

## Occurrence of the amphipod *Leucothoe spinicarpa* (Abildgaard, 1789) (Amphipoda) in the ascidian *Phallusia nigra* (Urochordata, Ascidiacea) in Southeastern Brazil

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### Abstract

Solitary ascidians are stable microhabitats potentially favorable for feeding, shelter and reproduction of amphipods. The occurrence and size range of *Leucothoe spinicarpa*, the symbiotic species of the solitary ascidian *Phallusia nigra*, was evaluated at Praia da Enseada, Ubatuba, Northern coast of São Paulo State. The amphipods, collected from the ascidians pharyngeal basket or atrium, were measured and sexed, and the ascidians were weighed. Juveniles, males and females were separated by differences in the excavations on the palm margin of gnathopods 2. The number of amphipods in each ascidian varied and there were few adults in opposition to a high number of juveniles. Males and females did not show difference in body size, but sexual dimorphism based on excavation of gnathopod and dactylus proportion was presented. Also, the ascidian weight was related with the number of associated adult amphipods but not with the juveniles. The high number and size range distribution of juveniles, the low number of ovigerous females, and even the presence of single adult in the ascidians, suggest the possibility of extended parental care.

Key words: symbiosis, abundance, Leucothoidae, sexual dimorphism.

### Introduction

Associations of different degrees of complexity established by crustaceans with hosts have been related to the need for shelter, foraging and breeding. The black solitary ascidian, *Phallusia nigra* (Savigny, 1816) is very abundant in the rocky shores of the coast of São Paulo State (Rodrigues *et al.*, 1998) and may provide potentially favorable and stable microhabitats for both breeding individuals and recruiting juveniles of amphipods.

The gammarid amphipod *Leucothoe spinicarpa* (Abildgaard, 1789), whose genus was recently revised by Serejo (1998), has been described as an endobiont of some species of ascidians and sponges (Ortiz, 1975; Vader, 1984a,b; Costello & Myers, 1987; Thiel, 1999, 2000). This interaction

provides not only food, but refuge for the juvenile amphipods, that can grow nearby the adults of the same species (Thiel, 1999, 2003). Furthermore, within this microhabitat, parental care may occur in amphipods both in embryonic and post embryonic stages (Dick *et al.*, 2002; Thiel 1997a,b, 1999). Once the adults are dead, juveniles may even 'inherit' the ascidian in which they were born (Thiel, 1999).

The aim of this study was to observe the occurrence, abundance and variation in size of the commensal gammarid amphipod *Leucothoe spinicarpa*, in all stages of its life cycle. Also, it intends to relate these observations to the biomass of the host: the solitary ascidian *Phallusia nigra*. A further goal is to contribute to the identification of both adult and young gammarids of this species.

## Material and Methods

Twenty-four individuals of *Phallusia nigra* were collected on the shallow infralittoral waters of Praia da Enseada (23°30'S; 45°00'W), Ubatuba, in the Northern coast of São Paulo State, Brazil, between November 1996 and January 1997. Every ascidian was carefully dissected so that its atrium and pharyngeal basket could be examined for the presence of amphipods. Each specimen was laid on absorbing paper for one hour before the total wet weight (including its pharyngeal basket) was obtained. The amphipods' head-to-telson length was measured using a micrometric ocular. Males were identified by the presence of prominent dentitions on the palms of gnathopods 2, which are less evident or absent in females and juveniles, respectively. Breeding females were identified by presence of oostegites and the ovigerous females by the presence of eggs in the brood pouch. The number of eggs in the brood pouch was recorded. The juveniles measured up to 4 mm in length and were sexually undifferentiated (Thiel, 1999). Biomass of ascidian and number of amphipods present in it were correlated using linear regression. Head-to-telson length was analyzed by Kruskal-Wallis test and the *post hoc* multiple comparison Dunn method (Zar, 1996).

