ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 46 (1): 83 - 90, 2005	UDC: 594.1 (611) 591.2(611) Original scientific paper

Infestation of the clam *Venus verrucosa* by Sipunculoidea and the lithophagus bivalve, *Gastrochaena dubia*

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From August 2003 to July 2004, specimens of the bivalve Venus verrucosa *(L.) were collected monthly in the channel connecting the lagoon of Bizerte (Tunisia) to the Mediterranean Sea. During the winter, 4% of the specimens had tiny perforations on the outer and inner faces of the valves; the perforations were connected to an intra-valve network of galleries, caused by 10-12 mm Sipunculoidea. Of specimens collected in February-March, 4% were infested with the lithophagous bivalve,* Gastrochaena dubia*, that lived within a cavity in the* V. verrucosa *valves. The cavity communicated to the outside through a calcareous tube developed by the* G. dubia *near the exit of the* V. verrucosa *siphons, indicating parasitism that can cause progressive perforation of the valve and lead to the death of the host.*

Key words: *Venus verrucosa;* Sipunculoidea, *Gastrochaenia dubia,* Bizerte Lagoon, Tunisia

INTRODUCTION

The clam, *Venus verrucosa (L.),* is a much appreciated marine product in Tunisia and the western Mediterranean. This species is widely distributed in coastal areas but the population density and flesh quality depend on the harvest site. In Tunisia, *V. verrucosa* is collected mainly in the north from the channel connecting Bizerte Lagoon to the Mediterranean Sea. *V. verrucosa* is protected by Tunisian authorities, but collection is not subject to declaration or supervision by state authorities. Consequently, no population statistics are available. However, our observations indicate that fishermen are able to collect 10-15 kg/3 h in the Bizerte Channel using scuba diving equipment. The clams are collected manually, according to requests of restaurants and hotels.

V. verrucosa lives buried 2 cm deep in mud, sand mixed with mud, or shell debris and gravel (TEBBLE, 1976). Specimens are rarely observed on the surface of the sediment and their emergence is usually due to unsuitable environmental conditions or the presence of a disturbing foreign organism. A link between heterospecific associations and emergence from sediment was observed in the cultured clam, *Ruditapes philippinarum*, infested by the *Vibrio tapetis* bacterium (PAILLARD *et al*., 1989).

Emergence was also observed in the bivalve, *Cerastoderma edule*, infested by the trematode *Curtuteria australis*. In this bivalve, the presence of trematode larvae cysts in the foot disturbs the organism, preventing it from obtaining nutrients from the sediment (THOMAS *et al*., 1998). The unusual emergence of clams favors the fixation of epibionts, generally sponges, *Cnidaria*, and sedentary polychaetes, on the shell, especially in the posterior-dorsal region. Because of its roughness and thickness, the clam shell can also allow fixation of larvae of lithophagous organisms searching for a favorable support for metamorphosis.

The aims of our study were to examine the unusual associates of the *V. verrucosa* host and discuss possible heterospecific relationships and their affect on emergence.

MATERIALS AND METHODS

From August 2003 to July 2004, scuba divers at a depth of 7-9 m collected 120 *V. verrucosa* clams monthly. The clams were harvested from a site located in the channel connecting the Bizerte Lagoon to the Mediterranean Sea (Fig. 1), an area characterized by strong hydrodynamism and high biodiversity. The channel is about 7 km long and 12 m deep.

Upon return to the laboratory, animals were sorted and those with epibionts were isolated and counted. Clams with epibionts in the shell that were connected to the outside by a pipe with two lodges were put into an aquarium for further study. Aquarium seawater was completely renewed every two days. The aquarium was continuously oxygenated. The *V. verrucosa* remained in the aquaria six months, from February to July, and were examined again with an optic binocular having a micrometric ocular.

