*Micronesica* 34(2):165-175, 2002

# An association between a syllid polychaete, *Haplosyllis basticola* n. sp., and the sponge *Ianthella basta*

RAFAEL SARDÁ, CONXITA AVILA

*Centre d'Estudis Avançats de Blanes (CSIC). Camí de Santa Bàrbara, s/n. 17300-Blanes, Girona (Spain)*

AND

VALERIE J. PAUL

*Marine Laboratory UOG. University of Guam. Mangilao, Guam 96923. USA*

**Abstract—**The association between a syllid polychaete and the marine sponge *Ianthella basta* (Pallas, 1776) is described from the island of Guam (Micronesia). The polychaete is a new species of *Haplosyllis* Langerhans, 1879, named *H. basticola.* The new species was observed infesting in extraordinary high numbers the inside of the sponge canals and mesenchyme without any specially built tube-like structure. The closest species to *H. basticola* is *H. anthogorgicola* Utinomi, 1956 but they clearly differ in size, morphology of the simple setae and the annulations of the dorsal cirri. *Haplosyllis basticola,* n. sp. is characterized by a maximum of 25 setigers, dorsal cirri of the anterior 1st to 4th setigers clearly annulated averaging 10, 3, 3, and 4 annulations respectively, and the presence of only one simple seta by parapodia with a large subterminal tooth and two smaller terminal teeth. A size frequency histogram of the population showed a unimodal distribution, which could be an indication of a continuous reproductive effort. The presence of female and male reproductive stolons gave us the opportunity to describe them. The chemical analysis of worm and sponge extracts and a Nuclear Magnetic Resonance Spectroscopy analysis done on *H. basticola* failed to confirm the presence of bastadins in the worm. We discuss evidence of a positive reciprocal relationship between the worm and the sponge.

## Introduction

During a research project to investigate the macrofauna associated with sponge species in tropical and temperate seas, a strict association between a small syllid and the large sponge *Ianthella basta* (Pallas, 1776) (Sponge, Ianthellidae) was observed on the island of Guam (Micronesia). A detailed examination of the syllid, found in hundreds of individuals per ml of sponge volume, confirmed its taxonomic status as a new species of *Haplosyllis*.

*Haplosyllis* spp have been suggested as obligatory commensals of several zoological taxa such as sponges, cnidarians, and ophiuroids (Martin & Britayev, 1998). However, whether these relationships are based on parasitism, mutualism or competition for resources is not so clear. Only *Haplosyllis spongicola* (or some synonimized organisms such as *H. cephalata*) have proved to be parasitic predators of their hosts. *Haplosyllis spongicola* grazes on many different prey species. In the rest of the cases, the relationship between the worm and the host is unknown. The presence of thousands of worms living inside *Ianthella basta* gave us the opportunity to describe the species, to investigate its reproduction, population dynamics and morphogenesis, and also to check the relationship between both species.

Usually, predator-prey relationships are established only by visually detecting the predator on the prey, finding them living both in close associations, or even finding grazing marks on the prey. Often, analysis of stomach contents is used to confirm these relationships. Another way to ensure the assignment of predator-prey associations is by chemical analysis of both prey and predator, since chemical-markers may be detected by many techniques, such as chromatography and spectroscopy. Many examples of chemically confirmed predator-prey associations exist in other marine invertebrates (e.g., opisthobranch mollusks; Avila 1995). Since there is a living association between the polychaete *Haplosyllis basticola* and the sponge *Ianthella basta*, and stomach contents were difficult to analyze due to the small size of the worm, before assuming a predator-prey relationship we found it useful to undertake a chemical analysis. It was our aim to know whether the chemical compounds of the sponge were found in the polychaete tissues.

