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

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To assess whether closely related host species harbour similar symbiotic communities, we studied two polychaetes, *Chaetopterus* sp. (n=11) and *Chaetopterus* cf. *appendiculatus* (n=83) living in soft sediments of Nhatrang Bay (South China Sea, Vietnam). The former harboured the porcellanid crabs *Polyonyx* cf. *heox* and *Polyonyx* sp., the pinnotherid crab *Tetrias* sp. and the tergipedid nudibranch *Phestilla* sp. The latter harboured the polynoid polychaete *Ophthalmonoe pettiboneae*, the carapid fish *Onuxodon fowleri* and the porcellanid crab *Eulenaios cometes*, all of which, except *O. fowleri*, seemed to be specialized symbionts. The species richness and mean intensity of the symbionts 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. may explain the higher number of associated symbionts observed, as well as the 100% prevalence (69.5% in *C.* cf. *appenciculatus*). Most *Chaetopterus* sp. harboured 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 shared a host and lived in pairs, thus partitioning resources. This led to the species coexisting in the tubes of *Chaetopterus* sp., establishing a tightly packed community, indicating high species richness and mean intensity, together with a low species dominance. In contrast, the aggressive, strictly territorial species associated with *C.* cf. *appendiculatus* established a symbiotic community strongly dominated by single species and, thus, low species richness and mean intensity. Therefore, we suggest that interspecific interactions are determining species richness, intensity and dominance, while intraspecific interactions are influencing only intensity and abundance. It is possible that species composition may have influenced the differences in community structure observed. 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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8 **Abstract**

9   To assess whether closely related host species harbour similar symbiotic communities, we

10 studied two polychaetes, *Chaetopterus* sp. (n=11) and *Chaetopterus* cf. *appendiculatus* (n=83)

11 living in soft sediments of Nhatrang Bay (South China Sea, Vietnam). The former harboured the

12 porcellanid crabs *Polyonyx* cf. *heox* and *Polyonyx* sp., the pinnotherid crab *Tetrias* sp. and the

13 tergipedid nudibranch *Phestilla* sp. The latter harboured the polynoid polychaete *Ophthalmonoe*

14 *pettiboneae*, the carapid fish *Onuxodon fowleri* and the porcellanid crab *Eulenaios cometes*, all of

15 which, except *O. fowleri*, seemed to be specialized symbionts. The species richness and mean

16 intensity of the symbionts were higher in *Chaetopterus* sp. than in *C.* cf. *appendiculatus* (1.8 and

17 1.02 species and 3.0 and 1.05 individuals per host respectively). We suggest that the lower

18 density of *Chaetopterus* sp. may explain the higher number of associated symbionts observed, as

19 well as the 100% prevalence (69.5% in *C.* cf. *appenciculatus*). Most *Chaetopterus* sp. harboured

20 two symbiotic species, which was extremely rare in *C.* cf. *appendiculatus*, suggesting lower

21 interspecific interactions in the former. The crab and nudibranch symbionts of *Chaetopterus* sp.

22 often shared a host and lived in pairs, thus partitioning resources. This led to the species

23 coexisting in the tubes of *Chaetopterus* sp., establishing a tightly packed community, indicating

24 high species richness and mean intensity, together with a low species dominance. In contrast, the

25 aggressive, strictly territorial species associated with *C.* cf. *appendiculatus* established a

26 symbiotic community strongly dominated by single species and, thus, low species richness and

27 mean intensity. Therefore, we suggest that interspecific interactions are determining species

28   richness, intensity and dominance, while intraspecific interactions are influencing only intensity

29   and abundance. It is possible that species composition may have influenced the differences in

30   community structure observed. We hypothesize that both host species could originally be

31   allopatric. The evolutionary specialization of the symbiotic communities would occur in

32   separated geographical areas, while the posterior disappearance of the existing geographical

33   barriers would lead to the overlapped distribution.

34   **1.- Introduction**

35   During the last few decades, significant efforts have been undertaken to study the species

36   composition and structure of marine symbiotic communities associated with different hosts taxa

37   such as scleractinian corals (Hoeksema et al. 2012; Stella et al. 2010), echiurans (Anker et al.

38   2005), hermit crabs (Williams & McDermott 2004) and echinoderms (Barel & Kramers 1977).

39   Despite this being an interesting aspect of marine ecosystems' functioning and the need to fill in

40   existing gaps in related knowledge, the current focus of scientific interests have shifted to

41   ecological and evolutionary aspects of the establishment of symbiotic communities (Baeza 2015;

42   Duffy 2002; Thiel & Baeza 2001). Accordingly, host characteristics (morphological, ecological

43   and physiological) have been considered as some of the most important parameters driving these

44   processes (e.g., Abele & Patton 1976; Deheyn et al. 2006; Goto & Kato 2011).

45   The coexistence of potential hosts that are taxonomically closely related taxonomically

46   (thus sharing similar morphological and physiological characteristics) may facilitate host

47   switching, leading to the infestation of different host species by the same species of symbiont, as

48   reported for example in freshwater fish (Poulin 1998). Accordingly, we may expect the

49   composition of symbiotic communities established on closely related hosts to be similar. Hence,

50   sympatric coral species belonging to the same family harbour symbiotic communities more

51   similar than those belonging to different families (Stella et al. 2010), while the symbiotic

52   communities associated with two starfish hosts from the same family living in the same area have

53   nearly identical species composition (Antokhina et al. 2012). There seems to be a correlation

54   between increasing taxonomic proximity between hosts and a higher similarity in species

55   composition of the respective symbiotic communities. In other words, we could expect that

56   closely related (i.e., belonging to the same genus) host species sharing the same habitat would

57   harbour very similar (or even identical) symbiotic communities. Therefore, the current study

58 investigated the symbiotic communities associated with two species of *Chaetopterus* in Nhatrang

59 Bay (Vietnam), to assess whether this hypothesis may apply to this particular situation.

60   These two species of *Chaetopterus* appeared to be excellent subjects for the intended

61 comparison due to their highly similar morphology. In fact, the genus has long been long

62 regarded as monospecific and, to date, the morphological identification of species is still

63 considered as rather complex (Britayev & Martin 2016; Nishi et al. 2009; Petersen 1984a;

64 Petersen 1984b). Moreover, these two species share the same habitat and, thus the influence of

65 environmental parameters can be excluded as influential factors on the associated symbiotic

66 communities.

67   The genus *Chaetopterus* (Annelida: Chaetopteridae) includes relatively large animals (up to

68 20−25 cm in length) living in roughly U-shaped tubes embedded into soft sediments or attached

69 to hard surfaces in shallow waters of temperate and tropical seas (Britayev & Martin 2016).

70 Morphologically, they are highly adapted for feeding on plankton using complex mucus-net

71 based mechanisms (Enders 1909). They are also well known as hosts harbouring numerous

72 symbiotic associates (often including complex communities) inside their parchment-like tubes.

73 These tubes provide well-protected shelter with continuous water flow bringing oxygen and food

74 items to the symbionts (Britayev & Martin 2016). To date, approximately 28 species of symbionts

75 have been reported living inside tubes of *Chaetopterus* (Britayev 1993). However, information on

76 the composition of associated communities is lacking, and is currently only available for two

77 species, *C. pergamentaceus* Cuvier, 1830 and *C.* cf. *cautus* Marenzeller, 1879, which are each

78 host to 3–5 species of crabs and polychaetes (Britayev 1993; Gray 1961).

79   A species of *Chaetopterus* (not confirmed but probably *Chaetopterus* cf. *appendiculatus*

80 Grube, 1874) inhabiting Vietnamese soft seabed sediments was previously reported as harbouring

81 three species of symbionts within its tubes: the polychaete *Ophthalmonoe pettiboneae* Petersen &

82 Britayev 1997, an unidentified carapid fish and a porcellanid crab (Britayev & Martin 2005). The

83 presence of a second, probably undescribed, species of *Chaetopterus* sharing the same habitat and

84 having its own associated symbiotic community allowed us to investigate the hypothesis that

85 postulates the similarity in composition of symbiotic communities associated with

86 morphologically similar hosts.