RESULTS

Infestation by Sipunculoidea

Four percent of the clams collected during the winter (14 of 360 specimens) had small perforations that measured 2-5 mm wide and were located mainly on the latero-dorsal edge of the shell (Fig. 2a). When opened, tiny blackish spots were revealed (Fig. 2b). When the mantle was detached from the interior layer and examined, abnormal structures were observed at the nodules, indicating injury to the host tissue (Fig. 2c). Without the mantle, perforations of different sizes were visible on the internal face of the clam shell (Fig. 2d-e). When the shell was broken, a network of communicating galleries was observed on the external shell face (Fig. 2f). Some contained mud, and appeared more or less black under the binocular. An unidentified Sipunculoidea, 10-12 mm long, was present

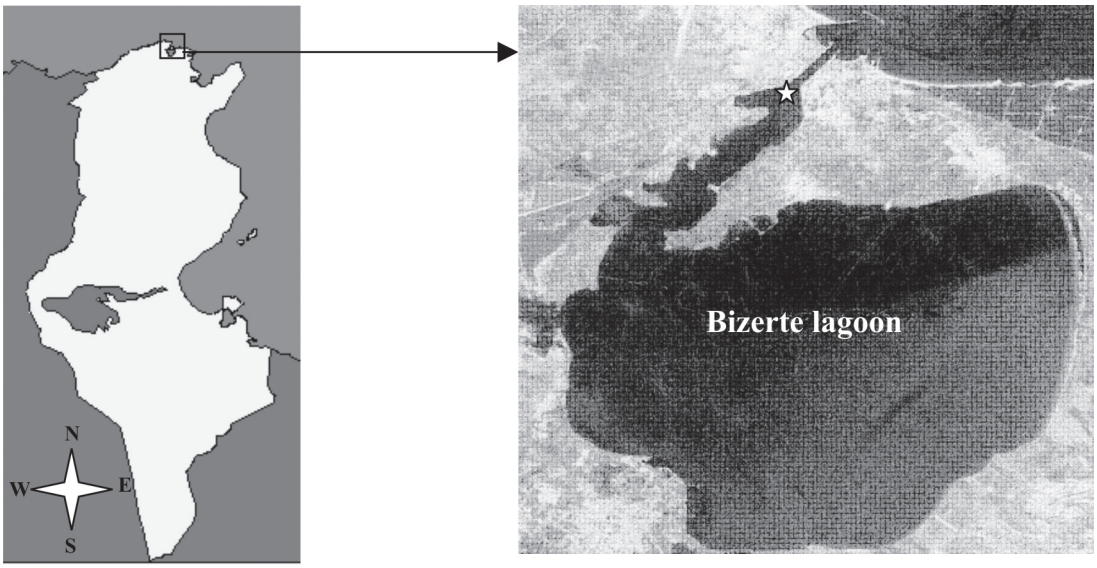

Fig. 1. Collection site (star)

