Density and adult ratio of the symbiotic harpacticoid copepod *Idomene purpurocincta* in the compound ascidian host *Aplidium yamazii*

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**Abstract:** Density and adult ratio of the symbiotic harpacticoid copepod, *Idomene purpurocincta*, living in the colony of the compound ascidian host, *Aplidium yamazii*, were investigated on the specimens taken from a boulder shore of Tosa Bay, western Japan, to know how these ecological parameters of the symbiont were related to the host size. Field collection of the host ascidian was carried out during the low tide in every spring tide from December 2004 to August 2006. All of the copepods isolated from the hosts using menthol were enumerated according to ontogenetic stages. The ascidian host colonies appeared from January to June 2005 and from December 2005 to August 2006. The symbiotic copepod was found in all of the hosts with few exceptions even at the initial appearance of the hosts in each season. These facts suggest that the copepod entered the host immediately after the colony formation. The density of the copepod in a host was usually $>10^4$ individuals per gram of host in dry weight. An exponential negative correlation was seen between the copepod density and the host size, and the density varied less as the host size increased. The adult ratio of the copepod also greatly varied in small hosts and tended to converge to about 25% with increase of the host size, and was negatively correlated with the copepod density. The reason for these host size-related copepod density and adult ratio may be explained by recruitment of nauplii in a host and migration of adults among hosts.

**Key words:** adult ratio, compound ascidian, density, harpacticoid copepod, symbiosis

**Introduction**

Copepods are the major animals symbiotic to many marine invertebrates and vertebrates (Gotto 1993, Kabata 1979). The relationships between them are generally considered parasitic or commensal. In marine invertebrates, ascidians serve as major hosts for many symbiotic copepod species (Dudley 1966, Dudley & Illg 1991, Illg & Dudley 1980, Ooishi & Illg 1977, Ooishi 1991). The population ecology of symbiotic copepods in a solitary ascidian has been studied, for example, as follows. Gage (1966) showed that seasonal variations in the population sizes of the cyclopoid copepods, *Notodelphys allmani* Thorell and *Ascidicola rosea* Thorell, which inhabited the pharyngeal cavity of the annual host ascidian, *Ascidia aspersa* (Müller), were closely related to that of the life history of the host, in which the new settlement of the ascidian during summer resulted in expansion of the copepod populations. A similar observation was made by Egan (1984) for the cyclopoid copepod *Pachyergus australis* Gotto in the ascidian *Pyura pachydermatina* (Herdman). Gage (1966) suggested that the individual number of *A. rosea* which feeds “food string”, a string-shaped food cohered by ciliary movement of a host, was limited by space availability of the string while that of *N. allmani* living in a large pharyngeal basket of the host is not limited by space.

In compound ascidians, there have been no studies on population ecology. A colony of compound ascidians is composed of hermaphroditic zooids embedded in common tunic, and grows by asexual budding of zooids. The zooids release planktonic larvae by sexual reproduction, and a new colony is formed by settlement of a single larva. The biological and ecological features of symbiotic copepods in compound ascidians are considered to be different from those in solitary ascidians since the solitary and compound ascidians have a quite different physical structure.

My study deals with the symbiotic life of harpacticoid copepod in the compound ascidian host in Tosa Bay, western Japan. In my preliminary observations, I found a symbiotic harpacticoid copepod *Idomene purpurocincta* (Norman & T. Scott), which had not been recorded in Japan according to the taxonomic key by Lang (1948), in the colony of the
compound ascidian host *Aplidium yamazii* (Tokioka) on the boulder shore of the bay. This ascidian has a common cloacal cavity, to which exhalent siphons of zooids open, with openings to the exterior. The copepod occurred densely in a common cloacal cavity.

In this paper, the density and adult ratio of the symbiotic harpacticoid copepod, *I. purpurocincta*, in the compound ascidian host, *A. yamazii*, are described to determine how these ecological parameters are affected by the host colony size, and the possible factors to determine the copepod density in a host colony are discussed. Because this study is the first record of a symbiotic harpacticoid copepod in a compound ascidian and also the first case of population ecology of a symbiotic copepod living in a compound ascidian, the present paper provides phylogenetically new information about the relationship between a symbiotic copepod and a host ascidian.

