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# New insights on the life style of the epibiotic amphipod crustacean *Tritaeta gibbosa* (Crustacea, Amphipoda, Dexaminidae)☆

Gerhard Scholtz

*Humboldt-Universität zu Berlin, Institut für Biologie/Vergleichende Zoologie, Philippstr. 13, 10115 Berlin, Germany*

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ABSTRACT

*Tritaeta gibbosa* is an epibiotic amphipod that lives in self-constructed invaginations in the tissue of various hosts as diverse as sponges, soft corals, ascidians and echinoderms. Here, some aspects of the relationship of *T. gibbosa* to different hosts are described. The main focus, however, is the colonisation of the holothurian *Ocnus planci*. The association between this holothurian species and *T. gibbosa* was studied by means of histology and scanning electron microscopy. The investigations revealed that *T. gibbosa* does not damage the host tissue. Only some stretching, thinning, thickening, and displacement of the holothurian cuticle, epidermis and dermis was observed. This suggests that the invagination is formed by pressure caused by a specific behaviour of *T. gibbosa*. The amphipod takes advantage of the properties of the mutable connective tissue characteristic for echinoderms. The majority of invaginations are situated at the holothurian's anterior end. Furthermore, most invaginations are perpendicularly oriented to the long axis of the holothurian body. This seems to improve the exploitation of food particles coming from the tentacles of *O. planci*. The amphipod specimens are subadult to adult and there is a strong bias toward females as is generally the case in benthic *T. gibbosa* populations. Although some new information about the ecology of *T. gibbosa* is added by this study, some general questions such as the tissue reactions of the various hosts, the ontogeny and juvenile behaviour, and the type of association to the host still need investigation.

## 1. Introduction

With an occurrence in the north Atlantic, the North Sea, the West African Atlantic, the Mediterranean, and the Black Sea, *Tritaeta gibbosa* (Spence Bate, 1862) is a widespread and abundant epibiotic amphipod (Fage, 1928; Ruffo, 1957; Peacock, 1972). *T. gibbosa* has been reported to inhabit a variety of species belonging to a number of higher metazoan taxa as diverse as sponges (e.g. Bate and Westwood, 1864; Carter, 1878; Vosmaer, 1887; Della Valle, 1877; Chevreux and Fage, 1925; Fage, 1928; Stephensen, 1929; Arndt, 1933; Bácescu and Mayer, 1960; Peacock, 1972; Peattie and Hoare, 1981; Costello and Myers, 1987; Çinar et al., 2002), alcyonarian corals (Fage, 1928; Arndt, 1933; Ruffo, 1957), ascidians (Della Valle, 1877; Millar, 1949; López-Legentil, 2005; Voultsiadou et al., 2007), and echinoderms, namely holothurians and an ophiurid (Ruffo, 1957; Barel and Kramers, 1977; Changeux, 1961; Laetz et al., 2013; this study). In addition to metazoan hosts, *T. gibbosa* has been found to live on algae and other benthic substrates (Jones et al., 1973; Sezgin, 1999), and even a pelagic occurrence of this species has been recorded (Fage, 1928; Peacock, 1972; Jones et al., 1973).

As epizoic species on various metazoan hosts, *T. gibbosa* shows a specific pattern of settlement. Individuals sit in small invaginations or pits on the surface of the host's tissue with their body bent dorsally and with the ventral side facing the environment (e.g. Fage, 1928; Ruffo, 1957; Scholtz, 2009; Laetz et al., 2013).

Most of the more detailed accounts of the ecology of *T. gibbosa* deal with the association of this amphipod with sponges (Della Valle, 1877; Fage, 1928; Peacock, 1972), whereas other hosts such as ascidians and echinoderms, not to speak of cnidarians (Arndt, 1933), have been somewhat neglected. Only recently, has new data on the relationship between *T. gibbosa* and holothurians been published (Scholtz, 2009; Laetz et al., 2013).

To shed more light on the biology of this interesting animal, a population of *T. gibbosa* housed by the holothurian species *O. planci* (Brandt, 1835) was investigated. A short summary of the

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☆ Dedicated to Prof. Peter Weygoldt on the occasion of his 85th birthday. Before he made his great contribution to our knowledge of chelicerate biology, Peter Weygoldt studied crustaceans under several aspects. His early work ranges from anecdotal faunistic and ecological observations to fundamentally important classic embryological investigations (e.g. Weygoldt, 1958).

*E-mail address:* gerhard.scholtz@rz.hu-berlin.de

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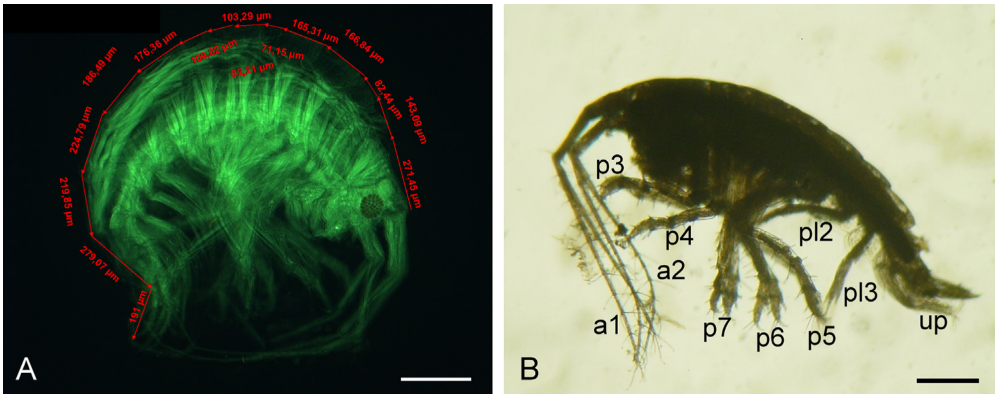

