Contributions to Zoology, 69 (1/2) 21-29 (2000)
SPB Academic Publishing bv, Amsterdam

The egg string attachment mechanism in salmon lice *Lepeophtheirus salmonis* (Copepoda: Caligidae)

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Key words: Salmon lice, *Lepeophtheirus salmonis*, genital complex, egg string attachment

Abstract

The anatomy of the genital complex and the hook apparatus that keeps the trailing egg strings in position, are illustrated and described. Based on light and scanning electron microscopy, it is shown how the sacs are mechanically secured by the penetration of a pair of hooks through the proximal ends of the strings. The muscle groups that move the hooks are described and a mechanism of operation is suggested.

Contents

Introduction

The salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837) is a well known parasite on wild and marine farmed salmon (*Salmo salar* L.), and a serious problem in European aquaculture. Although control measures presently include organophosphates, pyrethroids, hydrogen peroxide, and cleaner fish, the parasite continues to incur large costs for the fish farmers. A better control of sea lice depends on a thorough knowledge of its biology.

The fertilized embryos of the female louse are kept in two sacs or strings which are extruded through the gonopores in the genital complex. The strings are closed at both ends. Normal hatching occurs with the strings attached to the female, but if stressed, the female can release entire strings. This suggests that the strings are kept in place by an apparatus which is under muscular control. The present work describes this apparatus and explains how it is used to hold and release the egg strings.

Material and methods

Female salmon lice, with and without egg strings, were collected from farmed salmon on the west coast of Norway and brought in Thermos bottles to the Department of Biology, University of Oslo. The animals were studied alive under a binocular microscope, preserved in 4% formaldehyde or cleared in lactic acid for light microscopy. The parts of the parasite which were studied in detail were transferred to polyvinyl lactophenol for permanent mounting. Specimens for scanning electron microscopy were postfixed in 2.5% glutaraldehyde, and thereafter treated as described by Schram (1991).

Results

Anatomy of genital complex

The club-shaped ovaries are located in the prosome. The narrow oviducts pass posteriorly, and increase in diameter when they enter the genital complex

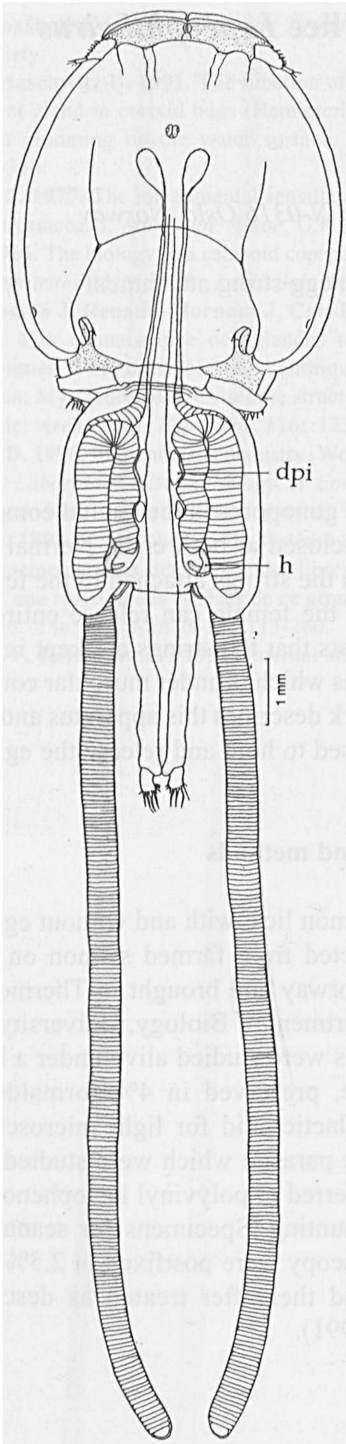


Fig. 1. Female *Lepeophtheirus salmonis* in dorsal view. The paired convoluted oviducts end above the hidden vitellaria. The hooks and the antra are visible, as are the origins of the four dorsoventral pillars between the oviducts and the gut. Abbreviations: dpi, dorsoventral pillar; h, hook.

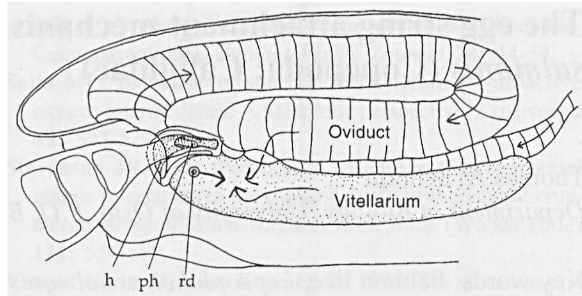


Fig. 2. Schematic illustration of genital complex of *Lepeophtheirus salmonis* in medial view, looking onto the point of the hook securing the egg string. The convoluted oviduct is visible opening into the more ventrally situated vitellarium. Arrows in the oviduct show the direction of the movement of the oocytes. Arrows from the receptacle duct, the oviduct, and the cement gland illustrate where their products meet in the genital antrum. Abbreviations: h, hook; ph, point of hook; rd, receptaculum duct.

