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Do syntopic host species harbor similar symbiotic communities? The case of *Chaetopterus* spp. (Annelida: Chaetopteridae)

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To assess whether closely related host species harbor similar symbiotic communities, we studied two polychaetes, *Chaetopterus* sp. (11 specimens) and *Chaetopterus* cf. *appendiculatus* (83 specimens) living in the soft bottoms of Nhatrang Bay (South China Sea, Vietnam). The former harbored the porcellanid crabs *Polyonyx* cf. *heox* and *Polyonyx* sp., the pinnotherid crab *Tetrias* sp. and the gastropod nudibranch Tergipedidae gen. sp. The latter harbored the polynoid polychaete *Ophthalmonoe pettiboneae*, the carapid fish *Onuxodon fowleri* and the porcellanid crab *Eulenaios cometes*. All them, except *O. fowleri*, seem to be specialized symbionts. Symbionts' species richness and mean intensity were higher in *Chaetopterus* sp. than in *C.* cf. *appendiculatus* (1.8 and 1.02 species and 3.0 and 1.05 individuals per host respectively). We suggest that the lower density of *Chaetopterus* sp. would lead to the observed higher number of its associated symbionts, as well as to the 100% in prevalence (69.5% in *C.* cf. *appenciculatus*). Most *Chaetopterus* sp. harbored two symbiotic species, which was extremely rare in *C.* cf. *appendiculatus*, suggesting lower interspecific interactions in the former. The crab and nudibranch symbionts of *Chaetopterus* sp. often share a host and lived in pairs. Thus, living in pairs and resource partitioning led to the species coexisting in the tubes of *Chaetopterus* sp. to establish a tightly packed community showing high species richness and mean intensity, together with a low species dominance. In contrast, the aggressive, strictly territorial species associated with *C.* cf. *appendiculatus* determine a symbiotic community strongly dominated by single species and, thus, with low species richness and mean intensity. Therefore, we suggest that the interspecific interactions are shaping species richness, intensity and dominance, while the intraspecific ones are just shaping intensity and abundance, and species composition may have been shaping the observed differences in community structure. We hypothesize that both host species could originally be allopatric. The evolutionary specialization of the symbiotic communities would occur in separated

geographical areas, while the posterior disappearance of the existing geographical barriers would lead to the overlapped distribution.

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Abstract

To assess whether closely related host species harbor similar symbiotic communities, we studied two polychaetes, *Chaetopterus* sp. (11 specimens) and *Chaetopterus* cf. *appendiculatus* (83 specimens) living in the soft bottoms of Nhatrang Bay (South China Sea, Vietnam). The former harbored the porcellanid crabs *Polyonyx* cf. *heox* and *Polyonyx* sp., the pinnotherid crab *Tetrias* sp. and the tergipedid nudibranch *Phestilla* sp. The latter harbored the polynoid polychaete *Ophthalmonoe pettiboneae*, the carapid fish *Onuxodon fowleri* and the porcellanid crab *Eulenaios cometes*. All them, except *O. fowleri*, seem to be specialized symbionts. Symbionts' species richness and mean intensity were higher in *Chaetopterus* sp. than in *C.* cf. *appendiculatus* (1.8 and 1.02 species and 3.0 and 1.05 individuals per host respectively). We suggest that the lower density of *Chaetopterus* sp. would lead to the observed higher number of its associated symbionts, as well as to the 100% in prevalence (69.5% in *C.* cf. *appenciculatus*). Most *Chaetopterus* sp. harbored two symbiotic species, which was extremely rare in *C.* cf. *appendiculatus*, suggesting lower interspecific interactions in the former. The crab and nudibranch symbionts of *Chaetopterus* sp. often share a host and lived in pairs. Thus, living in pairs and resource partitioning led to the species coexisting in the tubes of *Chaetopterus* sp. to establish a tightly packed community showing high species richness and mean intensity, together with a low species dominance. In contrast, the aggressive, strictly territorial species associated with *C.* cf. *appendiculatus* determine a symbiotic community strongly dominated by single species and, thus, with low species richness and mean intensity. Therefore, we suggest that the interspecific interactions are shaping species richness, intensity and dominance, while the intraspecific ones are just shaping intensity and abundance, and species composition may have been shaping the observed differences in community structure. We hypothesize that both host species could originally be allopatric. The evolutionary specialization of the symbiotic communities would occur in separated geographical areas, while the posterior disappearance of the existing geographical barriers would lead to the overlapped distribution.

1.- Introduction

During the last decades, significant efforts have been addressed to study the species composition and structure of marine symbiotic communities associated with different hosts taxa such as scleractinian corals (Hoeksema et al. 2012; Stella et al. 2010), echiurans (Anker et al. 2005), hermit crabs (Williams & McDermott 2004) and echinoderms (Barel & Kramers 1977). Despite this is an interesting aspect of the marine ecosystem's functioning and further research would be required to fill in the currently existing gap in the related knowledge, the current focus of scientific interests shifted to ecological and evolutionary aspects of the establishment of symbiotic communities (Baeza 2015; Duffy 2002; Thiel & Baeza 2001). Accordingly, host characteristics (morphological, ecological and physiological) have been considered as one of the most important parameters driving these processes (e.g., Abele & Patton 1976; Deheyn et al. 2006; Goto & Kato 2011).

The coexistence of potential hosts being taxonomically closely related (thus sharing similar morphological and physiological characteristics) facilitates host switching and leads to the infestation of different species by the same species of symbiont, as reported for instance in freshwater fish (Poulin 1998). Accordingly, we may expect the composition of the symbiotic communities established on closely related hosts to be similar. Hence, sympatric coral species belonging to the same family harbor symbiotic communities more similar than those belonging to different families (Stella et al. 2010), while the symbiotic communities associated with two starfish hosts from the same family living in the same area have nearly identical species composition (Antokhina et al. 2012). There seems to be a tendency linking an increasing taxonomical proximity between hosts to a higher similarity in species composition of the respective symbiotic communities. In other words, we could expect that closely related (i.e., belonging to the same genus) host species sharing also the same habitat would have to harbor very similar (or even identical) symbiotic communities. Therefore, we studied the symbiotic communities associated with two species of *Chaetopterus*, *Chaetopterus* sp. and *C.* cf. *appendiculatus* Grube, 1874 in Nhatrang Bay (Vietnam), to assess whether this hypothesis may apply to this particular situation.

Both species of *Chaetopterus* appeared to be an excellent subject for the intended comparison due to their very similar morphology. In fact, the genus has been long time considered as a monospecific and, to date, the morphological identification of the species is still being considered as rather complex (Britayev & Martin 2016; Nishi et al. 2009; Petersen 1984a; Petersen 1984b). Moreover, these two species share the same habitat, so that the influence of environmental parameters can be excluded as structuring factors for the associated symbiotic communities.

The genus *Chaetopterus* (Annelida: Chaetopteridae) include relatively large animals (up to 20−25 cm in length) living in roughly U-shaped tubes embedded into soft bottoms or attached to the hard surfaces in shallow waters of temperate and tropical seas (Britayev & Martin 2016). They are morphologically well adapted for feeding on plankton using complex mucus-net based mechanisms (Enders 1909). They are also well known as hosts harboring numerous symbiotic associates (often including complex communities) inside their parchment-like tubes that offers a well-protected shelter with continuous water flow bringing oxygen and food items to the symbionts (Britayev & Martin 2016). In fact, about 28 species of symbionts have been reported as living inside tubes of *Chaetopterus* (Britayev 1993). However, the information on the composition of the associated communities appears to be incomplete, as it is available only for two species, *C. pergamentaceus* Cuvier, 1830 and *C.* cf. *cautus* Marenzeller, 1879, which harbor to 3–5 species of crabs and polychaetes (Britayev 1993; Gray 1961).

The single currently known species of *Chaetopterus* inhabiting Vietnamese soft-bottoms, *C.* cf. *appendiculatus*, has been reported as harboring three species of symbionts inside its tubes: the polychaete *Ophthalmonoe pettiboneae* Petersen & Britayev 1997, and unidentified carapid fish and a porcellanid crab (Britayev & Martin 2005). The presence of a second, probably new, species of *Chaetopterus* sharing the same habitat and having its own associated symbiotic community allowed us to check the hypothesis postulating the similarity in composition of the symbiotic communities associated to taxonomically close hosts.

More specifically, in this paper we analyze: 1) The morphological and ecological characteristics of *C.* cf. *appendiculatus* and *Chaetopterus* sp.; 2) The composition, species richness and abundance of the symbiotic communities associated with the two host species; and 3) The host specificity of all symbiotic species.

2.- Material and methods

Sampling was conducted from March to April 2016 in four localities of Nhatrang Bay (Vietnam, South China Sea): the western coast of Mun Island, the southern coast of Mot Island, the western coast of Tre Island and Dam Bay (Fig. 1, Table 1). A copy of the letter of the Russian-Vietnamese Tropical Center supporting the collection of the samples and animals used in this paper has been provided to PeerJ as a supplementary file.

