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Morphology and reproduction in the *Hapalocarcinus marsupialis* Stimpson, 1859 species complex (Decapoda: Brachyura: Cryptochiridae)

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ABSTRACT

Symbiotic relationships contribute considerably to the high biodiversity found on coral reefs. Coral-dwelling gall crabs (Cryptochiridae) represent a prime example of coral-associated invertebrates that exhibit obligate relationships with their host. The induction of a skeletal modification in the coral, used as a dwelling by the crab, is the most remarkable aspect of this close association. Here we examined *Hapalocarcinus marsupialis* Stimpson, 1859, collected from *Pocillopora* and *Stylophora* corals in the Saudi Arabian Red Sea. Specimens were DNA barcoded, and five distinct clades were revealed, providing further evidence for the hypothesis that *H. marsupialis* is a species complex. Divergence (COI) between the clades ranged from 3.2 to 15.7%. The putative species were tested for differences in morphology and reproduction. Crabs were examined regarding morphometric characters (carapace length and width, pleon (abdomen), chelae, and growth patterns) and reproductive traits. The data were pooled and analysed by host genus and putative species, which revealed significant differences for most of the measured variables in female crabs. Specimens retrieved from *Pocillopora* were significantly larger (up to 49 %) and had higher fecundity than those inhabiting *Stylophora*. For reproductive output (RO) no differences at species- or host-genus level were detected. The average RO of ~70% over all specimens is high compared to other brachyurans, supporting the assumption that symbiotic brachyurans invest more energy in reproduction than their free-living counterparts. Discrepancies with published data on growth and reproduction of *Hapalocarcinus* are discussed. Our results demonstrate the usefulness of morphometric traits and fecundity in separating the clades in the *H. marsupialis* complex, and prepare the ground for further morphometric studies on the genus and other symbiotic brachyurans. Moreover, it highlights the need to check for the presence of cryptic species when studying aspects of the biology of a species.

Key words: associated coral fauna, cryptic species, Crustacea, gall crabs, genetic fingerprinting, host specificity, Pocilloporidae, scleractinian corals, symbiosis

INTRODUCTION

Symbiotic relationships are a well-documented phenomenon in tropical marine organisms (Castro, 1988). Living in symbiosis with a host species serves as a refuge from predation, food source, or mating site (Bauer, 2004; Stella *et al.*, 2011). The minute and easily overlooked coral-dwelling gall crabs of the family Cryptochiridae Paul'son, 1875, represent a prime example of obligate symbiotic relationships on coral reefs (Castro, 1976). A remarkable detail of the symbiotic relationship with their host is the modification of the coral tissue resulting in the development of a dwelling or so-called "gall" (Verrill, 1867; Calman, 1900; van der Meij & Hoeksema, 2013). Their predominant feeding mode was considered to be filter-feeding by Potts (1915) and Abelson *et al.* (1991), in contrast

to Kropp (1986), who suggested cryptochirids feed on coral mucus and coral tissue picked from the host surface. Furthermore, it remains unclear whether the relationship between gall crabs and corals is of a commensal, mutualistic, or parasitic nature (Castro, 1988; Simon-Blecher & Achituv, 1997, Terrana *et al.*, 2016).

First described from the Hawaiian Islands as living on branching corals by Stimpson (1859), the gall crab *Hapalocarcinus marsupialis* Stimpson, 1859 is known to inhabit corals of the genera *Pocillopora* Lamarck, 1816, *Stylophora* Schweigger, 1820, and *Seriatopora* Lamarck, 1816, all belonging to Pocilloporidae Gray, 1840 (Fize & Serène, 1957). *Hapalocarcinus marsupialis* is one of the most well-studied cryptochirid species (Potts, 1915; Kotb & Hartnoll, 2002) because their dwellings are often abundant, easily recognized, and their hosts can be encountered on the shallower sections of coral reefs (Terrana *et al.*, 2016).

After the description of *H. marsupialis* as the first gall-crab species, the subfamily Cryptochiridae was erected within the family Pinnotheridae by Paul′son (1875), which was subsequently elevated to family level by Richters (1880). The status of *H. marsupialis* was not affected by the subsequential substantial taxonomic revisions by e.g., Fize & Serène (1957) and Kropp (1990), however, several studies discuss the possibility of *H. marsupialis* being a species complex rather than a single species (Gore *et al.*, 1983; Kotb & Hartnoll, 2002; Castro, 2011; van der Meij & Schubart, 2014; Wei *et al.*, 2016). The first author suspecting the existence of two species based on their occurrence on two different coral hosts was Verrill (1869). MacNamee (1961) recorded different sizes for her Hawaiian specimens compared to material from Japan and Vietnam, suggesting there is 'racial variation' in the genus. Gore *et al.* (1983) examined the first two larval stages and noted discrepancies with earlier descriptions. Taking Verrill's findings into account they discuss the possibility of another species in the genus. More evidence supporting the hypothesis of *H. marsupialis* being a species complex comes from Kotb & Hartnoll (2002), and van der Meij & Schubart (2014). A combined biogeographic and host specificity approach, based on a large global dataset, indicates the presence of various cryptic species in *H. marsupialis* (SETM *et al.*, unpublished data).

Studies on the morphology of *H. marsupialis* confirmed the family's peculiarities compared to other brachyurans by reporting a lengthened carapace (cephalothorax) that is longer than wide, which allows the formation of a large brood pouch (Kotb & Hartnoll, 2002; McLay & Becker, 2015; Vehof *et al.*, 2016). Investigation of the reproductive aspects of *H. marsupialis* showed that this enlarged brood pouch (marsupium) is formed by the female pleon (abdomen). It enables the females to carry a considerable number of maturing embryos until hatching (Potts, 1915) and is a synapomorphy of Cryptochiridae (Vehof *et al.*, 2016). Kotb & Hartnoll (2002) recorded a high reproductive investment per brood of about 59% in Red Sea populations. Comparably high investment rates were found in pinnotherids (66%–97%), which also have a strongly broadened pleon similar to cryptochirids (Hines, 1992; Becker *et al.*, 2011). These results are particularly noteworthy because brood weight is generally constrained in non-symbiotic brachyuran crabs to about 20% of female body weight due to the limitation in space available for yolk accumulation within the calcified cephalothorax (Kotb & Hartnoll, 2002 and references therein).

The likely presence of cryptic species within *Hapalocarcinus* warrants the study of differences in morphology and reproduction among putative species. To address this question, we examined specimens of *H. marsupialis* collected in the Saudi Arabian Red Sea regarding differences in their 1) mitochondrial DNA using the COI barcoding gene, 2) morphological, and 3) reproductive characteristics. Various morphometric measurements were taken, including size of carapace, pleon, chelae, dactyl length and female weight, egg weight and size, brood mass volume, fecundity, and reproductive investment were examined in gravid females. Herein, we combine our findings with regard to genetic variability, morphometrics, and reproductive traits to test whether these characters can help separate clades and to reveal new details about the reproductive output of this genus.

MATERIAL AND METHODS

Sampling

Specimens were collected during two separate cruises (18–23 August 2018 and 14–17 August 2019) from 18 sites near Thuwal, Saudi Arabia (Supplementary material Table S1, Fig. S2). A total of 115 female (97 adult, 16 juveniles, and two damaged specimens) and 48 male specimens from 20 *Pocillopora* and 55 *Stylophora* colonies were collected between 1 and 32 m depth (Supplementary material Table S3). The gall crabs were individually placed in collection tubes and preserved in 70% ethanol for the duration of the study. Morphological examination was undertaken using an Olympus SZ51 stereomicroscope (Olympus, Tokyo, Japan). Crab sex was determined and females were classified into four colour groups (adapted from Kotb & Hartnoll (2002), who also studied Red Sea populations: white (nearly without any dark pigmentation), white-brown (more white than brown pigmentation), brown-white (more brown than white pigmentation), and brown (nearly without any bright pigmentation) (Supplementary material Fig. S4). All metadata, including the measurements below, are available in Supplementary material Table S5.

Genetic analyses

The fifth pereiopod of male and female gall crabs was used for molecular analysis based on the cytochrome oxidase subunit I (COI) barcoding gene (Folmer *et al.*, 1994), using a CTAB extraction protocol. First, the alcohol was removed by adding 0.5 ml CTAB (2%) together with 3 µm of proteinase K, followed by overnight incubation at 60 °C. The samples were subsequently extracted with a 24:1 solution of chloroform and isoamylacetate (0.5 ml), centrifuged at 8,000 rpm for 10 min, and the supernatant collected in a new collection tube. After precipitating the supernatant with 0.35 ml isopropanol, the samples were incubated overnight at 4 °C and subsequently centrifuged at 8,000 rpm for 10 min. The supernatant was carefully removed and 0.5 ml ethanol/NH_4- acetate solution added and the samples incubated at room temperature for 30 min. After centrifuging for 10 min at 8,000 rpm, the solution was carefully discarded and the samples dried overnight in the refrigerator to ensure the complete evaporation of the ethanol. After drying, 40 µm MilliQ was added. A polymerase chain reaction (PCR) was performed under the following conditions: 2.5 µl PCR-buffer, 2.5 µl DNTPS, 1 µl of primers LCO1490, and HCO2198 (Folmer *et al.*, 1994) respectively, 0.3 µl Taq, 18.7 µl MilliQ and 1 µl DNA template. Thermal cycling was performed as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 5 sec, 47 °C for 1 min, and 72 °C for 1 min and finalized by 10 min at 72 °C using AB Applied Biosystems Veriti 96 well thermocycler (Applied Biosystems, Foster City, CA, USA). PCR-product was cleaned and prepared for sequencing conducted by GATC using the SIGMA-Aldrich GenElute TM PCR Clean-Up Kit (SIGMA-Aldrich, St. Louis, MO, USA).

The resulting dataset of 106 sequences was aligned in BioEdit (Hall, 1999). A maximum likelihood analysis was conducted by IQ-TREE (Nguyen *et al.*, 2015), after performing a test of the best-fit model for nucleotide substitution using ModelFinder (Kalyaanamoorthy *et al.*, 2017). One thousand ultrafast bootstraps were performed using UFBoot (Hoang *et al.*, 2018). We selected the cryptochirids *Fungicola utinomi* (Fize & Serène, 1956), *Pseudocryptochirus viridis* Hiro, 1938, and *Utinomiella dimorpha* (Henderson, 1906) as the outgroups based on van der Meij &

Nieman (2016). In the phylogenetic tree, samples named 'GC' followed by a number are specimens specifically sampled for this study and for which morphometric and reproductive data is available. Samples with a five-digit code belong to previously collected *Hapalocarcinus* specimens stored at Naturalis Biodiversity Center, Leiden, The Netherlands (collection coded as RMNH.Crus.D), for these samples voucher specimens are available. Evolutionary divergence over sequence pairs (p-distance) within and between *a priori* determined groups was obtained using MEGA 7 (Kumar *et al.*, 2016).

Sequences have not been submitted to GenBank because of a lack of voucher specimens. The crabs were destroyed in the process of obtaining reproductive output data (see below); however, the sequences are available in Supplementary material Appendix S6.

Morphometric measurements

The following measurements were taken: carapace length (CL) from post-orbital margin to the posterior border of the carapace, carapace width (CW) as the distance between distal margins of the carapace, length of the chela (LC), width of the chela (WC), length of the dactylus (moveable finger) (LD) for females and males, pleon length (AL) as the distance between posterior margin of the carapace to the distal part of the pleon, and pleon width (AW) distance between lateral margins of the pleon were taken exclusively for female specimens. Variables of female body size (CL, CW, AL, AW) were measured to the nearest 10 µm using a Mitutoyo No. 500-311 digital calliper (Mitutoyo Corporation, Kanagawa, Japan). The remaining measurements were taken using a stereomicroscope equipped with a calibrated optical micrometer (accuracy 1 µm). Juveniles were excluded from the morphometric analyses.