87   More specifically, in this paper we analyse: 1) The morphological and ecological

88 characteristics of the two Vietnamese host species of *Chaetopterus*; 2) The composition, species

89 richness and abundance of the symbiotic communities associated with the two host species; and

90 3) The host specificity of all symbiotic species.

2.- Material and methods

91

92   Sampling was conducted between March and April 2016 in four localities of Nhatrang Bay

93   (Vietnam, South China Sea): the western coast of Mun Island, the southern coast of Mot Island,

94   the western coast of Tre Island and Dam Bay (Fig. 1, Table 1). A copy of the letter from the

95   Russian-Vietnamese Tropical Center supporting the collection of samples and animals used in

96   this paper has been provided to PeerJ as a supplementary file.

97   The chaetopterid hosts were collected by SCUBA diving at depths from 6 to 20 m. As their

98   tubes were embedded within the sediment to 15−20 cm depth, extraction was achieved by

99   washing out the sediments by hand. The tubes were then gently removed, immediately placed

100   into individual zip-lock plastic bags to avoid losing symbionts and transferred to seawater tanks,

101   where they were kept until reaching the laboratory facilities.

102   The density of the studied population of *Chaetopterus* was estimated along five 50 m long

103   and 2 m wide transects at Mun Island and Dam Bay. At each site, the transects followed the depth

104   profile and were placed parallel, each one immediately adjacent to the previous one. Two divers

105   were responsible for counting the number of chaetopterid tube syphons, each one on one side (1

106   m) of the transect. A second density estimate was based on the number of chaetopterids sampled

107   per hour at each sampling site (except when diving surveys were used for transect estimates).

108   In the laboratory, tube length was measured to the nearest 5.0 mm (Table S1). Then, tubes

109   were gently opened by hand and carefully checked for presence of symbionts. The species and

110   number of symbionts were recorded (Table S1). Water and sediment from the bag were sieved

111   through a 1 mm mesh and the retained sediments were carefully inspected by eye. The body in

112   *Chaetopterus* is divided into three differentiated regions: the nine-to-ten anterior-most segments,

113   the two-to-three mid-body segments, and an undefined (but usually very numerous) number of

114   posterior segments, which form the regions A, B, and C, respectively (Britayev & Martin 2016).

115   Hosts were extracted and measured either as length and width for region A (n=8) or as displaced

116   water volume in a graduate vessel to the nearest 1 ml (all remaining specimens) (Table S1). As

117   body volume showed a positive linear relationship with tube length (Tube length =

118   44.084+0.503*Body volume, F = 26.457, *P* < 0.0001; Table S2), this easy-to-obtain measurement

119   was used to study community structure.

120   All chaetopterid tubes, hosts and symbionts were photographed with Canon digital cameras

121   (G16 and EOS 6D). Selected hosts and all symbionts were fixed either in 70% or 99% ethanol, or

122   in a 4% formaldehyde/seawater solution for further studies. Small fragments of the ventral

123   uncinal tori of both host species have been dissected. To illustrate the shape of the uncini, these

124   fragments were squashed between slides, mounted in glycerine and photographed with the help

125   of a ProgRes C10 Plus digital camera (Jenoptics, Jena) attached to a Zeiss Axioplan compound

126   microscope.

127   All symbionts were measured to the nearest 0.1 mm, using a calibrated ocular micrometer

128   under an Olympus SZX9 stereomicroscope as body length from tip of prostomium to the end of

129   pygidium for polychaetes, as body length from tip of head to the end of caudal peduncle for fish,

130   and as carapace width for crabs. Crabs were sexed according to the abdominal shape.

131   DNA was extracted using Spin Columns Thermo Scientific GeneJET 50 kit, following the

132   manufacturer's standard protocol. 10 ng of genomic DNA was used as a template for polymerase

133   chain   reaction   (PCR)   with   special   mitochondrial   Cox1   primers:

134   TGTAAAACGACGGCCAGTGAYTATWTTCAACAAATCATAAAGATATTGG   and

135   CAGGAAACAGCTATGACTAMACTTCWGGGTGACCAAARAATCA (Carr et al. 2011). PCR

136   were set up in total volume of 20 μl. The PCR cycling profiles were as follows: initial

137   denaturation (95°C, 5 min); followed by 35 cycles of denaturation (95°C, 15 sec), annealing

138   (45°C, 15 sec) and extension (72°C, 60 sec). The resulting PCR products were purified by direct

139   purification from the PCR mixture and prepared for sequencing. Overlapping sequence

140   fragments were merged into consensus sequences using MEGA7 (Kumar et al. 2016), the protein

141   coding COI being simple to align. The obtained COI sequences and voucher paragenophores

142   (Pleijel et al. 2008) for the two species of *Chaetopterus* have been deposited in GenBank and in

143   the collections of the Severtsov Institute of Ecology and Evolution RAS, respectively. Seven host

144   specimens were used in genetic analyses to ensure species delineation (Table 2). The genetic

145   differentiation within and between species was assessed by pairwise genetic distances between

146   COI sequences using the Maximum Likelihood Model, which allowed us to show the percentage

147   of replicate trees in which the associated taxa clustered together in the bootstrap test (1000

148   replicates) next to each branch (Felsenstein 1985). The phylogenetic tree was drawn to scale,

149   with branch lengths in the same units as those of the evolutionary distances used to infer it, as

150   computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the

151   units of the number of base substitutions per site. The phylogenetic tree was built using the COI

152   sequences of *Chaetopterus* and *Mesochaetopterus* available from NCBI GenBank, using

153 *Spiocharopterus costarum* (Claparède 1869) as the outgroup, by means of the Neighbor-Joining

154 method (Saitou & Nei 1987) in MEGA7 (Kumar et al. 2016).

155  For the purposes of our study, the following terms are defined: Prevalence, as the ratio

156 between number of infested and total number of hosts; Intensity, as the number of symbionts

157 present in each infested host; Mean intensity, as the mean number of individuals of a particular

158 symbiotic species per infested host in a sample; Abundance, as mean number of symbionts per

159 examined host, infested and non-infested; and Species richness, as mean number of symbiotic

160 species per infested host.

161  The porcellanid crabs were identified by Prof. Bernd Werding, from the Institut für

162 Allgemenie und Spezielle Zoologie of the Justus-Liebig Universität (Giessen, Germany). The

163 pinnotherid crab was identified by Prof. Peter Ng from the Department of Zoology of the

164 National University of Singapore (Republic of Singapore). The carapid fish was identified by Dr.

165 Eric Parmentier from the Laboratoire de Morphologie Fonctionnelle et Evolutive of the Institut

166 de Chimie of the Université de Liege (Belgium). The tergipedid nudibranch was identified by Dr.

167 Irina Ekimova, from the Department of Invertebrate Zoology of the Lomonosov Moscow State

168 University (Russian Federation).

169  The relationship between host body volume and tube length were assessed by linear

170 regression. The species richness and mean intensity, as well as the average length of infested and

171 non-infested tubes of *Chaetopterus*, were compared by Student's t-test. Statistical analyses were

172 performed using Statistica 6.0 and PAST 2.17 software.

173 **3.- Results**

174 3.1.- Hosts characteristics

175  The two Vietnamese host species of *Chaetopterus* are morphologically similar. However,

176 one of them is significantly bigger than the other, both in terms of tube length (1.4:1, on average)

177 and body volume (2.7:1, on average) (t-test p<0.0001, Table 3). They also differ in the number of

178 chaetigers of region A (9 and 9-11, respectively) (Fig. 2A, 2D) and in the denticles of the

179 neuropodial uncini of region C (25-35 and 9, respectively) (Fig. 3A–D), as well as in tube

180 structure. Tubes of the bigger species are covered by silt, have a parchment-like appearance and

181 the inner lining is iridescent, silver or golden in colour, showing distinct transverse annulations

182 (Fig. 2C). In the smaller species, tubes are covered by sand and small coral and shell fragments,

183 have a paper-like appearance with a semi-transparent inner lining, whitish or brownish in colour

184 and lacking distinct annulations (Fig. 2F).