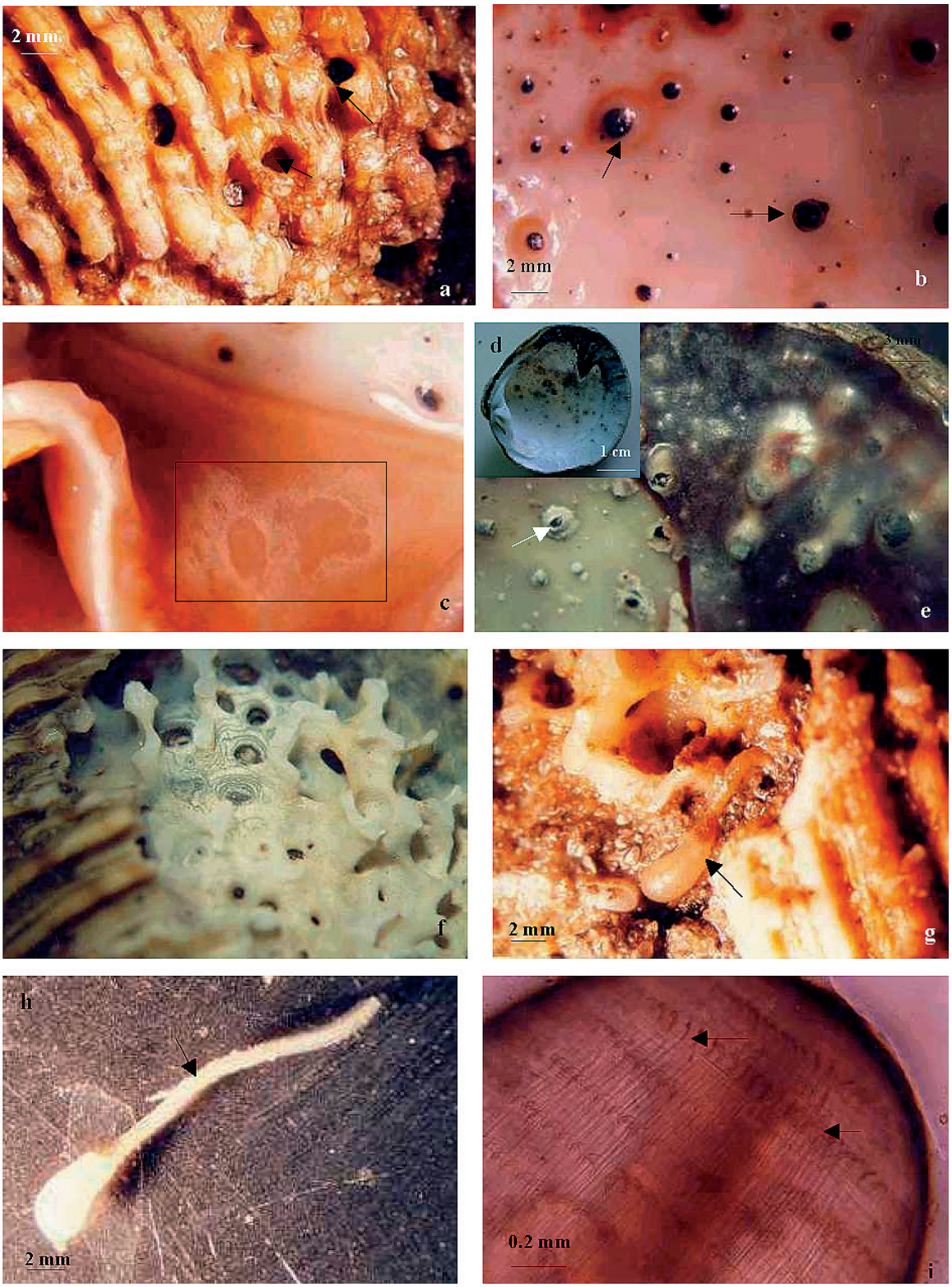

Fig. 2. Infestation of Venus verrucosa *by Sipunculoidae. (a) External view of the* V. verrucosa *shell with perforations caused by Sipunculoidea; (b) perforations observed through the mantle; (c) mantle injured by perforations; (d) internal view of the shell with perforations located mainly in the posterior part of the valve; (e) perforations of the nacreous layer; (f) intra-shell matrix network with external and internal communications; (g)* in situ Sipuncula*;(h) devaginated introvert of a sipuncula; (i) hooks on the introvert of a sipuncula*

in the gallery systems filled with black mud (Fig. 2g). The Sipunculoidea was sometimes up to 5 times the length of the clam body (Fig. 2h). Observations of the Sipunculoidea under a microscope revealed the presence of rows of hooks arranged in a circular pattern (Fig. 2i).