The chaetopterid hosts were collected by SCUBA diving at depths from 6 to 27 m. As their tubes were embedded into the sediment to 15−20 cm depth, they were extracted by washing out the sediments by hand. The tubes were then gently removed, immediately placed individually in zip-lock plastic bags to avoid losing symbionts and transferred to tanks with seawater on board, where they were kept until reaching the laboratory facilities.

The density of the studied population of *Chaetopterus* was estimated along five 50 m long and 2 m wide transects at Mun Island and Dam Bay. At each site, the transects followed the depth profile and were placed parallel, each ones immediately by the side of the previous one. Two divers were responsible of counting the number of chaetopterid tube syphons, each one on one side (1 m) of the transect. A second density estimate was based on the number of chaetopterids sampled per one hour at each sampling site (except when diving surveys were used for transect estimates).

In the laboratory, tube length was measured to the nearest 5.0 mm (Table S1). Then, tubes were gently opened by fingers and carefully checked for symbionts presence. The species and number of symbionts was recorded (Table S1). Water and sediment from the bag were sieved through 1 mm mesh and the retained sediments were carefully inspected with unaided eyes. Hosts were extracted and measured either as length and width of the region A (8 specimens) or as displaced water volume in a graduate vessel to the nearest 1 ml (all remaining specimens) (Table S1). As body volume shows a positive linear relationship with tube length (Tube length = 44.084+0.503*Body volume, F = 26.457, $P < 0.0001$; Table S2), this easy-to-obtain measure was used to study the community structure.

All chaetopterid tubes, hosts and symbionts were photographed with Canon digital cameras (G16 and EOS 6D). Selected hosts and all symbionts were fixed in 70% and 99% ethanol, as well as in a 4% formaldehyde/seawater solution for further studies. Small fragments of the ventral uncinal tori of both host species have been dissected. To illustrate the shape of the uncini, these fragments have been squashed between slides, mounted in glycerine and photographed with the help of a ProgRes C10 Plus digital camera (Jenoptics, Jena) attached to a Zeiss Axioplan compound microscope.

All symbionts were measured to the nearest 0.1 mm, using a calibrated ocular micrometer under an Olympus SZX9 stereomicroscope as body length from tip of prostomium to the end of pygidium for polychaetes, as body length from tip of head to the end of caudal peduncle for fish, and as carapace width for crabs. Crabs were sexed according to the abdominal shape.

DNA was extracted using Spin Columns Thermo Scientific GeneJET 50 kit, following the manufacturer's standard protocol. 10 ng of genomic DNA was used as a template for polymerase chain reaction (PCR) with special mitochondrial Cox1 primers: TGTAAAACGACGGCCAGTGAYTATWTTCAACAAATCATAAAGATATTGG and CAGGAAACAGCTATGACTAMACTTCWGGGTGACCAAARAATCA (Carr et al. 2011). PCR were set up in total volume of 20 µl. The PCR cycling profiles were as follows: initial denaturation (95°C, 5 min); followed by 35 cycles of denaturation (95°C, 15 sec), annealing (45°C, 15 sec) and extension (72°C, 60 sec). The resulting PCR products were purified by direct purification from the PCR mixture and prepared for sequencing. Overlapping sequence fragments were merged into consensus sequences using Mega version 6, the protein coding COI being trivial to align. The obtained COI sequences and voucher paragenophores (Pleijel et al. 2008) for the two Species of *Chaetopterus* have been deposited in the GenBank and in the collections of the Severtsov Institute of Ecology and Evolution RAS, respectively. Seven host specimens were used in genetic analysis to ensure species delineation (Table S3). The genetic differentiation within and between species was assessed by pairwise genetic distances between COI sequences using the Maximum Likelihood Model, which allowed us to show the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) next to each branch (Felsenstein 1985). The phylogenetic tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer it, as computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The phylogenetic tree was build using the COI sequences of *Chaetopterus* and *Mesochaetopterus* available from NCBI GenBank, using

Spiocharopterus costarum (Claparède 1869) as outgroup, by means of the Neighbor-Joining method (Saitou & Nei 1987) in MEGA7 (Kumar et al. 2016).

For the purposes of our study, the following terms are defined: Prevalence, as the ratio between number of infested and total number of hosts; Intensity, as the number of symbionts present in each infested host; Mean intensity, as the mean number of individuals of a particular symbiotic species per infested host in a sample; Abundance, as mean number of symbionts per examined host, infested and non-infested; and Species richness, as mean number of symbiotic species per infested host.

The porcellanid crabs were identified by Prof. Bernd Werding, from the Institut für Allgemenie und Spezielle Zoologie of the Justus-Liebig Universität (Giessen, Germany). The pinnotherid crab was identified by Prof. Peter Ng from the Department of Zoology of the National University of Singapore (Republic of Singapore). The carapid fish was identified by Dr. Eric Parmentier from the Laboratoire de Morphologie Fonctionnelle et Evolutive of the Institut de Chimie of the Université de Liege (Belgium). The tergipedid nudibranch was identified by Dr. Irina Ekimova, from the Department of Invertebrate Zoology of the Lomonosov Moscow State University (Russian Federation).

The relationship between host body volume and tube length were assessed by linear regression. The species richness and mean intensity, as well as the average length of infested and non-infested tubes of *C.* cf. *appediculatus*, were compared by Student’s t-test. Statistical analyses were performed using Statistica 6.0 and PAST 2.17 software.

3.- Results

3.1.- Hosts characteristics

The two Vietnamese host species of *Chaetopterus* are similar morphologically. However, one of them was significantly bigger than the other, both in terms of tube length (1.4:1, on average) and body volume (2.7:1, on average) (t-test $p<0.0001$, Table 2). They also differ in the number of chaetigers of region A (9 and 9-11, respectively) (Fig. 2A, 2D) and in the denticles of the neuropodial uncini of region C (25-35 and 9, respectively) (Fig. 3A–D), as well as in tube structure. Tubes of the big species are covered by silt, have a parchment-like aspect and the inner lining is iridescent, silver or golden in color and shows distinct transverse annulation (Fig. 2C). In the small species, tubes are covered by sand and small coral and shell fragments, have a paper-like aspect and the inner lining is semitransparent, whitish or brownish in color and lacks distinct annulations (Fig. 2F).

We tentatively identified the big species as *Chaetopterus* cf. *variopedatus* Grube, 1974 because, according to the original description, this species has the inner tube surface sand-colored and shows dense transverse annulations, but also because the neuropodial uncini from region C have more than 20 small denticles, the later being a singular feature among the species of *Chaetopterus*. In turn, the small host probably belongs to an undescribed species. The phylogenetic analysis including the COI sequences of the Vietnamese hosts (Fig. S1) shows low bootstrap values that do not allow us to correctly resolve the phylogeny of *Chaetopterus*. However, it clearly reveals the marked divergence between the two Vietnamese hosts, thus supporting our morphological inference that they are two different species.

Both host species were found at the same localities, with their tubes deeply embedded in silty sand bottoms. *Chaetopterus* cf. *appendiculatus* outnumbered *Chaetopterus* sp. in all samples, yet their proportion varied depending on the locality, with *Chaetopterus* sp. being relatively more abundant at Mot Islands (St. 2, 25.9%) and substantially less present in the other stations (5.0–9.1%) (Table 1).

The density *Chaetopterus* ranged from 0.6 to 1.0 specimens per 100 m² in the transects, while the number of collected worms per diving hour was lower at St. 4 in Dam Bay and higher at St. 1 in Point Nam, Tre Island (Table 1).

3.2.- Taxonomic composition of the symbiotic communities

91 specimens of 7 species of animals occurred in association with the two host species of *Chaetopterus*. Among them, the polynoid polychaete *Ophthalmonoe pettiboneae* (Fig. 4C), the tergipedid nudibranch *Phestilla* sp. (Fig. 5G), the carapid fish *Onuxodon fowleri* *(Smith 1964)* (Fig. 4D), and four species of decapods, three porcellanids, *Eulenaios cometes* (Walker 1887) (Fig. 4A, 4B), *Polyonyx* cf. *heox* Osawa & Ng, 2016 (Fig. 5A, 5B) and *Polyonyx* sp. (Fig. 5E, 5F), and the pinnotherid *Tetrias* sp. (Fig. 5C, 5D) (Table 3).

Four and three species were found inside the tubes of *Chaetopterus* sp. and *C.* cf. *appendiculatus*, respectively. Surprisingly, the symbiotic communities associated with the two hosts do not have any species in common, with the only similarity at a higher taxonomic level being the presence of porcellanid crabs (Table 3). Despite the lower sample size of *Chaetopterus* sp., the diversity of its associated community is higher than that of *C.* cf. *appendiculatus*. Accordingly, we may expect the number of species associated with *Chaetopterus* sp. to increase together with an increasing number of analyzed host specimens, while the diversity of the community associated with *C.* cf. *appendiculatus* shows an almost saturated species accumulation curve (Fig. 6A).