Reproduction analyses

The entire brood mass of the ovigerous females (N = 27) was gently removed from the brood pouch using soft tweezers. The eggs were classified into two stages following Zimmermann *et al.* (2015): stage I (early development, no eyespots visible) and stage II (late development, with visible eyespots) and subsequently counted. Fecundity was determined as the total number of eggs found in a brood pouch. Five eggs from each female were randomly chosen for measurement of the major and minor axis to the nearest 1 µm (including the chorionic membrane adhering tightly to the embryonic surface) using a stereomicroscope equipped with an optical micrometre. Using the formula of oblate spheroids (cf. Turner & Lawrence, 1979):

$$\mathrm{EV} = 4/3 * \pi * \mathrm{EL}/2(\mathrm{EW}/2)^2$$

where EV= egg volume, EL = egg length (major axis), and EW = egg width (minor axis), the volume of the eggs was calculated. The volume of the total egg mass was obtained by multiplying egg volume with the total number of eggs. Female specimens, together with their separated eggs, were dried at 60 °C for 48 h in an oven and weighed with a NewClassic MF MS104S analytical balance (Mettler Toledo, Columbus, OH, USA) with an accuracy of 0.1 mg. If a female specimen was also used for the genetic analysis, the fifth pereiopod was weighed separately to counterbalance for the one used in the DNA extraction. Dry weights were used to calculate the reproductive output (RO) by dividing the brood weight with female body weight (see Table 6).

Statistical analyses

We assessed normality with a Shapiro test and non-parametric data was log transformed for linear regression of the morphometric and reproductive variables. Count data was tested for potential zero-inflation using the R package DHARMa (Hartig, 2021). We examined whether size, carrying eggs or putative species (referred to as HM.01–HM.05) correlate with colour of female crabs using Chi-square and Fisher's exact tests. We used Pearson's correlations and, when assumptions of normality were not met, Spearman rank correlation to identify potential correlations between measured traits, and analysis of covariance using sex and host as factors.

Linear regressions of log transformed morphological and reproductive measurements were calculated for the allometric scaling analysis. The resulting relationships can be summarized as log Y = a ± b log X. We used analysis of covariance (ANCOVA) with CL, CW and female weight as covariates and CW, AL, fecundity, and brood weight as dependent variables. Host and sex served as factors in the analysis. When assumptions for parametric analysis were not met, we used Spearman rank correlations, Kendall-Theil Sen Seigel nonparametric regression using the R package mblm (Komsta, 2019), and a nonparametric ANCOVA following the Quade's method (Theil, 1950; Quade, 1967; Sen, 1968; Siegel, 1982). Allometry/isometry were examined by comparing the value of the regression slope (b) with the respective value for an isometric growth relationship of the crabs' bodies and reproductive traits (fecundity) with a t-test.

In order to test for size disparity between clades HM.01–HM.05, we used Kruskall-Wallis tests with subsequent post-hoc (Dunn Test) analysis (when normality was violated) or one-way ANOVA with Tukey post-hoc tests (Supplementary material Tables S9, S10). Potential size differences between specimens collected from the two host genera were investigated with t-tests or, when normality assumptions were not met, Wilcoxon tests (which is identical to Mann-Whitney U test in R; Ennos & Johnson, 2018) (Supplementary material Table S7, S8). A significance level of 0.05 was adopted. All statistical analyses were performed in R Studio (R Core Team, 2020).

RESULTS

Phylogeny

A phylogenetic tree was constructed based on the dataset containing 106 sequences, with *F. utinomi*, *P. viridis*, and *U. dimorpha* as outgroups, using a maximum-likelihood approach (Fig. 1). Five distinct clades, of which four have high supportive values, were retrieved (clades HM.01–HM.05). Most of our samples were retrieved in clades HM.01 and HM.02; all these specimens were collected from *Stylophora*. After comparing with results by SETM *et al.* (unpublished data), we found that sequences of specimens of clades HM.03, HM.04, and HM.05 clustered with those of other *H. marsupialis* individuals associated with *Pocillopora* corals, whereas specimens of clades HM.01 and HM.02 clustered with those inhabiting host corals *Stylophora* (and to a lesser extent *Seriatopora*, for which we had no samples in our study).

Within and between evolutionary divergence over sequence pairs was estimated for all five clades. For the analysis, the groups were determined *a priori* based on the grouping in the phylogeny reconstruction (Fig. 1). Table 1 shows intraspecific differences within clades HM.01, HM.02, and HM.03 ranging from 0.99 to 1.34%. The largest interspecific difference with 14.2% was estimated between clades HM.03 and HM.04 followed by 13.8% between clades HM.03 and HM.05. Clades HM.01 and HM.02 were with 3.3% the least divergent groups (Table 2).

Female colouration

The colour of female specimens varied greatly from completely white (no pigmentation) to dark brown (fully pigmented)

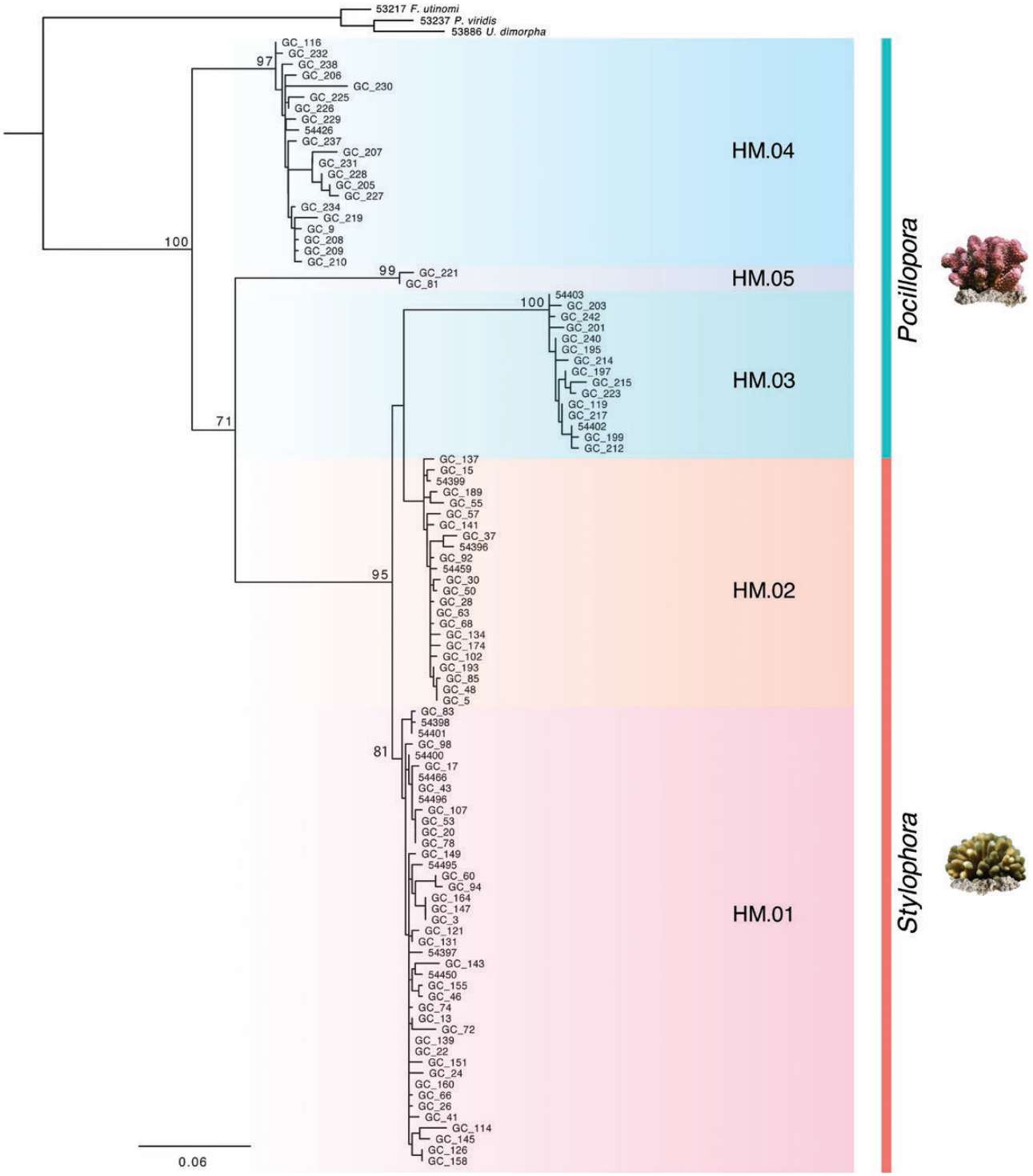

Figure 1. ML Tree (1,000 ultrafast bootstraps) containing 89 *Hapalocarcinus marsupialis* sequences from this study and additional sequences ($N = 14$) from SETM *et al.* (unpublished data). *Fungicola utinomi*, *Pseudochryptochirus viridis*, and *Utinomiella dimorpha* were used as outgroups.

(Supplementary material Fig. S4). Four different colour categories were tabulated in relation to crab size and assigned putative species (Table 3). Most individuals ($N = 55$) were retrieved in the mixed colour groups. Juvenile specimens were either coloured white ($N = 15$) or white-brown ($N = 1$). Before proceeding with the analysis, we tested count data for zero-inflation using the DHARMa package in R (Hartig, 2021). No zero-inflation of the data was detected. There was no association between egg-bearing and colour ($\chi^2(3) = 0.492$, $P = 0.921$). Fisher's exact test, however, revealed an impact of size on colour ($P < 0.001$). Furthermore, putative species and host genus were associated with colour (Fisher's exact test, $P = 0.004$ and $P < 0.001$, respectively). Even though they inhabit the same coral host, clade HM.02 specimens appeared to be darker than those belonging to clade HM.01.

Morphology of carapace and pleon

Carapace length varied 2.59–6.23 mm (mean 3.54 mm (SD ± 0.78)) in adult females and 1.08–2.47 mm (mean 1.79 mm (SD ± 0.32)) in males (Tables 4, 5). The size of the female pleon varied 2.27–9.79 mm (mean 4.57 mm (SD ± 1.37)) in length and 2.27–7.76 mm (mean 4.42 mm (SD ± 1.28)) in width (Table 4).

Females. We used Mann-Whitney U-test to test for morphological differences between *H. marsupialis* individuals collected from *Pocillopora* and *Stylophora* hosts. The tests revealed significant differences (Supplementary material Table S7) between the specimens from both host genera for all morphological traits that were measured (CL, CW, AL, and AW). The carapace of crabs retrieved from *Pocillopora* was on average 41.89% longer and 40.2% wider than that of crabs from *Stylophora* (Table 4; Fig. 2A, B). The size difference of the pleon showed a very similar pattern with crabs collected from *Pocillopora*, having a 49.4% longer and 42.4% wider pleon than those from *Stylophora* (Table 4; Fig. 3A, B).

For the subsequent analysis the specimens were further divided according to the putative species they were assigned to in the phylogenetic analysis. Overall, crabs belonging to clades HM.03, HM.04, and HM.05 (from *Pocillopora*) seemed at first to be bigger than those from clades HM.01 and HM.02 (from *Stylophora*) for all morphological traits that were measured (Table 4). Unlike in the previous analyses with host genus as grouping variable, not all of the observed differences between the clades were significant (Fig. 2C, D; Supplementary material Table S9). The statistical analysis revealed a significant difference between clades HM.04 and HM.01 for all measured traits, whereas clades HM.03 and HM.01 only differed in carapace length and width. Furthermore, there was no significant difference between clade HM.02 and either of the clades that inhabit pocilloporid corals (HM.03, HM.04, and HM.05). It is noteworthy that crabs belonging to clade HM.02 had a significantly longer and wider carapace and furthermore a wider pleon than those belonging to clade HM.01 (Figs. 2C, D, 3C, D).

Males. Differences in morphology between male specimens collected from both host genera were analysed using student's t-tests. Compared to the morphological analysis of the female specimens there seemed to be a similar pattern with males retrieved from *Pocillopora* having a longer and wider carapace, but with $P = 0.052$ and $P = 0.093$ for both respectively, t-tests indicate that the observed differences were not significant (Supplementary material Table S8). In the subsequent analyses of size disparity on clade level, no significant differences between male HM.01–HM.05 specimens were detected, which is most likely caused by the small sample size of males (Supplementary material Table S10).

