RESEARCH NOTE

DEVELOPMENT OF THE PHILOMETRIDS *PHILOMETRA OVERSTREETI* AND *PHILOMETROIDES PARALICHTHYDIS* IN THE EXPERIMENTALLY INFECTED COPEPOD *OITHONA COLCARVA*Timothy P. Bryan¹, Lam C. Tsoi^{1,2} and Isaure de Buron¹¹Department of Biology, College of Charleston, 58 Coming St., Charleston, South Carolina 29401, USA;²Current address: Medical University of South Carolina, Department of Bioinformatics, Charleston, South Carolina 29425, USA

Abstract. *Philometra overstreeti* Moravec et de Buron, 2006 and *Philometroides paralichthydis* Moravec et de Buron, 2006 are common parasites of the southern flounder, *Paralichthys lethostigma* Jordan et Gilbert. Because the life cycles of these parasites are unknown, our goal was to assess whether species of copepod commonly found in our estuaries could serve as intermediate hosts for these philometrids. Individuals of five species of copepods were collected and exposed to L₁ larvae of each philometrid species. The cyclopoid *Oithona colcarva* Bowman was the only species to become successfully infected. Successive moulting of philometrid larvae in the haemocoel of the copepods was studied using transmission electron microscopy. At 23°C the moult from L₁ to L₂ was observed for both species as early as 24 h post exposure to L₁ larvae. The moult from L₂ to L₃ was initiated within 2 days post exposure and completed by 6–7 days post exposure. Some parasite-induced tissue damage occurred in the copepod but no cellular response against larval philometrids was observed.

Philometrids are primarily histozoic nematodes that are exclusively fish parasites as adults. Although fairly common and potentially pathogenic to their hosts, our current knowledge of this group is quite limited (Moravec 2004, 2006). Whereas freshwater species are better understood (e.g., Anderson 2000, Moravec 2006), life cycles of marine philometrid species are rarely studied and only one such marine life cycle has been reported thus far, and so via the experimental infection of copepods (Sakaguchi and Fukuhara 1975). At this time all known life cycles of philometrids involve a copepod as a first intermediate host (Moravec 2006) and all involve copepods becoming infected after ingesting free-living L₁ larvae released from the fish-dwelling larvigerous adult. These L₁ larvae penetrate the intestinal wall of the copepod following ingestion and reach the haemocoel where they moult twice and develop into L₃ larvae, which are then infective to the definitive host (e.g., Moravec 2004). The study presented herein focused on identifying the putative intermediate host(s) of two species of brackish-water philometrids, *Philometra overstreeti* Moravec et de Buron, 2006 and *Philometroides paralichthydis* Moravec et de Buron, 2006 via experimental infection. Both species of philometrids are commonly found infecting the southern flounder *Paralichthys lethostigma* Jordan et Gilbert in the estuarine systems of South Carolina (SC), USA (Moravec and de Buron 2006).

Fish were collected by the staff of the SC Department of Natural Resources using electrofishing and trammel netting in the estuaries along the coast of SC (32°24'N to 33°24'N). Within 24 h upon return to the laboratory fish were dissected

and female philometrids were removed from two distinct microhabitats in the fish: *P. overstreeti* from among the teeth of the upper and lower jaw and *P. paralichthydis* from the joints between the bones forming the buccal cavity. Gravid female worms were placed individually in seawater and lanced to release the L₁ larvae to infect copepods within 1–3 h of fish dissection. Copepods were collected using a 150 µm mesh plankton net drawn horizontally within 2 m of the surface in the Charleston harbour (32°45'09"N, 79°53'56"W). Individuals of the following commonly found copepods were collected and sorted according to species: the calanoids *Acartia tonsa* Dana, *Parvocalanus crassirostris* (Dahl), and *Temora turbinata* (Dana); the cyclopoid *Oithona colcarva* Bowman; and the poecilostomatoid *Saphirella tropica* Wolfenden. Ten individuals of each species of copepod were then placed in separate 10-ml Petri dishes containing decanted seawater and exposed to over 500 L₁ larvae of either one or the other of the two species of philometrid for 24 h at 23°C. The experiment was repeated twice. After 24 h, copepods were crushed individually to check for the presence of larval philometrids in the haemocoel. Because only *O. colcarva* were infected 24 h post exposure following initial copepod infections, further experiments were pursued only with this species. In short, a total of 50–100 individuals of *O. colcarva* per Petri dish were exposed separately to L₁ larvae of each philometrid species as described above. Larvae were removed between 24 h and 38 h later to insure a high percent infection. Copepods were maintained at 23°C on a cycle of 12L:12D. The water in the Petri dishes was replaced every other day with freshly collected seawater filtered with a 70 µm mesh in order to assure the passage of the algae and protists upon which the copepods feed. Copepods were sub-sampled and crushed regularly every day to determine the time of philometrid larvae moulting. Days post exposure (d.p.e.) were calculated from the time copepods were first placed in contact with philometrid larvae. Because moulting was difficult to see in live animals and because we assumed that it could be mechanically induced by the pressure put on copepods to crush them to release the larvae, we used histology and transmission electron microscopy to confirm the time of moulting. In brief, two to three infected copepods at various d.p.e. as well as uninfected controls were fixed in 2% glutaraldehyde in 0.1 M cacodylate-sucrose buffer, rinsed in buffer, post-fixed in 1% osmium tetroxide in water, and processed according to classical techniques for Epon embedding. Serial 1 µm semithin sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a JEOL JEM 1210 transmission electron microscope.

