International Journal for Parasitology: Parasites and Wildlife 3 (2014) 6–11

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Phoretic interaction between the kangaroo leech *Marsupiobdella africana* (Hirudinea: Glossiphoniidae) and the cape river crab *Potamonautes perlatus* (Decapoda: Potamonautidae)

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A R T I C L E   I N F O

Article history:
Received 16 April 2013
Revised 1 October 2013
Accepted 2 October 2013

Keywords:
Intermediate host
Leech
Phoresy
Recruitment
Sex ratio

A B S T R A C T

The South African leech *Marsupiobdella africana* is a temporary ectoparasite of the amphibian *Xenopus laevis*, has a phoretic association with a freshwater crab *Potamonautes perlatus*, and exhibits advanced parental care by incubating its offspring in a brood pouch. Because phoretic associations are usually regarded to favor the phoront's dispersion, its occurrence within the biology of a parasitic species reflects an intimate context of interactions. In addition to phoresy, attachment to the crab may confer other advantages pertaining to offspring development and predator avoidance, dispersion and the parasitic life cycle. Two ponds where amphibian and crab hosts co-occur were sampled twice a month for a period of 1 year. The population dynamics of the leeches and their use of specific microhabitats as attachment sites on the crabs were also investigated. Results indicate a direct relationship between intra-specific variation in the sex ratio among captured crab hosts and the number of leeches recruited over time. The attachments to specific microhabitats on the hard surfaces of the host suggest a proximal proximate anti-predatory strategy. Finally, the importance of oxygen accessibility for the offspring development has been investigated experimentally. Results revealed a remarkable network of interactions linking all partners of this system raising the question as to whether the crabs merely act as a vehicle or play a role within the parasitic life cycle.

1. Introduction

Phoresy is defined as an intimate and temporary association when an individual, the phoront, is transported by another of a different species, the host, by the creation of ephemeral bridges between patchy habitats (Binns, 1982; Saul-Gershenz and Millar, 2006). This relationship between dispersal and phoresy has been extensively documented in arthropods (Binns, 1982; Tizo-Pedroso and Del-Claro, 2007; Krishnan et al., 2010; Sabagh et al., 2011). A typical example is the case of nematodes that attach preferentially to winged female wasps allowing dispersal between figs while wingless males are avoided (Krishnan et al., 2010). When the attachment to the host fails to provide all resources necessary for the phoronts' survival, phoretic interactions may diverge towards amensal or parasitic habits (Holte et al., 2001; Balashov, 2006; Saul-Gershenz and Millar, 2006). Before such evolution takes place, phoresy may become an interaction where attachment to the host is life-stage specific, temporary and/or optional but still provides significant advantages with regard to predation avoidance, dispersal and/or developmental success to the phoront. Recently, phoretic association was documented in free-living Oligochaeta (Lopez et al., 2005), but Maia-Carneiro et al. (2012) reported an unusual case of a leech transported by a vertebrate amphibian host without any deleterious observations. In this context, the case of the brooding leech *Marsupiobdella africana* (Glossiphoniidae) brings new insights to the role played by phoresy within the network of living interactions, especially when the carrier host is suspected to favor the development and parasitic transmission of the phoront.Within the Class Hirudinea (leeches), two independent adaptive trends influenced the diversification of their reproductive strategies and the kind of intimate interactions they may experience with other living partners. On the one hand leeches have undergone an adaptive diversification of their feeding strategies, *i.e.* from scavengers to parasitic blood feeders (Siddall and Burreson, 1998; Light and Siddall, 1999). An example is the kangaroo leech *M. africana*, which temporarily infects the amphibian host *Xenopus laevis* (see Van Der

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2213-2244
http://dx.doi.org/10.1016/j.ijppaw.2013.10.001

Lande and Tinsley, 1976). *M. africana* develops to maturity over a period of 2–3 weeks while attached to the host *X. laevis.* They then detach from the host to find a mate and copulate. Embryos are transferred externally to the brood pouch where up to 50 young develop over a period of up to 4 weeks. If the leech makes contact with a *Xenopus*, the young are discharged explosively on the surface of the host (Van Der Lande and Tinsley, 1976).The second adaptive trend involves the deposition of encapsulated eggs by the parental leech on the shells of arthropods and is described as a temporary and life stage specific phoretic strategy to protect the eggs from predation by snails (Sawyer, 1971; Sloan et al., 1984). Leech parental care evolved further from the direct protection of the cocoon by the parental body towards advanced stages of brooding behavior (Sawyer, 1971; Kutschera and Writz, 2001). The ultimate step in this evolutionary trend is reached in *M. africana*, where the parental leech transfers embryos from the female gonopore to a ventral brood pouch and incubates them until the final stages of their development (Van Der Lande and Tinsley, 1976). This evolution should have negated the need for phoretic strategies selected among related species to prevent egg predation. However, a phoretic relationship is maintained between the adult leeches of *M. africana* and a freshwater crab (Dick, 1959). This suggests that this relationship may confer advantages other than protection of the offspring, including improving parasite transmission. Studies on the biology of crab species revealed major seasonal variations in their spatial patterning and behavior depending on their sex and/or size (Trott, 1998; Unno, 2008; Smith et al., 2010; Di Virgilio and Ribeiro, 2012). These variations result in heterogeneity between individuals regarding their suitability for dispersion of leeches. Because most parasitic characteristics are largely governed by their ability to adapt transmission opportunities (Poulin, 1996; Combes, 2001), the choice of a parasitic leech for attachment/detachment to the crab host may result in several spin-offs. These include offspring development, predation avoidance, and dispersion.

