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The cercaria of *Lacunovermis macomae* (Lebour, 1908) (Trematoda: Gymnophallidae), and its penetration into the bivalve *Macoma balthica* (L.) in experimental conditions

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Daughter-sporocysts containing cercariae of *Lacunovermis macomae* occurred in 1–11% of *Macoma balthica*, with a shell length of over 10 mm, at a sampling site on the southwestern coast of Finland.

The cercaria and daughter-sporocyst are described, based on light microscopy, TEM and SEM. Some histochemical results are included. The cercaria has two pairs of penetration glands and 2 × 12 flame cells. The flame cell formula is $2\{[(2 + 2) + (2 + 2)] + (2 + 2)\} = 24$, differing slightly from a usual gymnophallid flame cell formula, viz. $2[(2 + 2 + 2) + (2 + 2 + 2)] = 24$. The cercaria is compared with other cercariae, described by various authors, from *M. balthica*.

The effects of ageing on behaviour, invasiveness and size of cercariae, obtained from daughter-sporocysts and kept in Petri dishes in natural brackish water (at 4–8°C), are reported. The cercariae lived for up to four weeks. Some invasive behaviour persisted for two weeks. Penetration into the mantle of *M. balthica* (with a shell length of 9–10 mm) lasted for about an hour at room temperature. The penetration is two-phasic, with a period of breakage of the surface layers and another period during which the cercaria pushes itself into the mantle.

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1. Introduction

The first record of daughter-sporocysts containing cercariae in *Macoma balthica* (L.) was a footnote in a study by von Siebold (1837). The cercariae were named *Cercaria Tellinae balticae* by Diesing (1850). Later, Pelseneer (1906) found in *M. balthica* from the English Channel off the French coast cercariae, which he believed to be *Cercaria dichotoma* Müller, 1850 (La Valette de St. George 1855). *Cercaria baltica* Markowski, 1936 was first described in *M. balthica* from the southern Baltic sea and later (Loos-Frank 1971a) from the North Sea. *C. baltica* was supposed by Cable (1953), Stunkard & Uzmann (1958) and Loos-Frank (1970, 1971a) to represent the larval stage of *Lacunovermis macomae* (Lebour, 1908) (= *Metacercaria mutabilis* Markowski, 1936).

In addition to the cercariae and metacercariae of *Parvatrema affinis* (Jameson & Nicoll, 1913), Reimer (1962, 1971) found two different cercariae — *Cercaria duoglandulosa* and *C. trioglandulosa* — in *M. balthica* on the GDR Baltic coast. He supposed that *C. duoglandulosa* belongs to *Gymnophallus macroporus* Jameson & Nicoll, 1913 (= *L. macomae*).

Cercariae and metacercariae of *P. affinis* also occur, albeit rarely, in *M. balthica* of the southwestern Finnish coast (Pekkarinen 1987a). In addition, the present author has found two other cercariae in *M. balthica*. One of these was suggested by Pekkarinen (1987b) to represent *Gymnophallus gibberosus* Loos-Frank, 1971. The more common cercaria was shown (Pekkarinen 1986a) to be the larval stage of *L. macomae*. The daughter-sporocysts of *P. affinis* usually also contain metacercariae, often in large numbers (Swennen & Ching 1974), by which they can be distinguished from the other above-mentioned larvae. In the present paper the cercaria of *L. macomae* is described and compared with the other cercariae and possible synonymies are discussed.

Metacercariae of *L. macomae* are common inhabitants of the extrapallial space of *M. balthica,* around the Tvärminne Zoological Station (SW Finland) (Pekkarinen 1984a). Therefore, the prevalence of the earlier larval stages (daughter-sporocyst/cercaria) is also considered. The cercaria is a stage responsible for transmission. Some notes on the behaviour, longevity and infectivity of the cercaria of *L. macomae* are also given. The penetration of the cercaria into a new host clam is described.

In order to study the host-parasite relationship, some histological and histochemical preparations on the daughter-sporocysts and cercariae *in situ* in *M. balthica* and also on free and penetrating cercariae, have been made.

2. Material and methods

2.1. The prevalence of the daughter-sporocysts

The prevalence of the daughter sporocysts was counted in samples of *M. balthica* collected from sites I–III around the Tvärminne Zoological Station (map in Pekkarinen 1984a). They were observed under the microscope in opened clams or in histological preparations.

2.2. Measurements and scanning electron microscopy (SEM) of the daughter-sporocysts and cercariae

For morphometric studies, daughter-sporocysts and cercariae were fixed in 4% formaldehyde in brackish water. For SEM, daughter-sporocysts and cercariae were fixed on membrane filters with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The samples were dehydrated with ethanol, critical point-dried and coated with gold.

2.3. Ageing and penetration experiments on the cercariae

Cercariae were released by crushing the daughter-sporocysts with watchmaker's forceps, in cold natural brackish water (Sal. 6–7‰). To remove remnants of the daughter-sporocysts, germinal balls and young cercariae, the brood of cercariae was washed several times with cold brackish water, using a Pasteur pipette. The ageing experiments were made in small Petri dishes, at 4–8°C, in the dark. The cercariae were washed daily to remove the last remnants of the embryos and dead cercariae. Sometimes all cercariae of a brood died within one or two days. There is some indication that care must be taken when handling and storing vapourable chemicals in the vicinity of cercariae. In later experiments, to ensure the maturity of the cercariae in the tests, swimming cercariae were separated from the remnants by transferring them with a pipette into another dish.

In the penetration experiments, small clams (< 5 mm in shell length) from site I or larger clams from site II (35 m) were used. The small clams were frequently already infected with small metacercariae, which confused the results. The clams were kept on a bare substratum in Petri dishes or beakers and, in some experiments, sand was used on the bottom to allow the clams to burrow. The beakers or dishes were kept at 4–8°C in the dark. The clams were examined at room temperature. In experiments where penetration time was recorded, opened clams from site I (about 9–14 mm in shell length) were used. The cercariae were guided with fine forceps through the water to the desired site on the mantle edge. In order to fasten their suckers, the cercariae needed to be pressed gently onto the mantle. They were, thus, placed between the sensory and muscular lobes and a "map" with setting times was drawn. The penetration was observed at room temperature for four hours.

2.4. Histological and histochemical methods

The penetration glands in living cercariae were stained with dilute, neutral red in brackish water. The stain, however, was toxic to the cercariae. Bouin-fixed clam bodies, containing daughter-sporocysts, were embedded in paraffin wax. Transverse sections, 7 µm in thickness, were stained with the following methods: HE (Mayer's haematoxylin – eosin), HCF (Mayer's haematoxylin – chromotrope 2R – fast green, Gray 1954: 339), Crossmon's staining, HAOL (Weigert's haematoxylin – acid fuchsin – orange – light green, Romeis 1968:371) and ABP (Mowry's method of Alcian blue – PAS – Mayer's haematoxylin after Pearse 1968:673). Some sections, with or without previous treatment in buffered (pH 6.0) 1% diastase solution, were stained with PAS and Mayer's haematoxylin. Calcium salts in the excretory corpuscles of cercariae and metacercariae were detected with alizarin red S, in paraffin sections of the samples fixed in 80% ethanol (method of McGree-Russell: Bancroft & Cook 1984:153).

Neutral lipids, activities of alkaline and acid phosphatases and ß-glucuronidase (ß-GU) were demonstrated in cryostate sections (Pekkarinen 1986a). Incubation times were 90 minutes for the phosphatases and 50 minutes for ß-GU at 37°C but also, sometimes, 20–22°C. Gelatinolytic activity was demonstrated in unfixed cryostate sections, on prestained gelatine films with Cunningham's method, as modified by Michel & Chrétien (1975). The following buffers were used in the dipping of the films: 0.02 and 0.1 M phosphate buffers, pH 6.8, 7.9; 0.1 M acetate-acetic acid pH 5; veronal-acetate-HCl pH 3.2, 6.8, 7.9. The sections were incubated at 37–40°C for 1.5–2 h.

For transmission electron microscopy (TEM), pieces of clam gonads containing daughter-sporocysts were fixed in glutaraldehyde (see above) and treated with 1.5% OsO_4 in phosphate buffer and, after dehydration, embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate.

3. Results

3.1. Daughter-sporocysts and their prevalence

The prevalence of the daughter-sporocysts of *L. macomae* in *M. balthica,* at different sampling sites around the Tvärminne Zoological Station, is given in

Table 1. Occurrence of daughter-sporocysts of *Lacunovermis macomae*, in *Macoma balthica*, collected around the Tvärminne Zoological Station (No of infected clams/No of clams examined). Capital letters indicate infection by species other than *L. macomae:* G = *Gymnophallus gibberosus*, P = *Parvatrema affinis*. E = Ekman sampler, V = vanVeen sampler, Tr = trawl, Prevalence = % infected clams with shell length over 10 mm.

Collecting site	Date	Collecting method	Shell length, mm								Prevalence
			6.0–7.9	8.0–9.9	10.0–11.9	12.0–13.9	14.0–15.9	16.0–17.9	18.0–19.9	20.0–21.9	
I, 7–8 m	Aug. 1983	E	0/2	0/16	1/21	0/24	1/24	1/14	0/9		3.26
	Sept. 1983	E	0/1	0/8	0/20	1/19	0/21	0/21	0/6	0/1	1.14
	Jan. 1984	E		0/22	0/20	2/61	2/8 1G	1/9	0/6		2.88
	April 1984	E + V	0/5	0/18	1/47	1/59	2/50	1/16	0/11	1/2	3.24
	June 1984	E	0/8	0/30	1/42	1/45	0/23	1/16	2/23	1/7	3.85
	July 1984	V	0/1	0/9	0/35	1/38	2/47	0/25	0/18	0/3	1.81
	July 1986	E	0/9	0/30	0/28	3/38	6/50	7/34	0/3	1/1	11.04
	Aug. 1986	V	0/21	0/35	0/27	0/41	1/71	1/41	1/15		1.54
	Sept. 1986	E	0/31	0/37	0/31	0/20	0/38	0/33	1/21	1/4	1.36
	Nov. 1986	E	0/23	0/53	0/32	0/26	0/33	3/34	0/11	0/1	2.19
II, 35 m	Aug. 1983	Tr				0/3	0/10	1/41 P	0/48	0/13	
	May–Aug. 1984	Tr					0/18	0/77	2/80 2G	0/20	
II, 40 m	July 1983	Tr					0/3	0/21	0/36	1/29 P	
III, 20–30 m	July 1983	Tr					0/9	1/39	0/18	0/5	
	July 1984	Tr					0/16	1/34	1/16	0/8	

Table 2. Dimensions (μm) of fixed (4% formaldehyde; No 1 fixed in 3% glutaraldehyde) daughter-sporocysts of *L. macomae* from *M. balthica*, collected from site I.

