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Invasive vermigon stage in the parasitic barnacles *Loxothylacus texanus* and *L. panopaei* (Sacculinidae): closing of the rhizocephalan life-cycle

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Abstract The parasitic barnacles, Rhizocephala, are unique in Crustacea by having an entirely endo-parasitic phase inserted into their lifecycle. A cypris larva, remarkably similar to the cypris of conventional acorn and goose barnacles (Thoracica), settles on the crustacean host and develops an infective stage, the kentrogon, underneath the exuviae of the cypris. The kentrogon penetrates the integument of the host by a hollow cuticle structure, the stylet, and injects the parasitic material into the hemocoelic fluid of the host. Although advanced stages of the internal development have been found and described several times, the nature of the originally injected parasitic material has remained obscure for decades. Recently, however, it was shown that the parasitic material was injected by the kentrogon in the form of a motile, multi-cellular and vermiform body. The present study demonstrates that the vermiform stage is an instar which forms the only and direct link between the kentrogon and the maturing internal parasite. The vermiform instar, or vermigon, is at all times clothed in a cuticle, contains several types of cells, including epidermis and the anlage of the later ovary, and stays intact while growing into the internal parasites with rootlets.

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Introduction

The Rhizocephala are barnacles that parasitize other crustaceans and exercise a profound control over their hosts by affecting them at morphological, physiological, and behavioural levels (O'Brien and van Wyk 1985; O'Brien and Skinner 1990; Høeg 1995; Høeg and Lützen 1995). Paramount among the numerous changes induced in the hosts is *parasitic castration*. Gonads of parasitized crabs do not mature and in consequence parasitized hosts do not reproduce (Reinhard 1956; Kuris 1974). The end effect could be described either colloquially as transforming the host into a robot that only serves the needs of the parasite or, more appropriately, as creating an organism possessing the phenotype of the host and the genotype of the parasite (O'Brien 1999).

Studies of rhizocephalan life-cycles have taken on a new significance in terms of their potential for resource management. Sacculinid rhizocephalans, which infest brachyuran decapods, have been suggested as biological control agents against introduced species of crabs, such as the green crab, *Carcinus maenas*, which are perturbing marine ecosystems throughout the world (Lafferty and Kuris 1996; Kuris 1997). These parasitic castrators are attractive as biological control agents because there is evidence to suggest that they may be specific in their choice of hosts; consequently, they might not present a threat to native crab species if introduced to control the pest crab (papers in Thresher 1997).

The life-cycle of sacculinids is direct; there is only one host. Nauplii, released regularly from the adult parasites, develop into typical cypris larvae. Sexes are separate in the Rhizocephala. Female cyprids settle on a vulnerable host and metamorphose into a kentrogon, which develops a hollow cuticular stylet with which it penetrates into the host and injects parasitic material. However, the nature of the injected material and the earliest internal development has until very recently remained obscure.

The kentrogon stage was first described by Delage (1884), but its anatomy remained entirely unknown until Høeg (1985) gave a complete TEM-based description of the kentrogon of *Lernaeodiscus porcellanae*, and its formation from the female cypris. While Høeg (1985) did not actually observe the injection of a parasite into the host, this crucial part of the life-cycle was finally documented by Glenner and Høeg (1995). These authors studied the sacculinid *Loxothylacus panopaei* parasitizing the mud crab *Rhithropanopeus harrisi* and observed the infection of the host in live material using video recordings followed by a TEM analysis (Fig. 1). Female cyprids of *L. panopaei* settle in the gill chamber just as is the case in *Lernaeodiscus porcellanae*. Following penetration of the stylet, the *Loxothylacus panopaei* kentrogon expels a large, vermiform body into the host, which after an initial quiet period passes through a