## Results

The biomass (wet weight) of specimens of the ascidian *Phallusia nigra* varied greatly ( $\bar{x} = 4.27 \text{ g} \pm 2.18$ , range = 1.32-9.57,  $n = 24$ ), as well as the number of gammarids inside the ascidians ( $\bar{x} = 5.00 \pm 6.905$ , range = 0-28,  $n = 122$ ). Only five of the 24 ascidians did not present endobiont leucothoids. In the remaining 19 ascidians, 122 individuals of *Leucothoe spinicarpa* were found, most of them ( $n = 83$ ) were juveniles. There were

also 13 males and 26 females from which only four were bearing eggs (Table I). The sex-ratio in the population was females biased (1♂:2♀).

The majority of *Leucothoe spinicarpa* specimens were found in the pharyngeal basket and a small number of them were lodged in the atrium. The number of juveniles and adults ranged from 0 to 28 and from 0 to 7, respectively. Males were found in 58% of the ascidians, while females in 79% of them. Males cohabitated with females in 38% of the hosts and in 8% with other males; the rest was inhabited only by females. The juveniles were in 17 ascidians and in three of those they were found with no adults. Four of the egg-bearing females presented 11 eggs each and were found in different ascidians.

Head-to-telson length varied in each gammarid group separated by sex and maturity (Table 1 and Figure 1). There was no significant difference between males and females' length, while juveniles were significant smaller than adults ( $H = 78.879$ ,  $df = 2$ ,  $p < 0.001$ ). Linear regression between ascidian biomass and number of adult *L. spinicarpa* present inside the ascidian showed significant positive relation, although not strong ( $R^2 = 0.31$ ). No relation was observed for the juveniles (Figure 2).

Sexual dimorphism in *Leucothoe spinicarpa* is not very evident (Figures 3-6). The dentitions on the palm margin of propod and gnathopod 2 are deeper and more evident in males (Figure 4), whereas they are superficial in females, hence less evident (Figure 5). The female gnathopod is more delicate than the male's (Figure 5). The surface of juveniles palm margin is almost completely even and smooth (Figure 6). Oostegites in pre-ovigerous or young females, eggs in marsupium in egg-bearing females and genital papillum and comparatively robust gnathopod 2 in males (Figures 4 and 5) were used to identify the sex of individuals of *L. spinicarpa*.

**Table I.** Number and head-to-telson length range for the amphipod *Leucothoe spinicarpa* maturity classes.

Maturity Classes	n	Head-to-telson ( $\bar{x} \pm \text{SD mm}$ )	Range
Juveniles	83	2.04 ± 0.71	1.00-4.12
Males	13	7.12 ± 0.92	5.06-8.56
All females	26	6.82 ± 0.96	4.96-9.04
Females with oostegites	5	6.03 ± 1.51	4.56-7.96
Ovigerous females	4	6.55 ± 1.76	6.32-8.88
Total	122	3.55 ± 2.41	1.00-9.04

## Discussion

The presence of *Leucothoe spinicarpa* in all life stages suggests that (a) the crustaceans spend their entire life cycle inside the host (Thiel, 1999, 2000); (b) adults can breed more than once while lodged there, which is evident from the array of lengths of the females bearing either oostegites or eggs. Juveniles presenting great size variation may

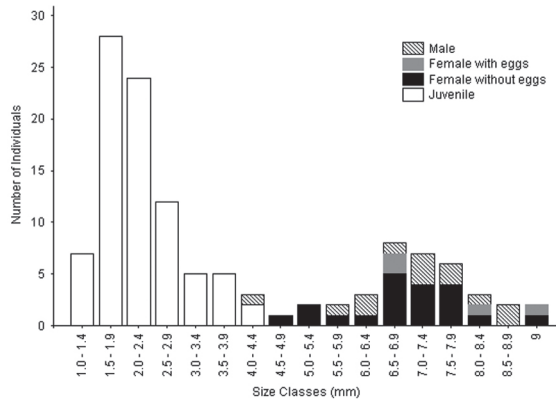


Figure 1. Relative abundance (%) of juveniles, females, females with oostegites, egg-bearing females and males of *Leucothoe spinicarpa* size classes (total length).