Ophthlmonoe pettiboneae is the single symbiotic species previously known from Vietnamese waters and from the same host species. The other six are here reported for the first time from the Vietnamese coasts. Moreover, *O. fowleri* is here reported as symbiont of chaetopterid for the first time, as well as *Tetrias* sp., *Polyonyx* sp., *P.* cf. *heox* and *Phestilla* sp., which also turned to be new for science and will be further described in specialized papers. The tergipedid nudibranch is also, to the best of our knowledge, the first known nudibranch living in symbiosis with a polychaete host. It shows a posterior end functioning as a sucker (Fig. 5F) allowing it to attach to the smooth inner surface of the host tube while the rest of the body can move detached (https://www.researchgate.net/publication/310159685_Phestilla_sp). Its flattened body, together with the lack of cnidosacs and the uniserial radula with long lateral denticles on the rachidian tooth clearly place it among the genus *Phestilla*. However, it differs from all known species of this genus by having a small central denticle in the radula, a wider foot and the cerata arranged as only one per row (Deart and Ekimova, personal communication). Moreover, its appearance and coloring (Fig. 5F) mimics, to some extent, those of the very posterior body end of the chaetopterid host.

3.3.- Structure of the symbiotic communities

56 (69.5%) over 83 specimens of *C. appediculatus* and all 11 (100%) *Chaetopterus* sp. were infested. Among the species associated with *C. appediculatus*, *O. pettiboneae* showed a higher prevalence than the two other symbionts (Table 3). Among the associates with *Chaetopterus* sp., the prevalence ranged from 11% to almost 90%, with the maximum one corresponding to *P.* cf. *heox* (Table 3).

The number of species inhabiting the same tube varied from 0 to 2 in *C.* cf. *appendiculatus*, and from 1 to 2 in *Chaetopterus* sp. However, the species richness was significantly higher in the latter ($p>0.001$, Fig. 6A, 6B) due to the common coexistence of two symbiont species in the same host. In fact, the small-sized *Polyonyx* sp. and *Phestilla* sp. (Fig. 5E–5G) were found in all cases living with other crab species, mostly with the large sized *P.* cf. *heox* (Fig. 5A, 5B). Only in one case, *Polyonyx* sp. shared the host tube with *Tetrias* sp. (Table 5). In contrast, most tubes of *C. appediculatus* were occupied by a single symbiotic species, either *O. pettiboneae* or *O. fowleri*.

Only in one case two species of symbionts (*O. pettiboneae* and *E. cometes*) shared the same host tube (Table 5).

The number of symbiont specimens infesting one host varied from 0 to 3 in *C.* cf. *appediculatus*, and from 1 to 5 in *Chaetopterus* sp., while the mean intensity was nearly three times lower in the former than in the later (p>0.001, Table 4). Accordingly, most *C.* cf. *appediculatus* were infested by one symbiotic specimen, while multiple infestation (two, three, or even four symbionts) was common in *Chaetopterus* sp. (Fig. 6B).

The distribution pattern of the symbionts among their hosts was primarily regular, as all. *O. pettiboneae* and most *O. fowleri* lived solitary inside their hosts. In turn, *Polyonyx* cf. *heox, Polyonyx* sp.*, E. cometes, Tetrias* sp. were found in host tubes usually as male/female pair. The number of nudibranches varied from 1 to 3 per hosts (Table 5) and, in one case, a couple was observed near to a recently spawned egg-mass attached to the inner side of the host tube (Fig. 5H).

The component communities differ also in the relative abundance of a particular species. In the community associated with *C.* cf. *appendiculatus*, *O. pettiboneae* was dominant in terms of both prevalence and abundance. In the community associated with *Chaetopterus* sp., the dominance of the most abundant symbiont, *P.* cf. *heox* is less distinctive, and the role the other species in the community structure is more relevant (Fig. 6C).

The average length of infested and non-infested tubes of *C. appediculatus* does not differ significantly (41.2–42.3, t-test, p=0.65). The number of both symbiotic species and specimens do not show any significant correlation with host tube length in both chaetopterid species.

4.- Discussion

4.1.- On the reasons of community dissimilarity

Our results demonstrate a strict segregation in species composition of the communities associated with the two Vietnamese syntopic species of *Chaetopterus*, which have no species in common. However, at higher taxonomic levels (i.e., family, order and class), they are similar each other and also resemble the symbiotic communities associated with other species of *Chaetopterus* and, even, echiuran worms in harboring scale-worms, pocellanid and pinnotherid decapods and fishes *(Anker et al. 2005; Gray 1961; Ng & Sasekumar 1993; Petersen & Britayev 1997)*.

The two Vietnamese species of *Chaetopterus* are very similar in body morphology and tube shape, as well as in their trophic-functional characteristics. Thus, no evident reasons linked to host morphology allow us to explain the dissimilarity in symbiotic species composition. We suggest that the community composition seems to be determined by historical events rather than by the physical or biological habitat characteristics. We may hypothesize that both host species were originally allopatric. Thus, the evolutionary establishment of the respective specialized symbiotic associations would likely occur in different, separated geographical areas, with the posterior disappearance of the geographical barriers leading to the current overlapping distribution. Once established, the respective symbiotic communities would be maintained by interspecific competition, leading to symbiont specialization to their respective host species as well as to preventing the exchange of symbionts between hosts when becoming sympatric, even if being as closely related as in the case of the two species of *Chaetopterus*. However, our hypothesis does not exclude the possible existence of differences in host physiology or behavior that would enhance the ability of the specialized symbionts to compete with possible invaders, thus contributing to maintain the differences in community composition.

Further assessment of this hypothesis would require an experimental approach to analyze the possible existence of a host-factor allowing the respective symbiont to recognize their own hosts, as well as to check the ability of the symbionts from one host to infest the other. In parallel, the regularity of the community segregation would have to be checked by more extensive field sampling addressed to discard (or reveal) the presence of additional symbionts on the alternative host species. This is particularly relevant for *Chaetopterus* sp. whose accumulation curve (Fig. 6A) supports an expected increase in the respective number of symbiotic species with sampling size. An additional, but not less pertinent question would be to assess the degree of specialization of the symbionts found in the two species of *Chaetopterus*, either based on previously published data or on our own observations. Therefore, it will be particularly relevant to consider whether they are obligatory or facultative and, in the case of obligatory symbionts, to further assess their degree of specificity (which may range from species-specific to opportunistic).

4.2.- Symbionts' specialization

Among the seven species of macroinvertebrates associated with *C.* cf. *appendiculatus* and *Chaetopterus* sp., four (i.e., one polychaete *O. pettiboneae*, one crab, *E. cometes*, and one fish, *O. fowleri*) are known as obligatory symbionts of chaetopterids and other benthic organisms. *Onuxodorn fowleri* also lives in the mantle cavity of bivalves and inside holothurians (Markle &

Olney 1990; Parmentier et al. 2002). In our samples, five of six specimens were juveniles, which allow us to suggest that they are employing *C.* cf. *appendiculatus* as temporal or intermediate host. The porcellanid *E. cometes* was reported from shallow waters off Singapore, living in association with a species of *Chaetopterus*, identified as *C. variopedatus* but certainly not this species (Ng & Nakasone 1993). In turn, the scale-worm *O. pettiboneae* was first reported from Ambon Island (Indonesia) and later from the coasts of Vietnam, always in association with *C.* cf. *appendiculatus* (Britayev & Martin 2005; Petersen & Britayev 1997; this paper).

The four other species appear to be new for science and are now being analyzed by the corresponding specialists. However, we may infer some considerations on their degree of specialization based on existing papers dealing with the ecology and morphology of some closely related taxa. Concerning the symbiotic crabs, the porcellanid *Polyonyx* sp. belongs to the "*Polyonyx sinensis*" species complex, usually obligatory associates of tubicolous polychaetes, mainly with species of *Chaetopterus* (B. Werding, unpublished data), while *Polyonyx* cf. *heox* belongs to the "*Polyonyx pedalis*" complex and the same or a very similar species has been recently reported from Singapore living in association with *Chaetopterus* cf. *pacificus* (Osawa & Ng 2016). This suggests that both porcellanids are obligate and, probably, specialized symbionts of *Chaetopterus* sp. In turn, *Tetrias* sp. belongs to the Pinnotheridae, a family that mainly includes symbiotic species living as endo- or ectosymbionts in mollusk mantle cavities, polychaete burrows, echinoid integuments or tunicate branchial sacs (Drake et al. 2014). Among them, two species of *Tetrias* are currently known. *Tetrias fischerii* (Milne-Edwards 1867) has been reported as symbiont of bivalves and annelids, while the host of *Tetrias scabripes* Rathbun, 1898 is unknown (Schmitt et al. 1973). Therefore, despite this cannot be assessed in light of our data, we suggest that the species associated with the Vietnamese *Chaetopterus* sp. is a specialized obligatory symbiont.

