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Exoskeletons of kinorhynchs in tissues of the bivalve *Macoma balthica* in the Baltic Sea, southwestern Finland

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Exoskeletons of kinorhynchs, resembling those of *Echinoderes levanderi* Karling, were encountered in tissues (most often in the gonad) of *Macoma balthica* (L.) taken from muddy bottoms at depths of 20-40 m. They could be found in up to 70% of individuals. The foreign bodies were usually surrounded by clam granulocytes. Resorption of the cuticular parts was difficult; such material was capable of surviving in the tissues for at least a year. In clams taken from a depth of 7-8 m no kinorhynchs were found.

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1. Introduction

Kinorhynchs are small superficially segmented and spined animals, which live in the muddy and sandy bottoms of coastal waters. Probably only one species, *Echinoderes levanderi* Karling, occurs in the southwestern coastal waters of Finland (Karling 1954). Karling found it in the Hanko-Tvärminne area at depths of 9-12 m. At one sampling site outside Tvärminne Zoological Station its population amounted to over 2700 individuals/m^2 in a muddy bottom, at a depth of 55 m, according to Purasjoki (1945:13). It may also even occur at a depth of 211 m (Levander 1900).

The author has collected samples of the clam *Macoma balthica* (L.) from different depths at and around Tvärminne Zoological Station for studies of their general condition and trematode parasites (Pekkarinen 1983, 1984 and unpublished work). The author's attention was aroused by peculiar nodules in the body of many clams from depths of 20-40 m. Many of the nodules appeared to contain remnants of kinorhynchs. At these greater depths the abundance of *Macoma balthica* is smaller than at shallower localities (Segerstråle 1960). Moreover, the clams grow more slowly but their life span is longer. Failures in their recruitment have been common for long periods (Segerstråle 1960). In this study the prevalence and effects of kinorhynchs in the tissues of *M. balthica* from different depths are assessed.

2. Material and methods

The *Macoma balthica* material was collected from sites I-III around Tvärminne Zoological Station (see map in Pekkarinen 1984) during 1983 and 1984. Histological preparations of 179 wild clams and 70 laboratory-maintained clams originating from site I, at a depth of 7-8 m, were examined for the assessment of the occurrence of kinorhynchs. The shell lengths of the clams ranged between 6 and 21 mm. The clams were maintained in the laboratory in pure sand under flowing natural brackish water, salinity about 6 ‰, temperature 5-15 °C, for 2-12 months. Similarly, 123 wild clams and 182 clams kept in the laboratory from collection sites II and III, at depths of 20-40 m, were used in the assessment of the prevalence and effects of kinorhynchs. The shell lengths of these clams were 13-24 mm.

The posterior bodies (gonad regions) of the clams were fixed in Bouin's fluid, and the cross sections, 7 μm in thickness, were stained with HE (Mayer's haematoxylin-eosin) and HCF (Mayer's haematoxylin-Chromotrope 2R-Fast green; Gray 1954). For examination purposes the kinorhynch parts were prepared out from the tissues of clams under a microscope.

3. Results

In clams from sampling site I (Sundholmen, depth 7-8 m) no remnants of kinorhynchs were found; they were not found in the histological preparations of the clams either. In opened clams from II and III (Storfjärden, 20-40 m) brownish yellow armours were sometimes readily seen in the dorsal body under a preparation microscope. Pink, whitish or brownish cellular nodes were seen many times through the body wall. In some individuals cell clumps could also be seen in the foot when illuminated from below. Some cell clumps also included dark green spots. A typical location of the cell clumps was posterior to the stomach or style sac. Often they became easily detached during the removal of the gonad tissue (i.e. they were interstitial). Many, but not all, of the cell masses enclosed chitinous armours (Fig. 1a) recognisable as being of kinorhynch origin. In some nodules there were glittering granules like minute sand grains. Living kinorhynchs were never found in *M. balthica.*

In the histological sections both of wild and laboratory-maintained (up to a year) clams from sites II and III parts of kinorhynchs could be found. About 28-70% of the wild clams harboured remnants of kinorhynchs in their tissues. The prevalence was greatest, i.e. 60-70% of clams, at a depth of 35 m (site II). It was possible to find the remnants of many kinorhynchs in one individual. These included entire armours and separate segments or spines. Most often the invaders were found in the gonads, between or in the follicles (Fig. 1). Occasionally they were also found in the gill vessels, kidneys, heart and pericardium, digestive gland and foot. One was recorded in the duct of the rudimentary byssus gland.

