (Lebour 1908 and Loos-Frank 1970 from the North Sea and Markowski 1936 and Reimer 1962 from the southern Baltic).

There are extremely numerous histological descriptions of common trematode species, e.g. *Fasciola* and *Schistosoma* species, but there are few about less common trematodes. Histological and histochemical outlines of the metacercaria of *Lacunovermis macomae* and of adults obtained experimentally in subcutaneous cultivation in the laboratory mouse are given here. Measurements of the adults are compared with those of metacercariae and with other descriptions of the adults of *L. macomae*. A comparison is also made with the close species *L. conspicuus* Ching. The possible existence of metacercariae of one or more other species in the extrapallial space of *M. balthica* is discussed.

2. Material and methods

Metacercariae were taken from the shells of *Macoma balthica* collected in the neighbourhood of Tvärminne Zoological Station, southwestern Finland (59°50′ N, 23°15′ E), from a depth of 7-8 m (salinity about 6‰). Measurements (see Loos-Frank 1970) were made on living untreated, CO_2-narcotized, and fixed metacercariae. From living worms measurements were made without excess pressure but to give a better view of internal organs and for preparing figures the cover glass was pressed gently. The worms were fixed with cold or hot 4% formaldehyde in brackish water. Total preparations were made with Loos-Frank's (1970) procedure 3.

Bouin-fixed metacercariae were dehydrated through an ethanol series and embedded in paraffin after butanol.

Table 1. Basic measurements (μm, mean and range) and derived measurements of metacercariae of *Lacunovermis macomae* (untreated or prepared with different methods) from *Macoma balthica* and of *L. conspicuus* (Ching 1965) from *M. inconspicua*; present study and data from the literature. Derived measurements within parentheses are calculated from the means of the original measurements.

Source Method of preparation *N*	Present study Untreated 17		Present study CO_2-narcotized 12		Lebour 1908 Living?	Present study Total preparation (Loos-Frank 1970) 21		Present study Hot 4% formaldehyde 12	
Length	438	319-542	412	363-465	700	310	267-422	482	412-542
Breadth	226	177-273	234	186-288		229	192-267	231	198-264
Oral sucker length (OSL)	108	87-127	105	84-115	220-260	89	71-112	100	84-115
Oral sucker breadth (OSB)	118	90-152	105	87-118		106	84-133	110	93-121
Pharynx length	40	34- 53	42	40- 43		36	28- 50	41	37- 43
Ventral sucker length (VSL)	65	59- 74	65	46- 71	60-70	52	28- 87	64	56- 71
Ventral sucker breadth (VSB)	66	59- 71						63	59- 71
Ventral sucker distance (VSD)	276	198-335	285	239-338		206	167-270	325	288-363
Length × breadth × 10^{-3}	99		96			71		111	
Length/breadth	1.94		1.76			1.35		2.09	
Length/OSL	4.12[1]		3.92			3.48		4.82	
OSL/VSL	1.69[2]		1.62			1.71		1.56	
OSB/VSB	1.82[3]							1.75	
VSD/length	0.63		0.69			0.66		0.67	

[1]N = 15; [2]N = 13; [3]N = 5.

Sections (7 μm) were stained using the following methods: Masson-Gomori (chromotrope-fast green, Gray 1954), Alcian blue-PAS-Mayer's haematoxylin, ABPM (Mowry 1963, after Pearse 1968) and Crossmon's haematoxylin-acid fuchsin-orange-light green, HAOL (Romeis 1968). Lipids were demonstrated in formalin-fixed entire worms with Fettrot (Barka & Anderson 1963).

Metacercariae were fixed in 3 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h and then a sandwich was made from the clam foot and the metacercariae and this was further fixed for 2 h. It was postfixed in 1.5 % OsO_4 for 1.5 h, dehydrated through an ethanol series and embedded in Epon. Semi-thin sections were stained with toluidine blue.

Because there was a suggestion of a mixed infestation of metacercariae in the extrapallial space of *M. balthica*, the metacercariae isolated were divided into three categories for SEM examination: large worms, which had very high surface coats; medium-sized worms, which were more active and more slender than the large ones; and small worms which were colourless. Both large and medium-sized worms had pink suckers. The medium-sized and small worms had very long digestive caeca. The worms were fixed on membrane filters in 3 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), dehydrated, dried through the critical point and coated with gold. The preparations were examined with a Jeol JSM-35C scanning microscope.

In order to obtain adult worms, plenty of metacercariae were fed repeatedly to laboratory mice and to young chickens. During the experiment both types of host animals were fed commercial food, respectively. Their guts and faeces were examined after different time elapses. No adults, eggs or even traces of fed metacercariae were found. Then metacercariae were cultivated in vitro in natural brackish water at 37°C on brackish-water agar or on agar with meat extract. No antibiotics were used. Stages in the development to maturity were examined under a microscope after different periods of cultivation. Finally, metacercariae were injected in a small amount of brackish water subcutaneously into laboratory mice (neck region). Adults were dissected from the mice 55–75 h after the inoculation and they were put in Ringer's solution. Measurements were made on living adults and on formaldehyde-fixed (4 %) adults. 5 eggs were measured in each individual. Mouse tissues containing adult worms were fixed in Bouin's fluid and processed in the same way as the metacercariae above in conjunction with stained preparations. Lipids in adults were stained in the same way as in the metacercariae.

Present study 4% formaldehyde 20		Loos-Frank 1970 Ethanol or formalin 13		Markowski 1936 Sublimate	Ching 1965 (*L. conspicuus*) Hot water	
409	329-542	431	352-560	450	479	393-541
238	198-279	234	165-319	270	230	199-291
110	99-124	116	81-134	112		
115	96-149	133	97-165	129	127	112-154
43	37-56	51	42-60	33	49	39-65
62	56-71	62	54-71	68		
71	62-81			71	72	68-78
261	226-344	316	248-378		288	228-336
97		(101)		(122)	(110)	
1.72		(1.84)		(1.67)	(2.08)	
3.72		(3.72)		(4.02)	(3.77)	
1.77		(1.87)		(1.65)		
1.61[4]				(1.82)	1.75	
0.64		(0.73)			(0.60)	

[4] $N = 13$.

3. Results

The metacercaria (Figs. 1 and 10a) is almost colourless, with two pink suckers, with white digestive caeca and conspicuous vitellariae. It is dorsoventrally flattened, the anterior end or midbody being broadest, the head rounded and the tail pointed.

The results from several basic measurements obtained for the metacercariae using different preparation methods are given in Table 1 and these are compared with those given earlier in the literature for *Lacunovermis macomae* and *L. conspicuus*. Smaller metacercariae (about 140-370 μm in length) in which the genital organs were less conspicuous were excluded from the tables. The dimensions of living metacercariae were very difficult to obtain. Narcosis with CO_2 slightly contracted the metacercariae and their outlines became irregular. When fixed in hot formalin, metacercariae appeared quite long in proportion to their breadth, and those fixed in cold formalin were slightly contracted in length. Worms in entire animal preparations were much fore-shortened and were also rounded in the dorsoventral direction. Assuming that the dorsoventral dimension does not change, the formula length (μm) × breadth (μm) × 10^{-3} could be used as an indication of the size of the worms, independent of different contraction states. The mean size was quite similar in all the groups apart from the entire animal preparations, i.e. from 96 to 111. Calculated from the data of Markowski (1936) and Reimer (1962), it is 122 and 94, respectively, and from the results of Loos-Frank (1970) it is 101, the last-mentioned fitting into the range of the means in my results. The dimension for *L. conspicuus* calculated from Ching's (1965) data also falls into this range.

Total length : oral sucker length varied between 3.48 and 4.82, depending on the preparation method. This range also embraces Loos-Frank's and Markowski's figures for *L. macomae* and Ching's figure for *L. conspicuus*. The oral sucker in Lebour's meta-