The third new species, the nudibranch *Phestilla* sp., has several behavioral (i.e., two or more specimens sharing the same host, egg-masses attached to the inner tube surface) and morphological (i.e., posterior end working as a sucker, overall body shape mimicking that of the host) features clearly pointing on a specialized symbiotic mode of life. This lead us to consider the species as the first know nudibranch living as symbiont with a marine annelid host. Nudribranches are well known as aposematic or mimetic organisms (Edmunds 1987; Gosliner & Behrens 1989; Rudman 1991), some of them being considered as true symbionts. Among them, there are some species of *Phestilla*, which lives in association with corals and are highly specialized predators (Faucci et al. 2007; Robertson 1970), while the exact nature of the association of the Vietnamese *Phestilla* sp. and *Chaetopterus* sp. would need in further research to be defined. The single related association occurred between the goniodorid nudibranch *Lophodoris scala* Er. Marcus & Ev. Marcus, 1970 and the innkeeper echiurid *Lissomyema exilii* (Müller 1883). In this case, the nudibranch lives inside the host burrow, sometimes creeping on the host trunk and feeding, possibly exclusively, on *Loxosomella* spp., an entoproct that colonizes the burrow walls (Ditadi 1982; Marcus & Marcus 1970), which seems not to be the case of the Vietnamese species.

Accordingly, all symbionts found in association with *Chaetopterus* sp. and *C.* cf. *appendiculatus* have to be considered as obligatory symbionts. Among them, the less specialized is *O. fowleri*, which is known to infested hosts belonging to different types of animals (mollusks and polychaetes). The porcellanid crabs *E. cometes* and *P.* cf. *heok* are probably genus-specific symbionts, while the scale-worm *O. pettiboneae*, together with the other porcellanid crab *Polyonyx* sp. and the tergipedid nudibranch *Phestilla* sp., must be considered as species-specific symbionts. The specificity of the pinnotherid crab *Tetrias* sp. seems not to be clear enough. However, taking into account the relative abundance of pinnotherids among symbionts of *Chaetopterus* species (Petersen & Britayev 1997; Schmitt et al. 1973), we could also propose it to be considered as specialized symbiont, at least at the family level.

Therefore, we consider all symbionts found in association with the two Vietnamese species of *Chaetopterus* as being, or tending to be, specialized symbionts, the single exception being the carapid fish.

4.3.- Possible causes of the observed differences in community structure

We found substantial differences in the structure of the symbiotic communities associated with *Chaetopterus* sp. and C. cf. *appendiculatus*. The first one shows a significantly higher species richness and mean abundance than the second, while the second one was clearly dominated by the presence of a single species, both in terms of abundance and frequency (Table 4, Fig. 5C). Taking into account that body size and tube length of *Chaetopterus* sp. are significantly lower than those of *C.* cf. *appendiculatus*, the situation certainly looks surprising. Usually, the species richness and abundance increase with the increasing host size (e.g., Abele & Patton 1976; Ribeiro et al. 2003). So, the sitiuation of the Vietnamese partnership requieres specific considerations.

We suggest that several factors are shaping the differences in the structure of the symbiotic communities associated with *Chaetopterus* sp. and C. cf. *appendiculatus*. Despite both host species have low population densities in Nhatrang Bay, that of *Chaetopterus* sp. was significantly lower, which would likely force the associated symbionts to use (and share) the few available hosts. This would possible explain the higher number of species in its associated community, as well as the fact that all host specimens of *Chaetopterus* sp. found in Nhatrang Bay harbored symbionts, in contrast to *C. appenciculatus* whose maximum prevalence was around 70%. Alternatively, the low density of both host populations may impede the secondary dispersion of the symbionts, which has been considered as a key mechanisms shaping the establishment and functioning of marine symbiotic communities (Mekhova et al. 2015) and raises the question on the adults' ability of long-distance migration.

Based on the species and specimens distribution among hosts, we also hypothesized that another factor determining the observed differences in symbiotic community structure could be the existence of inter- and intraspecific competition. In fact, whether most tubes of *Chaetopterus* sp. were occupied by a minimum of two symbiotic species, this co-occurence was very rare in *C.* cf. *appendiculatus*, where each host individual was infested by one symbiotic species (Fig. 6B). The single exception was a host tube shared by *O. pettiboneae* and *E. cometes*. Accordingly, we suggest that the main driving factors may be resource partitioning between symbiotic species having different sizes, in the case of *Chaetopterus* sp., and strong interspecific interactions, in the case of *C.* cf. *appendiculatus* as previously reported for holothurian hosts (Lyskin & Britayev 2005).

The characteristics of the structure of the symbiotic community associated with *C.* cf. *appendiculatus*, in which one host was usually occupied by one symbiotic species (Table 5), suggest the existence of interspecific competition between the polychaete and fish symbionts. In turn, the fact that there is a single symbiotic specimen per host (Table 5) supports the existence of intraspecific competition among polychaetes and fish individuals, respectively. At least for the polychaete, this hypothesis was supported by our direct observations in experimental aquaria, where specimens of *O. pettiboneae* where found fighting when trying to occupy the same host, as well as for the high frequency of presence of body traumas (Britayev and Martin, unpublished data). In contrast, the bulk of *Chaetopterus* sp. symbionts are crabs (Fig. 6C). Territorial defense is a well-known phenomenon in symbiotic decapods too (Baeza et al. 2002; Huber 1987; Vannini 1985). However, their behavior has a sexual component, as they often form heterosexual pairs consisting on gravid males and females co-inhabiting the same host (Castro 2015; Patton 1994), which was exactly the case of most porcellanid and pinnotherid crabs inhabiting the tubes of the two Vietnamese species of *Chaetopterus* (Table 5). This behavior leads to a significant increase in the abundance of symbiotic specimens in the community associated with *Chaetopterus* sp. Therefore, while interspecific interactions seemed to affect both species richness and abundance, the intraspecific ones just affect the abundance.

Our observations support that two main factors seemed to concur in determining the structure of the symbiotic communities associated with *Chaetopterus* sp. and C. cf. *appendiculatus* in Nhatrang Bay: the density of host populations and the competition (both inter- and intraspecific). Moreover, we may also state that the observed differences in community structure are strictly related to the respective species composition. Accordingly, living in pairs and resource partitioning led to the species coexisting in the tubes of *Chaetopterus* sp. to establish a tightly packed component community showing high species richness and mean intensity, together with a low species dominance. In contrast, the aggressive, strictly territorial species associated to *C.* cf. *appendiculatus* determine a component community strongly dominated by host inhabited by a single species and, thus, with low species richness and mean intensity.

The existence of two closely related host species with overlapping distributions but harboring very different symbiotic communities seems to be unusual. We suggest that it may probably be related with the scarcity of data currently available on the structure of symbiotic communities in marine environments. However, the situation is certainly not unique, as at least an additional example has been recently reported from Nhatrang Bay. In this case, the hosts were comatulid crinoids *Comanthus gisleni* Rowe, Hoggett, Birtles & Vail, 1986 and *C. parvicirrus* (Müller, 1841) (Mekhova & Britayev 2012). Accordingly, we expect further worldwide studies to discover more syntopic hosts harboring symbiotic communities with contrasted composition and structure.

5.- Conclusions

Two symbiotic communities inhabit the morphologically similar and syntopic species of the tube-dwelling chaetopterid polychaetes *Chaetopterus* sp. and *C.* cf. *appendiculatus* in Nhatrang Bay. They are mostly composed by specifically specialized species and show a very different composition. The current situation has been attributed to an initially allopatric host distribution allowing the symbiotic communities to be established independently, with the subsequent disappearance of the original geographical barriers leading to the current sympatry.

The present symbiotic communities differ in structural characteristics (i.e., species richness, mean intensity and species dominance) as a consequence of the differences in host density but also of the existing intra- and interspecific interactions that, in turn, depends on the behavior of the respective symbiotic species. Mating pairs and partitioned resources lead to high diversity and intensity in the community associated with *Chaetopterus* sp., while the aggressive and territorial species associated with *C.* cf. *appendiculatus* led to a community with low diversity and intensity but with a strong dominance of a single species.

The hypotheses postulating a similar composition for the symbiotic communities established on closely related hosts seems to reflect a rather common situation in marine ecosystems and, certainly, our results do not allow us to reject it. In fact, we are just describing the opposite situation, with two taxonomically related hosts living in the same habitat that harbor symbiotic communities with contrasted species composition. Therefore, we hypothesize on the possible reasons explaining their establishment. We also highlight that the situation of the Vietnamese partnerships is certainly not unique and should be considered as an interesting model to further assess different evolutionary and ecological aspects of the establishment of a symbiotic community.

Our results also point on the relevance of studying previously unknown symbiotic associations, which may provide key information allowing to understand the complex network of relationships driving the functioning of the marine ecosystems, particularly in benthic environments. Moreover, they are crucial in contributing to reveal the hidden biodiversity of the oceans, as supported by the fact that at least five over the nine species here studied turned to be new for the science.

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Table 1. Depth (m) and geographical coordinates of the studied locations, as well as density (chaetopterid specimens per 100 m² / per one diving hour), ratio of specimens (*Chaetopterus* sp. vs. *Chaetopterus* cf. *appendiculatus*) and infestation prevalence (%) of the respective host populations; nd: no data.