Morphology of chelae

Chelae length varied 1.16–2.54 mm (mean 1.66 mm (SD ± 0.31)) in adult females and 0.66–1.82 mm (mean 1.05 mm (SD ± 0.24)) in males (Tables 4, 5). For the character chelae width, a size range of 0.36–0.96 mm (mean 0.51 mm (SD ± 0.11)) and 0.33–0.83 mm (mean 0.53 mm (SD ± 0.10)) was recorded in adult females and males, respectively. The length of the dactyli varied 0.1–0.33 mm (mean 0.71 mm (SD ± 16)) in female specimens and 0.36–0.97 mm (mean 0.61 mm (SD ± 1.2)) in width (Tables 4, 5).

Females. Mann-Whitney U tests revealed significant differences for all three measured traits (LC, WC, LD). Crabs retrieved from pocilloporid hosts had a 30.2% longer and 43.9% wider chela compared to those collected from *Stylophora*. The difference in dactylus length was 48.3% (Fig. 4A, B; Supplementary material Table S7). Specimens attributed to clades HM.03 and HM.04 (*Pocillopora*) had significantly larger chaelae and dactyli than clades HM.01 and HM.02 specimens (*Stylophora*). Furthermore, individuals of clade HM.05 seem to grow larger chelae and dactyli compared to those in clades HM.01 and HM.02 but due to the low

Table 1. Estimates of evolutionary divergence (p distance) within groups based on COI, with the groupings of *H. marsupialis* determined *a priori* based on the phylogenetic reconstruction of Figure 1. Number of base substitutions per site averaging over all sequence pairs and standard error (SE) estimates are shown.

	d	SE
Clade HM.01	0.0076	0.0017
N = 34		
Clade HM.02	0.0103	0.0027
N = 20		
Clade HM.03	0.0236	0.0043
N = 13		
Clade HM.04	0.0124	0.0029
N = 19		
Clade HM.05	0.0074	0.0052
N = 2		

Table 2. Estimates of evolutionary divergence (p distance) between groups based on COI, with the groupings of *H. marsupialis* determined *a priori* based on the phylogenetic reconstruction of Figure 1. The number of base substitutions per site averaging over all sequence pairs shown below the diagonal. Standard error (SE) estimates shown above the diagonal.

	Clade HM.01	Clade HM.02	Clade HM.03	Clade HM.04	Clade HM.05
Clade HM.01		0.008	0.017	0.015	0.018
Clade HM.02	0.032		0.018	0.015	0.020
Clade HM.03	0.119	0.136		0.019	0.018
Clade HM.04	0.085	0.093	0.156		0.021
Clade HM.05	0.122	0.133	0.117	0.157	

Table 3. Number of female *H. marsupialis* individuals (juveniles and adults) of particular colours sorted regarding total number, species, and size class; three individuals could not be assigned to a clade due to missing genetic data, while determination of the size class of five individuals failed because of a damaged carapace.

		Host		Clade					Carapace width (mm)				
Colour	Total (ovigerous)	*Stylophora*	*Pocillopora*	HM.01	HM.02	HM.03	HM.04	HM.05	1.0-1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9
White	40 (6)	25	15	16	8	4	11	1	2	19	13	5	
White-brown	30 (7)	25	5	15	10	2	3			3	22	1	3
Brown-white	25 (7)	24	1	10	14		1			3	18	3	
Brown	20 (7)	20	0	10	10					4	7	7	

Table 4. Morphometric traits (mean ± SD) of female *Hapalocarcinus marsupialis* individuals for all specimens combined, as well as separated according to assigned coral host

	Carapace length (mm)	Carapace width (mm)	Pleon length (mm)	Pleon width (mm)	Chelae length (mm)	Chelae width (mm)	Dactylus length (mm)	Female dry weight(mg)
Total	3.54 ± 0.78	3.33 ± 0.72	4.57 ± 1.37	4.24 ± 1.28	1.66 ± 0.31	0.51 ± 0.11	0.71 ± 0.16	6.93 ± 4.9
	N = 92	*N* = 92	*N* = 89	*N* = 89	*N* = 93	*N* = 93	*N* = 92	*N* = 97
Clade HM.01	3.11 ± 0.35	2.97 ± 0.3	4 ± 0.75	3.53 ± 0.7	1.52 ± 0.12	0.46 ± 0.05	0.65 ± 0.09	4.76 ± 1.65
	N = 43	*N* = 43	*N* = 42	*N* = 42	*N* = 44	*N* = 44	*N* = 43	*N* = 47
Clade HM.02	3.64 ± 0.6	3.42 ± 0.59	4.67 ± 0.97	4.6 ± 1.08	1.66 ± 0.24	0.5 ± 0.06	0.69 ± 0.09	7.38 ± 4.07
	N = 36	*N* = 36	*N* = 34	*N* = 34	*N* = 36	*N* = 36	*N* = 36	*N* = 37
Clade HM.03	5.29 ± 1.06	4.95 ± 0.92	7.03 ± 3.09	5.84 ± 2.38	2.37 ± 0.15	0.86 ± 0.12	1.15 ± 0.11	14.43 ± 6.63
	N = 4	*N* = 4	*N* = 4	*N* = 4	*N* = 4	*N* = 4	*N* = 4	*N* = 4
Clade HM.04	5.04 ± 0.41	4.71 ± 0.47	7.02 ± 1.05	6.51 ± 0.89	2.32 ± 0.2	0.7 ± 0.07	1.06 ± 0.0	18.1 ± 5.98
	N = 5	*N* = 5	*N* = 5	*N* = 5	*N* = 5	*N* = 5	*N* = 5	*N* = 5
Clade HM.05	4.77	4.17	6.59	5.65	2.48	0.53	0.89	20
N = 1								
Pocillopora	5.11 ± 0.69	4.75 ± 0.66	6.98 ± 1.92	6.15 ± 1.54	2.36 ± 0.17	0.75 ± 0.14	1.08 ± 0.11	16.82 ± 5.93
	N = 10	*N* = 10	*N* = 10	*N* = 10	*N* = 10	*N* = 10	*N* = 10	*N* = 10
Stylophora	3.34 ± 0.53	3.16 ± 0.5	4.26 ± 0.92	4 1.02	1.57 ± 0.2	0.48 ± 0.06	0.66 ± 0.09	5.8 ± 3.24
	N = 82	*N* = 82	*N* = 79	*N* = 79	*N* = 83	*N* = 83	*N* = 72	*N* = 87

number of specimens ($N = 2$) no significant differences could be detected (Fig. 4C, D; Supplementary material Table S9).

Males. Congruent to the pattern observed for the females, males inhabiting *Pocillopora* had a significantly wider chela (16.8%) and a longer dactylus (14.6%) than those inhabiting *Stylophora*. The difference in LC was not significant (t-test, $P = 0.074$) (Supplementary material Table S8). One-way ANOVA revealed a significant size difference for WC between the five putative species ($F = 2.802$, $df = 4, 34$, $P = 0.0411$). A Tukey post-hoc analysis of specimens of clade HM.03 showed significantly wider chelae than those in clade HM.01. No size differences were detected for LC and LD (Supplementary material Table S10).

Allometric scaling of morphological traits

We analysed the relationship between carapace width and length in both sexes. Strong positive correlations were found for both females (Pearson correlation; $r = 0.981$, $t = 48.219$, $df = 90$, $P < 0.001$) and males ($r = 0.936$, $t = 17.458$, $df = 43$, $P < 0.001$). Linear regression analysis was used to examine this relationship further and sex was incorporated to test whether the regression slopes differed between sexes (Fig. 5). An ANCOVA model (adjusted $R^2 = 0.9836$, $F = 2713$, $df = 3, 133$, $P < 0.001$) indicated that there is no significant difference in the regression slopes ($F = 0.364$, $df = 1, 133$, $P = 0.548$) between both sexes and therefore there is no difference in the relationship of carapace length and width in the sexes (Fig. 5). We also tested whether crabs collected from two different coral-host genera (*Pocillopora* and *Stylophora*) differed in the growth relationship of their carapace. The model had a significant fit with adjusted $R^2 = 0.983$ ($F = 2631$, $df = 3, 133$, $P < 0.001$), but did not reveal a difference between the regression slopes ($t = –0.315$, $df = 1, 127$, $P = 0.753$) or intercepts ($t = 1.013$, $df = 1, 127$, $P = 0.313$). Linear regression without sex or host genus as covariates revealed a regression slope of $b = 1.003$, which indicated an isometric ($b = 1$) growth pattern of the carapace (Fig. 5). The growth relationship of pleon and carapace was examined in all females, showing a strong positive correlation (Spearman rank correlation; $rho = 0.79$, $S = 23831$, $P < 0.001$). This relationship was subsequently examined for potential differences between specimens collected from the two host genera following the Quade's method for a non-parametric ANCOVA analysis (because assumptions for parametric analysis were not met). Regression slopes of crabs from *Pocillopora* and *Stylophora* did not differ from each other ($F = 0.114$, $P = 0.737$), indicating that the growth relationship of pleon and carapace are the same for crabs from both host genera (Supplementary material Figure S11). In order to investigate the nature of this relationship (allometry/isometry), a Kendall-Theil Sen Seigel nonparametric regression was applied to the logarised data of carapace width and pleon length. A significant relationship of both body parts ($P < 0.001$) with a regression slope of $b = 1.178$ resulted, indicating positive allometry. A two-tailed t-test nevertheless failed to detect a significant difference between $b = 1.178$ and 1 ($t = 1.284$, $df = 84$, $P = 0.202$), showing that pleon length and carapace width have an isometric growth pattern (Supplementary material Figure S11).

Fecundity, egg size and reproductive output

Of 97 examined females, 27 were egg-bearing; 20 individuals carried eggs of stage I and seven carried eggs of stage II (Table 6). The number of eggs varied from 33 to 2,131 eggs per female (Fig. 6; Table 6). A t-test revealed a significant difference in egg volume between the two developmental stages, with stage II eggs being on average 12.9% larger than stage I eggs ($t = – 3.425$, $df = 23.218$, $P < 0.01$). The number of eggs per female varied widely from 33 to 2131 eggs (mean 492.11 (SD ± 523.68)). There was no significant difference in the number of eggs between the two stages (Wilcoxon test, $W = 103$, $P = 0.716$). The average brood-mass volume was 27.6 mm^3 (± 26.86 mm^3) with the lowest value at 2.39 mm^3 and the highest at 102.09 mm^3. The mean dry egg weight was 1.4 mg 100^{-1}. The RO values varied over a very broad range from 6.67% (CL = 3.03 mm) to 184.44% (CL = 5.9 mm), with a mean value of 69.62 ± 44.91% (Table 6).

As with the morphological analyses, specimens from the two host genera were examined for differences in reproductive traits using Mann-Whitney U-tests. Crabs collected from *Pocillopora* were significantly heavier and carried a considerably larger number of eggs compared to those from *Stylophora*, but no significant difference in the reproductive output of both groups was detected.