## Methods

### Field and laboratory procedures

The new species was observed infesting the interior part of the sponge *Ianthella basta* in extraordinary high numbers. The individuals were found at a site called Sponge Mound in Apra Harbour, Guam, Micronesia, inhabiting sponge colonies obtained from 25 to 35 m depth. We sampled parts of several sponge colonies of *Ianthella basta* to investigate the macrofauna associated on June 5, 1998. In the laboratory, samples of the sponges (Table 1) were maintained in seawater at room temperature to allow the fauna living inside the sponge to come out while another sample was frozen for chemical analysis. Immediately after the sponges were placed in jars with seawater, small white organisms rapidly came swimming out from the canals of the sponges. These were male and female reproductive bodies of a small species of *Haplosyllis*. Minutes later, young and adult individuals were seen coming out. We fixed the individuals that left the sponge during the next three days. No other organisms left the sponge or remained inside their canals. Around 12,000 specimens were fixed in formalin for taxonomic studies. Eight adult specimens of *Haplosyllis basticola*, three reproductive bodies and three juveniles were fixed in ethanol, dehydrated, brought to the critical point and

Table 1.- Sponge colonies investigated and associated *Haplosyllis basticola*.

| | Sponge 1 | Sponge 2 | Sponge 3 | Sponge 4 | Sponge 5 |
|---|---|---|---|---|---|
| Total sponge volume (ml) | 60.0 | 145 | 30.0 | 33.0 | 40.0 |
| Sponge volume for faunal analysis (ml) | 30.0 | 80.0 | 15.0 | 15.0 | 18.0 |
| Sponge wet weight for faunal analysis (g) | 13.8 | 63.8 | 2.3 | 6.7 | 9.3 |
| Sponge volume for chemical analysis (ml) | 30.0 | 65.0 | 15.0 | 18.0 | 12.0 |
| Sponge wet weight for chemical analysis (g) | 12.8 | 55.4 | 10.8 | 15.4 | 11.9 |
| Number of associated specimens | 2091 | 12775 | 2750 | 1791 | 2312 |
| Specimens used for chemical analysis | – | 8500 | – | – | – |

mounted for observation under a Scanning electron microscope (SEM). Finally, about 8,500 specimens from sponge # 2 were collected and freeze-dried for further chemical analysis. Table 1 shows the associated fauna, sponge volume, and wet weight of samples from the 5 sponge colonies that were investigated.

### HISTOLOGICAL ANALYSIS OF *IANTHELLA BASTA*

The host species, *Ianthella basta* (Porifera, Demospongiae, Ianthellidae) is a very peculiar fan-like sponge. Although it can have different colors the colonies we sampled were purple-blue. The fans of *I. basta*, a non-spiculate sponge with a lax mesenchyme inside, are thin with vertical ribbing on their surfaces, and with a large number of small inhalant pores (ostioles), oscules, and internal canals (Hooper & Wiedenmayer 1994). Tissue slices of the sponge *Ianthella basta* were processed for microscopy by fixing them in 2.5% glutaraldehyde in buffered seawater. Samples were postfixed for 2 h with 2% $OsO_4$, washed, dehydrated in a graded etahanol series, and embedded in Spurr's resin. Semi-thin sections (1 µm thick) were cut and stained with a solution of methylene blue (0.5%)-borax (0.5%) to observe the position of the worms inside the sponge.

### CHEMICAL ANALYSIS

In order to check whether *Haplosyllis basticola* contained any sponge metabolites, the polychaete and the sponge *Ianthella basta* in which the polychaete lives, were analyzed chemically. Chemical analyses were carried out by freeze-drying the samples, extracting them using acetone and then removing the solvent by evaporation under vacuum by rotary evaporator. Chemical composition of the extracts was compared by thin layer chromatography (TLC) (Table 2).

Table 2.- Quantitative data of the samples of *Haplosyllis basticola* and *Ianthella basta* used in the chemical analysis. n.a.: not available; (*): only a fragment of the sample was extracted.

| | *H. basticola* | *I. basta* |
|---|---|---|
| Total sample | 8.500 specimens | 80 ml volume |
| Wet weight (g) | n.a. | 63.8 |
| Dry mass (mg) | 160.1 | 266.4(*) |
| Acetone extract (mg) | 6.9 | 11.9(*) |

Further analysis of the polychaete extract consisted of proton nuclear magnetic resonance spectroscopy (NMR; located at the ICMIB-CNR in Arco Felice, Naples, Italy) of 3.6 mg of the extract in $CDCl_3$, and comparisons with NMR spectra of purified metabolites from *Ianthella basta*, which contains bastadins.