Locality	Station	Latitude	Longitude	Depth	Density	Ratio	Prevalence
Mun Island	1	12°10'10"N	109°17'46"E	13−16	1.0/3.8	2/20	65.2
Dam Bay	2	12°11'43"N	109°17'26"E	6−8	0.6/3.0	2/23	81.8
Mot Island	3	12°10'26"N	109°16'23"E	16−20	nd/4.7	7/20	90.9
Point Nam Tre Island	4	12°13'42"N	109°13'47"E	10−12	nd/7.1	1/19	60.0

Table 2. Number of specimens, mean tube length (min – max), cm and mean body volume (min – max) of *Chaetopterys* cf. *appendiculatus* and *Chaetopterus* sp.

Species	Number	Tube length (cm)	Body volume (cm^3)
Chaetopterus cf. *appendiculatus*	83	64.6 (41−88)	41.9 (23−72)
Chaetopterus sp.	11	44.8 (23−58)	15.8(2−32)

Table 3. Prevalence (%) and mean intensity (mean number of individuals per one infested host) of the symbiotic species associated with the two host *Chaetopterus*. P – Polychaeta, D – Decapoda, G – Gastropoda, A – Actinopteri.

Symbiont species	*Chaetopterus* sp.	*Chaetopterus* cf. *appendiculatus*
Ophthalmonoe pettiboneae (P)	-	64.1 (1.0)
Phestilla sp. (G)	22.2 (2.0)	-
Eulenaios cometes (D)	-	1.3 (2.0)
Polyonyx cf. *heox* (D)	88.9 (1.7)	-
Polyonyx sp. (D)	66.7 (1.3)	-
Tetrias sp. (D)	11.1 (2.0)	-
Onuxodon fowleri (A)	-	6.4 (1.2)

Table 4. Symbiotic community indexes: species richness (mean number of species per one infested host), infestation prevalence (%), and mean intensity (mean number of individuals per one infested host).

	Richness	Prevalence	Intensity
Chaetopterus sp.	1.8	100	3.0
Chaetopterus cf. *appendiculatus*	1.02	67.5	1.05

Table 5. Distribution among hosts (as number of host tubes found without and with 1, 2 and 3 individuals) for the five species associated with *Chaetopterus* spp. Cs: *Chaetopterus* sp.; Ca: *Chaetopterus* cf. *appendiculatus*.

Symbiont	*Chaetopterus*	0	1	2	3	4
Ophthalmonoe pettiboneae	Ca	21	56	0	0	0
Onuxodon fowleri	Ca	78	5	1	0	0
Eulenaios cometes	Ca	8	0	1	0	0
Polyonyx cf. *heox*	Cs	1	3	7	0	0
Polyonyx sp.	Cs	3	4	2	0	0
Tetrias sp.	Cs	10	0	1	0	0
Phestilla sp.	Cs	8	1	1	1	0

Table S1.- Main data on the two *Chaetopterus* hosts (tube length in cm, body volume in ml), symbionts' abundance and number of species, and number of specimens of each symbiotic species found in each individual host specimen.

Host			Symbiont		Polychaeta	Fish	Decapoda				Nudibranch
Specimen N	Tube length	Body volume	Abundance	N of species	*Ophthalmonoe pettiboneae*	*Onuxodon fowleri*	*Eulenaios cometes*	*Polyonyx* cf. *heox*	*Polyonyx* sp.	*Tetrias* sp.	*Phestilla* sp.

Chaetopterus sp.											
4	47	N/A	4	2	-	-	-	2	2	-	-
7	46	N/A	3	2	-	-	-	2	1	-	-
16	37	11	4	2	-	-	-	2	-	-	2
22	50	32	3	2	-	-	-	2	1	-	-
46	58	25	4	2	-	-	-	2	2	-	-
66	35	4	3	2	-	-	-	-	1	2	-
81	N/A	16	2	2	-	-	-	1	1	-	-
82	52	17	2	2	-	-	-	1	-	-	1
88	51		1	1	-	-	-	1	-	-	-
93	49	19	2	1	-	-	-	2	-	-	-
94	23	2	5	2	-	-	-	2	-	-	3
Chaetopterus cf. *appendiculatus*											
1	64	33	3	2	1	-	2	-	-	-	-
2	66	N/A	1	1	1	-	-	-	-	-	-
3	65	N/A	1	1	1	-	-	-	-	-	-
5	88	N/A	1	1	1	-	-	-	-	-	-
6	44	32	1	1	1	-	-	-	-	-	-
8	61	44	1	1	1	-	-	-	-	-	-
9	47	28	1	1	1	-	-	-	-	-	-
10	71	49	1	1	1	-	-	-	-	-	-
11	77	44	1	1	1	-	-	-	-	-	-
12	70	44	1	1	1	-	-	-	-	-	-
13	66	31	1	1	1	-	-	-	-	-	-
14	67	44	1	1	1	-	-	-	-	-	-
15	62	46	1	1	1	-	-	-	-	-	-
17	60	37	1	1	1	-	-	-	-	-	-

18	59	40	1	1	1	-	-	-	-	-	-
19	69	45	1	1	1	-	-	-	-	-	-
20	71	34	1	1	1	-	-	-	-	-	-
21	67	41	1	1	1	-	-	-	-	-	-
23	61	34	1	1	1	-	-	-	-	-	-
24	58	N/A	1	1	1	-	-	-	-	-	-
25	74	48	1	1	1	-	-	-	-	-	-
26	69	43	1	1	1	-	-	-	-	-	-
27	70	46	1	1	1	-	-	-	-	-	-
28	68	72	1	1	1	-	-	-	-	-	-
29	62	40	1	1	1	-	-	-	-	-	-
30	64	49	1	1	1	-	-	-	-	-	-
31	75	36	1	1	1	-	-	-	-	-	-
32	61	32	1	1	1	-	-	-	-	-	-
33	75	48	1	1	1	-	-	-	-	-	-
34	52	25	1	1	1	-	-	-	-	-	-
35	N/A	38	1	1	1	-	-	-	-	-	-
36	59	24	1	1	1	-	-	-	-	-	-
37	61	43	1	1	1	-	-	-	-	-	-
38	68	N/A	1	1	1	-	-	-	-	-	-

Table S1. Cont.

Host			Symbiont		Polychaeta	Fish	Decapoda				Nudibranch
Specimen N	Tube length	Body volume	Abundance	N of species	*Ophthalmonoe pettiboneae*	*Onuxodon fowleri*	*Eulenaios cometes*	*Polyonyx* cf. *heox*	*Polyonyx* sp.	*Tetrias* sp.	*Phestilla* sp.
Chaetopterus cf. *appendiculatus*											
39	72	50	1	1	1	-	-	-	-	-	-
40	59	32	1	1	1	-	-	-	-	-	-
41	58	28	1	1	1	-	-	-	-	-	-
42	65	40	1	1	1	-	-	-	-	-	-
43	64	50	1	1	1	-	-	-	-	-	-
44	71	49	1	1	1	-	-	-	-	-	-
45	79	62	1	1	1	-	-	-	-	-	-
47	66	48	1	1	1	-	-	-	-	-	-
48	76	51	1	1	1	-	-	-	-	-	-
49	72	51	1	1	1	-	-	-	-	-	-

50	64	45	1	1	1	-	-	-	-	-	-
51	56	41	1	1	1	-	-	-	-	-	-
52	55	49	1	1	1	-	-	-	-	-	-
53	63	52	1	1	1	-	-	-	-	-	-
54	70	44	1	1	1	-	-	-	-	-	-
55	69	45	1	1	1	-	-	-	-	-	-
56	56	39	1	1	1	-	-	-	-	-	-
57	74	47	1	1	-	1	-	-	-	-	-
58	59	39	1	1	-	1	-	-	-	-	-
59	48	39	1	1	-	1	-	-	-	-	-
60	67	56	1	1	-	1	-	-	-	-	-
61	50	23	1	1	-	1	-	-	-	-	-
62	66	N/A	-	-	-	-	-	-	-	-	-
63	76	N/A	-	-	-	-	-	-	-	-	-
64	N/A	N/A	-	-	-	-	-	-	-	-	-
65	61	N/A	-	-	-	-	-	-	-	-	-
67	77	N/A	-	-	-	-	-	-	-	-	-
68	60	31	-	-	-	-	-	-	-	-	-
69	67	50	-	-	-	-	-	-	-	-	-
70	50	N/A	-	-	-	-	-	-	-	-	-
71	N/A	36	-	-	-	-	-	-	-	-	-
72	76	54	-	-	-	-	-	-	-	-	-
73	68	45	-	-	-	-	-	-	-	-	-
74	78	57	-	-	-	-	-	-	-	-	-
75	66	40	-	-	-	-	-	-	-	-	-
76	81	N/A	-	-	-	-	-	-	-	-	-
77	69	40	-	-	-	-	-	-	-	-	-
78	62	47	-	-	-	-	-	-	-	-	-

79	55	34	-	-	-	-	-	-	-	-	-
80	57	35	-	-	-	-	-	-	-	-	-
83	59	44	-	-	-	-	-	-	-	-	-
84	64	N/A	-	-	-	-	-	-	-	-	-
85	59	51	-	-	-	-	-	-	-	-	-
86	76	45	-	-	-	-	-	-	-	-	-
87	41	23	-	-	-	-	-	-	-	-	-
89	78	43	-	-	-	-	-	-	-	-	-
90	56	39	-	-	-	-	-	-	-	-	-
91	52	33	-	-	-	-	-	-	-	-	-
92	56	36	-	-	-	-	-	-	-	-	-

Table S2.- Body volume (ml) and tube length (cm) of the 45 specimens of *Chaetopterus* cf. *appendiculatus* selected to estimate the relationship between these two measures in the analysis of the community structure.

