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## **Insights into the morphology of symbiotic shrimp eyes (Crustacea, Decapoda, Pontoniinae); the effects of habitat demands**

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Morphometric differences in the optical morphology of symbiotic palaemonid shrimps can be observed among species symbiotic with different host organisms. Discriminant functional analysis revealed three distinct groups within the species examined. Of these, bivalve symbionts appear to have an eye design that is solely unique to this host-symbiont grouping, a design that spans across multiple genera of phylogenetically unrelated animals. Although some taxonomic effects may be evident, this does not explain the difference and similarities in eye morphology that are seen within these shrimps. Therefore evolutionary pressures from their host environments are having an impact on the optical morphology of eyes however, as indicated by host-hopping events there ecological adaptations occur post host invasion.

1 Insights into the morphology of symbiotic shrimp eyes (Crustacea, Decapoda, Palaemonidae); the  
2 effects of habitat demands

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8 **Abstract**

9 Morphometric differences in the optical morphology of symbiotic palaemonid shrimps can be  
10 observed among species symbiotic with different host organisms. Discriminant functional  
11 analysis revealed three distinct groups within the species examined. Of these, bivalve symbionts  
12 appear to have an eye design that is solely unique to this host-symbiont grouping, a design that  
13 spans across multiple genera of phylogenetically unrelated animals. Although some taxonomic  
14 effects may be evident, this does not explain the difference and similarities in eye morphology  
15 that are seen within these shrimps. Therefore evolutionary pressures from their host environments  
16 are having an impact on the optical morphology of eyes however, as indicated by host-hopping  
17 events there ecological adaptations occur post host invasion.

## 18 1. Introduction

19 Symbiotic palaemonid shrimps are widespread and abundant in Indo-West Pacific reefal habitats,  
20 characterised by their affinity to form associations with a wide range of taxa. Until recently these  
21 shrimps were in the subfamily Pontoniinae. However in a recent phylogenetic study by De Grave  
22 *et al.*, (2015) this subfamily was synonymised with the family Palaemonidae, as were the related  
23 families Gnathophyllidae and Hymenoceridae. For the purposes of this investigation and  
24 throughout the remainder of this paper, we will refer to this group of shrimps as “pontoniine  
25 shrimps to avoid any systematic ambiguity. Members of the previously separate families  
26 Gnathophyllidae and Hymenoceridae were not included in the present analysis. Within the  
27 pontoniine shrimps, an estimated 60-70% (De Grave, 2001) are known to form associations with  
28 corals, sponges, ascidians, gorgonians, and so on. However this is likely to be an underestimate  
29 as the host association remains unknown for several species, but is inferred to be symbiotic due to  
30 their morphological similarity to other species. Pontoniine shrimps occur in a wider variety of  
31 tropical and subtropical habitats, and are known from deeper water, down to about 2000 m  
32 (Bruce, 2011). However, their highest species richness is on tropical coral reefs, down to about  
33 100 m. The most recent catalogue (De Grave & Fransen, 2011) lists 602 species, but numerous  
34 species have been described since then.

35 The traditional view of these shrimps as symbionts, has recently been challenged for a number of  
36 species dwelling in sponges, where diet studies revealed them to be parasites as their stomachs  
37 only contained host tissue and spicules (Ďuriš *et al.*, 2011). At present it is not known how  
38 widespread parasitism is in the group, and we thus refer to them as associates, inferring no  
39 trophic interaction with the host.

40 Morphological adaptation to an associated mode of life has been extensively noted in the  
41 taxonomic literature for pontoniine shrimps. Such adaptations include modified pereopods  
42 (Bruce, 1977; Patton, 1994) in addition to extensive modifications in general body plan and  
43 mouthparts (Bruce, 1966; Ďuriš *et al.*, 2011). Additionally, a range of ecologies are recognised,  
44 ranging from internally dwelling in small sized hosts like ascidians (e.g. species of the genus  
45 *Periclimenaeus*) to fish cleaning species, dwelling on anemones (e.g. *Ancylomenes* spp.). Despite  
46 this wealth of morphological and ecological disparity, few studies have been done linking  
47 morphological disparity with ecological constraints. A recent exception to this is the study by  
48 Dobson *et al* (2014) which examined gross eye morphology across four, broad, lifestyle

49 categories: ectosymbionts, bivalve endosymbionts, non-bivalve endosymbionts and free-living.  
50 Their results clearly demonstrated considerable differences in superficial optical parameters  
51 across various lifestyles. In many decapods, vision is thought to be an important feature of their  
52 morphology with variations in morphology and structure reflecting ecological habitat demands  
53 (Johnson, Shelton and Gaten, 2000). Differences in eye size, facet size and interommatidial angle  
54 have been observed in many marine species occupying different depths (Gaten, Shelton, and  
55 Herring, 1992; Johnson *et al.*, 2000). Eye parameter (EP) has been used by a number of  
56 researchers as a measure of determining the equipose between sensitivity and resolution of  
57 different organisms (Snyder, 1979; Stavenga & Hardie, 1989; Kawada *et al.*, 2006). For  
58 organisms occupying well-lit habitats EPs of between 0.45 and 1 rad- $\mu\text{m}$  have been recorded, 1-2  
59 for crepuscular and 2-3 for nocturnal species (Kawada *et al.*, 2006), however these values many  
60 vary in aquatic organisms due to the different refraction index of water. Pontonine shrimps are  
61 ideal study organisms for the relationship between eye morphology, vision and habitat demands,  
62 given their predilection for forming associations with a wide range of taxa.  
63 The current study builds upon this previous work, by focussing on and contrasting across actual  
64 host identities using a multivariate analytical framework and thus aims to further unravel  
65 potential differences in gross optical morphology of pontonine shrimps.

## 66 2. Methods

67 Optical characteristics of 96 species from 40 genera were examined from collections at the  
68 Oxford University Museum of Natural History. A copy of the dataset used in this paper can be  
69 accessed in the Supplemental Information. The work described in this paper was reviewed and  
70 approved by the Department of Biological Sciences, Faculty of Sciences ethics committee  
71 approval number U053. To understand differences in eye morphology between host categories,  
72 each species was classed into host-symbiont predefined groupings based on their most common  
73 host associations (Bruce, 1994); i.e. Actiniaria, Ascidiacea, Asteroidea, Bivalvia, Crinoidea,  
74 Echinoidea, Gorgonacea, Hydrozoa, Ophiuroidea, Porifera and Scleractinia or considered to be  
75 free-living. For all species, eye span (ES), diameter at the base of the eyestalk (DBES), facet  
76 diameter (FD) and eye diameter (ED) were measured using a dissecting microscope fitted with an  
77 ocular micrometer. To reduce scaling effects ES, DBES and ED were standardised by post orbital  
78 carapace length, whilst FD was standardised by eye diameter. A composite variable, ES-DBES  
79 (eye span minus diameter at base of eyestalk), was also formulated to provide an indication of

80 eye mobility, the greater mobility of the eyes the larger the value. In addition to the variables  
81 measured, eye parameter (EP) was calculated as an outcome of facet diameter ( $\mu\text{m}$ ) (FD) and  
82 interommatidial angle ( $\Delta\phi$  in radians) using Snyder (1979) equation (Equation 1).

83 Equation 1. 
$$EP = FD \Delta\phi$$

84 Interommatidial angle in radians, used in the calculation of EP, was estimated using an adaptation  
85 of Stavenga's (2003) formula (Equation 2).

86 Equation 2. 
$$\Delta\phi = 2 \left( \frac{FD}{ED} \right)$$

87 The presence or absence of the nebenaugae (see Dobson *et al.*, 2014) was also noted and when  
88 present the relative size was expressed after standardisation by eye diameter (ED). Our  
89 terminology follows Johnson *et al.*, 2015 who utilised nebenaugae for the structure previously  
90 referred to under several names.

91 Eye Parameter (EP) and standardised nebenaugae size was compared between hosts using a  
92 Kruskal Wallis test in the Statistical Software Package R 3.0.2 as this allowed for *Post Hoc*  
93 comparisons (R Core Team, 2013), whilst Eye Diameter (ED) was analysed by the means of an  
94 ANOVA.

95 Subsequently, the dataset was analysed with Discriminant Function Analysis (DFA), also known  
96 as Multiple Discriminant Analysis (MDA) or Canonical Variate Analysis (CVA). DFA extracts  
97 linear combinations of variables (known as roots) which maximise differences amongst a priori  
98 defined groups, in this case host categories, with the percentage correctly classified providing a  
99 goodness of fit measure, akin to more traditional P values.

100 As DFA requires the number of predictor variables to be fewer than the sample size of the  
101 smallest group, a number of host-categories could not be included in the analysis, namely  
102 Echinozoa, Hydrozoa, Ophiurozoa and Asterozoa, all of which are relatively infrequently  
103 inhabited by pontonine shrimp. Outliers were identified using within host category linear least-  
104 squares regression analysis, using post-orbital carapace length as the independent variable.

105 Individual outliers were corrected by re-measurement (where possible), and only excluded from  
106 the final dataset if their values still exceeded 3 standard deviation in residual plots. The final  
107 dataset analysed with DFA thus comprised of 83 species, across 7 host categories, as well as free-  
108 living taxa. Host categories herein analysed, comprise of Actiniaria (9 shrimp species),  
109 Ascidiacea (7), Bivalvia (12), Crinoidea (8), Gorgonacea (7), Porifera (14) and Scleractinia (13).  
110 Thirteen micro-predatory species, which are currently considered not to be host associated, i.e.  
111 free-living were also included in the analysis, a combination of species living on coral reefs and  
112 in seagrass beds.