## Results

Family Syllidae Grube, 1850
Subfamily Syllinae Grube, 1850
Genus Haplosyllis Langerhans, 1879
*Haplosyllis basticola* new species

### Material examined

More than 20,000 individuals were collected and some specimens have been deposited in the Marine Laboratory (Guam, USA), the “Museo Nacional de Ciencias Naturales” of Madrid (Spain) and the author’s collection. Apart from the holotype, 24 paratypes have been selected (collections of the CEAB) and more than 5,000 are present in the author’s collection (R. Sardá, CEAB, Spain). All the measurements given in the paper refer to the selected paratypes.

### Description

Length of 24 paratype complete specimens ranges from 0.95 mm (8 setigers) to 4.05 mm (23 setigers), width from 0.24 mm to 0.10 mm. Adult specimens (considered as individuals larger than 14 setigers) reach up to 25 setigers (Fig. 1a; 2a,b). Adult length averaging 2.57 mm and adult width averaging 0.20 mm. Body subcylindrical, strongly arched dorsally, flattened ventrally, being widest in the proventriculus region. All specimens colored brown-purple, exactly the same color as the sponge.

Prostomium oval, broader than long. Four eyes present in trapezoidal arrangement. The anterior ones slightly larger behind lateral antennae. Median antenna inserted near posterior margin of prostomium, averaging 11 annulations in adult specimens (range: 9 - 14) (Fig. 1b,d). Lateral antennae inserted near the basis of the palps, shorter than median antenna. Lateral antenna averaging 8 annulations in adult specimens (range: 6 -10). Palps almost twice as long as the prostomium, separate but in close contact in the base, bluntly subtriangular. Neither nuchal organs nor ciliary bands observed.

Proboscis terminates distally in a circlet of 9 soft papillae (Fig. 1c). Behind soft papillae a continuous circle of cilia with tufts between the soft papillae. The anterior margin of its inner wall is smooth and with one subdistal middorsal tooth. Proventriculus extends from setiger 3 to 6. It has about 25 to 30 rows of muscular cells. The peristomial segment is asetigerous and very small in length compared with the rest of the body setigers. Adult dorsal tentacular cirri averaging 7 annulations similar in length to median antennae. Ventral tentacular cirri shorter, averaging 4 annulations.

Parapodia uniramous (Fig. 1e). Dorsal cirri of the anterior 1st to 4th setigers are clearly annulated, those of the succeeding 3 or 4 setigers present 2-3 annulations at maximum (normally one), the rest of dorsal cirri diminishing in size until the pygidium and not annulated (Fig. 1b,d,e). Dorsal cirri longest on first setiger, averaging 10 annulations (range: 1 (juvenile specimen) - 14), shorter in the second setiger with 3 annulations (range: 1 (juvenile specimen) - 4), third setiger