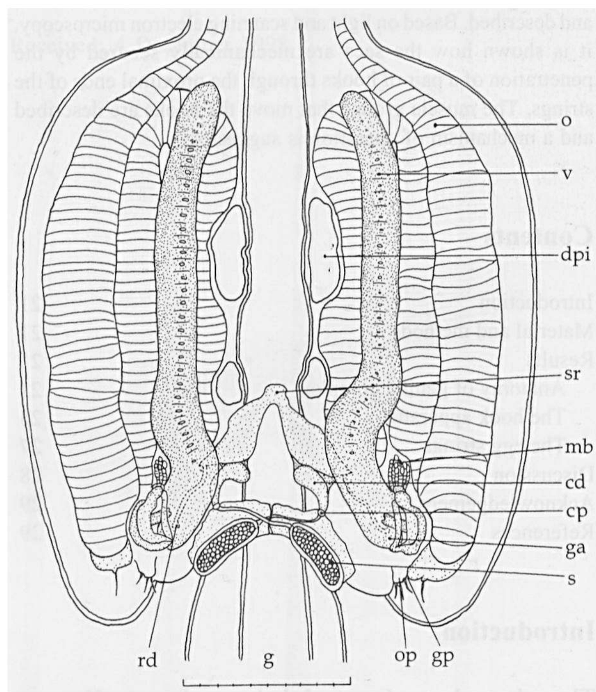


Fig. 3. Ventral view of genital complex of adult female *Lepeophtheirus salmonis* with internal organs. Abbreviations: cd, copulatory duct; cp, copulatory pore; dpi, dorsoventral pillar; g, gut; ga, genital