

# Supplementary Information

Trophic niche partitioning in giant clams

## **Summary:**

### **Materials and Methods**

Habitat (Supplementary Fig. 1-2)

### **Results**

Stable isotope analysis (Supplementary Tables 1-3, Supplementary Fig. 3)

Symbiodiniaceae diversity and community structure (Supplementary Fig. 4)

Phylogenetic signal analysis (Supplementary Fig. 5)

### **Discussion**

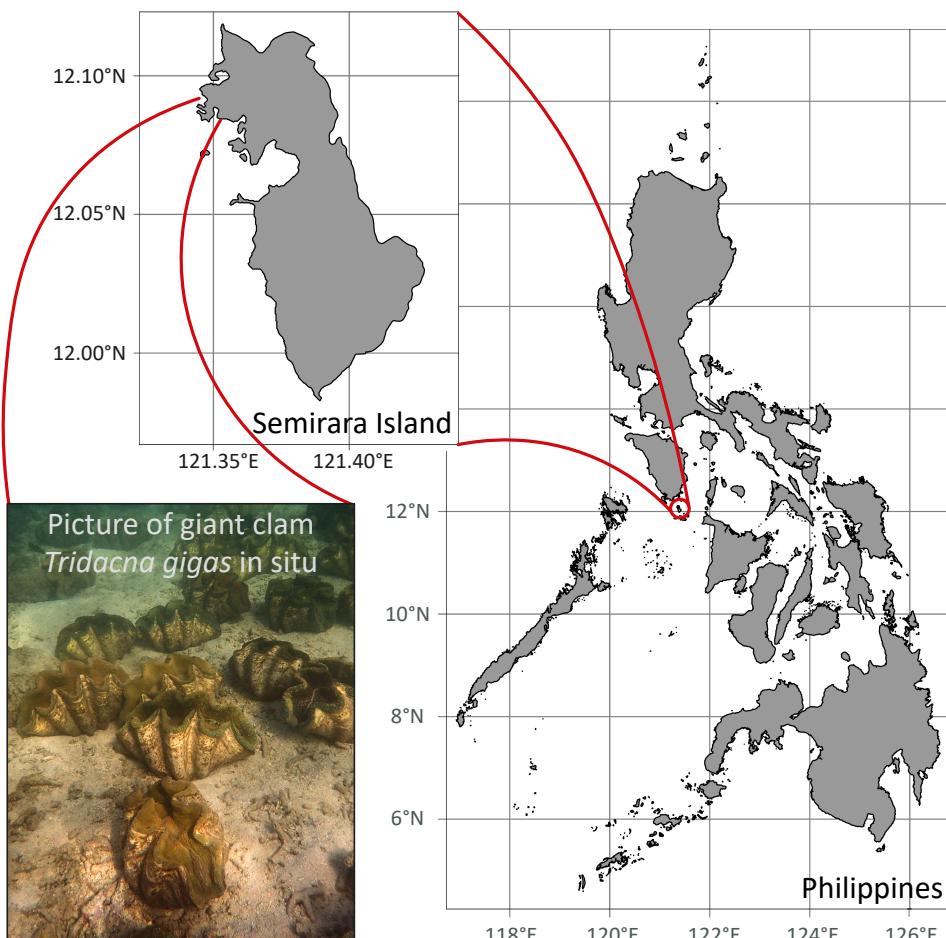
Geographic distribution of the 6 giant clam species of interest (Supplementary Fig. 6)