The same reproductive traits were compared between the five putative species HM.01–HM.05. Kruskall-Wallis tests were performed and if significant, post-hoc analysis (Dunn tests) was used to detect differences between the clades. The analysis revealed significant differences for the traits female weight and fecundity with clades HM.03 and HM.04 specimens having higher fecundity and

Table 5. Morphometric traits (mean ± SD) of male *H. marsupialis* individuals for all specimens combined, as well as separated according to assigned coral host

	Carapace length (mm)	Carapace width (mm)	Chelae length (mm)	Chelae width (mm)	Dactylus length (mm)
Total	1.79 ± 0.32	1.66 ± 0.30	1.05 ± 0.24	0.53 ± 0.10	0.61 ± 1.2
	N = 45	*N* = 45	*N* = 42	*N* = 42	*N* = 42
Clade HM.01	1.63 ± 0.25	1.51 ± 0.25	0.94 ± 0.16	0.46 ± 0.08	0.53 ± 0.10
	N = 14	*N* = 14	*N* = 12	*N* = 12	*N* = 12
Clade HM.02	1.83 ± 0.35	1.7 ± 0.30	1.05 ± 0.23	0.54 ± 0.08	0.61 ± 0.08
	N = 11	*N* = 11	*N* = 10	*N* = 10	*N* = 10
Clade HM.03	1.96 ± 0.36	1.78 ± 0.36	1.20 ± 0.30	0.60 ± 0.14	0.68 ± 0.18
	N = 7	*N* = 7	*N* = 8	*N* = 8	*N* = 8
Clade HM.04	1.87 ± 0.34	1.76 ± 0.29	1.11 ± 0.27	0.56 ± 0.09	0.64 ± 0.11
	N = 8	*N* = 8	*N* = 7	*N* = 7	*N* = 7
Clade HM.05	1.86 ± 0.57	1.69 ± 0.60	0.92 ± 0.37	0.56 ± 0.07	0.62 ± 0.22
	N = 2	*N* = 2	*N* = 2	*N* = 2	*N* = 2
Pocillopora	1.91 ± 0.35	1.76 ± 0.33	1.13 ± 0.29	0.58 ± 0.11	0.66 ± 0.15
	N = 17	*N* = 17	*N* = 17	*N* = 17	*N* = 17
Stylophora	1.71 ± 0.29	1.6 ± 0.28	1 ± 0.19	0.49 ± 0.08	0.57 ± 0.09
	N = 28	*N* = 28	*N* = 25	*N* = 25	*N* = 25

body weight than those of HM.01 clade. Interestingly, females attributed to clade HM.02 (same host as clade HM.01) were significantly heavier (43.16%) than clade HM.01 females. There seemed to be a similar pattern for the reproductive output, but with $P = 0.134$ no significant differences could be detected. The HM.05 clade contained only a single female that resembles an outlier in the dataset of reproductive traits. The female was the second heaviest (20 mg dry weight), but its reproductive output was the second lowest in the data set (7%) carrying only 42 eggs. Furthermore, the eggs were very late stage II that were partly opened by the hatching larvae.

The relationship between fecundity and carapace of all ovigerous females revealed a strong positive correlation between the two traits ($r = 0.772$, $t = 6.079$, $df = 25$, $P < 0.001$). The number of eggs increased with carapace width ($F = 36.958$, $df = 1, 25$, $P < 0.001$), and 58% of the variation could be explained by the width of the carapace (Fig. 7). A regression slope of $b = 3.498$ was calculated, with a value of isometry of 3. No significant difference between the slope and 3 was found ($t = 0.662$, $df = 26$, $P = 0.514$), hence the relationship between egg number and carapace width was isometric. Due to the small dataset and its impact on the mathematical accuracy (Clayton, 1990) on allometry/isometry analyses we did not perform an ANCOVA analysis with host as factor.

DISCUSSION

Phylogenetic relationships and host specificity

Hapalocarcinus marsupialis has so far been referred to as a single species, although there have been discussions about the presence of various species in the genus (e.g., Verrill, 1869; Gore *et al.*, 1983; van der Meij & Schubart, 2014; Wei *et al.*, 2016). The phylogenetic reconstruction (Fig. 1) revealed five distinct clades of *H. marsupialis* in the Red Sea, two of which are associated with *Stylophora* and the remaining three putative species with *Pocillopora*. This is in line with results by SETM *et al.* (unpublished data), who have found at least eight highly supported, host-genus specific clades in a global dataset. Given that the Hawaiian Islands is the type locality of *H. marsupialis*, we consider clades HM.01–HM.05 to be new species of *Hapalocarcinus*, but more research is needed to confirm the identity of the *H. marsupialis* sensu stricto species in the absence of a holotype (Kropp, 1990). Trees from initial runs revealed a somewhat unstable overall tree topology; however, support for the various putative species was always high (Fig. 1). Future approaches will focus on using multiple markers. Further research, taking the findings of Wei *et al.* (2016) and SETM *et al.* (unpublished data) into account, will help to determine which putative species are closely related. A recent study on the cryptochirid genus *Opecarcinus* Kropp & Manning, 1987 revealed that most Red Sea species have a sister species in the wider Indo-West Pacific (Xu *et al.*, 2021), hence we cannot assume the five Red Sea putative species are sister species. The within-species variation (p-distance) is low and the smallest difference between groups (3.2%) was found for clades HM.01 and HM.02 (Table 2). Divergence between the clades ranged between 3.2% and 15.7%, indicating cryptic speciation. Clades HM.03, HM.04, and HM.05 inhabit *Pocillopora*, whereas clades HM.01 and HM.02 inhabit *Stylophora*. This is in line with SETM *et al.* (unpublished data) which suggests a co-evolutionary host switch from *Pocillopora* to *Stylophora*. Such host-switches have been observed in other cryptochirid genera (e.g., *Fungicola* Serène, 1968), making Cryptochiridae an ideal model taxon for studies on the evolutionary history of such associations (van der Meij *et al.*, 2015a). Phylogenetic reconstructions of stony corals reveal *Pocillopora* as the more basal clade within Pocilloporidae, and *Seriatopora* as the most recent clade (Fukami *et al.*, 2008). We did not test for host-specificity beyond genus level in *H. marsupialis* sensu lato due to complex taxonomic issues in Pocilloporidae combined with high morphological plasticity marring coral host identification (e.g., Keshavmurthy *et al.*, 2013; Schmidt-Roach *et al.*, 2014). The six Red Sea morphospecies of *Stylophora* cannot be resolved based on mitochondrial regions (cytochrome oxidase I (COI)), clades control regions (CR), and open-reading frame of unknown function (ORF) (Arrigoni *et al.*, 2016), preventing the testing of possible host-specificity at species level. Given the strict host-specificity at genus level in clades HM.01 and HM.02, we can only compare our morphometric and reproductive output results with the data of Kotb & Hartnoll (2002), who only sampled species of *Stylophora* in the Red Sea.

Female colouration

The colour of female specimens of *Hapalocarcinus* range from white (no pigmentation) to dark brown (fully pigmented) (Supplementary material Fig. S4). Colour and size are significantly correlated (Table 3).

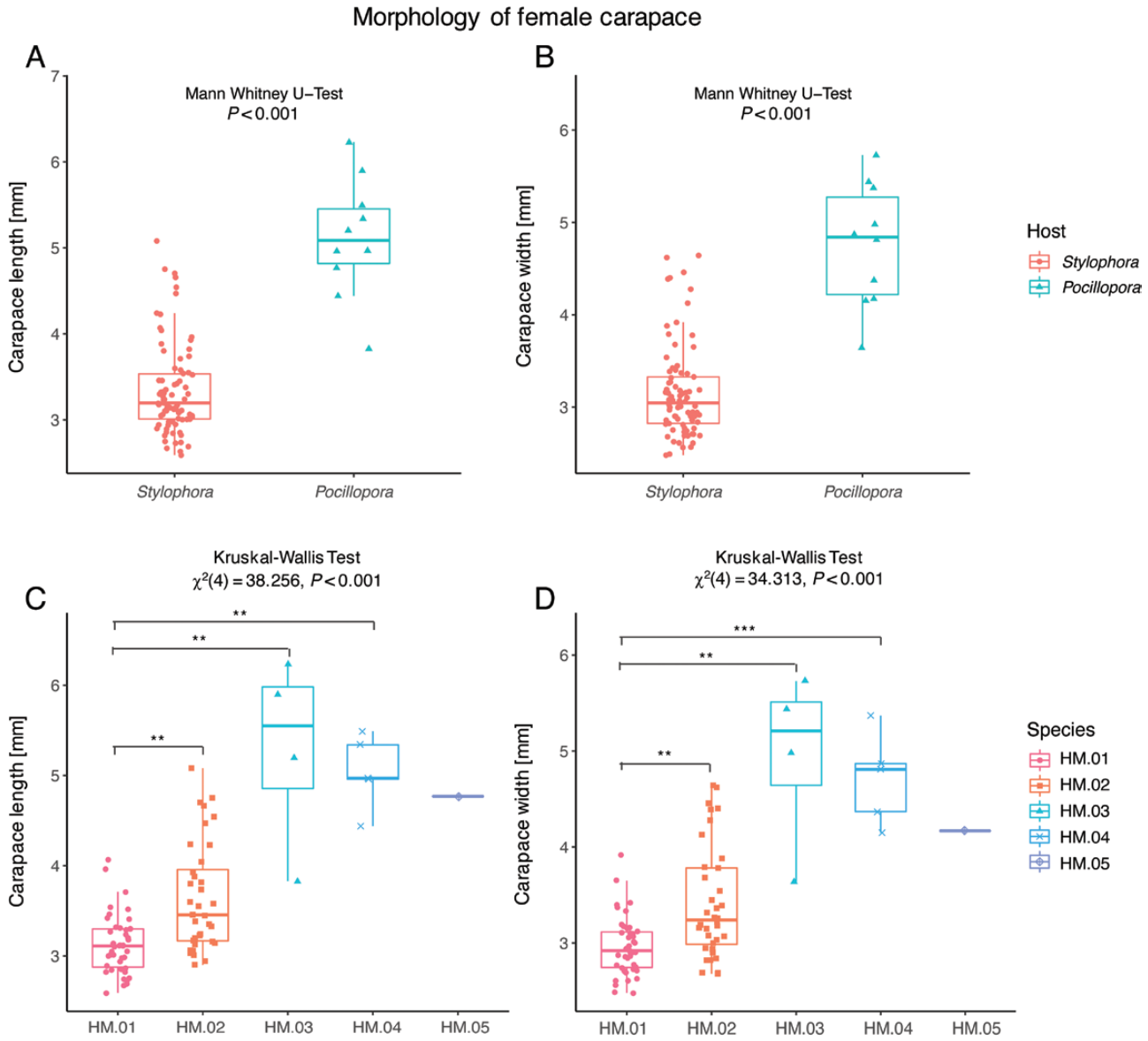

Figure 2. Carapace length (CL) and width (CW) of female *Hapalocarcinus marsupialis*, pooled by host genus (*Pocillopora* and *Stylophora*) and putative species HM.01–HM.05. Crabs retrieved from *Pocillopora* had significantly larger CL and CW than those inhabiting *Stylophora* (**A**, **B**). Putative species HM.01–HM.05 varied significantly in size. Asterisks indicate statistically significant differences between species calculated in post-hoc analysis (after Bonferroni correction; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (**C**, **D**).

The distribution of the four colour morphs over the five size classes reveals high colour variation in all sizes. This is in line with the hypothesis of Fize & Serène (1957), who suggested that colouration could be related to moulting. A possible connection between colouration and moulting has been observed in the ocypodid crab *Tubuca capricornis* (Crane, 1975), where Detto *et al.* (2008) observed a reduction in pigmentation with increasing carapace size and relation to moulting was observed. Kotb & Hartnoll (2002) qualitatively related size to colour in *Hapalocarcinus* females, and hypothesized that brown females have a lower predation risk because of their larger size and because they inhabit closed galls that restricts predators from entering. Moreover, they concluded that smaller females residing in open galls are brighter and, like their male conspecifics, better match the coral surface in the background. Our dataset, however, has brightly coloured large specimens (5.0–5.9 mm CW), contradicting their hypothesis (Table 3). MacNamee (1961) and Fize & Serène (1957) noted that colour variation occurred on a single colony, and hence cannot be attributed to habitat or other ecological factors.

Colour variation was high for specimens collected from *Stylophora*, whereas *Pocillopora* specimens were predominantly brightly coloured (white or white-brown) (Table 3; Supplementary material Fig. S4). Morphometric analyses (see below) indicated that crabs inhabiting *Pocillopora* are significantly bigger than those from *Stylophora*, which in combination with their light pigmentation disagrees with the findings of Kotb & Hartnoll (2002) that large crabs of Red Sea populations are darker coloured.

Our results show that colour correlates with the putative species (Table 3). HM.02 individuals were often dark and those in clade HM.01 were often brightly coloured. Both clades were collected from *Stylophora*, just like the Red Sea specimens studied by Kotb & Hartnoll (2002). Their results could have been impacted by their assumption that *H. marsupialis* represents a single species.

