COMMENSAL AMPHIPODS IN ASCIDIANS AND SPONGES OFF PHUKET ISLAND

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INTRODUCTION

Amphipods are often associated with invertebrates from which the host gains little or no advantage. Such an association may be epifaunal or endofaunal. In the latter instance the amphipod can be a parasite or commensal. Associates living within host organisms find a stable microhabitat that provides shelter from predation, and in some cases, nutrition (Thiel, 1998). Amphipod families regarded mainly as endofaunal associates are Leucothoidae, Anamixidae, Colomastigidae and Liljeborgiidae (Richmond, 1997). Of these four families only Liljeborgiidae was not found in this study.

Most of what is known about amphipod taxonomy, ecology and community structure is strongly biased toward temperate areas. The high diversity and small size of tropical amphipods, combined with difficulties in obtaining representative collections from reef sites, has hindered a taxonomic and systematic examination of tropical waters. Tropical waters though, offer promising research avenues with coral reefs representing a complex mosaic of macro- and microhabitats like live coral, coral rubble, turf algae, sessile invertebrates and reef derived sediments (Thomas, 1997).

The Andaman Sea, Thailand, was the object of our examination on cryptic and commensal amphipods. There have only been a few investigations of commensal amphipods in this area (Jansen and Dinesen, 2002) but none has considered the amphipod/host relationship.

MATERIALS AND METHODS

The project was carried out from February through April 2001 in the area around Phuket Island. Samples were collected by the use of SCUBA techniques, which has proved to be the only suitable method for collecting amphipods in their hosts (Thomas, 1997). Sixteen dives were made at the four different sampling stations (Fig. 1) at depths ranging between 0 and 25 meters: PMBC pier (7 dives), Koh Dok Mai (4 dives), Koh Hi (Coral Island) (4 dives) and Shark Point (1 dive). In total, more than 70 samples were...
collected from these stations. Furthermore, at the PMBC pier many samples were collected at low tide when the reef was exposed and samples could be collected easily.

The ascidian and sponge species were photographed for later identification and tissue samples were retained. The ascidians were anaesthetized with menthol to avoid contraction. Then the host was cut open, and the amphipods removed with a pair of fine forceps. The host and the amphipods were labelled and fixed in a 5% formalin borax-neutralised seawater solution, and after one week transferred to 70% ethanol. All samples were stored in the PMBC reference collection. A total of eight species of ascidians were collected during this study. They were identified using the keys of Monniot et al. (1991). In the case of *Polycarpa* sp. (Pl. 1 E) the length, width, weight, and displacement of water were measured. A total of 17 species of sponges were found of which four contained endobiont amphipods. The four species *Haliclona* sp. A., *Amphimedon* sp., *Xestospongia* sp. and *Haliclona* cf. *pigmentifera* (Pl. 1A, B, C and D), were identified to genus by Associate Professor Ole S. Tendal, Zoological Museum, Copenhagen, Denmark. All amphipods were examined under a dissecting microscope, sorted into host groups, and identified to family using keys in Barnard (1969) and Barnard and Karman (1991). Only the endobiont amphipods of the families Leuchotoidae, Anamixidae and Colomastigidae were identified to species.

**SYSTEMATIC ACCOUNT**

**Family Colomastigidae**

*Colomastix lunalilo* Barnard, 1970

**Material examined:** PMBC reef flat, Phuket, Thailand (Fig. 1); 34 adult and 3 immature specimens: The adults consisted of 25 females and 9 males. Size: smallest 2.6 mm and largest 5.1 mm. Immatures all 1 mm. Shark Point, Phuket, Thailand; 5 specimens, all males; Size: between 1.9 and 2.5 mm. Koh Dok Mai, Phuket, Thailand; 1 specimen, immature.

**Identification:** *Colomastix lunalilo* was identified using keys in Barnard (1970, 1971), Ledoyer (1978, 1979) and Hirayama (1990). It was first described by Barnard in 1970 and re-described by Hirayama (1990) based on mature specimens.

