International Journal for Parasitology: Parasites and Wildlife 4 (2015) 142–147

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

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

Reproductive strategies of the kangaroo leech, *Marsupiobdella africana* (Glossiphoniidae)

Natasha Kruger, Louis Du Preez *

Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa

ARTICLE INFO

Article history:
Received 7 November 2014
Revised 14 January 2015
Accepted 20 January 2015

Keywords:
Cape River crab
Clawed frog
Leech
Reproduction
Spermatophore

ABSTRACT

The Kangaroo Leech, *Marsupiobdella africana*, is a hermaphroditic organism, with insemination taking place by the planting of a spermatophore on another leech. Spermatophores are mostly planted on the anterior of the recipient leech, but not always. Several spermatophores may be planted by different leeches on a single recipient. The spermatophore consists of two side by side lobes. Within minutes from planting of the spermatophore, the contents are squeezed out and into the body of the recipient. Sperm are believed to find the way to the ova by following chemical cues. Kangaroo Leeches display advanced parental care by transferring fertilized eggs from the reproductive opening to a brood pouch on the ventral side. Fully developed leeches may copulate after detaching from the amphibian host *Xenopus laevis*, or from the Cape River Crab *Potamonautes perlatus* with which it maintains a phoretic association.

1. Introduction

Leeches are often viewed as nasty blood sucking creatures and most people display an inherent fear and dislike of them. This perception is illustrated in many phrases in literature. Even the former British Prime Minister, Margaret Thatcher, on occasion, stated "To cure the British disease with socialism was like trying to cure leukemia with leeches". Despite their close association with medieval medicine, leeches are today widely used in the treatment of various circulatory disorders (Wright and Finical, 2000). In addition to their medical uses, leeches display some amazing behavior and reproductive strategies.

Many leeches are devoted parents, caring for their young in a manner that resembles the care normally associated with higher chordates. Whilst some leeches protect a cocoon containing fertilized eggs (Sawyer et al., 1981; Kutschera and Wirtz, 1986), others deposit encapsulated eggs on the shells of arthropods as a temporary and life stage specific phoretic strategy to protect the eggs from predation by snails (Sawyer et al., 1981). Others build nests for their young or carry eggs or young on their ventral surfaces. The ultimate step in this evolutionary trend is displayed by *Marsupiobdella africana* Goddard and Malan (1913) (Glossiphoniidae), where the parental leech transfers as many as 50 fertilized eggs from the female gonopore to its own ventral brood pouch, incubating them for over a four week period (Van der Lande and Tinsley, 1976; Badets and Du Preez, 2014). This is a very effective form of parental care as the eggs and developing young are safe against predation.

Marsupiobdella africana temporarily infests the amphibian host *Xenopus laevis* (see Fig. 1a). If the gravid leech with fully developed embryos makes contact with a *Xenopus*, the young are discharged explosively onto the surface of the host (Van der Lande and Tinsley, 1976). Whilst they remain attached to the host, they feed and develop to maturity over a period of two to three weeks. Initially the testes are rudimentary, but as they reach maturity the testes and genital area in general enlarge. There is no trace of any connection between vas deferens and atria, which makes it typically glossiphoniid in form. The atria are conspicuous structures with globular, thick walls. Inside the atria an aggregation of spermatozoa enclosed in a membrane can occasionally be recognized (Van der Lande and Tinsley, 1976).

After reaching maturity they detach from their hosts to find a mate and copulate (Fig. 1b). The ova start to develop as soon as they are transferred to the brood pouch. Over a period of about a month, the pouch becomes progressively bigger as the young develop and grow on the inside. Such growth occurs until the pouch occupies the majority of the body, thus distorting the organ systems in its vicinity. The nerve cord is forced to fold back double on itself, at both anterior and posterior walls of the pouch, being pushed dorsally against the gut by the pressure of the growing juveniles. The space occupied by the pouch diminishes after the young have been discharged and the pore lengthens (Van der Lande and Tinsley, 1976). Leeches then find a Cape River Crab (*Potamonautes perlatus*) that acts as a phoront (Fig. 1c). Leeches

* Corresponding author. Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa. Tel.: +27 82 455 5275; fax: +27 18 299 2370.
E-mail address: Louis.duPreez@nwu.ac.za (L. Du Preez).