Fize & Serène (1957) proposed that female colouration could be related to egg-laying, but we found no correlation between

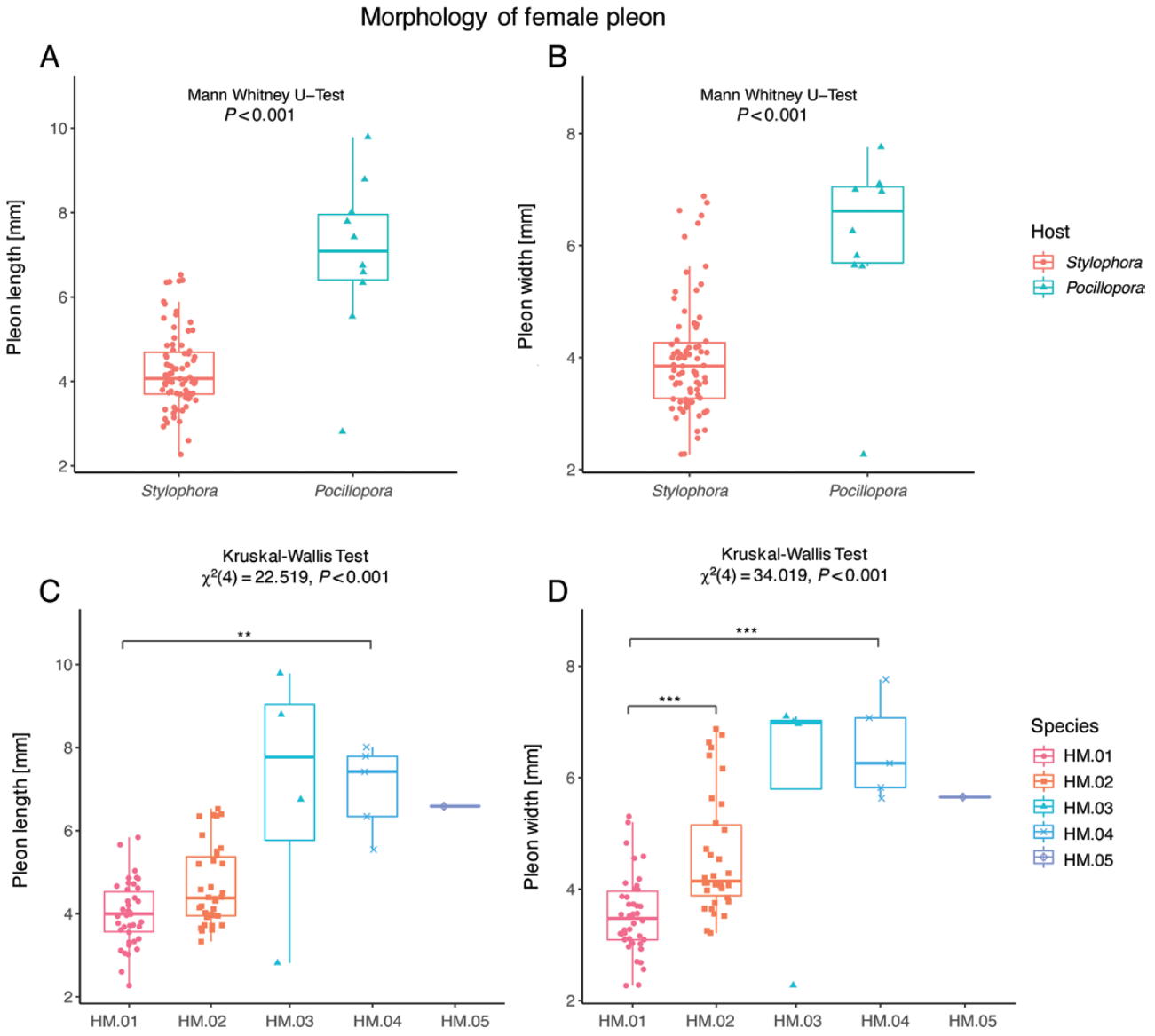

Figure 3. Pleon length (AL) and width (AW) of female *Hapalocarcinus marsupialis*, pooled by host genus (*Pocillopora* and *Stylophora*) and putative species HM.01–HM.05. Crabs sampled from *Pocillopora* had significantly larger AL and AW than those inhabiting *Stylophora* (**A**, **B**). Putative species HM.01–HM.05 varied significantly in size. Asterisks indicate statistically significant differences between species calculated in post-hoc analysis (after Bonferroni correction; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (**C**, **D**).

egg-bearing and colour (Table 3). Kotb &Hartnoll (2002) also stated that egg-bearing does not correlate with the colour of crabs, but provided no statistical test to confirm this statement. A Chi-square test based on their female colouration data (Kotb & Hartnoll, 2002: Table 3) does reveal a significant effect of egg-bearing on colour ($\chi^2(3) = 54.67$, $P < 0.001$), which is in disagreement with our findings.

Morphologies of the carapace, chelae, and pleon

Sexual dimorphism is common in many decapod crustaceans, including cryptochirids (Kropp & Manning, 1987). Differences vary between species, but the most obvious dimorphic character in cryptochirids is size, with females being larger than males (e.g., van der Meij *et al.*, 2015b). No significant difference in allometric growth patterns between the sexes was detected in *Hapalocarcinus* (Fig. 5). Specimens were examined for morphological differences in the carapace, chelae, and pleon (females only). For the analyses, data were pooled by host genus and putative species (based on the genetic analysis; Fig. 1). The host genus analyses showed that female specimens retrieved from *Pocillopora* attain a larger size for all of the measured traits compared to those collected from *Stylophora* (Fig. 2–4). A similar pattern was observed for males ($N = 45$) (Supplementary material Table S8); however, not all traits were significant. Nonetheless, the low P values indicate that males from *Pocillopora* reach a larger size than those from *Stylophora* while retaining isometric growth. In the closely related species *Fungicola fagei* (Fize & Serène, 1956) and *F. syzygia* van der Meij, 2015, associated with different mushroom coral hosts (Fungiidae Dana, 1846), minor differences in carapace shape (CL:CW) were also observed (van der Meij, 2015).

Analyses of clades showed that not all comparisons of carapace and pleon measurements were significant (Figs. 2, 3), possibly linked to the low number of specimens for some of the clades (Table 4). Females of clades HM.01 and HM.04 significantly differed in all four measured morphological traits, and females of

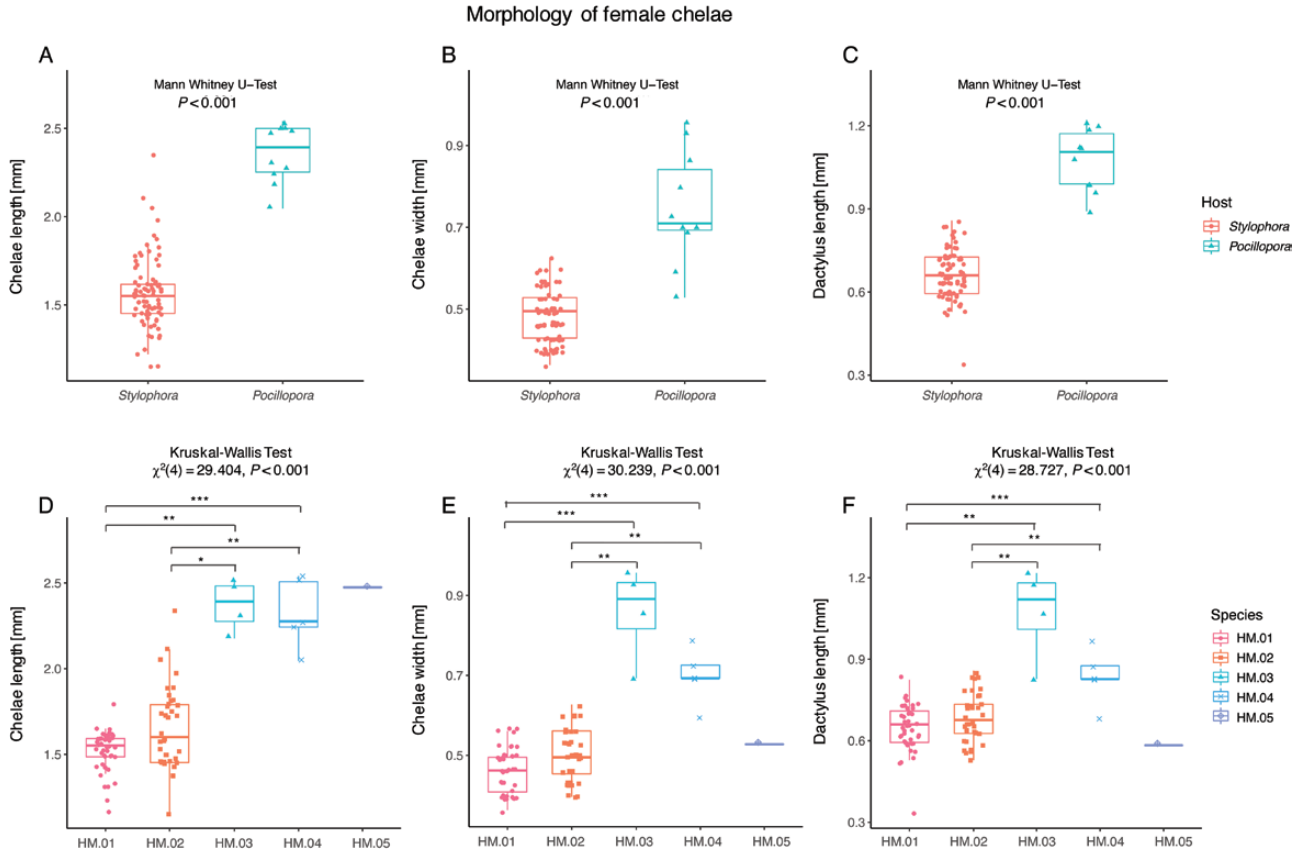

Figure 4. Log carapace width *versus* log carapace length. No significant differences in the relationship of carapace length and width were detected between females (closed circles) and males (crosses). The linear regression fitted for both sexes together revealed a regression slope of b = 1.003, indicating an isometric (b = 1) relationship of the variables.

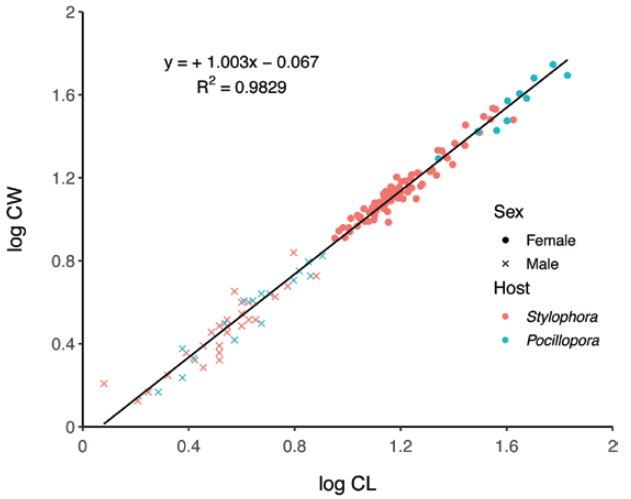

Figure 5. Morphological characteristics of the chelae of female *Hapalocarcinus marsupialis* pooled by host genus (*Pocillopora* and *Stylophora*) and putative species HM.01–HM.05). Crabs sampled from *Pocillopora* had significantly larger chelae than congeners inhabiting *Stylophora* (**A**–**C**). Putative species HM.01–HM.05 varied significantly in size. Asterisks indicate statistically significant differences between species calculated in post-hoc analysis (after Bonferroni correction; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (**D**–**F**).

clade HM.03 had larger carapaces than those of clade HM.01 (Figs. 2, 3). HM.02 specimens attained a larger size than those of clade HM.01 (traits CL, CW, AW) despite the low genetic distance (Table 2) as well as small differences in the means of the measured traits (Figs. 2–4; Table 4), highlighting that the recent separation of the putative species might have already resulted in minor morphological differences. The small sample size of the *Hapalocarcinus* individuals sampled from *Pocillopora*, however, likely impacted the detection of statistically significant differences in morphology. Sampling efforts for *Hapalocarcinus* inhabiting *Pocillopora* should be increased to better compare size differences among the putative species. Similarly, more data are required on male specimens, and the size and shape of dwellings belonging to either *Pocillopora* or *Stylophora* provide interesting avenues for further research. There is supporting evidence for size diversification in cryptic species based on habitat properties in other taxa. Wellborn *et al.* (2005), for example, found cryptic amphipods of the *Hyalella azteca* (Saussure, 1858) species complex to have diverged into a large and a small ecomorph as an adaptive response to disparate regimes of predation pressure present in their habitats.