185   We tentatively identified the bigger host species as *Chaetopterus* cf. *appendiculatus*

186 because, according to the original description, this species has a sand-coloured inner tube surface,

187 showing dense transverse annulations. Additionally, it is the only described species of

188 *Chaetopterus* possessing neuropodial uncini from region C with more than 20 small denticles.

189 *Chaetopterus appendiculatus* was already reported as host of *O. pettiboneae* from the Banda Sea

190 (Indonesia) by Petersen & Britayev (1997). Petersen (1997) proposed the redescription of *C.*

191 *appendiculatus* as a valid species, based on the type material from Ceylon. The fact that formal

192 redescription has never been published does not prevent us from considering the species as valid,

193 whose formal redescription is far beyond the scope of this paper. However, the long geographical

194 distance between Ceylon/Indonesia and Vietnam prevents us in fully assigning the Vietnamese

195 specimens to *C. appendiculatus*, and we refer to the species as *C.* cf. *appendiculatus* in this paper.

196 The smaller host is likely undescribed.

197   The phylogenetic analysis including the COI sequences of the Vietnamese hosts (Fig. 4)

198 showed low bootstrap values that did not allow us to fully resolve the phylogeny of

199 *Chaetopterus*. However, it clearly revealed that the two Vietnamese hosts are different species

200 included within two separate monophyletic clades (with 100% bootstrap support), thus

201 confirming our morphological inference. Although with low support, the closest clades to those

202 of the two Vietnamese *Chaetopterus* belong to *C. variopedatus* (Renier 1804). However, the

203 specimens joining the *Chaetopterus* sp. clade (42% bootstrap support) originate from the

204 Mediterranean, while those joining the *C.* cf. *appendiculatus* clade (54% bootstrap support)

205 originate from the Atlantic. As indicated by Martin et al. (2008), our results support the inference

206 that the two populations of *C. variopedatus* belong to different species, with the Mediterranean

207 species described and the Atlantic species still undescribed. The results also confirm that *C.*

208 *variopedatus* sensu Hartman (1959) is not a single cosmopolitan species, but a complex including

209 more than 20 different species (Bhaud 1998; Osborn et al. 2007; Petersen 1984a; Petersen 1984b;

210 Petersen 1997). As is the case for *C. appendiculatus*, some of these species have not yet been

211 formally redescribed. However, as many as nine species have recently been described, and five

212 have been redescribed in the recent literature (Nishi 2001; Nishi et al. 2000; Nishi et al. 2009;

213 Osborn et al. 2007; Sun & Qiu 2014).

214   The two Vietnamese *Chaetopterus* host species were found at the same localities, with their

215 tubes deeply embedded in silty sand sediments. *Chaetopterus* cf. *appendiculatus* outnumbered

216   *Chaetopterus* sp. in all samples, yet their proportion varied depending on the locality, with
217   *Chaetopterus* sp. being relatively more abundant at Mot Islands (St. 2, 25.9%) and substantially
218   less abundant at the other stations (5.0–9.1%) (Table 1).
219   The density of *Chaetopterus* ranged from 0.6 to 1.0 individuals per 100 m² in the transects,
220   while the number of collected worms per diving hour was lower at St. 4 in Dam Bay and higher
221   at St. 1 in Point Nam, Tre Island (Table 1).

222   3.2.- Taxonomic composition of the symbiotic communities
223   91 individuals of seven species of animals occurred in association with the two host species
224   of *Chaetopterus*. Among them, the polynoid polychaete *Ophthalmonoe pettiboneae* (Fig. 5C), the
225   tergipedid nudibranch *Phestilla* sp. (Fig. 6G), the carapid fish *Onuxodon fowleri (Smith 1964)*
226   (Fig. 5D), and four species of decapods, three porcellanids, *Eulenaios cometes* (Walker 1887)
227   (Fig. 5A, 5B), *Polyonyx* cf. *heox* Osawa & Ng, 2016 (Fig. 6A, 6B) and *Polyonyx* sp. (Fig. 6E,
228   6F), and the pinnotherid *Tetrias* sp. (Fig. 6C, 6D) (Table 4).
229   Four and three species were found inside the tubes of *Chaetopterus* sp. and *C.* cf.
230   *appendiculatus*, respectively. Surprisingly, the symbiotic communities associated with the two
231   hosts did not have any species in common, with the only similarity at a higher taxonomic level
232   being the presence of porcellanid crabs (Table 4). Despite the lower sample size of *Chaetopterus*
233   sp., the diversity of its associated community was higher than that of *C.* cf. *appendiculatus*.
234   Accordingly, it may be expected that the number of species associated with *Chaetopterus* sp.
235   would increase with an increasing number of analysed host individuals. Conversely, the diversity
236   of the community associated with *C.* cf. *appendiculatus* showed an almost saturated species
237   accumulation curve (Fig. 7A).
238   *Ophthlmonoe pettiboneae* is the single symbiotic species previously known from
239   Vietnamese waters and from the same host species. The other six are here reported for the first
240   time from the Vietnamese coasts. Moreover, *O. fowleri* is herein reported as a symbiont of
241   chaetopterids for the first time, as well as *Tetrias* sp., *Polyonyx* sp., *P.* cf. *heox* and *Phestilla* sp.,
242   which are new to science and will be described at later dates in specialized papers. The tergipedid
243   nudibranch is also, to the best of our knowledge, the first known nudibranch living in symbiosis
244   with a polychaete host. It shows a posterior end functioning as a sucker (Fig. 6F) allowing it to
245   attach to the smooth inner surface of the host tube, while the rest of the body can move freely
246   (https://www.researchgate.net/publication/310159685_Phestilla_sp). Its flattened body, together
247   with the lack of cnidosacs and the uniserial radula with long lateral denticles on the rachidian

248   tooth clearly place it within the genus *Phestilla*. However, it differs from all known species of

249   this genus by having a small central denticle of the radula, a wider foot and cerata arranged one

250   per row only (Deart and Ekimova, personal communication). Moreover, its appearance and

251   colouring (Fig. 6F) mimics, to some extent, that of the very posterior end of the chaetopterid host.

252   3.3.- Structure of the symbiotic communities

253   56 (69.5%) out of 83 individuals of *C. appediculatus* and all 11 (100%) *Chaetopterus* sp.

254   were infested. Among the species associated with *C. appediculatus*, *O. pettiboneae* showed a

255   higher prevalence than the two other symbionts (Table 4). Among the associates with

256   *Chaetopterus* sp., the prevalence ranged from 11% to almost 90%, with the maximum

257   corresponding to *P.* cf. *heox* (Table 4).

258   The number of species inhabiting the same tube varied from 0 to 2 in *C.* cf. *appendiculatus*,

259   and from 1 to 2 in *Chaetopterus* sp. However, the species richness was significantly higher in the

260   latter (p>0.001, Fig. 7A, 7B) due to the common coexistence of two symbiont species in the same

261   host tube. In fact, the small-sized *Polyonyx* sp. and *Phestilla* sp. (Fig. 6E–6G) were found in all

262   observed cases living with other crab species, mostly with the large sized *P.* cf. *heox* (Fig. 6A,

263   6B). Only in one case, *Polyonyx* sp. shared the host tube with *Tetrias* sp. (Table 6). In contrast,

264   most tubes of *C. appediculatus* were occupied by a single symbiotic species, either *O.*

265   *pettiboneae* or *O. fowleri*. Only in one case two species of symbionts (*O. pettiboneae* and *E.*

266   *cometes*) were present sharing the same host tube (Table 6).

267   The number of symbiont individuals infesting one host varied from 0 to 3 in *C.* cf.

268   *appediculatus*, and from 1 to 5 in *Chaetopterus* sp., while the mean intensity was nearly three

269   times lower in the former than in the later (p>0.001, Table 5). Accordingly, most *C.* cf.