**Remarks:** Hirayama (1990) listed seven morphological variants which were not given by Barnard (1970) and are interpreted as variations due to growth. Our material differs both from that of Barnard (1970) and that of Hirayama (1990). It matches with the description by Hirayama in that...
Plate 1. Hosts; A: Haliclon a sp. A; B: Amphimedon sp.; C: Xestospongia sp.; D: Haliclon a cf. pigmentifera; E: Polycarpa sp.
the outer rami of uropod 3 are nearly equal to half the length of the inner rami (Fig. 2). Hirayama mentioned that the length of propodus and carpus differed between the sexes. In our material the propodus and carpus are sub equal and there were no differences between the sexes. Coxae 1–2 had nipple-like anterior points, telson lacking penicillate setae midmarginally and the palm of the male gnathopod 2 defined by two large excavations where the dactyl is slightly geniculate near the apex, which is all similar to Barnard’s description (1970).

Distribution: Colomastix lunalilo has been recorded from Hawaii (Barnard, 1971), Mauritius (Ledoyer, 1978), Madagascar (Ledoyer, 1979), Fiji, Red Sea (Lyons and Myers, 1990) and New Caledonia (Hirayama, 1990).

Habitat: This species was found in two different sponge species at three different locations. At the PMBC pier (Fig. 1) it was found in Haliclona sp. A. (Pl. 1A). Several specimens of this sponge were located in shallow water on the reef top, which was exposed at low tide. Two of the sponges contained C. lunalilo and no other amphipods. One sponge contained 12 mature females and 4 mature males. The other contained 12 mature females, 5 mature males and 3 immature specimens. All mature females carried eggs or had well developed brooding pouches. Four samples did not contain any amphipods. The sponge was rod-shaped and hollow with one oscular opening. The amphipods were located in the bottom of the hollow cylinder inside the sponge.

At Shark Point (Fig. 1) a single specimen of the sponge Amphimedon sp. (Pl. 1B) contained several specimens of C. lunalilo, all mature males and no other amphipods. This sample was taken from 15 m depth. The sponge had several large holes on the surface, in which the amphipods were located. It was uncertain if these holes were oscula or whether they were made by amphipods.

Another specimen of Amphimedon sp. was found at 12 m depth at Koh Dok Mai (Fig. 1) and contained one immature C. lunalilo.

Family Anamixidae

Nepanamixis sp.

Material examined: Koh Dok Mai, Phuket, Thailand. 99 specimens; 13 anamorphs, length 2–3 mm and 86 leucomorphs, length 1–2 mm.

Anamorph specimens: Eyes composed of 13 ommatidia; antenna 1 longer than antenna 2; coxa 1 triangular; gnathopod 1 carpus basally inflated with 1 distinct seta; setae on carpus of gnathopod 2 in distinct rows; propodus of gnathopod 2, 3 times longer than wide (Fig. 3 B).

Figure 3. Nepanamixis sp.; A: adult leucomorph; B: anamorph, both from Koh Dok Mai. Scale bars = 1 mm.
Identification: Anamorph specimens were identified to *Nepanamixis* sp. after Thomas (1997) because article 5 on gnathopod 1 was basally inflated (Fig. 4B). However, they can be distinguished from all the *Nepanamixis* species described in Thomas (1997) by the following morphological characters: 1) length of the second gnathopod is 3 times its width (Fig. 4A); 2) triangular process on coxa 1; 3) the shape of carpus and propodus on gnathopod 1 does not resemble any of the other anamixid species (Fig. 4B).

The specimens resemble a *Nepanamixis* sp. found in 1998 in Phuket, Thailand (Jansen and Dinesen, 2002), that has not been given a name to date. We have not found any morphological differences in our specimens, and considered them, therefore, to be identical.

Leucomorph specimens (Figs. 3A; 4C, D) were identified to family by using the key in Barnard and Karman (1991).