Brood number	Date	Host shell length, mm	*N*	Mean length	Length range	Mean width	
						smallest	largest
1	June 1984	12.8	11	862	696–1020	93	208
2	June 1984	13.2	8	855	660–960	165	252
3	July 1984	14.1	12	813	648–900	124	221
4	Sept. 1984	13.0	16	922	576–1200	96	217
5	Sept. 1984	13.3	15	784	456–996	96	185
6	July 1986	15.7	14	827	636–1010	94	207
7	July 1986	16.3	16	746	444–1040	95	216
8	July 1986	17.4	21	809	576–1030	111	233
9	July 1986	16.6	15	1075	540–1720	101	318
10	Nov. 1986	16.2	20	1431	1044–1836	163	298

Table 1. At site I, daughter-sporocysts were found in clams with over a 10 mm shell length (10.3 mm was the smallest one infected), among which the prevalence was 1–4% during 1983–1984. Infected clams were exceptionally common, 11% in July 1986, decreasing thereafter. The clams infected by the daughter-sporocysts, with cercariae, did not harbour more metacercariae of the same species than clams without daughter-sporocysts. Although absent in this study, at site II, daughter-sporocysts of *L. macomae* do sometimes occur at this sampling site (Pekkarinen 1987b).

The daughter-sporocysts of *L. macomae* occur mainly in the haemolymph spaces of the clam gonad. The germinal tissue of the gonad has often almost totally atrophied. To a lesser extent, daughter-sporocysts sometimes also occupy the region of the digestive gland and, occasionally, the mantle, gills, kidney and pericardial gland. The birth pore end of the daughter-sporocyst is narrower than the other (Fig.

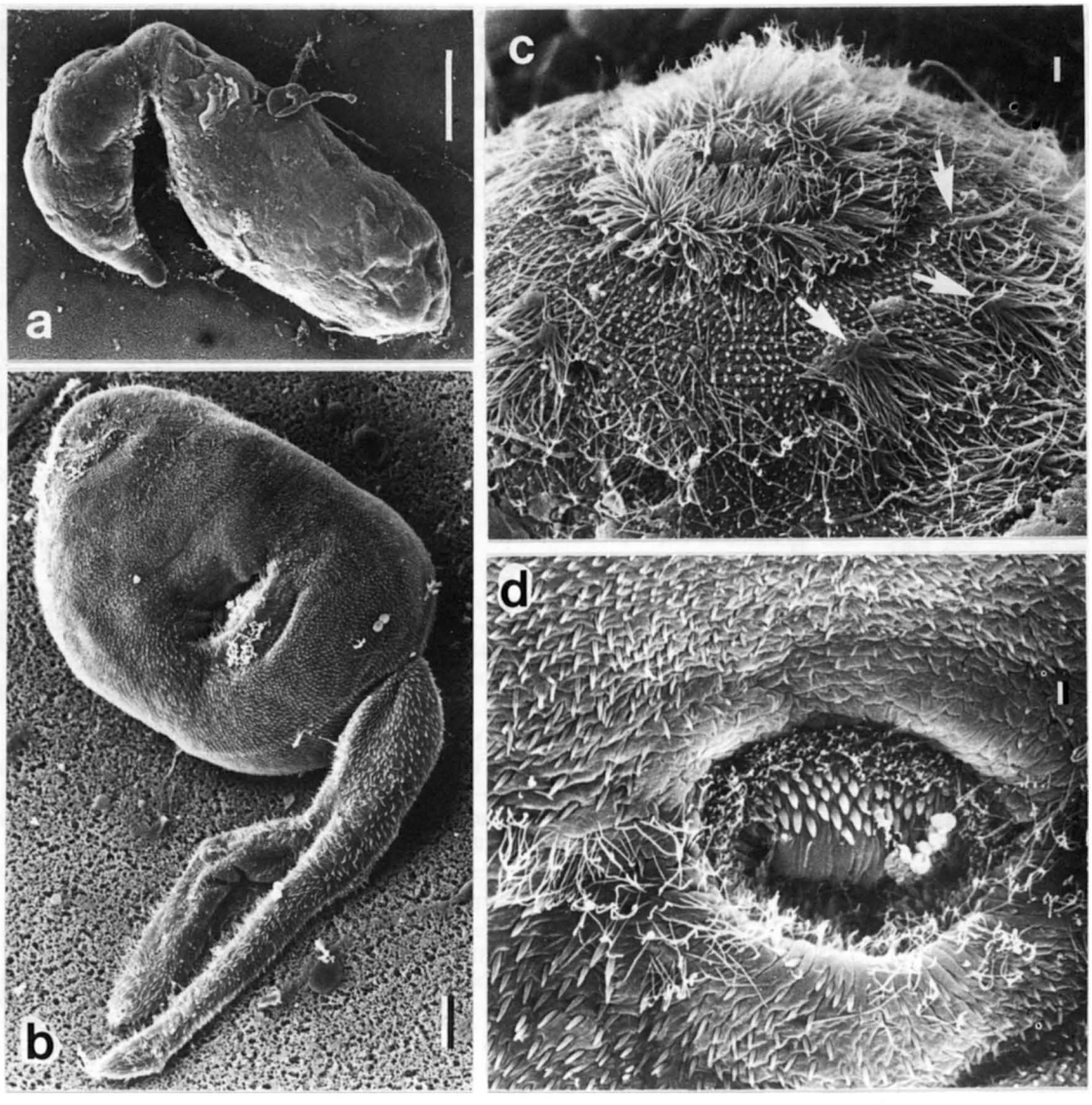

Fig. 1. The daughter-sporocyst and cercaria of *Lacunovermis macomae*. — a. Daughter-sporocyst. — b. Cercaria. — c. Anterior end of a cercaria, with the mouth surrounded by a zone of microvilli and scattered microvilli on the forebody. The arrows point to sensory setae surrounded by tufts of microvilli. The longer flagella at the lower corners belong to clam spermatozoa. — d. The region of the ventral sucker. There are microvilli and heavy spines in the sucker. Scale bars 100 μm in a, 10 μm in b and 1 μm in c and d.

1a). The daughter-sporocysts often have one or more constrictions in the body.

The dimensions of fixed daughter-sporocysts from different clams are given in Table 2. The mean length was between 746 and 1431 μm, the longest sporocyst measured being 1836 μm. The mean maximum widths were 185–318 μm. The sporocysts of brood No 10 contained, on average, 43 cercariae and a larger number of embryos.

3.2. The cercaria

The cercaria of *L. macomae* (Figs. 1b, 3a and 4a) has a proportionally long tail stem and furcae (Table 3). The oral sucker is withdrawn, and so the mouth opening is usually small. The ventral sucker is also usually embedded. There are microvilli around the mouth and on the forebody (Fig. 1c), in and around the ventral sucker (Fig. 1d) and on the tail (Fig. 2e).