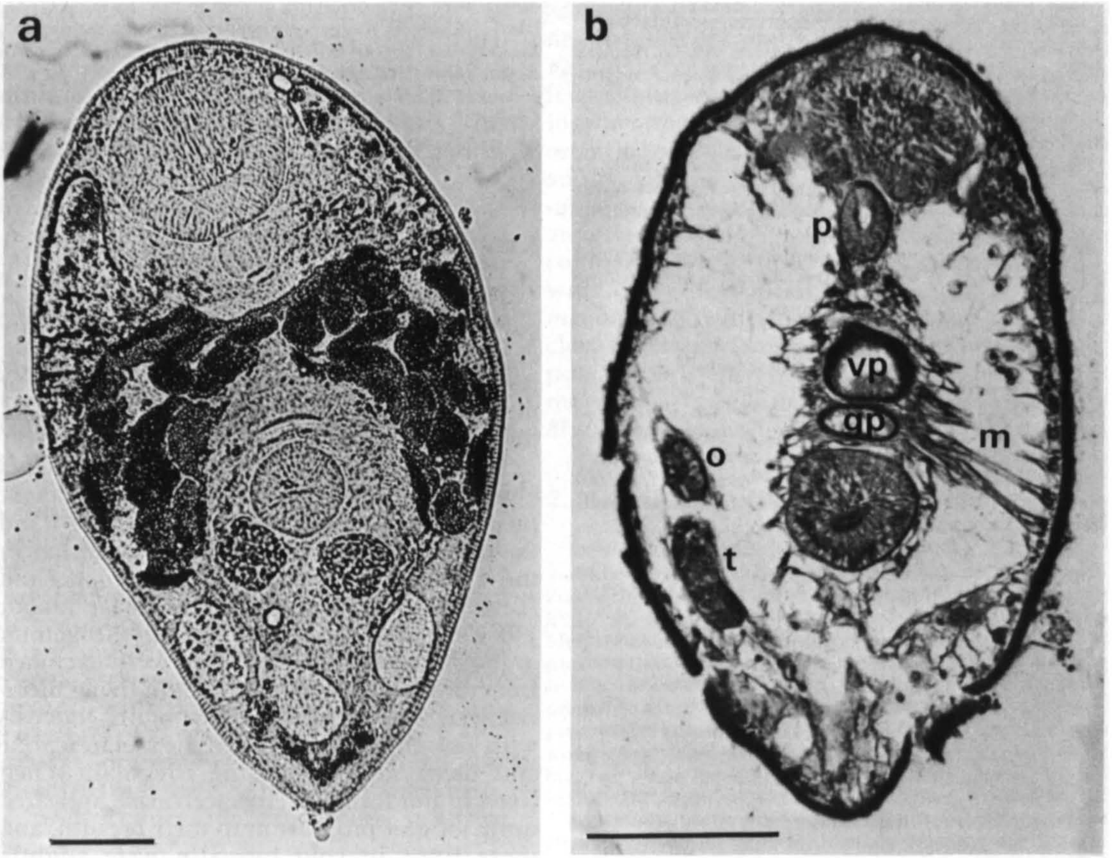

Fig. 1. — a. Ventral view of the metacercaria of *Lacunovermis macomae* from *Macoma balthica* pressed under a cover glass. Due to pressing the size has increased and the gonads and vitellariae have moved towards the rear. — b. Horizontal section, stained with Alcian blue-PAS-Mayer's haematoxylin, from the metacercaria. gp = genital pore, m = muscle, o = ovarium, p = pharynx, t = testis, vp = ventral pit. Scale bars 100 μm.

cercaria was larger. The pharynx is slightly shorter but similar in breadth to that of Loos-Frank's metacercaria. However, in Markowski's metacercaria it is shorter still. The ventral sucker is very slightly more anterior than in Loos-Frank's description, but the size of the sucker is quite similar.

Other dimensions of the metacercariae are compared below as the range of the means of three different preparations, i.e. living, living-CO_2 and formalin-fixed metacercariae, with the mean value of Loos-Frank's fixed metacercariae in parentheses. The digestive caeca are usually very long, being 114-177 μm (127). They usually extend behind the anterior margin of the ventral sucker. The caecum in front of the ovarium often extends slightly further backwards than the other one. The vitellarium towards the ovarium is usually slightly more anterior than the other one. Its size is 39-45 × 35-38 μm (46 × 41). The ovarium, being 41-48 × 34-38 μm (58 × 42), is not very much larger than the testis, 35-45 × 35-37 μm (45 × 36), as in Loos-Frank's data. The position of the ovarium in 51 % of individuals is on the right side and in 49 % on the left side (n = 110). In 5 % of metacercariae the ovarium was behind the testis. Exact measurements were very difficult to make on the gonads because they were somewhat overlapping and in living worms their shape altered. Other internal genital organs are as Loos-Frank describes. The beginning of the uterus leaving the Mehlis' gland is quite large, with circular streaks. The uterus first coils in the hindbody and then coils laterally to the

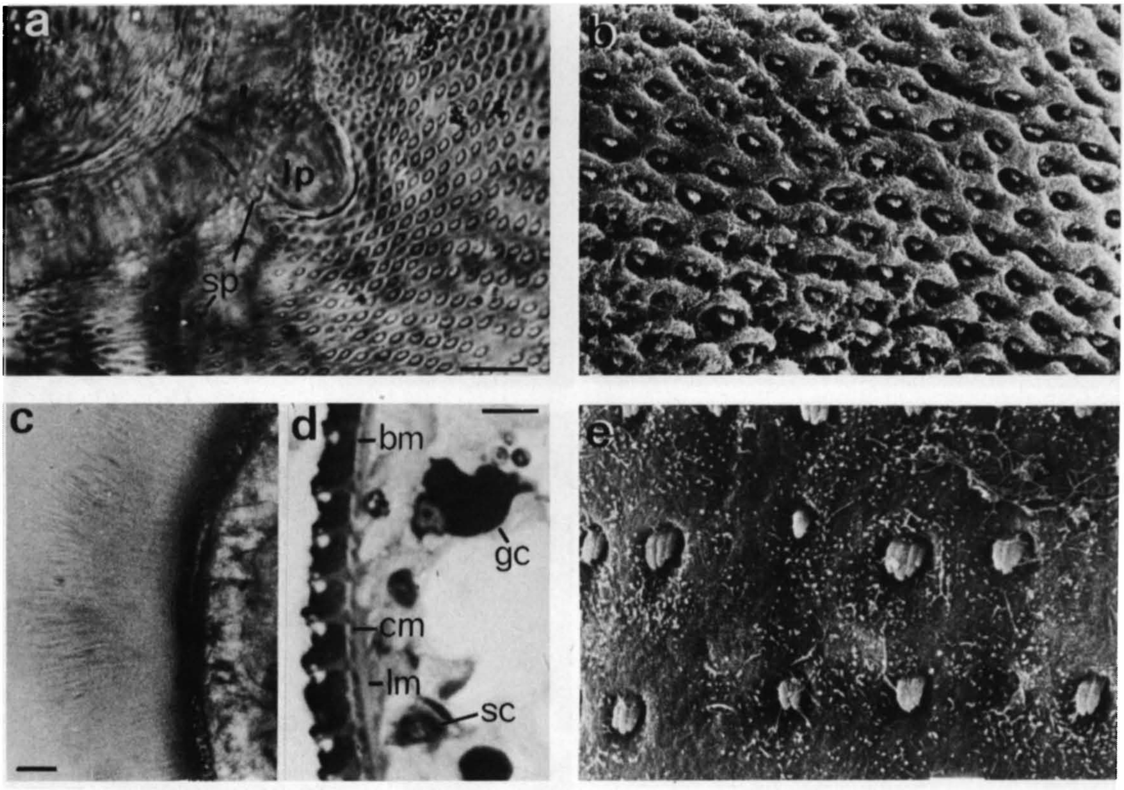

Fig. 2. The tegument of the metacercaria of *Lacunovermis macomae*. — a. Tegument near the oral sucker and its lateral projection (lp) in living animal. sp = sensory papilla. — b. A SEM picture of the tegument. — c. Filamentous surface coat. — d. Longitudinal Epon section stained with toluidine blue from the tegument. Anterior side uppermost. bm = basement membrane, cm = circular muscle, lm = longitudinal muscle, gc = granular cell (single granules not visible), sc = subtegumentary cell (tegumentary cell body). — e. Spines in stretched ventral posterior surface. Scale bars 10 μm in a and c, 1 μm in b and e, and 5 μm in d.

ventral sucker on the opposite side to that on which the ovarium is situated. The seminal vesicle is club-shaped and situated anteriorly, laterally or overlapping the ventral sucker. The vasa efferentia join together just at the pouch of the seminal vesicle.

The outer tegument is decorated with minute pits so that it has the appearance of a meshwork (Fig. 2a-b). Each pit is equipped with a spine, about 3-4 μm long. The spines are broad on their face and wedge-shaped in profile (Fig. 2d) with an oblique base. The posterior corner of the base projects through the basement membrane. The spines do not usually protrude much out of the tegument. When they are examined at a greater magnification with the SEM, they are found to be "multiple" spines, i.e. each apparent spine usually consists of 2-4 individual spines attached to each other side by side (Fig. 2e). The tegument is covered with a filamentous surface coat (Figs. 2c and 10a), 3-20 μm high, sometimes still higher. It was not visible on all individuals.

The oral sucker is equipped with two retractile lateral papillae (Figs. 2a, 3a and 10a). Minute ciliated sensory papillae are concentrated especially in the head region and on paired longitudinal areas on the ventral surface from head to tail. The ventral sucker has an outer circle of six papillae (arranged as three on each side) (Fig. 3b) and an inner circle of six papillae. The genital pore has two larger papillae on the posterior wall (Fig. 5a-b) and smaller papillae are also present around the genital pore and on the lateral sides of the

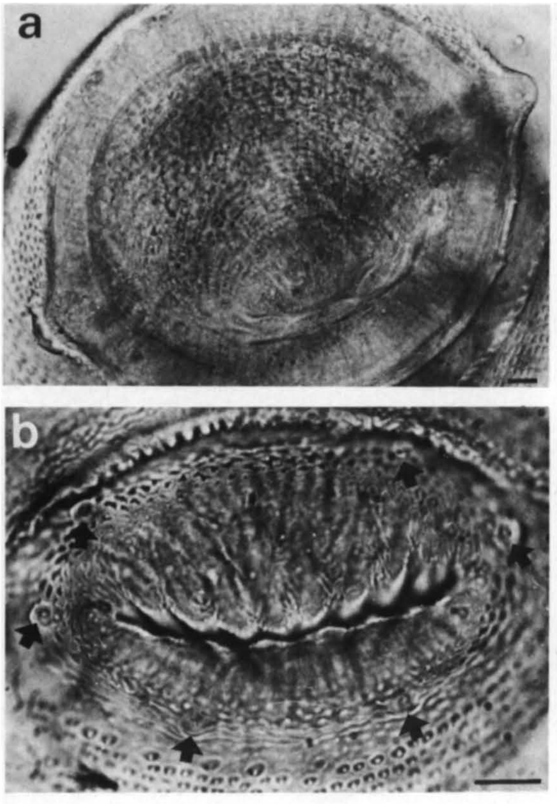

Fig. 3. The suckers of *L. macomae.* — a. Oral sucker with its lateral papillae. — b. Ventral sucker with six outer papillae (arrows). Scale bars 10 μm.