## **Other Supplementary Data for this manuscript include the following:**

Data S1 to S3

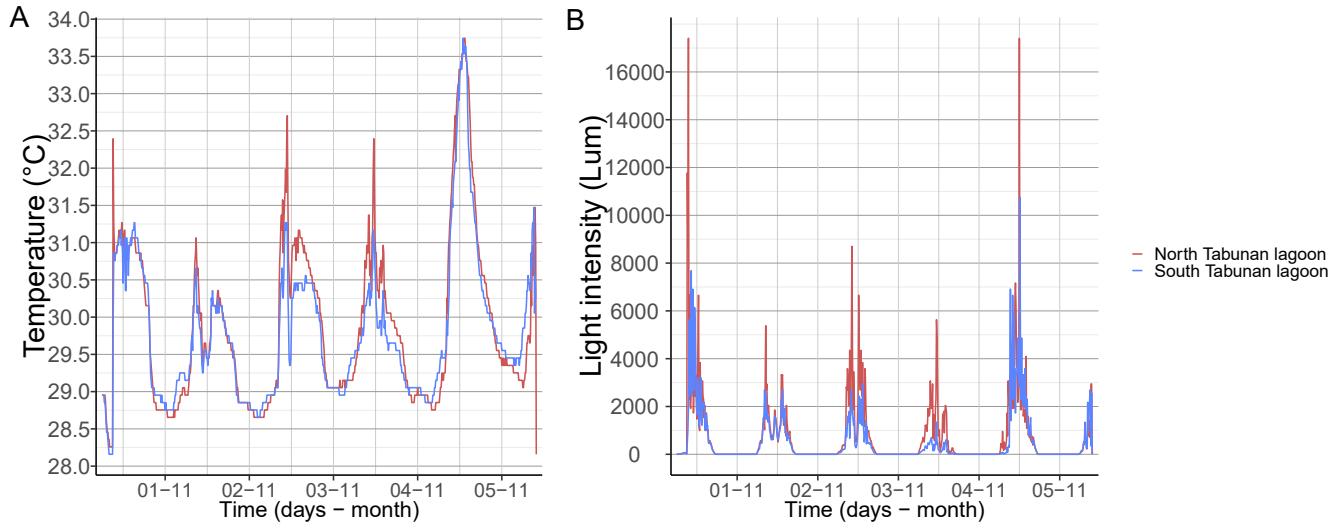
## **Materials and Methods**

### Habitat



**Supplementary Figure 1:** Map of the study site highlighting Semirara Island and the Tabunyan lagoon.  
Picture: Isis Guibert.

Light intensity and temperature were highly similar in both areas of the Tabunyan lagoon (Supplementary Fig. 2). Visual observations showed that both areas were characterized by sandy sediment, 2-5 m depth and patches of seagrass around the sampled areas.



**Supplementary Figure 2.** Environmental conditions in the two sampled areas sampled in the Tabunan Lagoon in November. A) Light intensity and B) temperature were recorded in the north Tabunan lagoon (red) and the south Tabunan lagoon (blue).

## Results

### Stable isotope analysis

**Supplementary Table 1.** Summary statistics of stable isotope analysis of paired clam host and algal symbiont samples. Mean ( $\pm$ SD) carbon to nitrogen ratio (C:N), carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope values, and difference between host and symbiont isotope values ( $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$ ) of six giant clam species collected from Cover Bay, Semirara Island, Philippines.

Species	n	C:N		$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$
		Host	Symb	Host	Sym	Host	Sym	(‰)	(‰)
<i>T. gigas</i>	27	5.7 $\pm$ 1.3	5.7 $\pm$ 1.3	-14.4 $\pm$ 1.2	-15.0 $\pm$ 1.1	4.9 $\pm$ 0.3	4.9 $\pm$ 0.1	0.5 $\pm$ 0.5	0.1 $\pm$ 0.3
<i>T. derasa</i>	28	5.6 $\pm$ 1.4	6.0 $\pm$ 0.7	-12.3 $\pm$ 0.9	-13.1 $\pm$ 0.7	4.7 $\pm$ 0.3	4.3 $\pm$ 0.2	0.8 $\pm$ 0.7	0.4 $\pm$ 0.3
<i>H. porcellanus</i>	23	6.0 $\pm$ 0.7	5.7 $\pm$ 0.3	-16.7 $\pm$ 0.4	-17.8 $\pm$ 0.5	4.8 $\pm$ 0.1	4.4 $\pm$ 0.3	1.1 $\pm$ 0.4	0.4 $\pm$ 0.3
<i>T. maxima</i>	9	7.0 $\pm$ 2.2	6.4 $\pm$ 1.5	-17.7 $\pm$ 0.7	-18.9 $\pm$ 0.9	4.7 $\pm$ 0.2	4.2 $\pm$ 0.2	1.2 $\pm$ 0.9	0.5 $\pm$ 0.3
<i>H. hippopus</i>	25	5.5 $\pm$ 1.2	5.5 $\pm$ 0.5	-15.2 $\pm$ 0.9	-16.5 $\pm$ 0.8	5.2 $\pm$ 0.3	4.4 $\pm$ 0.4	1.3 $\pm$ 0.6	0.8 $\pm$ 0.4
<i>T. squamosa</i>	26	4.8 $\pm$ 1.7	6.1 $\pm$ 0.6	-14.9 $\pm$ 1.3	-16.7 $\pm$ 1.4	5.0 $\pm$ 0.3	4.2 $\pm$ 0.3	1.7 $\pm$ 0.9	0.7 $\pm$ 0.3

**Supplementary Table 2.** Standard ellipses areas and overlap metrics of six giant clam hosts and their associated algal symbionts in isotopic space. Stable Isotope Bayesian Ellipses in R (SIBER) analysis was used to fit standard ellipses area corrected for sample size ( $\text{SEA}_{\text{C}}$ ) that captured 40 % of the variation of

the data to host (Host  $\text{SEA}_C$ ) and symbiont (Symbiont  $\text{SEA}_C$ ) isotope values. The mode of the last 100 posterior ellipses from a Bayesian generated distribution was used as to compute the Bayesian standard ellipse area of the host group (Host  $\text{SEA}_B$ ), the symbiont group (Symbiont  $\text{SEA}_B$ ), and the area of overlap between the two. The area of overlap of host and symbiont was expressed as a proportion of Host  $\text{SEA}_B$  ( $\text{SEA}_B\text{H}$ ) and Symbiont  $\text{SEA}_B$  ( $\text{SEA}_B\text{S}$ ).