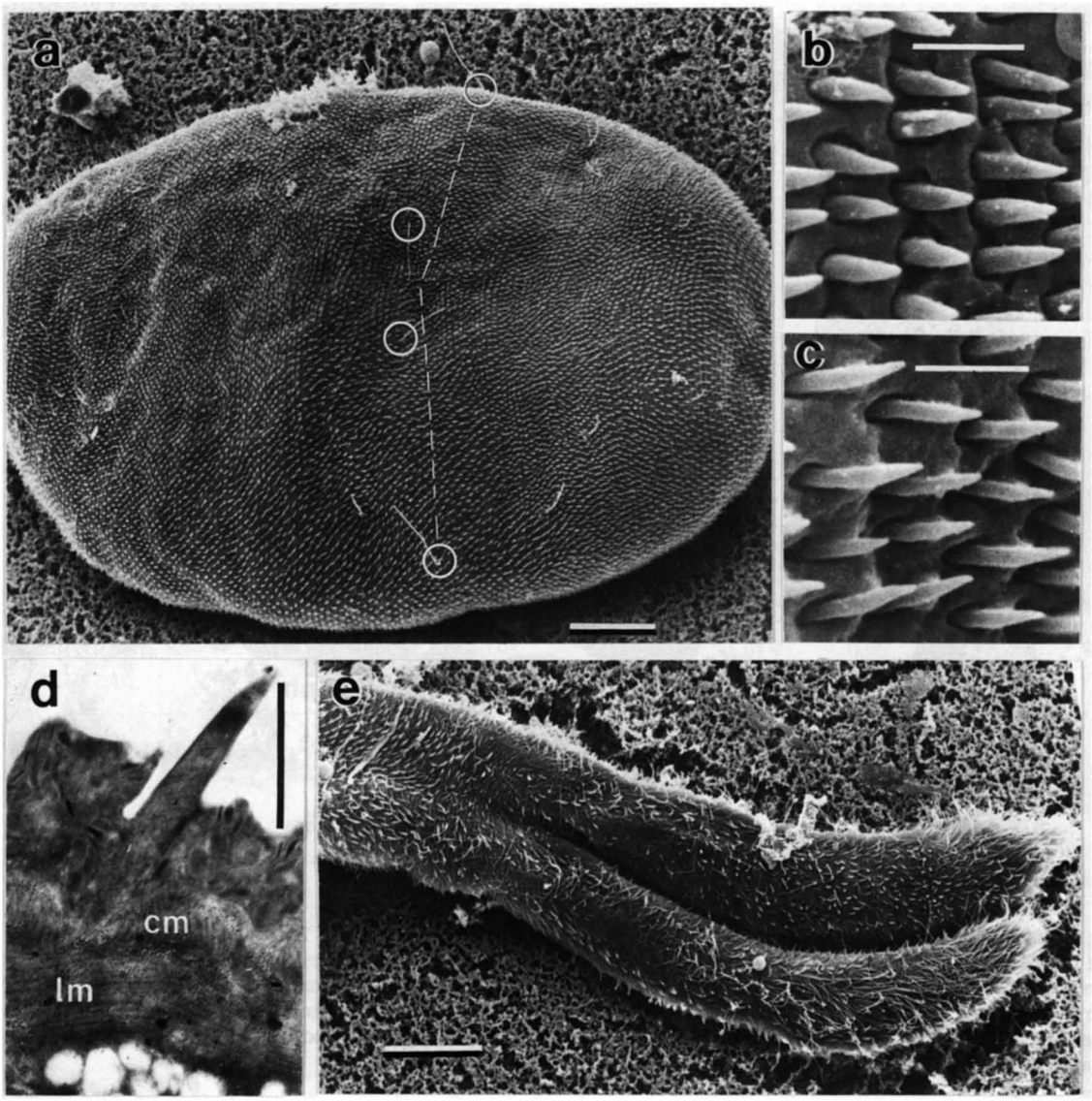

Fig. 2. Tegumental structures of the cercaria of *L. macomae*. — a. Dorsal surface of a cercaria with spines, sensory cilia, and microvilli (the anterior end points to the left). The sensory structures with the longest cilia are marked with circles. The two middorsal ones are slightly anterior to the broken line drawn following the annulation of the tegument from one of the lateral sense organs to the other. — b. Spines on the anterior body. — c. Spines on the posterior body. — d. A TEM section from the tegument through a spine. The clear vacuoles below the circular (cm) and longitudinal muscles (lm) probably belong to a subtegumentary cell. — e. Ventral view of the tail. Scale bars 10 μm in a and e and 1 μm in b—d.

The tegument on the body is faintly cross-striated (annulated) but otherwise smooth. The body and tail are covered by spines, of which about 1 μm is visible outside the tegument (Fig. 2). The spines are sometimes slightly flattened or have a shallow, longitudinal groove. Spines are lacking at the site of the bifurcation. In the ventral sucker, within the zone of microvilli, there is a zone of heavy spines pointing to the centre of the sucker (Fig. 1d) and sometimes, next to them, there are also a few microvilli. Spines in the oral sucker are sparse.

There are 2 × 6 papillae on the ventral sucker. Around the oral sucker there are prominent sensory setae with surrounding tufts of microvilli (Fig. 1c). On the body there are small sensory papillae, with a shorter or longer cilium (Fig. 2a), and few or many

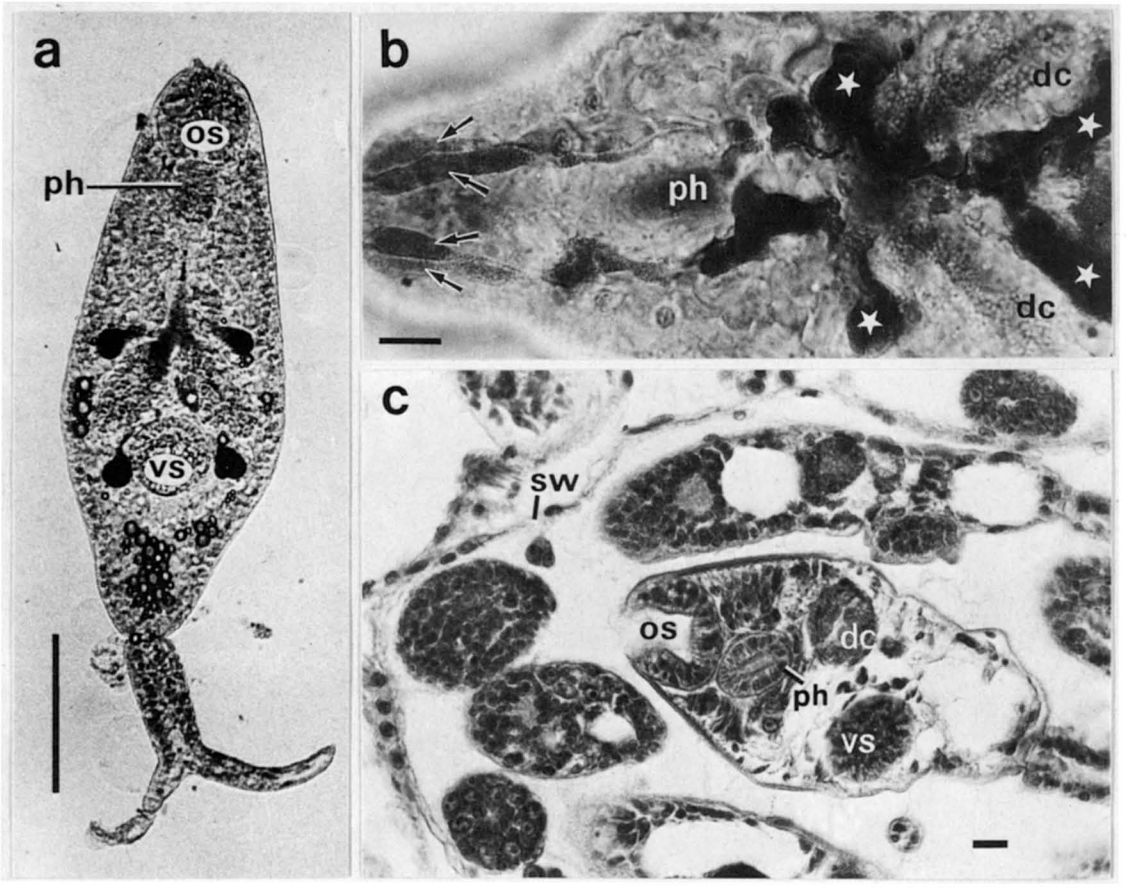

Fig. 3. Internal structures of the cercaria of *L. macomae*. — a. A cercaria slightly pressed. The penetration glands and part of the gut stained intensively with neutral red. The excretory vesicle contains large round corpuscles. os = oral sucker, ph = pharynx, vs = ventral sucker. — b. Dorsal view of the anterior body with the penetration glands (marked with asterisks) and the gland ducts (arrowed) stained with neutral red. The digestive caeca (dc) between the anterior and posterior pairs of the penetration glands are finely granular. — c. Embryos and a cercaria in a daughter-sporocyst. sw = daughter-sporocyst wall. HCF staining. Bars: 100 μm in a, 10 μm in b and c.

surrounding microvilli. Four papillae on the dorsal side, at the level of the ventral sucker, bear the longest cilia (at least up to 12 μm) (Fig. 2a). The two middorsal ones are slightly more anterior than the latero-dorsal.

The cercaria has two pairs of dorsally located penetration gland cells, one anterior to and the other posterior to, the digestive caeca (Figs. 3a, b and 4a). The ducts open onto the dorsal lip of the oral sucker. The ducts and the gland bodies are often very diverticulate. The cells of the digestive caeca are finely granular. The lumen of the caeca is small or even inconspicuous (in many cercariae of one brood the lumen was wider). Rudiments of the genital organs are already visible in the cercariae.

The excretory vesicle is Y-shaped, with a short and usually broad stem, and arms reaching to the level of the oesophagus or pharynx (Fig. 4a). The vesicle contains large corpuscles (diameter up to 10–12 μm) (Fig. 3a). Usually up to eight flame cells were found on each side of the cercaria, but in numerous cercariae 2 × 12 flame cells could be traced. Because the location of the flame cells suggested a formula different from that currently established in later stages of *L. macomae,* the protonephridial system of the metacercaria was re-examined. The formula was found to be $2\{[(2 + 2) + (2 + 2)] + (2 + 2)\} = 24$ (Fig. 4).

The mean body lengths of fixed cercariae, from different clams, were 169–209 μm (Table 3). The

Table 3. Basic measurements (in μm) and derived measurements of fixed cercariae of *L. macomae* from broods 1–5 (see Table 2).

	$Mean_1$	$Mean_2$	$Mean_3$	$Mean_4$	$Mean_5$	Individual
N	8	11	10	10	9	$range_{1-5}$
Body length (BL)	169	177	193	198	209	124–229
Body breadth (BB)	96	85	92	94	83	74–115
Oral sucker length (OSL)	41	43	43	40	41	31–50
Oral sucker breadth (OSB)	38	36	37	36	35	31–43
Pharynx length	27	28	26	31	32	22–37
Pharynx breadth	20	19	17	17	18	15–23
Ventral sucker length (VSL)	34	36	35	33	32	28–40
Ventral sucker breadth (VSB)	40	39	38	36	36	31–47
Ventral sucker distance (VSD)		115	130	124	131	81–115
Digestive caecum length	36		35	38	40	25–56
Digestive caecum breadth	29		25	29	27	19–37
Tail stem length	50	43	53	51	63	31–81
Tail stem breadth	22	26	26	26	23	19–34
Furca length	66	79	68	66	77	50–102
$BL \times BB \times 10^{-3}$	16.35	15.00	17.59	18.50	17.42	10.42–21.74
BL/BB	1.78	2.09	2.17	2.11	2.59	1.48–2.78
BL/OSL	4.10	4.16	4.49	4.98	5.06	3.30–5.94
OSL/VSL	1.20	1.18	1.22	1.23	1.30	0.92–1.54
OSB/VSB	0.98	0.93	0.96	1.01	0.96	0.79–1.29
VSD/BL		0.65	0.68	0.63	0.62	0.54–0.77

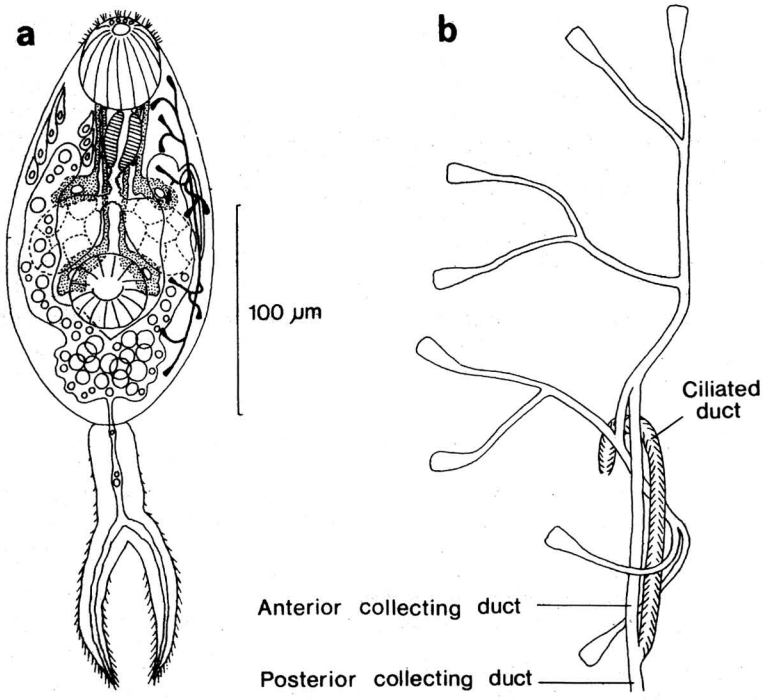

Fig. 4. — a. Ventral view of the cercaria of *L. macomae* drawn after combination of measurements of fixed cercariae and photographs and sketches of living cercariae. — b. Schematic drawing of part of the right protonephridial system in the metacercaria of *L. macomae* viewed from the dorsal side.

