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The cercaria and metacercaria of *Gymnophallus gibberosus* Loos-Frank, 1971 (Trematoda: Gymnophallidae) in *Macoma balthica* (L.) (Bivalvia) in the Baltic brackish water (southwestern Finland)

Marketta Pekkarinen

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Metacercariae, which sometimes occur at the pallial line and adductor muscles inside the shells of *Macoma balthica,* were identified as *Gymnophallus gibberosus.* Some similarity with *G. somateriae* (Levinsen) was also observed. The flame cell formula was found to be $2\{[(2 + 2) + (2 + 2)] + (2 + 2)\} = 24$.

Long daughter-sporocysts (up to 2.7 mm) containing cercariae were rarely found in *M. balthica* of the same locality. Metacercariae which were experimentally reared from the cercariae in *M. balthica* developed slowly. Although immature, they already showed some similarity with the metacercariae of *G. gibberosus,* and are thus suggested as belonging to this species.

The cercaria has four pairs of penetration glands and $2 \times 12 = 24$ flame cells. It is compared with another cercaria — namely, of *Lacunovermis macomae* (Lebour) — occurring in *M. balthica.* The concept of *Cercaria baltica* Markowski may, besides *L. macomae,* include *G. gibberosus.*

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1. Introduction

Metacercariae of *Gymnophallus gibberosus* Loos-Frank, 1971 inhabit both *Macoma balthica* (L.) and *Cerastoderma* spp. (*C. edule* (L.) and *C. lamarcki* (Reeve)) (Loos-Frank 1971a, Lauckner 1971). According to Lauckner (1983), they probably also occur in *Mytilus edulis* L. Lauckner suspected that some of the records of *G. strigatus* (Lebour, 1908) in various bivalves, and particularly *Brachycoelium* sp. Giard, 1897 in *Tellina solidula* (= *Macoma balthica*), may include larval *G. gibberosus.*

In the extrapallial space of *M. balthica* from southwestern Finland, there occasionally occur, besides metacercariae of *Lacunovermis macomae* (Lebour, 1908), metacercariae of another species (Pekkarinen 1984a). This was supposed by Pekkarinen (1986a) to be *Gymnophallus gibberosus.* In the present paper the metacercaria is described, compared with the earlier descriptions (Loos-Frank 1971a, Lauckner 1971), and identified as *G. gibberosus.*

The cercaria of *G. gibberosus* is previously unknown. Apart from the larval stages of *Parvatrema affinis* (Jameson & Nicoll, 1913) (Pekkarinen 1987a), the author has found daughter-sporocysts and cercariae of two different trematode species in *M. balthica,* in southwestern Finland. One cercaria (Pekkarinen 1987b) was experimentally shown by Pekkarinen (1986a) to belong to *L. macomae.* In the present study the cercariae of the other species were reared into metacercariae in the laboratory, by natural and experimental infections, in *M. balthica.* Based on the present data, these cercariae are suggested to be those of *G. gibberosus.*

Cercaria baltica, described by Markowski (1936) from the southern Baltic *M. balthica* and by Loos-Frank (1971b) from the North Sea *M. balthica* , had been supposed by Loos-Frank to belong to *L. macomae.* According to Pekkarinen (1987b), both descriptions of *C. baltica* disagree in some features with *L. macomae.* Comparison of the cercaria of this paper with *Cercaria baltica* suggests that, besides *L. macomae, G. gibberosus* may be another species involved in the descriptions of *C. baltica.*

2. Material and methods

The prevalence of the daughter-sporocysts was assessed in *Macoma balthica* samples, collected at three sites (I–III) around the Tvärminne Zoological Station (see map in Pekkarinen 1984b). The sporocysts were detected in opened clams under the dissecting microscope and/or in histological preparations. For the histological methods see Pekkarinen (1987b).

For the measurements the daughter-sporocysts and cercariae were fixed in 4 % formaldehyde in brackish water or in 3 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Glutaraldehyde-fixed samples were dehydrated with ethyl alcohol, dried through the critical point and coated with gold for scanning electron microscopy (SEM).

Most of the cercariae aborted from the daughter-sporocysts in one clam from site II (depth of 35 m), in September 1984, were used in an infection experiment. The experiment was carried out as reported previously for rearing metacercariae of *L. macomae* (Pekkarinen 1986a). Another experiment of metacercarial development was made in June–December 1986. During dissection of individuals of *M. balthica* for other studies, exceptionally many young metacercariae were revealed in the extrapallial space, and swimming cercariae similar to the metacercariae were found in the water. It was reasoned that the sample included a clam which harboured daughter-sporocysts and cercariae, and that it had infected the others. The clams, over fifty in number, were divided into two aquaria. The infected clam could emit cercariae throughout the experiment. Prior to division the clams had been in the same aquarium for ten days. The age of the growing metacercariae in that aquarium, which did not include the infected one, was known within ten days. After their discovery (four months later), the daughter-sporocysts and cercariae were examined. The penetration ability of the cercariae was tested, as described in Pekkarinen (1987b). The metacercariae growing in the laboratory, and those from natural infections in wild clams, were examined alive and fixed as indicated above for measurements and SEM.

Rearing of metacercariae to the adult stage was tested in the subcutaneous tissue of the laboratory mouse, as described in Pekkarinen (1984a), and *in vitro* in Tyrode's solution containing glucose and a small amount of egg albumen and yolk at 37–40°C. Fresh medium was provided daily.

3. Results

3.1. Daughter-sporocysts and their prevalence

A total of 791 clams with shell lengths of over 10 mm, collected from sampling site I (depth 7–8 m) between the summers of 1983 and 1984, included one (0.13 %) infected with daughter-sporocysts of the species tentatively identified as *Gymnophallus gibberosus* (this name will be used henceforth for this species). Samples from the summer and autumn of 1986 (633 individuals of shell lengths 10–22 mm) were free of infection.

The samples from site II (depth 35 m) totaling 310 individuals (shell lengths 14–22 mm), collected during the summers of 1983 and 1984, included two clams (0.65 %) infected with daughter-sporocysts of *G. gibberosus*.

In experiments where clams from sites II (depth about 40 m) and III (depth 20–30 m) were sampled immediately (in summer 1983) or after maintenance in the laboratory (at 5–8°C or 5–15°C) for up to twelve months, daughter-sporocyst infections were also recorded. Among 465 clams (shell lengths 13–24 mm) from site II (40 m), three individuals were infected by daughter-sporocysts of *L. macomae,* and one (0.22 %) by those of *G. gibberosus*. These daughter-sporocysts had survived in the laboratory for twelve months. Among 330 clams (shell lengths 13–22 mm) from site III, three harboured daughter-sporocysts of *L. macomae,* and two (0.61 %) those of *G. gibberosus*. The clams which died during the experiments were not examined, and they are not included in the total numbers.

