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Influence of the endo-symbiont *Leucothoe wuriti* (Thomas & Klebba, 2007) (Crustacea, Leucothoidae) on the biomass of *Phallusia nigra* (Savigny, 1816) (Tunicata, Ascididae), in the northeastern coast of the São Paulo State, Brazil

Influência do endobionte Leucothoe wuriti *(Thomas & Klebba, 2007) (Crustacea, Leucothoidae) sobre a biomassa de* Phallusia nigra *(Savigny, 1816) (Tunicata, Ascididae) no litoral norte do Estado de São Paulo, Brazil*

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Abstract
The ascidian *Phallusia nigra* shows a large geographical distribution on the tropical belt over the world. It is a solitary benthic filter-feeder species, black color and so commonly founded attached on the natural and artificial reefs. It is sessile nature became this animal a suitable substratum to the development of symbiotic organisms as the amphipod *L. wuriti*. However, this relationship could bring some disadvantages to the tunicate, once the presence of the amphipod into the ascidian may block its food intake. The main purpose of this investigation is evaluating the effects of the presence of the *L. wuriti* on the biomass of the ascidian *P. nigra* in the southeastern Brazilian coast. Thirty ascidian were caught fouling on the wharf columns at Itaguá beach,Ubatuba municipality, in the northeastern coast of São Paulo State (23°27'07.S 45°02'49.W). A total of 142 individuals of *L. wuriti* were obtained from the atrium and oral siphon of *P. nigra*, within average mean number of 4.96 ± 2.96 amphipods by tunicate. A positive association between ascidian biomass and amphipod biomass was verified, suggesting that the presence of amphipods inside ascidians seems cause no problems concerning to the food intake and growth for these tunicates.
Keywords: interspecific association, Ubatuba -SP, filter-feeding

Resumo
Com ampla distribuição geográfica, *Phallusia nigra* é uma ascídia solitária de coloração enegrecida e facilmente encontrada submersa e incrustada em portos e costões rochosos. Devido ao seu hábito bentônico e filtrador, essa ascídia pode servir como substrato favorável para o desenvolvimento de organismos simbiontes, como o crustáceo *Leucothoe wuriti*. Entretanto, o fato desses organismos habitarem seu interior, obstruindo a passagem ou alimentando-se das partículas pode representar uma desvantagem, prejudicando o aproveitamento de alimento pelo tunicado. O principal objetivo desse estudo foi avaliar os efeitos da presença de *L. wuriti* sobre a biomassa da ascídia *P. nigra* na costa sudeste do Brasil. Foram coletadas 30 ascídias incrustadas no cais da Sardinha da praia Itaguá, no município de Ubatuba, costa sudeste do estado de São Paulo (23°27'07.S 45°02'49.W). Um total de 142 anfípodes *L. wuriti* foram encontrados habitando o saco branquial e o sifão inalante de *P. nigra*. O número médio de crustáceos associados foi de 4,96 ± 2,96 indivíduos por ascídia. Foi verificada uma associação positiva entre a biomassa das ascídias e a biomassa dos anfípodes sugerindo que a presença desses anfípodes no interior das ascídias parece não causar danos no aproveitamento do alimento ou crescimento desses tunicados.
Palavras-chave: Associação interespecífica, Ubatuba-SP, filtradores

INTRODUCTION

Crustacean amphipods represent a large animal group that exhibits a strong adaptive success, exploiting efficiently a variety of habitats. In the marine environment, they are found living in the benthos, associated to the sandy and muddy bottoms, swimming through the algae and even as symbiotic of sessile invertebrates (Leite, 2011).

In the family Leucothoidae there are common gammaridean amphipods that maintain symbiotic relationship with benthic organisms as algae, sponges, cnidarians and ascidians (Thomas, 1979; Costello & Myers, 1987; Thiel 1999; Poore et al., 2000; Buhl-Morthensen & Morthensen, 2004, Thomas & Klebba 2007; White & Thomas 2009). This family consists in more than 130 species distributed into six genera *Anamixis, Nepanamixis, Paranamixis, Leucothoe, Leucothoella* and *Paraleucothoe* (White & Thomas, 2009; White, 2011).

Species of the *Leucothoe* genus are described as symbiotic of some sponge and ascidians, highlighting *Leucothoe spinicarpa* (Abildgaard, 1789), that livies into the sponges *Spheciospongia vesparia* (Westinga & Hoetjes, 1981), *Jophon spatulatus* and *Microxina simplex* (Lörz, 2001), besides the ascidians *Ecteinascidia turbinata, Styela plicata, Microcosmos exasperatus, Clavelina oblonga* (Thiel, 1999), and *Ecteinascidia thurstoni* (Chavanich et al., 2007), among other symbiotic relationships involving species of this genus (Thomas & Klebba, 2007).

Symbiotic *Leucothoe* is also found in Brazilian coast, as reported by Cantor et al. (2009) for *L. spinicarpa* on the northeastern coast of São Paulo State, where it was found a large number of juveniles and a small amount of ovigerous females of this species on the *P. nigra*, besides an expected positive relationship between size and fecundity, and the continuous breeding period for these amphipods.