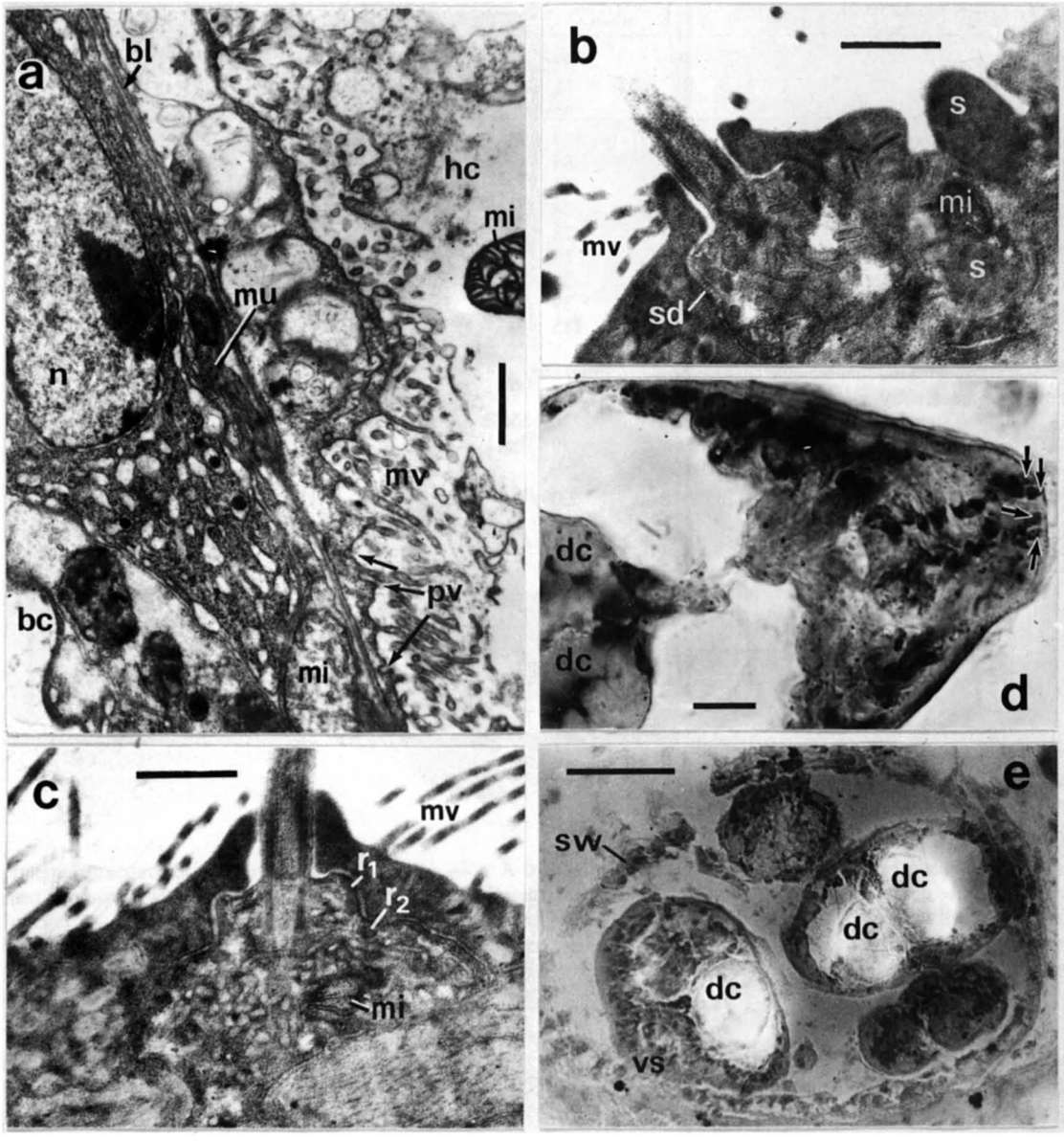

Fig. 5. Ultrastructure and histochemistry of the daughter-sporocyst and intrasporocysteal cercaria of *L. macomae*. — a. Daughter-sporocyst wall. Host side with a disintegrating cell (hc) with pseudopodia and a free mitochondrion (mi) is at the right, brood chamber (bc) to the left. Tegument with microvilli (mv) and pinocytotic vesicles (pv), basal lamina (bl), muscle (mu) and part of a subtegumentary cell and its nucleus (n) are visible. — b and c. Sensory structures in the tegument of the anterior body of a cercaria. The nervous bulb is circled by a septate desmosome (sd) and two dense rings (r_1 and r_2). s = spine. — d. Activity of acid phosphatase in the anterior body of a cercaria. The ducts of the penetration glands are marked with arrows. — e. A daughter-sporocyst with two cercariae cross-sectioned. Pre-stained gelatine film has been digested at the sites of the caeca (dc). Veronal-acetate-HCl buffer, pH 3.2. Bars: 1 µm in a, 0.5 µm in b and c, 10 µm in d and 50 µm in e.

mean body size, expressed as length × breadth (in μm) × 10^{-3}, in different broods was 15.0–18.5. The pharynx is longer than is wide (Fig. 3c). The length of the oral sucker is about 1/4–1/5 of the body length. The oral sucker is slightly longer than the ventral one (the mean sucker length ratio is about 1.2–1.3), but their breadths are quite similar.

A few exceptionally large cercariae, with morphological anomalies, were found. They had at least three, probably four, pairs of penetration glands and a great number (at least up to 17) of flame cells on a side.

3.3. Histology and histochemistry of the daughter-sporocysts and cercariae

In light microscope preparations the daughter-sporocyst walls were usually thin (Fig. 3c) but for example, near the birth pore end, they were thicker with an irregular and vacuolated inner lining. The walls were (even after diastase treatment) PAS-positive. They are composed of tegument, circular and longitudinal muscles, tegumentary cells and probably a thin cell layer lining the brood chamber (Fig. 5a). Pinocytotic vesicles were sometimes detected between the microvilli of the tegument. The tegument contained vacuoles and membraneous bodies and a few mitochondria. The (sub)tegumentary cells had cisternae of rough endoplasmatic reticulum (RER). Some cells included lipid droplets, as shown with Fettrot. Mitochondria, in the subtegumentary layer, were often large with granular content and tubular cristae. Host granulocytes and other cells were seen in close contact with daughter-sporocysts. They were sometimes disintegrating, and free mitochondria were occasionally present outside the daughter-sporocysts (Fig. 5a). Free mitochondria were also, sometimes, found in the brood chamber. Sometimes, the chamber contained acidophilic and PAS-positive matter.

The tegument and tegumentary cells of the cercariae were PAS-positive after diastase. Some cells stained with alcian blue or both PAS and alcian blue. Numerous subtegumentary cells contained clear vacuoles (0.2–0.5 by 0.1–0.3 μm), which sometimes showed a slightly electron-opaque centre. The tegument contained mitochondria, dense rods and lighter vacuoles (Figs. 2d, 5b and c). The sensory receptors, so far varified with TEM, are sheathed ciliary receptors (Fig. 5b–c). The tegumentary sheath or collar around a central cilium, on the nervous bulb, is short and bears microvilli. The bulb is attached to the surrounding tegument with a circular septate desmosome and two dense rings.

The digestive caeca of the cercariae and the penetration glands, including their ducts, were acidophilic and strongly PAS-positive (diastase-resistant). Positive PAS reactions of these organs appeared early in development. The gastrodermal cells contained globules of moderate density (diameter about 0.5–1.5 μm). Between the globules there were cisternae of RER. Parallel sheets of RER were present in the lateral parts of the cells. Distal parts of the cells beared microvillous or lamellar projections, and a few pedicels were present at the proximal part of the cells. The cells were attached to each other by septate desmosomes near the apex. Glycogen was present in the muscle and parenchyma cells of the cercariae, as well as of the daughter-sporocysts. In the germinal balls it was scarce. Cells with dense granules (about 0.3–1.2 by 0.2–0.6 μm) were found in the forebody of the cercariae. Fettrot-stainable material was not detected in the cercariae, although a few small lipid droplets were revealed with TEM.

Acid phosphatase activity was evident in some tegumentary cells of the daughter-sporocysts and cercariae. In the cercariae (Fig. 5d), activity of this enzyme was also revealed in the pharynx, oesophagus and distal parts of the gastrodermal cells or in the lumen of the caeca. Some activity was found in the tegument and, sometimes, in the penetration glands (Fig. 5d). Activity of ß-glucuronidase was not detected in the cercariae or germinal balls but it occurred in some cells, probably host amoebocytes, on the sporocyst walls. Indication of alkaline phosphatase activity was found in the excretory corpuscles of the cercariae but this was not certain because of the presence of Ca^{++}, as shown by alizarin red S.

Some amoebocytes, the kidney, part of the style sac epithelium, intestine and stomach, and especially the digestive diverticula of the host clam, digested gelatin at pH 3.2. There was some digestion also at pH 6.8 and 7.9. However, control sections first kept at 100°C for 15 minutes also showed some digestion. In many cercariae similar gelatine digestion was observed at the site of the digestive caeca (Fig. 5e). In some preparations the activity was localized in the distal parts of the cells and in the lumen.