The daughter-sporocysts (Fig. 1a) measured up to 2.7 mm in length (Table 1). The mean maximum width was 161–208 μm in different broods of daughter-sporocysts. They occur mostly in the haemolymph spaces of the gonadal region of the clam, where they more or less replace its germinal tissue. The outer surface of the daughter-sporocysts stained slightly with alcian blue. The medium in the brood chamber was sometimes alcianophilic.

3.2. Cercaria

In the forebody of the cercaria there are four pairs of dorsal penetration gland cells (Figs. 1c–e and 3a) with their ducts opening on the dorsal lip of the oral

Table 1. Dimensions (in μm) of fixed (1: 3 % glutaraldehyde, 2 and 3: 4 % formaldehyde) daughter-sporocysts from broods 1–3, in different individuals of *Macoma balthica* from site II (depth 35 m).

Brood number	Date	Host shell length, mm	*N*	Mean length	Length range	Mean smallest width	Mean largest width
1	VI.1984	18.0	12	1697	924–2280	76	161
2	IX.1984	19.7	21	1632	1164–2052	65	177
3	XI.1986	19.3	23	1977	1152–2724	62	208

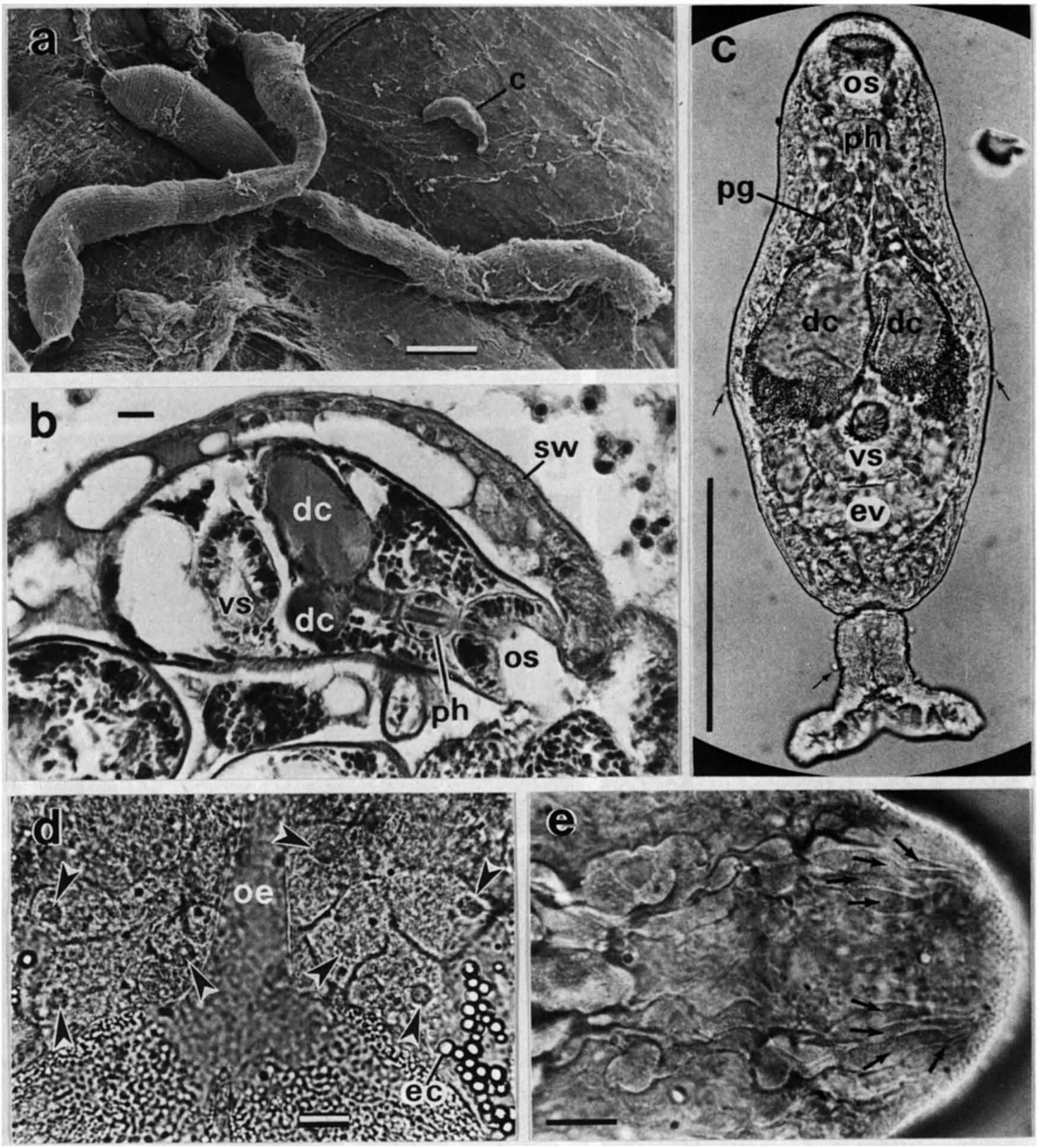


Fig. 1. The daughter-sporocyst and cercaria of *Gymnophallus gibberosus*. — a. Two daughter-sporocysts (one probably broken, lower left-hand corner) and a cercaria (c). — b. A cercaria and embryos within the daughter-sporocyst. Note the rounded pharynx (ph) and digestive caeca (dc) with wide lumen in the cercaria. Alcian blue — PAS — Mayer's haematoxylin staining. os = oral sucker, vs = ventral sucker, sw = daughter-sporocyst wall. — c. A fixed cercaria. ev = excretory vesicle, pg = penetration gland. The small arrows point to sensory papillae. — d. The four penetration glands of one side (with arrowed nuclei) and three of those at the other side, beyond the oesophagus (oe), at focus. ec = excretory corpuscles in the anterior end of the excretory arm. — e. The ducts of the penetration glands in the dorsal forebody. Scale bars: 100 µm in a and c, and 10 µm in b, d and e.