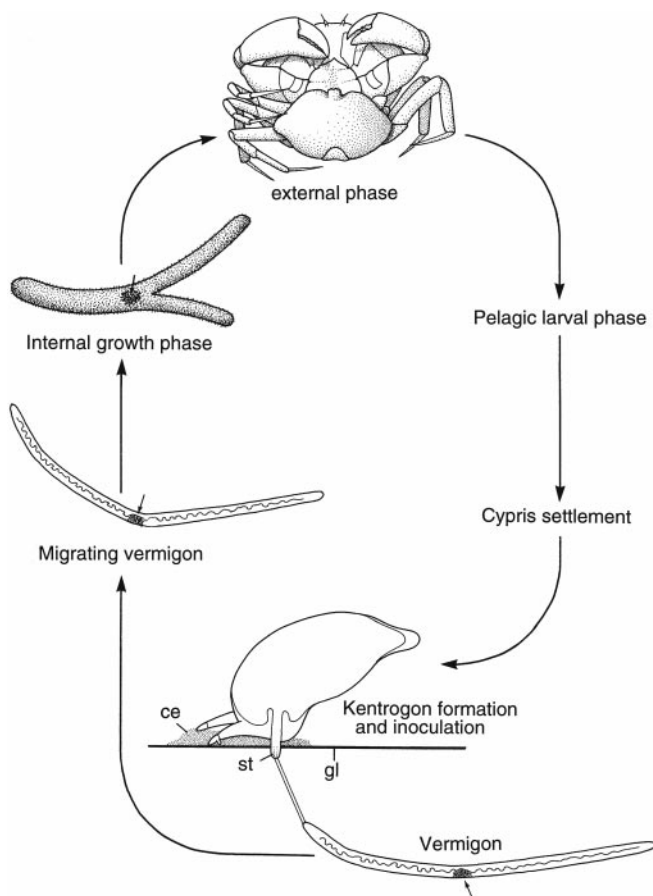


Fig. 1 The life-cycle of *Loxothylacus panopaei* parasitizing the mud crab *Rhithropanopeus harrisi*. Nauplii, released by the external reproductive body, develop into cyprids. The female cypris settles in the gill chamber of the crab and metamorphoses (moult) into a kentrogon, which penetrates the integument and injects a vermigon into the blood spaces. The vermigon migrates through the crab and grows into an internal parasite, which eventually erupts with an external reproductive body. The cells of the primordial ovary (arrows) can be traced as a globular body of cells already from the kentrogon. All stages in the parasite life-cycle, including the early vermigon, are enveloped by epidermis and cuticle. Male parts of life-cycle excluded. Modified from Glenner and Høeg (1995); drawn by Beth Beyerholm (ce cement; st stylet; gl gill)

phase of undulatory body motions. Glenner and Høeg reported that between 8 and 10 h following injection, the vermiform bodies begin to disintegrate and liberate numerous large cells exhibiting amoeboid motions. They took this as evidence that the “amoeboid” cells corresponded to the single invasion cell, which Høeg (1985) suspected to be the only element passed into the host from the *Lernaeodiscus porcellanae* kentrogon. The difficulty with this explanation revolves around how such a stage with naked, undifferentiated cells could have become intercalated into the life-cycle of an otherwise highly evolved crustacean. In addition, Glenner and Høeg (1995) did not observe the injected parasites in situ in the host, but instead in preparations cultured in artificial sea water, and thus could not observe the parasite for any extended period of time.

The purpose of the work presented here was to examine the vermiform stage in its natural environment within the tissues of the crab. We achieved this by infesting megalopa larvae and subjecting them to light and electron microscopy and by observing recently injected parasites in vivo within the crab. This approach demonstrates that the vermiform body never breaks up but instead develops directly into a single internal parasite while always being clothed in a cuticle.

Materials and methods

Rearing and infection experiments

Mud crabs, *Rhithropanopeus harrisi*, parasitized with the sacculinid rhizocephalan *Loxothylacus panopaei* were sampled near the Duke University Marine Laboratory, Beaufort, NC, USA. Larvae released from the parasite were cultured to the cypris stage and broods of female cyprids were used for infection experiments (Glenner and Brodin 1997). Larvae of *R. harrisi* were reared to the megalopa stage as in Forward and Wellins (1989) and then exposed to female *L. panopaei* cyprids. Larvae of *Loxothylacus texanus* were reared in a comparable fashion and female larvae used to infest blue crabs, *Callinectes sapidus*. In this parasite species the cyprids settle on the external body surface, where they attach at the base of setae, especially on the appendages and on intersegmental membranes. Numerous cyprids attached to the last, fifth, pair of thoracopods (the paddle).

Video recordings

Using light microscopy on live *Callinectes sapidus* immobilized to glass microscope slides by rubber bands, we observed the development of parasite larvae attached to the paddle and made video recordings of the injected vermiform parasites in situ within the tissues of the limb.

Immunostaining

A few limbs of *Callinectes sapidus* containing vermiform parasite bodies were removed, and stained for actin using TRITC-phalloidin (final concentration of approximately 20 μM) or for nuclei with the addition of Hoechst 33258 (final concentration of approximately 1 $\mu\text{g ml}^{-1}$) in the solution containing crab tissue. Staining was performed after having opened the cuticle to allow observation and provide access for the stain. The stained preparations were then observed with a Leitz epifluorescence microscope.