 Remarks: The *Nepanamixis* species found in this study varies in one point from all other members of the genus. Its eyes are composed of 13 ommatidia instead of 9. This character is normally used in separating the genera *Anamixis* and *Paranamixis* but is invalid in this case (Thomas, 1997).

Since it is impossible to identify the leucomorphs to species and also different species of anamixids can be found in the same host at the same time, it is possible that the anamorph (Fig. 3B) and the leucomorph (Fig. 3A) specimens examined belong to different species. This can only be resolved by molecular biological methods or by observing a moult in the laboratory.

Distribution: This species has only been recorded from Phuket at two locations, Coral Island (Fig. 1) (1 anamorph, 25 leucomorphs) (by Jansen and Dinesen, 2002), and Koh Dok Mai (Fig. 1) (this study). These two locations are only 5 to 10 km apart.

Habitat: *Nepanamixis* sp. was taken from five samples of the sponge *Xestospongia* sp. Some of the anamixids were located in the numerous small oscula, with a diameter of only 1–2 mm, scattered over the surface of the sponge. However, most of them were found swimming or crawling on the...
surface of the sponge. There was much variation in the ratio of anamorphs to leucomorphs for each sponge sample (Fig. 5). Examination of the samples in the laboratory showed that the specimens located in the oscula would leave their host quickly when disturbed. All the anamixid specimens found had the same red colour as the sponge from which they were taken.

Family Leucothoidae

*Leucothoe furina* (Savigny, 1816)

*Leucothoe hornelli* (Walker, 1904)

*Leucothoe furina* (Stebbing, 1906; Barnard, 1932; 1937)

**Material examined:** PMBC pier (Fig. 1); 85 specimens found in *Polycarpa* sp. (Pl. 1E), largest 6.5 mm and smallest 1.5 mm; 24 specimens found in samples of different sponges and ascidians.

**Identification:** The identification is based on Walker (1904, p. 258, pl. 3, fig. 17), Schellenberg (1928, p. 635–637), Imbach (1967a, p. 79, pl. 21), Nayar (1967) and Sivaprakasam (1967, p. 387–388, fig. 2). We were not able to distinguish between the sexes.

**Distribution:** Red Sea, Suez, South Arabian Coast, Ceylon, Maldives, East Indies, Australia, Gambier Archipelago (Imbach, 1967a,b), India (Sivaprakasam, 1967), and Thailand (Bussarawit, 1984).

**Habitat:** *Leucothoe furina* was endobiont in one species of host, an ascidian of the genus *Polycarpa*. Furthermore it was found on several different hosts as an epifaunal amphipod and on ropes covered with invertebrates. These specimens were, however, not as large as the adults found in *Polycarpa* sp. None was found in mud/bottom samples taken from near the PMBC pier. However, a small *L. furina* individual was found among coral rubble near the PMBC pier. *Leucothoe furina* were found at several locations as an epifaunal amphipod: PMBC pier, Coral Island (southern bay, western side), Koh Dok Mai and the Pearl farm (Fig. 1).

Position of *L. furina* in *Polycarpa* sp.: When the ascidian was dissected, *L. furina* was mostly found in the branchial chamber and sometimes in the inhalant opening. In actively filtering *Polycarpa* sp., *L. furina* was sitting 5–10 mm inside the inhalant opening with their heads orientated towards the exterior. The amphipods remained motionless in that position and apparently did not change position when the ascidian closed up temporarily. There could be up to 10 amphipods in the inhalant opening.