Actually, the taxonomic status of *L. spinicarpa* is being questioned. The Taxonomists suspect of the cosmopolitan distribution of this animal, suggesting that a pool of species was incorrectly identified as *L. spinicarpa* (Crowe, 2006; White, 2011), what probably have occurred with *Leucothoe wuriti* (Thomas & Klebba, 2007).

Previous accounts suggest that the symbiotic relationships between *Leucothoe spinicarpa* (=*Leucothoe wuriti*, White, 2011) and ascidians are based on shelter from predation, reproduction and feeding sites, provided by the tunicate for the amphipod that spend its entire life into the host, and even left the tunicates for the juveniles after the adult's death (Thiel, 1999).

The tunicate *Phallusia nigra* (Savigny, 1816) is a black solitary ascidian, with large geographic distribution over the world, being found in Brazilian coast from Ceará to São Paulo states. It is, mentioned as an alien species to the occidental Atlantic Ocean (Rocha et al., 1999; Fofonoff et al., 2014), commonly found attached on rocky shores and submerse buildings, as harbors and wharf columns along the Brazilian coast (Cantor et al., 2009; Dias & Duarte, 2011; Rocha et al., 2012).

The sessile nature of the *P. nigra* makes this animal a suitable substratum to the development of endo-symbiotic organisms as it is verified for the amphipod *L. wuriti*. However, this relationship could bring some disadvantages to the tunicate, once the presence of the amphipod into the ascidian may reduce the amount of food available for the ascidian. Thus, the main purpose of this investigation is to evaluate the effects of the presence of L. *wuriti* on the biomass of the ascidian *P. nigra* in the southeastern Brazilian coast.

MATERIAL & METHODS

Ascidians (fig.1A) were caught manually on the wharf columns, at depths between 3.0 and 5.0 m, at Itaguá beach, Ubatuba municipality, in the northeastern coast of São Paulo State (23°27'07.S 45°02'49.W) (fig. 2), during daylight snorkeling sessions, in March, 2013.

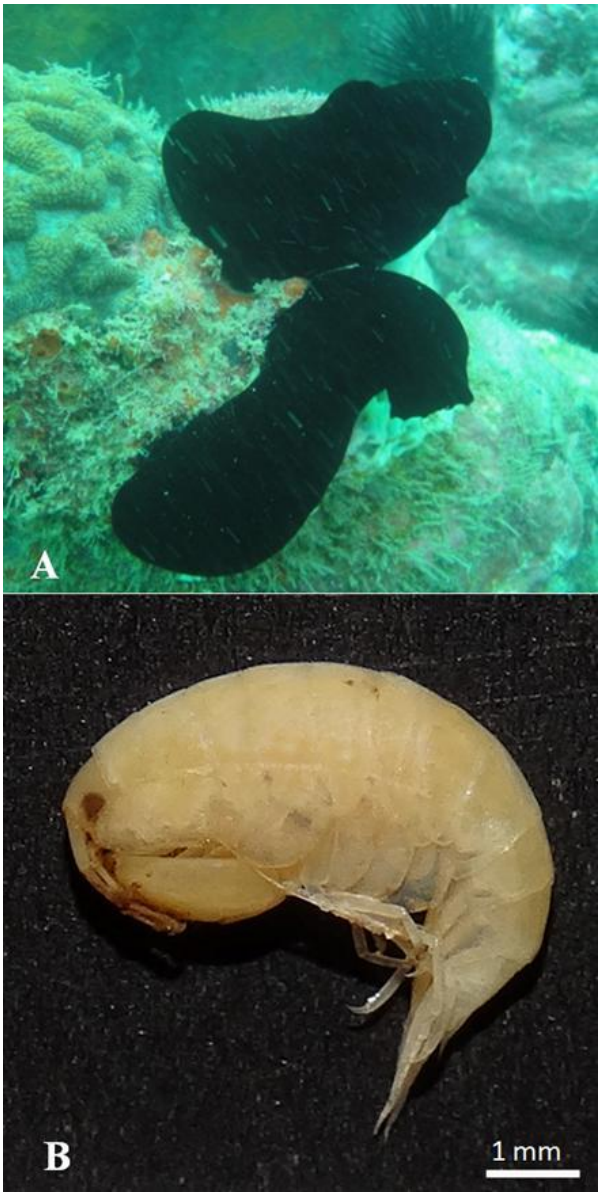

Figure 1. A. *Phallusia nigra* (credits; Cobo, V.J.). B. Adult male of *Leucothoe wuriti* (credits: Rosa, A.H.B.).

The sampled ascidians were dissected and the amphipods removed, counted and preserved in 70% ethylic alcohol and stored before weight measurements. The ascidian internal organs were removed and dehydrated at 40°C by 24h, and weighted. *Leucothoe wuriti* (figure 1B) recognition was made according to the remarks pointed in Thomas & Klebba (2007).