113 For consistency, statistical analysis of eye size, Eye Parameter and nebenaugae was carried out on  
114 the reduced dataset.

115 Prior to DFA, proportions were arcsine-transformed to meet the assumptions for statistical  
116 analysis of normality and homogeneity (Zuur, Ieno and Elphick, 2010). All DFA analysis was  
117 performed in SPSS 18. In all DFA analysis, all variables were entered simultaneously, with the  
118 contribution of each variable assessed on the basis of discriminant loadings (structure  
119 correlations, rather than discriminant coefficients, as those are considered more valid when  
120 interpreting the relative contributions of each variable).

### 121 **3. Results**

#### 122 **3.1 Eye size, Eye Parameter and nebenaugae presence**

123 Across all species examined, mean relative ED (Fig. 1) ranged from 0.09 to 0.27, with  
124 significantly smaller eyes occurring in bivalve associated species (ANOVA,  $F_{7,75} = 9.26$ ,  $P < 0.001$ ,  
125 Tukey  $P = 0.05$ ). Although the analysis deemed none of the remaining differences to be  
126 statistically significant, ascidian ( $\bar{x} = 0.19$ ,  $SD \pm 0.06$ ) and sponge symbionts  
127 ( $\bar{x} = 0.19$ ,  $SD \pm 0.06$ ) were also found to possess some of the smallest relative EDs whilst  
128 gorgonian symbionts ( $\bar{x} = 0.28$ ,  $SD \pm 0.11$ ) and free-living shrimps ( $\bar{x} = 0.26$ ,  $SD \pm 0.06$ ) had  
129 the largest relative EDs.

130 Eye parameter (EP) (Fig. 2) ranged from 0.44 – 8.06 rad- $\mu\text{m}$ , with a significantly larger EP found  
131 in ascidian, bivalve and sponge associates (Kruskal Wallis,  $H$  (adjusted for ties) = 43.62,  $df = 7$ ,  
132  $P < 0.001$ , *Post hoc* pairwise comparisons  $P = 0.05$ ). The smallest EP values were found in  
133 associates of crinoid, gorgonians and in free-living shrimps. Associates of sea anemones and  
134 corals were not significantly different to any other host category in terms of EP (Fig. 2), whilst  
135 the widest range of values is present in sponge associates. Although not statistically considered as  
136 outliers in within-host category regression analysis, three species exhibited an aberrant EP, all of  
137 the genus *Pontonia*. *Pontonia panamica* an ascidian commensal has the largest EP in the dataset  
138 (EP = 7.45), whilst *P. mexicana* and *P. pinnophylax* exhibited considerable larger values than  
139 other species associated with bivalves.

140 A significant association was found between the presence/absence of the nebenaug and host  
141 category (Chi-squared test,  $\chi^2 = 24.777$ ,  $df = 7$ ,  $P < 0.001$ ). High absence rates of the nebenaug  
142 were observed among ascidian, bivalve and poriferan symbionts (Fig. 3), whilst it is prevalent in  
143 sea anemone associates and free-living shrimps. However, the relative size is not different across  
144 host categories (Kruskal Wallis test,  $H = 8.93$ ,  $df = 6$ ,  $P = 0.178$ ), with ascidians excluded as only  
145 one species, *Periclimenaeus hecate*, had a nebenaugen.

### 146 3.2 Multivariate analysis

147 Discriminant function analysis revealed only two significant roots (Table 1), which cumulatively  
148 explain 94.6% of total variance. Examination of the structure matrix (Table 2) revealed that three  
149 variables were highly loaded on to the first root (EP, FD, ED), whilst a fourth variable (ES-  
150 DBES) displayed greatest loading on the second function.

151 A classification matrix indicates that overall 50.6% of shrimp species were correctly classified in  
152 respect to their priori defined groups (host classification) (Table 3), but with significant variation  
153 as to within-group classification. Bivalve associates were 100.0% correctly classified, with a high  
154 number also correctly classified for sponge associates (78.6%). Over half of the free-living  
155 species (61.5%) were correctly classified to their priori group, with other species classified as sea  
156 anemone, crinoid and coral associates. Gorgonian associates correctly classified in 42.9% of  
157 cases, with misclassified taxa allied to free-living, coral and crinoid associates. Coral associates  
158 correctly classified in 38.5% of cases with species misclassifying as associates of sponges, sea



159 anemones, crinoids and free-living species. Sea anemone and crinoid associates were only 22.2  
160 and 25.0% correctly classified. All ascidian symbionts were found to misclassify, with 71.4% of  
161 them misclassified as sponge associates.

162 When comparing the relative position of the centroids for each host category (Fig. 4) it is  
163 obvious, that the eyes of ascidian and sponge associated species are very similar to each other, as  
164 are the eyes of crinoid and coral associates, both of which also group with the free-living species.  
165 Although broadly similar to the latter grouping, the eyes of gorgonian and sea anemone  
166 associates are somewhat divergent as well as divergent to each other, as evidenced by the position  
167 of their centroids. Bivalve associates clearly occupy an isolated position, relative to the other  
168 host categories.

169 When plotting only the ascidian associates in the DFA analysis (Fig. 5), a divergent position of *P.*  
170 *panamica* is evident, whilst the other taxa form a loose grouping. The positions of sponge  
171 associates (Fig. 6) reveal two distinct, but loose groupings, as well as a divergent species,  
172 *Thaumastocaris streptopus*. Membership of either of the two groups does not appear influenced  
173 by phylogeny, as either group contains species belonging to the genera *Typton* and  
174 *Periclimenaeus*. The positions of the individual bivalve associates (Fig. 7) reveals a relatively  
175 tight grouping, but with an isolated position occupied by *Conchodytes nipponensis*. The  
176 positions of individual crinoid associates (Fig. 8) are rather scattered, but with a very isolated  
177 position for *Laomenes nudirostris*. A similar scattered pattern is observed for the coral associates  
178 (Fig. 9) and the free-living species (Fig. 10). Gorgonian associates also demonstrate this pattern  
179 (Fig. 11), but with a significant, isolated position for *Pontonides loloata*. A similar pattern is  
180 observed for sea anemone associates (Fig. 12), with an isolated position for *Periclimenes*  
181 *scriptus*.

#### 182 4. Discussion

183 Multivariate analysis clearly reveals that three distinct eye types are present in pontoniine  
184 shrimps, with bivalve associates comprising a type on their own. Sponge and ascidian associates  
185 have remarkably similar eyes, to the point that the majority of ascidian associates were  
186 misclassified as sponge associates in the analysis. A third eye type is present in a range of

187 ectosymbiotic taxa, associated with sea anemones, gorgonians, corals, crinoids, as well as free-  
188 living species.

189 An examination of the structure loadings reveals that along the first root, both facet diameter  
190 (FD) and Eye Parameter (EP) increases, but with a concomitant decrease in eye diameter (ED),  
191 whilst along the second root eye mobility (as measured by ES-DBES) decreases. Broadly  
192 speaking, the ectosymbiotic and free-living taxa thus have smaller facet diameters, a lower EP  
193 and bigger eyes, than their endosymbiotic counterparts in bivalves, sponges and ascidians.  
194 Equally, bivalve associates display more mobile eyes than ascidian and sponge associates, but  
195 with roughly similar facet diameter and EP. It should be noted that the relative eye size of  
196 bivalve associates is significantly smaller than all other host groupings, this may be as a result of  
197 their comparably larger body sizes (e.g. mean average 6.9 mm CL versus 3.0 mm CL for  
198 Actiniaria, 2.5 mm CL for Porifera and 1.34 mm CL for Gorgonacea symbionts).

199 Within deep sea caridean species the nebenauge has been suggested to have an important role in  
200 diurnal migrations (Johnson *et al.*, 2015). The concept that orientation to light is aided by the  
201 presence of the nebenauge is further supported by these results with it being highly abundant  
202 within sea anemone, crinoid, free-living and coral associates. However for bivalve, ascidian and  
203 sponge associates both diurnal migrations and orientation to light would be of little significance  
204 for species with an endosymbiotic mode of life.

205 This result is not surprising, given the clear relationship between gross eye morphology of  
206 pontonine shrimps and life style already demonstrated in Dobson *et al.* (2014). Therein, based on  
207 a range of optical parameters, the eyes of free-living and ectosymbiotic species were found to be  
208 very similar, and clearly different from both types of endosymbiotic species considered, bivalves  
209 and non-bivalve associates. Further, bivalve endosymbionts exhibited an intermediary group  
210 between free-living/ectosymbionts and non-bivalve endosymbionts, potentially linked to their  
211 presumed more active lifestyle, with bivalve associated documented to move hosts in search of a  
212 mate (Baeza *et al.*, 2011).

213 Whilst the relationships between optical parameters and lifestyle in Dobson *et al.* (2014) appears  
214 clear-cut and supported by the present analysis, by including actual host identity, rather than  
215 lifestyle in the current analysis, a number of surprising findings emerge.