Allometric scaling

Variation in shape is often characterized by differences in the relative size of body parts (Thompson, 1942), hence scaling relationships can be used to distinguish, for example, different morphotypes. A study by McCullough *et al.* (2015) on male dimorphism in rhinoceros beetles found that 30 out of 31 of the examined species had a discontinuous breakpoint allometry regarding the relationship between horn length and body size, indicating the existence of two male morphotypes ("major and minor males"). By using the principle of allometric scaling to detect potential differences in carapace growth of the *H. marsupialis* clades from *Pocillopora* and *Stylophora*, we found that there were no differences in the growth relationship of either carapace or pleon

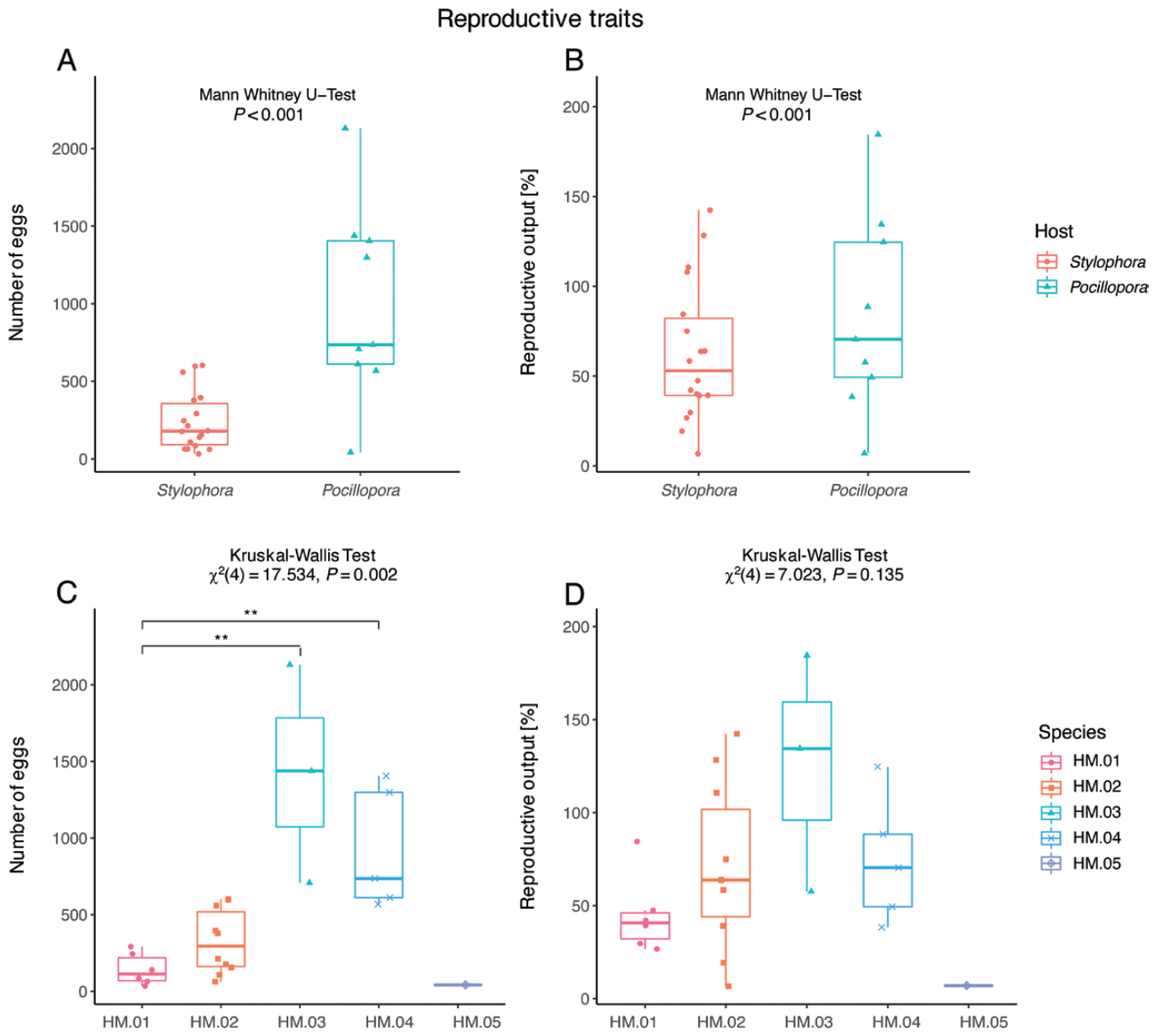

Figure 6. Reproductive traits for female specimens grouped by host genus (*Pocillopora* and *Stylophora*) and putative species HM.01–HM.05. Females collected from *Pocillopora* had a significantly higher number of eggs. No significant difference was detected in the reproductive output (**A, B**). The number of eggs varied significantly between putative species HM.01–HM.05. Asterisks indicate differences between species identified in the post-hoc analysis (after Bonferroni correction; *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (**C, D**).

(Fig. 5; Supplementary material Fig. S11). To avoid false detection of differences between clades due to mathematical constraints (small sample size, see Clayton, 1990), we did not conduct allometric scaling with species as covariate.

The combined logarithmic regression analysis of all adult specimens (male and females) revealed isometric carapace growth (b = 1) (Fig. 5); carapace proportions do not change while the crab grows. This is in disagreement with Kotb & Hartnoll (2002), who found a positive allometric growth relationship (b = 1.15) in Red Sea populations indicating that the carapace becomes relatively wider with increasing size. El-Damhougy *et al.* (2018: fig. 3) agreed with Kotb & Hartnoll (2002); however, their results showed isometric carapace growth (b = 1.009) and not allometric growth. We also examined the relationship of AL and CW. The regression analysis revealed a slope of b = 1.178 (Supplementary material Fig. S11), indicating an isometric growth relationship of both traits, which is also in disagreement with Kotb & Hartnoll (2002), who showed a slope of b = 1.06 (adult specimens) that, according to the authors, is statistically different from 1 ($t = 3.32$, $P < 0.001$). Kotb & Hartnoll (2002) concluded that the growth relationship of carapace and pleon is of allometric nature. Even though our slope has a considerably higher value (b = 1.178), a t-test failed to detect a significant departure from 1. With ImageJ (Rueden *et al.*, 2017) we extracted the data from Kotb & Hartnoll, 2002: fig. 2 and reran their analyses. With y = 1.067x + 0.21 our model had a highly similar fit to the original model (y = 1.065x + 0.022) of Kotb & Hartnoll (2002). A subsequent t-test showed that the slope of b = 1.067 did not significantly differ from 1 (SE = 0.102, $df = 57$, $t = 3.045$, $P = 0.515$). Furthermore, the conclusions of Kotb & Hartnoll (2002) are based on a model with $R^2 = 0.4$, where CW only explains 40% of the variation of AL for adult females, indicating a great amount of unexplainable variation. Because of the non-parametric method used, we are not able to provide a comparative R^2 value, but the results of the Spearman's rank correlation (rho = 0.79) indicate a strong relationship of both traits. Kotb & Hartnoll (2002) stated that growth in *Hapalocarcinus* is in line with general growth patterns of female crabs noted by Hartnoll (1974, 1982). By comparing the level of

Table 6. Reproductive traits (median ± 95% CI) of *H. marsupialis* individuals for all specimens combined, sorted regarding egg stage as well as according to the assigned species; no sequence could be obtained for two females with eggs.

	Egg number	Egg volume (mm^3)	Brood-mass volume (mm^3)	Brood-mass dry weight (mg)	Egg dry weight (mg 100^{-1} eggs)	Female dry weight (mg)	Reproductive output (%)
Total	292	0.06	19.29	3.8	1.39	5.6	58.46
N = 27	(124–607)	(0.05–0.07)	(8.07–37.57)	(1.45–8.5)	(1.1–1.55)	(4.2–7.3)	(39.32–98.21)
Egg Stage I	564	0.06	30.7	6.9	1.42	6.65	72.7
N = 20	(179–660)	(0.05–0.06)	(9.96–40.9)	(2.4–8.85)	(1.21–1.55)	(4.1–15.8)	(44.7–109)
Egg Stage II	156	0.07	10.9	1.8	1.16	6.50	39.3
N = 7	(62–378)	0.06–0.07)	(4.47–24.4)	(1.3–3.8)	(1–1.53)	(3.3–7.8)	(7–58.5
Clade HM.01	113	0.06	6.48	1.2	1.4	3.5	40.73
N = 6	(69–219)	(0.05–0.07)	(4.05–11.81)	(0.88–3.1)	(0.91–1.55)	(2.25–4.3)	(32.13–46.10)
Clade HM.02	296	0.06	17.64	3.7	1.24	6.2	63.77
N = 10	(161–518)	(0.06–0.07)	(9.34–28.71)	(1.88–6.63)	(1.04–1.4)	(4.65–6.68)	(43.97–101.71)
Clade HM.03	1438	0.05	71.99	25.80	1.56	18	134.38
N = 3	(1073–1784)	(0.05–0.05)	(55.71–87.04)	(17.45–29.50)	(1.42–1.68)	(16.9–18.6)	(95.98–159.41)
Clade HM.04	735	0.06	42.39	9.30	1.52	16.1	70.45
N = 5	(611–1298)	(0.05–0.06)	(32.16–67.03)	(7.80–13.77)	(1.14–1.55)	(15.8–16.9)	(49.37–88.42)
Clade HM.05	42	0.07	2.97	1.4	3.33	20	7
N = 1							
Pocillopora	735	0.06	42.4	9.3	1.55	16.9	70.5
N = 9	(568–1440)	(0.05–0.06)	(32.2–83.9)	(6.5–25.8)	(1.14–1.79)	(15.8–19.2)	(38.5–134)
Stylophora	179	0.06	10.3	2.4	1.29	4.55	52.9
N = 18	(96.5–335)	(0.06–0.07)	(6.48–21.8)	(1.3–3.8)	(1–1.46)	(3.15–6.55)	(39.3–75)

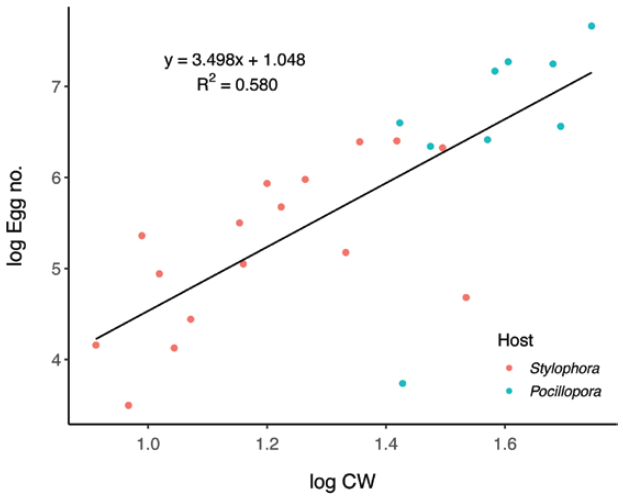

Figure 7. Log egg number *versus* log carapace width. Linear regression revealed a significant relationship between number of eggs and carapace width in specimens of *Hapalocarcinus marsupialis*. A regression slope of b = 3.498 was retrieved and tested against 3 as value for isometry. The t-test showed that there was no significant departure from 3 (t = 0.662, *df* = 26, *P* = 0.514), indicating an isometric relationship of the two variables.

allometry in *Hapalocarcinus* (1.06) with the value of 1.16 noted for other female crab species, they concluded that disproportionate growth is limited after maturity in *Hapalocarcinus*. This comparison is nevertheless disputable because the value of 1.16 was calculated as the average of growth patterns of 14 non-cryptochirid crab species that differ greatly regarding habitat and lifestyle (Hartnoll, 1974: Table 1), and cannot be compared with the coral-dwelling *Hapalocarcinus* species. The contradictory results between our findings and those of Kotb & Hartnoll (2002) illustrate that it can be dangerous to draw conclusions based on scaling relationships. Moreover, Clayton (1990) discussed discrepancies in the interpretation of allometry determined using statistical methods and states that mathematical findings can easily be altered through the size of the data set, outliers, and use of factors such as size or species groups.

