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Internal reproductive anatomy of the praying mantid *Ciulfina klassi* (Mantodea: Liturgusidae).

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ABSTRACT

Using light and scanning electron microscopy, the internal male and female reproductive anatomy of the praying mantid *Ciulfina klassi* is identified and described. This is the first detailed study to investigate the internal reproductive morphology of any Mantodea. The female structures identified were (1) paired ovaries with primitive panoistic type ovarioles, (2) a single blind-ended spermatheca with secretory gland cells and surrounding layer of striated muscle, and (3) female accessory glands associated with the production of the ootheca (the egg casing). The male structures identified were (1) paired multi-tubular testes, in which different stages of spermatogenesis were observed, (2) tubular vasa deferentia, (3) seminal vesicles, (4) male accessory glands and (5) a single muscular ejaculatory duct. Knowledge of basic reproductive morphology can be used to infer function and so provide key information for future research into reproductive behavior and physiology in the Mantodea

1. Introduction

Praying mantids (Mantodea) are an abundant group of predatory insects, with approximately 2300 described species, spread across 434 genera (Ehrmann, 2002). Mantids are a common subject in behavioral studies and display intriguing mating behavior and morphology (Maxwell, 1999). For example males have been shown to respond to perceived risk of sperm competition (Prokop and Vaclav, 2005), biased sex ratios (Lawrence, 1992; Hurd et al., 1994; Moran and Hurd, 1998) and variation in mate quality (Lawrence, 1992). Furthermore studies have investigated mate attraction (Robinson and Robinson, 1979; Perez, 2005), and antennal sensory morphology, in order to receive sex pheromones (Hurd et al., 2004; Holwell et al., 2007). Mantids are also renowned for their sexual cannibalism, which may be an adaptive behavior for females (Birkhead et al., 1988). Male condition (Prokop and Vaclav, 2005), male morphology and behavior (Elgar, 1992; Maxwell, 1998; Maxwell, 1999) may influence chances of being cannibalized. Although praying mantids are a diverse group of insects, and are of interest to biologists, to date there have been no detailed studies on their internal reproductive morphology. This is surprising as information on morphology can be vital for understanding mating behavior in any group of organisms.

Based on morphological and molecular analyses, the order Mantodea is commonly grouped together in the monophyletic insect superorder Dictyoptera, together with Isoptera (termites) and Blattaria (cockroaches) (Lo et al., 2000, 2003; Klass, 2001; Grimaldi and Engel, 2005; Klass and Meier, 2006). However, the relationships among these taxa are still under debate. A review by Eggleton (2001) outlines the major hypotheses for the relationships among these insect groups. Previous morphological studies determining the relationships within the Dictyoptera have not considered internal reproductive morphology, due presumably to a lack of data for the Mantodea. The only histological study on the internal reproductive anatomy of a praying mantid was by Sathe and Joshi (1986), which briefly described the spermatheca of *Hierodula coarctata*. Due to this lack of information on mantid reproductive anatomy, this study aims to provide the first detailed morphological study of the internal reproductive anatomy of a praying mantid.

Ciulfina klassi is a small predatory, non-sexually cannibalistic mantid endemic to north-eastern Australia. This species exhibits a preference for the lower reaches of narrow, smooth-barked trees that are low in structural surface complexity (Hill et al., 2004). *Ciulfina* display a generalist mode of hunting, actively pursuing and intercepting prey (Svenson and Whiting, 2004). Like in many insects, *Ciulfina* males deliver sperm via an externally attached spermatophore that is affixed to the female's genital opening during copulation. After copulation, the female will remove and consume the spermatophore; *Ciulfina* are the only mantids known to exhibit this behavior (Holwell, 2007).

The *Ciulfina* genus has been the focus of several research projects in recent years including projects studying genital

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morphology, copulation and sperm transfer (Holwell, 2006; Holwell et al., unpublished data) spermatophore feeding and mating behavior (Holwell, 2007) and mate location and antennal morphology (Holwell et al., 2007). This study will be the first to describe the morphology of the internal reproductive anatomy of *C. klassi*, by means of light and scanning electron microscopy (SEM). Microscopy elucidates the microanatomy of cells, tissues, and organs and their functioning in structural terms (Grimstone, 1976; Ross et al., 1989). By obtaining structural information on the male and female reproductive structures, we are better able to understand their function in reproduction, and elucidate how these structures might influence reproductive strategies of *C. klassi*.

2. Methods

2.1. Collection of specimens

Ciulfina klassi were collected in March–April 2006, from open *Eucalyptus* woodland in far north Queensland, between Mission Beach and Townsville, Australia. They were individually housed in inverted cups fitted with a fabric window to allow airflow. A piece of bark was glued inside the cup to provide the mantid with a perch. They were watered daily and fed *Drosophila melanogaster* three times a week. This study used specimens that were either collected as adults in the field, or reared to maturity in the laboratory. The observations and results of this study are based on the dissecting, processing and imaging of at least 15 males and females.