3.4. Behaviour, ageing and penetration of the cercariae

After the abort from the daughter-sporocysts, under illumination and during warming up to room

temperature, the cercariae swim vigorously. The swimming movements are typical of gymnophallid cercariae (see Bartoli 1974). The body is broad and flattened, somewhat elongate, with a slightly pointed anterior end. The tail is bent forwards ventrally, the bifurcation reaching the level of the oral sucker. The tail stem undulates as small lateral waves and the furcae beat by turns. The cercariae showed periods of swimming, sinking and resting. Time spent swimming decreased and resting increased, with age. Resting cercariae are globular (about 80–100 µm in diameter), they lie on their dorsal side, with the shortened tail bent tightly against the ventral surface of the body. A resting cercaria, when disturbed, may begin to swim.

Ageing cercariae (over a week old) began to make creeping movements. The tail of many cercariae degenerated, and decaudation sometimes occurred. The body of a resting tailless cercaria was not usually globular, but resembled that of a swimming cercaria. The body could react to vibrations by shrinking.

During the second week, mortality increased. During the third week, some cercariae still showed swimming behaviour but with impaired efficiency. Aged, resting cercariae made only a few slow swimming movements after being stimulated by mechanical vibrations. Many cercariae, from two broods, lived in Petri dishes (at 4–8°C) for three to four weeks in winter. Many of the survivors were then tailless. The cercariae were active, they crept and attached firmly to the glass by their suckers. The penetration glands and digestive caeca of the cercariae seemed to be unchanged.

Table 4. The mean body length/body breadth ratio of the cercariae and metacercariae (Mc) (see Fig. 6).

	Time		
Brood No.	0 days	3 days	7 days
6	1.71	1.68	1.59
7	1.83	1.66	1.64
7 Mc		1.63	
8	1.93	1.58	1.79
9	1.77	1.73	1.80
10	2.05	1.72	1.71
11	2.13		
11 Mc		2.07	
12	1.64		
12 Mc		1.76	1.61
13	2.30	1.67	
13 Mc		1.55	

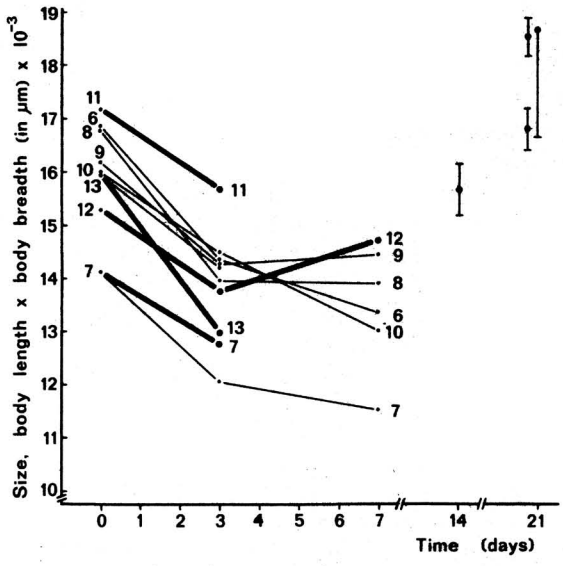

Fig. 6. The body size in different broods (Nos. 6–13) of cercariae of *L. macomae* which were obtained from daughter-sporocysts and kept in natural brackish water (4–8°C) or allowed to penetrate into *M. balthica*. After the penetration they are called metacercariae in this connection (large dots and bold lines). *N* was always 25 except for a few groups of metacercariae, where it was smaller. The SEs were 0.13–0.48 during the first week (omitted to simplify the figure).

The size of the cercariae, expressed as body length x body breadth, decreased during ageing after release from the daughter-sporocysts (Fig. 6); but different stages of contraction or extension of the body, when fixed, may bias the size measurements. However, in some broods, the mean length/breadth ratio did not change or changed only slightly with time (Table 4). The size of those cercariae which were allowed to penetrate into clams also decreased by the third day (Fig. 6). Size reduction was most marked in those cercariae which penetrated a larger clam (No. 13). The size of young metacercariae did not exceed the general range in the *L. macomae* cercariae, until after a few weeks. In the two- and three-week-old metacercariae the mean length/breadth ratio was 1.77–1.87.

In the experiments where clams were exposed to cercariae in a Petri dish, cercariae swam randomly — no orientation towards the clams could be noticed. Cercariae entered the mantle cavity or siphonal space along with water currents. A usual route was with the inhalant current through the siphon. A clam opening its shells, during digging, for example, caused inhalant currents of water through the pedal and siphonal gapes. The cercariae which already attached

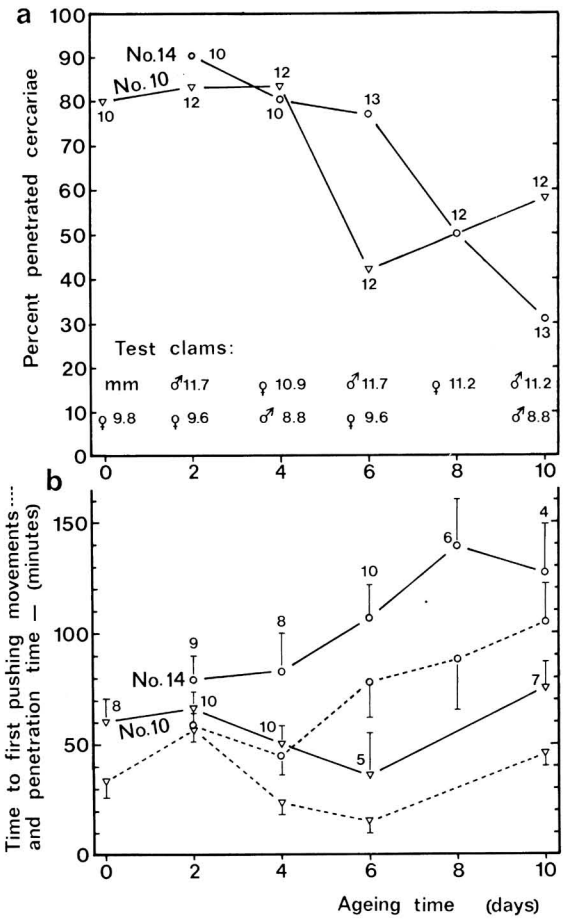

Fig. 7. Effect of ageing (at 4–8°C) on penetration of cercariae of *L. macomae*, obtained from two broods (Nos. 10 and 14) of daughter-sporocysts and tested at the mantle edge of *M. balthica*. The cercariae of brood No. 10 were tested with the smaller clams (shell lengths 8.8–9.8 mm). Numbers of cercariae tested are also given in a and penetrated cercariae in b. The vertical bars in b indicate SEs.

their suckers or stuck to the surfaces, or cilia, of the clam, remained in the mantle cavity in spite of exhalant currents during closure. Cercariae were also carried in along with the foot.

Penetrating cercariae were often seen at the mantle edge, for example, near the periostracal groove. The clams sometimes swept the mantle edge with their foot, but this did not detach the penetrating cercariae. In small clams, which were transparent enough, cercariae were seen to creep in the mantle cavity to the palp and gill's point of contact. From there, they often crept dorsally, along the border of the gill. Due to the irritation, the clams tried to clean the mantle cavity by bending their foot dorsally and sweeping with it. Cercariae in the suprabranchial chamber and in the inhalant siphon often elicited a "coughing" reflex.

Just after release cercariae, which swam vigorously, resisted being set on the mantle of a clam. When set on the mantle and gently pressed, a cercaria usually makes a swinging movement with the forebody and immediately attaches its suckers. If the site is not accepted the cercaria, using its suckers, migrates to a new site. Sometimes the anterior body gropes upwards, while the ventral sucker is holding fast. The oral sucker, may also, test the surface around the cercaria until a good site is found.

During the first phase of penetration both suckers are attached to the surface (Fig. 8a). The tail is bent dorsally. The oral sucker makes minute dilation and contraction movements. After some time, when the oral sucker has pierced through the surface and anchored in the mantle, the cercaria detaches the ventral sucker and begins to push into the mantle (Fig. 7b). The body and tail extend until extremely thin. Every now and then, the cercaria thus, pushes itself into the host. When the posterior body relaxes, the anterior body paves the way.

The invasiveness of the cercariae was 80–90% during 4–6 days after the release, decreasing thereafter (Fig. 7a). The unsuccessful cercariae detached voluntarily or accidentally. Efforts lasting over four hours during the 6th–10th days were also classified as unsuccessful. In another experiment several cercariae were still capable of penetration on the 13th day after the release.

The penetration time could be as short as six minutes. The mean penetration time of cercariae from brood No. 10, into clams with a shell length of 9–10 mm, was about an hour (Fig. 7b). Cercariae from brood No. 14 penetrated into clams with a shell length of 11–12 mm more slowly, at least when aged. In another experiment, when a 14-mm clam was used as a host, penetration of cercariae was still slower. When the penetration lasted over three hours, detached host epithelial cells or amoebocytes often formed a cloud around the cercaria. Penetrating cercariae caused long-lasting or intermittent muscular contractions in the mantle.