antrum; gp, gonopore; mb, muscle bands; o, oviduct; op, operculate plate; rd, receptaculum duct; s, spermatophore; sr, seminal receptacle; v, vitellarium. Scale bar 1 mm.

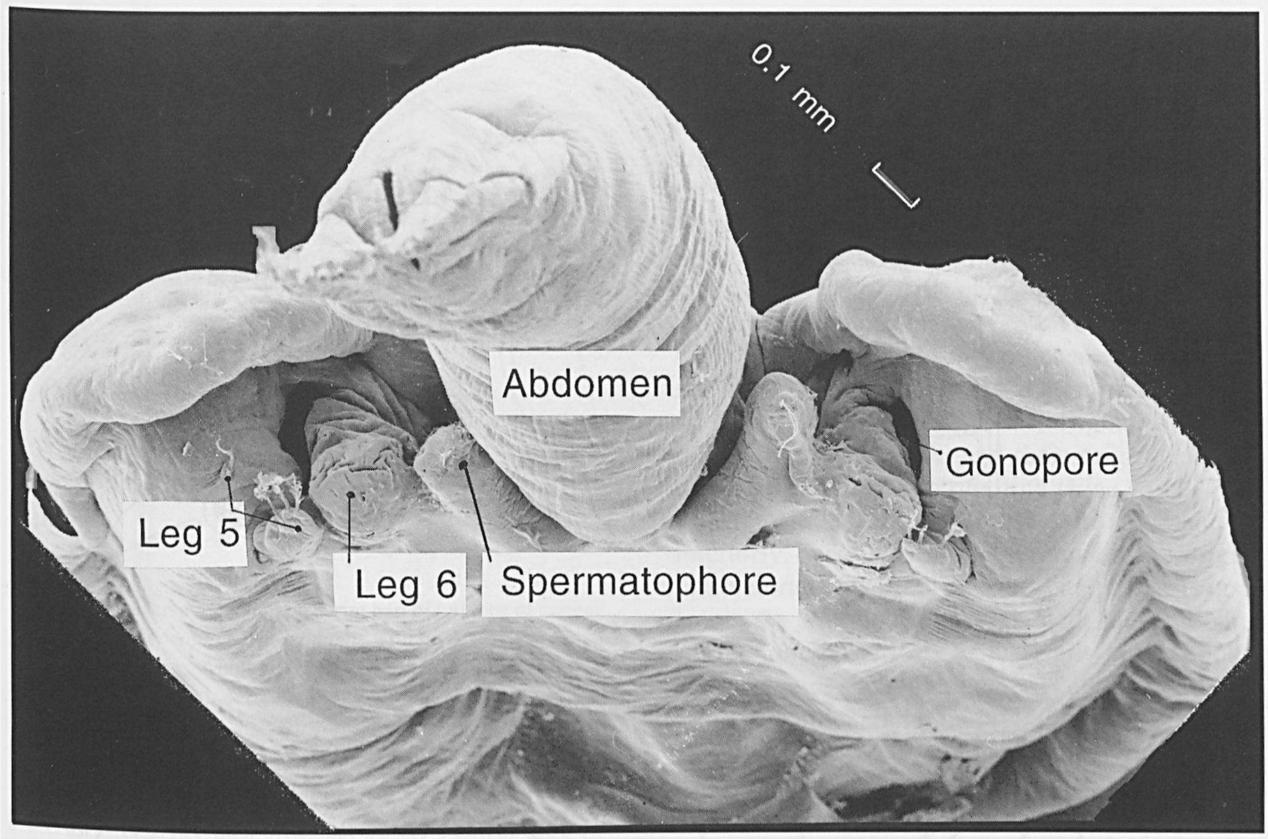
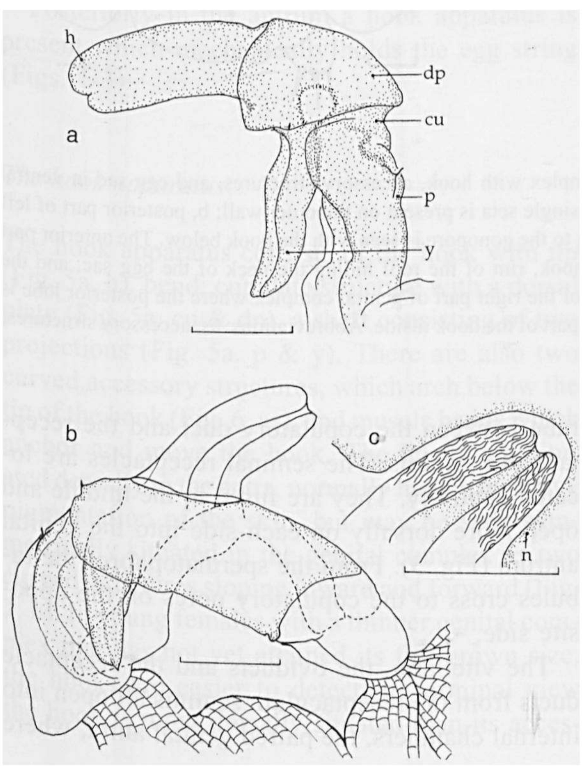