Figure 2. Studied area, indicating the sample site, Ubatuba, northeastern coast of São Paulo State.

Associations between ascidian and amphipod biomass, as well as the relationship between ascidian biomass and number of amphipods, were assessed by linear regressions ($p < 0.05$) (Zar, 1999).

RESULTS

Thirty individuals of *P. nigra* were collected and 142 individuals of *L. wuriti* (4.79 ± 2.98 amphipods *per* ascidian) were removed from the ascidians pharyngeal basket or atrium. From the amphipods obtained all the demographic categories were present. Adults

and juveniles of both sexes, and some eggs were found. Among the 30 sampled ascidians only one had no amphipods inside the atrium or the oral siphon.

No significant association was verified between the ascidian biomass and number of amphipods, although there was a tendency of the heavy ascidians hosting more amphipods (figs. 3 and 4, respectively).

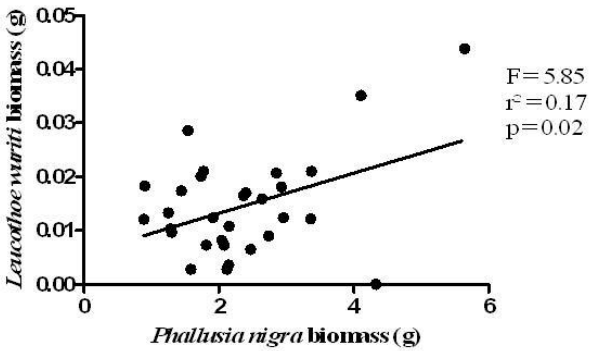

Figure 3. Association between ascidian and amphipod biomass.

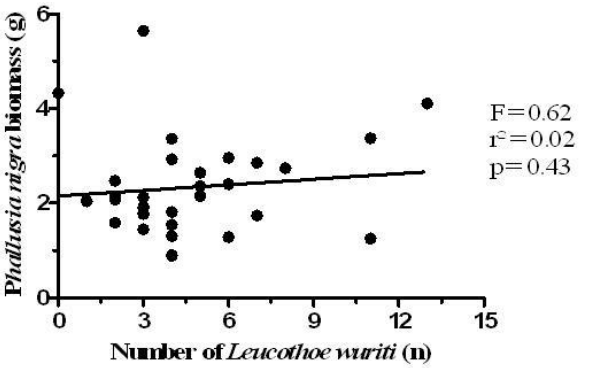

Figure 4. Relationship between number of amphipods and ascidian biomass.

DISCUSSION

Previous accounts suggest that there is no relationship between the number of symbiotic individuals and the biomass of the ascidian *P. nigra* (Thiel, 1999), as verified in the present study, pointing out that the tendency of heavier ascidians hosting a higher number of amphipods is not obligatory. However, this tendency was verified for *L. spinicarpa* (=*Leucothoe wuriti*, White, 2011) and *P. nigra*, as reported by Cantor et al. (2009) in some sites of the coast of São Paulo State, although the association is not so strong (r^2= 0.31).

According to Chavanich et al. (2007), some factors such as host abundance or the symbiotic species richness may cause variations on the density of *Leucothoe* spp. in its hosts, which could explain the contrasting results obtained from the study of Cantor et al. (2009).

Thus, this tendency in direction of a positive association between ascidian and amphipod biomass suggests that the symbiotic relationship seems not to be prejudicial to the host concerning specially to food intake. Some accounts concerning the association between the amphipods of the *Leucothoe* genus and sponges pointed out that this relationship cause no damages to the hosts, as reported for *Leucothoe luquei* and the sponge *Calyx podatypa* (Ortiz & Winfield, 2012), and between *Leucothoe* sp. and the sponges *Phorbas* sp., *Iotrochopsamma arbuscula*, *Holopsamma laminaefavosa* and *Halichondria* sp. (Poore et al., 2000), suggesting that these relationships must represent commensalism interactions, which seems to be the same situation verified in the present investigation.

However, the establishment of a positive association between *P. nigra* and *L. wuriti*, may also indicate a mutualism interaction, if these amphipods are acting as cleaners, and not only as consumers of the food obtained by the tunicates. According to Côte (2000), many cleaner associations are found between marine animals and Dvoretsky & Britayev (2009) suggested a relationship of cleaning symbiosis between the amphipod *Ischyrocerus commensalis* and the red king crab *Paralithodes camtschaticus*. This amphipods are found living on exoskeleton, gills, mouth appendages and egg masses under abdomen of female of the red king crab and may feed on dead tissues, pathogenic bacteria and fungi on sites of damaged carapace of this crabs. In this sense, it is possible that *L. wuriti* feeds on the particles not used as food for *P. nigra*, removing these

materials from the siphons, preventing the obstruction of these ascidians channels.

Despite these assumptions, more detailed investigations are required in order to identify precisely what kind of symbiotic interaction is in fact established between the amphipod *L. wuriti* and the ascidian *P. nigra*, on the northeastern coast of São Paulo State, as a model for the entire distribution range of these animals.

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