2.2. Light microscopy (LM)

Light microscopy (LM) was used to identify and describe the composition and structure of the different internal reproductive organs of male and female *C. klassi*. The female reproductive structures examined were the ovaries, oviduct and spermatheca. The male structures studied were the testes, vas deferens, ejaculatory duct and accessory glands. Carbon dioxide was used to anesthetize adult male and female mantids, and their reproductive structures were dissected and fixed overnight in 4% paraformaldehyde, 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The specimens were washed with 0.1 M phosphate buffer and were post fixed for 1 h with 1% osmium tetroxide. The specimens were washed with water, and dehydrated through a series of graded ethanol baths (from 50 to 100% (v/v)). The tissues were then infiltrated with LR White Resin (medium grade, London Resin Company, London). Once infiltrated with resin, the specimens were placed in embedding gelatin capsules and flat moulds, and incubated at 60 °C overnight to polymerize the resin.

The tissues were sectioned using glass knives on a Reichert Ultracut microtome (Leica, Austria). Semi-thin sections were cut (1.0 μm) and mounted on glass slides and stained with 1% (w/v) methylene blue in 40% (v/v) glycerol and 0.6% sodium bicarbonate. Sections were mounted using Biomount™ mounting medium (ProSciTech, Thuringgowa, Australia) and viewed under a bright field Olympus BX-50 microscope. Images of the sections were taken with a Sony Digital Interface DFW-X700 camera and the images were adjusted in the BTV-Pro 5.4 (for Macintosh, Ben Software) and Image J 3.16 (Image Processing and Analysis in Java) computer programs.

2.3. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to obtain detailed images of the external surfaces of the reproductive structures. Carbon dioxide was used to anesthetize adult male and female mantids, and their reproductive structures were dissected and fixed overnight in 4% paraformaldehyde, 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The specimens were washed with 0.1 M phosphate buffer and were post fixed for 1 h with 1% osmium tetroxide. The specimens were washed with water, dehydrated through a series of graded ethanol baths (from 50 to 100% (v/v)). The specimens were critical point dried with the K850 Critical Point Drier (Emitech, UK). They were then mounted on 10 mm stubs using carbon adhesive tabs, sputter-coated with gold (20 nm thick coat), using the Emitech K550, and viewed with a JEOL JSM 6480LA analytical scanning electron microscope.

3. Results

3.1. Female reproductive system

The internal female reproductive anatomy of sexually mature *C. klassi* (Fig. 1) consists of three main structures that meet at the genital chamber: (1) paired panoistic ovaries open into lateral oviducts that lead to the genital chamber; (2) a single blind-ended spermatheca attached to the genital chamber via a spermathecal duct; (3) paired, branched female accessory glands lie ventral to the ovaries and open into the genital chamber.

3.1.1. Ovaries

Ciulfina klassi females possess two ovaries approximately 10 mm in length that extend the length of the abdomen dorsal and lateral to the digestive tract. The ovaries consist of 7–9 panoistic ovarioles that join to the lateral oviducts (Fig. 2A). The panoistic ovarioles have three main regions: (1) a terminal filament that extends from the apex and suspends the ovariole within the body cavity, the terminal filaments are not fused in this species (Fig. 2B); (2) the germarium, the apex of the ovariole, is the site of female germ cell (oogonia) proliferation (Fig. 2B,C); (3) the vitellarium, the long proximal part of the ovariole, where the primary oocytes begin to enlarge through the uptake of yolk, and align in a single row (Fig. 2B,D).

The panoistic ovarioles of *C. klassi* are covered by an external sheath, which is well supplied with tracheoles (Fig. 2A,D). The tracheoles are the branching finger-like tubes of the tracheoblasts that lead from the cavity of the tracheal tubules, and are commonly bound to organs to provide them with oxygen. Within the external sheath of the ovariole are mycetocytes (bacteria-containing cells) (Fig. 2F). The heavily stained line surrounding the outer edge of the ovariole is a structureless membrane, known as either the tunica

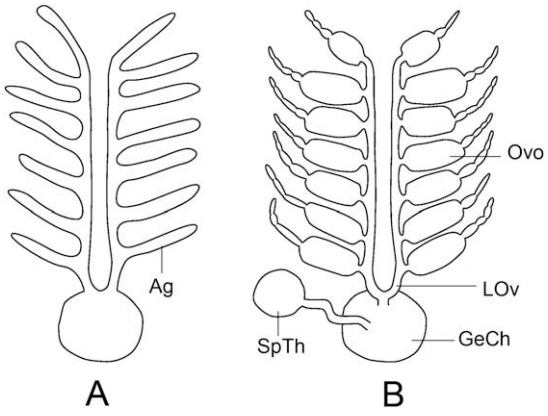

Fig. 1. Schematic drawing representing the gross anatomy of the *Ciulfina klassi* female reproductive anatomy. (A) Female accessory glands. These accessory or colleterial glands are paired structures of the female reproductive anatomy that lie ventral to the ovaries. (B) Ovaries and spermatheca. These structures are dorsal to the female accessory glands. Abbreviations: Ag, accessory glands; GeCh, genital chamber; LOv, lateral oviduct; Ovo, ovariole; SpTh, spermatheca.