Fig. 4. Terminal view (SEM) of the posterior end of the genital complex of *Lepeophtheirus salmonis* with crescent shaped oviduct openings. Posterolateral lobes are seen above the vestibules, leg 5 venterolaterally and leg 6 medially.



and become convoluted (Fig. 1). They continue ventrally and turn dorsally at the end of the genital complex, proceed forward until they make a second turn inward and go backwards again until they reach the genital antra, in which they terminate from the dorsal side (Figs. 1; 2). The paired vitellaria (cement glands) are situated ventrally in the genital complex. These are sausage-shaped organs which are closed off anteriorly, and extend posteriorly until they merge in the genital antra. In the antrum, the oocytes, vitelline and sperm meet before being passed through to the exterior via the gonopores (Fig. 3. gp). The gonopores are located ventrolaterally at the posterior end of the genital complex. In ventral view, opercular plates with three setae, which are rudimentary fifth legs (Huys & Boxshall, 1991, fig. 2.9.25B), hide them. In ter-

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Fig. 5. Lepeophtheirus salmonis. Hook in terminal (a) and lateral view (b); c, point of the hook and notch. Abbreviations: cu, cupulate structure; dp, dorsal plate; h, hook; n, notch; p & y, ventral projections. Scale bars 0.1 mm.

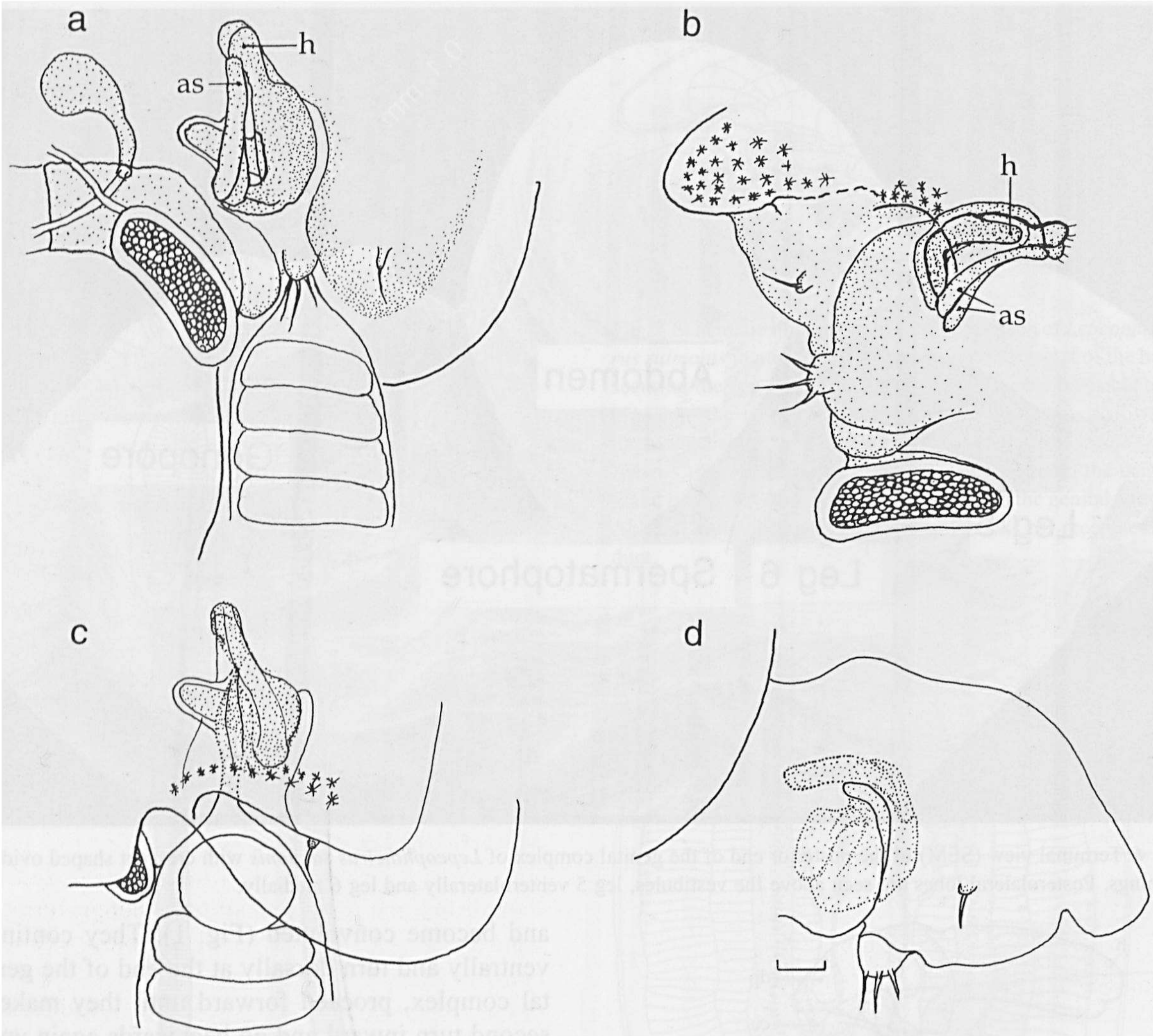

Fig. 6. Lepeophtheirus salmonis. a, left posterior end of genital complex with hook, accessory structures, and egg sac in ventral view. An operculate plate with three setae overlies part of the sac. A single seta is present on the outer wall; b, posterior part of left lobe of genital complex in medial view. The roof of the funnel leading to the gonopore is seen with the hook below. The anterior part of the hook is more dorsally situated than its posterior end; c, the hook, rim of the roof hiding the neck of the egg sac, and the overlying genital complex, are seen in dorsal view; d, terminal view of the right part of genital complex where the posterior lobe is cut away. The crescent-shaped opening of the gonopore is shown with part of the hook inside. Abbreviations: as, accessory structures; h, hook. Scale bar 0.1 mm.

minal view, the gonopores are seen as two crescent-shaped openings (Fig. 4). One seta is situated on the cuticule laterally to the oviduct openings on each side (Figs. 4; 6). These represent part of the fifth legs whereas the unarmed lobe located medial to the fifth leg represents the sixth leg (Boxshall, 1974).

The two copulatory pores lie on either side of the midline, ventrally on the genital complex (Fig. 3, cp). There is a mesenterium-like compressed tube between the copulatory duct and the receptaculum seminis. The seminal receptacles are located ventrally. They are fused in the middle and open more dorsally on each side into the genital antrum (Fig. 3). From the spermatophores the tubules cross to the copulatory pores on the opposite side.

The vitellaria, the oviducts and the receptacle ducts from the receptaculum seminis all open into internal chambers, the paired genital antra, where