Amphipod-host relationship: We never found other amphipod species in *Polycarpa* sp., no matter whether *L. furina* was present or not. Small juveniles were never found alone in *Polycarpa* sp. Mostly we observed cohorts of small juveniles of the same size together with a few adults. Eighty percent of the *Polycarpa* sp. collected contained *L. furina*. The mean number of amphipods per ascidian was 5.67 ± 6.89 s.e., n = 15. No significant linear relationship was found between the number of amphipods inside the host and host size (measured as displacement of water) \( r^2 = 0.039 \), \( p > 0.05 \), n = 15) indicating that all ascidians examined seemed equally suitable to be colonized by *L. furina* regardless of size.
Commensal amphipods in ascidians and sponges

**Leucothoe tridens** Stebbing, 1888

Stebbing (1906), Schellenberg (1938), Barnard (1970)

**Material examined:** PMBC pier; 15 specimens found in samples of the sponge *Haliclona cf. pigmentifera* (Pl. 1D), Size: 1.5 mm, furthermore 3 were found in another sponge species and 1 in an ascidian. Koh Dok Mai; 10 specimens found in an ascidian and all amphipods collected were of similar size.

**Identification:** The identification was based on Stebbing (1888, p. 777, Taf. 47; 1906, p. 166), Schellenberg (1938, p. 21, fig. 11), Barnard (1965, p. 492; 1970, p. 211, fig. 137; 1971, p. 102–103; 1972, p. 81). We were not able to distinguish between the sexes.

**Distribution:** New Zealand (Stebbing, 1888), Philippines (Schellenberg, 1938), Micronesia (Barnard, 1965).

**Habitat:** *Leucothoe tridens* was found in three sponge species and two ascidians. It seems that *L. tridens* was only found as an endobiont amphipod in the sponge *Haliclona cf. pigmentifera* at PMBC pier. The amphipods were sitting inside the channels of the sponge.

**DISCUSSION**

**Colomastix lunalilo**

*Colomastix lunalilo* is reported from the Andaman Sea, Thailand, for the first time and was found in two sponges, *Haliclona* sp. A (Pl. 1A) and *Amphimedon* sp. (Pl. 1B). These sponges are also recorded as host species for *C. lunalilo* for the first time. Other hosts for *C. lunalilo* include the sponge *Siphonochalina* sp. (Hirayama, 1990) and the algae *Sargassum* sp. (LeCroy, 1995). Thus *C. lunalilo* is a generalist tolerating a range of conditions. While Shark Point and Koh Dok Mai were typical tropical coral reef sites, with clear water and low nutrient concentration, PMBC pier had turbid water with a high concentration of plankton and dissolved organic material. 

*Colomastix lunalilo* does not leave *Haliclona* sp. A during low tide even when the sponge is exposed to air for several hours. The opposite behaviour was reported by Rützler (1995) in most littoral sponge-endobiont amphipods. We believe that *C. lunalilo* is able to stay in its host when exposed to air because the water-filled interior of the host protects it from radiation and desiccation. Female inquiline amphipods are normally very aggressive towards each other causing high intraspecific competition, which prevents high numbers of mature females occupying the same host (Thiel, 2000). The high percentage of females and the high concentration of amphipods in *Haliclona* sp. are very unusual for inquiline amphipods indicating that intraspecific competition between individuals of *C. lunalilo* is low.

At PMBC pier the occupancy of potential hosts was two out of six (33%). This suggests that the local distribution of *C. lunalilo* probably is not limited by host availability.

**Nepanamixis sp.**

*Nepanamixis* sp. was only found in the sponge *Xestospongia* sp. (Pl. 1C) at one location (Koh Dok Mai). Here it occurred in great numbers, being the dominant amphipod found in the host. All the specimens of *Xestospongia* sp. contained *Nepanamixis* sp., and the amphipod had the same red colour as its host, suggesting that *Nepanamixis* sp. may be host specific. This is consistent with descriptions of host associations for other members of the family which are highly host specific (Thomas, 1997).

The ratio of leucomorphs to anamorphs found in this study was 10:1. This ratio has also been found among other anamixids (Thomas, 1997). In the individual host species, however, the ratio is very different from sample to sample and there was no clear pattern between numbers of leucomorphs and anamorphs. The lack of previous
knowledge and our limited data prevent us from further interpretation.

