



## Structural and ultrastructural characteristics of male reproductive tract and spermatozoa in two Cryptinae species (Hymenoptera: Ichneumonidae)

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### ARTICLE INFO

#### Article history:

Received 28 September 2009

Received in revised form 12 November 2009

Accepted 17 November 2009

#### Keywords:

Accessory gland

Testes

Phylogeny

*Lymeon dieloceri*

*Pachysomoides* sp.

### ABSTRACT

This study represents the first characterization of male reproductive tracts and ultrastructural description of sperm of Cryptinae species. In *Lymeon dieloceri* and *Pachysomoides* sp., the male reproductive tract is formed by a pair of testis, two deferent ducts, two accessory glands and one ejaculatory duct. The spermatozoa are similar to those described for other Hymenoptera, with: (1) the acrosome formed by the acrosomal vesicle covering the perforatorium, which has its base inserted in a cavity located in the nucleus point; (2) a thin nucleus with electron-dense chromatin; (3) an electron-dense centriolar adjunct located between the nucleus and one of the two mitochondrial derivatives; (4) an axoneme with a 9 + 9 + 2 microtubule arrangement; (5) two long mitochondrial derivatives with peripheral cristae and; (6) two accessory bodies located between the two mitochondrial derivatives and the axoneme. These ichneumonids present structural characteristics similar to other parasitic wasps, such as presence of a single follicle per testis, layer of extracellular material enveloping the acrosome and accessory microtubules ending before others in final portion of the flagellum. However, male reproductive system and the spermatozoa presented morphological characteristics that allowed their differentiation, such as oval shapes accessory glands and the symmetric mitochondrial derivatives in *L. dieloceri* compared to the spherical accessory glands and asymmetrical derivatives observed in *Pachysomoides* sp. Taken together, data presented here demonstrates that diversity of morphological characteristic from the male reproductive tract and spermatozoa in Hymenoptera might provide a character system that can be used, in association with other systems, to resolve various uncertainties about the evolutionary relationships of this insect group.

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### 1. Introduction

Hymenoptera, traditionally divided in “Symphyta” (sawflies and woodwasps), “Parasitica” (parasitic wasps) and “Aculeata” (bees, ants and wasps), have passed through numerous taxonomic and phylogenetic studies in recent years (Brothers, 1999; Vilhelmsen, 1999, 2001; Fernández and Sharkey, 2006). These studies often present “Symphyta” and “Parasitica” as paraphyletic groups and, for this reason, lose their taxonomic category status. Currently, it is recommended that Hymenoptera should be classified in superfamilies, since the taxon below the category of

order has maintained itself relatively stable (Fernández and Sharkey, 2006).

Ichneumonidae is one of the largest families of Hymenoptera, and a number of taxonomic (Townes, 1969, 1970a,b; Yu and Horstmann, 1997a,b; Triplehorn and Johnson, 2005) and phylogenetic studies (Wahl and Sharkey, 1993; Quicke et al., 1994, 1999a,b,c; Belshaw et al., 1998; Wahl and Gauld, 1998; Sharkey, 2007) have been performed analyzing the evolution of this group. Most authors acknowledge that some of the Ichneumonidae 37 subfamilies can be organized into three groups: Ophoniforme, Ichneumoniforme and Pimpliforme (Fernández and Sharkey, 2006). However, a large external and behavioral morphology, accompanied by a appreciable number of homoplasies, complicates the understanding of the phylogenetic relationships between these three groups (Quicke et al., 1994; Wahl and Gauld, 1998; Quicke et al., 1999a,b,c).

Cryptinae is a large subfamily of Ichneumoniforme. These parasitoids are found most frequently in the larva of Lepidoptera

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and other Hymenoptera (sawflies and social vespids). Although a considerable amount of information is available about the host relationships of some Ciptinae, virtually nothing is known about the biology of most tropical genera (Gauld and Hanson, 1995).

Morphological differences in the reproductive system and spermatozoa in Ichneumonidae might contribute as possible tools to phylogenetic analysis matrices whose systematics remain uncertain (Jamieson et al., 1999; Quicke et al., 1992). In the present study we describe the morphology of the male reproductive tract and the spermatozoa ultrastructure from the two species of Cryptinae, in order to contribute to future systematic analysis of this family.