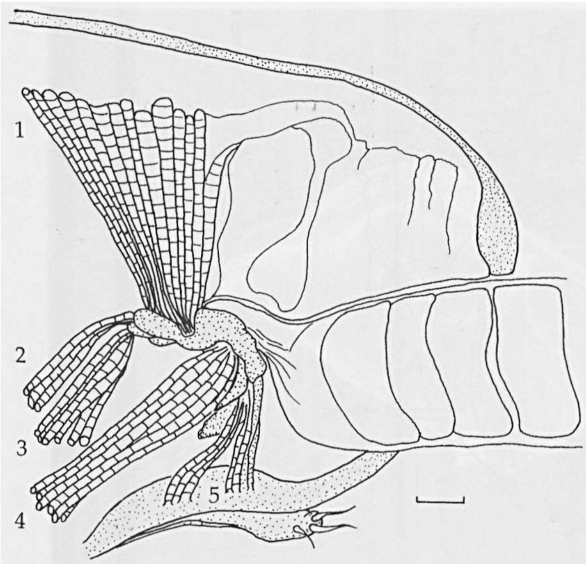

Fig. 7. Transverse section of posterior part of left lobe of genital complex of *Lepeophtheirus salmonis* with hook and egg sac in situ, lateral view. Abbreviations: 1 & 4, muscle groups that move the hook; 2, 3, & 5, anchoring muscle groups. Scale bar 0.1 mm.

the fertilization takes place. The entrance from the oviduct is wide and situated on its ventral side to the underlying antrum, which seems to be an expanded continuation of the vitellarium (Fig. 2).

Posteriorly in the antrum a hook apparatus is present which mechanically holds the egg string (Figs. 1-3).

The hook apparatus

The hook apparatus consists of the hook with tip (Fig. 5a. h), bend: cupulate structure with a dorsal plate (Fig. 5a. cu & dp), a shaft consisting of two projections (Fig. 5a. p & y). There are also two curved accessory structures, which arch below the tip of the hook (Fig. 6. as), and muscle bands which anchor and move the hook. The hooks are situated dorsally to the antra, normally hidden by heavy pigmentation of the skin, but may be seen symmetrically situated in the genital complex as two curved structures sloping inward and forward (Fig. 1. h). In young females with a thinner genital complex that has not yet attained its full-grown size, the hooks are easier to detect. In terminal view the hook is more dorsally situated than its accessory structures (Fig. 6b). Part of the main body of the hook may be seen in terminal view as a yellow chitinous structure through the crescent shaped gonopore (Fig. 6d). The hook with its accessory structures takes up a space c. 400 μm long, 300 μm wide and 300 μm deep.

Lateral to the vitellaria there are 5-6 muscle bands on each side inserted onto the anterior end of the hooks (Fig. 3. mb). This muscle group passes forward and originates on the ventral cuticle. In Fig. 7, the hook and its muscle groups are seen in lateral view. The muscle group that contracts when the hook penetrates the egg sac, originates dorsal and lateral to the hook, and their origin extends along an anterior-posterior line (Fig. 7. 1). The apparatus is anchored by muscle groups 2 and 3 anteriorly, and 5 posteriorly. Group 2 and 3 originate ventrally, just outside the vitellarium (Figs. 3. mb; 7). Muscle group four, with longer fiber, inserts more posteriorly and laterally on the hook, and originates toward the inner ventral side of the genital complex. This muscle seem to be an antagonist to group 1, i. e. it contracts when the egg sac is to be released. Muscle group 5 and connective tissue are secured between the posterior end of the hook and the inner ventral surface of genital complex. The second, third and fifth muscle group keep the hook in position, whereas those of the first and fourth group operate the movement of the hook.

The muscle attachment to the body wall is different from the four more conspicuous hyaline origins which are symmetrically situated pillars of connective tissue in the genital complex, and clearly visible both in dorsal and ventral view (Figs. 1; 3). The fact that the exoskeleton above these origins is without the brown pigment, which is otherwise distributed all over the parasite, makes it easier to observe these structures in transmitted light (Schram, 1993).

Medial to the hook there are two curved accessory structures (Figs. 6a-b; 8a. as) that seem to be connected to the genital antrum.

The hook and its accessory structures may be compared to a torso (Fig. 8f) in which the trunk corresponds to the cupulate centrally situated base (Figs. 5a; 8e. cu). The shoulder would be the somewhat curved rectangular plate (c. 350×90 μm), which has a fingerprint structure dorsally (Fig. 5a; 8b. dp).