Of the initial five copepod species experimentally exposed to the L₁ larvae of *P. overstreeti* and *P. paralichthydis*, only individuals of the cyclopoid *O. colcarva* were successfully

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Fig. 1. Transmission electron micrographs of larvae of *Philometroides paralichthydis* showing the continuous process of moulting in the haemocoel of an individual *Oithona colcarva*. **A** – L₂ in L₁ moult (arrow) 2 days post exposure (d.p.e.) – see damage of tissues in contact (star); **B** – L₃ in L₂ moult (arrow) 3 d.p.e.; **C** – L₃ in L₂ moult (arrow) 5 d.p.e.; **D** – L₃ in thin and fully detached L₂ moult (arrow) 7 d.p.e. Scale bars: A = 2 µm; B, D = 500 nm; C = 1 µm.

infected. Within 1 d.p.e., larvae of both philometrid species were seen inside the guts of the copepod. Although most infected copepods harboured one to two philometrid larvae, we also observed higher numbers of larvae in the haemocoel of some copepods, with nine larvae being found in one instance. In both species moulting from L₁ to L₂ occurred within 2 d.p.e. (Fig. 1A) and a second moult from L₂ to L₃ was initiated by 3 d.p.e. (Fig. 1B). The moulting process was continuous and showed progressive detachment and thinning of the cuticle (Fig. 1C). In both species the cuticle completely separated from the larvae by 7 d.p.e. (Fig. 1D). All L₃ larvae remained encased in the loose L₂ cuticle for as long as the host copepod could be maintained, which on average was 11 d.p.e.

but in one case was 31 d.p.e. Although soft tissues of infected copepods directly in contact with the philometrid larvae were progressively degraded (Fig. 1A), no obvious or overt host cellular defence reaction was observed.

The observation of two successive moults occurring in each species of philometrid within individuals of *Oithona colcarva* and the repeatability of these experimental infections indicate that this species of copepod is a possible intermediate host for both philometrid species. Successful experimental infection of *Paralichthys lethostigma* is needed to confirm this conclusion and we are currently attempting to carry out these infections in our laboratory. Support for *O. colcarva* as the natural host for these nematodes comes from the fact that the

range of this copepod species (Razouls et al. 2008) encompasses the range of the southern flounder, which is found from about Cape Hatteras, North Carolina, through the northern part of the Gulf of Mexico (Froese and Pauly 2008), as well as the fact that no other copepod species examined was capable of being infected. Whether more intermediate hosts occur is a recognized possibility and is unknown at this time. Significantly, *O. colcarva* is a euryhaline species (Bowman 1975) and its selection as a potential vector available over a broad range of salinities may be a reflection of the fact that throughout their life in the estuarine system, southern flounders perform small-scale migrations up and down the tidal rivers feeding the estuaries (Prentice 1989). In effect, although it is likely that the life cycle of *P. overstreeti* occurs at a higher salinity because of its absence in smaller fish that live higher in the river in low salinity waters, it is possible that infection by *P. paralichthydis* is acquired at lower salinities by young-of-the-year flounders (pers. obs.). Further, although *O. colcarva* populations display a seasonal occurrence in temperate regions (Lonsdale 1981a), it is known to occur in the SC estuarine system throughout the year, with its highest abundance occurring from June through mid-July (Lonsdale and Coull 1977, Knott 1980).

Since development of the larvae of *P. paralichthydis* and *P. overstreeti* requires only about 7 days at 23°C in the copepod and because the maximum life span of an individual *O. colcarva* is known to be only about 53 days at 20°C and 39 days at 25°C (Lonsdale 1981b), the presence of paratenic hosts in these philometrids' life cycles is highly likely. This contention is supported by the occurrence of infection of both species in large piscivorous flounder, which do not feed upon copepods, and the fact that such transport hosts are a well-known and important component in the life cycle of numerous other piscine parasites (Morand et al. 1995).

It is important to note that the L₃ larvae observed herein remained in the cuticle shed during the second moult, which has also been reported for *Philometra ovata* by Moravec (1980). It is possible that this conserved cuticle allows the philometrid larvae to evade the copepod immuno-defence system, since no obvious host defence reaction was observed. Significantly, the juveniles of both *P. paralichthydis* and *P. overstreeti* remained very active and thrashed inside the haemocoel of the copepods at all times observed. Whereas this thrashing may have induced at least part of the destruction of tissues in direct contact with the larvae (Fig. 1A), it cannot be discounted that the observed damage may also have been induced biochemically. Although, it is not known if philometrid larvae release excretory-secretory products in their copepod hosts, such a phenomenon is common in nematode larval stages found in vertebrate hosts (Abollo et al. 2001, Lun et al. 2003) and it would not be unexpected to find that such products play a significant role in causing host damage in the copepod. The impacts of infection on the copepod species composition of the estuarine plankton community are unknown at this time.

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