http://dx.doi.org/10.1016/j.ijppaw.2015.01.005

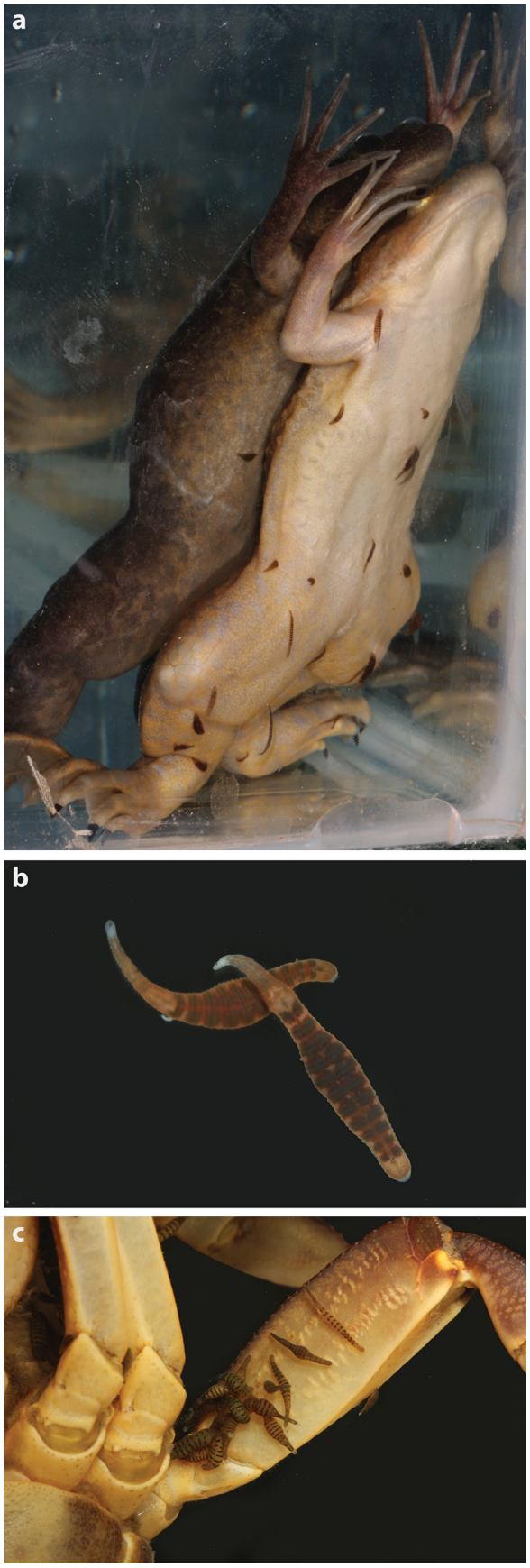

Fig. 1. Light micrographs of (a) two Clawed Frogs *Xenopus laevis* infected with Kangaroo Leeches *Marsupiobdella africana*; (b) leeches on the legs of a Cape River Crab *Potamonautes perlatus*; and (c) two Kangaroo Leeches copulating.

keep to the ventral and lateral sides of the crab body and proximal sections of the legs (Badets and Du Preez, 2014).

Although the deposition of the spermatophore on the surface of the leech has been reported before (Van der Lande and Tinsley, 1976), this study aims to provide information on the copulatory behavior and mechanism involved, as well as the morphology and ultrastructure of the spermatophore.

2. Materials and methods

2.1. Obtaining specimens

Two adjacent artificial ponds (26,68297S; 27,09519E) in the North-West University Botanical Garden in Potchefstroom, South Africa, sustain populations of Clawed Frogs (*Xenopus laevis*) and the Cape River Crab (*Potamonautes perlatus*). Both ponds are between 0.2 and 0.8 m deep, well vegetated with a variety of aquatic and semi-aquatic plants and contain a muddy bottom with ample organic debris.

Clawed Frogs were collected using baited bucket traps with an inwardly directed funnel (North-West University ethical clearance no. NWU-00005-14-S3 and Department of Development, Environment, Conservation and Tourism of the North-West Province of South Africa collection permit no. 028 NW-11). Traps were baited with chicken liver and left overnight. Crabs were collected at night using a dip net. To avoid leeches detaching, frogs and crabs were minimally handled and scientists wore latex surgical gloves. Leeches were removed from the surface area of the hosts using forceps and placed in a plastic tub containing pond water. The host individuals were released where collected.

2.2. Fixing specimens

Leeches collected were observed using a stereo microscope. Specimens were preserved for whole mount preparations, histological sectioning and/or scanning electron-microscopy. Immediately after collecting, leeches were examined for the presence of spermatophores using a dissection microscope and those with spermatophores were fixed. Leeches without spermatophores were then placed together in a small tub of 100 mm diameter containing pond water and closely observed under the dissecting microscope. As soon as a spermatophore was planted on the surface of another leech it was removed and fixed.

For whole mounts, specimens were fixed in 10% neutral buffered formalin (NBF) and the coverslip was weighted down using a 14 g lead weight. Specimens were stained using acetocarmine and following dehydration in a graded ethanol series and clearing in xylene, specimens were permanently mounted in Canada balsam. Specimens for histological sectioning were fixed in Bouin's fixative, dehydrated, cleared in xylene, impregnated with paraffin wax and imbedded in paraffin wax using a Slee embedding center. Serial sections of 8 μm were prepared, mounted on glass slides and routinely stained in Harris hematoxylin and eosin. Sections were examined using a Nikon Eclipse E800 compound microscope, fitted with a Nikon DXM1200 digital camera. For observing surface morphology, specimens were fixed in hot 70% ethanol or NBF, critically point dried and mounted on carbon tape on 12 mm SEM stubs. Before coating, specimens were studied and composite focused images obtained using a Nikon AZ100 microscope fitted with a motorized Z-drive and a high resolution digital camera. Images were captured on a personal computer with Nikon NIS Elements imaging software. Specimens were subsequently coated using a SPI Module sputter coater fitted with a gold-palladium source, and studied using a Phenom Pro-desktop scanning electron microscope at a power of 5 kV.