270   *appediculatus* were infested by one symbiotic individual, while multiple infestation (two, three,

271   or even four symbionts) was common in *Chaetopterus* sp. (Fig. 7B).

272   The distribution pattern of the symbionts among their hosts was primarily regular, as all. *O.*

273   *pettiboneae* and most *O. fowleri* lived solitary inside their host tubes. In turn, *Polyonyx* cf. *heox,*

274   *Polyonyx* sp*., E. cometes, Tetrias* sp. were found in host tubes usually as male/female pair. The

275   number of nudibranchs varied from 1 to 3 per hosts (Table 6) and, in one case, a couple was

276   observed near to a recently spawned egg-mass attached to the inner side of the host tube (Fig.

277   6H).

278   The component communities differed also in the relative abundance of a particular species.

279   In the community associated with *C.* cf. *appendiculatus*, *O. pettiboneae* was dominant in terms of

280 both prevalence and abundance. In the community associated with *Chaetopterus* sp., the

281 dominance of the most abundant symbiont, *P*. cf. *heox* is less distinctive, with the role that other

282 species had in the community structure being more relevant (Fig. 7C).

283   The average length of infested and non-infested tubes of *C. appediculatus* does not differ

284 significantly (41.2–42.3, t-test, p=0.65). The number of both symbiotic species and individuals do

285 not show any significant correlation with host tube length in both chaetopterid species.

286 **4.- Discussion**

287 4.1.- Community dissimilarity

288   Our results demonstrate a strict segregation in species composition of the communities

289 associated with the two Vietnamese syntopic species of *Chaetopterus*, which had no species in

290 common. However, at higher taxonomic levels (i.e., family, order and class), they were similar to

291 each other and also resembled the symbiotic communities associated with other species of

292 *Chaetopterus* and, even, echiuran worms in harbouring scale-worms, pocellanid and pinnotherid

293 decapods and fishes *(Anker et al. 2005; Gray 1961; Ng & Sasekumar 1993; Petersen & Britayev*

294 *1997)*.

295   The two Vietnamese species of *Chaetopterus* are very similar in body morphology and tube

296 shape, as well as in their trophic-functional characteristics. Thus, no reasons linked to host

297 morphology were evident allowing us to explain the dissimilarity in symbiotic species

298 composition. We suggest therefore that community composition appears to be determined by

299 historical events rather than by the physical or biological habitat characteristics. We may

300 hypothesize that both host species were originally allopatric. Thus, the evolutionary

301 establishment of the respective specialized symbiotic associations would likely occur in different,

302 separated geographical areas, with the posterior disappearance of geographical barriers leading to

303 the current overlapping distribution. Once established, the respective symbiotic communities

304 would be maintained by interspecific competition, leading to symbiont specialization to their

305 respective host species as well as to preventing the exchange of symbionts between hosts when

306 becoming sympatric, even being as closely related as is the case for these two species of

307 *Chaetopterus*. However, our hypothesis does not exclude the possible existence of differences in

308 host physiology or behaviour that would enhance the ability of the specialized symbionts to

309   compete with possible invaders, thus contributing to maintain the differences in community

310   composition.

311   Further assessment of this hypothesis would require an experimental approach to analyse

312   the possible existence of a host-factor allowing the respective symbiont to recognize their own

313   hosts, as well as to check the ability of the symbionts from one host to infest the other. In parallel,

314   the regularity of the community segregation would have to be checked by more extensive field

315   sampling addressed to discard (or reveal) the presence of additional symbionts on the alternative

316   host species. This is particularly relevant for *Chaetopterus* sp. whose accumulation curve (Fig.

317   7A) supports an expected increase in the respective number of symbiotic species with sampling

318   size. An additional, but not less pertinent question would be to assess the degree of specialization

319   of the symbionts found in the two species of *Chaetopterus*, either based on previously published

320   data or on our own observations. Therefore, it would be particularly relevant to consider whether

321   they are obligatory or facultative and, in the case of obligatory symbionts, to further assess their

322   degree of specificity (which may range from species-specific to opportunistic).

323   4.2.- Symbionts' specialization

324   Among the seven species of macroinvertebrates associated with *C.* cf. *appendiculatus* and

325   *Chaetopterus* sp., four (i.e., one polychaete *O. pettiboneae*, one crab, *E. cometes*, and one fish, *O.*

326   *fowleri*) are known as obligatory symbionts of chaetopterids and other benthic organisms.

327   *Onuxodorn fowleri* also lives in the mantle cavity of bivalves and inside holothurians (Markle &

328   Olney 1990; Parmentier et al. 2002). In our samples, five of six individuals were juveniles, which

329   allows us to suggest that they are employing *C.* cf. *appendiculatus* as temporal or intermediate

330   hosts. The porcellanid *E. cometes* was reported from shallow waters off Singapore, living in

331   association with a species of *Chaetopterus*, identified as *C. variopedatus* but this identification is

332   undoubtedly incorrect (Ng & Nakasone 1993). In turn, the scale-worm *O. pettiboneae* was first

333   reported from Ambon Island (Indonesia) and later from the coasts of Vietnam, always in

334   association with *C.* cf. *appendiculatus* (Britayev & Martin 2005; Petersen & Britayev 1997; this

335   paper).

336   The four other species appear to be undescribed and are now being analyzed by the

337   corresponding specialists. However, we may infer some considerations on their degree of

338   specialization based on existing papers dealing with the ecology and morphology of some closely

339   related taxa. Concerning the symbiotic crabs, the porcellanid *Polyonyx* sp. belongs to the

340   "*Polyonyx sinensis*" species complex, usually obligatory associates of tubicolous polychaetes,

341   mainly with species of *Chaetopterus* (B. Werding, unpublished data), while *Polyonyx* cf. *heox*

342   belongs to the "*Polyonyx pedalis*" complex and the same or a very similar species has been

343   recently reported from Singapore living in association with *Chaetopterus* cf. *pacificus* (Osawa &

344   Ng 2016). This suggests that both porcellanids are obligate and, probably, specialized symbionts

345   of *Chaetopterus* sp. In turn, *Tetrias* sp. belongs to the Pinnotheridae, a family that mainly

346   includes symbiotic species living as endo- or ectosymbionts in mollusc mantle cavities,

347   polychaete burrows, echinoid integuments or tunicate branchial sacs (Drake et al. 2014). Among

348   them, two species of *Tetrias* are currently known. *Tetrias fischerii* (Milne-Edwards 1867) has

349   been reported as symbiont of bivalves and annelids, while the host of *Tetrias scabripes* Rathbun,

350   1898 is unknown (Schmitt et al. 1973). Although this cannot be assessed from our data, we

351   suggest that the species associated with the Vietnamese *Chaetopterus* sp. is a specialized

352   obligatory symbiont.

353   The third undescribed species, the nudibranch *Phestilla* sp., has several behavioural (i.e.,

354   two or more individuals sharing the same host, egg-masses attached to the inner tube surface) and

355   morphological (i.e., posterior end working as a sucker, overall body shape mimicking that of the

356   host) features clearly pointing toward a specialized symbiotic mode of life. This lead us to

357   consider the species as the first know nudibranch living as a symbiont with a marine annelid host.

358   Nudibranchs are well known as aposematic or mimetic organisms (Edmunds 1987; Gosliner &

359   Behrens 1989; Rudman 1991), some of them being considered as true symbionts. Among them,

360   there are some species of *Phestilla*, which lives in association with corals and are highly

361   specialized predators (Faucci et al. 2007; Robertson 1970), while the exact nature of the

362   association of the Vietnamese *Phestilla* sp. and *Chaetopterus* sp. would need further research to

363   be defined. The single related association occurred between the goniodorid nudibranch

364   *Lophodoris scala* Er. Marcus & Ev. Marcus, 1970 and the innkeeper echiurid *Lissomyema exilii*

365   (Müller 1883). In this case, the nudibranch lives inside the host burrow, sometimes creeping

366   along the host trunk and feeding, possibly exclusively, on *Loxosomella* spp., an entoproct that

367   colonizes the burrow walls (Ditadi 1982; Marcus & Marcus 1970), which seems not to be the

368   case for the Vietnamese species.