Light and electron microscopy

Megalopa larvae were fixed for TEM as in McDowell and Trump (1976) at various time intervals following exposure to the parasite larvae. Fixed megalopae were rinsed in buffer, dehydrated with dimethoxypropane and embedded in TAAB 812. Serial sections were first cut at 2 μm , stained with toluidine blue and mounted on microscope slides. All sections of a series were observed through a Leica DM RXA, and photographs of sections stored digitally in LIDA (Leica Image Database) on a PC system. We also photographed selected sections on 35 mm negative film. This allowed us to detect, count and follow vermignons in a fast and easy manner. Exceptionally informative sections were extracted from the slides and remounted on an TAAB 812 block for TEM analysis as follows: we carefully removed the coverslip and then used fresh, unpolymerized TAAB 812 to glue an elongate piece of resin onto the section in question. Subsequently we immersed the slide in liquid nitrogen enabling removal of the slide from the resin block by shaking. The section then adhered to the top of the resin block and could be remounted for ultrathin sectioning for TEM. This technique enabled us to scan through very long series of 2- μm -thick sections in a fast and easy manner using light microscopy and to study selected sections with internal stages using TEM.

Results

Observations on *Loxothylacus panopaei*

We observed numerous parasite larvae settled on the gills of *Rhithropanopeus harrisii* on sections of megalopae fixed 2 to 4 d after exposure to parasite cyprids. Sometimes the parasite larvae occurred in such numbers that they almost occluded the chamber. Numerous vermiform bodies, henceforward called *vermignons*, occurred within the blood spaces of the gills having been injected by the attached kentrogons (Fig. 2A, B). Occasionally the parasite larvae could actually cause the carapace covering the branchial chamber to bulge out as is sometimes seen in decapods infested with isopod gill parasites.

From the positions of the vermignons in megalopae fixed at increasing time intervals after infection it follows that they are initially located in branchial sinuses, whence they migrate through the heart and thence into other body parts. Fig. 2D shows two vermignons in the process of migrating from the pericardium and into the heart through ostia. The vermignons were always lying free in the branchial blood sinuses without any signs of having attached to the wall. Eventually the vermignons migrate to many parts of the body and we even detected one lodged in the blood spaces of an eyestalk. During this migratory phase the distinct spiralling body described by Glenner and Høeg (1995) remains clearly visible extending the entire length of the vermignon (Fig. 2C, arrows).

The vermignons located in the blood spaces of the gills and the heart had a size and morphology comparable to that of recently injected ones (Glenner and Høeg 1995). Those present elsewhere in the body were mostly larger, sometimes considerably so, and showed clear signs of growth and maturation. The exceedingly thin "sheath" surrounding the very recently injected vermignon

(Glenner and Høeg 1995) remains intact at all times, but it increases in thickness and reveals itself to be a true cuticle, which was seen with LM on 2- μm sections (Fig. 2C, E).

At all stages the vermignon consists of at least three cellular components: an outer epidermis, a subjacent layer of large cells (the "amoeboid cells" of Glenner and Høeg 1995), and a very distinct globular body of small cells (Fig. 2C). The globular body has a diameter of half the width of the vermignon and is situated midway along the length of the vermignon. With size and age these three cellular components become increasingly distinct from each other; the ultrastructural details of this growth process will be published elsewhere (Glenner in preparation).

The oldest vermignon bodies developed projections that represent the earliest part of the rootlet system by which rhizocephalans absorb nutrients from their hosts. The cuticle surrounding these stages has increased in thickness and can easily be seen in the light microscope, although it still remains a delicate epicuticle (Fig. 2F). The thickness of this cuticle is only slightly thinner than the so-called homogenous layer of the cuticle covering the fully developed rootlet system of later stages (Høeg 1992). The above-mentioned globular body has developed into the primordium of the external reproductive sac (externa). This primordium, very confusingly called "nucleus" in much older literature, consists of a central anlage to the ovary surrounded by an incipient but quite distinct mantle cavity and its accompanying epithelia. It is this primordium that will eventually emerge as the externa beneath the abdomen of the host, while the rootlet system remains within the crab. In the entire vermignon body, the tissue beneath the epidermis has loosened up leaving large, sinusoidal spaces (Fig. 2E). At this stage, our internal *Loxothylacus panopaei* specimens are comparable in degree of development to those Rubiliani et al. (1982) observed with light microscopy in the sacculinid *Sacculina carcini*. The succeeding internal development is quite well documented in several species (Høeg and Lützen 1995).

Observations on *Loxothylacus texanus*

Despite a supposed close relationship between *L. panopaei* and *L. texanus*, the latter has not been observed to attach in the branchial chamber, but instead upon the appendages just as in the "classical" rhizocephalan *Sacculina carcini*. After exposure of *Callinectes sapidus* to female cyprids, we obtained numerous kentrogons attached to the edge of the "paddle" of the last, fifth, thoracopod. The kentrogons ejected vermignons, which we observed in vivo and in situ with light microscopy in the limbs of crabs immobilized with rubber bands.