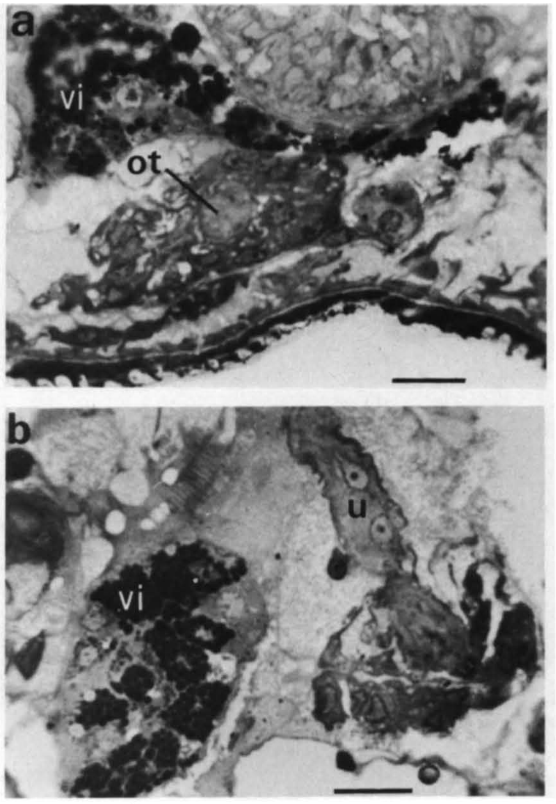

Fig. 4. Semi-thin sections through the Mehlis' gland of the metacercaria stained with toluidine blue. — a. Ootype (ot) cross sectioned and the Mehlis' gland surrounding it. Ventral side uppermost. — b. Mehlis' gland and longitudinal section of the uterus (u). Anterior side to the left. vi = vitellarium. Scale bar 10 μm.

ventral pit anterior to it. The most prominent nerves leaving the cerebral ganglia are the ventral nerve cords and anterior nerves. Branches from these run, for example, to the suckers.

The ventral pit is an invagination of the ventral surface. There is an outbulging of the ventral surface between the ventral pit and the genital pore (Figs. 5d and 10a). The genital pore (Fig. 5) is a transverse slit, as wide as the ventral pit, leading to the genital atrium, in the posterior wall of which the gonoduct opens. The excretory system with its 2 × 12 flame cells is as Loos-Frank (1970) describes. The excretory vesicle contains refractile granules (Fig. 6).

In the body wall there are circular, longitudinal and diagonal muscle fibres. The longitudinal ones are the most prominent of these (Fig. 2d). In the parenchyma there are dorsoventral (Fig. 5d) and transverse muscles (Figs. 1b and 5d). The transverse muscles are attached to the sides of the genital pore and the ventral pit. The muscular suckers are well equipped with muscles moving them in different directions. The paired muscles running from the oral sucker to the neighbourhood or to the sides of the ventral sucker are strong.

The tegument, including the insides of the suckers, ventral pit, genital atrium and the gonoduct opening, is very alcianophilic (Figs. 1b and 5c), as is the surface coat. A slight PAS-positive shade may also exist in the tegument of some individuals. In toluidine blue stained Epon sections the tegument appeared blue to violet and the surface coat violet. Also, the noncellular cyst walls around the metacercariae on the inner surface of clam shells stained meta-

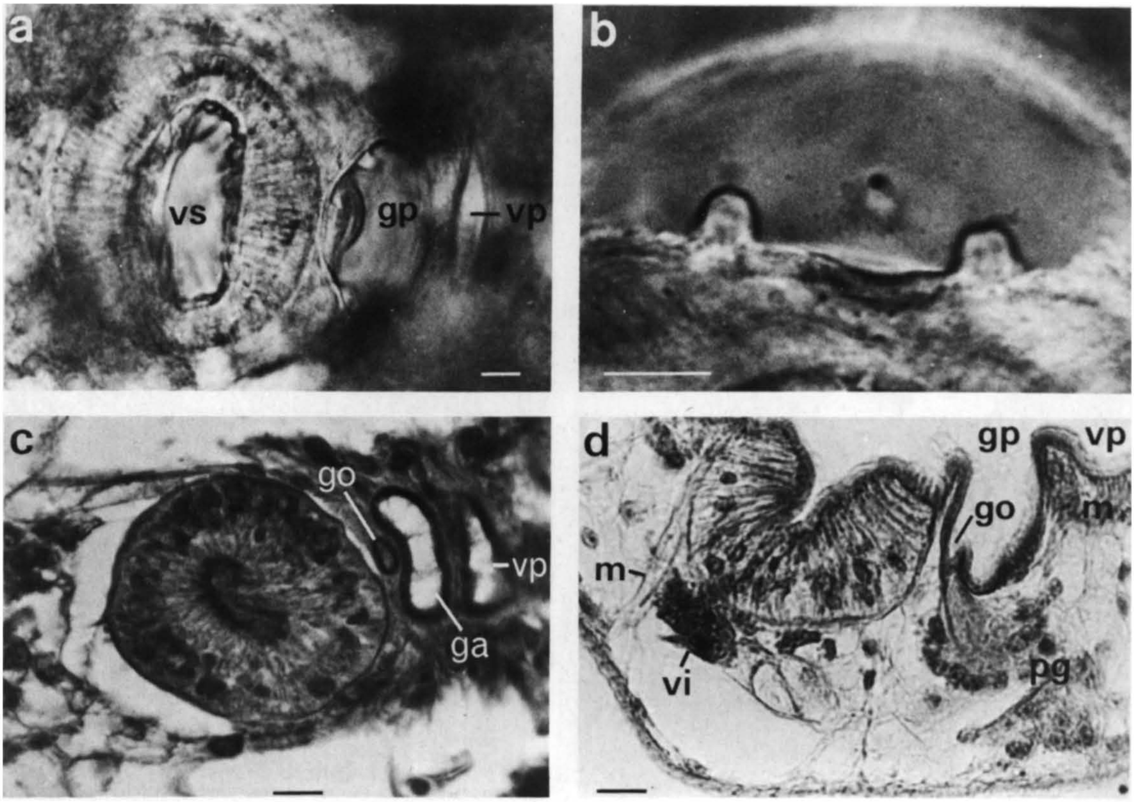

Fig. 5. Genital pore and its associate structures in the metacercaria of *L. macomae*. — a. Ventral sucker, genital pore and ventral pit. Anterior side to the right. The small elevation in the genital atrium is the site where the gonoduct opens. — b. Genital pore and its papillae. Anterior uppermost. — c. Horizontal section from the same place as in a. Alcian blue-PAS-Mayer's haematoxylin. — d. Midsagittal section from the same site as in a, stained with Masson-Gomori. Key to symbols: ga = genital atrium, go = gonoduct opening, gp = genital pore, m = muscle, pg = prostatic gland, vs = ventral sucker, vi = vitellarium, vp = ventral pit. Scale bars 10 μm.

chromatically reddish. Masson-Gomori and HAOL specifically stain spines orange-red, while the tegument itself appears greenish-orange or greyish. There are numerous alcianophilic cells under the outer tegument and between the muscle cells of the suckers. Apparently the same cells have violet flecks in their cytoplasm when stained with toluidine blue. Some cells with pink homogenous cytoplasm and light nucleus were occasionally seen among them. Such cells were twice encountered in anaphase. In the head region there are sometimes cells which show both alcianophilia and PAS-positivity. Granular gland cells, which may often be very large, stain only yellow-brownish in ABPM but intense orange-brown in Masson-Gomori triple staining and red in HAOL. The granules stained with toluidine blue (Fig. 2d).

PAS-positivity was seen in the muscles, parenchyma cells and gonoduct walls. The contents of the digestive caeca were PAS-positive, as were long microvillus-like projections of the digestive cells. The proximal parts of the prostatic gland cells opening into the ejaculatory duct were PAS-positive. The cells were long and very finely granular. The cells of the Mehlis' gland were moderately PAS-positive. Their nuclei were small and irregular. In Epon sections the cells appeared to contain vacuoles (Fig. 4). Vitelline gland cells stained intensively orange-brown or orange-red in Masson-Gomori and HAOL. In ABPM they appeared only brownish-yellow. In toluidine blue stained Epon sections of vitelline glands there were cells with intensively staining large globules in pink cytoplasm and cells with small granules or with no granules

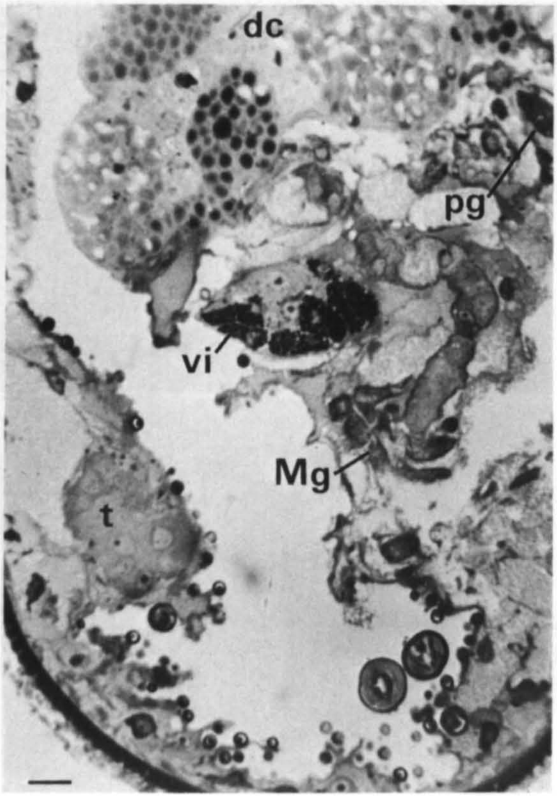

Fig. 6. Hindbody of the metacercaria showing the distal part of the digestive caecum (dc), excretory vesicle, Mehlis' gland (Mg), vitellarium (vi), testis (t) and part of the prostatic gland (pg). Toluidine blue. Scale bar 10 μm.