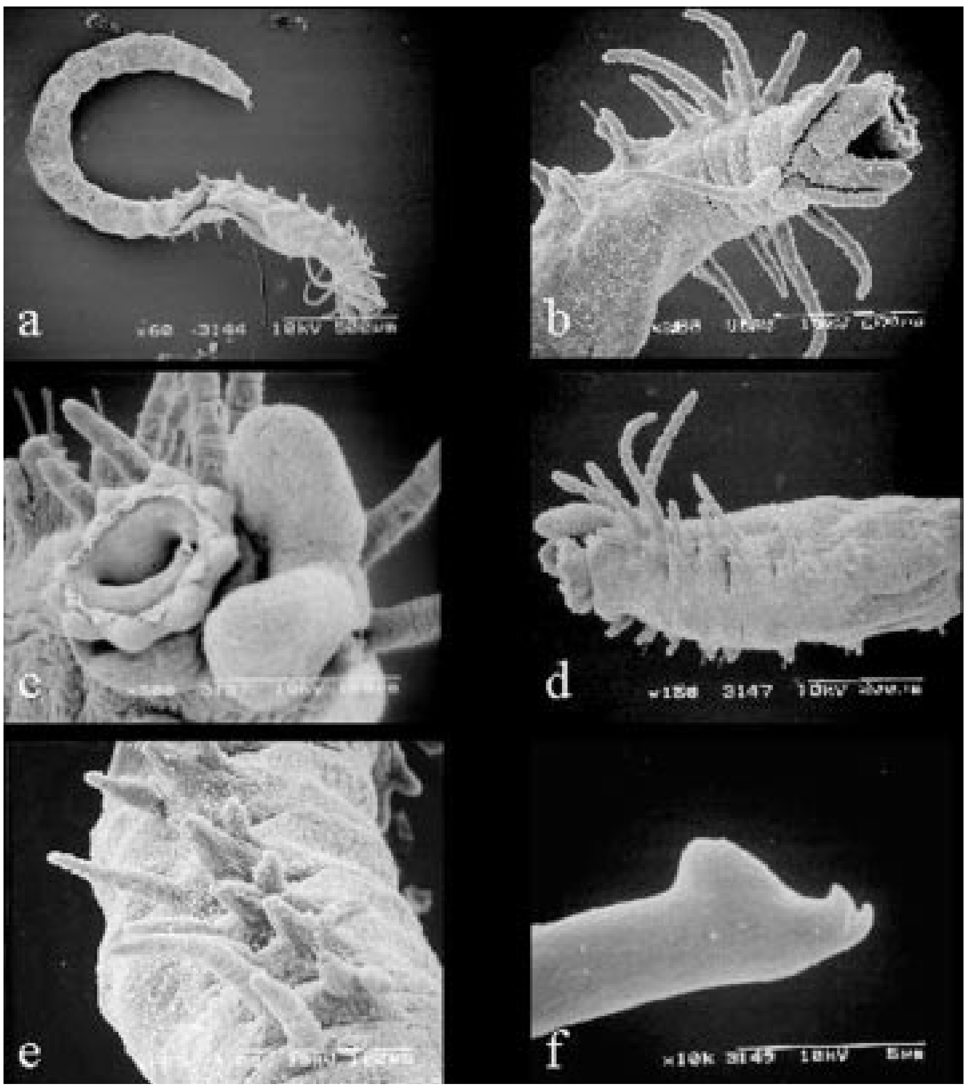


Figure 1. *Haplosyllis basticola* (n. sp.). a) General view. b) Anterior end, dorsal view, showing the lateral and median antennae, tentacular cirri, and dorsal cirri of the first four setigers. c) Proboscis with the circlet of 9 soft papillae and one tooth. d) Anterior end, ventral view. e) Parapodium of the first setigers. f) Dorsal simple seta.