Morphology of the vermignon

Just as in *Loxothylacus panopaei*, the *L. texanus* vermignon showed no signs of breaking up but remained

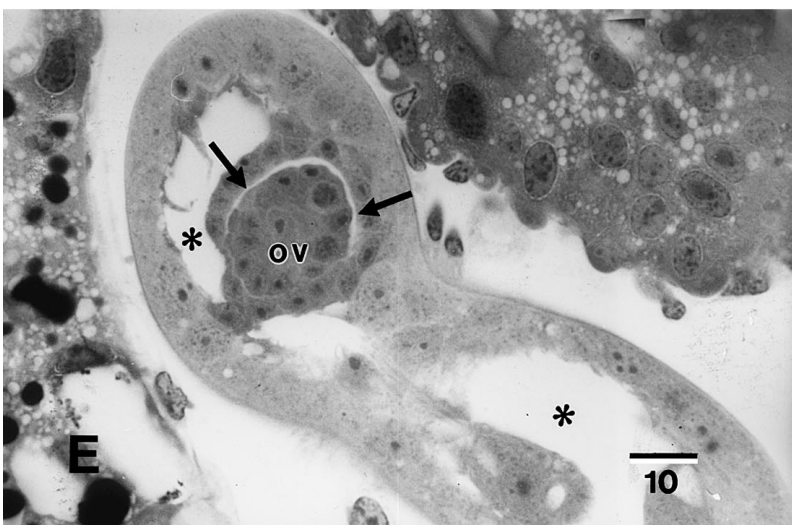
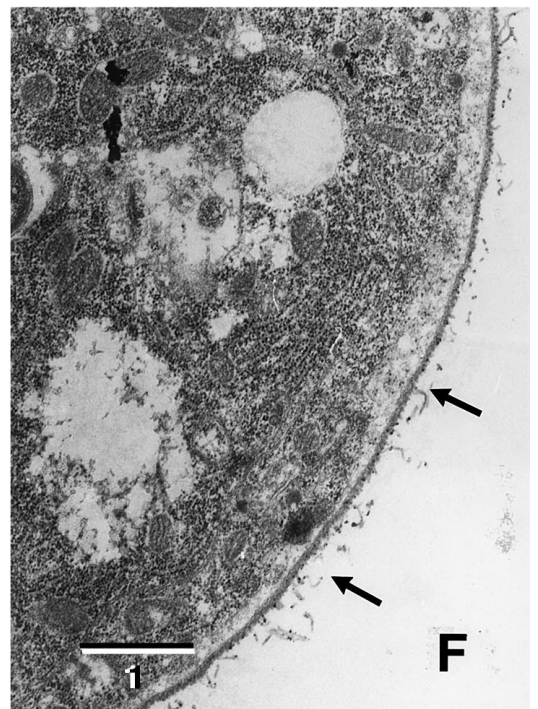
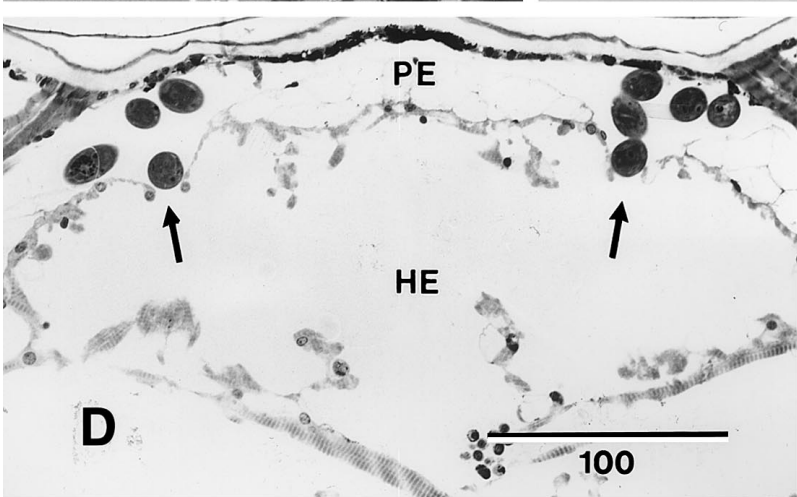
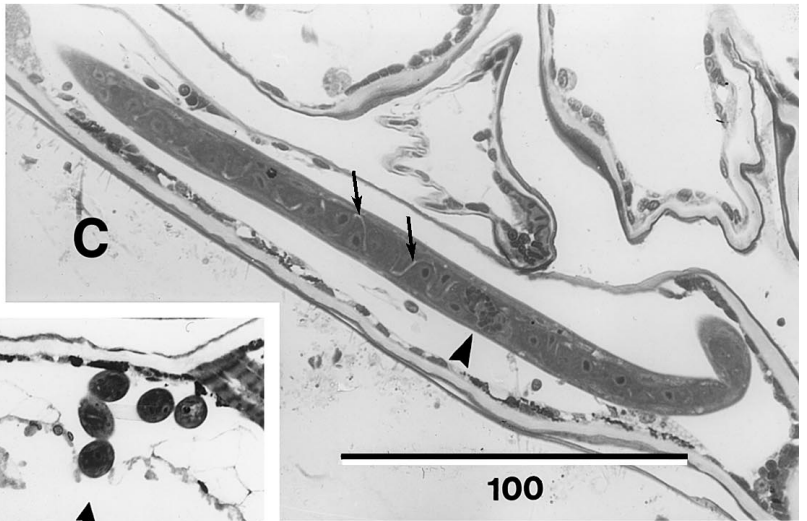