Fig. 7. Four individuals of *L. macomae* in the subcutaneous tissue of a mouse surrounded by infiltrated mouse white blood cells. The uppermost individual is seen in horizontal section, the one next to it in cross section. Only a tangential section of the back of the third one is visible. The fourth one, which has been invaded by mouse cells, has probably been damaged during the injection. Masson-Gomori. Scale bar 100 μm.

(Figs. 4 and 6). The globules contained small granules at the periphery. The nuclei were central.

The excretory granules had shades varying from pink to violet or blue when stained with ABPM or toluidine blue (Fig. 6). Smaller granules along the walls of the excretory vesicle had a slightly more red tone in toluidine blue than the larger ones. In Masson-Gomori and HAOL the granules were greenish. Flame cells stained bright red in HAOL, and in Masson-Gomori the basal plate of the cell stained green and the flagellae reddish-brown. In ABPM the flagellae were totally colourless (Fig. 8b).

The digestive cell droplets, which appeared white in paraffin sections, stained with OsO_4 and toluidine blue (Fig. 6). Using Fettrot, these droplets were found to consist of lipid. Lipid droplets were also present among the musculature of the suckers and in the ovarium. In some individuals small lipid granules were also present in the excretory vesicle. The vitelline gland cells did not stain with Fettrot.

When metacercariae were incubated in vitro at 37° C in brackish water, sperm tails appeared in the testes within 12 h so that some of the testis lobules appeared fibrous. The digestive caeca showed a great reduction in size in 12 h. Within 24 h the seminal vesicle had distended and was full of spermatozoa (c.f. Fig. 8c). The common vitelline reservoir was also distended and full of vitelline cells (c.f. Fig. 8a). Finely granular gland ducts were very prominent in the tegument. Sperms were also seen in some individuals in the uterus and in the ootype. The eggs were, however, smaller than those cultivated in vivo, and abnormal eggs and vitel-

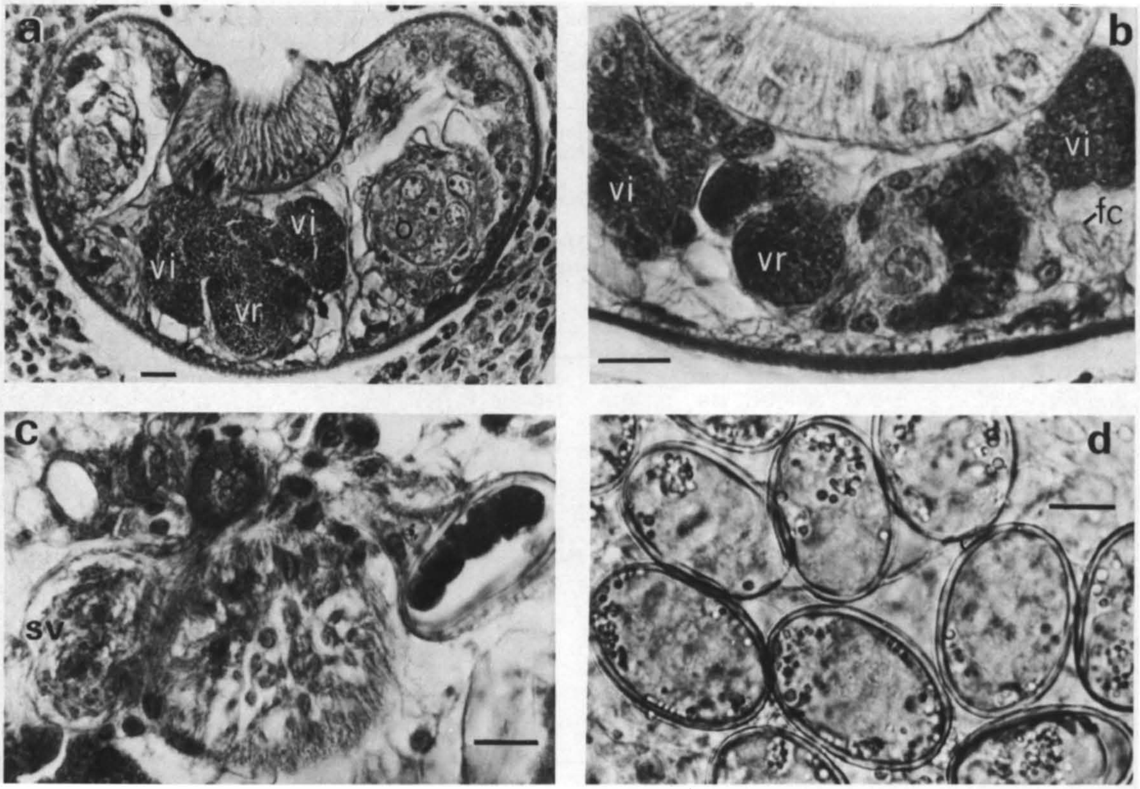

Fig. 8. Signs of maturation in *L. macomae* incubated in tissues of mouse for 55 h. — a. Cross section of an individual showing vitelline glands (vi) and the distended full vitelline reservoir (vr). o = ovarium. Ventral side uppermost. ABPM staining. — b. Cross section at the site of the Mehlis' gland, ootype and vitellariae. The darker cells of the Mehlis' gland are Alcianophilic and the lighter cells PAS-positive. Ventral side uppermost. fc = flame cell. — c. Horizontal section showing the base of the ventral sucker at the centre and to the leaf of this the full seminal vesicle (sv) and the right uterus containing an egg. HSOL staining. — d. Embryonated eggs in a living individual. Scale bars 10 μm in all pictures.

line clumps were sometimes present in the uterus. Heavy microbial infestation occurred in the medium. Mortality was great, and those alive remained quiescent with the oral sucker gaping.

When cultivated subcutaneously in mice for 55 h the number of eggs in the worms was usually greater than in vitro and their size was more even (Fig. 8d). The eggs with yellow capsules contained vitelline cells together with an ovum or embryo. Continuing the incubation time up to 75 h did not lead to an increase in the mean number of eggs developed. There was some infiltration of mouse white blood cells around the worms and some fibrous capsule formation could be seen (Fig. 7).

In the adult worm (Fig. 9) the tegument was less alcianophilic than in the metacercaria (c.f. Figs. 1b and 9b). The spines of the adults were similar to those of the metacercariae. Alcianophilic cells had appeared in the Mehlis' gland (Fig. 8b). In Masson-Gomori the Mehlis' gland was greenish and in HAOL reddish with a blue cast. Lipids in the digestive caeca had greatly decreased, but in some individuals they had increased a lot in other tissues and in the excretory vesicle.

Of the adults, 52 % were right-sided (i.e. the ovarium was on the right side) and 48 % left-sided ($n = 21$). Measurements on the adults are given in Table 2. The adults obtained from mice were on average smaller than the metacercariae. The mean length × breadth was the same as Loos-Frank (1970) obtained. The worms in this study were, however, shorter and broader (the length : breadth ratio was smaller than in the earlier study by Loos-Frank). The oral sucker in proportion to body length was

Table 2. Basic measurements (μm, mean and range) and derived measurements of adult *Lacunovermis macomae* (untreated or prepared with different methods) and of *L. conspicuus* (Ching 1965); present study and data from the literature. Derived measurements within parentheses are calculated from the means of the original measurements.

Source	Present study				Jameson & Nicoll 1913	Loos-Frank 1970		Sulgostowska & Grytner-Ziecina 1974	Ching 1965 (*L. conspicuus*)	
Infection	Subcutaneous cultivation 2-3 days				Natural	Experimental 9 days		Natural	Experimental 5 days	
Host	Laboratory mouse				*Melanitta nigra*	*Somateria mollissima*		*Clangula hyemalis*	*Peromyscus maniculatus*	
Method of preparation	Living		4% form-aldehyde		?	Ethanol or formalin		Ethanol-glycerol-formalin	Hot water	
N	8		9			11			7	
Length	335	279-453	342	291-474	400-500	449	350-581	310-390	431	388-496
Breadth	202	146-229	216	167-270	140-230	164	130-176	110-170	196	165-256
Oral sucker length (OSL)	116	99-130	110	87-133		126	111-141	90-126		
Oral s. breadth (OSB)	122	108-130	128	108-149	140-170	133	111-148		131	114-154
Pharynx length	39	37-43	43	35-49	40	47	44-50	23-28	47	39-52
Ventral s. length (VSL)	62	43-74	61	46-71		64	57-74	42-58	74	68-91
Ventral s. breadth (VSB)	67	59-78	74	60-81	65-75					
Ventral s. distance (VSD)	233	192-310	222	180-291	300-400	310	274-391		234	205-279
Range of egg size	29-31 × 16-19		26-32 × 16-18		29-34×15-20	33-37 × 18-22		28-30 × 13-16	26-34 × 12-16	
Mean egg size	30 × 18		30 × 16		32×18	34 × 21				
Number of eggs	15 - 36		13 - 23		30 - 100				9 - 33	
Length × breadth × 10^{-3}	68		74			(74)			(84)	
Length/breadth	1.66		1.58			(2.74)			(2.20)	
Length/OSL	2.89		3.11			(3.56)				
OSL/VSL	1.87		1.80			(1.97)		2.50		
OSB/VSB	1.82		1.73		2.25	(2.08)			1.80	
VSD/length	0.70		0.65			(0.69)			(0.54)	

larger than in the metacercaria. The ratio of body length : oral sucker length was about 3, while in the metacercaria it was around 4. Some increase in the size of the vitellariae was noted (size 44-47 × 37-42 μm as the range of the means in living and in fixed adults). This also occurred in the *L. macomae* studied by Loos-Frank (1970) (size 55 × 46 μm) and in the *L. conspicuus* studied by Ching (1965).