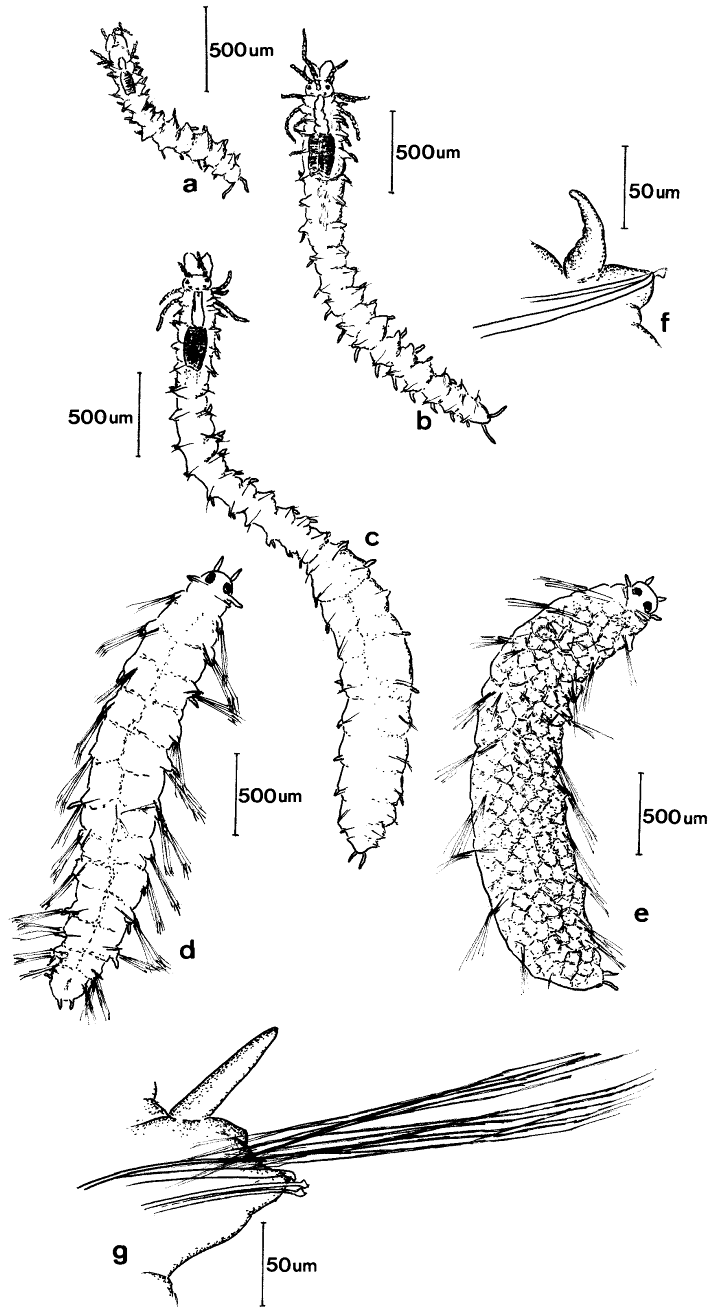


Figure 2. *Haplosyllis basticola* (n. sp.). a) Juvenile specimen (12 setigers). b) Adult specimen (18 setigers). c) Adult specimen with stolon segments ready to separate (25 setigers). d) Male reproductive body. e) Female reproductive body. f) Medium parapodium, lateral view of adult specimen. g) Medium parapodium, lateral view of reproductive body.

with 3 annulations (range: 1 (juvenile specimen) - 4), and fourth setiger with 4 annulations (range: 1 (juvenile specimen) - 7). Ventral cirri short and digitate. Each parapodium supported by a single aciculum which is hooked at the tip (Fig. 2f). Only one simple seta per parapodium from the first until the last setiger. Setae with large subterminal tooth and two smaller terminal teeth (Fig. 1f).

Pygidium narrower than preceding segments, with two relatively long anal cirri, without annulations.

*Etymology*.- The species is named after its host, the sponge *Ianthella basta.*

## Reproductive female and male bodies

*Haplosyllis basticola* reproduces by schizogamy in which existing segments of the worm are transformed into stolon segments, which is common in the Syllinae subfamily. Stolon segments develop swimming setae, a new thin acicula and a second simple seta (Fig. 2g). Reproductive bodies are bigger in size than adult specimens (Fig. 2a-e). The resulting stolons do not show sexual dimorphism besides the recognition inside of both types of gametes. Male and female reproductive bodies have 11 or 12 setigers (Fig. 2d,e). Reproductive bodies contain a clearly distinguished head with 2 antennae. In the specimen drawn in Fig. 2c, almost ready to be released based on its size (11 setigers), neither eyes nor antennae could be observed indicating that may be the head is developed after separation as has been observed in other syllid species (Garwood 1991). Only one pair of tentacular cirri present. Each parapodium has two acicula, one similar to the adult specimen and another thinner one that supports the swimming setae. In addition to the bunch of swimming setae observed for each parapodia, two simple setae with the same morphology as those observed in adult individuals are present. The pygidium bears two anal cirri without annulations.

## Population biology of *Haplosyllis basticola*

The population is present inside the sponge in very high numbers, average density of 132 individuals per ml of sponge volume. The histological semi-thin sections of the sponge showed the worms living inside the sponge canals and mesenchyme without any specially built structure (tube or similar).

Our observations on more than 900 individuals show that *Haplosyllis basticola* did not have more than 25 setigers. The size frequency histogram of the population related to the number of setigers (Fig. 3) showed a unimodal distribution, which could be an indication of a continuous reproductive effort. The most abundant class of the population was the one containing 14 setigers. Due to the fact that reproductive bodies contains 11 or 12 setigers, it seems that reproductive processes could occur regularly in these populations.