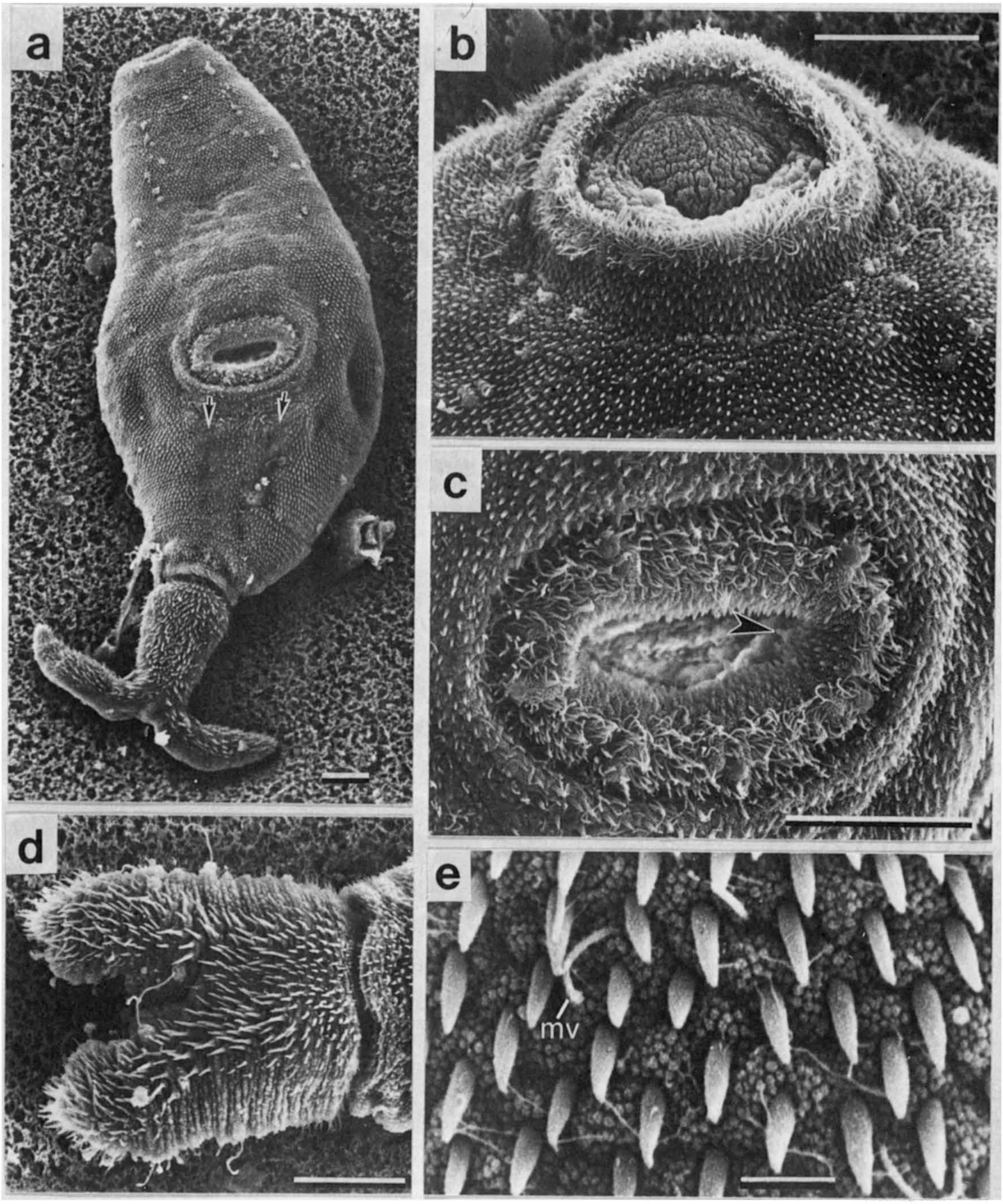
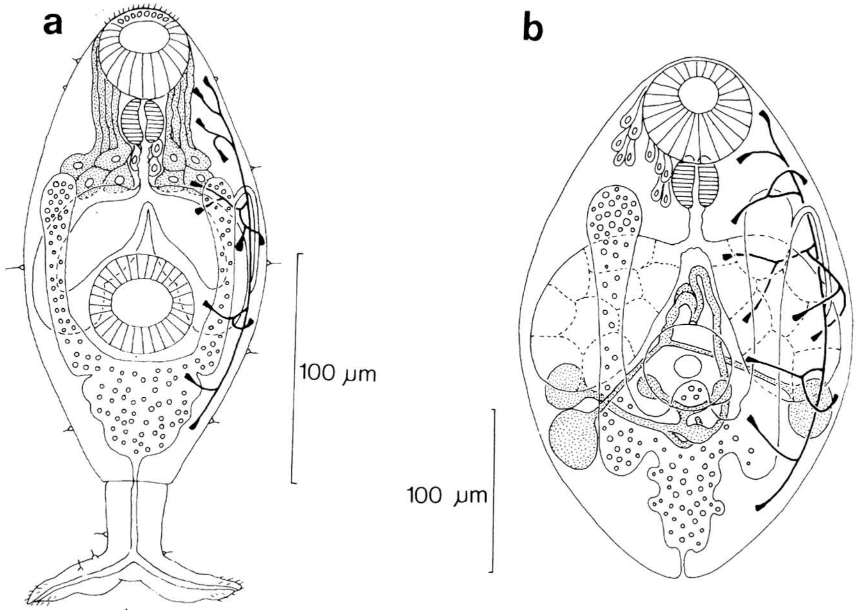


Fig. 2. The surface of the cercaria of *G. gibberosus*. — a. Ventral side, with prominent sensory papillae. Note the fields with sparser spination, posterior to the ventral sucker (arrows), and the unspined area at the site of bifurcation in the tail. — b. Ventral view of the anterior end. Microvilli are present, around and on, the oral sucker. There are spines pointing into the sucker and several papillae on the inner wall of the sucker. — c. Ventral sucker with an outer zone of papillae doublets and microvilli, and an inner zone of spines pointing into the sucker. One of the six inner papillae is visible (arrowed). — d. Ventral view of a tail. Sensory papillae are usually fewer on the ventral, than on the dorsal side. Spines are small or absent laterally. — e. Tegument and spination of the ventral forebody. mv = microvillus. Scale bars: 1 μm in e and 10 μm in the others.

Fig. 3. Ventral view of the cercaria (a) and metacercaria (b) of *Gymnophallus gibberosus*, drawn after a combination of measurements, of fixed specimens, and photographs and sketches of living specimens.



sucker. The digestive caeca are large with wide lumina (Fig. 1b–c) and granular gastrodermis (granule diameter about 0.5–3 μm). The length of the oesophagus is variable due to different stretching or contraction. The penetration glands stained only slightly with neutral red, but the contents of the digestive caeca stained intensively red. Both the penetration glands, gastrodermal cells and contents of the caeca showed PAS-positivity, which was diastase-fast.

The excretory vesicle is Y-shaped (Fig. 3a), with a triangular or variable stem. The slightly dilated distal ends of the arms of the excretory vesicle reach the anterior ends of the digestive caeca, or the level of the oesophagus. The excretory vesicle contains calcareous (stained with alizarin red S) corpuscles, similar in size to the gastrodermal granules. Usually 2×8 flame cells were observed but 2×12 flame cells were actually present in swimming cercariae, which had been aborted from daughter-sporocysts a week earlier. The 2×12 flame cells are arranged as six pairs on each side of the body with a formula of $2\{[(2+2) + (2+2)] + (2+2)\} = 24$ (Fig. 3a).