During penetration the tail is usually left outside, but may be drawn along with the cercaria. A cercaria which has reached the haemolymph space of the mantle, does not directly penetrate the extrapallial space but migrates laterally within the mantle, using its suckers and making peristaltic movements. The

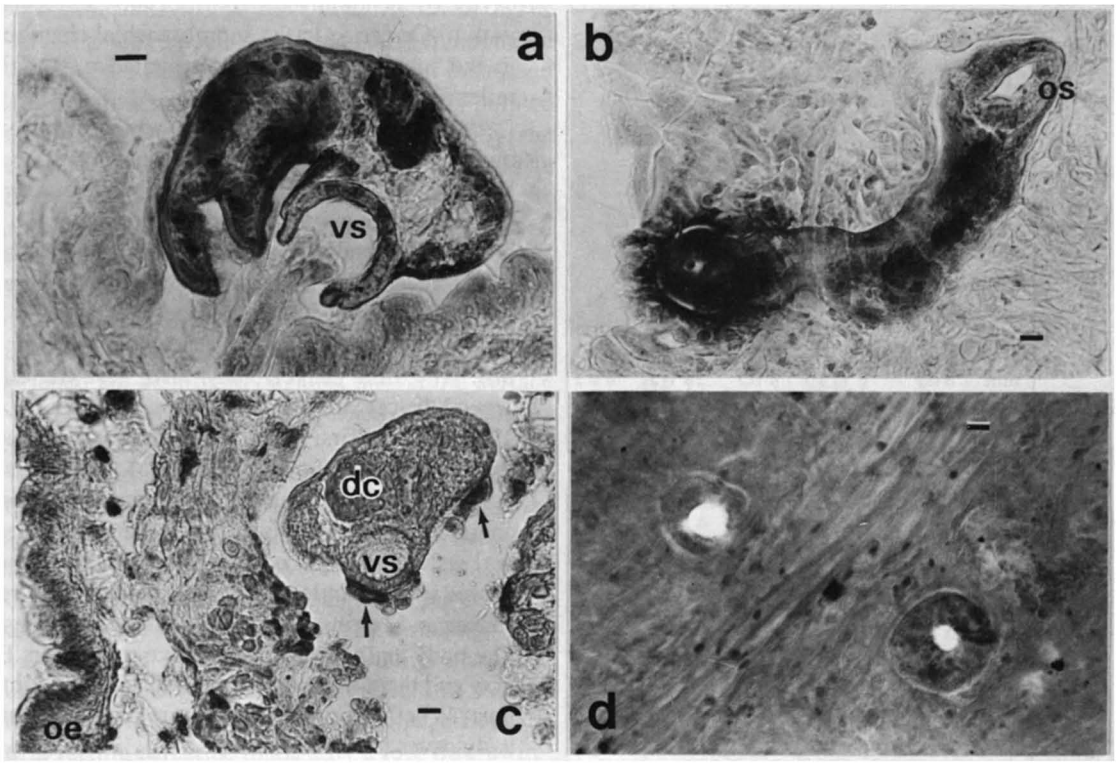

Fig. 8. Enzyme histochemistry of penetrating cercariae of *L. macomae* on or within the mantle of *M. balthica*. — a. Acid phosphatase activity in a cercaria during the first phase of penetration of the mantle. The picture was combined from the photographs of two sequential sections. The tail is still out of the plane. — b. A cercaria penetrated further into the mantle. The ventral sucker is probably at the site where acid phosphatase has diffused to the surrounding tissue. — c. β-GU activity in the outer epithelium (oe) of the mantle and in amoebocytes, which have also attached to the surface of a migrating cercaria (arrowed) in the haemolymph space. — d. Digestion of pre-stained gelatine film is apparent in the oral suckers (cross-sectioned) of two cercariae among mantle muscles. Veronal-acetate-HCl buffer pH 3.2. Bars 10 μm.

digestive caeca of many cercariae dilated within a day.

In histological preparations of small clams kept in contact with cercariae for 6–48 h, cercariae were found in the extrapallial space and in almost all tissues: the mantle, palps, gills, heart-pericardium-kidney complex, stomach, appendix, intestine, digestive diverticula, gonad and the foot. In histological preparations of wild clams from sites II (40 metres) and III (20–30 metres), migrating cercariae/metacercariae were found in tissues of 15–25% of individuals in July (in 1983 and 1984, respectively). Most often they occurred in the heart-pericardium-kidney complex. Amoebocytes often surrounded them. They were also found in the gonad, foot, gills and the sheath of the siphon. In the samples from site I, migrating cercariae were found rarely. Calcified corpuscles ("pearls") are often found in the kidney or in the pericardial gland. In large clams, aged metacercariae of *L. macomae*, are common in the pallial isthmus under the ligament or hinge. They are surrounded by an epithelial layer and are sometimes calcified.

3.5. Histochemistry of penetrating cercariae

The distribution of acid phosphatase activity in penetrating cercariae (Fig. 8a) was similar to that in the cercariae in daughter-sporocysts. There was only one case where acid phosphatase had diffused to the surroundings (Fig. 8b). β-GU activity was not found in the penetrating cercariae. Amoebocytes with strong β-GU activity were common in the mantle. Sometimes they were found attached on the cercariae (Fig. 8c). Digestion of gelatine took place in the digestive caeca, pharynx and mouth of some, but not all, penetrating cercariae (Fig. 8d). Diffusion of the active

agent from the mouth to the surroundings was not evident. However, outside the mantle edge, where numerous cercariae had been set, some liquefaction of gelatine was observed.

4. Discussion

4.1. Comparison of the cercaria of *L. macomae* with the other cercariae described occurring in *M. balthica*

Cercaria dichotoma, as described by Pelseneer (1906) from *M. balthica,* resembles the cercaria of *L macomae.* In the drawing by Pelseneer, the cercaria is small. Another cercaria within a daughter-sporocyst drawn by him is, however, three times the length of the separate cercaria. The daughter-sporocyst lengths agree with the present measurements. The oral sucker of the cercaria is longer than the ventral one. The pharynx, tail stem and furcae are long and digestive caeca short. The exretory vesicle contains large corpuscles ('á grosses concretions'), the actual size of which, however, remains unknown. In spite of the meagre information available on this cercaria, the description, however, greatly suggests that it is *L. macomae* in question. Cercariae identified to be *C. dichotoma,* by various authors, from other host species may be different (see for example, Campbell 1985).

The mean length, 1.2 mm, given by Markowski (1936) for daughter-sporocysts of *Cercaria baltica* is within the range in the present study. If the dimensions of the daughter-sporocysts of *C. baltica* given by Loos-Frank (1971a) (length nearly 2 mm, breadth 0.3 mm) are mean figures, in comparison with the present data they are proportionally large. Their length agrees with the daughter-sporocyst length of *Gymnophallus gibberosus* (Pekkarinen 1987b). They are wider, however. Immature sporocysts of *G. gibberosus,* which have not for the present been described, may be misidentified as those of *L. macomae.*

Both Markowski and Loos-Frank found four pairs of penetration glands in *C. baltica.* The number agrees with that in *G. gibberosus* (Pekkarinen 1987b). Loos-Frank described the penetration glands as being arranged pre- and postcaecally, as in the present cercaria. The penetration glands of *L. macomae* are lobular and can, thus, be erroneously counted as four pairs. Loos-Frank described only two pairs of gland ducts.

The long pharynx in living cercariae, described by Loos-Frank, agrees with that in *L. macomae* and so does the dense "hair" (= microvilli) on the head region and tail. The pharynx length in the fixed cercariae was very small, resembling the cercaria of *G. gibberosus* (Pekkarinen 1987b). Markowski gave very small dimensions for the oral sucker, 11 × 11 µm. The dimensions may represent the mouth. The oral sucker of *L. macomae* looks small externally, but the retracted sucker is larger. It is slightly longer than the ventral sucker, the breadths being nearly similar. In *G. gibberosus* the ventral sucker is usually larger, as it was also in fixed *C. baltica* described by Loos-Frank.

The tail stem of *C. baltica* and usually also of *L. macomae,* is longer than that of *G. gibberosus.* Similarly, the furcae are longer in *L. macomae* and in *C. baltica* described by Loos-Frank. In Markowski's data the furca length is short; 38 µm. There is some discrepancy in the description of *C. baltica* in that the tail stem in figure 6 of Markowski (1936) is even shorter than the furcae (which are longer than 38 µm), although the stem length is reported to be 90 µm. Markowski referred to the poor swimming of *C. baltica.* This is characteristic of *G. gibberosus.*

The digestive caeca of *L. macomae* are smaller and have a narrower lumen than those of *G. gibberosus.* Markowski described a similar narrow lumen in the caeca of *C. baltica.* Corpuscles in the excretory vesicle are larger in *L. macomae* and in *C. baltica,* studied by Loos-Frank, than in *G. gibberosus.* Loos-Frank described prominent sensory papillae in *C. baltica.* Sensory papillae are minute in *L. macomae* but more conspicuous in *G. gibberosus* .

The cercaria of *G. gibberosus* was not known when *C. baltica* was described. Since the descriptions of *C. baltica* agree in some features with the cercaria of *L. macomae* and in some features with *G. gibberosus* (Pekkarinen 1987b), it is possible that both Markowski and Loos-Frank have studied cercariae of the two species without knowing it.

Cercariae of the two species, *L. macomae* and *G. gibberosus,* cannot be distinguished from each other on the basis of the body size or the appearance of the tegumental spines. The flame cell numbers were also similar. The flame cell numbers are not reliable characteristics because all flame cells are not always discernible and the number is dependent on the age of the cercariae, until a final number is attained. In a mature cercaria of *L. macomae,* the final number can even be the same as in the metacercaria, namely, 2 × 12 = 24. Thus, in contrast to the earlier statement (Pekkarinen 1986a), no increase in the number of flame cells needs to take place during the metacercarial transformation.

Cercaria duoglandulosa (Reimer, 1962, 1971) resembles the cercaria of *L. macomae* in the number and location of the penetration glands and in the form of the excretory vesicle. Its excretory vesicle contains prominent corpuscles. The cercaria had only 2 × 6 = 12 flame cells. The pharynx of *C. duoglandulosa* was slightly shorter and the tail stem and furcae were even longer than in *L. macomae*. The daughter-sporocysts of *C. duoglandulosa,* with their length of 400–530 µm, overlap the lower length limit of the *L. macomae* daughter-sporocysts measured in the present study. Reimer (1962) supposed that *C. duoglandulosa* might belong to *Metacercaria mutabilis* Markowski, 1936 (= *L. macomae).* Due to the resemblance with the present cercaria it may really be *L. macomae*. The specimens which Reimer studied may have been young — the flame cells of the cercariae could therefore be fewer and the daughter-sporocysts shorter.

Cercaria trioglandulosa, which according to Reimer (1962) may belong to *Gymnophallus deliciosus* (Olsson) Odhner, has more penetration glands than the cercaria of *L. macomae* has. They all are precaecal. Because this cercaria is distinct from *C. duoglandulosa*, it must thus be a species different from *L. macomae*. One possible candidate might also be *G. gibberosus* (Pekkarinen 1987b).