Growth of the polychaete could also be observed either by the number of setigers or by the annulations present in the first dorsal cirri. There was a clear relationship between both anatomical figures. Figure 3 shows this relationship in a linear regression:

annulations first d.c. = -5.21 + (0.774* number of setigers), ($r^2$= 0.81).

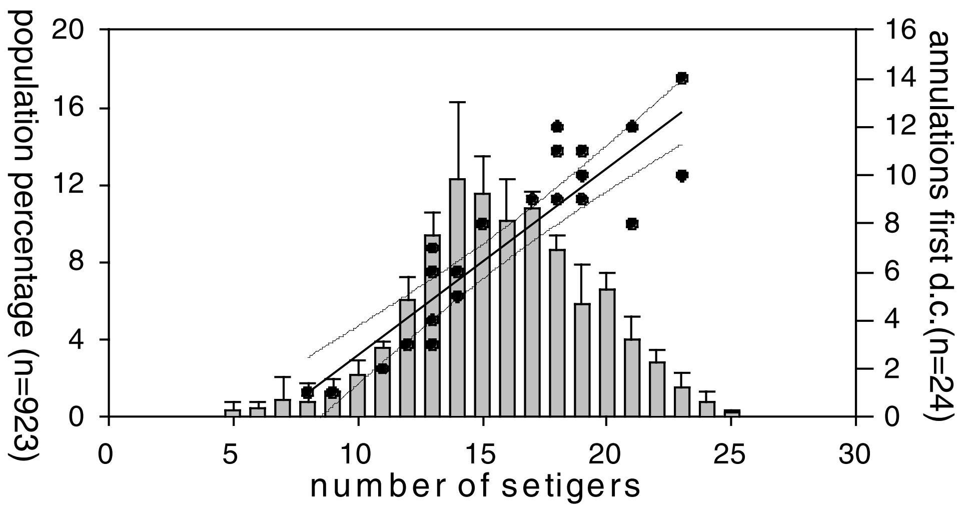


Figure 3. The relationships between the annulations of the first dorsal cirri and setiger number in *Haplosyllis basticola.* Heavy line is the linear regression between both variables. Grey bars indicate the percentage of the population (± s.d. of three sponge colonies) containing different number of setigers.

### Chemical analysis

Thin layer chromatography indicated a similar chemical pattern between the sponge and the polychaete. The sponge extract contained several polar, UV-active compounds that could be present also in the polychaete extract, although only as traces. Although the chemical analysis by TLC of the sponge and the polychaete extracts could indicate a predator-prey relationship between the worm and its host sponge, *Ianthella basta*, the more precise, NMR analysis did not confirm this relationship. Bastadins, a complex mixture of macrocyclic compounds previously described for *I. basta* (Kazlauskas et al. 1980, 1981, Pordesimo & Schmitz 1990) were not observed in the polychaete NMR spectrum.

## Discussion

Ten species and three subspecies are currently recognised in the genus *Haplosyllis*. All of them are typically small and cited as commensal and/or parasitic of sponges, corals, and echinoderms. These include *H. uncinigera* Grube, 1878; *H. anthogorgicola* Utinomi, 1956, from Japan; *H. bisetosa* Hartmann-Schröder, 1960, from the Red Sea; *H. chamaleon* Laubier, 1960, from the Western Mediterranean; *H. dollfusi* (Fauvel, 1933), from the Gulf of Suez; *H. spongicola* (Grube, 1855), from the Adriatic Sea; *H. spongicola brevicirra* Rioja, 1941, from Western Mexico: *H. spongicola tentaculata* (Marion, 1879), from France; *H. trifalcata* (Day, 1960), from South Africa; *H. agelas* Uebelacker, 1982 from Bahamas; and *H. hainanensis* Sun, 1996 from Hainan