369   Accordingly, all symbionts found in association with *Chaetopterus* sp. and *C.* cf.

370   *appendiculatus* have to be considered as obligatory symbionts. Among them, the less specialized

371   is *O. fowleri*, which is known to infest hosts belonging to different types of animals (molluscs

372   and polychaetes). The porcellanid crabs *E. cometes* and *P.* cf. *heok* are probably genus-specific

373   symbionts, while the scale-worm *O. pettiboneae*, together with the other porcellanid crab

374   *Polyonyx* sp. and the tergipedid nudibranch *Phestilla* sp., must be considered as species-specific

375   symbionts. The specificity of the pinnotherid crab *Tetrias* sp. is not clear at this time. However,

376   taking into account the relative abundance of pinnotherids among symbionts of *Chaetopterus*

377   species (Petersen & Britayev 1997; Schmitt et al. 1973), we could also propose that it should be

378   considered as a specialized symbiont, at least at family level.

379   Therefore, we consider all symbionts found in association with the two Vietnamese species

380   of *Chaetopterus* as being, or tending to be, specialized symbionts, the single exception being the

381   carapid fish.

382   4.3.- Possible causes of observed differences in community structure

383   We found substantial differences in the structure of the symbiotic communities associated

384   with *Chaetopterus* sp. and C. cf. *appendiculatus*. The first shows a significantly higher species

385   richness and mean abundance than the second, while the second was clearly dominated by the

386   presence of a single species, both in terms of abundance and frequency (Table 5, Fig. 6C). Taking

387   into account that body size and tube length of *Chaetopterus* sp. are significantly lower than those

388   of C. cf. *appendiculatus*, this situation is particularly unexpected. Usually, species richness and

389   abundance increase with the increasing host size (e.g., Abele & Patton 1976; Ribeiro et al. 2003).

390   Thus, the sitiuation of the Vietnamese partnership requires specific considerations.

391   We suggest that several factors are shaping the differences in the structure of the

392   symbiotic communities associated with *Chaetopterus* sp. and C. cf. *appendiculatus*. Despite both

393   host species having low population densities in Nhatrang Bay, that of *Chaetopterus* sp. was

394   significantly lower, which would likely force the associated symbionts to use (and share) the few

395   available hosts. This would possible explain the higher number of species in its associated

396   community, as well as the fact that all host individuals of *Chaetopterus* sp. found in Nhatrang

397   Bay harboured symbionts, in contrast to *C. appenciculatus* whose maximum prevalence was

398   around 70%. Alternatively, the low density of both host populations may impede the secondary

399   dispersion of the symbionts, which has been considered as a key mechanisms shaping the

400   establishment and functioning of marine symbiotic communities (Mekhova et al. 2015) and raises

401   the question on the adults' ability of long-distance migration.

402   Based on species and individual's distributions among hosts, we also hypothesized that

403   another factor determining the observed differences in symbiotic community structure could be

404   the existence of inter- and intraspecific competition. In fact, most tubes of *Chaetopterus* sp. were

405   occupied by a minimum of two symbiotic species, this co-occurence being very rare in C. cf.

406   *appendiculatus*, where each host individual was infested by one symbiotic species (Fig. 7B). The

407   single exception was a host tube shared by *O. pettiboneae* and *E. cometes*. Accordingly, we

408   suggest that the main driving factors may be resource partitioning between symbiotic species

409   having different sizes, in the case of *Chaetopterus* sp., and strong interspecific interactions, in the

410   case of *C.* cf. *appendiculatus* as previously reported for holothurian hosts (Lyskin & Britayev

411   2005).

412   The characteristics of the symbiotic community structure associated with *C.* cf.

413   *appendiculatus*, in which one host was usually occupied by one symbiotic species (Table 6),

414   suggest the existence of interspecific competition between the polychaete and fish symbionts. In

415   turn, the fact that there was a single symbiont per host (Table 6) supports the existence of

416   intraspecific competition among polychaetes and fish individuals, respectively. At least for the

417   polychaete, this hypothesis was supported by our direct observations in experimental aquaria,

418   where individuals of *O. pettiboneae* where found to fight when trying to occupy the same host

419   tube, as well as by the high frequency of body traumas present (Britayev and Martin, unpublished

420   data). In contrast, the bulk of *Chaetopterus* sp. symbionts were crabs (Fig. 7C). Territorial

421   defence is a well-known phenomenon in symbiotic decapods too (Baeza et al. 2002; Huber 1987;

422   Vannini 1985). However, their behaviour has a sexual component, as they often form

423   heterosexual pairs consisting of gravid males and females co-inhabiting the same host (Castro

424   2015; Patton 1994), which was exactly the case of most porcellanid and pinnotherid crabs

425   inhabiting the tubes of the two Vietnamese species of *Chaetopterus* (Table 6). This behaviour

426   lead to a significant increase in the abundance of symbiotic individuals in the community

427   associated with *Chaetopterus* sp. Therefore, while interspecific interactions seemed to affect both

428   species richness and abundance, the intraspecific ones only affected the abundance.

429   Our observations support two main factors determining the structure of symbiotic

430   communities associated with *Chaetopterus* sp. and C. cf. *appendiculatus* in Nhatrang Bay: the

431   density of host populations and competition (both inter- and intraspecific). Moreover, the

432   observed differences in community structure appear to be strictly related to the respective species

433   composition. Accordingly, living in pairs and resource partitioning led to species coexisting in the

434   tubes of *Chaetopterus* sp. and establish a tightly packed component community showing high

435   species richness and mean intensity, together with a low species dominance. In contrast, the

436   aggressive, strictly territorial species associated with *C.* cf. *appendiculatus* established a

437   component community strongly dominated by host being inhabited by a single species and, thus,

438   low species richness and mean intensity.

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

448   **5.- Conclusions**

449   Two symbiotic communities inhabit the morphologically similar and syntopic species of
450   the tube-dwelling chaetopterid polychaetes *Chaetopterus* sp. and *C.* cf. *appendiculatus* in
451   Nhatrang Bay. They are mostly composed of specifically specialized species and show a very
452   different composition. The current situation has been attributed to an initially allopatric host
453   distribution allowing the symbiotic communities to be established independently. This is then
454   followed by the subsequent disappearance of the original geographical barriers leading to the
455   current sympatry. The present symbiotic communities differ in structural characteristics (i.e.,
456   species richness, mean intensity and species dominance) as a consequence of the differences in
457   host density but also of the existing intra- and interspecific interactions that, in turn, depends on
458   the behaviour of the respective symbiotic species. Mating pairs and partitioned resources lead to a
459   high diversity and intensity in the community associated with *Chaetopterus* sp., while the
460   aggressive and territorial species associated with *C.* cf. *appendiculatus* led to a community with
461   low diversity and intensity but with a strong dominance of a single species.
462   The hypotheses postulating a similar composition for the symbiotic communities
463   established on closely related hosts seems to reflect a rather common situation in marine
464   ecosystems and, certainly, our results do not allow us to reject it. In fact, the opposite situation
465   was observed within our data, with two taxonomically related hosts living in the same habitat that
466   harbour symbiotic communities with contrasted species composition. Therefore, we hypothesize
467   on the possible reasons explaining their establishment. We also highlight that the situation of the
468   Vietnamese partnerships is certainly not unique and should be considered as an interesting model

469   to further assess different evolutionary and ecological aspects of the establishment of a symbiotic
470   community.
471   Our results also highlight the importance of studying previously unknown symbiotic
472   associations, which may provide key information allowing the complex network of relationships
473   driving the functioning of the marine ecosystems, particularly in benthic environments, to be
474   understood. Moreover, they are crucial in revealing the hidden biodiversity of the oceans, as
475   supported by the fact that at least five of the nine species herein studied are currently
476   undescribed.