The eggs were quite similar in size to those which Jameson & Nicoll (1913) obtained from *Melanitta nigra*, but smaller than those obtained experimentally by Loos-Frank in *Somateria mollissima*. The ova measured by Sulgostowska & Grytner-Ziecina were narrower than those in the other studies, as were the eggs of *L. conspicuus*.

The smaller metacercariae, which were colourless and measured about 140-370 μm in length, obtained from the extrapallial space of *M. balthica*, had small suckers (Fig. 10b) and a proportionally large pharynx (Fig. 11a), 31-41 μm in length. Their length × breadth was about 20-60. In living metacercariae the length of the oral sucker was 34-84 μm and that of the ventral sucker 31-56 μm. Total length : oral sucker length was 4.3-7.2. The sucker ratio was 0.7-1.4. The ventral sucker is situated at a point 2/3rds of the total length from the anterior end, and it has 6 + 6 papillae. The musculature of the suckers is not highly developed. In the smallest specimens the genital pore and lateral projections of the oral sucker were not visible. Rudiments of lateral papillae may be present in larger specimens. The tegument is puckered anterior to the ventral sucker (Fig. 10b) so that the presence of any rudimentary ventral pit cannot be ascertained. The outer tegument is much thinner than that in *L. macomae* described above. Large round nuclei are found in the cells of the sucker tissue and under the tegument. The tegument is covered with slender spines (Fig. 10b), of which, when pressed with a cover glass, about 1.5-2.5 μm are visible. The spines are actually "double" spines, as shown in Fig. 11b. The digestive caeca are enormous (Fig. 11a), being 87-133 × 40-62 μm. Due to this, flame cells could not be seen and the gonads were not visible either in the smallest individuals.

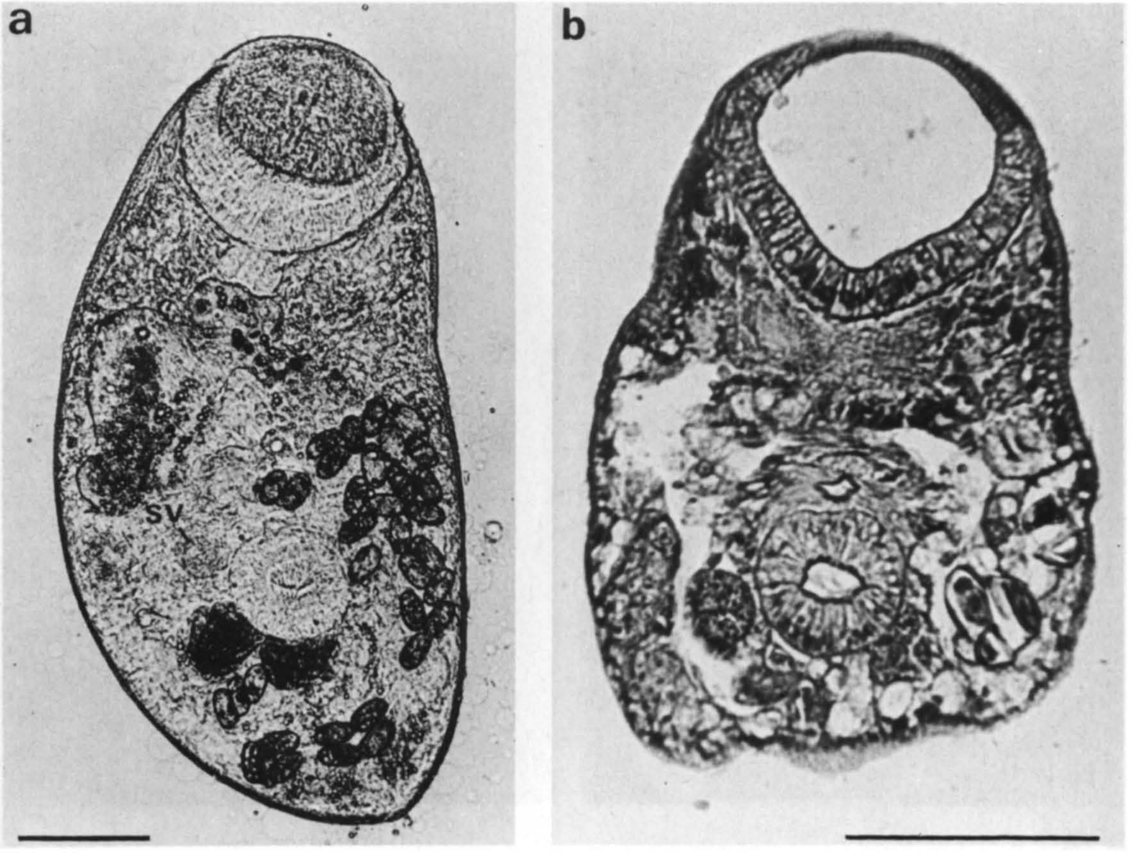

Fig. 9. Ventral views of mature *L. macomae* dissected from the mouse. — a. Slightly pressed living individual. sv = seminal vesicle. The distribution of eggs shows the situation of the uterus. —b. Horizontal section stained with ABPM. Scale bars 100 μm.

Four long- and sharp-spined adults were obtained from the mice besides typical *L. macomae*. Their lengths were 280-290 μm and sizes 42-69. The oral sucker length was 56-87 μm, pharynx length 31-41 and ventral sucker length 46-59 μm. Total length : oral sucker length was 3.2-5.2 and the sucker ratio 1.0-1.2. One of them was immature, with a voluminous excretory vesicle. The eggs of the mature worms were 27 × 18 μm (range of individual eggs 24-32 × 17-21 μm). The spines of the tegument (Fig. 11c) were as long as 3.0-4.7 μm.

4. Discussion

The presence of the ventral pit anterior to a wide genital pore distinguishes the genus *Lacunovermis* from all other gymnophallid genera (Ching 1965, 1972, 1973a). The type species is *L. conspicuus* Ching. As early as 1908 Lebour depicted a "peculiar structure X" with a rim and muscles radiating from it in front of the ventral sucker of the metacercaria of *L. macomae* ("Cercaria macomae"). It may have been the ventral pit or the genital pore. According to Markowski (1936) there were small folds laterally to the genital pore. When the metacercaria contracts, the ventral tegument really becomes puckered, and when much contracted, the genital pore and ventral pit may become nearly hidden. Loos-Frank (1970) has drawn the genital pore of *L. macomae* in her figure rather small, but this probably represents the gonoduct opening in the genital atrium. In my examinations the genital pore of the metacercaria is wide transversally and often slit-like. Lateral projections

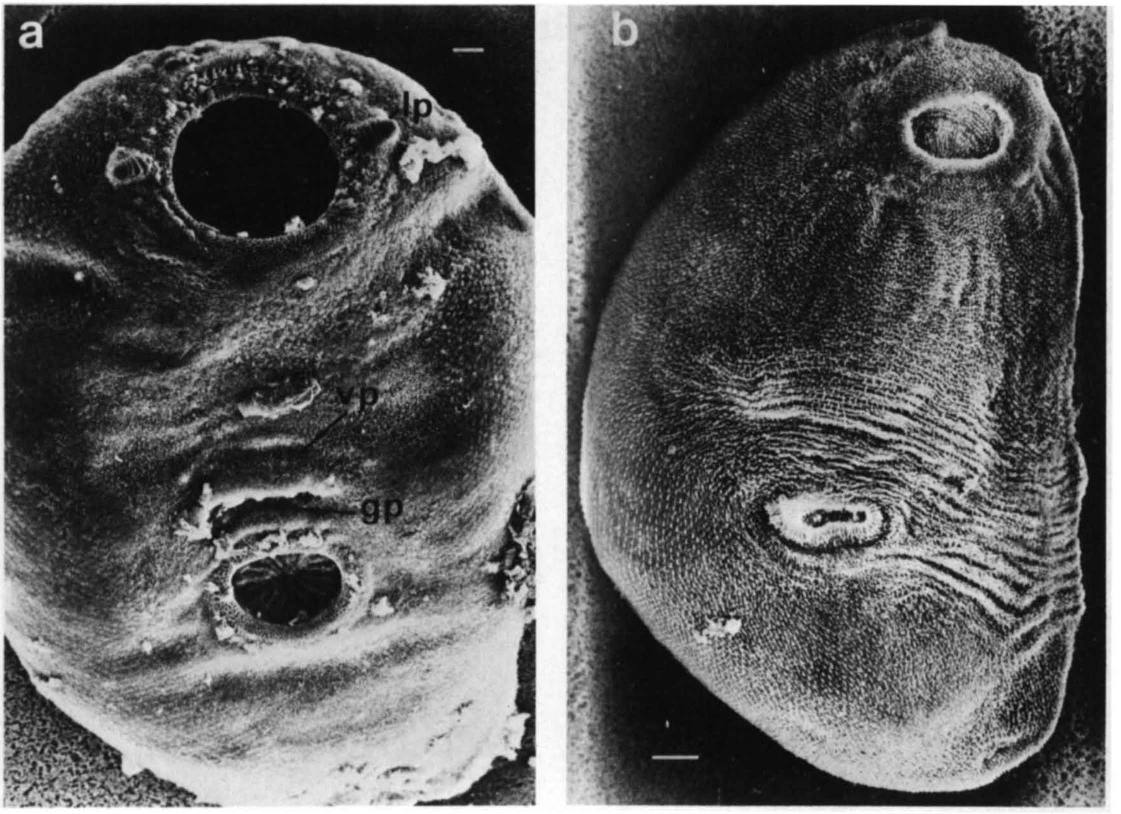

Fig. 10. Surface views of the metacercaria of *Lacunovermis macomae* (a) and the unknown smaller metacercaria (b) from the extrapallial space of *Macoma balthica*. The surface coat hides the details of the tegument of this individual of *L. macomae*. gp = genital pore, lp = lateral papilla, vp = ventral pit. The tegument of the metacercaria in b is covered with slender spines. Scale bars 10 μm.

are present on the oral sucker in both *L. conspicuus* and in *L. macomae*. They are retractile and thus may go unnoticed in the retracted state.