**Materials and Methods**

**Study site**

The present study site is located at Nomi-wan (33°21’28”N, 133°18’36”E), a cove of Tosa bay, in Kochi Prefecture, Shikoku, Japan (Fig. 1), which has a shoreline about 100 m in length. The colonies of the compound ascidian host, *Aplidium yamazii*, are found on the boulder bottom in the area from the shore to about 25 m offshore, where the water depth is less than 0.5 m at the low tide in spring tide.

**Density and adult ratio of the copepod**

Field collection of *A. yamazii* colonies was carried out during the low tide in all of the spring tides from December 2004 to August 2006. Each collection was started one hour before the lowest tide and continued at most for two hours. The colonies of *A. yamazii* were collected from the surface of boulders using a pallet and forceps. However, the number of colonies collected at each sampling occasion was limited to less than six individuals to avoid disturbance on the ascidian host population due to field collection. Water temperature at the sampling site was measured with a probe (YSI, Model 85).

Immediately after sampling, the ascidian colonies were put into individual glass bottles (500 ml) filled with 32 μm-mesh filtered sea water, and about 40 mg L⁻¹ of crystal menthol was added to the bottles to let the symbiotic harpacticoid copepod, *Idomene purpurocincta*, out from their common cloacal cavities. A couple of hours later, the ascidian colony in each bottle was rinsed with tap water, and the copepods in the water of the bottle were collected with 32 μm-mesh cloth. Dry weights of the host ascidian colonies were measured by drying at 60°C for 12 hours. All copepods were enumerated under a binocular according to three different ontogenetic stages (viz. nauplii, copepodids and adults). The adults were readily distinguished by the presence of dark-brown pedigers. Adult ratios (the number of adults to the total one of the copepods including nauplii) in individual hosts were counted. The copepod density of all three stages in a host ascidian colony is expressed as the number of individuals per gram of host in dry weight.

**Results**

The ascidian host *Aplidium yamazii* occurs on the surface of the boulders at the study site from the winter to the
summer of the next year. In this study, the colonies were first found on 12 January 2005 when the water temperature was 14.3°C, and they continued to appear for about six months up to 21 June 2005 at 24.9°C (Table 1, Fig. 2A); henceforth this period is referred to as the 2005 season. Decay of the colonies was observed on the last sampling day. A total of 55 colonies were collected in this season. The colonies of the next season, referred to as the 2006 season, occurred for eight months from 31 December 2005 at 16.5°C to 9 August 2006 at 32.1°C, and 39 colonies in total were collected. No seasonal changes were seen in the host colony size in both the 2005 and 2006 seasons (Fig. 2B). In the 2005 season, large colonies (>0.5 g in dry weight) were collected together with small ones at the five sampling occasions (12 January, 11 February, 25 February, 12 March, 10 May). The mean dry weight of colonies in the 2005 season was 0.26±0.28 (mean±S.D.) g. All of the colonies in the 2006 season were less than 0.5 g in dry weight, and the mean dry weight of colonies (0.08±0.08) was significantly smaller than that in the 2005 season (U-test, p<0.01).

*Idomene purpurocincta* was collected from all of the ascidian host colonies except two hosts collected on 12 January 2005 and one host on 11 February 2005. In total, 32,499 and 15,493 individuals of the copepod were collected from 52 and 39 individuals of the ascidian host colonies in the 2005 and 2006 seasons, respectively. The mean numbers of the copepod in a host were 625±847 (mean±S.D.) in the 2005 season and 397±357 in the 2006 season.

In the 2005 season, the mean copepod density in a host was extremely low (49 to 88 indiv. g⁻¹) at the first three sampling occasions until 11 February, suddenly increased
by two orders, $3.10 \times 10^3$ indiv. g$^{-1}$, on 25 February, and fluctuated in the high density range ($1.70 \times 10^7$ to $1.29 \times 10^4$ indiv. g$^{-1}$) until the end of the season (Fig. 3). The mean copepod density in all host ascidians except those collected at the first three sampling occasions was $4.58 \times 10^3 \pm 5.98 \times 10^1$ (mean ± S.D.) indiv. g$^{-1}$.