*Nepanamixis* sp. does not rely solely on retraction into its host for physical protection against predators since some specimens were observed outside the sponge. Individual *Nepanamixis* sp. were located in the tiny oscula scattered over the surface. The oscula were only fairly shallow offering little shelter from predators. However, the amphipods were well camouflaged against the sponge both in terms of colour and shade. When disturbed, *Nepanamixis* sp. left its host and swam actively. This behaviour is in sharp contrast with other endobiont amphipods such as *L. furina* and *C. lunalilo* that move slowly and awkwardly outside their hosts.

**Leucothoe furina**

It is difficult to judge whether *L. furina* is an obligate symbiont or not. The genus *Leucothoe* has been reported from a wide range of hosts. Most authors have suggested that these amphipods are obligate symbionts (Biernbaum, 1981; Voultriadou-Konkoura et al., 1987). We found juveniles but no mature adults outside hosts in coral-rubble. Since *L. furina* was collected from other invertebrates and ascidians, it is clearly not host-specific in the area around Phuket. This is in agreement with the literature concerning Leucothoidae (Richmond, 1997). Whilst not an obligate symbiont it clearly gains some advantage from its hosts.

Host relationship: Although not obligate endobiont, *L. furina* had a preference for the big solitary ascidian *Polycarpa* sp. occupying 80% of the collected specimens. Since up to 10 specimens of *L. furina* were observed inside the inhalant opening of *Polycarpa* sp., it seems likely that the amphipods may be affecting the inhalant stream. *Leucothoe furina* was not found in any other large solitary ascidians apart from *Polycarpa* sp. though the preferred host was found among other large solitary ascidian species. Large solitary ascidians live for several years and constitute a stable microhabitat for the amphipods. The ascidians provide shelter from predation, a reproduction habitat, and a strong feeding current supplying the amphipods with food (Thiel, 1998).

Demography and life history: *Polycarpa* sp. provide a single chamber with one inhalant opening. *Leucothoe furina* monopolize this host suggesting that intra- and interspecific competition might be high. The low number of *L. furina* adults, and the fact that no other species were found in each *Polycarpa* sp., support this hypothesis. These results match well with the observations of Thiel (2000) on the undescribed species *Leucothoe* “ascidicola” an ascidian-dwelling amphipod.

The population demography of *L. furina* where few adults and cohorts of young juveniles dominate the host shows that *Polycarpa* sp. is an important reproductive habitat. Furthermore, the presence of cohorts of relatively large juveniles indicates that *L. furina* reproducing inside *Polycarpa* sp. may have an extended parental care. This seems likely that large solitary ascidians constitute a stable habitat for reproduction suitable for extended parental care. Our observations are analogous to those of Thiel (1998) though he made his observations on *L. spinicarpa*.

We only found large adults and juveniles in the ascidian. Subadults were found in coral-rubble and as epifaunal amphipods. The reason for the absence of subadults in *Polycarpa* sp. could be that the high occupancy rate of *Polycarpa* sp. creates strong intraspecific competition. By seeking less favourable habitats as coral-rubble and epifaunal hosts the subadults could avoid competition.

Thiel (1998) hypothesized that low host-specialization by *L. spinicarpa* enables subadults of this endobiotic amphipod to avoid intraspecific aggression by seeking alternative habitats such as coral-rubble and zooids. This seems plausible and could explain why we found subadults in coral-rubble and as epifauna, which seem to be less attractive habitats. However, it may be a valuable alternative for juveniles and subadult amphipods when adults occupied as much as 80% of the hosts collected.
**Leucothoe tridens**

*Leucothoe tridens* was recorded from the Andaman Sea, Thailand, for the first time. It was common at two locations, PMBC pier and Koh Dok Mai (Fig. 1). *Haliclona cf. pigmentifera* (Pl. 1 D) is reported as host for *L. tridens* for the first time. However, *L. tridens* was also found in samples of other sponge species and ascidians. It is therefore difficult to say how obligate this relationship may be. However, *L. tridens* was only found as an endofaunal associate in this sponge. Previous literature does not mention hosts for *L. tridens.*

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