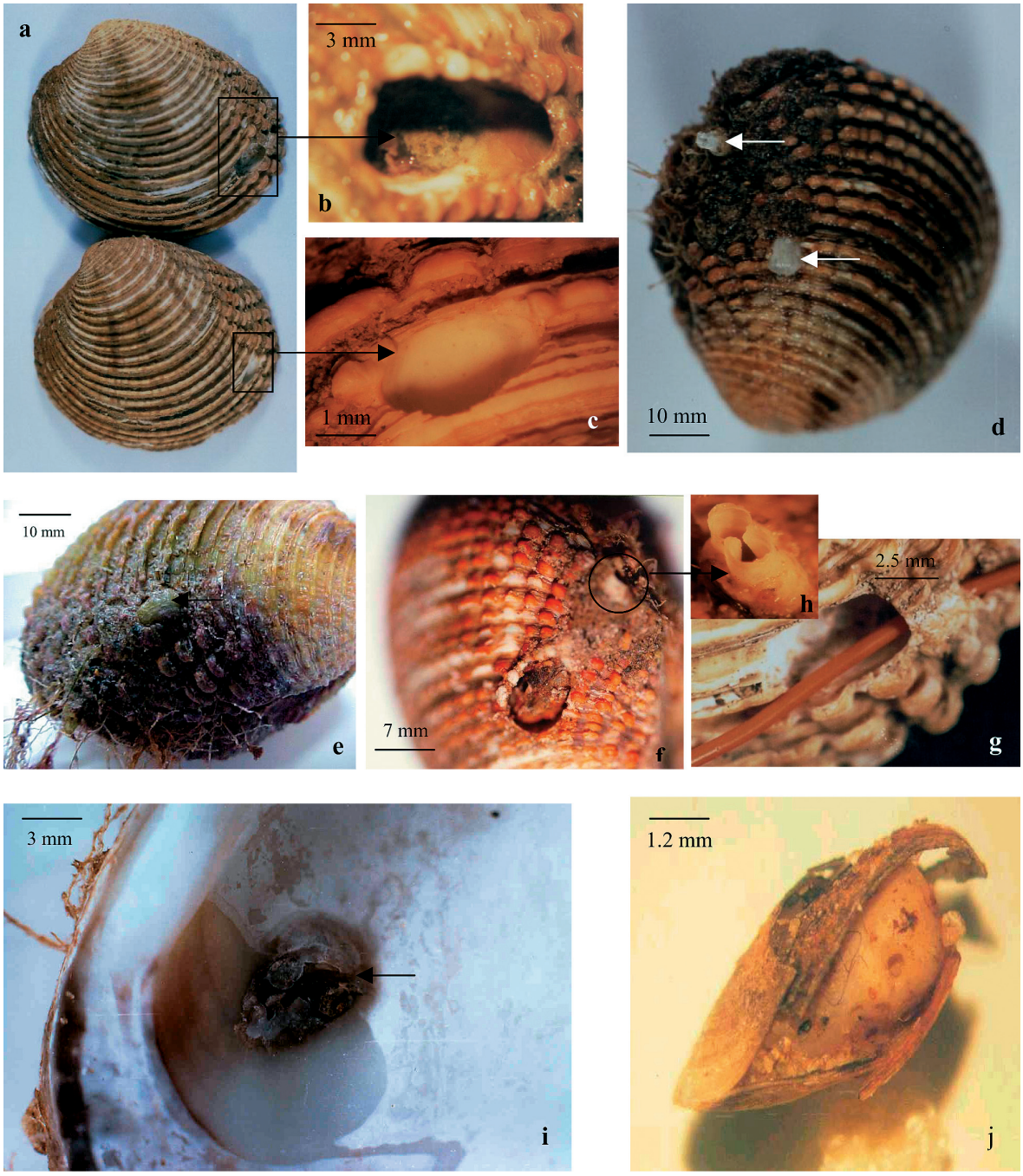

Fig. 3. Infestation of Venus verrucosa *by* Gastrochaena dubia. *(a-c) Localization and size of* V. verrucosa *shell injuries; (d) formation of a tube and covering of the cavity by a calcareous secretion by epibiont; (e) covering algae of the fine calcareous layer; (f)* in situ *epibiont after removal of the thin nacreous layer; (g) communication between intra-valve cavity and posterior perforation; (h) two galleries of the epibiont formed in the posterior part of the* V. verrucosa *shell; (i) injury in the nacreous layer leading to shell perforation; (j)* G. dubia *epibiont out of its gallery.*