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671   diversity and natural history of hermit crab associates. *Journal of Experimental Marine*
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673 Table 1. Depth (m) and geographical coordinates of the studied locations, as well as density

674 (chaetopterid individuals per 100 m² / per diving hour), ratio of individuals (*Chaetopterus* sp. vs.

675 *Chaetopterus* cf. *appendiculatus*) and infestation prevalence (%) of the respective host

676 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

677 Table 2. Specimens list for the two Vietnamese host species used in the molecular analyses,

678 detailing the GenBank accession numbers and the collection references for the voucher

679 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

680 Table 3. Number of individuals, mean tube length (min – max), cm and mean body volume (min

681 – max) of *Chaetopterus* cf. *appendiculatus* and *Chaetopterus* sp.

682

Species	Number	Tube length (cm)	Body volume (cm³)
Chaetopterus cf. *appendiculatus*	83	64.6 (41−88)	41.9 (23−72)
Chaetopterus sp.	11	44.8 (23−58)	15.8(2−32)

683   Table 4. Prevalence (%) and mean intensity (mean number of individuals per infested host) of the

684   symbiotic species associated with the two host *Chaetopterus*. P – Polychaeta, D – Decapoda, G –

685   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)

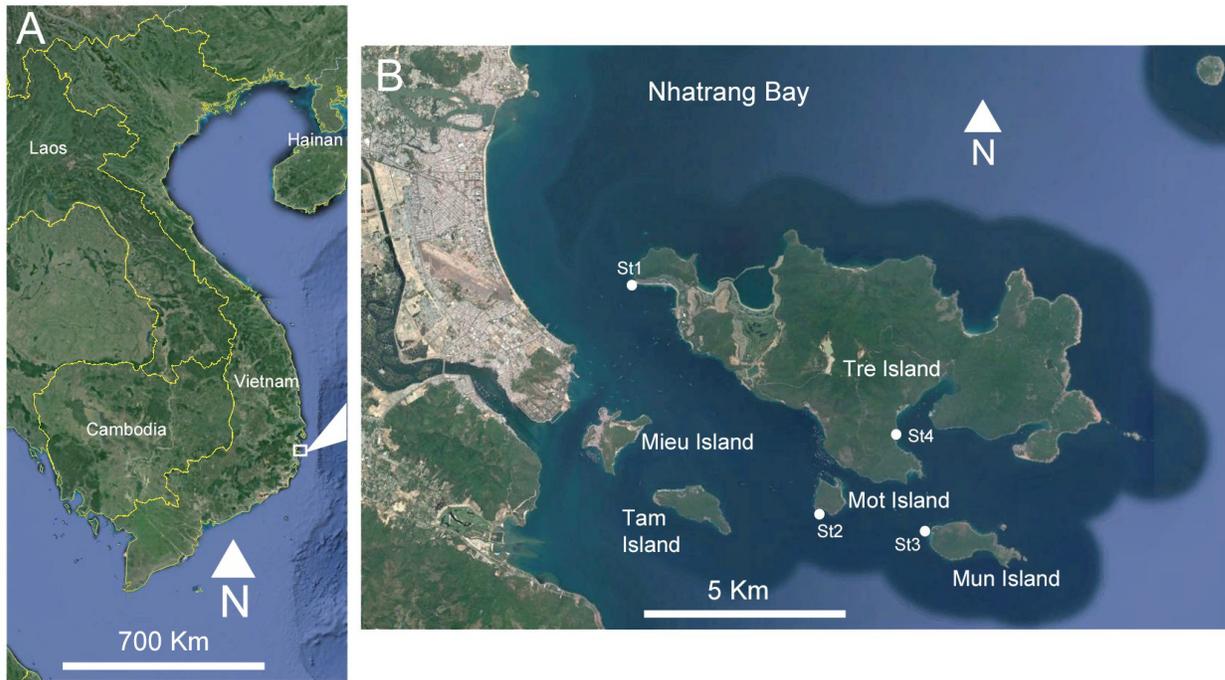
686 Table 5. Symbiotic community indexes: species richness (mean number of species per one
687 infested host), infestation prevalence (%), and mean intensity (mean number of individuals per
688 infested host).

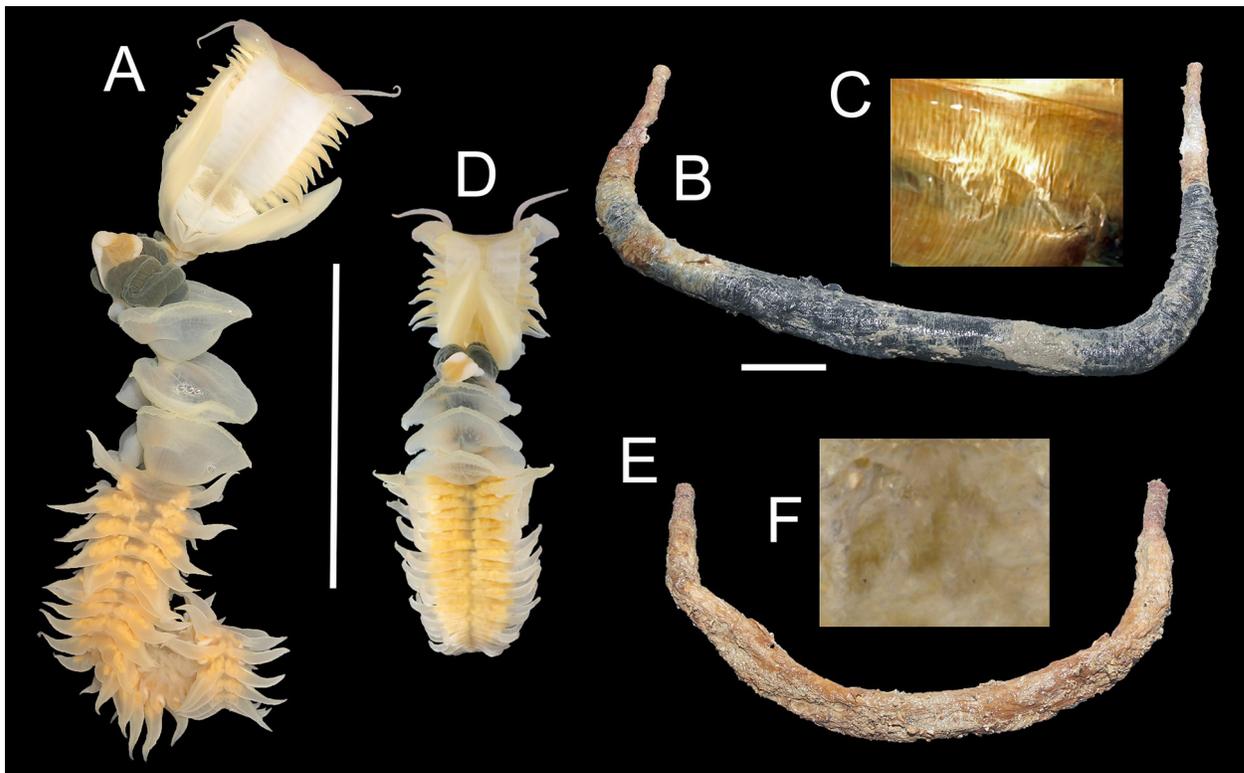
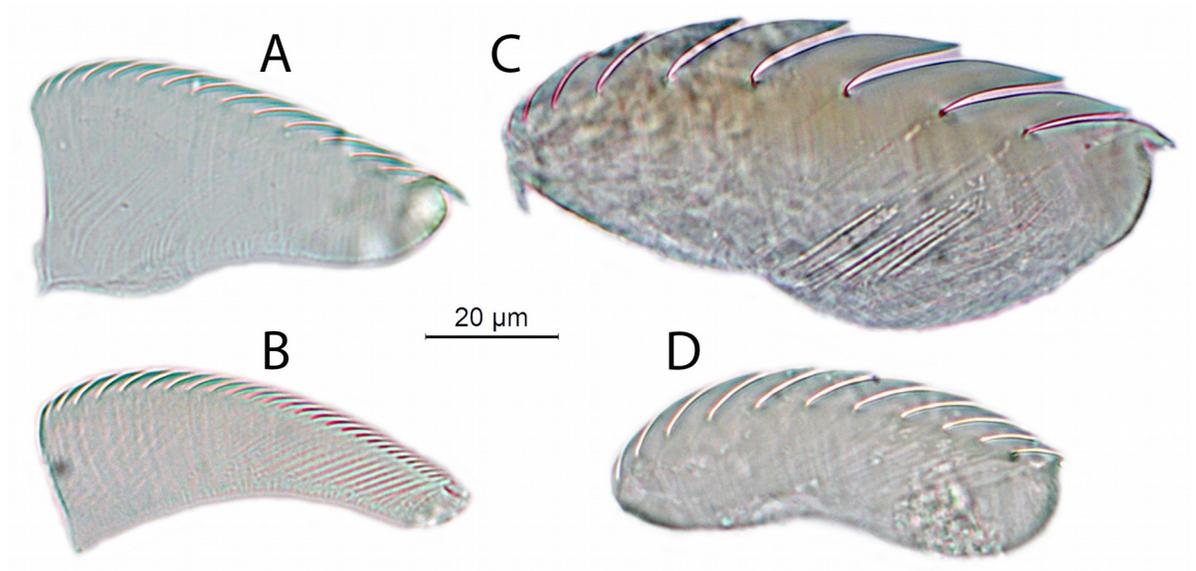
	Richness	Prevalence	Intensity
Chaetopterus sp.	1.8	100	3.0
Chaetopterus cf. *appendiculatus*	1.02	67.5	1.05