## 2. Material and methods

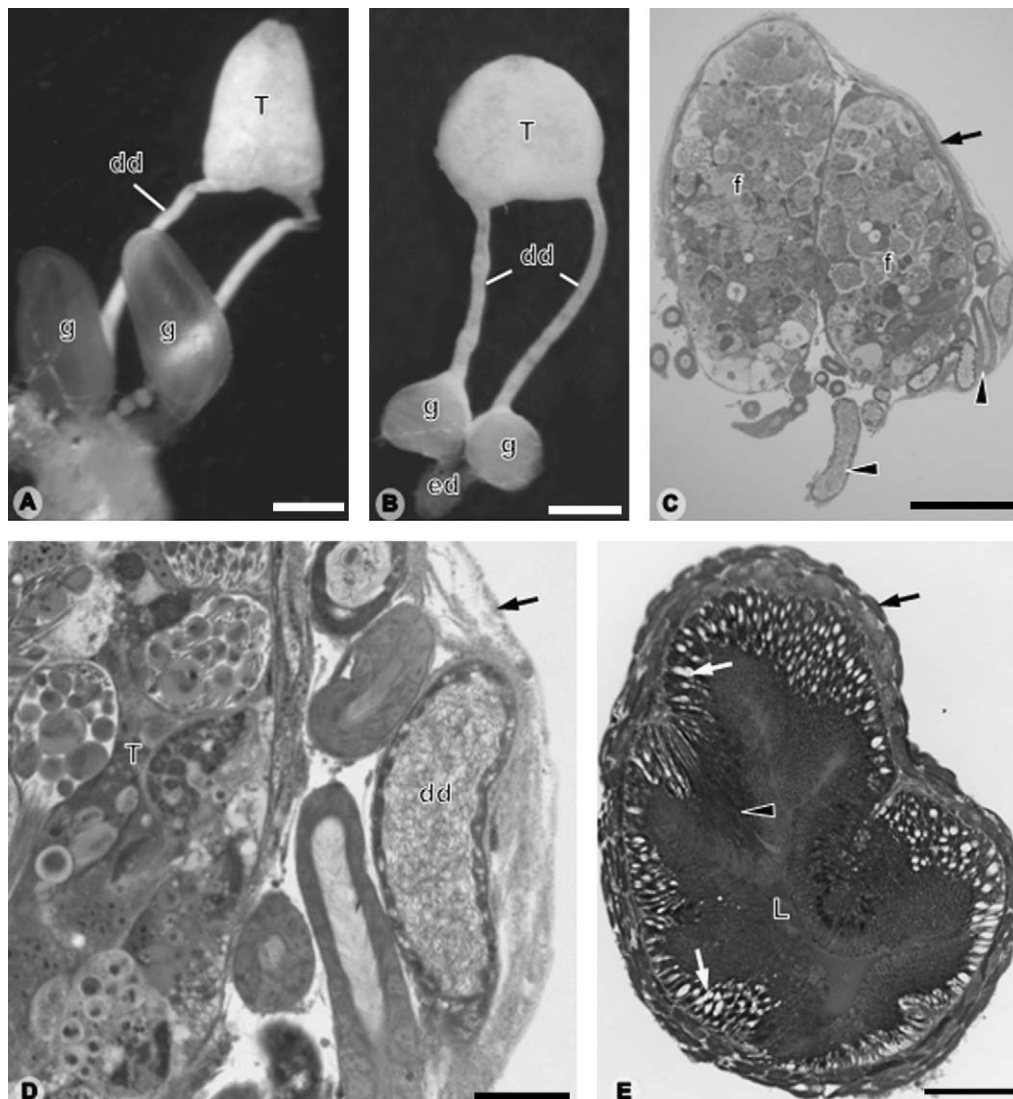
Adult males of *Lymeon dieloceri* were sampled in the nests of *Digelasinus diversipes* (Hymenoptera: Argidae) and adult males of *Pachysomoides* sp. were sampled in the nests of *Mischocyttarus cassununga* (Hymenoptera: Vespidae). Hives of both species were

collected in forest fragments areas near the city of Viçosa, MG, Brazil and maintained in the Structural Biology Laboratory of the Universidade Federal de Viçosa until the emergence of the adults.

### 2.1. Light microscopy

The reproductive systems of five males of each species were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 and post-fixed in 1% osmium tetroxide in the same buffer, for 2 h. They were then dehydrated in increasing alcohol series and embedded in Histo-resin<sup>®</sup> (GMA, Leica). Semithin sections (2  $\mu$ m) were stained with 1% toluidine borate and mounted in Entelan<sup>®</sup> (Merck). The analysis and photographs were made with an Olympus BX-60 microscope.

For each male, the suspension of spermatozoa extracted from one of the seminal vesicles was spread on clean glass microscope slides and fixed for 20 min in a solution of 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. After drying at room temperature, preparations were observed under a photo-



**Fig. 1.** Light microscopy of the reproductive system of *Lymeon dieloceri* (A, C–E) and *Pachysomoides* sp. (B). (A and B) Anatomy of the male reproductive system showing the testes (T) and deferent ducts (dd) surrounded by a common capsule, accessory glands (g) and the ejaculatory duct (ed). (C) Longitudinal section of the testes showing the presence of a single follicle (f) per testis. The arrow indicates the capsule which surrounds the testes and the arrowheads indicate the deferent ducts enlarged in testes basal region. (D) Region where spermatozoa are stored in the deferent ducts (dd). The arrow indicates the capsule which surrounds the ducts and the testes (T). (E) Longitudinal section of the accessory glands showing the epithelial folds (arrowhead), the narrow lumen (L), the secretion vesicles at the base of the epithelium (white arrow) and a muscle layer (black arrow). Bars: A and B, 0.5 mm; C, 100  $\mu$ m; D and E, 35  $\mu$ m.

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