The tegument of the cercaria is rough and spined (Fig. 2). It was alcianophilic, sometimes also PAS-positive (diastase-resistant). Both suckers have a zone of microvilli (Fig. 2b–c), which are also present around the suckers and occasionally elsewhere on the body. There is also a zone of spines inside the suckers. The ventral sucker bears six inner papillae and six outer papillae, which appear to be doublets.

The spines of the tegument are thick, with about 1 μm protruding through the tegument (Fig. 2e). On the tail, the spines are longer (Fig. 2d) but at the site of the bifurcation they are absent. Sensory papillae of the cercaria are numerous and many of them are prominent (Figs. 1c, 2a, b and d). Four dorsal sense organs of a cycle, at the level of the ventral sucker, bear the longest sensory cilia (about 10 μm in length, maybe much longer).

Measurements of cercariae from three *M. balthica* individuals are given in Table 2. The mean body length of the cercariae in three broods was 156–199 μm. The cercariae of the third brood were more contracted (length/breadth ratio smaller). Swimming cercariae from this brood, in June, were slightly smaller (length x breadth 14.43; length/breadth 1.39). The tail stem and furcae of the cercariae are proportionally short, although in living specimens they can be longer. The ventral sucker is usually larger than the oral sucker. The pharynx is roundish (Fig. 1b).

A few exceptionally large cercariae, with some morphological differences from normal cercariae, were found. They could have up to seven pairs of penetration glands.

The cercariae of this species swam poorly. They barely rose up from the bottom. The cercariae lived in Petri dishes in natural brackish water (salinity 6–7‰) at 20–22°C for several days, and at 6–8°C for up to four weeks. The water was changed daily. Swimming movements gradually decreased, and creeping and quiescence increased. When the water was

Table 2. Basic measurements (in µm, mean and range), and derived measurements of fixed cercariae C_1–C_3 (see Table 1), and laboratory-reared (4 months) metacercariae MC_2 and MC_3 from the respective cercariae, as well as metacercariae from naturally infected wild *Macoma balthica* (MC_4). For comparison, mean measurements of metacercariae of *G. gibberosus* found by Loos-Frank (1971a) from *Cerastoderma edule* (*C.e.*) and *Macoma balthica* (*M.b.*) are given. Most of the derived measurements in the two latter columns were calculated from the means in Loos-Frank's data. OL and OB = ovarium length and breadth, TL and TB = testis length and breadth.

	Present study									Loos-Frank (1971a)	
Host:	*Macoma balthica*									*C.e.*	*M.b.*
	C_1	C_2	C_3	$Range_{1-3}$	MC_2	MC_3	$Range_{2-3}$	MC_4	$Range_4$	MC	MC
N:	13	15	18		7	10		24		16	29
Body length (BL)	193	199	156	143–236	243	264	170–329	319	260–372	373	280
Body breadth (BB)	100	99	99	87–112	117	138	96–164	219	136–260	221	163
Oral sucker length (OSL)	38	36	36	32–40	39	54	34–59	66	46–74	79	48
Oral sucker breadth (OSB)	40	38	37	36–43	47	55	40–62	70	53–79	75	52
Pharynx length	20	19	18	16–22	25	28	22–33	33	24–40	29	24
Pharynx breadth	20	20	18	16–22	27	29	25–32	31	22–37	37	28
Ventral sucker length (VSL)	38	39	42	32–45	38	45	31–48	51	43–62	58	41
Ventral sucker breadth (VSB)	48	46	50	41–54	42	47	37–49	55	43–69		
Ventral sucker distance (VSD)	123	121	95	87–146	147	166	115–211	187	161–214	200	146
Digestive caecum length	65	62	62	53–77	73	83	37–98	94	36–140		
Digestive caecum breadth	40	33	38	21–50	41	43	35–50	58	35–90	49	37
Tail stem length	40	42	37	31–53	OL 15	19	11–27	30	22–39	28	19
Tail stem breadth	27	27	25	19–34	OB 14	19	9–22	26	18–32		
Furca length	41	46	44	26–63	TL 19	25	13–34	37	28–52	47	35
Furca breadth	18	16	11	10–22	TB 13	22	9–28	34	20–43	37	25
BL × BB × 10^{-3}	19.37	19.62	15.42	13.58–23.76	28.15	36.16	19.04–42.15	70.13	35.36–96.72	82.43	45.64
BL/BB	1.94	2.02	1.57	1.39–2.51	2.11	1.96	1.41–2.92	1.48	1.25–1.91	1.69	1.72
BL/OSL	5.14	5.47	4.38	3.92–6.46	6.20	4.94	4.47–7.78	4.89	4.06–5.65	4.72	5.83
OSL/VSL	1.00	0.94	0.84	0.71–1.19	1.02	1.18	0.92–1.44	1.28	1.07–1.58	1.33	1.22
OSB/VSB	0.83	0.82	0.74	0.68–0.93	1.14	1.14	0.93–1.38	1.28	1.10–1.47	1.29	1.27
VSD/BL	0.64	0.61	0.61	0.52–0.76	0.62	0.63	0.52–0.73	0.59	0.50–0.67	0.54	0.52

strongly disturbed swimming cercariae became quiescent and nearly spherical. The cercariae use their furcae as additional suckers. They can even "stand" up on the tips of the furcae, or they make swimming movements with one furca or both attached to the bottom.

In the penetration tests on the mantle edge of *M. balthica,* the cercariae could only attach their suckers weakly, or they did not even try. Only several days after removal from the daughter-sporocyst did some cercariae settle on the mantle, but successful penetration was limited. After some efforts they usually migrated.

3.3. Metacercariae

The metacercariae found occasionally in *Macoma balthica* around the Tvärminne Zoological Station were pink in colour. They could contract until spherical in shape. They were usually found in and around the anterior adductor and foot retractor muscles, but also inhabited regions of the pallial line (including the pallial sinus) and the posterior adductor muscle. Calcified concretions ("pearls") were often found in the adductors.

The length of the metacercariae varied between 260 and 372 µm (Table 2). The sucker ratio was 1.1–1.6. The pharynx was roundish. The digestive caeca, with granular gastrodermis, were long and they crossed the arms of the excretory vesicle dorsally (Fig. 3b). Sometimes the gastrodermal granules had coalesced into larger globules (Fig. 4a). The caeca could extend to the posterior border of the ventral sucker. Fragments of the gastrodermal cells with projecting microvilli were readily pinched off, and thus the caeca diminished in size. The oesophagus often pulsated, mixing the contents of the caeca. The forebody was occupied by numerous gland cells.

