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Morphology of the male reproductive ducts of *Clibanarius erythropus* (Anomura Diogenidae)

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Abstract

The male reproductive ducts of Clibanarius erythropus are observed at stereomicroscope and interference phase microscope. The results obtained are compared with data on C. erythropus specimens observed at optical microscope (Mouchet, 1931). The paired lobe-like testes merge into vasa deferentia made up of 4 zones. The stage of maturation of spermatophores is described according to the different zones. Ducts, isolated in toto from the crab, keep their orientation in the space. The materials secreted by the ducts form the different portions of the spermatophore.

Introduction

Clibanarius erythropus (Latreille, 1818), a common Mediterranean hermit crab, lives on the rocky bottom in the upper infralittoral zone. Its larval development (Thiriot, 1974), population structure, shell use, growth (Benvenuto and Gherardi, 2001; Gherardi, 1991), behaviour (Pessani and Tirelli, 2002; Tirelli et al., 1998; Gherardi, 1990) and epibiontic relationships (Tirelli et al., 2006a; Basile et al., 2004) are well known, while no recent research is available as regards the morphology of the male apparatus.
The male reproductive apparatus of hermit crabs is composed by testes and vasa deferentia, the latter forming and containing spermatophores. The morphology of testes and vasa deferentia connected to the stages of maturation of spermatophores have been studied, only at optical microscope, in few hermit crab species (Greenwood, 1972; Matthews, 1956, 1953; Mouchet, 1931). Only Mouchet (1931) analysed the reproductive apparatus of *C. erythropus*. Aims of this research are to describe the gonad morphology of *C. erythropus*, the orientation in the space of the ducts and the stages of spermatophores maturation, using both the stereomicroscope and the interference phase-contrast microscope; the obtained results are compared with the observations by Mouchet (1931).

Materials and methods

Five males of *C. erythropus*, collected at -0.5 m at Balzi Rossi (Western Liguria, Mediterranean Sea), were immediately preserved in 4% buffered formalin. In the laboratory, the male reproductive system was dissected. The male apparatus was *in toto* viewed under a Leica Zoom 2000 stereomicroscope, therefore it was placed onto microscope slides and clarified with a couple of drops of lactophenol. Spermatophores were teased out of the distal part of the vas deferens and placed onto further microscope slides to make temporary squashes. Slides were viewed and photographed with a Wild Leitz GMBH 020-437.035 interference phase-contrast microscope. Both the microscopes were equipped with a Sony SSC-DC58AP videocamera interfaced with a PC, and the photos were taken using the Matrox PC-VCR Remote Programme.

Results

The paired lobe-like testes of *C. erythropus* are constituted by seminal tubules, placed dorso-lateral to the large epatopancreas and dorsal to the midgut. The tubular, paired and sinuous vasa deferentia deepen into the acini of the epatopancreas before opening to the exterior via the paired gonopores. The paired, lobe-like testes, composed of cystic structures, each merge into a tubular duct made up of four zones (Fig. 1): 1) highly coiled narrow collecting tubule, 2) proximal, 3) medial, and 4) distal zone. In the collecting tubule the sperm mass is amorphous and embedded in seminal fluid. In the proximal zone the sperm mass becomes subdivided into fragments by the dense primary spermatophore layer (Fig. 2). In between the proximal and the medial zone the sections of aggregated spermatozoa are shaped by secretory products to form the ampulla of the spermatophore. In the medial portion, the spermatophore stalk and foot are formed and the stalk progressively lengthens. In the distal zone the mature spermatophore are stored, enveloped into a mucilage-like

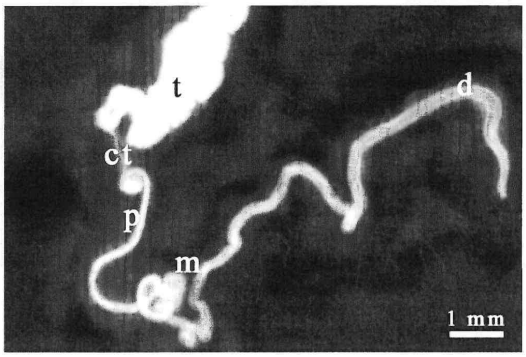

Fig. 1 - Clibanarius erythropus: male reproductive apparatus in toto at stereomicroscope. Testes (t), coiled collecting tubule (ct), proximal (p), medial (m), and distal (d) zones.