Specimen	Tube length	Body volume
1	44	32
2	61	44
3	47	28
4	71	49
5	64	33
6	77	44
7	60	31
8	70	44
9	66	31
10	67	44
11	67	50
12	62	46
13	60	37
14	59	40
15	69	45
16	71	34
17	67	41
18	61	34
19	76	54
20	74	48
21	69	43
22	70	46
23	68	72
24	68	45
25	78	57
26	62	40
27	64	49
28	75	36
29	66	40
30	61	32
31	75	48
32	52	25
33	59	24
34	69	40
35	61	43
36	62	47
37	55	34
38	74	47
39	72	50
40	59	32
41	58	28
42	65	40
43	57	35
44	64	50
45	59	44
46	71	49

Table S3. List of the specimens of the two Vietnamese host species used in the molecular analyses, detailing the GenBank accession numbers and the collection references for the voucher paragenophores.

Chaetoperus	Specimen number	Accession number	Voucher
cf. *appendiculatus*	14	KY124465	sevin Pl/Vn 2016Ch0001
cf. *appendiculatus*	76	KY124466	sevin Pl/Vn 2016Ch0002
cf. *appendiculatus*	77	KY124467	sevin Pl/Vn 2016Ch0003
cf. *appendiculatus*	80	KY124468	sevin Pl/Vn 2016Ch0004
sp.	16	KY124469	sevin Pl/Vn 2016Ch0005
sp.	82	KY124470	sevin Pl/Vn 2016Ch0006
sp.	93	KY124471	sevin Pl/Vn 2016Ch0007

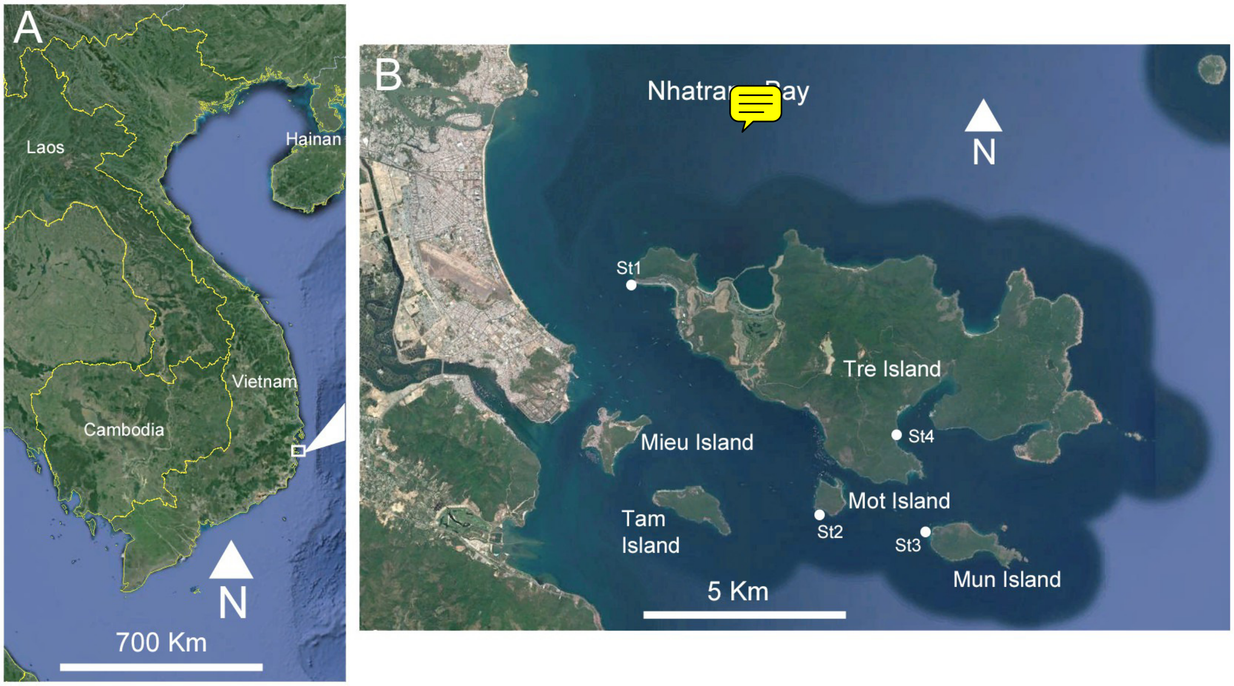


Figure 1. Location of the study area in the Vietnamese coast of the South China Sea (A), indicating the sampling sites in Nhatrang Bay (B). Map data from Google Earth Pro, (c) 2016 Google.

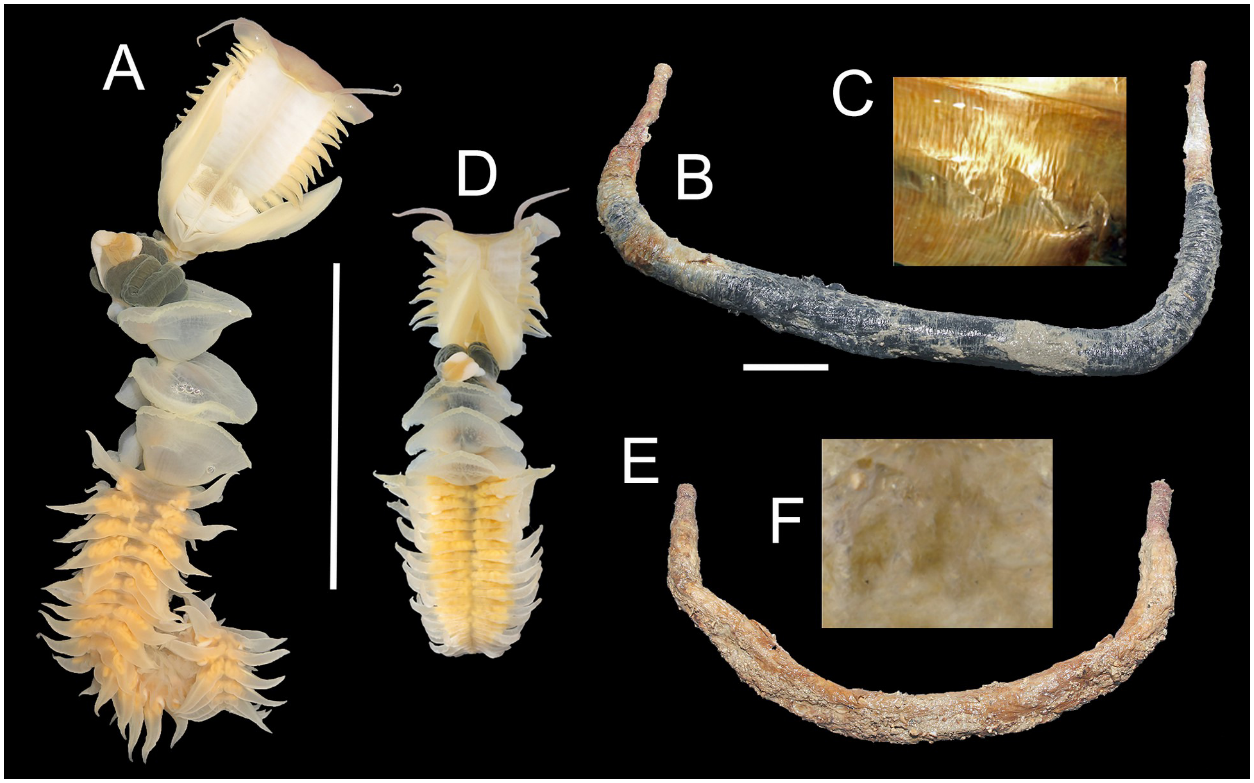


Figure 2.- *Chaetopterus* cf. *appendiculatus*: (A) whole worm; (B) tube; (C) detail of inner tube surface. *Chaetopterus* sp.: (D) whole worm; (E) tube; (F) detail of inner tube surface. Scale bars are 5 cm.