The excretory vesicle was shaped like a tuning fork, with diverticula reaching posteriorly from the bases of the arms (Figs. 3b and 4a). The distal ends

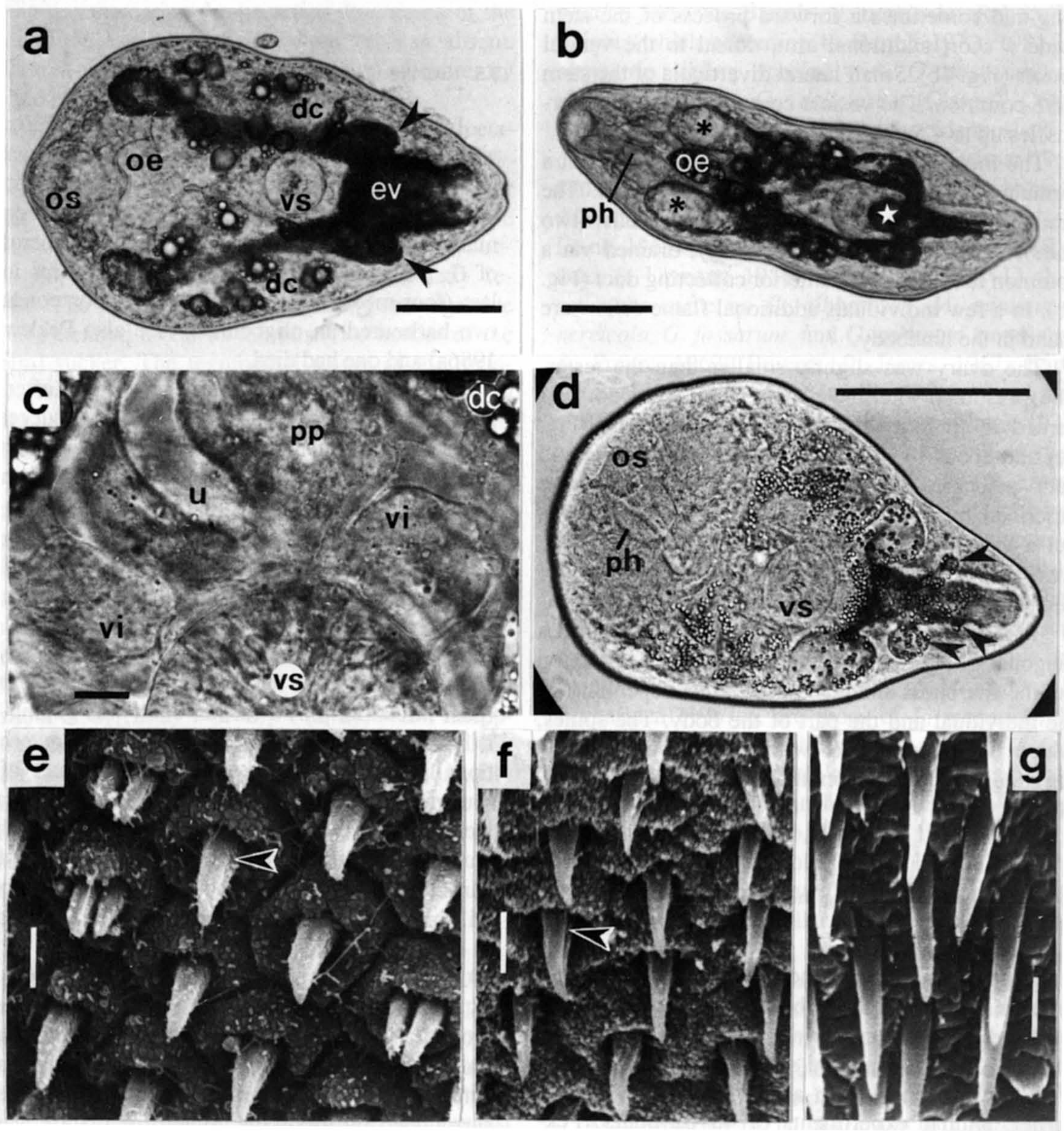


Fig. 4. The metacercaria of *Gymnophallus gibberosus*. — a. A living individual, flattened with a coverslip. The oesophagus (oe) is in a dilated state. The arrows point to the backward diverticula of the arms of the excretory vesicle (ev). — b. An individual with a third arm of the exretory vesicle (white asterisk, the other arms with black asterisks). — c. Part of a flattened metacercaria showing the vitelline glands (vi) in front of the ventral sucker (vs). pp = pars prostatica, u = uterus. — d. A metacercaria three months post infection from *M. balthica* (flattened). The arrows point to small diverticula on the stem of the excretory vesicle. — e. Ventrolateral tegument of a metacercaria. The arrow points to a groove on the side of a spine. Two spines only occasionally exist in the same check. — f. Tegumental spines from the ventral posterior body of another metacercaria. — g. Tegumental spines of an immature individual from the laboratory-mouse. One deformed spine is visible in the lower right-hand corner. Scale bars: 100 µm in a–b and d, 10 µm in c and 1 µm in e–f.

of the arms were somewhat dilated and reached the level of the oesophagus or pharynx. The stem was long and sometimes a forward process of the stem made a short additional arm, dorsal to the ventral sucker (Fig. 4b). Small lateral diverticula of the stem were common. The vesicle contained excretory corpuscles up to 4.5 µm in diameter.

The metacercariae had 2 × 12 flame cells with a formula of 2{[(2+2) + (2+2)] + (2+2)} = 24. The tubules from the four posterior flame cells (two pairs), on each side in the forebody, drained via a common tubule into the anterior collecting duct (Fig. 3b). In a few individuals additional flame cells were found in the hindbody.

The ovary was slightly smaller than the testes. The presumed vitelline glands (they were not discerned in all individuals) were oval or round (Fig. 4c) and about 14 × 14 µm. The seminal vesicle and pars prostatica appeared to be similar to those described by Loos-Frank (1971a) in *G. gibberosus*, but a vas deferens was not found. The genital pore was inconspicuous and opened medially at the anterior border of the ventral sucker.

The tegument of the metacercariae usually had a diagonal checkered pattern. The spines were sharp but the sharpness and thickness varied, depending on the individual and the part of the body. The spines, which were examined with SEM, had shallow longitudinal grooves on the face and sides (Figs. 4e–f).

3.4. Experiments in rearing metacercariae into adults

Metacercariae were inoculated into the subcutaneous tissue of the laboratory mouse, but the worms did not mature during an incubation period of 55 hours. The tegumental spines of these immature worms were long, sharp and smooth (Fig. 4g). In earlier, similar experiments of 48–62 hours (Pekkarinen 1984a) the sizes of the eggs (in µm) of three mature individuals were: one egg 24 × 18, four eggs measured among eleven (24–30) × (17–21), and five eggs measured among twenty-two (24–26) × (17–20). The mean size of ten eggs was 26 × 18 (the mean length 27, and maximum length 32, were incorrect in Pekkarinen 1984a). The eggs of the third individual were, surprisingly, found near the pharynx. The vitelline glands were similar to those of *G. gibberosus* Loos-Frank. Their sizes (µm) were (32–43) × (40–53). During one week's incubation *in vitro,* a few individuals produced spermatozoa. The worms were in good condition.