Infestation by the bivalve *Gastrochaena dubia*

Only six of the 1440 collected specimens (0.41%) were infested by *Gastrochaena dubia*. However, the six infested specimens were collected in the February and March samples, raising the infection rate to 2.5% (6/240 specimens) during these months. Infested clams were 35-42 mm long, had breaches (4-10 mm long, 2-6 mm wide, 1.5-5 mm deep) in the latero-dorsal edge of the left valve (Fig. 3a-c), and had a cavity sealed with a thin smooth stiff deposit, essentially white in color (Fig. 3d) but often greenish (Fig. 3e) due to micro-alga growth. The *G. dubia* lived in the thin cover of the cavity. When the cover was removed, the *G. dubia* produced a new deposit of whitish secretion within 2-3 days. The diameter of the cavity grew from about 4 mm in February to 8 mm in July and communicated to the external surface through a perforation (Fig. 3f-g) located in the posterior part of the clam shell. Two small inter-communicating tubes emerged near the exit of the host's siphons (Fig. 3h). After six months of maintenance in the aquarium, degradation of the nacreous layer of one clam was noticed, perforating the shell of the host (Fig. 3i). The *G. dubia* that caused this disturbance was 10.52 mm (Fig. 3j).

DISCUSSION

In this study, we revealed the presence of two unusual infestants of *V. verrucosa*, an unidentified Sipunculoidea and the lithophagous bivalve *G. dubia*. Most other studies on parasitism in bivalves describe non-molluscan organisms that infest bivalve host tissues: Trematoda (MARTINEZ, 1972; BARTOLI, 1973; MONTAUDOUIN *et al*., 2000; TRIGUI EL MENIF *et al.*, 2004), *Turbellaria* and Cestoda (MONTAUDOUIN *et al.*, 2000), copepod (BERNARD, 1969; MONTAUDOUIN *et al*., 2000; LE PENNEC & LE PENNEC, 2003), fungi (JOLY & COMPS, 1980), and protozoa (COMPS & CHAGOT, 1987; KWANG-SIK *et al.,* 2002; ATTIA EL HILI *et al*., 2004; MIRELLA DA SILVA & VILLABA, 2004). Most of these organisms infest the gonad tissues of the bivalves, causing physiologic disturbances as well as decreasing the number and quality of oocytes. In some cases, the bivalve hosts become sterile (MARTINEZ, 1972; EL MENIF *et al.,* 2004).

BARTOLI (1973) showed that the metacercarian *Gymnophallus fossarum* that resides in the extrapallial compartment of *Cardium glaucum* is responsible for shell anomalies such as the formation of protuberances parallel to growth ridges. Research rarely describes the presence of an invertebrate living totally inside the valves of bivalves. Nevertheless, this is the case with the Sipunculoidea that we isolated in the winter in intra-valve galleries of *V. verrucosa*. The Sipunculoidea was the only invertebrate living in the gallery system and the perforations were the same size as the Sipunculidae body. We presume that, since the shells of the dead infested clams are severely deteriorated, the divers collected only living clams during other seasons. The Sipunculoidea was undoubtly responsible for creating the gallery network by producing secretions that dissolved the host shell or by a mechanical process. The hooks in the introvert sipunculien were used as perforators.

GRASSÉ (1959) wrote that some species of Sipunculoidea have hooks on their introvert and are able to excavate the host shell. D'HONDT (2000) suggested that Sipunculoidea occupies but does not excavate the galleries and that the build-up of intra-valve galleries makes the host shell fragile and prone to damage. A similar phenomenon was observed in the oyster *Crassostrea gigas* (CATHERINE *et al.,* 1990) when infested by a polychaete worm of the Spionidae family (genus *Polydora*). According to CATHERINE *et al.* (1990), the worm drilled into the oyster shell to form galleries that disfigure the host shell. A similar disturbance was found in the bivalve *Lithophaga lithophaga*, also from the northern coast of Tunisia, by JAAFAR *et al*. (2004) who removed two forms of a Cirratulidae known as the perforator polychaetous *Dodecaceria concarum* from intra-shell galleries of this species,.