The mean copepod density at each sampling occasion in the 2006 season ranged between $2.70 \times 10^3$ and $1.10 \times 10^4$ indiv. g$^{-1}$. The mean copepod density in all hosts collected in the 2006 season was $6.42 \times 10^3 \pm 4.34 \times 10^3$ indiv. g$^{-1}$, which was approximately 1.4 times higher than that in the 2005 season except the first three sampling occasions (statistically significant, $U$-test, $p < 0.01$).

The mean adult ratio of the copepod (including nauplii) remained in a high range, 61% to 100% at the first three sampling occasions (12 January, 26 January, 11 February) in the 2005 season, but decreased markedly on 25 February, and fluctuated in a range between 17% and 48% by the end of this season (Fig. 4). In the 2006 season, the mean adult ratio fluctuated in a low range between 17% and 28% in the first approximately seven months (31 December 2005 to 11 July 2006), but high adult ratios (54% and 57%) were noted at the last two sampling occasions (16 July and 9 August), respectively.

The relationship between the individual weight of the ascidian host and the copepod density in a host colony is shown in Fig. 5. In this study, 91 pairs of data on the individual weight of the ascidian host and the copepod density in a host colony were collected, but 78 of them were used for the figure. The remaining 13 pairs of data were removed from the plots for the following four reasons.

1. Seven pairs of data with extremely low copepod densities (4 to 103 indiv. g$^{-1}$, see Fig. 3) at the first three sampling occasions in the 2005 season (12 January, 26 January, 11 February). These data presumably resulted from incomplete propagation of the copepods due to the limited time after they entered into the body of the hosts.

2. Two pairs of data with extremely low copepod density (43 and 143 indiv. g$^{-1}$, see Fig. 3) and high adult ratios (71% and 100%, see Fig. 4) on 26 April and 24 May 2005. These results may be caused by bad physiological conditions of the host.

3. A pair of data with an extremely high copepod density on 21 June 2005 ($3.97 \times 10^4$ indiv. g$^{-1}$, see Fig. 3). It is likely that the decay of host ascidian body already started, and the copepods concentrated on the remaining intact parts of the body.

4. Three pairs of data with extremely high adult ratios of the copepods (54% to 75%, see Fig. 4) at the last two sampling occasions in the 2006 season (16 July and 9 August 2006), which are possibly explained by inactive reproduction of the copepod due to high water temperature.

The copepod density showed an exponential negative correlation ($r^2 = 0.30$, $p < 0.01$) with the host size expressed by dry weight (Fig. 5). The variation of the copepod density was apparently larger in smaller hosts; the standard deviation of the density of the smallest 10 hosts ($4.87 \times 10^3$ indiv. g$^{-1}$) was about 4.4 times greater than that of the largest 10 hosts ($1.11 \times 10^5$ indiv. g$^{-1}$). The exponential regression line shows that the copepod density decreased as the host size...
increased, and was less than $5 \times 10^4$ indiv. g$^{-1}$ in hosts larger than 0.3 g in dry weight.

The relationship between the individual weight of the ascidian host and the adult ratio of the copepod is plotted in Fig. 6 with the copepods collected from 78 ascidian hosts shown in Fig. 5. The ratio did not correlate significantly with the host size ($r^2=0.02$, $p>0.05$). However, the standard deviation of the smallest 10 hosts (10.8%) was about 1.6 times greater than that of the largest 10 hosts (6.7%), indicating that, as the host size increased, the variation of the adult ratio decreased, and the ratio converged to around 25%. Figure 7 indicates the relationship between the copepod density and the adult ratio. The adult ratio was negatively correlated with the copepod density in a host colony ($r^2=0.33$, $p<0.01$).

**Discussion**

Seasonal changes in the number of symbiotic cyclopoid copepods within a solitary ascidian host have been investigated by Gage (1966), Egan (1984), Schmidt (1984) and Svavarsson et al. (1993). Copepods observed in the first three studies usually lived solely in a host. In the last study, two different species of copepod (Gunenotophorus globularis Buchholz and Doropygus pulex Thorell) were observed in the host. The former species occurred most often singly in each host, and the latter was found usually with several individuals (Svavarsson et al. 1993). By contrast, the number of the harpacticoid copepod, Idomene purpurocincta, occurring in the compound ascidian host, Aplidium yamazii, in this study was quite large (527 individuals in mean in a host throughout the study period).