216 The eyes of ascidian associated species emerges as being remarkable similar to the eyes of  
217 sponge associated species, to the point that the majority of a priori classified species in this group  
218 were misclassified as sponge eyes by the multivariate analysis. This is herein interpreted being  
219 likely a significant signal of phylogenetic constraint, as four out of the seven species in this host  
220 category belong to a primarily sponge dwelling genus, *Periclimenaeus* (see below) with generally  
221 conservative eye morphology, potentially indicative of recent host switching event(s). Two  
222 further species in this host category, phylogenetically unrelated to *Periclimenaeus*, *Dactylonia*  
223 *okai* and *Odontonia katoi* are thought to be closely related species (Fransen, 2002), but with  
224 significantly different gross eye morphology. *Dactylonia okai* possesses stout triangular shaped  
225 eyes, whereas the eyes of *Odontonia* species are small and hemispherical (Fransen, 2002).  
226 Whilst *D. okai* and *O. katoi* are found living within large solitary ascidians, species of  
227 *Periclimenaeus* are found living within both ascidians and sponges. Species such as  
228 *Periclimenaeus orbitocarinatus* and *Periclimenaeus ascidiarum* live in association with  
229 compound ascidians that are structurally similar in morphology to the canals of sponges occupied  
230 by, for example, *Periclimenaeus maxillulidens*. The structural similarity in hosts between the  
231 symbionts of compound ascidians and sponges could be a plausible explanation for the high  
232 misclassification of ascidian symbionts to sponges. Two species were misclassified as either a sea  
233 anemone or bivalve associate. Although DFA does not provide information on individual  
234 classified species, it is evident from Fig. 5 that *P. panamica* is the species misclassified as a  
235 bivalve associate. The genus *Pontonia* comprises of 11 species (De Grave & Fransen, 2011) and  
236 is morphologically very conservative. Although the host for one species, *P. longispina*, is not  
237 known, the majority of species associate with bivalves in the families Pinnidae and Pteriidae,  
238 whilst one poorly known species *P. chimaera*, is thought to be an associate of large gastropods of  
239 the genus *Strombus*. *Pontonia panamica* is the only species to associate with ascidians, the  
240 solitary species *Ascidia interrupta* in the eastern Pacific. Although Marin and Anker (2008)  
241 speculate that a host switch to ascidians occurred early on in the evolutionary history of this  
242 genus, the retention of essentially a “bivalve” eye is perhaps indicative of a more recent host  
243 switching event. However, on balance the differences in eye morphology between the  
244 phylogenetically not related genera herein analysed as ascidian associates suggests that despite  
245 occurring in a similar host environment, their enclosure inside ascidians has not provided  
246 pressure on their eyes to become optically similar. As to whether this lack of overall evolutionary  
247 pressure is imparted by distinctive host morphologies (compound, solitary) or habitats (intertidal,

248 subtidal) or indeed is determined by differential behavioural attributes (social biology) of the  
249 associates themselves remains unclear.

250 Notwithstanding their close similarity to ascidian associate eyes, the eyes of sponge associated  
251 species appear to be quite uniform, with the majority being correctly classified in their a priori  
252 defined host group, but seemingly forming two distinct subgroups in the analysis, in addition to  
253 the outlying *T. streptopus*. We infer here that the classification into two subgroups is putatively  
254 related to host morphologies, as sponge species exhibit a discrete and distinct range of canal  
255 sizes. Space partitioning, as well as individual host selection is indeed known to play a  
256 significant role in the sponge-dwelling gambarelloides group of *Synalpheus* (Duffy, 1992;  
257 Hultgren and Duffy, 2010; 2012). The speculation that canal sizes of the host may play a  
258 significant role in optical acuity of pontoniine species, can however not be substantiated, as the  
259 host range of most species remains unknown, with even the identity of many hosts simply not  
260 being known. For instance, for many species of *Periclimenaeus*, a primarily sponge associated  
261 genus, the hosts are not known (Bruce, 2006). Of particular interest are the three ectosymbiotic  
262 species included in this primarily endosymbiotic group in the present analysis, *T. streptopus*,  
263 *Periclimenes harringtoni* and *Periclimenes incertus*. *Thaumastocaris streptopus* is an Indo-  
264 Pacific species, which dwells in the central atrium of vase-shaped sponges like *Siphonochalina*  
265 and *Callyspongia* (see Bruce, 1994). Based on the present suite of optic parameters, this species  
266 does not cluster with the rest of the sponge associates. Although Ďuriš *et al.* (2011) consider the  
267 species to be parasitic, in common with several other sponge associates, the isolated position of  
268 the species in the present analysis, combined with their asymmetrical first pereopods and a  
269 segmented carpus (both unique within the family) is indicative perhaps of a different behavioural  
270 niche. The Indo-Pacific, *P. incertus* dwells on the outside of a variety of sponges, and clusters  
271 reasonably close to the other sponge associates in the present analysis, potentially indicative of  
272 similar relationship to the host, if external. The Caribbean *P. harringtoni* dwells in the atria of  
273 *Neofibularia nolitangere* and based on the optical parameters studied herein, appears to have an  
274 eye structure very similar to that of endosymbiotic species, potentially an example of habitat  
275 driven adaptation, despite the significant difference in position on the host.

276 The sea anemone associates included in the present analysis, fall into four ecological/systematic  
277 groups, *Ancylomenes* and three different species groups of *Periclimenes*. *Ancylomenes* species are  
278 on the whole considered to be fish cleaners, who only utilise the sea anemone as an advertisement

279 for their services to client fish (Huebner & Chadwick, 2012). It should be noted that this is  
280 potentially a generalisation, as direct observation of fish cleaning behaviour is not available for  
281 all species, with this information lacking for one species herein included *A. tosaensis*.  
282 *Periclimenes yucatanicus* and *Periclimenes rathbunae* are active large bodied species, associated  
283 with a variety of sea anemones in the Caribbean. Fish cleaning has not been observed for either  
284 species, with Limbaugh *et al.*, (1961) considering *P. yucatanicus* a fish-cleaning mimic.  
285 *Periclimenes ornatus* and *P. inornatus* belong to the same species complex, and are smaller  
286 bodied species which hide in between the tentacles of a variety of Indo-Pacific sea anemones.  
287 Finally, *P. scriptus*, a Mediterranean and subtropical Northeast Atlantic species which is not  
288 phylogenetically closely related to the other two groups, is an active species, associated with long  
289 tentacle sea anemones, with no known fish cleaning behaviour. With the exception of *P. scriptus*  
290 (see below) these species exhibit a scattered grouping in the DFA analysis, and as a group have a  
291 low percentage correctly classified, at 22%. It thus appears that despite their broad ecological  
292 niche similarity as sea anemone associates, insufficient convergent pressure on their optical  
293 parameters is noted, indicative of differential usage of their eyes.

294 In contrast to sea anemone associates, coral associates exhibit a reasonable level of correctly  
295 classified in the DFA analysis, at 38.5%, despite the large variety of host morphotypes involved  
296 in this association. Several species *Coralliocaris* spp., *Harpilius* spp. and, *Harpiliopsis* spp. are  
297 associated with branching corals of the families Pocilloporidae and Acroporidae. Other species  
298 in this group are associated with corals which extend their polyps during the day, either short  
299 polyps (e.g. *Hamopontonia corallicola* on *Goniopora*) or long polyp forms, such as *Cuapetes*  
300 *kororensis* on *Heliofungia actiniformis*. Morphologically heavily modified taxa are also present  
301 in this group, such as the laterally flattened *Ischnopontonia lophos* which moves between the  
302 corallites of *Galaxea*. It thus appears that the habitat and/or behaviour in the case of coral  
303 associates is a significant driver in optical parameters, akin to the free-living species, which had  
304 an approximately similar level of correctly classified species (53.8%). However, in contrast to  
305 free-living taxa, which are considered to be micro-predators, several of the coral associates are  
306 potentially parasites (Stella *et al.*, 2011). The common functionality of their optic parameters (to  
307 a degree) remains unclear, although it is known that several species, e.g. *Coralliocaris* defend  
308 their coral host against predators (Marin, 2009a; Stella *et al.*, 2011), perhaps necessitating the  
309 need for similar optical acuity to free-living micro predators.

310 Bivalve associates exhibited a 100% correct classification in the DFA analysis, although with  
311 reasonable scatter in the scatter plot, and a significant outlier (*C. nipponensis*). Yet the group  
312 consists of several genera, including *Conchodytes* and *Anchistus*, which are phylogenetically  
313 distant (Kou *et al.*, 2014). Furthermore these species can be differentiated by general bauplan  
314 morphologies, ranging from relatively unspecialized (*Anchistus* and *Paranchistus* for example) to  
315 dorso-laterally compressed (e.g. *Conchodytes*) (Bruce 1981; Fransen & Reijnen, 2012). Their  
316 phylogenetic distance is evidence of multiple host invasions (Kou *et al.*, 2014), but the present  
317 analysis reveals considerable convergence in optical parameters, indicative of profound habitat  
318 induced restraints.

319 A number of species occupy isolated positions within their respective groups, notably *P. loloata*,  
320 *P. scriptus*, *C. nipponensis* and *L. nudirostris*. Although we cannot discount variation in optical  
321 parameters of individual eyes, which may have lowered the percentage correctly classified and  
322 induced a higher degree of scatter, two species are worthy of further discussion. The corneal part  
323 of the eye of *Laomenes* species is characterised by an apical papilla (see illustrations for several  
324 species in Marin, 2009b) which contains functional facets, but which are somewhat different in  
325 shape to facets elsewhere on the cornea. The relative size as well as the exact position of the  
326 papilla has been used as a minor taxonomic character to differentiate between species (Marin,  
327 2009b). However, it is known that a large degree of infra-specific variation is present, which  
328 unquestionably would influence some of the herein included optical parameters. *Periclimenes*  
329 *scriptus* appeared isolated within the sea anemone grouping however due to the small size of the  
330 specimen (CL 1.25 mm) it is possible that this animal was not fully mature as ovigerous females  
331 have a reported CL of 5.0 mm (Đuriš *et al.*, 2013).