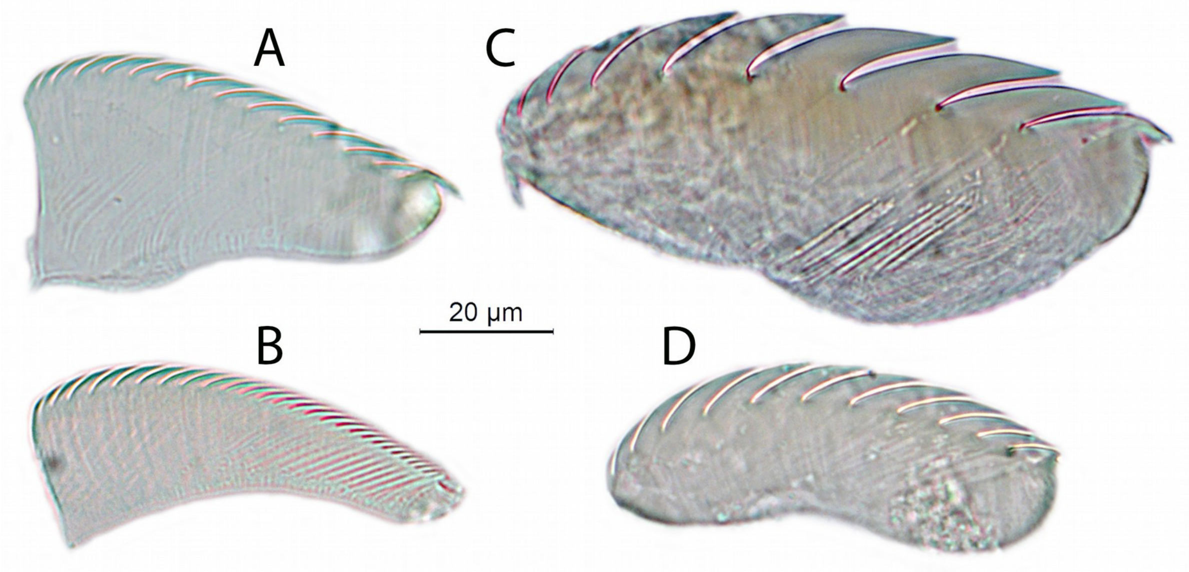


Figure 3. Uncini from ventral neuropodial tori of region C. *Chaetopterus* cf. *appendiculatus*: (A) upper tori; (B) lower tori. *Chaetopterus* sp.: (C) upper tori; (D) lower tori.

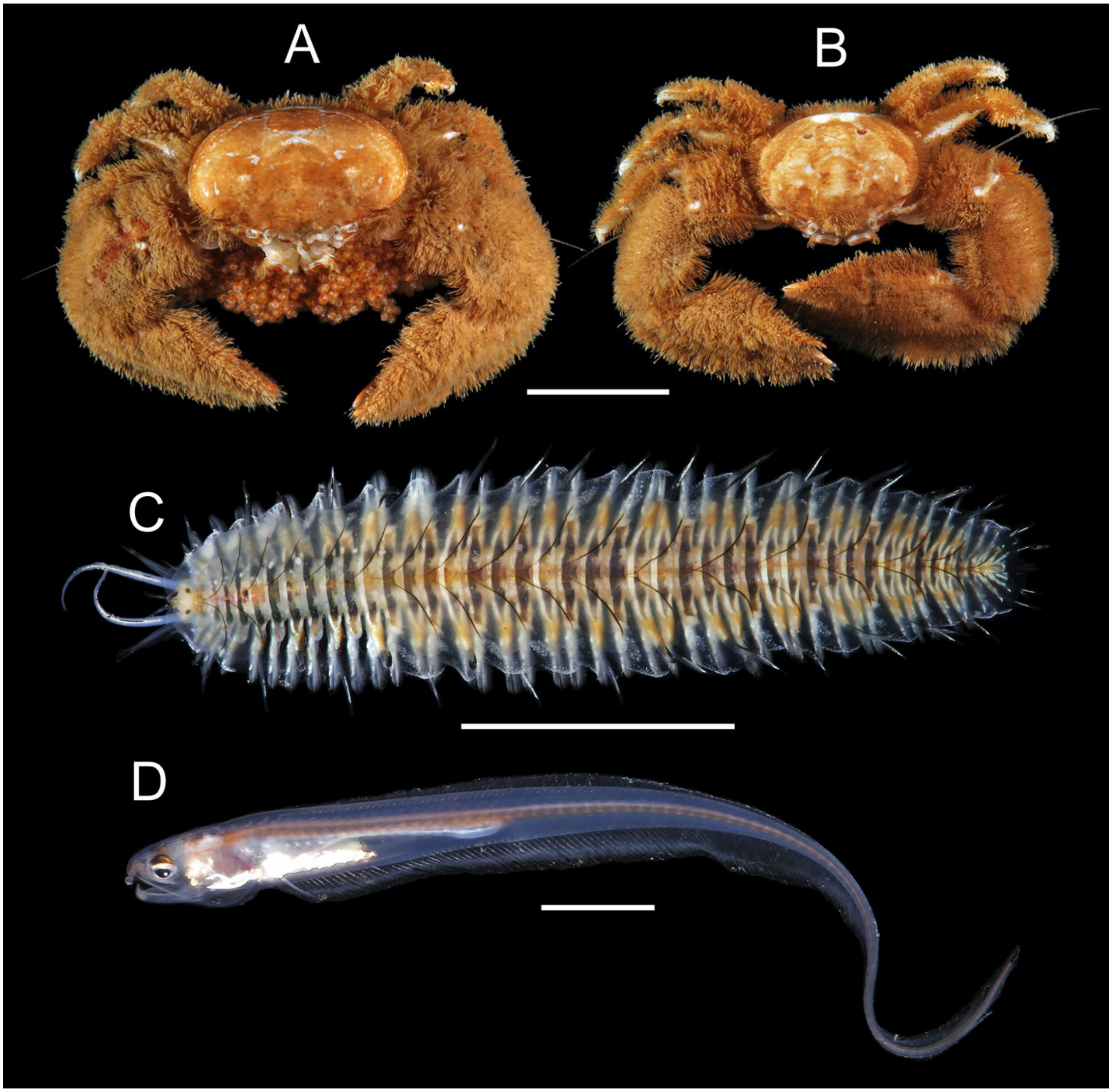


Figure 4. Symbiotic community associated to *Chaetopterus* cf. *appendiculatus*: (A, B) *Eulenaios cometes* (female and male, respectively); (C) *Ophthalmonoe pettibonneae*; (D) *Onuxodon fowleri*. Scale bars are 1 cm.