The high copepod number of this species in a host is attributed partly to the size of the copepod (about 0.5 mm in adult body length), which is much smaller than cyclopoid copepods occurring in the solitary ascidians. The control of copepod number by the body size of ascidian host is suggested in the case of small-sized poecilostomatoid copepod, Lichomolgus canus Sars (Svavarsson 1990), although its number (less than $2 \times 10^2$ indiv. host$^{-1}$) was rather smaller than that of the present study.

Another possible factor that determines the copepod number in a host is food supply for the copepod. In a solitary ascidian host, symbiotic copepods feed mucus or “food string” of the host (Gage 1966, Gotto 1957, Svavarsson 1990). In the data used for the analysis of the relationship between the copepod density and the size of ascidian host in the present study, the mean and highest densities of the copepod were $5.10 \times 10^3$ and $1.76 \times 10^4$ indiv. g$^{-1}$, respectively. The volume of a host with 1-g dry weight can be estimated as 15 cm$^3$, since a round host of 4 cm in diameter was measured 0.25-g dry weight and thickness of the host was generally 3 mm regardless of its size. Therefore, the above densities can be converted to those per the host volume of $3.4 \times 10^3$ and $1.2 \times 10^4$ indiv. cm$^{-3}$, respectively. Considering that the copepods in a host are restricted to the common cloacal cavity, the densities per space of the cavity are at least 1.3 $\times 10^3$ and 4.3 $\times 10^3$ indiv. cm$^{-3}$, respectively, because the volume of the common cloacal cavity can be safely estimated to be at most 27% of the host volume. This ratio (27%) of the cavity volume is calculated from the covering ratio (<90%) of the cavity in plane view based on dye-injection observation and the ratio (<30%) of the cavity depth to the host depth based on observation on host sections.

In my direct observation of the common cloacal cavity of a living host under a binocular, copepods of various stages were so crowded that they frequently contacted with each other as shown in Fig. 8. Such a high copepod density ($>10^3$ indiv. cm$^{-3}$) has never been reported from free-living copepods; e.g. the highest density of planktonic copepods in nature was recorded as $10^4$ indiv. m$^{-3}$ (=10$^2$ indiv. cm$^{-3}$) in a swarm (Haury & Yamazaki 1995). Although the feeding habit of the present copepod is unknown, enough food and limited space in a host are considered possible factors that lead to the high densities.

In the present study site, I. purpurocincta was found in the host throughout the 2005 and 2006 seasons. On the first
The compiled data of the copepod density and the adult ratio showed that the density and the ratio tend to vary in smaller hosts (Figs. 5, 6). Ontogenetic behaviors of the copepod may account for the large variation of these parameters in smaller hosts. In the tank where host ascidian colonies were reared, I observed that adult copepods often crawled on the surface of the host body, and swam occasionally in the water apart from the hosts. These observations suggest that the adult copepods frequently migrate among the hosts. Frequent migrations of the adults among the hosts would result in higher variations of the adult ratio, because emigration and immigration rates probably varied depending on the location and other conditions of the hosts such as size. In a small host, the large variations of the adult ratio are more expected due to high emigration rate because the high copepod density will encourage the adults to venture from the host (see Fig. 7). The large variations of the adult copepod numbers in the small hosts would cause the large variations of the copepod density, because of varied recruitment of nauplii.

The negative relationship between the copepod density and the host size in the present study (Fig. 5) indicates that the density tends to converge to a value less than about $5 \times 10^3$ indiv. g$^{-1}$. The abundance of immature copepod in a host is controlled by the number of recruitment as nauplii and losses in the process of development to adults such as predation and physiological death, whereas that of adult is determined by recruitment of developed copepodids, immigration and emigration, loss by predation, and physiological death. The convergence of density in large hosts indicates that the factors to increase copepod density are balanced with those to decrease the density. This equilibrium of density may occur more clearly in larger hosts due to their larger capacity.

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