Fig. 2 *Loxothylacus panopaei*. Early development of the vermigon within megalopae of *Rhithropanopeus harrisi*. Light micrographs from 2- μ m epoxy resin sections. **A** Cyprids (arrowheads) within the gill chamber and a vermigon (arrow) within a blood space. The vermigon is still within a gill filament. Both cyprids and vermignons in cross-section. **B** Detail of left side scene in A, but from an adjacent section. Cypris (arrowhead) in gill chamber and vermigon (arrow) within blood space of a gill filament; both in cross-section. **C** Semilongitudinal view of a recently injected vermigon within a dorsal blood sinus. The body is principally filled with large-sized cells. The distinct globular body (arrowhead) of small-sized cells will develop into the visceral sac and ovary of the mature parasite. An elusive spiralling structure (arrows) extends throughout the length of the vermigon. **D** Cross-section through the heart of a megalopa. Several vermignons in cross-section within the pericardium (PE), two of these (arrows) penetrating into the lumen of the heart (HE) through ostia. **E** Late “vermigon” developing adjacent to the hepatopancreas. This internal parasite has grown significantly compared to the recently injected ones in A to D. The globular body in C has matured into the primordium of the visceral sac, surrounded by an incipient mantle cavity (arrows) and containing the anlage to the ovary (ov). Compared to C, the interior of the parasite has opened up into a loose system of lacunae (asterisks). **F** TEM section through integument of an only minutes-old vermigon. Note that the body is enveloped by a delicate, but distinct cuticle (arrows); Scales in μ m

intact during 4 h of observation following liberation from the kentrogon. The *L. texanus* vermigon is very similar to the one in *L. panopaei*. One distinct similarity is the presence of a central spiralling rod, which extends throughout the length of the body, except for the most distal regions at either end (Figs. 2C, 3A, D).

Immunostaining

Specimens stained for actin with phalloidin showed an intense positive reaction both peripherally in the vermigon and in the central spiralling structure (Fig. 3A). Focussing at high magnification at the level of the epidermis revealed a dense layer of actin filaments oriented parallel with the long axis of the vermigon (Fig. 3B). These peripheral actin filaments are most likely located in the epidermal cells. Such a concentration of epidermal actin filaments occurs quite commonly in larvae of marine invertebrates, where they can play an important role in rapid changes of form during metamorphic processes, e.g. in the retraction of the tail in ascidian tadpole larvae (Cloney 1982). The pronounced reaction in the central spiralling structure is more elusive. Our LM and TEM micrographs failed to reveal a single cell or any row of cells that could correspond to the spiral structure, and we remain confident that it is not a muscle cell. Further TEM observations are needed to elucidate the morphology of this structure.

Motility of the vermigon

We videotaped the movement of *Loxothylacus texanus* vermignons in the distal segment (“paddle”) of the fifth thoracopod of *Callinectes sapidus* and replayed the re-

cordings at normal and fast-forward speeds. Most, if not all, larvae were located in the tissues exterior to rather than inside blood spaces and left open “channels” behind them as they forced their way slowly through the dense tissue of the limbs. During these movements, the diameter of the vermigon body changed only slightly, i.e. there was no pronounced peristalsis as seen in *L. panopaei* by Glenner and Høeg (1995). Just as portions of the rod performed irregular meandering movements in either direction along the long axis, the vermignons also repeatedly reversed the direction of their movements. In general, a wave of movement would pass down the entire rod, but not in a very organized fashion, and movement could stop and reverse direction at any time.