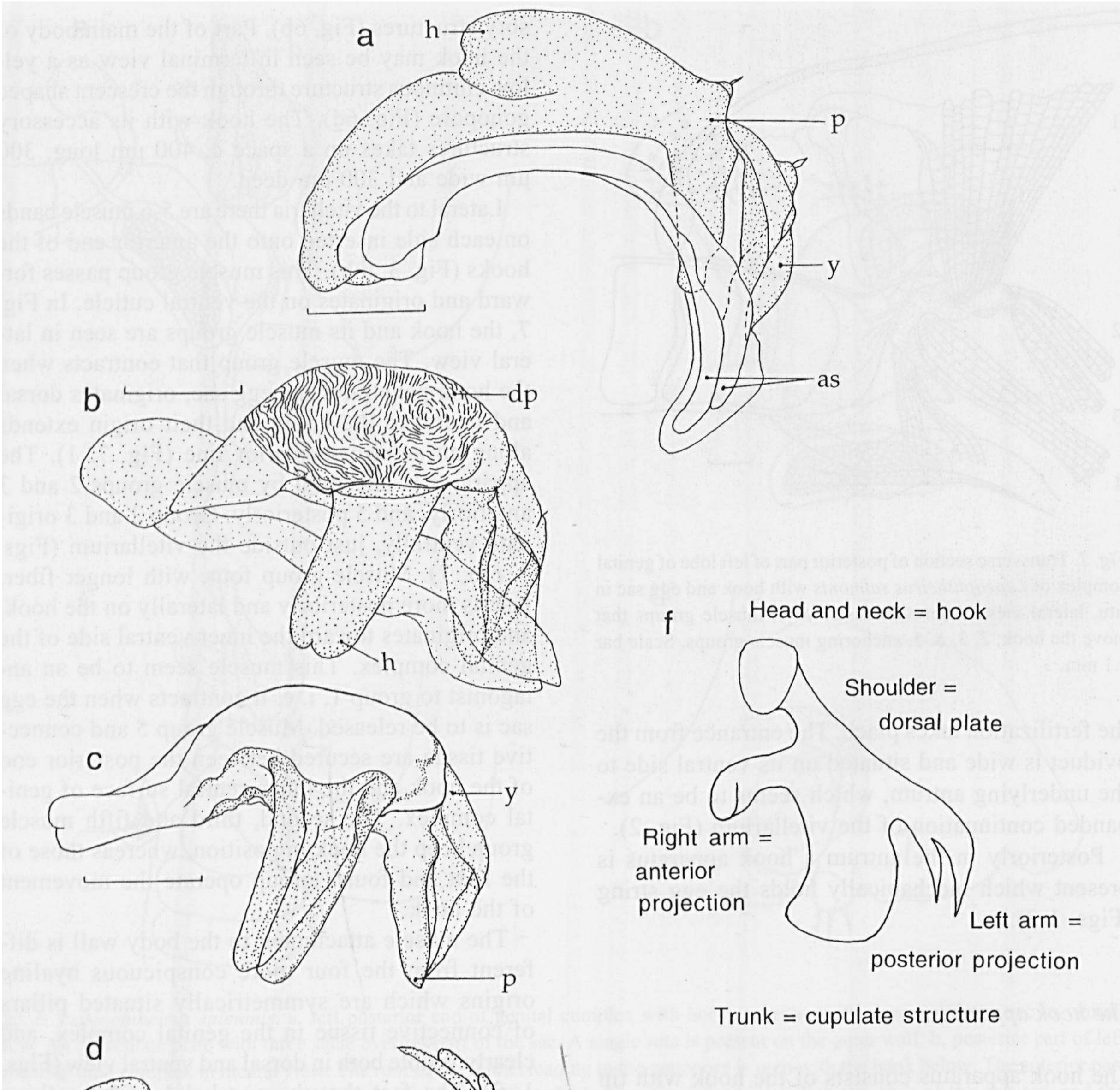

Fig. 8. Lepeophtheirus salmonis. The same hook in different view, anterior end to the left; a, medial view of right hook, facing toward the tip of the hook. The two arch-shaped accessory structures are situated more medially than the hook; b, dorsal view, the arches are below the pointing hook; c, somewhat lower focus, the root of the hook is seen. The arches omitted; d, more lateral view, and the tip of the hook touching the object glass; e, ventral view; f, schematic drawing of a torso with explanations. Abbreviations: as, accessory structures; cu, cupulate structure; dp, dorsal plate; h, hook; p & y, ventral projections. Scale bars 0.1 mm.