## 332 5. Conclusion

333 Overall, our analysis demonstrates that there is a significant evolutionary pressure of the host  
334 environment on the optic parameters of associate shrimp species, with in many cases congruence  
335 being evident between phylogenetically unrelated taxa. This is especially evident in bivalve and  
336 sponge associates, and to a lesser extent in other host taxa. This result is in sharp contrast to the  
337 disparate morphology of many other body parts of pontoniine shrimps, with significant variation  
338 in mouthparts, pereopods and even general body shape between genera, inhabiting the same  
339 host. At the same time, evidence emerges from the optical analysis of recent host switching

340 events in certain lineages, where the optical parameters have not evolved to a communality yet,  
341 especially in the genera *Periclimenaeus* and *Pontonia*, where taxa living in different hosts appear  
342 to retain a close optical similarity to those living in other taxa.

## 343 6. Acknowledgements

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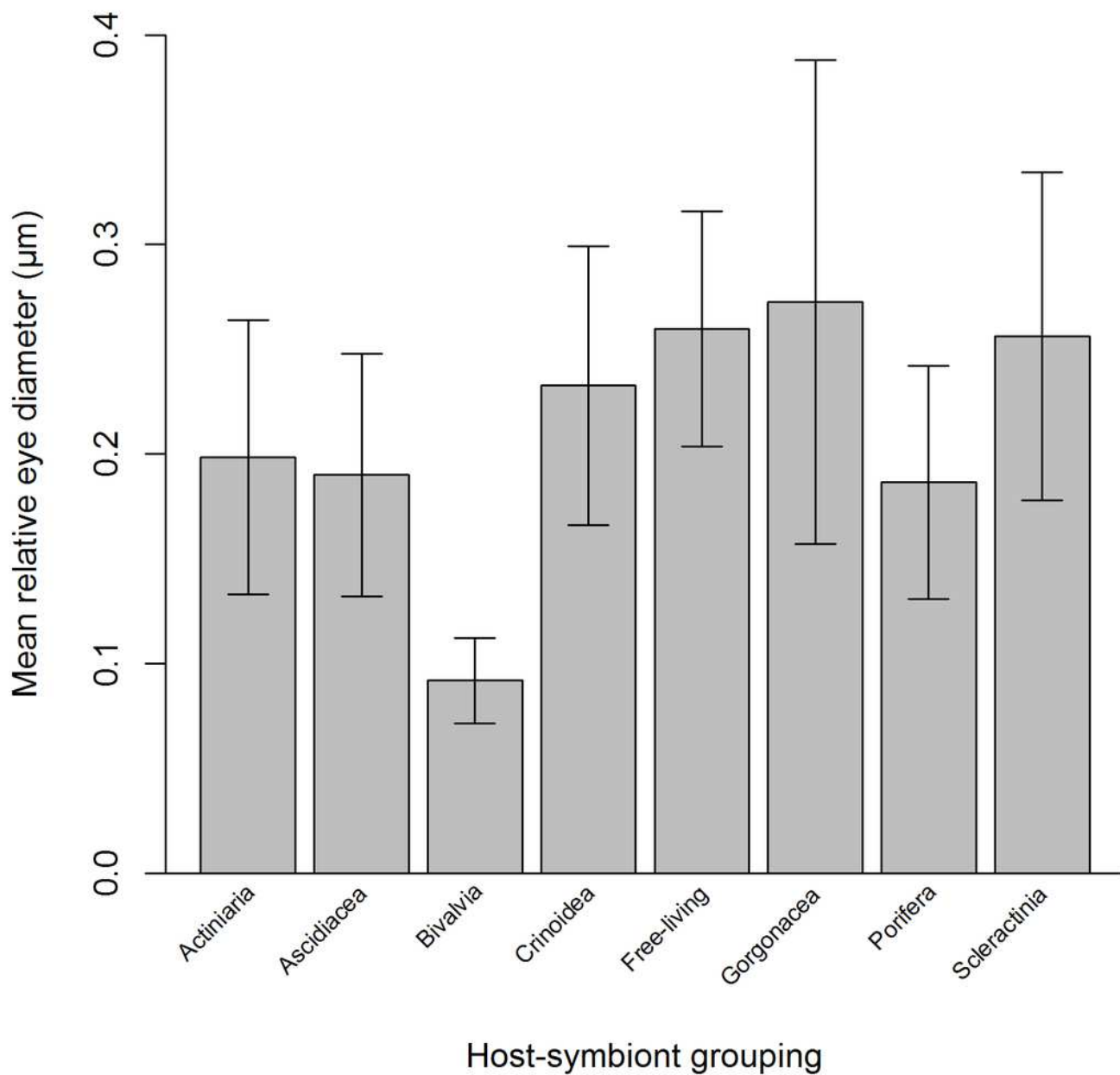


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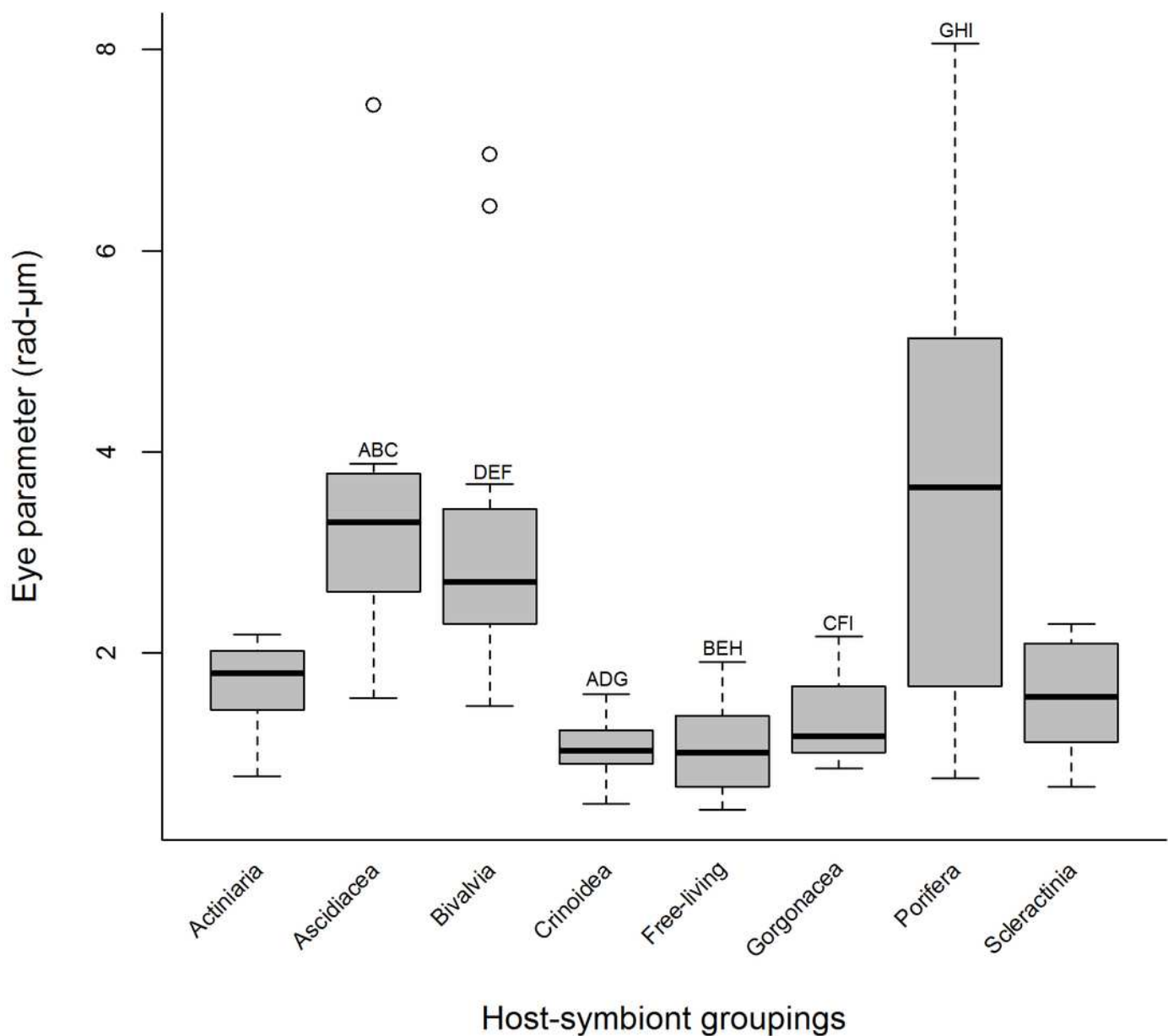
## 1

Figure 1. Mean relative eye diameter (standardised by post-orbital carapace length) for 83 species of Pontoniinae associated 8 host-symbiont groupings.