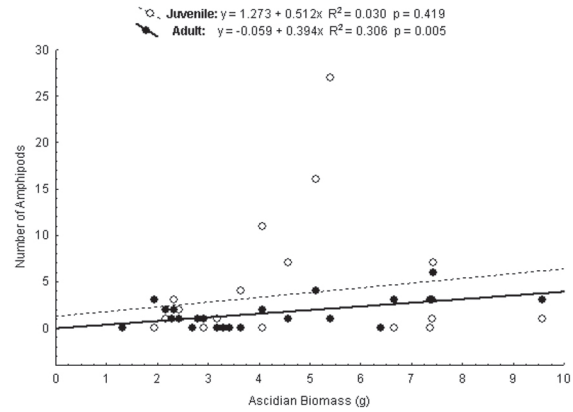


Figure 2. Relation between biomass (g) of the ascidian *Phallusia nigra* and the number of guest juveniles (white circles) and adults (black circles) amphipods *Leucothoe spinicarpa*.

indicate extended parental care, which enhances survival and growth rates (Thiel, 2003) as nursed younglings are bestowed with protection both from physical stress and potential predators (Thiel, 1997a, b; 1999). More evidence of parental care

can be found in the presence of both egg-bearing females and juveniles inside of ascidians and in the greater number of juveniles compared to eggs. Also, the great size variation of juveniles may indicate continuous reproduction as observed in *Leucothoe*