Discussion

Our study confirms and extends the observations of Glenner and Høeg (1995) except for one crucial observation. The injected vermigon does not break up and liberate its cellular contents following injection into the host. Instead it stays intact and enveloped within its original sheath, now interpreted as a cuticle, and develops directly into the internal parasite equipped with a system of nutrient-absorbing rootlets. Since the injected parasite stays intact and is never devoid of a cuticle, it corresponds to a true instar following the kentrogon. We therefore name this internal parasitic stage “the vermigon”. The etymology derives from Greek “vermes” (worm) and “gonos” (larva) and is given to comply with the kentrogon stage in females and the trichogon stage in males. We emphasize, however, that none of these three “-gons” may necessarily represent larvae in the strict sense.

The break up of the vermigon and the liberation of its cells observed by Glenner and Høeg (1995) is now interpreted as an artifact probably caused by incubating the vermignons in sea water rather than in hemocoelic fluid. Glenner and Høeg (1995) chose this procedure because settlement occurred on gills that had earlier been dissected from the gill chambers of the crabs, in order to facilitate videotaping emergence of the subsequent stage. In the current study, cyprids were allowed to settle and infect megalopa larvae and juvenile crabs, and larvae were never exposed to artificial solutions. Consequently the migration and development of the vermignons was monitored in their natural environment.

It is surprising that *Loxothylacus panopaei* cyprids will settle on megalopae of *Rhithropanopeus harrisi*, since it is normally believed that infection of rhizocephalan hosts occurs only in postlarval stages. In *R. harrisi*, however, the megalopae spend most of their time on the bottom and are morphologically very close to the first crab stage. Walker et al. (1992) observed the presence of an *L. panopaei* externa on a small Stage 1 crab, which can only mean that this host was infected while still a megalopa. These authors also noted cyprids