Island (China). Although these similar species possess different types of setae and annulations, they can be initially separated into two groups based on size. There is a group of species relatively small in size (from 2 to 6 mm length at maximum). This group includes *H. bisetosa* associated with an alcyonarian anthozoan in the Red Sea (Hartmann-Schröder 1960), and *H. anthogorgicola* associated with the gorgonacean *Anthogorgia bocki* in the Pacific Coast of Southern Japan (Utinomi 1956). Together with these two species, one species belonging to a similar genus, *Haplosyllides floridana* Augener, 1924 (formerly named *Haplosyllis aberrans*), also has a similar morphology and size. *H. floridana* has been associated with *Ophiocoma pumila* (Ophiurida) in Puerto Rico, observed in Indochina and the Marshall Islands (Fauvel 1939, Hartmann-Schröder 1978), and recently found invading the sponge *Xetospongia muta* in Cuba (San Martín et al. 1997). There are large differences between the shape and number of the simple setae, dorsal cirri, and shape of the acicula between *H. floridana* and *H. bisetosa* and our species *H. basticola.* The closest species to this new one would be *H. anthogorgicola.*

We had the opportunity to study 15 paratypes of the type series of *H. anthogorgicola* described by Utinomi (S.M.B.L., Type 161). Although both species are similar in size and body shape, and they only have an aciculum and one single seta on each parapodium, they clearly differ in size, morphology of the simple setae and the number of annulations of their dorsal cirri. (Table 3). *H. anthogorgicola* is a larger species with more setigers than *H. basticola.* The setae of *H. anthogorgicola* have a large subterminal tooth and only one smaller terminal tooth instead of two as in *H. basticola.* Finally, the formula of the number of annulations in dorsal cirri stated in the paper of Utinomi or in the studied paratypes of *H. anthogorgicola* (Table 4) is quite different from the one observed in the adult paratypes of *H. basticola* that were examined.

The NMR analysis done on *H. basticola* failed to confirm the presence of bastadins, typical macrocyclic compounds of *I. basta.* Although a possible (but not probable) explanation for this could be an alteration of these compounds during the preparation and transfer of the polychaete extract for the NMR, there are other facts suggesting we should reject the hypothesis of a predator-prey associa-

Table 3.- Summary of statistical tests performed for different morphological variables between *Haplosyllis anthogorgicola* and *H. basticola* (n. sp.). When the assumptions of normality were not met by our data, Mann-Whitney Rank Sum Test (T) was used instead of t-test.

| | *H. anthogorgicola* | *H. basticola* | statistical test |
|---|---|---|---|
| Number of setigers | 39.9 ± 4.85 | 19.7 ± 1.97 | $t_{18}$= 13.0 (p<0.0001) |
| Adult length (mm) | 3.04 ± 0.40 | 2.57 ± 0.28 | |
| Adult width (mm) | 0.21 ± 0.02 | 0.20 ± 0.03 | |
| Dorsal cirri 1st setiger | 27.0 ± 1.77 | 9.9 ± 2.02 | $t_{18}$= 19.4 (p<0.0001) |
| Dorsal cirri 2nd setiger | 9.0 | 2.5 | T= 132.0 (p<0.0001) |
| Dorsal cirri 3rd setiger | 10.0 | 3.0 | T= 132.0 (p<0.0001) |
| Dorsal cirri 4th setiger | 17.8 ± 2.71 | 3.8 ± 1.90 | $t_{18}$= 13.5 (p<0.0001) |
| Dorsal cirri 5th setiger | 8.0 | 1.0 | T= 132.0 (p<0.0001) |

Table 4. Number of annulations in dorsal cirri in *Haplosyllis anthogorgicola* versus *H. basticola*

| Body segment | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th | 11th→ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *H. anthogorgicola* | 33 | 7 | 7 | 15 | 6 | 10 | 5 | 3 | 3 | 3-1 | 1… |
| *H. basticola* | 10 | 3 | 3 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1… |

tion. The presence of thousands of individuals of *H. basticola* in small portions of the sponge colonies would result, if these were in a predator-prey relationship, in the sponge suffering severe damage inside. We could not confirm any damage to the sponge either underwater or in the laboratory.