Reproductive output

We found that only 20.8% of the females were ovigerous in the 2018 samples and 42.2% in those from 2019. These numbers differ greatly from previous studies on the reproductive output of *H. marsupialis* in the Red Sea. Kotb & Hartnoll (2002) found about 80% and El-Damhougy *et al.* (2018) reported 84.9% ovigerous females, both from the Egyptian Red Sea. El-Damhougy *et al.* (2018: fig 5) also reported that the number of egg-bearing females in *Stylophora* showed moderate variation throughout the year, with the lowest number reaching 57% in October and a maximum of 100% in December. Since the sampling for our study took place during one week in August 2018 and one week in August 2019 no predictions about the number of ovigerous females for the rest of the year in the Saudi Arabian Red Sea are possible; however, the low number of gravid females in our dataset is remarkable.

It is noteworthy that we found up to 2,231 eggs per female, nearly tripling the maximum number of Kotb & Hartnoll (2002) and El-Damhougy *et al.* (2018), who observed 10–700 and 10–740, respectively.

Allometry was defined by Huxley & Tessier (1936) as the scaling relationship between the size of a body part and the body as a whole, but since then the concept of allometric analysis has been expanded to also include general biological scaling relationships such as physiological or ecological traits (Shingleton, 2010). Here we used allometric scaling analyses to examine the relationship of reproductive and morphometric traits in female *Hapalocarcinus* specimens. The analyses revealed a positive correlation of carapace width and egg number (Fig. 7), with a regression slope of 3.4 that indicated isometric relationship of both variables. This contradicts Kotb & Hartnoll (2002), who found a negative allometric relationship between size and fecundity, and

suggested that relative fecundity decreased with size due to increasingly poor fertilisation success because of sperm depletion and food limitation via the small pores in the gall. Our results indicate an isometric relationship between CW and egg number, thus disagreeing with the above assumptions.

Our results for the reproductive output (69.6%) are in approximate agreement with the 59% found by Kotb & Hartnoll (2002). Their calculation, however, differs greatly from the one we used. Firstly, Kotb & Hartnoll (2002) only calculated the reproductive investment for females with a CW of 3–4 mm, the modal class of mature females in their dataset. This selection was based on the presence of eggs or mature ovaries as well as swollen spermathecae, which led to the exclusion of individuals in size class 2.5–2.9 mm of which two-thirds were ovigerous. Subsequently, they used a 3.6 mm CW to calculate the mean dry body weight (4.39 mg, $N = 8$) and the equivalent of brood size (180 eggs). Their calculations are based on models of the allometric scaling analysis (log egg number *versus* log carapace width; model of carapace size and female weight was not provided) which, based on carapace width and fecundity, shows a wide scatter ($R^2 = 1.06$). The modelled number of eggs was then multiplied with the mean dry egg weight per 100 eggs to determine the brood weight. The value of 1.43 mg per 100 eggs that was used for this calculation, however, was a mean based on only three calculations of their total dataset. Lastly, Kotb & Hartnoll (2002) used brood weight and female dry weight to calculate RO. Using the same approach on our dataset resulted in an RO of 49% for a CW of 3–4 mm and 66.5% for 4–5 mm CW (our modal class of ovigerous females). Considering the mathematical constraints of allometric scaling (see Clayton, 1990), this approach to calculate the reproductive investment of *H. marsupialis* is incorrect.

Our 69.6% value for the reproductive investment of *H. marsupialis* sensu lato shows that symbiotic brachyurans can invest more energy in reproduction than their free-living counterparts. For the latter, relatively low RO values have been reported, ranging 3%–22% (Hines, 1982, 1992), whereas the symbiotic pinnotherids *Zaops ostreum* (Say, 1817) and *Fabia subquadrata* Dana, 1851 were observed to have similar or higher RO values (66% and 97% respectively, using the same approach we used to calculate RO). Salas-Moya *et al.* (2014) found a RO of 76.7% for the pinnotherid *Austinotheres angelicus* (Lockington, 1877), but this phenomenon seems not to be restricted to symbiotic brachyurans. Van der Meij *et al.* (2018) found similar results for a symbiotic (commensal) caridean shrimp, *Pontoniopsis comanthi* Borradaile, 1915, and obtained the highest so far recorded RO (31%), which far exceeds the values for free-living caridean species, which strongly suggests that living in a sheltered environment enables them to allocate more energy towards reproduction. It should however be noted that *Hapalocarcinus*, and possibly other symbiotic crustaceans, are less calcified than (most) free-living species, which increases their relative brood weight (Kotb & Hartnoll, 2002).

Our results show that *H. marsupialis* should be considered a species complex in the Red Sea, containing at least five putative species (HM.01–05) inhabiting two different host genera. Because the Hawaiian Islands is the type locality of *H. marsupialis*, it is plausible that all five Red Sea putative species are new to science (SETM *et al.*, unpublished data). Because of the strong morphological similarities between the putative species, and the previous classification as a single species, *H. marsupials* should be considered a "cryptic species" complex (see Bickford *et al.*, 2007). Using basic morphometric values, we could at least clearly separate clades HM.01 and HM.04. Differences in carapace size (CL, CW, AL, AW) and chelae size (length and width) are useful characters to differentiate between the putative species. Female dry weight is also variable between the putative species, but less practical to use for species identification. In addition, there are indications of differences in the reproductive traits (fecundity, RO), albeit based on relatively few gravid females and hence further data is needed to test this further.

Female *H. marsupialis* from *Pocillopora* and *Stylophora* were tested for differences in reproductive traits and body weight. Results were congruent with those of the morphological analysis, except for the comparison of RO values (Fig. 6; Supplementary material Table S7). *Pocillopora* crabs had a significantly higher number of eggs and thus a higher brood weight; however, these females were also significantly heavier than those from *Stylophora*. An additional allometric scaling analysis correlating brood weight and female weight revealed an isometric relationship of both variables (slope b = 1.219; Supplementary material Fig. S12), indicating that the mean brood weight increases proportional with body size. Such a result is well in line with Hines (1992), who found an isometric relationship of female body weight and brood weight for 35 crab species, which could explain the similar RO values for crabs inhabiting *Pocillopora* and *Stylophora*. This assumption is further supported by the isometric relationship of fecundity and body size: relative fecundity increases proportionally with body size. The analysis at putative-species level revealed a similar pattern with similar RO values for all five clades. This supports the assumption that brood weight increases proportional with body weight. Furthermore, this is in line with the isometric relationship of fecundity and carapace width described above. For the other reproductive traits, we found clade HM.01 to differ significantly from clades HM.03 and HM.04, which assorts well with the findings of the morphological analysis. The small number of ovigerous females in the data set might have hampered detection of minor differences between the putative species.

Our results question the results from previous studies on reproductive traits of *H. marsupialis* sensu lato (e.g., Kotb & Hartnoll, 2002; El-Damhougy *et al.*, 2018). Furthermore, the results of studies examining the global distribution of *H. marsupialis* (Utinomi, 1944), or their distribution in coral reefs (Mohammed & Yassien, 2013 (who only recorded *H. marsupialis* sensu lato, not *O. aurantius* as stated; fig. 3a–e); Terrana *et al.* 2016) are likely to be impacted by the results of our study. More specimens from *Pocillopora* are needed to test whether growth relationships of carapace, pleon, or chelae are also suitable traits to distinguish clades HM.01-05. Further research exploring the link between the size of dwellings in *Pocillopora* and *Stylophora* and the size of their respective *Hapalocarcinus* associates is needed to determine whether the maximum size species can attain is influenced by dwelling morphology in their various host corals, and whether other factors, such as currents, affect gall morphology.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Sample locations.

S2 Figure. Map of sample locations

S3 Table. Pictures and identification (genus level) of host coral colonies.

S4 Figure. Colour groups of female *Hapalocarcinus marsupialis* individuals.

S5 Table. Metadata of all measurements

S6 Appendix. Sequences of 103 *H. marsupialis* individuals and three outgroups.

S7 Table. Results of statistical analysis (Mann-Whitney U test) used to detect differences in morphometric and reproductive characters of female *H. marsupialis* pooled by host genus.

S8 Table. Results of statistical analysis (t-test) used to detect differences in morphometric characters of male *H. marsupialis* pooled by host genus.

S9 Table. Results of statistical analysis (Kruskall-Wallis test) used to detect differences in morphometric and reproductive characters of female *H. marsupialis* pooled by species.

S10 Table. Results of statistical analysis (one-way ANOVA) used to detect differences in morphometric characters of male *H. marsupialis* pooled by species.

S11 Figure. Allometric analysis of log CW and log AL.

S12 Figure. Allometric analysis of log female weight and log brood weight.

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REFERENCES

Abelson, A., Galil, B.S. & Loya, Y. 1991. Skeletal modifications in stony corals caused by indwelling crabs: hydrodynamical advantages for crab feeding. *Symbiosis*, **10**: 233–248.

Arrigoni, R., Benzoni, F., Terraneo, T.I., Caragnano, A. & Berumen, M.L. 2016. Recent origin and semi-permeable species boundaries in the scleractinian coral genus *Stylophora* from the Red Sea. *Scientific Reports*, **6**: [https://doi.org/10.1038/srep34612].

Bauer, R.T. 2004. *Remarkable shrimps: Adaptations and natural history of the carideans*. University of Oklahoma Press, Norman, OK, USA.

Becker, C., Brandis, D. & Storch, V. 2011. Morphology of the female reproductive system of European pea crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae). *Journal of Morphology*, **272**: 12–26.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, **22**: 148–155.

Borradaile, L.A. 1915. Notes on Carides. *Annals and Magazine of Natural History*, Series 8, **15**: 205–213.

Calman, W.T. 1900. On a collection of Brachyura from the Torres Straits. *Transactions of the Linnean Society, Zoology*, Series 2, **8**: 1–50.

Castro, P. 1976. Brachyuran crabs symbiotic with scleractinian corals: A review of their biology. *Micronesica*, **12**: 99–110.

Castro, P. 1988. Animal symbioses in coral reef communities: a review. *Symbiosis*, **5**: 161–184.

Castro, P. 2011. Catalog of the anomuran and brachyuran crabs (Crustacea: Decapoda: Anomura, Brachyura) of the Hawaiian Islands. *Zootaxa*, **2947**: 1–154.

Clayton, D.A. 1990. Crustacean allometric growth: a case for caution. *Crustaceana*, **58**: 270–290.

Crane, J. 1975. *Fiddler crabs of the world: Ocypodidae: genus* Uca. Princeton University Press, Princeton, NJ, USA.

Dana, J.D. 1851. On the classification of the Grapsoidea. *American Journal of Science and Arts*, Series 2, **12**: 283–290.

Detto, T., Hemmi, J.M. & Backwell, P.R. 2008. Colouration and colour changes of the fiddler crab, *Uca capricornis*: a descriptive study. *PLoS ONE*, **3**: [https://doi.org/10.1371/journal.pone.0001629].

El-Damhougy, K.A., Salem, E.S.S., Fouda, M.M. & Al-Hammady, M.A. 2018. The growth and reproductive biology of the coral gall crab, *Hapalocarcinus marsupialis* Stimpson, 1859 (Crustacea: Cryptochiridae) from Gulf of Aqaba, Red Sea, Egypt. *Journal of Basic and Applied Zoology*, **79**: [https://doi.org/10.1186/s41936-017-0010-6].

Ennos, R. & Johnson, M.L. 2018. *Statistical and data handling skills in biology*. Pearson, London.

Fize, A. & Serène, R. 1956. Note préliminaire sur huit espèces nouvelles, dont une d'un genre nouveau, d'Hapalocarcinidae (1ère partie). *Bulletin de la Société zoologique de France*, **80**: 375–378.

Fize, A. & Serène, R. 1957. Les Hapalocarcinides du Vietnam. *Archives du Muséum d'Histoire naturelle (Paris)*, Série 7, **5**: 3–202.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299

Fukami, H., Chen, C.A., Budd, A.F., Collins, A., Wallace, C., Chuang, Y.Y., Chen, C., Dai, C.-F., Iwao, K., Sheppard, C. & Knowlton, N. 2008. Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). *PLoS ONE*, **3**: [https://doi.org/10.1371/journal.pone.0003222].