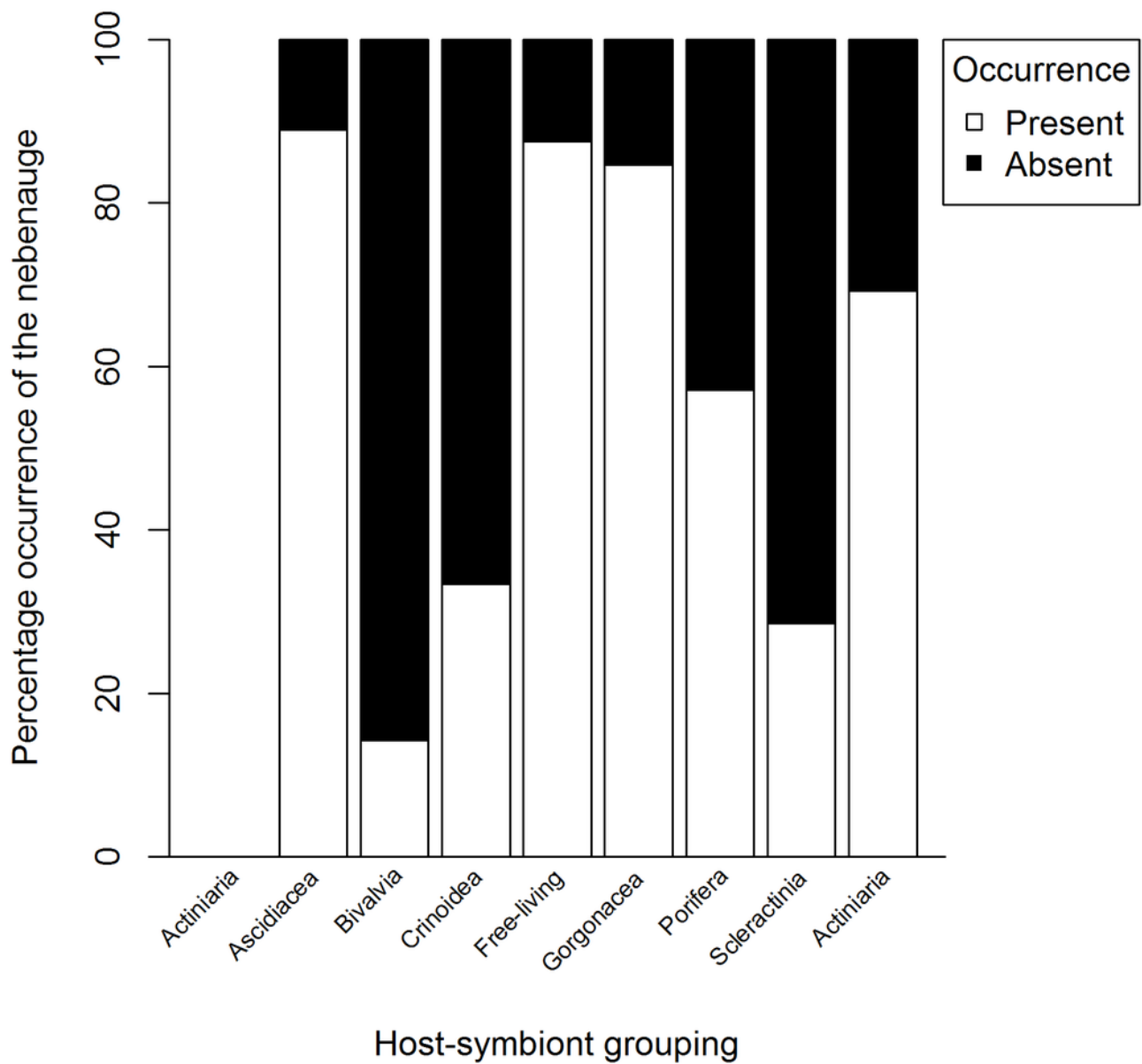
## 2

Figure 2. Median eye parameter for 83 species of Pontiinae from 8 host-symbiont groupings. Significant differences are represented by hosts possessing the same letter A-I (Tukey HSD  $P < 0.05$ ).



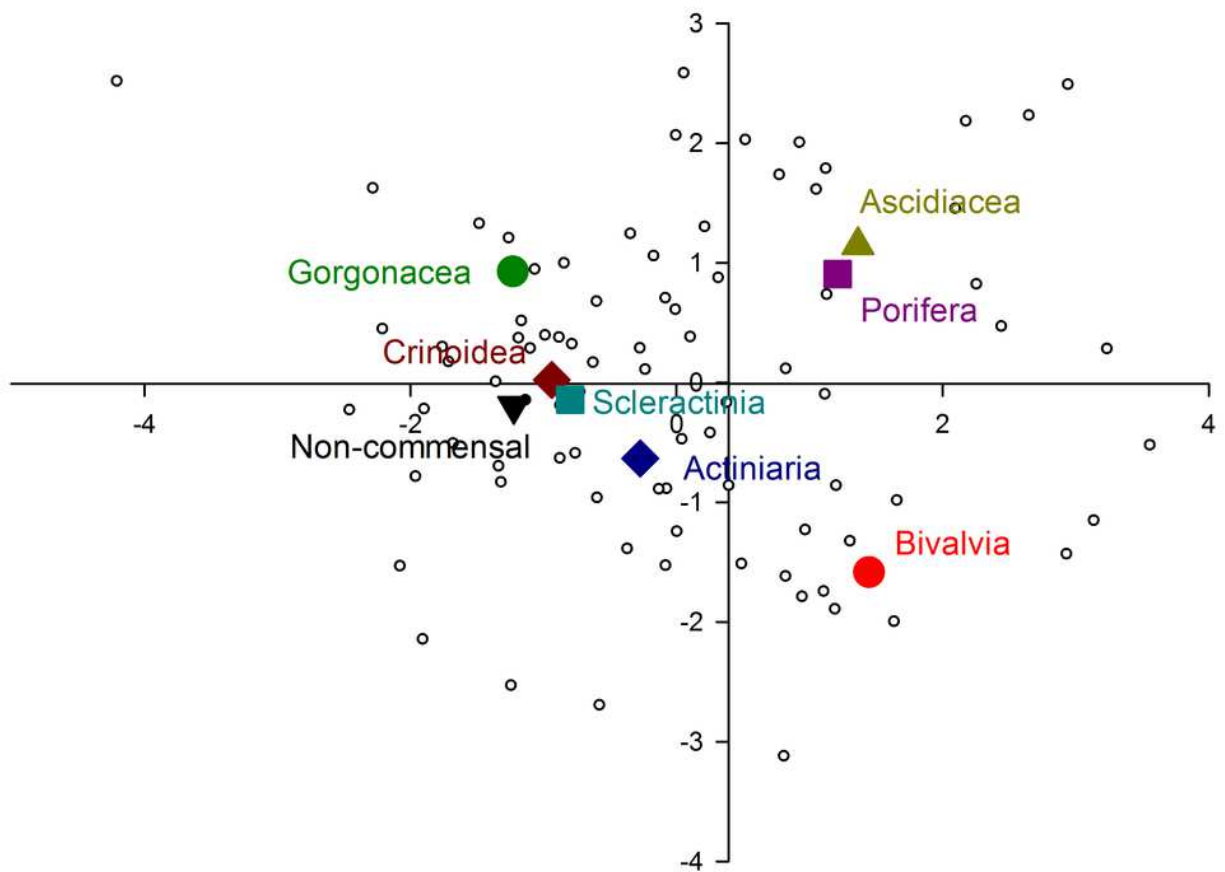
## 3

Figure 3. Percentage occurrence of the nebenauge for 83 species of Pontoniinae from 8 host-symbiont groupings.



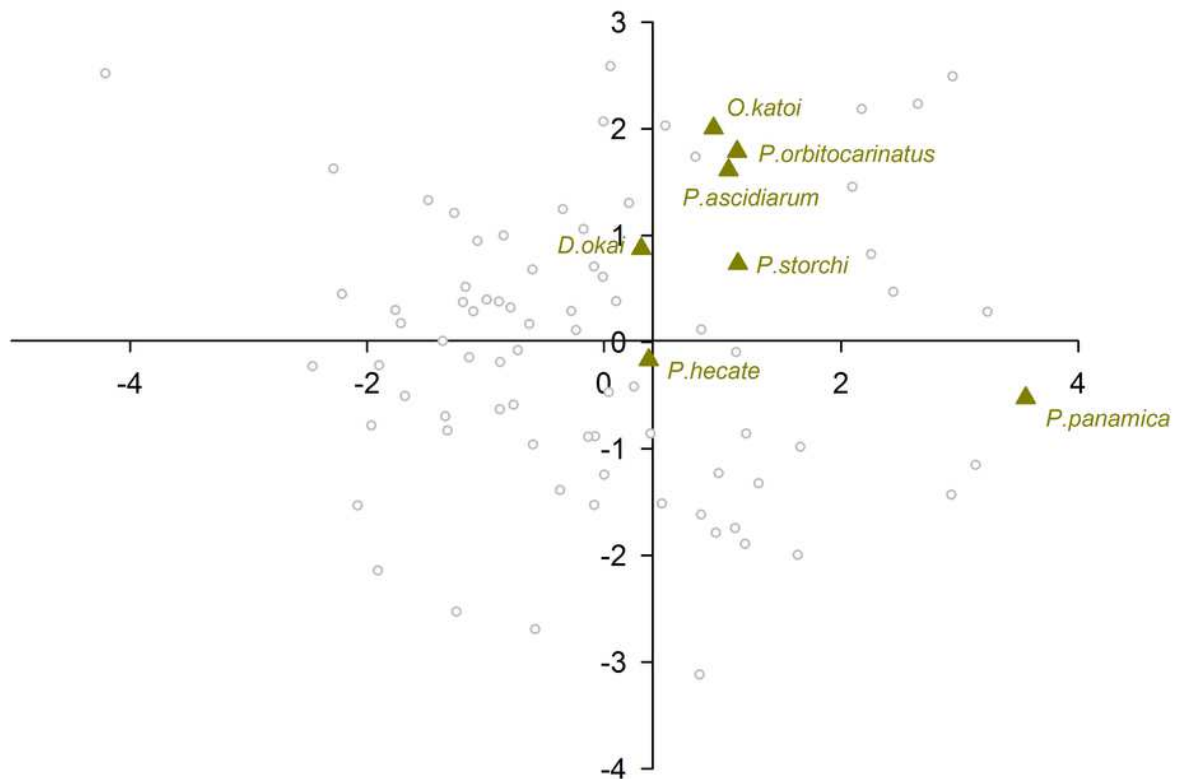
## 4

Figure 4. Morphological variation demonstrated by the DFA scores (first and second root only) of all 83 species of pontonine shrimps (grey circles) displaying the positioning of the centroids for each of the 8 hosts-symbionts groups.