Sponges are not very efficient at capturing large particles of food (>5 µm) and they need to spend some energy to expel such particles again. If the worm population could be maintained preying upon these particles, it could be possible that the sponge could also use the digested material excreted by the worm. In this way a positive reciprocal relationship between the worm and the sponge could be obtained. Several authors have noticed the presence of a small goby fish, *Pleurosicya elongata,* living on the surface of *I. basta* (Colin & Arneson 1995). Gobiids are very territorial fishes. The presence of the worm population, living inside the sponge in millions of individuals, occasionally going out of it and sometimes expelling reproductive stolons, may also serve as a good food resource for the fish. These symbiotic relationships between sponges or cnidarians and small syllids, such as *Haplosyllis basticola* or *H. anthogorgicola,* could be frequent in tropical environments.

## Acknowledgements

We thank the U.S.-Spain Joint Comission on Scientific and Technological Cooperation for funding this project and the travel to Guam in 1998. We acknowledge Dr. T. Miura who helped us find the type specimens of *H. anthogorgicola* and we thank the curator of the Seto Museum (Seto Marine Biological Laboratory) for the loan of Utinomi specimens of the species. We thank Dr. Dieter Fiege, Dr. Guillermo San Martín, and Dr. Manuel Maldonado for their useful comments. We thank the ICMIB (CNR) at Naples, and specially Dr. G. Cimino, for the NMR analysis. Thanks are also due to M. Puglisi, S. Taboada and A.M. Domènech for their help during this study. Scanning electron microscopy was carried out at the Servei de Microscopia of the University of Barcelona, and we are thankful to their technical staff.

## References

Avila, C. 1995. Natural products of opisthobranch mollusks: a biological review. Oceanography and Marine Biology Annual Review 33: 487–559.

Colin, P. L. & C. Arnesson. 1995. Tropical Pacific Invertebrates. 296 pp. Coral Reef Press. Irvine, CA (USA).

Fauvel, P. 1939. Annélides Polychètes de l'Indochine recueillis par M.C. Dawydoff. Commentations. Pont. Acad. Sci. Ann. III 3: 243–368.

Garwood, P. 1991. Reproduction and classification of the family Syllidae (Polychaeta). Ophelia. Suppl. 5: 81-87.

Hartmann-Schröder, G. 1960. Polychaeten aus dem Roten Meer. Kieler Meeresforsch. 16: 69–125.

Hartmann-Schröder, G. 1978. Einige Sylliden-Arten (Polychaeta) von Hawaii und aus dem Karibischen Meer. Mitt. Hamb. Zool. Mus. Inst. 75: 49–61.

Hooper, J. N. A. & F. Wiedenmayer. 1994. Porifera. *In* A. Wells (ed.) Zoological Catalogue of Australia. Volume 12. Melbourne: CSIRO Australia xiii, 624 pp.

Kazlauskas, R., R.O. Lidgard, P.T. Murphy, & R.J. Wells. 1980. Brominated tyrosine-derived metabolites from the sponge *Ianthella basta*. Tetrahedron Letters 21: 2277–2280.

Kazlauskas, R., R.O. Lidgard, P.T. Murphy, R.J. Wells & J. F. Blount. 1981. Brominated tyrosine-derived metabolites from the sponge *Ianthella basta.* Australian Journal of Chemistry 34: 765–786.

Martin, D. & D. T. Britayev. 1998. Symbiotic Polychaetes: Review of known species. Oceanography and Marine Biology Annual Review 36: 217–340.

Pordesimo, E.O. & F.J. Schmitz. 1990. New bastadins from the sponge *Ianthella basta.* Journal of Organic Chemistry 55: 4704–4709.

San Martín, G., D. Ibarzábal, M. Jiménez & E. López. 1997. Redescription of *Haplosyllides floridana* Augener, 1924 (Polychaeta: Syllinae), with notes on morphological variability and comments on the generic status. Bulletin of Marine Science 60: 364–370.

Utinomi, H. 1956. On the so-called "Umi-Utiwa", a peculiar flabellate gorgonacean, with notes on a syllidean Polychaete commensal. Publication of the Seto Marine Biology Laboratory 5: 243–250.

Received 19 Feb. 2001.