Loos-Frank (1970) did not mention large papillae on the posterior wall of the genital pore of *L. macomae*. In the metacercaria here studied they are present and according to Ching (1965) they are also present in *L. conspicuus*.

The digestive caeca reach beyond the anterior margin of the ventral sucker in the metacercaria studied here, as in Loos-Frank's description of *L. macomae*. Also in *L. conspicuus* they extended posteriorly to the level of mid-acetabulum. Large vitellariae, also ascertained in this study, are typical to both *L. macomae* and *L. conspicuus*.

There are some differences between *L. macomae* and *L. conspicuus*. One is found in the excretory system. The stem of the Y-shaped excretory vesicle is somewhat distended in *L. macomae* and the arms reach dorsally to the oral sucker, also in the metacercaria studied here, while in *L. conspicuus* the excretory vesicle is V-shaped and the arms reach to the level of the oeasophagus. The proximal parts of the arms are narrower in *L. macomae* than in *L. conspicuus*. Ching (1965) found only 2((2 + 2) + (2 + 2)) flame cells in the metacercaria of *L. conspicuus*, while the metacercaria studied here and *L. macomae* have 2((2 + 2 + 2) + (2 + 2 + 2)) flame cells. Because of these differences from *L. conspicuus* and the similarities with *L. macomae*, the metacercaria studied here is suggested as belonging to *L. macomae*.

The length alone does not reliably charac-

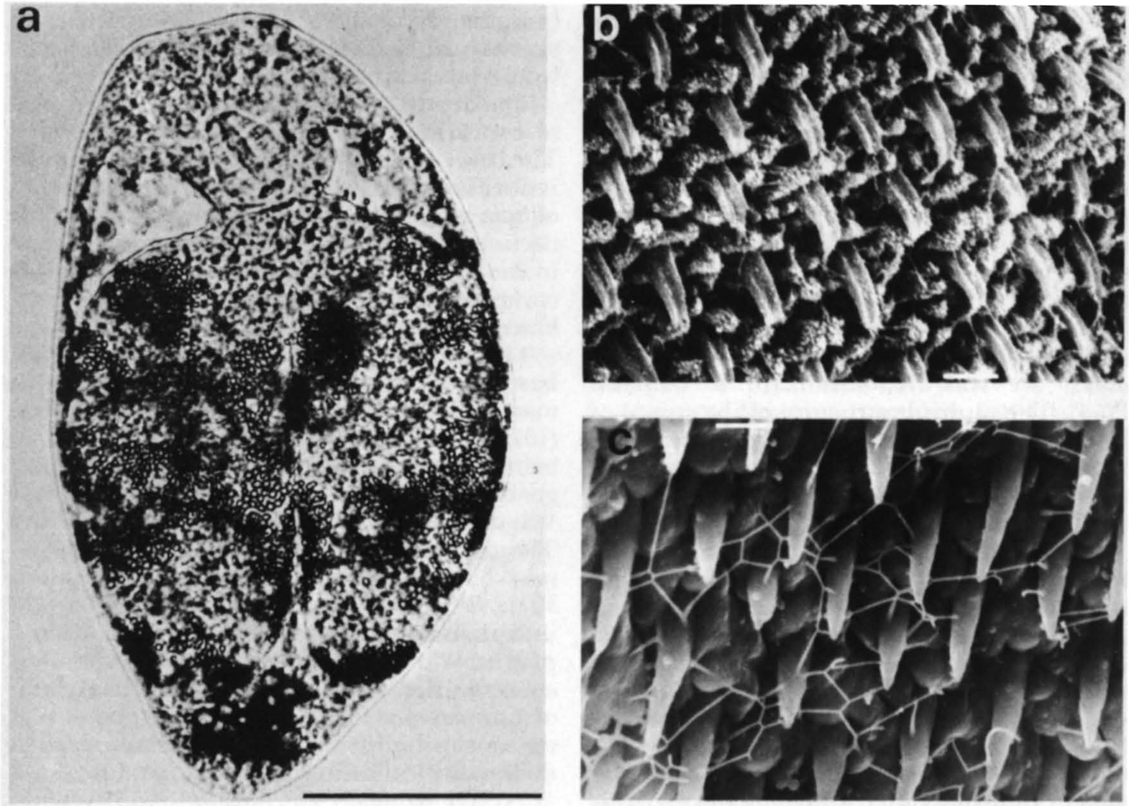

Fig. 11. — a. The "small metacercaria" from *M. balthica* pressed under a cover glass. — b. Spines of the small metacercaria (c.f. Fig. 2e). — c. The surface of a "long- and sharp-spined adult" matured in the mouse (c.f. b above). — Scale bars: 100 μm in a, 1 μm in b and c.

terize worm sizes. In addition to different degrees of shrinkage due to different fixation methods, the length in proportion to breadth of the worms varies due to varying degrees of contraction. If the dorsoventral dimension does not vary, rough estimates of their sizes can be calculated by multiplying the length by the breadth. The metacercariae in this study thus calculated were of similar size to those studied by Loos-Frank. However, Loos-Frank mentions that the worms are almost round in cross section, while in my study they are flattened.

The size of the testis in my study was quite similar to that in Loos-Frank's study, but the ovarium was not larger than the testis to the degree that Loos-Frank states. This may result from the difficulties in measuring exact sizes of the gonads.

It was earlier supposed that the ovarium is situated on the right side of the worm (Lebour 1908, Jameson & Nicoll 1913). Markowski (1936) also occasionally found it on the left side. Here it was estimated to be randomly situated on the right, or on the left, side. The occasional location of the ovarium behind the testis may result from the great mobility of the gonads. According to Ching (1965), the ovarium of *L. conspicuus* is usually dextral.

Lebour (1908) and Markowski (1936) found minute spines on the tegument of *L. macomae*. Loos-Frank (1970) does not refer to the tegumental structure. The surface of *L. conspicuus* is also covered entirely with spines (Ching 1965). The entire tegument of "Cercaria baltica" which is suggested as the cercaria of *L. macomae* is equipped with minute spines (Markowski 1936, Loos-Frank 1971a).

The spines of *L. macomae* were found in this study to be broad on the face, and wedge-shaped in profile. *Meiogymnophallus minutus* (Cobbold) (= *Gymnophallus oedemiae* Jameson & Nicoll in partim) also has broad spines, rounded at the tips (Loos-Frank 1971a), although Jameson (1902) depicts them as broad but pointed. The body of *Paragymnophallus odhneri* (= *G. somateriae* sensu Odhner) is heavily spined, the spines being broad and slightly pointed and arranged in alternate transverse rows (Ching 1973a). The spines of *Parvatrema borealis* Stunkard & Uzmann are scale-like (Stunkard & Uzmann 1958). The multiple structure of the spines of *L. macomae* now seen under the SEM cannot be seen with the light microscope. Thus far the author has not seen any SEM pictures of the spines of other gymnophallid species.

The small metacercariae in this study, despite having long and slender spines, cannot with certainty be distinguished as a different species owing to the double nature of the spines. The individual spines were not as tapering and sharp-ended as those of *Schistosoma* species (Kuntz et al. 1977, Sakamoto & Ishii 1977). A question is posed as to whether the double spines could broaden so as to later possess four individual spines as the metacercaria grows. The spines of *Haematoloechus medioplexus* are intracellular organelles and consist of crystalline protein (Burton 1964). The tegument of the cercaria of *Schistosoma mansoni* resembles that of the adult in its basic organisation. The outer layer of the cercarial tegument is thinner than that of the adult. Although the cercarial spines are smaller than those of the adult, they possess an identical structure (Morris 1971). The tegumental spines of the metacercaria of *Parvatrema homoeotecnum* James are 2.5–3 μm long (James 1964). The spines of *G. gibberosus* Loos-Frank depicted by Loos-Frank (1971b) are pointed and 3–4 μm in length. The spines of the metacercaria of *G. somateriae* (Levinsen) are also long and sharp (Ching 1973b). The spines of *Meiogymnophallus jamesoni* Bowers (= *G. oedemiae* Jameson & Nicoll in partim) are 4 μm long and transverse (Bowers 1965), while those of *M. minutus* are only 1 μm long (Bowers & James 1967). The long sharp-pointed spines of some adults, obtained in the mice, differ from the double spines of the small metacercariae to such a degree that these specimens may represent different species. Further SEM examination of the surface spines of different species at different developmental stages would be valuable.

The depth to which the bases of the spines reach varies between species (Burton 1964). The base of the spine in profile in *L. macomae* is not as rounded as Morris (1971) depicts those of *Schistosoma mansoni*. There may be a connection from the spine base to the muscle layer in the tegument of *L. macomae*; the posterior corner of the spine base at least traverses the basement membrane.