**Fig. 1.** Whole mounts of *T. gibbosa* specimens. A) A specimen showing auto-fluorescence. Lines and numbers indicate the method of length measurement. B) A specimen seen with back-light under a dissecting microscope showing the long setae of the two pairs of antennae (a1, a2), the setose pereopods (p3-p7), and the large second and third pleopods (pl2, pl3) (the first pleopod is covered by the last pereopod, likewise the gnathopods are not recognizable). Up: uropods. Scale bars 250 µm.

major SEM and histological results of this study has been previously presented (Scholtz, 2009). However, a more detailed account was lacking. In the meantime, Laetz et al. (2013) published a paper applying a partly similar approach. The results reported here confirm some aspects of Laetz et al. (2013). Furthermore, they add new information on the biology of *T. gibbosa*.

## 2. Materials and methods

### 2.1. Animals

Specimens of the holothurian *Ocnus planci* were collected with dredges and ground nets at various depths off the coast of Banyuls sur Mer/France on the occasion of several zoological students' excursions during early November in the 1970s to the 1980s. The holothurians and the epizoic amphipod specimens of *Tritaeta gibbosa* were preserved and stored in 5% formaldehyde.

### 2.2. Histology and measurements

Whole mounts of *T. gibbosa* individuals were used for species and sex determination and for body length measurements (Figs. 1 and 2). The amphipod specimens were carefully removed from *O. planci*, dehydrated with an ethanol series, mounted on microscopic slides, embedded with Roti®-Histokitt, and covered with a cover slip. In addition to bright field light microscopy and differential interference contrast, auto-fluorescence (Figs. 1 and 2) of the animals allowed careful and detailed examination of the features relevant for species and sex determination (filters with excitations at 365 nm, 450–490 nm, and 546 nm). The occurrence of a deep cleft on the anterior margin of the propodus of the first gnathopods of males served as the main criterion to discriminate sexes (Chevreux and Fage, 1925).

Body length was measured from the basis of the first antennae to the end of the telson following the curved back of the animals (Fig. 1). Measurements were carried out with the measurement tool implemented in the software AxioVision 4.8.

Histological sections were carried out from rectangular cuts of holothurian tissue circumscribing an invagination which contained an amphipod specimen. The whole probe was dehydrated via an ethanol series and transferred into a methacrylate embedding medium (Kulzers Technovit®). The blocks were serially semi-thin sectioned at a thickness of 3 µm (Microtome Zeiss HM 2165). The sections were transferred onto microscopic slides and stained with a mixture of methylene blue and Azur-II (1% methylene blue in 1% aqueous borax solution, 1% Azur-II in aqua dest. 1:1) for about 5 min. In order to counterstain the preparations, the slides were transferred to a basic fuchsine solution for about 30 s. The sections were analysed with a light microscope (Zeiss Axioskop 2 plus) and photographed with an AxioCam HRc using the software AxioVision 4.8.

### 2.3. SEM (scanning-electron-microscopy)

For SEM some isolated specimens of *T. gibbosa* or the excised tissue of the holothurian with the invagination hosting the amphipod were removed and dehydrated with a series of increasingly concentrated ethanol. The objects were then dried at the critical point (Balzers Union) and sputter coated with gold (Balzers Union). Observation and micrographs were carried out with a LEO 1450 VP scanning electron microscope.

### 2.4. Counts

*O. planci* specimens were checked for the number of invaginations housing *T. gibbosa*. These were counted irrespective of whether they were empty or contained an amphipod specimen. To quantify the colonisation of the regions of the holothurian body by amphipods three classes were created: 'anterior' covers the area surrounding the mouth until one third of the total length, 'median' comprises the middle third, and 'posterior' is the third of total length around the anus region. The orientation of the slit-like invaginations was determined with respect to the longitudinal axis of the holothurian. Two classes were established. All slits less that 45° to the longitudinal axis of the host were classified as longitudinal those that were more that 45° were classified as oblique to the longitudinal axis of the holothurian.

## 3. Results

### 3.1. Infested species

In the Mediterranean, *Tritaeta gibbosa* has been found to colonize a number of species from different major metazoan taxa, namely the sponge *Suberites domuncula* (Olivi, 1792), the holothurian *Ocnus planci*, and the colonial ascidian *Cystodytes dellechiajei* (Della Valle, 1877) (Fig. 2). The infested hosts show characteristic slit-like invaginations on their surface, although surface structure and texture of the sponge tissue, the ascidian tunic, and holothurian skin are very different. Yet, all host species share the feature of suspension feeding. However, the mechanism creating particle flow are very different between sponges, ascidians, and holothurians. In the following, the focus has been placed on the relationship between *T. gibbosa* and the holothurian *Ocnus planci*.

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