3.5. Rearing of metacercariae from cercariae

In the first experiment the intensity of infection was low. Among the recipient clams there was an individual which carried cercariae of *L. macomae.* It infected the other clams with young metacercariae, making it difficult to distinguish young metacercariae of *G. gibberosus* from them. The five clams in the last (four-month) sample were in a poor condition: two harboured an oligochaete (see also Pekkarinen 1986a) and one had died.

The second experiment, carried out after the natural emergence of cercariae, was more successful in terms of the intensity of infection. When the metacercariae were observed for the first time their mean number per clam was 18 in eight clams. After two months, a mean number of 18 (range 10–33) was also observed in three individuals. Two additional clams with open shells, in the two-month sample, harboured 77 and 24 metacercariae. Histological sections of the latter revealed that it was ill and dying. Three months after the infection with the cercariae (post infection, p.i.) a further clam was gaping, and it harboured 28 metacercariae. These were proportionally well developed. In four individuals of the four-month sample, the numbers of metacercariae were 8, 15, 38 and 60 (in that aquarium which included the clam with the cercariae, three individuals harboured 23, 37 and 52 metacercariae). Six months p.i., one clam was dead. Numbers of metacercariae in the living clams were 9, 20, 21, 23, 30, 41 and 59.

The metacercariae were found, sometimes in groups, in and around the adductor and foot retractor muscles and along the pallial line and the pallial sinus. At the pallial line the metacercariae were not surrounded by the tissue. When the mantle was removed, the worms detached easily, or remained on the mantle or shell in mucus. Early metacercariae were sometimes also free in the central extrapallial space. Small round or oval imprints, which were calcified, had appeared on the inner surface of the shell.

In the second experiment, 0–10 days p.i., the size of the young metacercariae was about 16 (length × breadth in µm × 10^{-3}). The ratio body length/body breadth (BL/BB) was 1.80. The sucker length ratio was 0.99, and the sucker width ratio was 0.90. In both experiments, 1.5–2 months p.i., the mean metacercarial sizes were about 20 (BL/BB 1.53–1.84), and the mean sucker ratios were 0.91–1.15. In these young metacercariae the posterior diverticula of the arms of the excretory vesicle were small or absent. The three-month old metacercariae had an excretory vesicle typical of *G. gibberosus* (Fig. 4d).

At four months p.i., the mean sizes of the metacercariae were 28–36 (range 19–42, BL/BB 1.96–2.11) (Table 2: MC_2 and MC_3). The mean sucker ratios of these metacercariae were 1.02–1.18. The ducts of the penetration glands were usually unchanged, and the glands proper were slightly reduced. The pharynx was roundish and the digestive caeca extended as far as the posterior border of the ventral sucker. The tegumental spines of the metacercariae had a longitudinal groove on the face. In some individuals the tegument had a checkered pattern.

Two months later (six months p.i.), no clear size increase or changes in the other measurements were found. The mean sizes of metacercariae in three clams were 35–39 (range 26–51). The mean ratio BL/BB was 1.39–1.52. Remnants of the penetration glands with their ducts were still present. Finely granular gland cells had appeared in the forebody.

The metacercariae had 2 × 12 flame cells, with a formula of $2\{[(2+2) + (2+2)] + (2+2)\} = 24$. Exceptional formulae were, however, noticed in about 10% of the five- and six-month-old metacercariae. Usually the protonephridia of the other side were normal. The anomalous formulae varied considerably, one example being $\{[(4+4) + (4+2)] + (2+2)\}$.

4. Discussion

The site preference of the metacercariae found in *Macoma balthica*, and also of those raised experimentally from the cercariae, is similar to that found by Lauckner (1971) for *Gymnophallus gibberosus* in *Cardium edule.* The calcareous concretions ("pearls"), often found in the adductors, also suggest former presence of *G. gibberosus* (see Lauckner 1971). The presence of these worms may easily be underestimated — namely, the metacercariae may remain in the adductor muscles taken along with the soft part, in spite of vigorous shaking of the detached body in water.

The excretory vesicle of both metacercariae was typical of *G. gibberosus* (see Loos-Frank 1971a, Lauckner 1971). Other species which, at some developmental stage, may have a similar excretory vesicle are: *Meiogymnophallus minutus* (Cobbold) (according to Bowers & James 1967 it is synonymous with *Gymnophallus nereicola* Rebecq & Prévot), *Gymnophallus fossarum* Bartoli, *G. nereicola* Rebecq and Prévot (which according to Bartoli 1972, 1981 is a sympatric sibling species with *G. fossarum*), *G. rebecqui* Bartoli, *G. deliciosus* (Olsson) Odhner (which is sometimes regarded as identical to *G. choledochus* Odhner, for example, by Reimer 1962), and *G. bursicola* Odhner. All of these species have 2 x 8 flame cells, except for *G. choledochus* (Loos-Frank 1969) and probably also *G. bursicola* (Stunkard & Uzmann 1958) which have 2 × 12 flame cells, the same as *G. gibberosus. M. minutus, G. nereicola, G. fossarum* and *G. rebecqui* (see Rebecq & Prévot 1962, Bowers & James 1967, Bartoli 1965, 1972, 1974) are smaller in size and have smaller eggs than *G. gibberosus* (Loos-Frank 1971a) and the present species. The uterus in all of these, including *G. gibberosus*, is mostly in the anterior two thirds of the body.

Metacercariae of *Meiogymnophallus minutus* inhabit the subarticular extrapallial space of *Cerastoderma edule* (Bowers & James 1967, Loos-Frank 1971b, Lauckner 1971). They are usually surrounded by mantle tissue. Metacercariae, which have been found within the pallial isthmus of *M. balthica* by Pekkarinen (1987b), are those of *L. macomae.* The spines of *M. minutus* differ from those of *G. gibberosus* and the trematode of the present study. They are short and rounded at the apex (Bowers & James 1967, Loos-Frank 1971b, Lauckner 1971).

According to Bartoli (1972, 1974, 1981), the only second intermediate host for *G. nereicola* is *Nereis diversicolor* Müller (or other nereids). There is a peculiarity in the life cycle of *G. nereicola.* Sometimes, a small number of cercariae develop into metacercariae within daughter-sporocysts in the first intermediate host, *Abra ovata* (Philippi) (Bartoli 1974, 1981). The cercariae (Bartoli 1972) differ from the ones examined in the present study.

Metacercariae of *G. fossarum* in *Cerastoderma glaucum* (Bruguière), settle below the hinge (subarticular extrapallial space) and at the margin of the mantle (peripheral) (Bartoli 1973a). In *Venerupis decussata* (L.) they prefer the mantle at the pallial line (Bartoli 1973b). The daughter-sporocysts of *G. fossarum* are, in contrast to the present species, short, and its cercariae have no penetration glands (Bartoli 1972).