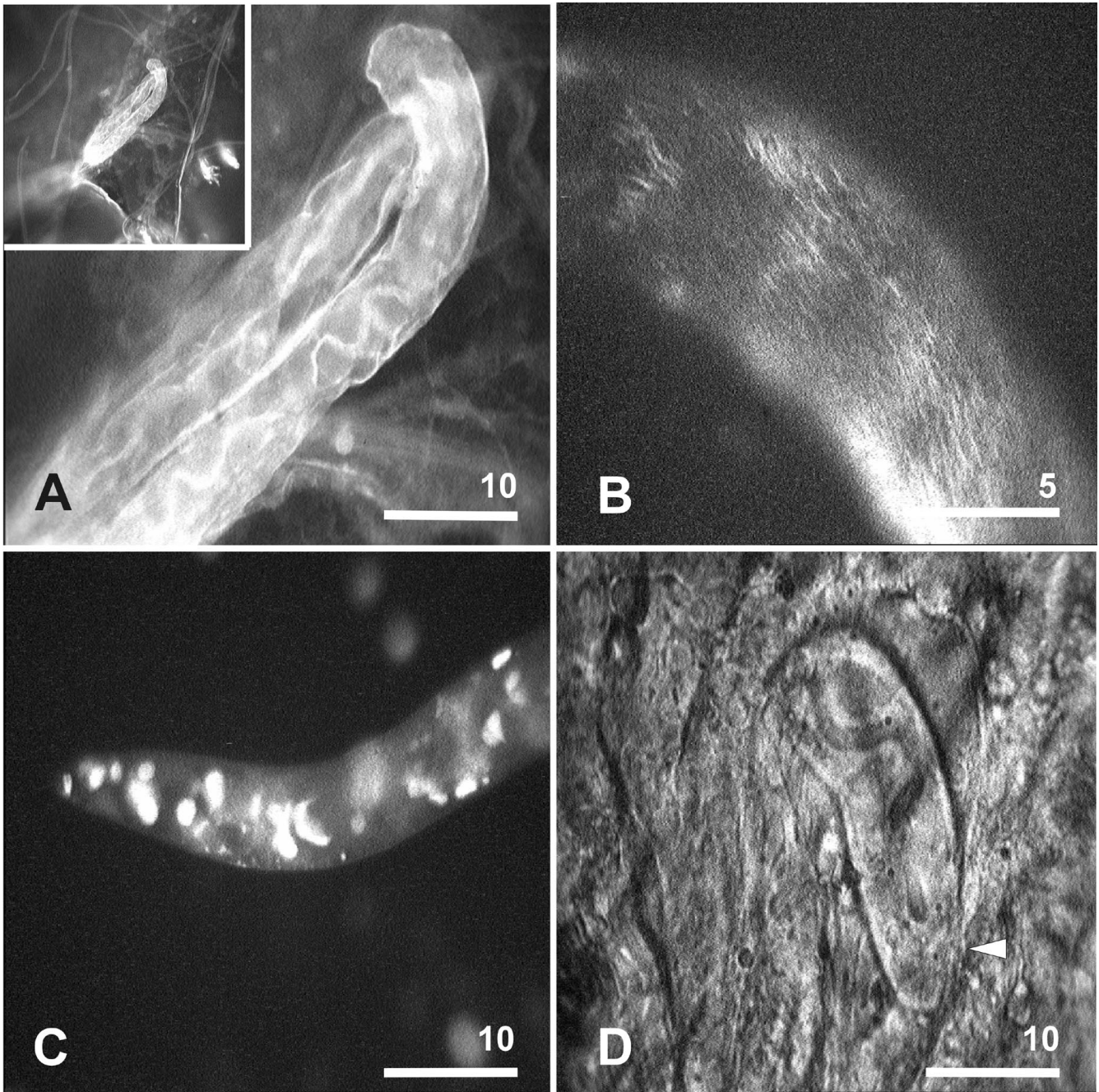


Fig. 3 *Loxothylacus texanus*. **A** Vermigon in situ in tissue of fifth thoracopod (paddle) of *Callinectes sapidus* and stained with phalloidin. Cuticle of limb has been opened to allow view of parasite and access of stain. Positive stain for actin filaments occurs along surface of vermigon and in central spiralling structure. Inset shows whole vermigon. **B** Vermigon prepared and stained as in A, but picture focussed on epidermis, showing numerous actin filaments running parallel to the longitudinal axis. **C** Vermigon dissected out of crab thoracopod and stained for nuclei (DNA) (stained areas correspond to nuclei of large cells that fill the vermigon in Fig. 2C). Specimen has been punctured with a needle allowing the central spiralling structure to disintegrate or escape. **D** Unstained vermigon in situ in tissue of fifth thoracopod (paddle). Note that central spiralling structure terminates just short of distal end of vermigon (*arrow*). Video observations confirm that vermigons can move proximally in the limbs even through such dense tissue as seen here; Scales in μm

in the gill chambers, although settlement was not observed. The internal anatomy of the *R. harrisii* megalopa is similar to that of early instar juvenile crabs, and we are therefore confident that the development of the vermigons observed here would be the same as that occurring in later crab stages.

Motility of the vermigon

Our observations on *Loxothylacus texanus* vermigons under in vivo conditions confirm that the motility of the vermigon observed in vitro by Glenner and Høeg (1995) is real. These authors found that the vermigon lacked

muscles and suggested that the motility was caused by actin filaments inside some vermigon cells. The presence of phalloidin-stained material within the *L. texanus* vermigon strongly supports this conclusion. Glenner and Høeg (1995) observed that the vermigon remains immotile until ca. 15 min after its expulsion from the kentrogon. Therefore it seems that the motility has no function during the injection process but serves to propel the vermigon through the tissues and blood system of the host.

The sheer size of the *Loxothylacus texanus* vermigon impedes its passage proximally in the limbs, whether it travels through the muscle tissue or within one of the narrow blood spaces (Fig. 3D). The *L. panopaei* vermigon must likewise pass through the narrow blood spaces of the gills and through a minute ostium before it reaches the heart and can enter one of the major vessels leading to other parts of the body (Fig. 2D). We therefore believe that the movements observed in *L. panopaei* and *L. texanus* vermigons are crucial for reaching their ultimate destinations in their hosts. Although we did not observe peristalsis in *L. texanus* vermigons we are uncertain whether this signifies a true difference to *L. panopaei*.

The reaction with phalloidin in *Loxothylacus texanus* vermigons strongly suggests that actin fibers located in the epidermis play an important part in the observed motility. The video recordings indicate that the central spiralling structure also plays a part, but whether it is a passive or an active one remains obscure until new TEM studies have clarified its nature.

Growth of the vermigon into an interna

It is now clear that the sheath enveloping the early vermigon is a very thin cuticle that later develops into the cuticle covering the internal parasite and its rootlet system. It therefore follows that kentrogonid rhizocephalans are never devoid of a cuticle at any stage of their life-cycle.

Growth of the internal parasite apparently starts almost immediately after infection as many of the vermigons studied here had increased substantially in size and reached a stage, at which they are more appropriately called "interna" than vermigon.