This study focuses on the distribution of leeches on the Cape river crab *Potamonautes perlatus*. Whereas the role of the crab has previously been restricted to that of a vehicle for the leech (Dick, 1959; Van Der Lande and Tinsley, 1976), we tried to determine its importance within the "life cycle" of the leech and especially the nature of the factors governing attachment and detachment. We investigated whether seasonal variations in the dynamics of the crab population could impact on the survival and transmission of the leech species. The use, by the leech, of specific microhabitats on the crab was also considered. Ultimately the environmental factors that may account for the detachment of leeches from the crab were explored experimentally.

2. Materials and methods

2.1. Study site

Two artificial ponds in the botanical garden of the North-West University in Potchefstroom (North-West Province, South Africa), that support large populations of *P. perlatus* and *X. laevis*, were selected for this study. The bottom of the first pond (15 m × 6 m) sloped gradually, to reach a maximum depth of 0.9 m. Mosquito fish (*Gambusia affinis*), a potential predator of *M. africana*, and tadpoles of the common river frog (*Amietia angolensis*) were abundant in this pond. The second pond was round (3 m diameter) and sloped rapidly to a depth of 80 cm. Fish and *Amietia* tadpoles were absent from this pond. In both ponds mud and organic debris, up to 30 cm deep, covered the bottom. After heavy rains, overflow from the first pond sometimes reached the second. A preliminary survey showed that the pH, conductivity and temperature variations were very similar in both ponds.

2.2. Host and phoront sampling

Twice a month between March 2011 and March 2012, the two ponds were sampled at night (8–10 pm). Chicken liver in a gauze bag attached to a line was used as bait and lowered to a depth of 20–30 cm in the water. Bait bags were randomly distributed around the ponds. Due to the difference in the size of the two ponds, 12 and 4 bait bags were used for ponds 1 and 2, respectively to equilibrate sampling efforts. Crabs were collected using a dipnet and transferred to a bucket containing pond water. Crabs were sexed, the largest width of the upper carapace was measured and the number of leeches per crab counted by naked eye for each segment of the crab. The presence of lesions that could have been associated with the occurrence of leeches was checked under a dissecting microscope. All captured crabs were released after the last one had been caught in order to avoid overnight pseudo-replication. A sub-sample of 21 crabs was used to determine which microhabitats on the surface of the host are utilized by the leeches. A group of 15 individuals was also used in the lab experiment carried out during November 2011.

2.3. Experimental test for oxygen dependence

We tested the hypothesis that dissolved oxygen acts as a factor driving the interaction between crabs and leeches. As a preliminary condition, the borehole water used in this experiment was boiled to remove dissolved oxygen and then allowed to cool for one day. Three groups of 5 crabs were maintained individually at 20 °C for four days in identical tanks containing 7 L of this water. Due to the dimensions of the tanks (30/20/20 cm), the depth of the water (12 cm) was enough to prevent the crabs from reaching the surface, where both crabs and leeches would have been directly exposed to oxygen. The first group was maintained in continuously aerated water using air stones. The second group was maintained in water that was aerated for only one day prior to the experiment and then left without an external air supply, to simulate oxygen depletion by the host individual's consumption. The third group of crabs was maintained in water without any aeration. At 24 h intervals following the onset of the experiment, the leeches that detached from the crab were counted and removed. After 96 h of exposure the remaining leeches on the crabs were counted.

2.4. Statistical analyses

We performed a GLM (Poisson error and log link function) to investigate the relationship between the mean number of leeches per host individual and the host gender across the different ponds and months of sampling. A similar analysis was performed to compare the variations in the size of host individuals. ANOVA and post hoc testing (HSD Tukey) was performed to investigate the variations in the size of carrier hosts across gender for each month of sampling. Linear Pearson and nonlinear correlations between leech numbers, crab size and the host sex ratio were also tested. The proportions of leeches recovered from parts of the body and segments of the appendages, were compared between male and females hosts using a chi-square test. Finally, non-parametric ANOVA (Friedman test) was performed to test the differences in the number of leeches detached from host crabs depending on experimental conditions for each day of counting.