Metacercariae of *G. rebecqui* parasitise the dorsal parts of, or general, extrapallial space of *Abra tenuis* (Montagu) and *Cerastoderma glaucum* (Bruguière)

(Bartoli 1983, Campbell 1985). Its tegumental spines are multi-pointed, and its cercaria is also different from the present cercaria.

Gymnophallus choledochus, G. deliciosus and *G. bursicola* (Odhner 1900, Loos-Frank 1969) all have larger eggs than the other above-mentioned species, the egg size being similar or close to that in *G. gibberosus* and the present species. When full-grown adults they are quite large and their vitelline glands consist of many distinct follicles or lobules. Reimer (1962) supposed that *Metacercaria tertia,* found by him in *Cerastoderma edule*, could be identical to *G. deliciosus*. Its vitelline glands consisted of several separate follicles, even in the metacercarial stage. The vitelline glands of the metacercaria of *G. gibberosus* (Loos-Frank 1971a) and the present metacercaria are small and compact.

In *G. deliciosus* the ventral sucker is anterior to the mid-point of the body and its testes are larger than the oral sucker (Odhner 1900, Loos-Frank 1969). *G. bursicola* can be distinguished from *G. gibberosus,* as described by Loos-Frank (1971a), and present trematode by its elliptical pharynx, short caeca, large seminal vesicle and by the smaller size of its suckers in relation to its body size (see Odhner 1900).

The metacercariae of *G. choledochus* occur in polychaetes and sometimes also develop within the daughter-sporocysts in the first intermediate host, *Cerastoderma edule* (Loos-Frank 1969). Its daughter-sporocysts (Loos-Frank 1969, Lauckner 1971) are strikingly different from the daughter-sporocysts in the present study. Reimer (1962) supposed that *Cercaria trioglandulosa*, which he found in *Macoma balthica*, might be the larval form of *G. deliciosus* (according to him *G. deliciosus* and *G. choledochus* were identical). The daughter-sporocysts of *C. trioglandulosa* were different from those of *G. choledochus*. In fact, they were more similar to the daughter-sporocysts, described here, as those of *G. gibberosus*.

The metacercariae of *G. gibberosus,* found by Loos-Frank (1971a), in *M. balthica* were smaller than those she found in *Cerastoderma edule*. According to Lauckner (1971), the metacercariae in *C. lamarcki* (Reeve) were also smaller than those in *C. edule*. The metacercariae of the present study were, however, on the average larger than those which Loos-Frank found in *M. balthica* (Table 2) but the maximum length was smaller. Maybe Loos-Frank's sample included more, younger individuals. Comparisons of the sizes are, of course, affected by the age distribution of the individuals in the sample. The oral sucker in the metacercariae found by Loos-Frank in *M. balthica,* was proportionally small. The upper limit of the sucker ratios calculated by the present author was slightly greater, about 1.6, while in Loos-Frank's data it was 1.45. In the experimentally grown metacercariae of the present study the sucker ratios gradually increased from that in the cercariae, thus, the relationship between the two suckers is allometric. In the present metacercariae the gastrodermis was not longitudinally ridged, pharynx was not clearly wider than its length and a vas deferens was not discerned. Otherwise, the present species agrees with the data presented by Loos-Frank (1971a) and Lauckner (1971) on *G. gibberosus*.

Another species, viz. *Gymnophallus somateriae* (Levinsen, 1881) in connection with *G. gibberosus* needs to be considered. Levinsen found the adults in *Somateria mollissima,* in Greenland. Metacercariae of *G. somateriae* occur in a clam closely related to *Macoma balthica*, viz. *M. inconspicua*, on the Pacific coast of Canada (Vancouver) (Ching 1973). The metacercarie of the present study are similar in size, and in many other characteristics to *G. somateriae.* The only differences appear to be that *G. somateriae* has fewer (2 x 10 = 20) flame cells, a pharynx wider than its length (as it was in Loos-Frank's data of *G. gibberosus*) and possibly a different excretory vesicle. The excretory vesicle of *G. somateriae* (Ching 1973) was described as Y-shaped, with 'small lobes near the base of the stem', but the site of the lobes is not clear. According to the figure, they cannot be anything but the dilated bases of the arms. In *G. gibberosus* there are typically posterior diverticula at the bases of the arms but they, however, can disappear due to fixation (Loos-Frank 1971a). The body size of adult *G. gibberosus* from *Melanitta fusca* (Loos-Frank 1971a), is not significantly different from *G. somateriae* as described by Ching (1973). The range of egg sizes, in both species, overlap in great part. Only the proximal part of the seminal vesicle in adult *G. somateriae* is very large, even larger than the ventral sucker. Obtaining mature specimens of both species has been difficult. For a better comparison more material is needed.

The flame cell formula of the present species was slightly different from that reported by Loos-Frank (1971a) in *G. gibberosus*: there were four pairs of flame cells on each side in the forebody and only two pairs in the hindbody, instead of three pairs in both the fore- and hindbody. In *Lacunovermis macomae* (see Pekkarinen 1987b) the flame cell formula was found to be similar — namely, 2{[(2+2) + (2+2)] +

(2+2)} = 24. Both species have 24 flame cells, even in the cercaria. The cercaria of *G. choledochus* may have the same number of flame cells (Loos-Frank 1969). The formula for flame cells in many species ought to be re-examined. Uneven distribution of the flame cells, with the anterior collecting duct receiving more tubules, occurs in such trematodes which have 2 × 6 or 2 × 10 flame cells (James 1964, Bartoli 1965, Ching 1973, Shimazu 1975).

Deviation from the normal flame cell formula (often with an increase in number) was rarely found in wild *G. gibberosus* (see also *L. macomae* cercariae: Pekkarinen 1987b), the occurrence being more frequent in the laboratory-reared metacercariae of *G. gibberosus*. Adult *Fasciola* can have hundreds of flame cells in its protonephridial system. It is not known how the individual flame cells arise (Wilson & Webster 1974). Repeated divisions of certain former flame cells might be a way of increasing their number: this may explain why flame cells often occur in pairs.