Sometimes the kinorhynch parts had little or no effect on the surrounding tissue (Fig. 1c). Often, however, they were surrounded by clam granulocytes (Fig. 1b and d-f). The cuticular parts usually remained yellow but sometimes they stained intensively with eosin or chromotrope (Fig. 1e). In some individuals the cellular clumps consisted of large vacuolated cells with triangular bright red-staining grains within. Such grain-containing masses could exist in almost all tissues. Occasionally the triangular grains were present in association with kinorhynch parts (Fig. 1f). Dark green spots were common in cell clumps. In a few cases the gonoducts seemed to be obstructed and they were filled with cellular debris and granulocytes.

The kinorhynch exoskeletons (Fig. 1a) resembled those of *Echinoderes levanderi* Karling. The length of the body of 11 individuals ranged between 391 and 636 μm (523 μm mean). All the spines were no longer present in every exoskeleton, and during preparation the armours tended to disintegrate readily. Deformation of spines, hairs and the teeth of the zonite combs had taken place in those armours which had been in the clams for longer times. Often they were swollen. The lateral terminal spines were longer (329, 350 and 394 μm, measured in three individuals) or shorter (96-97 μm). The shorter lateral terminal spines had rounded or broken ends. The accessory lateral terminal spines of a female were 102 μm in length. The most posterior dorsal spine measured 98 and 100 μm in two individuals.

4. Discussion

Kinorhynchs are not known to act as parasites; most kinorhynchs feed on diatoms, the others are deposit-feeders (Barnes 1980:288). Why then are they found in the tissues of *Macoma balthica*? Possibly they arrive there accidentally. The frequent occurrence of the exoskeletons in the clams from the deeper sampling sites II and III may reflect a dense population of kinorhynchs in the sediment. Near the Askö Laboratory on the Swedish coast of the northern Baltic proper kinorhynchs, probably *E. levanderi,* occur over a depth range of at least 10-50 m, but reach their greatest abundance below 15 m (Ankar & Elmgren 1976).

It is not known whether the kinorhynchs were living animals or mere empty armours when they entered the clams. Entering as a result of suction by the siphon or through the pedal gape into the mantle cavity, owing to their spinous surface they could have injured the body surface and penetrated it. The body wall lateral to the pericardium just under the separation of the mantle is thin. The gills may trap kinorhynchs readily. It is also possible that the kinorhynchs had come through the natural openings. Perhaps they were ingested or burrowed through the mouth, or they burrowed or were forced in through the