Gore, R.H., Scotto, L.E. & Reed, J.K. 1983. Early larval stages of the Indo-Pacific coral gall-forming crab *Hapalocarcinus marsupialis* Stimpson, 1859 (Brachyura, Hapalocarcinidae) cultured in the laboratory. *Crustaceana*, **44**: 141–150.

Henderson, J.R. 1906. On a new species of coral-infesting crab taken by R.I.M.S "Investigator" at the Andaman Islands. *Journal of Natural History*, **18**: 211–219.

Hartig, F. 2021. *DHARMa: Residual Diagnostics for Hierarchical (Multi-level/ Mixed) Regression Models. R package version 0.4.3* [https://CRAN.R-project.org/package=DHARMa].

Hines, A.H. 1982. Allometric constraints and variables of reproductive effort in brachyuran crabs. *Marine Biology*, **69**: 309–320.

Hines, A.H. 1992. Constraint on reproductive output in brachyuran crabs: Pinnotherids test the rule. *American Zoologist*, **32**: 503–511.

Hiro, F. 1938. A new coral-inhabiting crab, *Pseudocryptochirus viridis* gen. et. sp. nov. (Hapalocarcinidae, Brachyura). *Zoological Magazine* (Tokyo), **50**: 149–151.

Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q. & Vinh, L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, **35**: 518–522.

Huxley, J. S. & Tessier, G. 1936. Terminology of relative growth. *Nature* **137**: 780–781.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., Von Haeseler, A. & Jermiin, L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, **14**: 587–589.

Keshavmurthy, S., Yang, S.Y., Alamaru, A., Chuang, Y.Y., Pichon, M., Obura, D. & MacDonald, A. 2013. DNA barcoding reveals the coral "laboratory-rat", *Stylophora pistillata* encompasses multiple identities. *Scientific Reports*, **3**: [https://doi.org/10.1038/srep01520].

Komsta, L. 2019. *mblm: median-based linear models*. R package version 0.12.1. [https://CRAN.R-project.org/package=mblm].

Kotb, M.M. & Hartnoll, R.G. 2002. Aspects of the growth and reproduction of the coral gall crab *Hapalocarcinus marsupialis*. *Journal of Crustacean Biology*, **22**: 558–566.

Kropp, R.K. 1986. Feeding biology and mouthpart morphology of three species of coral gall crabs (Decapoda: Cryptochiridae). *Journal of Crustacean Biology*, **6**: 377–384.

Kropp, R.K. 1990. Revision of the genera of gall crabs (Crustacea: Cryptochiridae) occurring in the Pacific Ocean. *Pacific Science*, **44**: 417–448.

Kropp, R.K. & Manning, R.B. 1987. The Atlantic gall crabs, family Cryptochiridae (Crustacea: Decapoda: Brachyura). *Smithsonian Contributions to Zoology*, **462**: 1–21.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**: 1870–1874.

Lockington, W.N. 1877. Remarks on the Crustacea of the west coast of North America, with a catalogue of the species in the Museum of the California Academy of Sciences. Grapsoidea, or Ocypodidae. *Proceedings of the California Academy of Sciences*, **7**: 145–156.

MacNamee, C.G. 1961. *Life history, morphology, habits and taxonomy of Hapalocarcinus marsupialis Stimpson* (Arthropoda, *Crustacea, Decapoda)*. M.S. thesis, University of Hawaii, Honolulu.

McLay, C.L. & Becker, C. 2015. 2015. Reproduction in Brachyura. In: *Treatise on zoology – anatomy, taxonomy, biology. The Crustacea* (P. Castro, P.J.F. Davie, D. Guinot, F.R. Schram & J.C. von Vaupel Klein, eds.), **Vol. 9C-I**, pp. 185–243. Brill, Leiden, The Netherlands.

McCullough, E.L., Ledger, K.J., O'Brien, D.M. & Emlen, D.J. 2015. Variation in the allometry of exaggerated rhinoceros beetle horns. *Animal Behaviour*, **109**: 133–140.

van der Meij, S.E.T. 2015. Host relations and DNA reveal a cryptic gall crab species (Crustacea: Decapoda: Cryptochiridae) associated with mushroom corals (Scleractinia: Fungiidae). *Contributions to Zoology*, **84**: 39–57.

van der Meij, S.E.T. & Hoeksema, B.W. 2013. Distribution of gall crabs inhabiting mushroom corals on Semporna reefs, Malaysia. *Marine Biodiversity*, **43**: 53–59.

van der Meij, S.E.T. & Nieman, A.M. 2016. Old and new DNA unweave the phylogenetic position of the eastern Atlantic gall crab *Detocarcinus balssi* (Monod, 1956) (Decapoda: Cryptochiridae). *Journal of Zoological Systematics and Evolutionary Research*, **54**, 189–196.

van der Meij, S.E.T. & Schubart, C.D. 2014. Monophyly and phylogenetic origin of the gall crab family Cryptochiridae (Decapoda: Brachyura). *Invertebrate Systematics*, **28**: 491–500.

van der Meij, S.E.T., Fransen, C.H.J.M., Pasman, L.R. & Hoeksema, B.W. 2015a. Phylogenetic ecology of gall crabs (Cryptochiridae) as associates of mushroom corals (Fungiidae). *Ecology and Evolution*, **5**: 5770–5780.

van der Meij, S.E.T., Berumen, M.L. & Paulay, G. 2015b. A new species of *Fizesereneia* Takeda and Tamura, 1980 (Crustacea: Brachyura: Cryptochiridae) from the Red Sea and Oman. *Zootaxa*, **3931**: 585–595.

van der Meij, S.E.T., Johnson, M.L. & De Grave, S. 2018. Reproductive strategies of the crinoid-associated shrimps *Cristimenes commensalis* (Borradaile, 1915) and *Pontoniopsis comanthi* Borradaile, 1915 (Crustacea: Palaemonidae). *Crustacean Research*, **47**: 89–99.

Mohammed, T.A.A. & Yassien, M.H. 2013. Assemblages of two gall crabs within coral species Northern Red Sea, Egypt. *Asian Journal of Scientific Research* **6**: 98–106.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**: 268–274.

Paul′son, O. 1875. *Studies on Crustacea of the Red Sea with notes regarding other seas. Part I. Podophthalmata and Edriophthalmata (Cumacea)*. S.V. Kul′zhenko, Kiev. [in Russian, English translation by the Israel Programme for Scientific Translations, Jerusalem, 1961].

Potts, F.A. 1915. *Hapalocarcinus*, the gall forming crab, with some notes on the related genus *Cryptochirus*. *Carnegie Institute of Washington, Papers from the Department of Marine Biology*, **8**: 35–69.

Quade, D. 1967. Rank analysis of covariance. *Journal of the American Statistical Association*, **62**: 1187–1200.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna [https://www.R-project.org/].

Richters, F. 1880. Decapoda. *In: Beiträge zur Meeresfauna der Insel Mauritius und der Seychellen bearbeitet von K. Möbius, F. Richters und E. von Martens nach Sammlungen, angelegt auf einer Reise nach Mauritius von K. Möbius* (K. Möbius, ed.), pp. 139–178, pls. XV–XVIII. Verlag der Gutmann'schen Buchhandlung, Berlin.

Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T. & Eliceiri, K.W. 2017. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, **18**: 1–26.

Salas-Moya, C., Mena, S. & Wehrtmann, I.S. 2014. Reproductive traits of the symbiotic pea crab *Austinotheres angelicus* (Crustacea, Pinnotheridae) living in *Saccostrea palmula* (Bivalvia, Ostreidae), Pacific coast of Costa Rica. *ZooKeys*, **457**: 239–252.

Saussure, H D. 1858. Mémoire sur divers Crustacés nouveaux des Antilles et du Mexique. *Mémoires de la Société de Physique et d'Histoire naturelle de Genève*, **14**: 417–496.

Say, T. 1817. An account of the Crustacea of the United States. *Journal of the Academy of Natural Sciences of Philadelphia*. **1**: 57–63, 65–80, 97–101, 155–169.

Schmidt-Roach, S., Miller, K.J., Lundgren, P. & Andreakis, N. 2014. With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological Journal of the Linnean Society*, **170**: 1–33.

Sen, P.K. 1968. Estimates of the regression coefficient based on Kendall's tau. *Journal of the American Statistical Association*, **63**: 1379–1389.

Shingleton, A. 2010. Allometry: The study of biological scaling. *Nature Education Knowledge*, **3**(10): 2 [https://www.nature.com/scitable/knowledge/library/allometry-the-study-of-biological-scaling-13228439/].

Siegel, A.F. 1982. Robust regression using repeated medians. *Biometrika*, **69**: 242–244.

Simon-Blecher, N. & Achituv, Y. 1997. Relationship between the coral pit crab *Cryptochirus coralliodytes* Heller and its host coral. *Journal of Experimental Marine Biology and Ecology*, **215**: 93–102.

Stella, J.S., Pratchett, M.S., Hutchings, P.A. & Jones, G.P. 2011. Coral-associated invertebrates: diversity, ecological importance and vulnerability to disturbance. *Oceanography and Marine Biology*, **49**: 43–104.

Stimpson, W., 1859. *Hapalocarcinus marsupialis*, a remarkable new form of brachyurous crustacean on the coral reefs of Hawaii. *Proceedings of the Boston Society of Natural History*, **6**: 412–413.

Terrana, L., Caulier, G., Todinanahary, G., Lepoint, G. & Eeckhaut, I. 2016. Characteristics of the infestation of *Seriatopora* corals by the coral gall crab *Hapalocarcinus marsupialis* Stimpson, 1859 on the Great Reef of Toliara, Madagascar. *Symbiosis*, **69**: 113–122.

Theil, H., 1950. A rank-invariant method of linear and polynomial regression analysis. *Indagationes Mathematicae*, **12**(85): 173–177.

Thompson, D.W. 1942. *On growth and form*, Edn. 2. Cambridge University Press, Cambridge, UK.

Turner, R.L. & Lawrence, J.M. 1979. Volume and composition of echinoderm eggs: implications for use of egg size in life-history models. In: *Reproductive ecology of marine invertebrates* (S.E. Stancky, ed.), pp. 25–40. University of South Carolina Press, Columbia, SC, USA.

Utinomi, H. 1944. Studies on the animals inhabiting reef corals. III. A revision of the family Hapalocarcinidae (Brachyura) with some remarks on their morphological peculiarities. *Palao Tropical Biological Station Studies*, **2**: 687–731.

Vehof, J., Meij, S.E.T., van der Türkay, M. & Becker, C. 2016. Female reproductive morphology of coral-inhabiting gall crabs (Crustacea: Decapoda: Brachyura: Cryptochiridae). *Acta Zoologica*, **97**: 117–126.

Verrill, A.E. 1867. Remarkable instances of crustacean parasitism. *American Journal of Science*, **44**: 126.

Verrill, A.E. 1869. On the parasitic habits of Crustacea. *American Naturalist*, **3**: 239–250.

Wei, T.P., Hwang, J.S., Peng, S.H., Chen, H.C. & Lee, Y.C. 2016. Genetic diversification of the gall crab, *Hapalocarcinus marsupialis* (Crustacea: Decapoda: Cryptochiridae), related to their host corals (Scleractinia: Pocilloporidae). *Journal of Fishery Society of Taiwan*, **43**: 135–144.

Wellborn, G.A., Cothran, R. & Bartholf, S. 2005. Life history and allozyme diversification in regional ecomorphs of the *Hyalella azteca* (Crustacea: Amphipoda) species complex. *Biological Journal of the Linnean Society*, **84**: 161–175.

Xu, T., Bravo, H., Paulay, G. & van der. Meij, S.E.T. 2021. Diversification and distribution of gall crabs (Brachyura: Cryptochiridae: *Opecarcinus*) associated with Agariciidae corals *Coral Reefs* [https://doi.org/10.1007/s00338-021-02163-1].

Zimmermann, U., Carvalho, F.L. & Mantelatto, F.L. 2015. The reproductive performance of the Red-Algae shrimp *Leander paulensis* (Ortmann, 1897), (Decapoda, Palaemonidae) and the effect of post-spawning female weight gain on weight-dependent parameters. *Brazilian Journal of Oceanography*, **63**: 207–216.