The second infestant was the lithophagous bivalve *G. dubia*. This species bores into various substrata: sand, limestone, sandstone, and dead mollusk shells (TEBBLE, 1976). At the end of the planktonic phase, the pediveliger larvae of *G.*

dubia settle down on a substratum which, in the present case, was the rough shell of the emerged *V. verrucosa*. Using chemical processes, *G. dubia* gradually perforates coral substrata (CARTER, 1978), or the host shell in our case. Then it uses an invaginated and devaginated organ to bore into the shell region of the internally naked host. This infesting bivalve is often associated with other lithophagous species in calcareous rocks such as *L. lithophaga, L. aristata, Petricola lithophaga*, and *Striarca lactea.* The fixation of *G. dubia* larvae does not occur randomly. All infested specimens have a cavity dug into the posterior part of the shell that contains a tube connecting to the external environment and located near the outlet of the host's siphons, suggesting assumes that the clam emerged from the sediment during the fixation of the pediveliger larva of *G. dubia*. The strategic position of the infestant on the latero-dorsal edge of the shell means that the parasite takes advantage of the clam, consuming part of the nutrients intended for this species. As long as the width of the *G. dubia* is less than the thickness of the host shell, we consider this association a form of commensalism. However, growth leads to progressive internal perforation of the clam shell, disturbing the host and eventually leading to a parasitic association. After reaching a certain size of opening cavity (8 mm), the parasite can prevent the clam from hermetically closing its valves, making the host more vulnerable to further attacks from pathogenic agents and predators.

In conclusion, we revealed the presence of two taxa (Sipunculoidae and Bivalvia) in the valves of *V. verrucosa*. The relationship between *V. verrucosa* and these species is probably time-dependant on the development of the association. As the organisms grow and mature, they weaken the valve structure by multiplying the number of galleries and/or size of excavated cavities, increasing the possibilities of infection by pathogenic agents or physiological exhaustion of the host by monopolizing food resources and limiting the ability of the host to reconstruct its nacreous layer. The presence of these parasites on *V. verrucosa* valves probably causes some form of competition for nutrition, especially in the case of *G. dubia* whose siphons are near those of the *V. verrucosa*.

ACKNOWLEDGEMENTS

We are grateful for the English translation help and advice provided by Dr. Fredj ALAYA (Tunis) and Dr. Roger FLOWER (UCL, London, UK).

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Received: 27 December 2004
Accepted: 04 April 2005

Zaraženost školjkaša (ladinke) *Venus verrucosa* sipunkuloidnim crvima i litofagnim školjkašem *Gastrochaena dubia*

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SAŽETAK

Između kolovoza 2003. i srpnja 2004. u kanalu koji povezuje lagunu Bizerte (Tunis) s Mediteranom, mjesečno su sabirani primjerci školjkaša *Venus verrucosa* (L.). Na 4% primjeraka zapažene su sićušne perforacije s vanjske i unutarnje strane školjke, koje su bile povezane u mrežu hodnika. Promatranjem binokularnim mikroskopom kod nekih je primjeraka zapaženo prisutstvo sipunkuloidnog crva, dužine od 10 do 12 mm. Drugi školjkaši (0,41%) bili su zaraženi školjkašem *Gastrochaena dubia*, koji živi čitav u šupljini unutar školjkaševih valva. Šupljina komunicira sa sredinom pomoću vapnenčaste cijevi koju je stvorio školjkaš. Otvor ove cijevi smješten je blizu sifona školjkaša, ukazujući na parazitizam. Rast *G. dubia* može prouzročiti progresivnu perforaciju valve što dovodi do postepenog uginuća domaćina.

Ključne riječi: *Venus verrucosa,* Sipunculoidea, *Gastrochaenia dubia,* Laguna Bizerte, Tunis