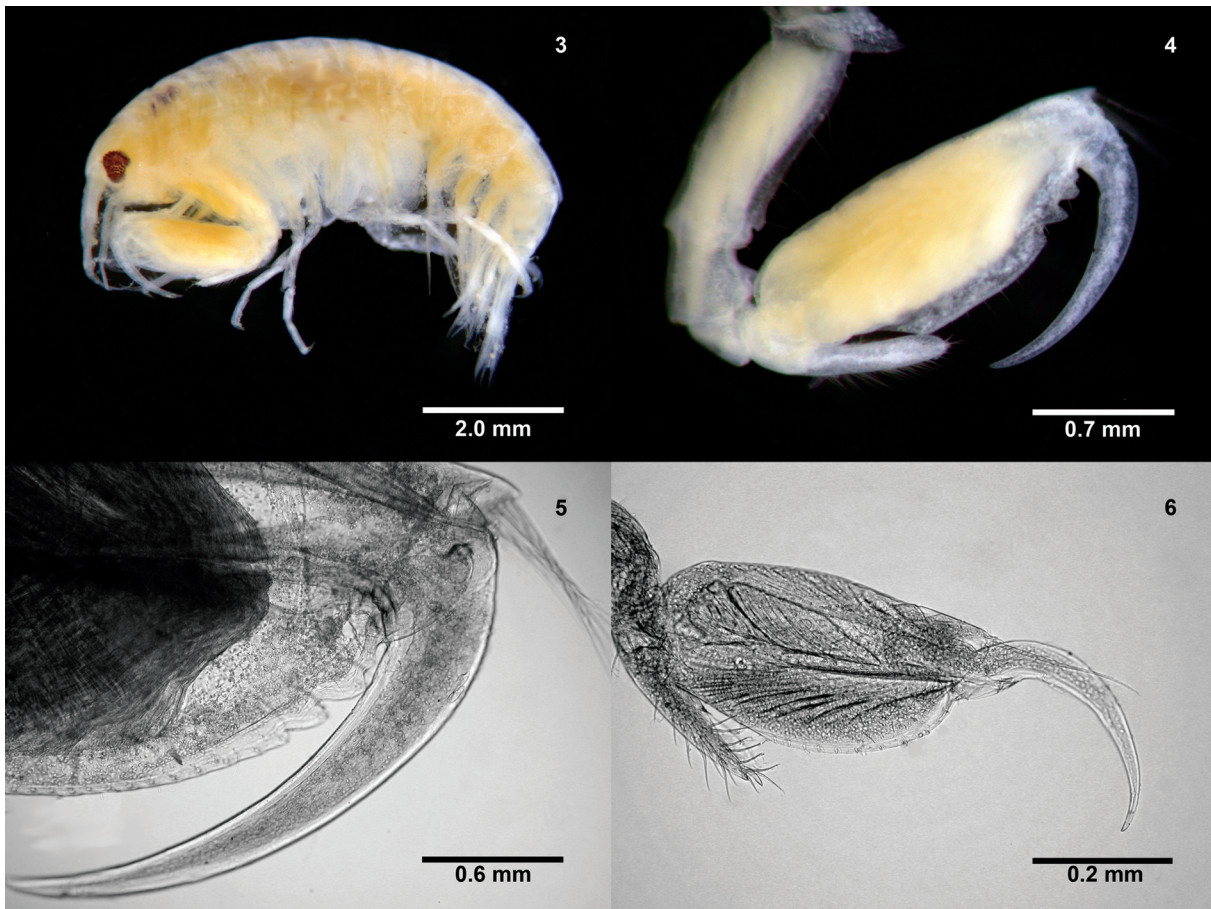


Figure 3-6. *Leucothoe spinicarpa*: (3) adult male; (4) male gnathopod 2; (5) close view of the palm margin on female gnathopod 2; (6) close view of the palm margin on gnathopod 2 of juvenile.

sp. from the ascidian *P. nigra* of the São Sebastião canal. A recent study indicated the occurrence of 6 cohorts throughout the year and that the most part of the population was formed by juveniles (Siqueira, 2007). The amphipods of tropical and subtropical regions were generally characterized by multivoltinisms, continuous reproduction, small body size, rapid maturity, and many broods with few, relatively small embryos; converse sets of traits characterized high latitude species (Saint-Marie, 1991). Multivoltinism and continuous reproduction has been reported in other species in tropical areas such as *Pseudorchestia brasiliensis* in Rio de Janeiro, Brazil (Cardoso & Veloso, 2001).

Sex ratio skewed towards adult females has been detected for amphipods (Moore, 1981; Borowsky, 1983; Thiel, 1997c; Appadoo & Myers, 2004) and it has been related to a number of factors. These factors include food availability, parasitic relations, sexual reversion, longevity between the sexes, body size (Wenner, 1972), local concentration of females due to male migration or predation. The sexually biased predation was observed for *Corophium volutator* and *Corophium arenarium* (Fish & Mills, 1979), and *Leptocheirus pinguis* (Thiel, 1997c), when they emerge from sediment in search of female burrows. Possibly, males of *L. spinicarpa* were predated when they left the host for reproduction. Also, the males may be leaving the ascidian to live in the nearby macroalgae (Leite *et al.*, 2002) or sponges (Pavani & Leite, 2007). Gammarids probably lodge themselves in ascidians because of the stable microhabitat inside. Furthermore, the latter are long-lived, offer reasonable inner space and intense alimentary current (Thiel, 1999).

However, the food and shelter provided by the ascidians may be limited resources for the gammarids. Smaller ascidians seem to present less adult amphipods living inside than the bigger ones, suggesting that *Phallusia nigra* biomass might influence its potential to become a host for adult *L. spinicarpa*. On the other hand, Thiel (1999) did not observe this correlation and suggested that the selection of hosts might be random. Other biotic interactions, such as competition and predation, as already suggested for gammarids associated with sponges (Ribeiro *et al.*, 2003), or parasitism (Ortiz & Lalana, 1992) may also interfere in the number of hosted adults. There are strong indications that the presence of the parasite suppresses oviposition and development of secondary sexual characters in female gammarid hosts (Vader & Berge 2004).

In general, there are few studies that provide information about the gammaridean population biology, especially those associated with other invertebrates. Therefore, we suggest that further studies deal with long-term observations and focus on the development of more accurate morphological criteria for sexual identification, in order to enable better understanding of this animal's life cycle.

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