The structure of sense organs in trematodes has been studied by e.g. Morris (1971), Nuttman (1971), Lyons (1972, 1973), Brooker (1972). Sensory papillae have been described as being present on the tegument of many monogenetic and digenetic trematode cercariae (e.g. Wagner 1961, Nuttman 1971, Lyons 1972). The number and distribution of the papillae may be of some taxonomic value (Richard 1977). The papillae are grouped according to underlying nerve courses in the genera *Microphallus* Ward and *Maritrema* Nicoll. A similar concentration was noticed in the metacercaria of *L. macomae* in the head region and along the most prominent ventral nerve courses and their ramifications. The body and furcae of "Cercaria baltica" bear sensory papillae with bristles 14 μm in length (Loos-Frank 1971a). Sensory papillae have been recorded on the forebody of the metacercaria of *L. conspicuus* by Ching (1965) and of "Metacercaria I" from *Mya arenaria* by Stunkard & Uzmann (1958). Sensory papillae around the mouth opening have been recorded in the metacercaria of *Parvatrema affinis* (Jameson & Nicoll) by Swennen & Ching (1974) and *P. homoeotecnum* by James (1964).

The arrangement of six papillae on the ventral sucker of "Cercaria baltica" depicted by Loos-Frank (1971a) is very similar to that of the six papillae on the ventral sucker of the metacercaria studied here. In "Metacercaria I" of Stunkard & Uzmann (1958), however, six papillae on the ventral sucker have a different arrangement at least in the diagram. Again, the small slender-spined metacercaria in this study has a similar arrangement of 6 + 6 papillae on the ventral sucker to that of *L. macomae*. The cercaria of *L. conspicuus* has no sensory papillae on the acetabulum (Ching 1965).

There is short "hair" in front of the oral sucker of "Cercaria baltica" and longer "hair" on the furcae (Loos-Frank 1971a). It is not known whether this refers to cilia or to the so-called surface coat. The external filamentous surface coat or glycocalyx is highly developed, e.g. in the cercaria of *Schistosoma mansoni* (Morris 1971, Hockley 1973). It occurs also on monogeneans (Lyons 1973). On the metacercaria of *L. macomae* it is sometimes extremely high in comparison to that of *Schistosoma.* Acid mucosubstances, which show β-metachromasia with toluidine blue and alcianophilia, are present in the surface coat of some species (Hockley 1973). The surface coat of the metacercaria of *L. macomae* shows this with great intensity. It has been suggested that the surface coat functions as exchange resins and regulates the flow and concentration of cations, or it may be protective (Morris 1971). The capsule which usually closed the metacercariae of this study against the shell of the clam is noncellular and this, too, stains metachromatically. Whether it is formed by the clam or by the metacercaria is not known. The presence of a membrane between the metacercaria and the shell suggests that this is formed at least partly by the metacercaria itself. Could the coat also function in the formation of this capsule? The cyst wall of *Fasciola hepatica* consists of many layers of protein and mucosubstances (Dixon 1965). Alcianophilia of the tegument decreased in the adults in mouse tissues. Their site of infection was, however, abnormal. No protection against digestive enzymes of the gut was needed. The origin of the surface coat is obviously in the "subtegumentary cells" (perinuclear region of the tegument), which are alcianophilic and contain β-metachromatically staining flecks. Cells with only slight γ-metachromasia may be germinative subtegumentary cells. Occasionally they were seen in anaphase. Cheng & Provenza (1960) also found mitotic telophases in the so-called beta cells of *Haematoloechus confusus* Ingles. The granular gland cells may represent the alpha cells designated by Cheng & Provenza.

The gastrodermis of *L. macomae* fits that category of trematodes to which *Haematoloechus medioplexus* (Stafford), *H. cylindracea, Opisthioglyphe ranae* (Frölich), *F. hepatica* and *S. mansoni* belong (Halton 1967). The gastrodermis of these species is composed of cells of irregular size and shape with microvilli that vary in length, and not a regular columnar epithelium with a striated border. There are large neutral lipid reserves in the cells of the gastrodermis in *L. macomae.* It is not known whether these lipids are used by the metacercaria when it develops into an adult. At any rate they diminish in the digestive caeca but may accumulate elsewhere in the tissues, e.g. in the excretory vesicle. The excretion of lipids into the incubation medium has been detected in the adults of *F. hepatica* and *Echinostoma revolutum* (Frölich) (Fried & Appel 1977).

The Mehlis' gland of the metacercaria is not as voluminous as that of *F. hepatica,* which was described by Threadgold & Irwin (1970). *F. hepatica,* however, produces hundreds of eggs (Erasmus 1972). In *F. hepatica* two types of cells were present in the Mehlis' gland (Threadgold & Irwin 1970). In one of these granular endoplasmic reticulum formed distended cisternae. Expanded cisternae are also present in one cell type of the gland in the frog lung-fluke *Haematoloechus medioplexus* (Burton 1967). Vacuoles seen in the Mehlis' gland cells of *L. macomae* in semi-thin sections may be such distended cisternae. Some cells in the Mehlis' gland of *H. medioplexus* were strongly PAS-positive even after diastase treatment (Burton 1963). The cells of the Mehlis' gland in the metacercaria of *L. macomae* were moderately PAS-positive. In adult *L. macomae* many cells of the gland showed alcianophilia.

The vitelline cells of the metacercaria of *L. macomae* differ from those in *Diplodiscus amphichrus* (Tubangi) in having the globules evenly distributed around the nucleus and not around a central vacuole (c.f. Kanwar & Agrawal 1977). The structure of the globules was, however, observed in toluidine blue stained semi-thin sections to be similar. The globules consisted of smaller granules along their outer rim. Neutral lipids were not present in the cells in demonstrable amounts. The vitelline cells of trematodes have been reported as comprising a lot of protein but with other materials also present (Burton 1963, Kanwar & Agrawal 1977).

It seems that a rise in temperature triggers the maturing of the metacercaria of *L. macomae.* Sperms developed one day earlier than the eggs, so that they had sufficient time to pass through the uterus into the oviduct to meet the ova. Also, vitelline cells had descended into the vitelline reservoir within one day. The development occurred at a similar speed in seawater cultured *L. conspicuus*. It produced 1-2 eggs at 37° C in 48 h (Ching 1965). Attempts by Bowers & James (1967) to bring metacercariae of *Meiogymnophallus minutus* to maturity in diluted seawater were unsuccessful.

Many attempts have been made to infect natural and experimental hosts with gymnophallid metacercariae. In spite of numerous experiments by James (1964) with feeding metacercariae of *Parvatrema homoeotecnum* to different animals, no adults were revealed. According to James, *P. homoeotecnum* grows to twice its original length in the final host and it may be very host-specific. *P. boringuenae* Cable does not increase in size and its adults were recovered from domestic fowl chicks by Cable (1953). Metacercariae of *P. affinis* matured very rapidly in ducklings (egg production began in 4 hours) (Swennen & Ching 1974). No mature adults of *Gymnophallus somateriae* were obtained when metacercariae were fed by Ching (1973b) to ducklings, chicks and field mice (*Peromyscus maniculatus*). Ching (1965) recovered adult *L. conspicuus* after experimental feeding to field mice. When metacercariae of *L. macomae* were fed to laboratory mice and chickens in the present study, no adults were obtained. As unsuccessful were Reimer's (1962) attempts to infect domestic and wild birds with *L. macomae*. Bowers & James (1967) obtained adults of *Meiogymnophallus minutus* in ducklings only when the birds were fed on minced cockles and not on commercial baby chick crumbs prior to, and during, the experiment. The metacercariae also required to be fed in situ in the cockles. Loos-Frank (1970) fed infected *M. balthica* to *Somateria mollissima* and thus succeeded in recovering adults of *L. macomae*. The recovery of *Gymnophallus gibberosus* adults in laboratory raised natural hosts was slight although heavily infected clams were fed (personal communication by Loos-Frank to Ching 1973b). The simple subcutaneous cultivation technique used in this study is suggested when adults are required.

When the mean sizes of the metacercariae and that of adults as length × breadth are compared, the size decreases in adults both in this and Loos-Frank's (1970) study. The mean sizes of the adults were quite similar in both. A similar size decrease occurred in experimentally maturing *L. conspicuus* (Ching 1965). However, *L. conspicuus* obtained from natural infection in *Melanitta nigra* were of a similar size to the metacercariae. In naturally infected adults of *L. macomae* in *Melanitta nigra* the number of eggs was very great (30-100) (Jameson & Nicoll 1913). Loos-Frank did not give the number of eggs in adults cultivated in *Somateria mollissima*. The size of the eggs was, however, larger than in other records. *L. macomae* obtained from *Clangula hyemalis* were small (Sulgostowska & Grytner-Ziecina 1974). Their eggs were as narrow as those of the *L. conspicuus* studied by Ching (1965). *Gymnophallus gibberosus*, which exists both in *Cardium edule* and in *M. balthica*, attains a greater size in *C. edule* than in *M. balthica*. Its adults were larger in *Melanitta fusca* than in *Somateria mollissima* (Loos-Frank 1971b). Bowers & James (1967) suggest that species of *Gymnophallus*, which are usually comparatively large and occur in the bursa Fabricii or gall bladder of the natural host, may be small and occur in the intestine of experimental hosts. Erasmus (1972) remarks that specific diagnoses based on measurements of parasites from experimental and possibly abnormal hosts must be treated with caution.

The sizes of the digestive caeca and excretory vesicle are also affected by the worm's environment. Both of them increase in size in older metacercariae of *Meiogymnophallus minutus* (Jameson 1902). The distended excretory vesicle laden with granules may be due to the metacercaria being blocked by the host tissue (Jameson 1902, Bowers & James 1967, Loos-Frank 1971a). Most of the excretory granules are discharged when the metacercaria is loosened from the cockle (Bowers & James 1967). The author of this study has occasionally also observed metacercariae from *M. balthica* to expell granular fragments from the gastrodermis.