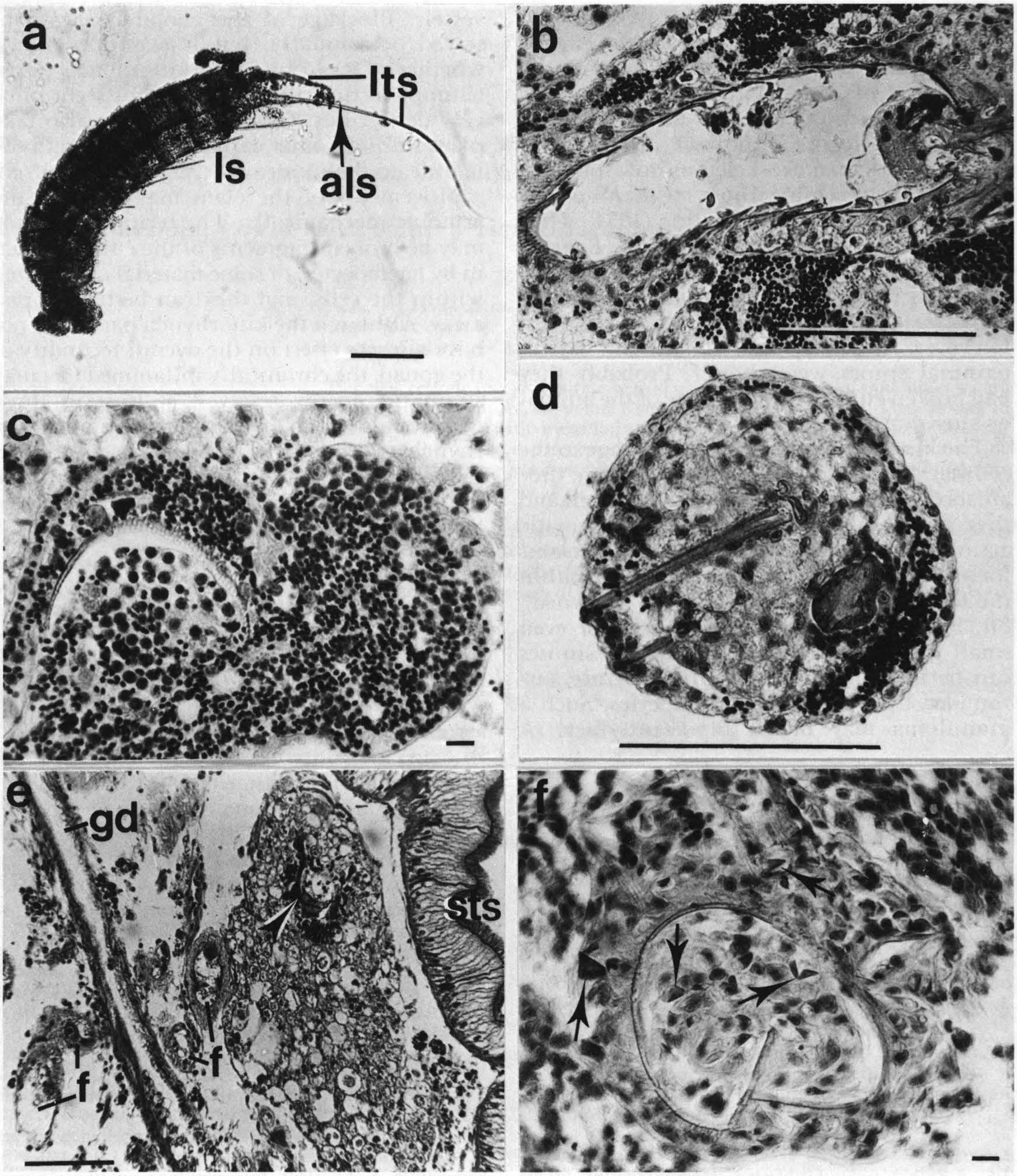

Fig. 1. Parts of kinorhynchs in tissues of *Macoma balthica*. - a. A female exoskeleton prepared out from the gonad of a clam. als = the tip of an accessory female lateral spine, ls = lateral spines, lts = lateral terminal spines (one broken). Scale bar 100 μm. — b. Longitudinal section of a kinorhynch exoskeleton in the testicular area of a clam. Clam granulocytes surround the chitin and occupy the former body space, which is nearly devoid of residual tissue. HCF staining. Scale bar 100 μm. — c. A tergal plate of a kinorhynch cross-sectioned in a testis follicle. Spermatogenesis is only slightly disturbed. HE staining. Scale bar 10 μm. — d. A granuloma around parts of a kinorhynch in a testis follicle. HE staining. Scale bar 100 μm. — e. A granuloma with residual parts of a kinorhynch intensively stained with chromotrope (arrowed) in an ovary between the style sac (sts) and gonoduct (gd). f = follicle. Scale bar 100 μm. — f. Cross section of a segment, and triangular fragments (arrowed) in the infiltrated clam cells. HCF staining. Scale bar 10 μm.

gonopore, uropore or the opening of the duct of the rudimentary byssus gland. Movements of the clam and the pumping locomotory movements of the kinorhynchs, if they are alive, may force the armours forwards in the tissues.

The kinorhynch exoskeletons now found in *M. balthica* resemble those of *Echinoderes levanderi* as described by Karling (1954). They also resemble those of *Echinoderes* (*Echinoderella*) spec. as described by Reimer (1963). Common features are, for instance, the large body size and the lengths of the spines. However, in some individuals the lateral terminal spines were shorter. Probably they had broken either during the life of the animal or after death.

The kinorhynch parts are a challenge to the cellular defence mechanisms of the clam: they are too large to be engulfed by a single cell and they may be hard to dissolve by enzymatic action. Kinorhynch armours survived in clams for at least one year. Separate segments within the follicles of the gonad may be "spawned" off, but larger parts in the follicles or even small fragments in the haemolymph sinuses can be more harmful. Usually they are surrounded by a clump of granulocytes. Such a granuloma may block important ducts or vessels. Blockage of the gonoducts was observed occasionally, but it was not known whether it was caused by kinorhynch parts. Clumps in the gill vessels did not seem to be very deleterious. Gas exchange can also take place through other surfaces (e.g. through the mantle epithelium).

Movements of the clam may "grind" the armours mechanically. The triangular grains may be broken fragments of the cuticle, taken in by haemocytes, or some material crystallized within the cells, and they can be transported away. Although the kinorhynch parts may not have a severe effect on the overall fecundity of the gonad, the chronically inflammed foci may strain the energy reserves, at least in those individuals which are harbouring many kinorhynchs. Perhaps this small strain contributes to the already poor recruitment of the *Macoma* population living at greater depths (see Segerstråle 1960).

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