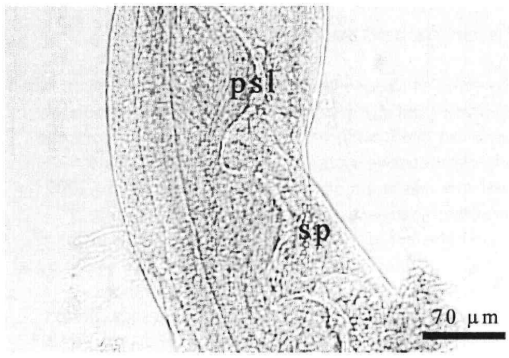

Fig. 2 - Clibanarius erythropus: stages of maturation of spermatophores at interference phase-contrast microscope. Proximal zone with sperm (sp) mass subdivided by the primary spermatophore layer (psl).

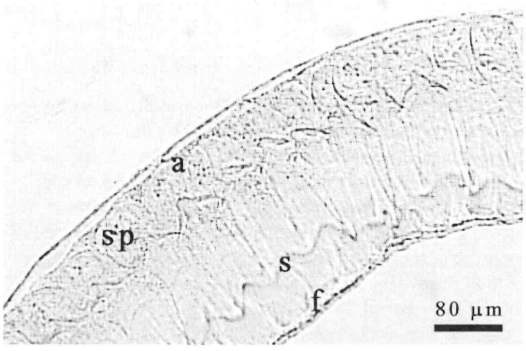

Fig. 3 - Clibanarius erythropus: stages of maturation of spermatophores at interference phase-contrast microscope. Distal zone with mature spermatophores made up of ampulla (a) with spermatozoa (sp), stalk (s) and foot (f).

secretion and connected at the base by a ribbon-like secretion (Fig. 3). From the collecting tubule to the distal zone, 6 mucilage-like secretions, constituting different portions of the spermatophore, were observed.

Discussion

The reproductive organs of *C. erythropus* are placed in the abdomen, dorsal to hepatopancreas and midgut, as in other hermit crabs (Tirelli et al., 2006b; Manjón-Cabeza and García Raso, 2000; Greenwood, 1972; Matthews, 1956, 1953; Mouchet, 1931); this disposition differs from the one observed in the Anomura *Galathea intermedia*, where the reproductive ducts are placed in the cephalothorax (Kronenberger et al., 2004).

Coiled vasa deferentia have already been described in hermit crabs (Tirelli et al., 2006b; Manjón-Cabeza and García Raso, 2000; Greenwood, 1972; Matthews, 1956, 1953; Mouchet, 1931). We noted they deepen into the acini of the epatopancreas, in agreement with Mouchet (1931). The 4 zones making up the vasa deferentia are the same described for the shrimp *Pleoticus muelleri* (Diaz et al., 2002), the crayfish *Cherax* sp. (Talbot and Beach, 1989), the diogenids *Diogenes pugilator* (Manjón-Cabeza and García Raso, 2000) and *Calcinus tubularis* (Tirelli et al., 2006b). The wall lining the collecting tubule produces the secretion which envelops the spermatic mass and conveys sperms towards the proximal zone, in agreement with the observations on *Homarus americanus* (Kooda-Cisco and Talbot, 1986), *Penaeus setiferus* (Ro et al., 1990) and *Calcinus tubularis* (Tirelli et al., 2006b). In the proximal zone, the sperm mass is subdivided in fragments, as in *Enoplometopus* sp. (Haley, 1984), *Homarus* sp. (Kooda-Cisco and Talbot, 1986), *Galathea intermedia* (Kronenberger et al., 2004), and *Calcinus tubularis* (Tirelli et al., 2006b). In the medial zone, the ampulla is completed, the stalk lengthens and the foot is formed (Tirelli et al., 2006b; Ro et al., 1990; Talbot and Beach, 1989; Kooda-Cisco and Talbot, 1986). For the first time in the present research it was possible to see the foot of the spermatophores produced by *C. erythropus*, because, differently from Mouchet (1931), the spermatophores were teased out of the ducts. The series of loose coils of the medial zone has never been reported previously in the literature. We suppose that, as the ducts were isolated *in toto* from the hermit crab, they maintained their orientation in the space. Mouchet (1931), instead, subdivided the reproductive duct in portions: doing so, the orientation in the space has been missed. In the distal zone, mature spermatophores are stored (Dudenhausen and Talbot, 1983; Talbot and Beach, 1989; Ro et al., 1990; Tirelli et al., 2006b).

Based on their different refractivity to light (method followed by Mouchet, 1931), we observed 6 mucilage-like secretions. Teasing the spermatophores out of the ducts allowed to identify one secretion more than Mouchet (1931). Nevertheless this result is only indicative because no chemical analysis of the produced substances has been performed.

In conclusion, the redescription of the morphology of the reproductive tract of *C. erythropus* using stereomicroscope and phase-contrast microscope only in part confirms data by Mouchet (1931), improving the knowledge of the reproductive biology of Anomura.

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