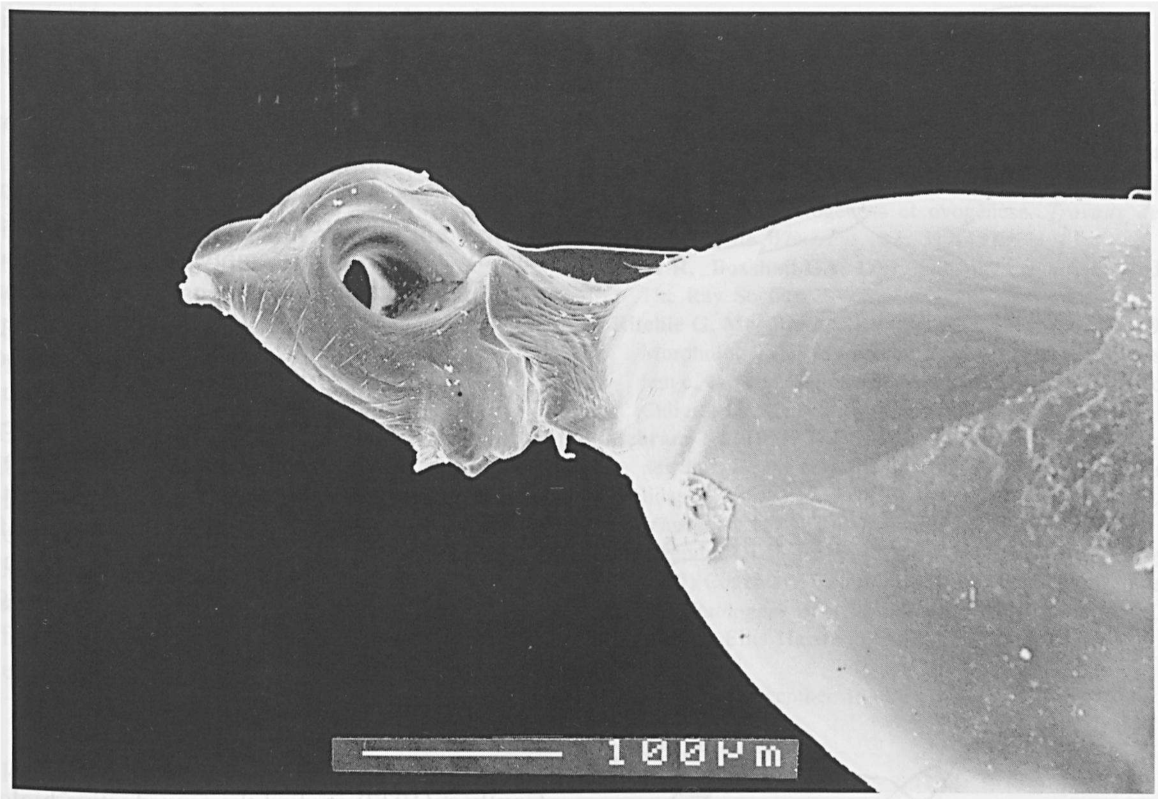

Fig. 9. SEM photo of the proximal end of the egg string of *Lepeophtheirus salmonis*.

The head and neck correspond to the hook, which is slightly curved as seen in terminal view (Fig. 5a), conical (Fig. 8), and equipped with lateral furrows (Fig. 5b). Its surface has also a fingerprint-like surface, which may assist in perforating the egg string (Fig. 5c). The hook moves into a notch in the antrum wall when the egg string is perforated (Fig. 5c). The hook does not project perpendicularly from its base, but has a curved and oblique orientation (Fig. 8b). The hook proceeds anteriorly and medially about 180 µm from its attachment as shown in Fig. 5b, but only 160 µm penetrates the egg sac. The outer diameter near the apex is 60 µm and more basally c. 75 µm.

The shaft of the hook corresponds to the arms, or rudiments of arms, of the torso. The right one stretches anteriorly and has a bony end. The left arm proceed posteriorly and ends in two prominent projections which point down- and outward (Fig. 8. p & y). Projection y is approximately 130 µm long and situated beside the most posterior one (Fig. 8).

The egg strings

The proximal end of the egg string is laterally compressed when kept in position in the gonopores, and firmly secured to the louse with the hook (Fig. 6c). In lateral view the proximal end of the egg sac has a beak-like apex, an arched dorsal surface, a straighter ventral one, and a conical hole joining the sides (Figs. 9; 10). The laterally compressed neck is asymmetrically connected to the egg string. The conical hole has its greatest diameter laterally, and a sloping side, c. 100 µm long, through the egg string (Fig. 9). This mechanical locking device gives the egg string a certain freedom of movement in the vertical plane. The diameter of the hole in the egg string is on the lateral and the median side, approximately 100 µm and 50 µm, respectively. The diameter of the egg sac proper is c. 0.5 mm, and the length of the constricted proximal end 0.3-0.4 mm.

The embryos in the trailing egg strings are oriented. Their anterior ends are situated laterally and