689   Table 6. Distribution among hosts (as number of host tubes found without and with 1, 2 and 3

690   individuals) for the five species associated with *Chaetopterus* spp. Cs: *Chaetopterus* sp.; Ca:

691   *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

692   Figure 1. (A) Location of the study area on the Vietnamese coast of the South China Sea. (B)

693   Sampling sites, Nhatrang Bay. Map data from Google Earth Pro, (c) 2016 Google.

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

697

698   Figure 3. Uncini from ventral neuropodial tori of region C. *Chaetopterus* cf. *appendiculatus*: (A)

699   upper tori; (B) lower tori. *Chaetopterus* sp.: (C) upper tori; (D) lower tori.

X16 *Chaetopterus* sp.

X93 *Chaetopterus* sp.

X82 *Chaetopterus* sp.

AM503094 *Chaetopterus variopedatus*

AM503095 *Chaetopterus variopedatus*

DQ209253 *Chaetopterus* cf. *luteus*

DQ209254 *Chaetopterus* cf. *luteus*

DQ209256 *Chaetopterus pugaporcinus*

DQ209257 *Chaetopterus pugaporcinus*

AM503096 *Chaetopterus variopedatus*

DQ087501 *Chaetopterus* sp.

X14 *Chaetopterus* cf. *apendiculatus*

X76 *Chaetopterus* cf. *apendiculatus*

X77 *Chaetopterus* cf. *apendiculatus*

X80 *Chaetopterus* cf. *apendiculatus*

DQ209251 *Mesochaetopterus taylori*

KP222296 *Mesochaetopterus tingkokensis*

DQ209252 *Chaetopterus* sp. 1

DQ209255 *Chaetopterus* sp. 2

AM503097 *Mesochaetopterus xerecus*

AM503098 *Mesochaetopterus rogeri*

KT307698 *Spiochaetopterus costarum*

0.05

700   Figure 4. Preliminary phylogenetic tree for species of *Chaetopterus* and *Mesochaetopterus* based
701   on the COI sequences obtained from NCBI GenBank and our data (in bold). The sequences for
702   the two Vietnamese species are listed in Table 2.

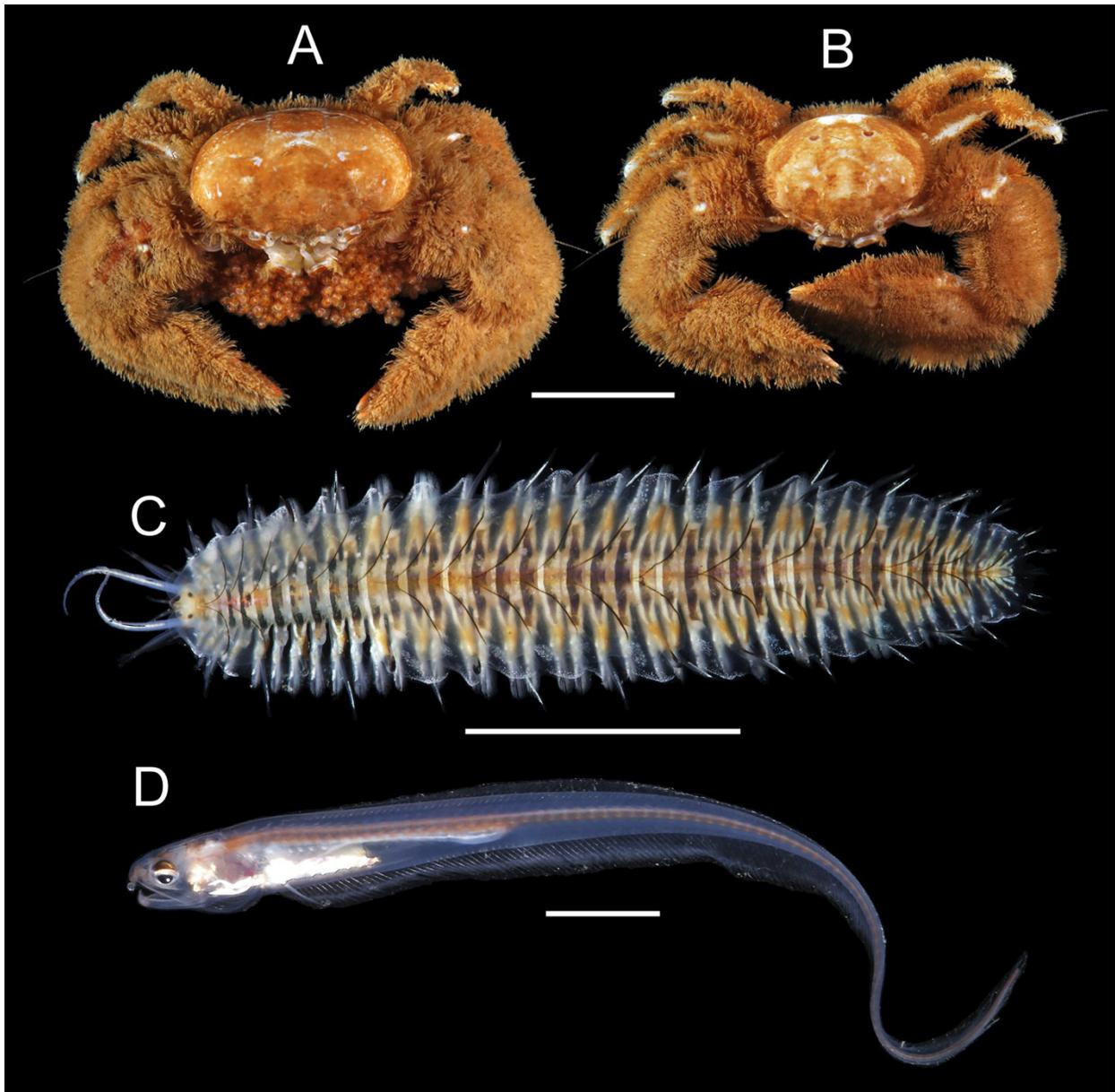
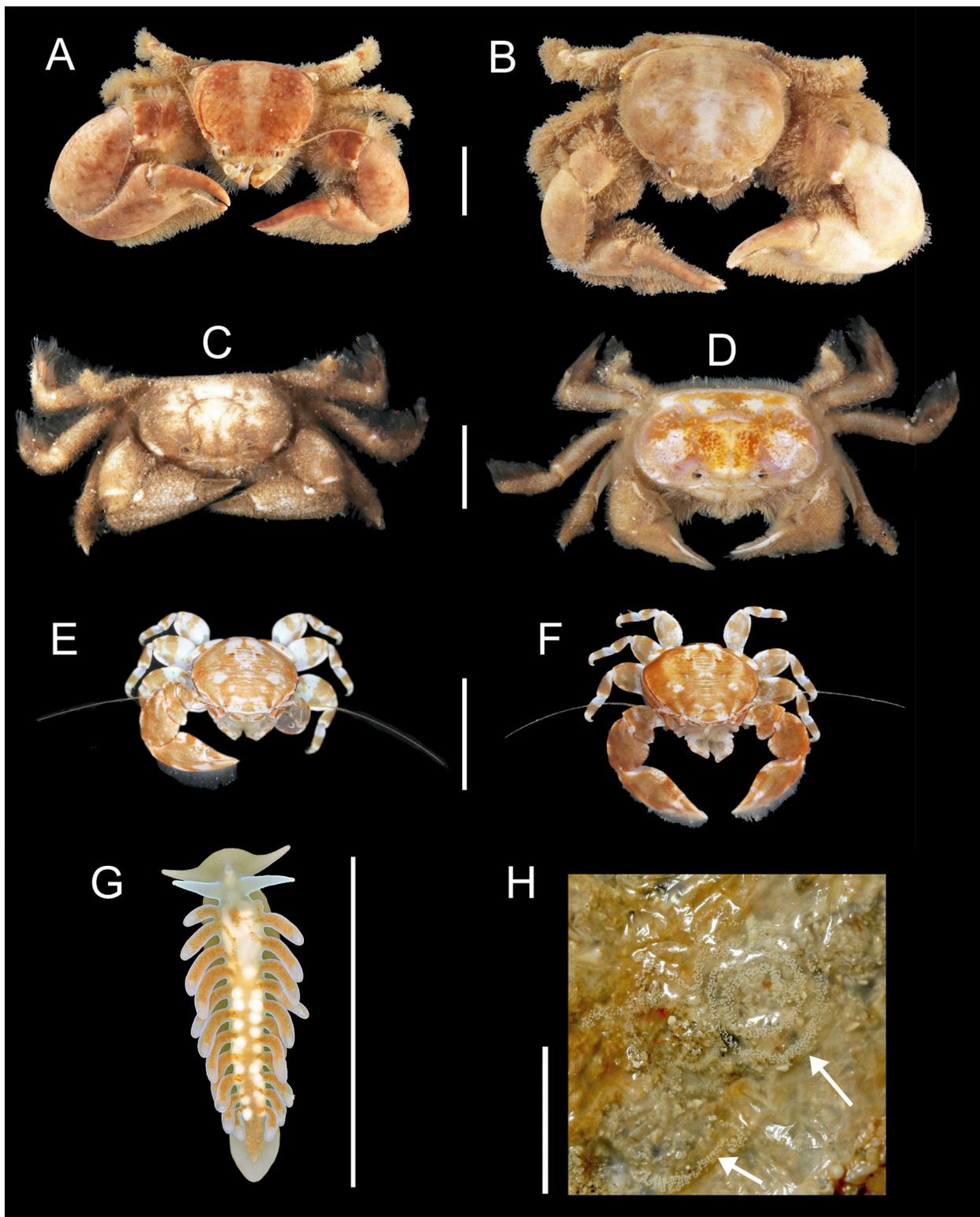