According to Lauckner (1971), young metacercariae of *G. gibberosus* are free in the extrapallial space of *Cerastoderma edule*, but older ones are often covered by a thin membrane. Similarly, the young experimental metacercariae in the present study were free. The calcified imprints inside the central parts of the shells, which were normally clean, signified the former presence of young metacercariae. These had probably migrated to the muscular sites or died. Asymmetrical gaping of the valves — with a larger gap in the anterior region — is characteristic of *Cerastoderma edule* and *C. lamarcki* infected with metacercariae of *G. gibberosus* (Lauckner 1983). The survival of cockles, heavily infected with *G. gibberosus*, is substantially reduced. A few individuals of *M. balthica* showed gaping two to three months after infection with the cercariae. One individual was in very poor condition and moribund, but the reason for this disease is not known. Clams in the sampling site where the recipient clams were collected, often had anomalies (Pekkarinen 1985, 1986b and unpublished data), which may affect their viability. The metacercariae of *G. gibberosus* actively digest tissues of the host — probably aided by proteolytic enzymes — and penetrate the muscle tissue, causing the host to form calcareous concretions (Lauckner 1971). The sharp spines, described in the present study, may also contribute to these processes. The metacercariae which Pelseneer (1906) described as gnawing the anterior adductor muscle of *M. balthica* may have, thus, been those of *G. gibberosus*.

The metacercariae develop slowly in *M. balthica*. It is likely that many metacercariae, even in the wild clams, were immature, and therefore the vitelline glands could not always be distinguished. For a similar reason, sexual maturation of many metacercariae in experimental final hosts and *in vitro* was poor. Maturation may also take a longer time, even in natural final hosts. Loos-Frank (1971a) obtained sexually mature individuals five to six days post infection in *Somateria mollissima*. In the wild they occurred in *S. mollissima* and in the caecum (and hindgut) of *Tadorna tadorna, Melanitta fusca* and *M. nigra*.

The differences between the present cercaria, which is considered to be *G. gibberosus*, and *Lacunovermis macomae* (Pekkarinen 1987b) also occurring in *M. balthica* are as follows. Both suckers of *L. macomae* are similar in size, or, the longitudinal diameter of the oral sucker is greater. In *G. gibberosus* the ventral sucker is usually larger. The pharynx of *L. macomae* is clearly longer than its breadth, whereas in *G. gibberosus* it is rounded. The former species has two pairs of penetration glands, whereas the latter has four pairs. The digestive caeca of *G. gibberosus* are larger, with a wider lumen, and there are usually larger granules in the gastrodermal cells. The tail stem and furcae were usually shorter in *G. gibberosus*, and had few microvilli. Microvilli were present around the suckers but they were less frequent than in *L. macromae*. Instead, sensory papillae on the body and tail of *G. gibberosus* were more prominent. The excretory vesicle of *G. gibberosus* contained smaller excretory corpuscles. Furthermore, the daughter-sporocysts in which the cercariae of *G. gibberosus* develop are usually longer and narrower, although some overlapping of the size ranges exists. Immature daughter-sporocysts can, of course, be shorter, and they can be confused with those of *L. macomae*. The size of the cercaria body cannot be used as a distinguishing characteristic. The size and form of the tegumental spines do not differ greatly either. The flame cell numbers of mature cercariae were identical, namely, 2 × 12.

The ability of the cercariae of *G. gibberosus* to penetrate the mantle edge of *M. balthica* was poor. The cercariae may have been exhausted, or the usual route of penetration and migration is different. The cercariae, however, lived in the laboratory as long as the cercariae of *L. macomae*, namely, one month. The only clear histochemical difference observed between these two cercariae was the alcianophilia of the tegument in *G. gibberosus*.

The cercaria (and daughter-sporocyst) of *G. gibberosus* has many characteristics similar to, but also different from, *Cercaria baltica* described by Markowski (1936) and Loos-Frank (1971b). The daughter-sporocysts of *C. baltica* fall within the length range of the sporocysts of the present species although the sporocysts studied by Markowski, with a length of ca 1.2 mm, are near the lower limit of the range, and the daughter-sporocysts described by Loos-Frank are wider (0.3 mm).

Markowski refers to the poor swimming of *Cercaria baltica,* which agrees well with the present cercaria. The former cercaria has a round pharynx, as does the cercaria in this study. According to Loos-Frank's description, the pharynx of *C. baltica* is long, nearly twice its breadth in living specimens, but shorter than its width in fixed ones. The suckers of the present cercaria are similar in size to those studied by Loos-Frank in fixed cercariae, but the tail stem and furcae of the latter are longer. In the data given by Markowski the tail stem of the cercariae was very long (90 µm), but the furcae were short, similar to those in this study. In figure 6 of Markowski (1936) the tail stem and furcae of the cercaria are inconsistent with the given measurements, the tail stem being shorter than the furcae. The form of the excretory vesicle, in the figures of creeping cercariae, resembles that of present cercariae.

According to both descriptions, *C. baltica* has four pairs of penetration glands. The number agrees with the present cercaria, but in the specimens studied by Markowski they were all more anterior, and in those of Loos-Frank two pairs were more posterior, namely, between the caeca and ventral sucker. Loos-Frank reports microvilli ('Haare') on the anterior end and tail of the cercaria, and papillae with long sensory setae on its body and tail. The prominent sensory papillae are consistent with *G. gibberosus*. According to Pekkarinen (1987b), the cercaria of *Lacunovermis macomae* resembles *C. baltica,* in some characteristics. Thus, both earlier workers may have had cercariae of the two species in their samples.

The daughter-sporocysts of *Cercaria trioglandulosa* from *Macoma balthica* (Reimer 1962, 1971) agree in their dimensions with the lower part of the size range given for the daughter-sporocysts of the present species. The body size of the cercaria was similar to *G. gibberosus*. The pharynx was rounded, as in *G. gibberosus*, but slightly smaller. The digestive caeca were described as quite large, with a wide lumen, as is the case in the present cercaria. The oral sucker of *C. trioglandulosa* is similar to that in *G. gibberosus*, the ventral one being smaller. As indicated by their name, the cercariae had only three pairs of penetration glands, and they had 2 × 8 flame cells. Reimer (1962, 1971) also described another cercaria in *M. balthica* — *C. duoglandulosa* which according to Pekkarinen (1987b) is identical to *L. macomae*. *C. trioglandulosa* uses the same host as *G. gibberosus*, thereby, making it tempting to try and find some similarity in these cercariae. The penetration glands of *G. gibberosus* are crowded before the caeca and, thus, difficult to count. Some of the flame cells may easily be missed, and if the cercariae examined by Reimer were young, the flame cell number might therefore be small. The cercariae were fixed in hot water, which may cause the tail to be extended. Since some differences still remain, and as Reimer (1962) already supposed, the cercaria may belong to *G. deliciosus*.

Metacercariae of many gymnophallid species (if they all are valid species) are much alike, and their cercariae may also resemble each other. Many gymnophallids use several secondary host species, and it is difficult to predict in which species the daughter-sporocysts and cercariae will be found. There is, therefore, much work yet to be done. The daughter-sporocyst and cercarial stages of *G. gibberosus* have previously been unknown. In this paper, the daughter-sporocysts and cercariae found in *Macoma balthica* are considered to belong to *G. gibberosus*, the metacercariae of which are also found in *M. balthica* of the same locality. Another possible host species in the same locality, namely *Cerastoderna glaucum* (Reeve), has not been examined in relation to this problem.

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