4.2. Prevalence, histology and effect of daughter-sporocysts on their host

Hardly 1% of *M. balthica* individuals in the southern North Sea were infected by daughter-sporocysts of *Cercaria baltica* (Loos-Frank 1971a), whereas the prevalence among individuals with a shell length of 10–20 mm in the southern Baltic Sea (Poland) was 6% (Markowski 1936). The figures given by Markowski and Loos-Frank may include daughter-sporocysts of *Gymnophallus gibberosus* (see section 4.1.). *Cercaria duoglandulosa* occurred in 3.27% of *M. balthica* individuals on the southern (GDR) Baltic coast (Reimer 1971).

In a sampling site, in the northern part of the Baltic Sea (site I), the prevalence of the daughter-sporocysts of *L. macomae* among clams with a shell length of over 10 mm was 1–4%, during the years 1983–1984. Later, a high prevalence — 11% — was recorded in July 1986 at the same site. The following low percentage — 1.5% — in August, was first thought to result from the larger and heavier sampling grab (Table 1). Stratification of clams in the sediment has not been studied but if the infected clams are, in some circumstances, in the top layer or on the sediment, then samples taken with a lighter and smaller grab may include proportionally more of these clams from the top layer. Hulscher (1973) frequently found moribund individuals of *M. balthica,* infected by *Parvatrema affinis,* on the sediment in summer. The prevalence of *L. macomae* was still low in September and November, when the sampling was similar to July. The decrease in prevalence may have resulted from clam deaths. Athough variations were not always great, the lowest prevalence was found in late summer or early autumn. It is not known whether the clams, or the daughter-sporocysts in them, are evenly distributed in the study area. An uneven distribution could lead to biased results.

I have, so far, noticed daughter-sporocyst infections only in clams with a shell length of over 10 mm. Clams above this size are nearly always infected by the metacercariae of the same species (Pekkarinen 1984a). The daughter-sporocysts were found to occur mainly in the gonad region, and only to a lesser extent in the digestive gland of the clam. Similar tissue preference was also noticed with daughter-sporocysts of *Parvatrema affinis* (Pekkarinen 1987a) and *Gymnophallus gibberosus* (Pekkarinen 1987b). The digestive gland is of vital importance for the host and thus, also, for the developing parasites. Therefore, it is advantageous for the parasites to suppress the functions of the digestive diverticula to a small extent only. A severe infection leads to castration of the gonad. Possible mechanisms of parasitic castration of molluscs are discussed and schematically summarized by Sullivan et al. (1985).

Constrictions in daughter-sporocysts in *M. balthica,* were noticed even by Pelseneer (1906) and Markowski (1936). The daughter-sporocysts of *L. macomae* described here, and of *Gymnophallus gibberosus* (Pekkarinen 1987b), have thinner and wider sectors. The sectors did not suggest multiplication (cf. *G. fossarum*: Bartoli 1974). Fournier & Théron (1985), who studied the sectorization of the daughter-sporocysts of *Schistosoma mansoni,* supposed that the narrow anterior zone with its nervous supply might control chronobiological emission of cercariae.

The daughter-sporocyst tegument of many trematode species bears microvilli (Bils & Martin 1966, Køie 1971a, Gibson 1974, Fournier & Théron 1985, Göbel & Pan 1985). The wall of the daughter-sporocysts of *L. macomae* was similar to that described by Køie (1971a) for *Cercaria buccini* Lebour (= in part *Podocotyle reflexa* (Creplin) (Køie 1981), in part *Anomalotrema koiae* Gibson & Bray, 1984). Lipid

droplets were detected in the subtegumentary cells of the sporocysts of *L. macomae*. They have also been found in other species (Køie 1971a, Ždárská & Soboleva 1982, Fournier & Théron 1985).

Acid and alkaline phosphatases have been detected at the surface of the tegument of larval and adult trematodes (Lumsden 1975). Phosphatases are believed to function in digestive processes, carbohydrate metabolism and active transport of metabolites (Halton 1967, Lumsden 1975). Køie (1971a) found high activity of acid and alkaline phosphatases in the tegument of the daughter-sporocysts of *Podocotyle reflexa,* in *Buccinum undatum*. The activity was weaker in the subtegumentary layer. In the daughter-sporocyst body wall of *Cercaria helvetica* XII (Dubois) acid phosphatase activity was found in vesicles and vacuoles and alkaline phosphatase along membranes of these vacuoles and in apical and basal plasma membranes of the tegument (Reader 1975). Some subtegumentary cells of the daughter-sporocyst walls of *L. macomae* showed activity of acid phosphatase.

Trematode daughter-sporocysts may secrete hydrolytic enzymes via the tegument to the surroundings. Daughter-sporocysts break down exogenous carbohydrates and absorb them through the body wall (Thomas & Pascoe 1973, Popiel & James 1976). Histolysis of host tissues was evident around daughter-sporocysts of *Podocotyle reflexa* (Køie 1971a). Tissues of *M. balthica* were also found to be in stages of degradation around daughter-sporocysts of *L. macomae*. Daughter-sporocyst infections can even alter enzyme activities in host tissues (Moore & Halton 1973, Marshall et al. 1974).

4.3. Histology and function of different organs of cercariae

Microvilli are quite common on the suckers of cercariae of different families (Køie 1971b, 1976, 1978, 1981, 1985, Gibson 1974, Pariselle & Matricon-Gondran 1985), although they do not occur on all species. The present author has found them in both *L. macomae* (present study) and in *G. gibberosus* (Pekkarinen 1987b). Microvilli ("bristles", "hair") on the tegument of the body and/or tail have been recorded in several cercariae (Loos-Frank 1969, 1971a, Køie 1971b, 1981, Rees 1971a, Bartoli 1983, Ždárská 1983, Campbell 1985, Pekkarinen 1987b and present study). In some species microvilli occur in young cercariae but in older ones they decrease in number or disappear (Køie 1971b, Ždárská 1983).

The trematode tegument has been supposed to function in digestion, transport, osmoregulation, excretion, secretion, protection and support (Halton & McCrae 1985). The microvilli increase the surface area and help in absorption of nutrients and uptake of oxygen (Rees 1971a). According to Køie (1971b), the zones of microvilli on the suckers may function similarly to those in the adhesive organ of the Strigeoidea, namely, in digestion and absorption. They are important during the period in the daughter-sporocyst (Gibson 1974), during the free-living stage, penetration (Køie 1976) and migration (Køie 1981). Later on, the microvilli usually disappear. In the cercaria of *L. macomae* the microvilli may be important even when the cercaria is within the daughter-sporocyst. The tegument and its associated cells of the cercaria of *Zoogonoides viviparus* (Olsson), reacted positively for acid and alkaline phosphatases (Køie 1971b). Acid phosphatase activity was intense in the subtegumentary cells but less so in the tegument of the cercaria of *L. macomae*.

Spines inside the suckers are also common in cercariae (Køie 1973, 1981, Ebrahimzadeh 1974, Gibson 1974, Shoop & Corkum 1984, Pariselle & Matricon-Gondran 1985, Pekkarinen 1987b and present study). The spines usually disappear during later development. Adults of *Schistosoma* species, however, still have spines in their suckers (Kunz et al. 1977, Sakamoto & Ishii 1977). The cercaria of *L. macomae* has a zone of heavy spines in its ventral sucker. During the first phase of penetration the cercaria uses the oral sucker for piercing, while holding on the mantle with its ventral sucker. Due to the spines pointing into the sucker the attachment of the ventral sucker can be firm. In *Podocotyle staffordi* Miller the spines in the suckers may erode cells of the daughter-sporocysts for nutrition (Gibson 1974).

The tegumental spines of the trematode body have been supposed to help in emergence through the birth pore and from the host, in attachment to the host, maintaining position, penetration, migration, and in the abrasion of nutrients (Rees 1971a, 1974, Bennett 1975a, b, Køie 1977, 1982, 1985). The tegumental spines of the *L. macomae* cercaria may be important in anchoring, during peristaltic movements in the later stage of penetration and also during migration. During the metacercarial development of *L. macomae* the spines become broad and multi-pointed (Pekkarinen 1984b, 1986a).

Ciliated sensory receptors are common in Platyhelminthes. Pariselle & Matricon-Gondran (1985) described six types of sensory receptors in the cercaria of *Nicolla gallica* (Dollfus). In addition to simpler types, they found receptors which had a sheath of tegumentary origin covered with short or long villi. The receptors described here in the cercaria of *L. macomae* resemble 'type b' (with short collar and long villi) of Pariselle & Matricon-Gondran, although there were different inclusions in the sensory bulb. Pariselle & Matricon-Gondran believed that the stereocilia-like villi on the collar of sheathed receptors could have a mechanoreceptive function, namely, they are able to transmit movements to the collar and to the bulb. These sensory receptors are different from the collar receptors found earlier in different animal classes (discussed in Pekkarinen 1986b).

The cercarial gastrodermis has ultrastructural characteristics of a synthesizing and secretory epithelium (Køie 1971b, Bennett & Threadgold 1973, Erasmus 1977, Halton & McCrae 1985 and present study). However, the gut of a free-swimming cercaria is apparently non-functional (Erasmus 1977). In the cercaria of *Fellodistomum fellis* (Olsson) the lumen of the caeca develops later between the future gastrodermal cells (Halton & McCrae 1895). In *Cryptocotyle lingua* the digestive system has not developed in the cercaria (Rees 1974). In the cercaria of *Fasciola hepatica* the lumen of the caeca is absent (Bennett & Threadgold 1973). The caecal lumen of the *L. macomae* cercaria is small, suggesting only a minor function in digestion and absorption. Lipids are present in certain cercariae but they are probably fatty acids rather than glycerides (Cheng 1963). Neutral fats were not detected in the gastrodermal cells of the cercariae of *L. macomae* with light microscopy, in contrast to the metacercariae (Pekkarinen 1984b, 1986a). There were small globules in the cercarial gastrodermis but their chemical composition is not known.

Various enzymes have been reported to occur in the caeca of some cercariae (Erasmus 1977). Køie (1971b) found acid and alkaline phosphatase activity in the gastrodermis of *Zoogonoides viviparus*. Acid phosphatase activity is more commonly found in trematode gastrodermis (Halton 1967, Bogitsh 1975). In the cercaria of *Echinostoma revolutum* acid phosphatase acitivity is strong in the oesophagus and intestine, but weaker in the pharynx (Fried et al. 1984). Acid phosphatase activity was also found in the gut of *L. macomae*. Some agent in the caeca of *L. macomae* digested gelatine.