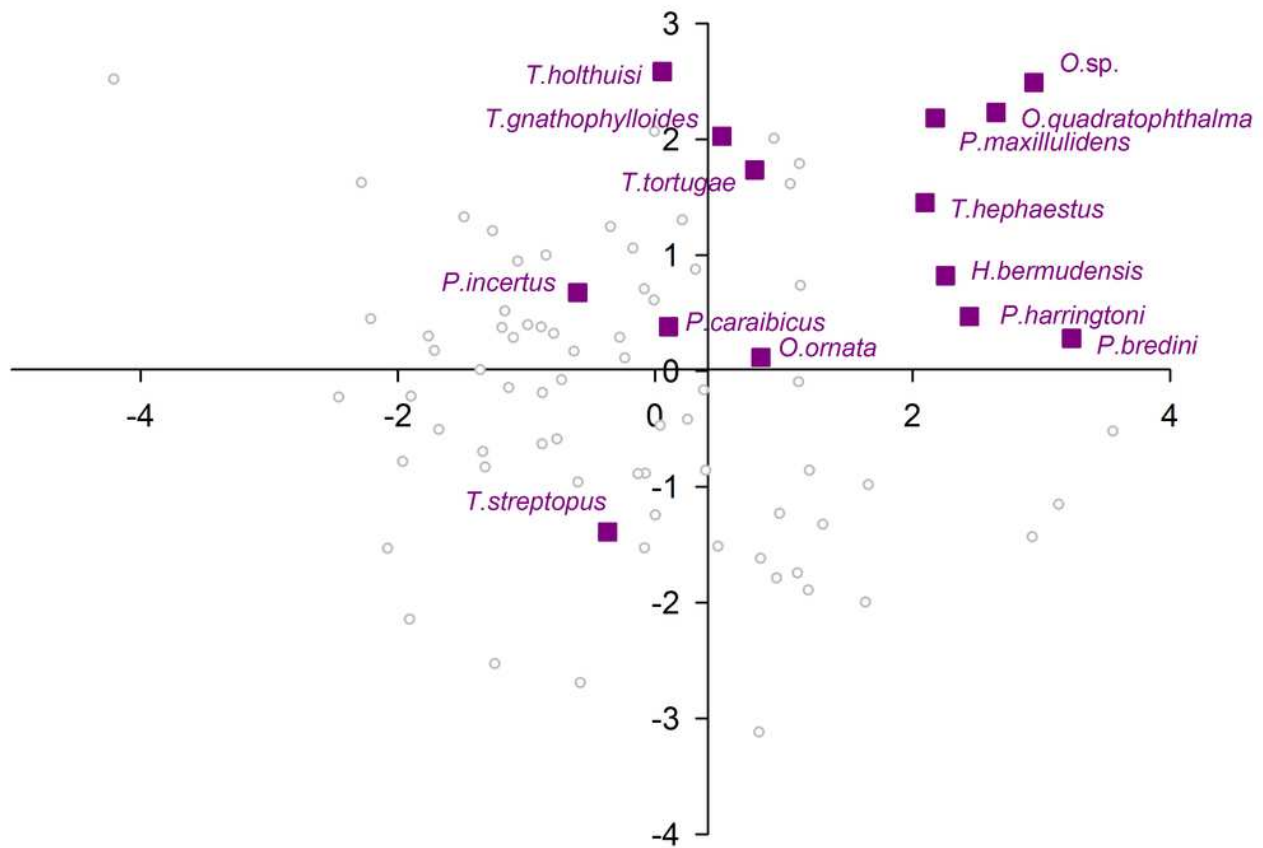
## 5

Figure 5. Morphological variation demonstrated by the DFA scores (first and second root only) of Ascidiacea associates.



## 6

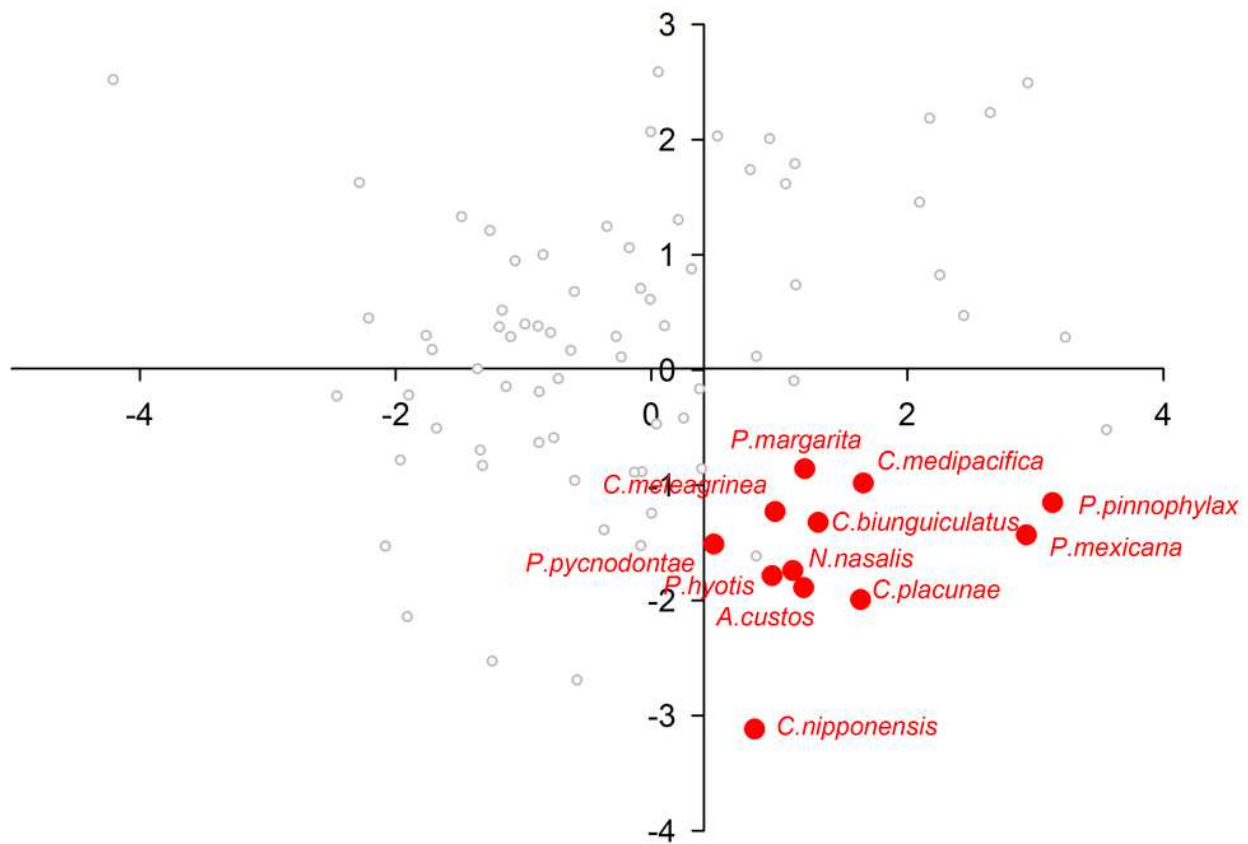
Figure 6. Morphological variation demonstrated by the DFA scores (first and second root only) of Porifera associates.