The small metacercaria in this study in some respects (tegument, spines, sucker sizes, sucker muscles) differs from, but also shows some similarity (spines, pharynx size, structure of digestive caeca, site and papillae of ventral sucker) to, *L. macomae*. The large digestive caeca may be associated with a highly assimilative growing phase of the metacercaria (c.f. *Leucithodendrium somateriae* in *Mytilus edulis*; Jameson 1902). Two gymnophallid trematodes besides *L. macomae* have been recorded in *M. balthica*: *Gymnophallus gibberosus* by Loos-Frank (1971b) and *Parvatrema affinis* by e.g. Swennen & Ching (1974). From these *G. gibberosus* is extrapallial and *P. affinis* intravisceral. *M. inconspicua* also harbours two other gymnophallids in addition to *L. conspicuus: Gymnophallus somateriae* (Levinsen) according to Ching (1973b) and *Meiogymnophallus multigemmulus* Ching according to Ching (1965). The still scanty data on the small metacercaria does not make it possible to decide whether this belongs to *L. macomae* or to some other species. The sharp-spined adults, however, suggest a different species.

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References

Barka, T. & Anderson, P. J. 1963: Histochemistry. Theory, practice and bibliography. — 660 pp. Harper & Row, Publishers, Inc. New York.

Bartoli, P. 1981: Ségrégation biologique et écologique de deux espèces jumelles sympatriques de Trématodes (Trematoda, Gymnophallidae). — Zeitschr. Parasitenk. 65:167-180.

Bowers, E. A. 1965: A description of Meiogymnophallus jamesoni sp. nov. (Trematoda: Gymnophallidae) from the intestine of the common scoter, Melanitta nigra L. — Ann. Mag. Nat. Hist. 8 (13th series): 277-283.

Bowers, E. A. & James, B. L. 1967: Studies on the morphology, ecology and life-cycle of Meiogymnophallus minutus (Cobbold, 1859) comb. nov. (Trematoda: Gymnophallidae). — Parasitology 57:281-300.

Brooker, B. E. 1972: The sense organs of trematode miracidia. — Zool. J. Linn. Soc. 51 (Suppl. 1):171-179 + 7 pl.

Burton, P. R. 1963: A histochemical study of vitelline cells, egg capsules, and Mehlis' gland in the frog lungfluke, Haematoloechus mediplexus. — J. Exp. Zool. 154:247-257.

—"— 1964: The ultrastructure of the integument of the frog lung-fluke, Haematoloechus medioplexus (Trematoda: Plagiorchiidae). — J. Morphol. 115:305-318.

—"— 1967: Fine structure of the reproductive system of a frog lung-fluke. I. Mehlis' gland and associated ducts. — J. Parasitol. 53:540-555.

Cable, R. M. 1953: The life-cycle of Parvatrema borinquenae gen. et sp. nov. (Trematoda: Digenea) and the systematic position of the Gymnophallinae. — J. Parasitol. 39:408-421.

Cheng, T. C. & Provenza, D. V. 1960: Studies on cellular elements of the mesenchyma and of tissues of Haematoloechus confusus Ingles, 1932 (Trematoda). — Trans. Amer. Micr. Soc. 79:170-179.

Ching, H. L. 1965: Life cycles of Lacunovermis conspicuus n. gen., n. sp. and Meiogymnophallus multigemmulus n. gen., n. sp. (Gymnophallidae: Trematoda) from Macoma inconspicua and diving ducks from Vancouver, Canada. — Proc. Helminthol. Soc. Washington 32:53-63.

—"— 1972: A redescription of Gymnophalloides tokiensis Fujita, 1925 (Trematoda: Gymnophallidae). — Can. J. Zool. 50:1299-1302.

—"— 1973a: Paragymnophallus odhneri gen. n., sp. n. (Trematoda: Gymnophallidae) for Gymnophallus somateriae sensu Odhner (1900, 1905). — Can. J. Zool. 51:807-810.

—"— 1973b: Description of Gymnophallus somateriae (Levinsen, 1881) from Macoma inconspicua and diving ducks from Vancouver, Canada. — Can. J. Zool. 51:801-806.

Dixon, K. E. 1965: The structure and histochemistry of the cyst wall of the metacercaria of Fasciola hepatica L. — Parasitology 55:215-226 + 1 pl.

Erasmus, D. A. 1972: The biology of trematodes. — 312 pp. Edward Arnold, London.

Fried, B. & Appel, A. J. 1977: Excretion of lipids by Echinostoma revolutum (Trematoda) adults. — J. Parasitol. 63:447.

Gray, P. 1954: The microtomist's formulary and guide. —794 pp. Blakiston Company, Inc., New York.

Halton, D. W. 1967: Observations on the nutrition of digenetic trematodes. — Parasitology 57:639-660.

Hockley, D. J. 1973: Ultrastructure of the tegument of Schistosoma. — Adv. Parasitol. 11:233-305.

James, B. L. 1964: The life cycle of Parvatrema homoeotecnum sp. nov. (Trematoda: Digenea) and a review of the family Gymnophallidae Morozov, 1955. — Parasitology 54:1-41 + 1 pl.

Jameson, H. L. 1902: On the origin of pearls. — Proc. Zool. Soc. Lond. 1:140-166 + pl. XIV-XVII.

Jameson, H. L. & Nicoll, W. 1913: On some parasites of the scoter duck (Oedemia nigra), and their relation to the pearl-inducing trematode in the edible mussel (Mytilus edulis). — Proc. Zool. Soc. Lond. 1913:53-63.

Kanwar, U. & Agrawal, M. 1977: Cytochemistry of the vitelline glands of the trematode Diplodiscus amphichrus (Tubangi, 1933) (Diplodiscidae). — Folia Parasitol. 24:123-127.

Kuntz, R. E., Tulloch, G. S., Huang, T. & Davidson, D. L. 1977: Scanning electron microscopy of integumental surfaces of Schistosoma intercalatum. — J. Parasitol. 63:401-406.

Lebour, M. V. 1908: Trematodes of Northumberland coast, No. II. —Trans. Nat. Hist. Soc. Northumberland (Durham, Newcastle-upon-Tyne) (N.S.) 3:28-45 + pl. I-III.

Loos-Frank, B. 1970: Zur Kenntnis der gymnophalliden Trematoden des Nordseeraumes. II. Lacunovermis macomae (Lebour, 1908) n. comb. (Syn.: Gymno-

phallus macroporus Jameson & Nicoll, 1913) und seine Metacercarie. — Zeitschr. Parasitenk. 35:130-139.
—"— 1971a: Zur Kenntnis der gymnophalliden Trematoden des Nordseeraumes. IV. Übersicht über die gymnophalliden Larven aus Mollusken der Gezeitenzone. — Zeitschr. Parasitenk. 36:206-232.
—"— 1971b: Zur Kenntnis der gymnophalliden Trematoden des Nordseeraumes. III. Gymnophallus gibberosus n. sp. und seine Metacercarie. — Zeitschr. Parasitenk. 35:270-281.
Lyons, K. M. 1972: Sense organs of monogeneans. — Zool. J. Linn. Soc. 51 (Suppl. 1):181-199 + 3 pl.
—"— 1973: The epidermis and sense organs of the Monogenea and some related groups. — Adv. Parasitol. 11:193-232.
Markowski, St. 1936: Über die Trematodenfauna der baltischen Mollusken aus der Umgebung der Halbinsel Hel. — Bull. Int. Acad. Pol. Sci., Ser. B, Sci. Nat. (II):285-317 + pl. 13-15.
Morris, G. P. 1971: The fine structure of the tegument and associated structures of the cercaria of Schistosoma mansoni. — Zeitschr. Parasitenk. 36:15-31.
Nuttman, C. J. 1971: The fine structure of ciliated nerve endings in the cercaria of Schistosoma mansoni. — J. Parasitol. 57:855-859.
Pearse, A. G. E. 1968: Histochemistry. Theoretical and applied. I. 3rd ed. — 759 pp. J. & A. Churchill, Ltd, London.
Pekkarinen, M. 1983: Seasonal changes in condition and biochemical constituents in the soft part of Macoma balthica (Lamellibranchiata) in the Tvärminne brackish water area (Baltic Sea). — Ann. Zool. Fennici 20:311-322.
—"— 1984: Trematode metacercariae in the extrapallial space of Macoma balthica (Bivalvia) in brackish water (southwestern Finland, Baltic Sea). — Ann. Zool. Fennici 21:473-480.
Reimer, L. 1962: Die digenetischen Trematoden de Wasservögel der Insel Hiddensee und ihre Larvalstadien aus den die Insel umgebenden Brackgewässern. — Diss., Univ. Greifswald, 128 pp.
Richard, J. 1977: Cercariae of Microphallidae: determination of the genera Microphallus Ward, 1901 and Maritrema Nicoll, 1907 according to chaetotaxy. — Parasitology 75:31-43.
Romeis, B. 1968: Mikroskopische Technik. 16 ed. — 757 pp. R. Oldenburg Verlag, München.
Sakamoto, K. & Ishii, Y. 1977: Scanning electron microscope observations on adult Schistosoma japonicum. — J. Parasitol. 63:407-412.
Stunkard, H. W. & Uzmann, J. R. 1958: Studies on digenetic trematodes of the genera Gymnophallus and Parvatrema. — Biol. Bull. 115:276-302.
Sulgostowska, T. & Grytner-Ziecina, B. 1974: Trematodes of Clangula hyemalis (L.) from the Baltic coast. — Acta Parasitol. Pol. 22:401-413.
Swennen, C. & Ching, H. L. 1974: Observations on the trematode Parvatrema affinis, causative agent of crawling tracks of Macoma balthica. — Neth. J. Sea Res. 8:108-115.
Threadgold, L. T. & Irwin, W. B. 1970: Electron microscope studies of Fasciola hepatica. IX. The fine structure of Mehlis' gland. — Zeitschr. Parasitenk. 35:16-30.
Wagner, A. 1961: Papillae on three species of Schistosoma cercariae. — J. Parasitol. 47:614-618.

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