Figure 5. Symbiotic community associated with *Chaetopterus* cf. *appendiculatus*: (A, B) *Eulenaios cometes* (female and male, respectively); (C) *Ophthalmonoe pettibonneae*; (D) *Onuxodon fowleri*. Scale bars are 1 cm.

706   Figure 6. Symbiotic community associated with *Chaetopterus* sp.: (A, B) *Polyonyx* cf. *heox* (male
707   and female, respectively); (C, D) *Tetrias* sp. (male and female, respectively); (E, F) *Polyonyx* sp.,

708 (male and female, respectively); (G, F) *Phestilla* sp. (whole body and egg-mass, respectively);

709 egg-mass indicated by arrows. Scale bars are 0.5 cm.

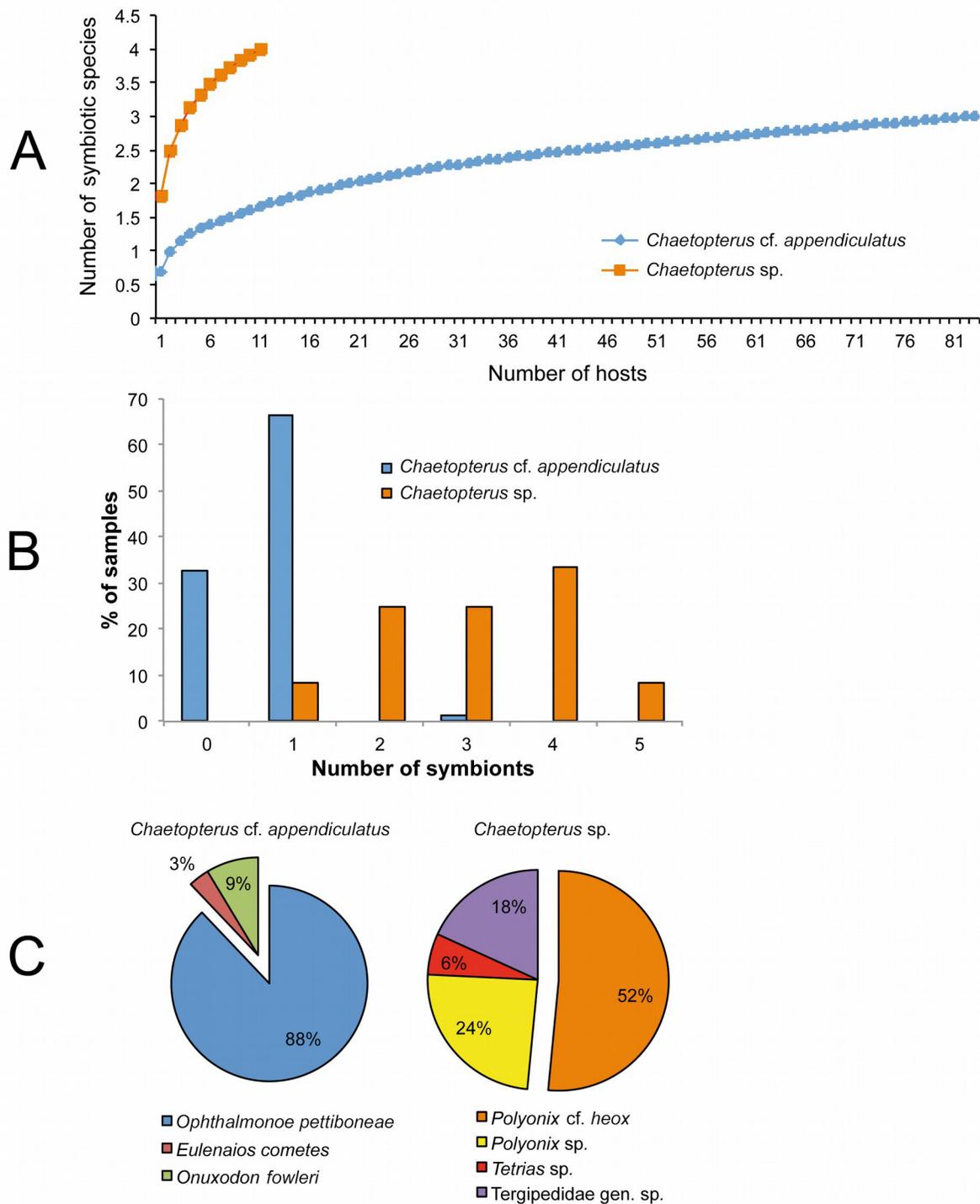



Figure 7. Characterization of the symbiotic assemblages associated with the two host species of *Chaetopterus*: (A) rarefaction curve; (B) distribution of symbionts per host; (C) relative abundance of the symbiotic species.