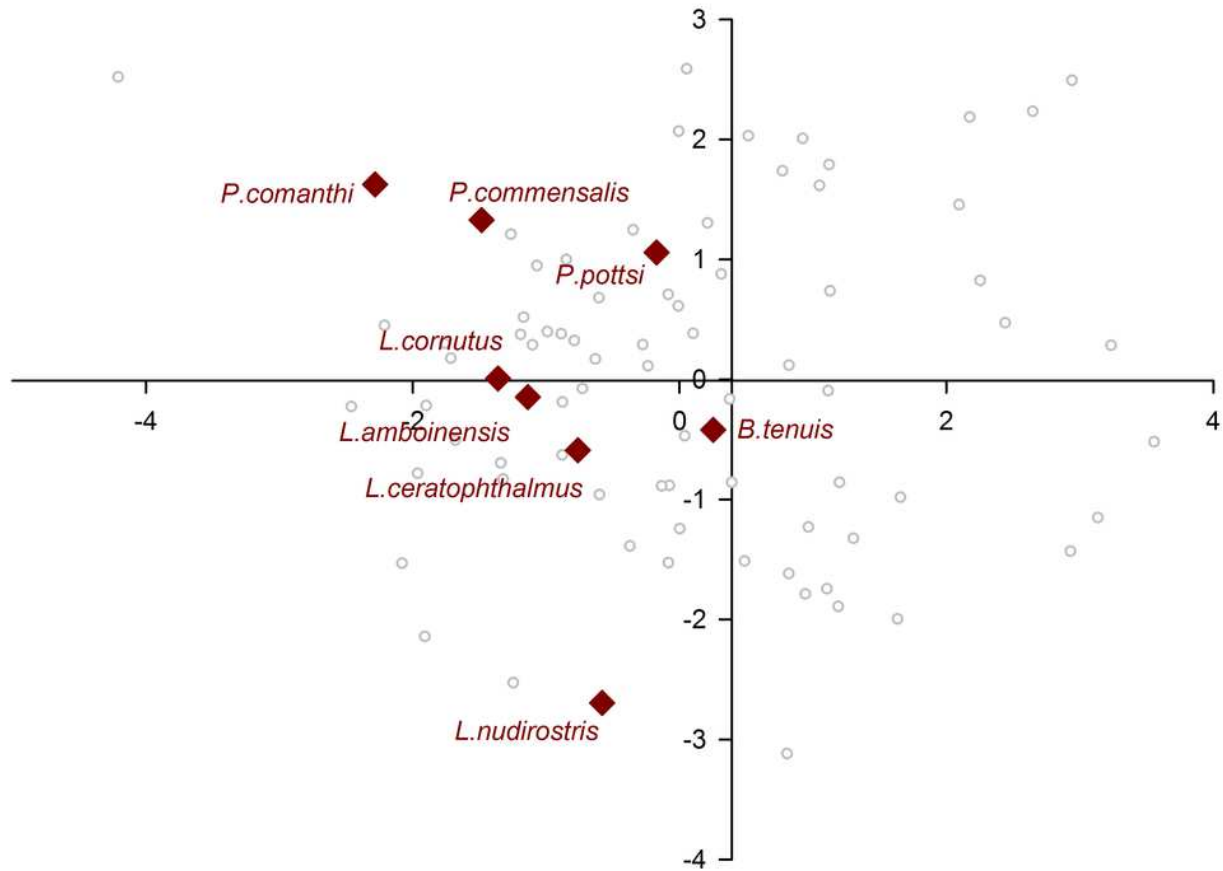
## 7

Figure 7. Morphological variation demonstrated by the DFA scores (first and second root only) of Bivalvia associates.



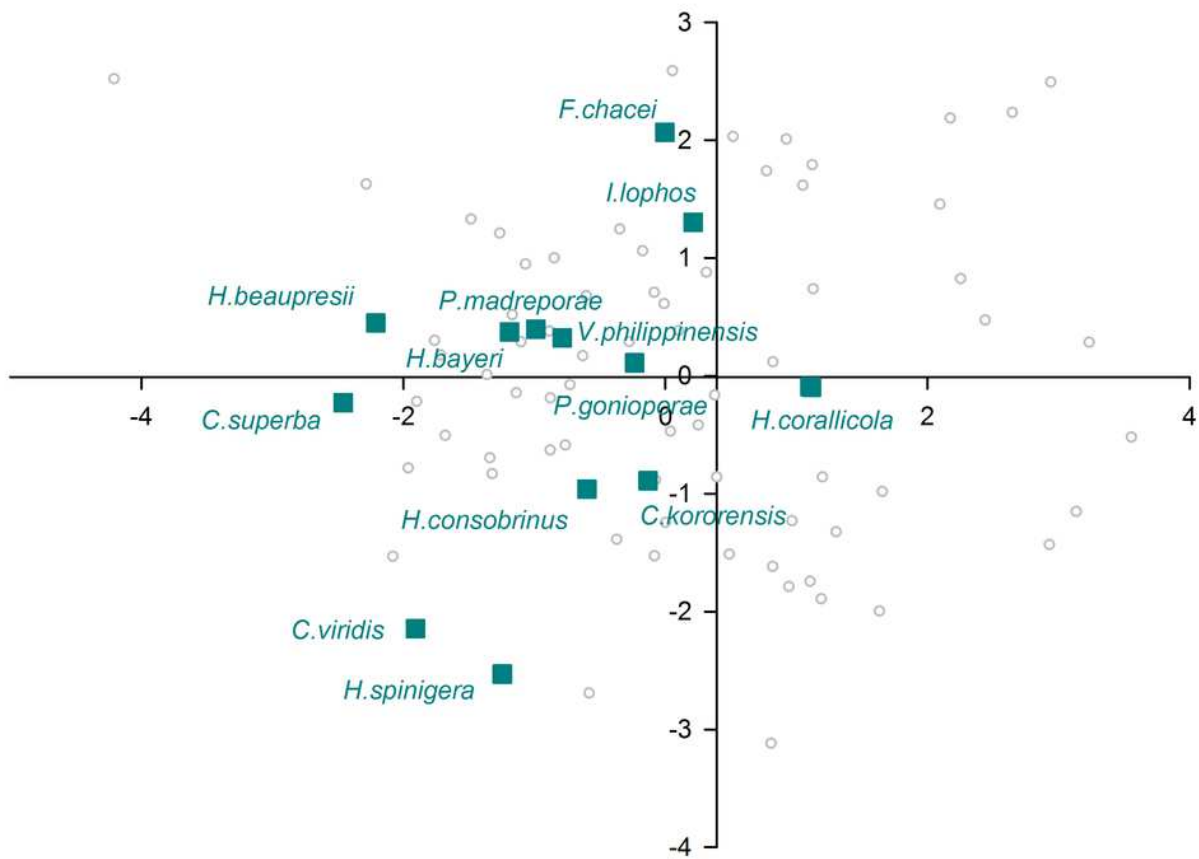
## 8

Figure 8. Morphological variation demonstrated by the DFA scores (first and second root only) of Crinoidea associates.



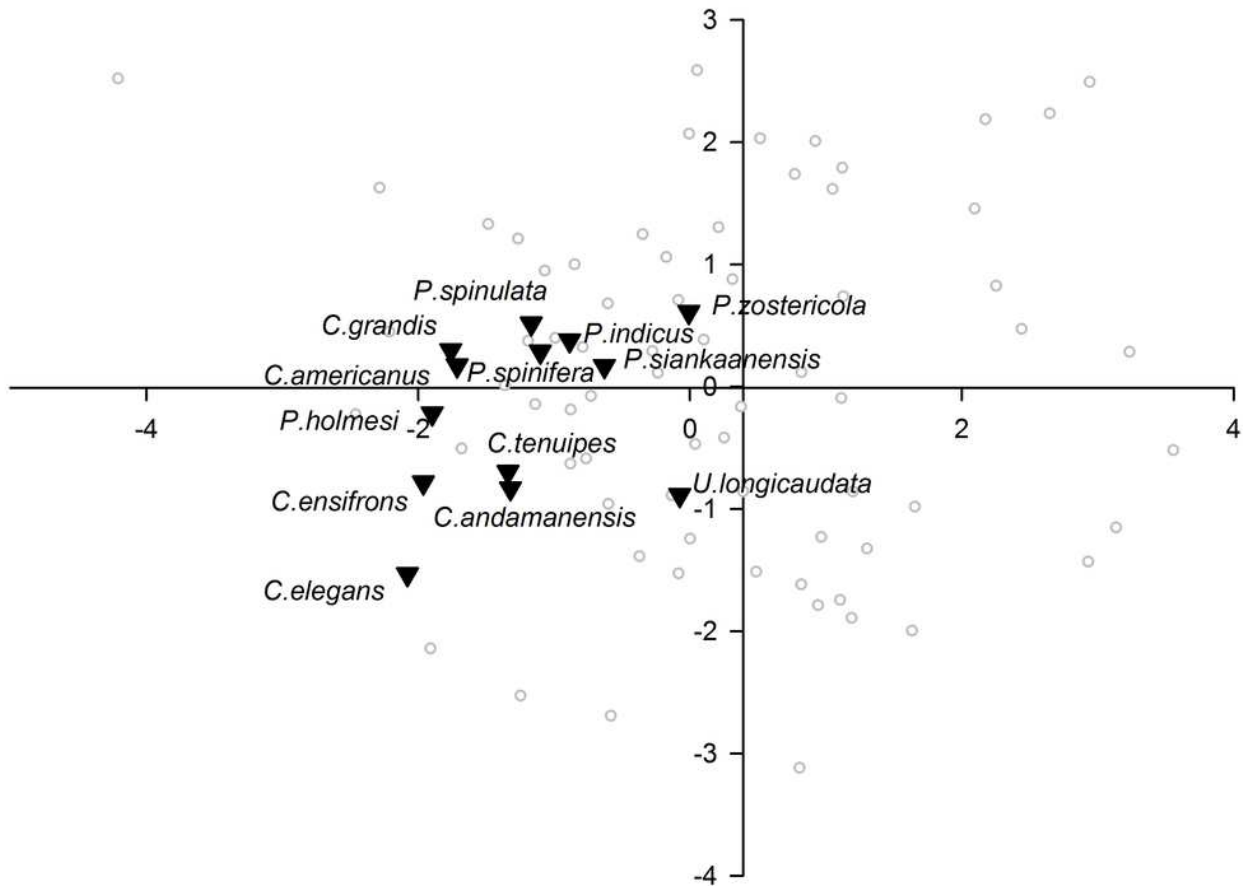
## 9

Figure 9. Morphological variation demonstrated by the DFA scores (first and second root only) of Scleractinia associates.