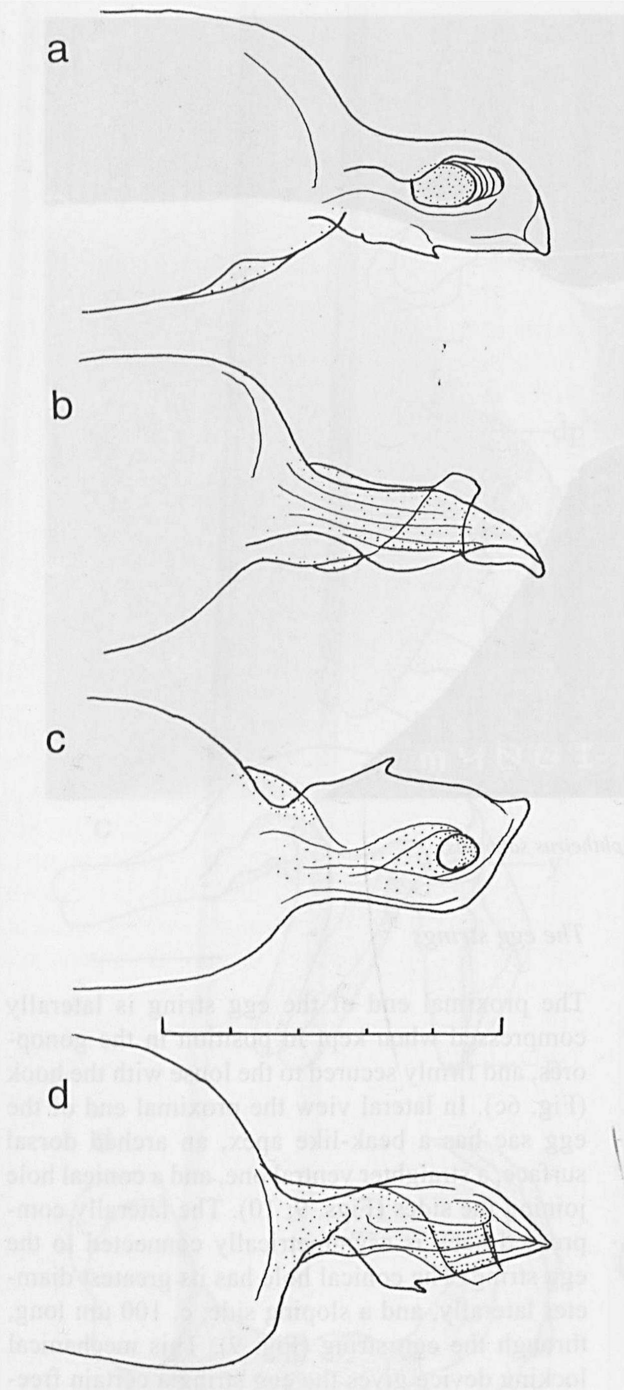

Fig. 10. Proximal end of right egg string of *Lepeophtheirus salmonis* in different positions; a, lateral view; b, dorsal view; c, medial view; d, ventral view. Scale bar 0.05 mm.

in such a manner that the front normally points towards the outer side of the string. The eye pigment and the black colour on the antennules stands out along the whole lateral margin of the string. By the use of these criteria it is easy to orientate a loose egg string.

Normally, the egg strings are secured to the louse until all, or nearly all, eggs are hatched and the empty strings have contracted to irregular bands. Then the strings are released simultaneously, and shortly after two new empty sacks are extruded and filled with fertilized eggs. A stressed louse may, however, release its strings. When clasped proximally with a forceps and turned forward above the genital complex and pulling sharply backwards, the string comes off intact. This may be due to mechanics of the hook apparatus, or the procedure may initiate a louse response similar to that performed when the empty strings are set free after hatching. Just pulling the string backwards usually results in a broken egg string, or a damaged conical hole.

Discussion

Goulliart (1937) studied the reproductive biology of many parasitic copepods, and also illustrated and described the eggsac attachment mechanisms of *Lepeophtheirus pectoralis*. As a pioneer his description of a crooked cupulate structure with a hook that keeps the egg sac in position is principally correct, but has morphologically only superficial resemblance to the present description.

Neither Huys & Boxshall (1991) in their description of the genital complex of *L. pectoralis*, nor Ritchie et al. (1996) who has recently described the morphology and ultrastructure of the reproductive system of salmon lice, have mentioned the hook apparatus. Although Ritchie et al. (1996) have not focused on the attachment apparatus, the hooks in *L. salmonis* are clearly visible on a light microscope photograph of the genital complex of a preadult II female (Ritchie et al., 1996, fig. 1D).

The copulatory duct in *L. pectoralis* has a narrow opening to the seminal receptacle as shown by Huys & Boxshall (1991). In salmon lice only a compressed tube can be seen and it is difficult to see where it enters the receptaculum seminis. This opening may, however, only be apparent when the receptaculum is in the process of being filled up.

As found in *L. pectoralis* and a wide range of families (Huys & Boxshall, 1991), the tubules from the spermatophores cross before they enter the receptaculum seminis.

It appears from the illustration in Huys & Boxshall (1991: Fig. 2.9.25A) that the vitellaria of *L. pectoralis* has a narrow inlet to the oviduct some distance before the genital antrum. In *L. salmonis,* however, the oviducts empty their eggs through a wide opening from above into the underlying antrum, which in turn is an expansion of the vitellarium (cement gland).

Ongoing studies on other copepods have shown that species belonging to the parasitic families Pennellidae and Hatschekiidae also have hooks that mechanically holds on the egg strings of reproductive females. Although the shape, size, position, and accessory structures of the hooks are different, they all penetrate the egg sacs from the side leaving a tapering conical imprint.

Acknowledgment

I express my sincere thanks to Peter Andreas Heuch for valuable criticism of the manuscript and correcting the English.

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Received: 4 December 1998