The penetration glands of some cercariae have been reported to be PAS-positive, as were those of *L. macomae*. The post-acetabular glands of *Schistosoma mansoni* are PAS-positive (Ebrahimzadeh 1970) and pre-acetabular glands stain with purpurin (alizarin), suggesting the presence of inorganic calcium (Stirewalt & Kruidenier 1961). The function of the post-acetabular glands is thought to be primarily adhesive, the mucous secretion helping in attachment, during looping movements, onto the skin. Lubricative, protective and enzyme directive functions have also been suggested (Stirewalt & Kruidenier 1961). Enzyme activities have been described in the secretions of the pre-acetabular glands. Stirewalt (1963, 1973) has summarized the reported lytic activities of cercariae and their extracts.

Alkaline and acid phosphatase reactions were negative in the penetration glands of *S. mansoni* (Ebrahimzadeh 1970). Hydrolytic enzyme activities were not found in the penetration glands of *Z. viviparus* (Køie 1971b). Activity of acid phosphatase has been histochemically detected in the glands and associated ducts of developing and free-swimming cercariae of *Cryptocotyle lingua* (Krupa et al. 1967). The penetration glands and their ducts in *L. macomae* sometimes showed acid phosphatase activity. The fact that it was not found in all individuals may be due to the cercariae being immature, or to the inactivity of the glands within daughter-sporocysts.

In the excretory system of helminths activity of, for example, alkaline phosphatase has been found (Wilson & Webster 1974, Parshad & Guraya 1977). The excretory corpuscles of the cercaria of *Echinostoma revolutum* showed strongly positive reaction for alkaline phosphatase (Fried et al. 1984). Alkaline phosphatase may be active in the excretory corpuscles of *L. macomae,* too, but the positive reaction may also result from calcium salts.

In *L. macomae,* Loos-Frank (1970) gave the formula of the flame cells as $2[(2 + 2 + 2) + (2 + 2 + 2)] = 24$. There is a slight contradiction with the present study, where the formula was found to be $2\{[(2 + 2) + (2 + 2)] + (2 + 2)\} = 24$. A similar formula was found for *G. gibberosus* (Pekkarinen 1987b). The anterior part of the excretory system in *Parvatrema rebunense* Shimazu, 1975 is similar. Variation from the normal formula was found rarely in cercariae of *L. macomae,* but more commonly in laboratory-reared metacercariae of *G. gibberosus* (Pekkarinen 1987b). The protonephridial systems of gymnophallid species ought to be re-examined.

4.4. Behaviour, ageing and penetration of cercariae

Swimming cercariae of many species show negative geotaxis, migrating to the surface of water (Smyth & Halton 1983). Cercariae can swim rhythmically with intermittent sinking periods (Styczyńska-Jurewicz 1961, Chapman 1974). During their life, cercariae of *Opisthioglyphe ranae* show periods of: 1) free, active swimming; 2) swimming with effort as a result of stimulation; and 3) passive rest at the bottom. The activity can be increased by, for example, mechanical vibration, increasing temperature, decreasing light intensity and the presence of host extract. Finally the cercariae lie on the bottom and, even when strongly stimulated, show only weak swimming movements (Styczyńska-Jurewicz 1961).

The longevity of cercariae depends on the height of the water column utilized for swimming. In a 20° water column of 200 mm cercariae of *O. ranae* lived for 48 h, but at a water depth of 15 mm they lived for 80 h (Styczyńska-Jurewicz 1961). The longevity of cercariae also depends on the water temperature. Køie (1975, 1978, 1979, 1980, 1981, 1982) has reported the viabilities of several cercariae. The cercariae of *Aporocotyle simplex* lived only for three days at 6°C (Køie 1982). Cercariae of some species lived for a week at 4–10°C. At higher temperatures cercariae of *Podocotyle reflexa* died within 24 h (Køie 1981). Decaudation of cercariae was sometimes observed (Køie 1975, 1980, 1982). Among gymnophallid cercariae, those of *G. fossarum* and *G. rostratus* lived at 12– 13°C (Sal. 38‰) for nearly six days and 9–10 days, respectively (Bartoli 1974). At 20°C, *G. fossarum* and *G. nereicola* lived only for three days.

The proportionally long life span (up to four weeks at 4–8°C) of the cercariae of *L. macomae,* and also of *G. gibberosus* (Pekkarinen 1987b), is partially due to the low water depth in the Petri dishes. It is not known whether the cercariae swam in the dark between examinations. The cercarial size, indicated as body length × body breadth, decreased during ageing. The size reduction may be due to consumption of glycogen reserves. Exclusion of the third dimension — namely, the thickness of the body dorsoventrally — causes some inaccuracy in the comparison with cercariae at different stages of contraction. When the ratio of body length/body breadth is small, the size may be slightly underestimated. Length × breadth is, however, a better indication of the size than the length alone. In routine measuring it is not possible to measure the thickness of the worms.

During ageing the cercariae of *L. macomae* showed behaviour typical of many cercariae. The periods of swimming and resting are appropriate for dispersal and making contact with *M. balthica.* Trematode cercariae are thought to be sucked in through the siphon of bivalves, their own role being passive (Bartoli 1974, Allison 1979, Campbell 1985). The spherical resting cercariae of *L. macomae* are easily sucked in through the siphon of the clam. As the clam is normally buried, the siphon is the only route into the mantle cavity.

Styczyńska-Jurewicz (1961) did not refer to another period of activity in *Opisthioglyphe ranae* cercariae, the creeping period, which aged cercariae of *L. macomae* exhibited. In *Parorchis acanthus,* creeping movements preceded cyst formation (Rees 1971b). Cercariae of *Opechona bacillaris* sometimes, especially after decaudation, crawled on the bottom of a dish (Køie 1975). Cercariae of *Cryptocotyle lingua* have been observed to crawl on the body of a fish (Chapman 1974). The creeping period of *L. macomae* cercariae may approximate to the seeking of the site of penetration or to the later, migratory part of the penetration. The creeping of aged cercariae may also be locomotion typical of metacercariae.

The cercariae of *Opisthioglyphe ranae,* kept in a water depth of 15 mm (20°), showed complete invasiveness even after 78 h (Styczyńska-Jurewicz 1961). Cercariae of *Stephanostomum caducum* were infective up to a week at 5°C (Køie 1978). The success of infection of *Venerupis aurea* (Gmelin) (shell length 24 mm) by cercariae of *Gymnophallus rostratus* was 36–40%, during three days post emergence, then decreased until totally abolished after six days (Bartoli 1974). The ability of the cercariae of *L. macomae* to penetrate the mantle of *M. balthica* was good for at least four to six days. Some invasiveness could persist at 4–8°C for two weeks. The penetration tests were, however, made at room temperature. If wild cercariae are infective for an equally long period, they can infect clams far from their primary host clam. Autoinfestation of the host clam of the cercariae of *G. rostratus* is somehow suppressed (Bartoli 1974). Individuals of *M. balthica* harbouring daughter-sporocysts and cercariae of *L. macomae* did not carry more metacercariae than other clams. The vigorous swimming of the cercariae of *L. macomae,* just liberated from daughter-sporocysts, may restrict the possibility of autoinfection.

At the start of penetration — namely, the piercing of the surface layers of the mantle — the cercaria of *L. macomae* used its ventral sucker as a holdfast. The

dilating and constricting movements of the oral sucker, with its few spines, may function in the mechanical breaking of the tissue. Enzymes from the digestive caeca, as well as secretions from the penetration glands (although no clear evidence was obtained), may contribute to the penetration. Gelatine digestion occurred in the mouth opening of some migrating cercariae. Because gelatinolytic activity was also shown in the pharynx and caeca, it is suggested that the agent had been extruded from these organs. The gelatinolytic activity may be associated with feeding and/or penetration. The lumen of the caeca dilated during or after penetration. The migrating cercariae probably ingest nutrients from the host. In *Fasciola hepatica* juveniles the secretory product of the gastrodermis is presumed to be hydrolytic and to be utilized in excystment, penetration and migration within the host (Bennett & Threadgold 1973).

Stirewalt (1973) found protease activity (with the gelatine film technique) in the pre-acetabular glands of *Schistosoma mansoni*. The secretion of these glands is emptied into the skin during penetration. The major skin components susceptible to attack by the proteases of *S. mansoni* cercariae are probably keratin, non-collagenous proteins of the basement membrane and the proteinaceous backbone of the proteoglycan matrix of the dermis (Dresden et al. 1977). In *Lepidapedon elongatum* (Lebour) Nicoll most of the contents of the penetration glands disappear after penetration and encystment, but traces of the glands were still visible in two-week-old metacercariae (Køie 1985). The penetration glands of *L. macomae* are not emptied during penetration and so aiding penetration may not be their only function. The cercariae were dissected from daughter-sporocysts and, thus, did not normally emerge. The glands may also function during the emergence from the host. In some species, remnants of the glands can persist for several months (Køie 1982, Pekkarinen 1987b). It is not known whether the secretions of the glands have some function during early metacercarial life, for example, digestive, adhesive or protective.

The penetration of *Gymnophallus nereicola* into *Nereis diversicolor* (Bartoli 1974) is principally similar to the invasive behaviour of *L. macomae* in *M. balthica*. First it dissolves the cuticle of the polychaete, aided by the secretion from the penetration glands. The entrance is, however, extremely rapid. The pushing movements of the cercariae of *L. macomae* are, obviously, energy-consuming. In intact clams penetration in the narrow parts of the mantle cavity is aided by the pressure of the opposite surfaces. The principal route of the cercariae of *Gymnophallus fossarum* to the extrapallial space of *Cerastoderma glaucum* is through the labial palps and gills, although they also use other routes (Bartoli 1973, 1974). The cercariae of *L. macomae* were seen migrating in many tissues of *M. balthica*. The migratory period may be quite long if the cercariae have missed the easiest route to the extrapallial space. Such cercariae can be trapped by host amoebocytes.

The penetration and migration of the cercariae of *L. macomae* seem to be slower in older clams than in younger ones. The cercariae/metacercariae found in the tissues of the clams from sites II and III may have been unsuccessful penetrators. The clams at these sampling sites were larger and probably old (see Segerstråle 1960). Possibly all of these: swimming, penetration and migration in the tissues of the clams, contribute to the small size of early metacercariae.

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