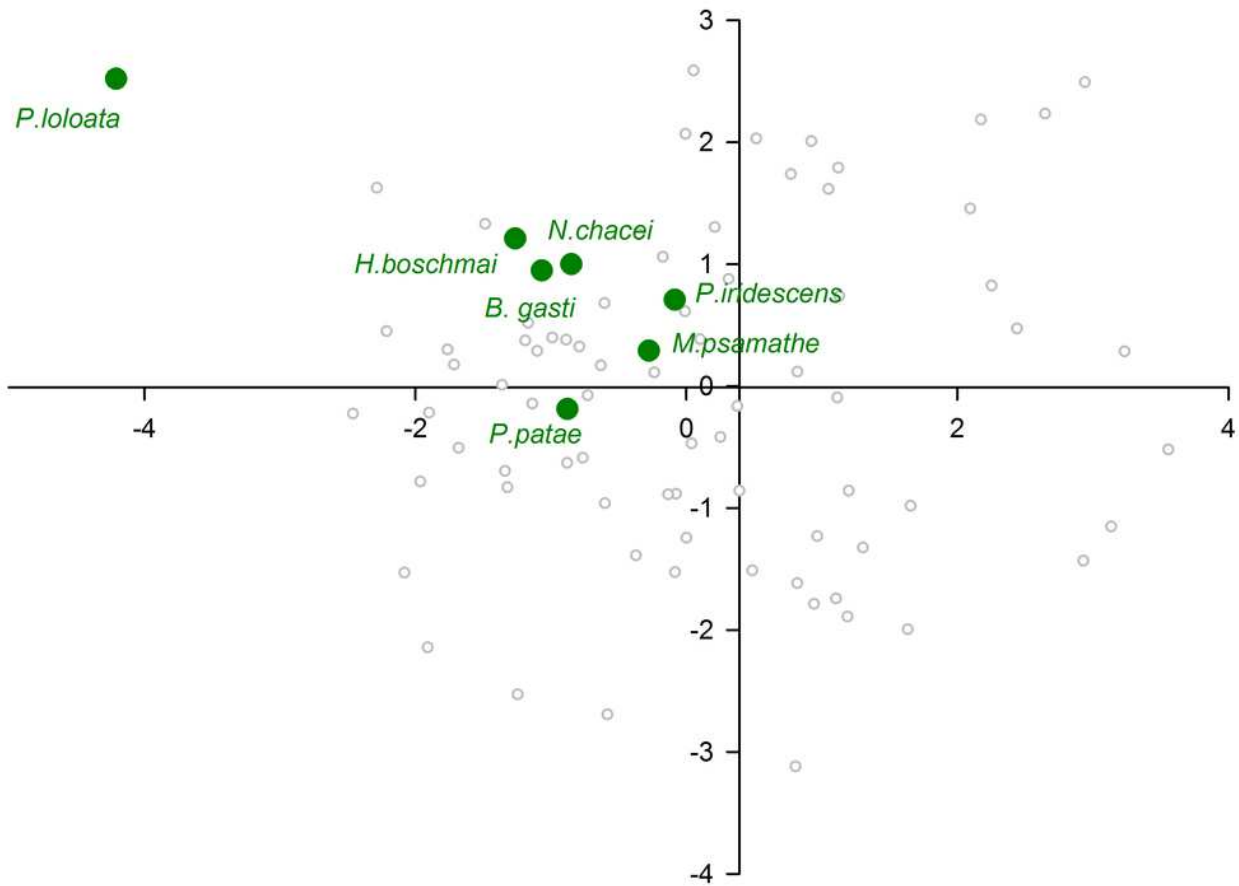
## 10

Figure 10. Morphological variation demonstrated by the DFA scores (first and second root only) of non-commensal species.



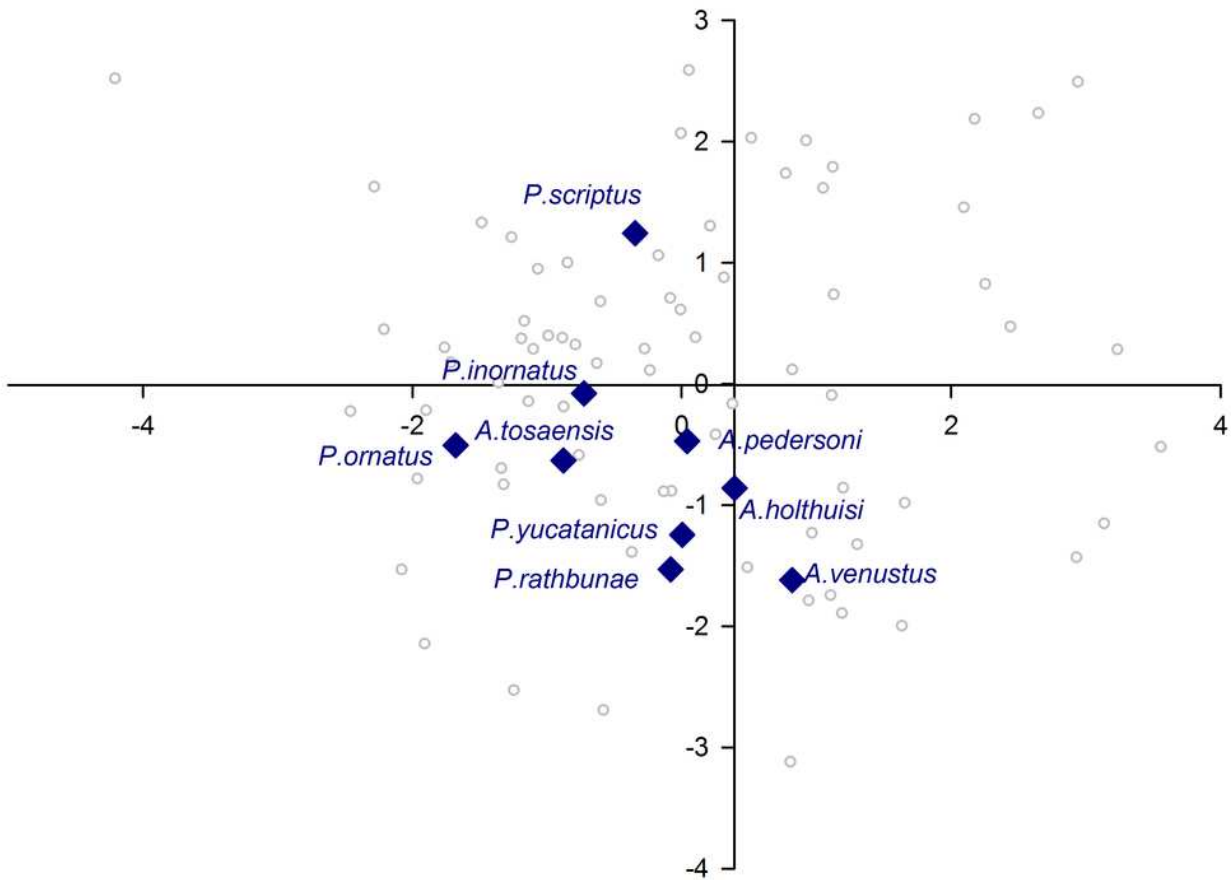
## 11

Figure 11. Morphological variation demonstrated by the DFA scores (first and second root only) of Gorgonacea associates.



## 12

Figure 12. Morphological variation demonstrated by the DFA scores (first and second root only) of Actiniaria associates.



**Table 1** (on next page)

Table 1. Summary statistics for DFA analysis.

1 Table 1. Summary statistics for DFA analysis

2

	Eigenvalue	% of variance	Cumulative %	Canonical correlation	Wilks's $\lambda$	<i>P</i> value
Root 1	1.436	59.1	59.1	0.768	0.194	<0.005
Root 2	0.864	35.5	94.6	0.681	0.473	<0.005
Root 3	0.090	3.7	98.4	0.288	0.882	0.482
Root 4	0.040	1.6	100	0.196	0.962	0.561

3



**Table 2** (on next page)

Table 2. Structure matrix of discriminant loadings, with the largest absolute correlation between each variable and any discriminant function indicated by \*. All variables were entered simultaneously.

1 Table 2. Structure matrix of discriminant loadings, with the largest absolute correlation between  
2 each variable and any discriminant function indicated by \*. All variables were entered  
3 simultaneously.

4

	Function 1	Function 2
ArcsinFD	0.808*	0.482
EP	0.718*	0.166
ArcsinED	-0.657*	0.481
ES-DBES	-0.158	-0.695*

5

**Table 3** (on next page)

Table 3. DFA Classification matrix, showing number of species correctly and incorrectly classified into a priori defined groups, expressed as a percentage of within group species numbers.

Table 3. DFA Classification matrix, showing number of species correctly and incorrectly classified into a priori defined groups, expressed as a percentage of within group species numbers.

		DFA classification							
		Actiniaria	Ascidacea	Bivalvia	Crinoidea	Non-commensal	Gorgonacea	Porifera	Scleractinia
A p r i o r i g r o u p s	Actiniaria	22.2	-	11.1	11.1	22.2	-	-	33.3
	Ascidacea	14.3	-	14.3	-	-	-	71.4	-
	Bivalvia	-	-	100.0	-	-	-	-	-
	Crinoidea	25.0	-	-	12.5	12.5	37.5	-	12.5
	Non-commensal	7.7	-	-	15.4	61.5	-	-	15.4
	Gorgonacea	-	-	-	28.6	14.3	42.9	-	14.3
	Porifera	7.1	-	-	14.3	-	-	78.6	-
	Scleractinia	15.4	-	-	7.7	15.4	-	23.1	38.5