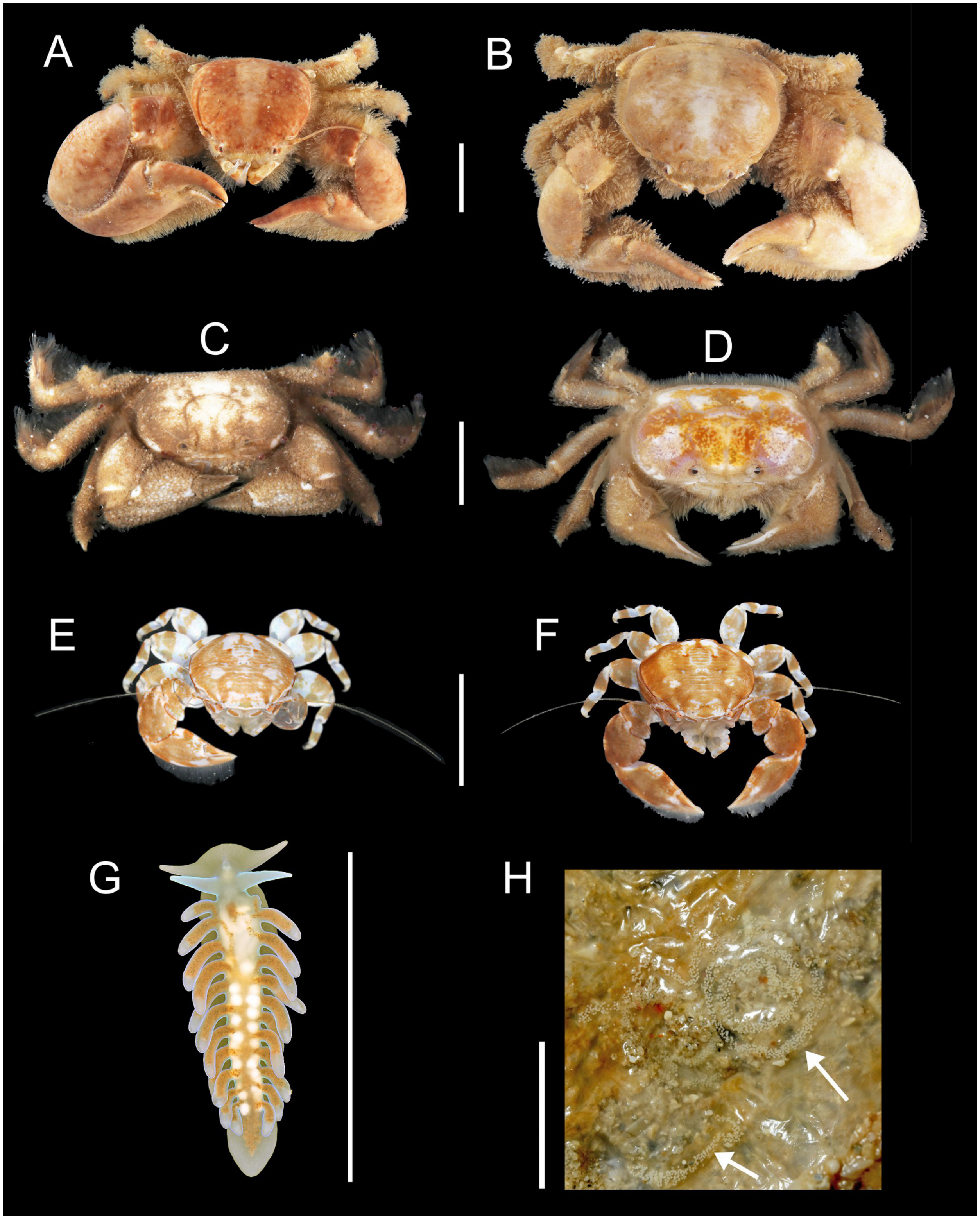
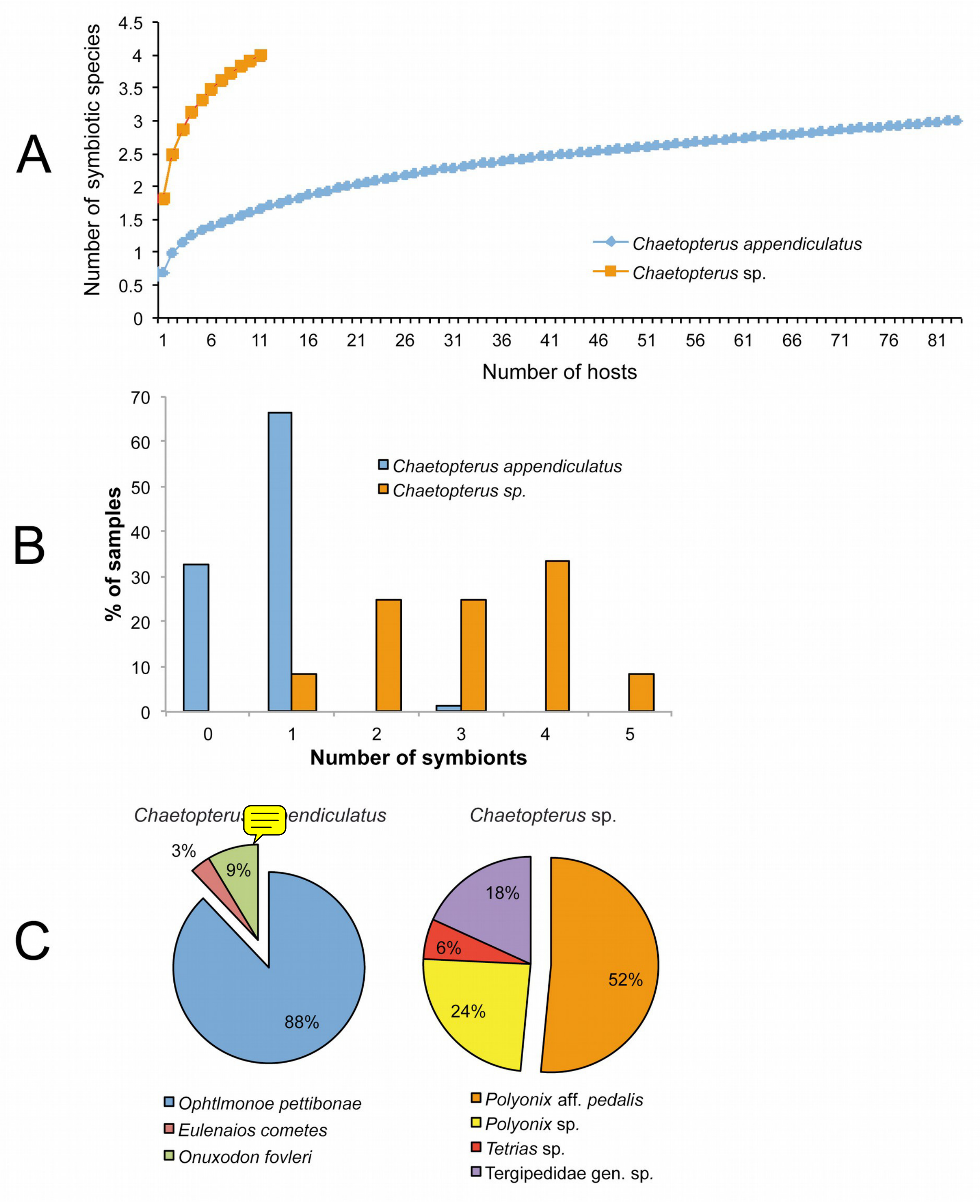
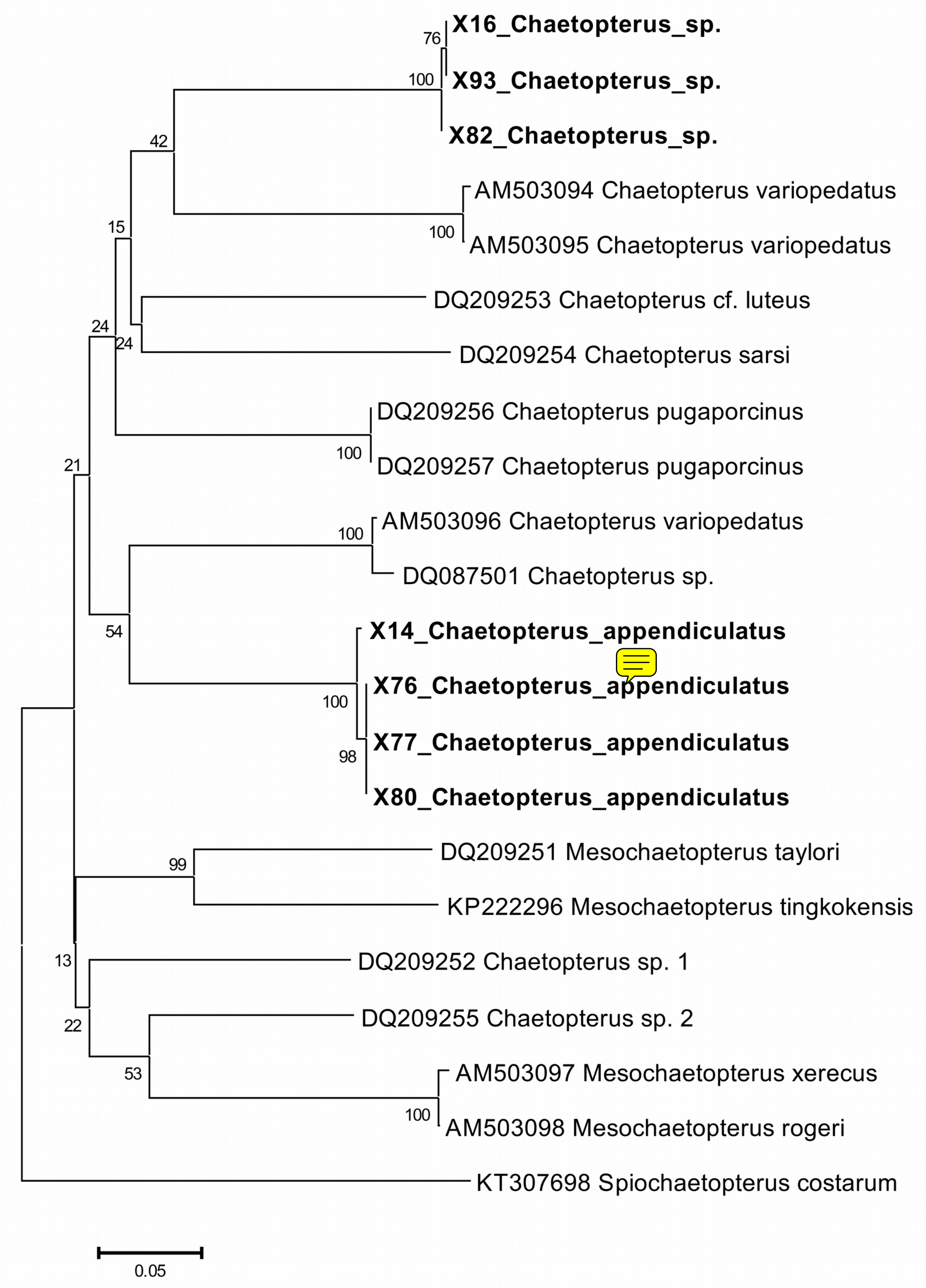


Figure 5. Symbiotic community associated to *Chaetopterus* sp.: (A, B) *Polyonyx* cf. *heox* (male and female, respectively); (C, D) *Tetrias* sp. (male and female, respectively); (E, F) *Polyonyx* sp., (male and female, respectively); (G, F) *Phestilla* sp. (whole body and egg-mass, respectively); arrows pointing on egg-mass. Scale bars are 0.5 cm.



668 Figure 6. Characterization of the symbiotic assemblages associated with the two host species of 669 *Chaetopterus:* (A) rarefaction curve; (B) distribution of symbionts per host; (C) relative 670 abundance of the symbiotic species.



671 Figure S1. Preliminary phylogenetic tree for the species of *Chaetopterus* and *Mesochaetopterus* 672 based on the COI sequences obtained from NCBI GenBank and our original ones data. The 673 sequences for the two Vietnamese species are listed in Table 2.