The oldest stages studied by us, with clear signs of formation of the externa anlage and a developing rootlet system, overlap with those that previously have been observed by other workers using both light and electron microscopy (Smith 1906; Brinkmann 1936; Rubiliani et al. 1982; Høeg 1990). Rubiliani et al. (1982) exposed small *Carcinus maenas* to female cyprids of the "classical" sacculinid *Sacculina carcini* and later produced histological sections of the infected crabs. The earliest stages found were comparable to our oldest stages in *Loxothylacus panopaei*. In both species, the interna had a primordium, or "nucleus", beginning to differentiate into the tissues and organs of the externa. In *L. pan-*

opaei, most if not all vermigon cell types, including those of the ovary, can be traced back to the kentrogon and even to the cypris using TEM (Glenner 1998).

Vermigons in other Rhizocephala

Our study revealed the presence of almost identical vermigons in *Loxothylacus panopaei* and *L. texanus*, both belonging to the Sacculinidae. Since *L. panopaei* cyprids attach on gills and those of *L. texanus* on the appendages, we can infer that the presence of a vermigon stage is not coupled to any particular site of settlement. The observation by Walker et al. (1992) that *L. panopaei* cyprids can settle also externally on megalopae supports this conclusion.

Veillet (1963) was probably the first who directly observed host infection in the Rhizocephala. He studied the process in the sacculinid *Drepanorchis neglecta*, which infects mud crabs. His micrograph clearly shows a kentrogon within a spent cypris and an elongate body, somewhat more bulbous than *Loxothylacus* spp., emanating from the injection stylet. In hindsight, there is no doubt that this depicts the injection of the vermigon of *D. neglecta*.

In the classical rhizocephalan *Sacculina carcini*, Høeg (1992: Fig. 17D) observed and illustrated a bulbous body emanating from the tip of a kentrogon. We have (Glenner and Høeg unpublished) repeated this observation numerous times in this species and always seen the ejection of such bodies. From comparison with *Loxothylacus* spp. it is obvious that kentrogons of both *Drepanorchis neglecta* and *S. carcini* expel vermigon stages, although of a somewhat more bulbous shape than those found in *L. panopaei* and *L. texanus*.

In *Lernaeodiscus porcellanae* (Lernaeodiscidae), Høeg (1985) used TEM to study the female cypris and the kentrogon in great detail. Høeg did not actually observe the infection process but from a comparison of kentrogons fixed before and after infection he concluded that only a single, large cell was injected into the host. Glenner and Høeg (1995) documented that the injection of the vermigons lasts but a couple of minutes, so fixation for TEM of a specimen during this event is a daunting task. We still believe that Høeg's single "inoculation cell" is injected into the host of *L. porcellanae*, but we also predict that a renewed study will show that it is part of a true vermigon, which could be exceedingly small in this species. If true, the presence of a vermigon would characterize all three families of the suborder Kentrogonida and perhaps also the groundplan of all Rhizocephala.

Closing the rhizocephalan life-cycle

The observation that the kentrogon injects a vermigon, which migrates through the crab tissues or blood spaces and grows directly into the internal parasite has closed the life-cycle of the kentrogonid Rhizocephala (Fig. 1)

after more than 100 years of study (Delage 1884). At present, all stages in the life-cycle have been subjected to histological study on parasites reared under controlled laboratory conditions. Until now it was assumed that the early rhizocephalan parasite developed de novo from undifferentiated cells injected by the kentrogon (Delage 1884; Smith 1907; Høeg 1985; Glenner and Høeg 1994). The break up of the vermigon observed by Glenner and Høeg (1995) initially seemed to confirm this view but was, as shown here, probably due to an artifact caused by experimental conditions.

Our observations remove a crucial obstacle for understanding the evolution of the rhizocephalan life-cycle, and future theories can now proceed from a solid observational basis. There is no "second embryogenesis" or "naked, undifferentiated stage" in rhizocephalan ontogeny, neither during host infection nor during the early internal development. The demonstration that the cuticle-covered vermigon originates from the kentrogon and develops directly into the adult parasite allows a much more conventional scenario for the evolution of the rhizocephalan life-cycle. The vermigon instar has admittedly an exceedingly simplified structure, but the now well-known trichogon instar formed by male cyprids is actually even less complex (Høeg 1987). The vermigon already includes the most important cellular elements for forming the adult parasite, including cuticle, epidermis and the body of cells that later mature into the ovary.

Rhizocephala is the sister group of the cirripede order Thoracica (Spears et al. 1994; Høeg 1995), and the urrhizocephalan was most likely a setose-feeding animal comparable to pedunculated barnacles. Accordingly, Glenner and Høeg (1994) have suggested that the kentrogon evolved as an extreme specialization of the first juvenile stage in setose-feeding barnacles. The ontogenetic continuity between the pelagic rhizocephalan larvae and the internal parasitic phase has now been largely clarified, but we still need to understand the selective pressures that led to the evolution of an internal migratory stage (the vermigon) and resulted in the development of the parasite far from the site of infection.

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