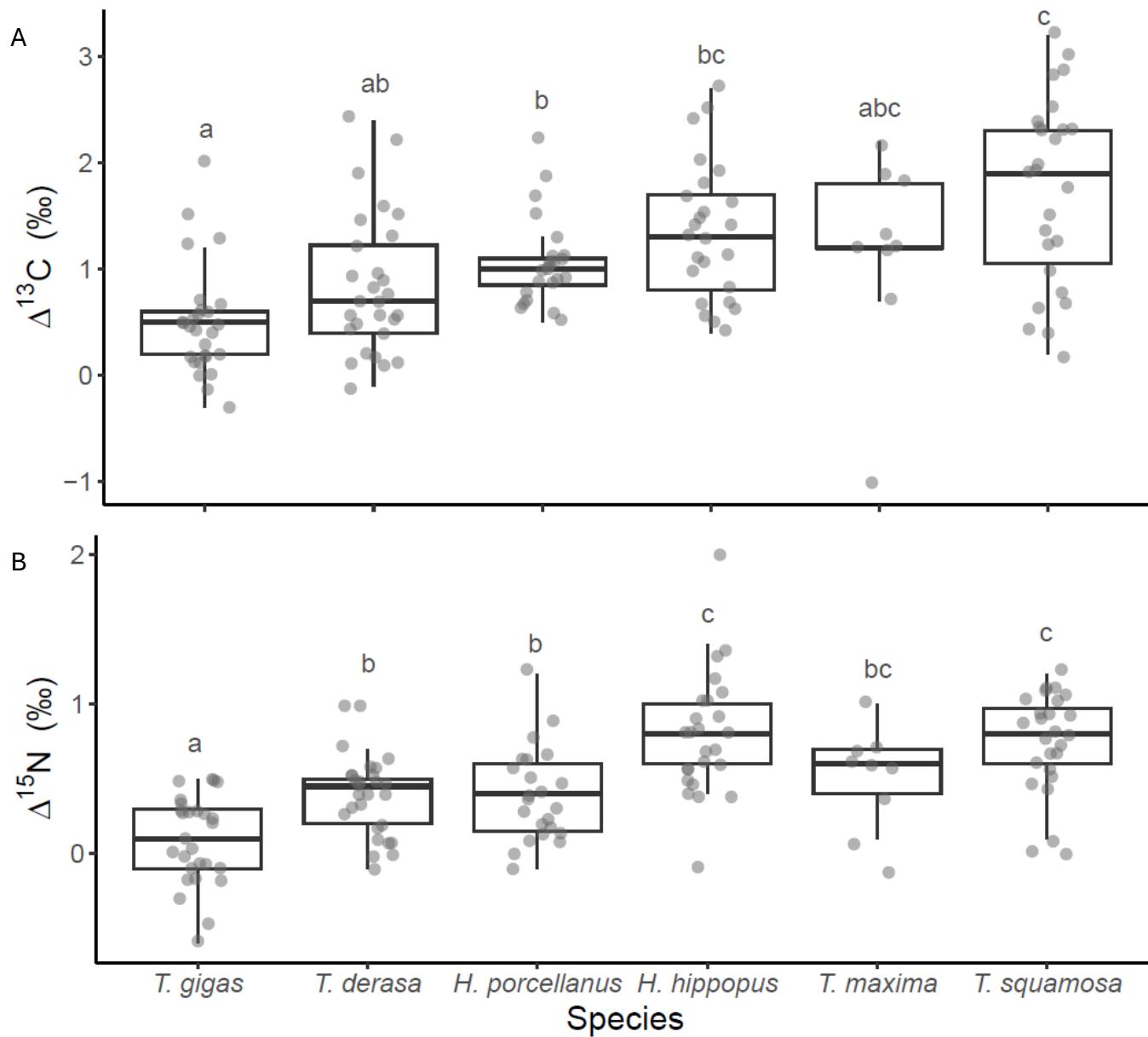
Species	Host $\text{SEA}_C$ (‰²)	Host $\text{SEA}_B$ (‰²)	Symbiont $\text{SEA}_C$ (‰²)	Symbiont $\text{SEA}_B$ (‰²)	$\text{SEA}_C$ area of overlap (‰²)	$\text{EA}_B$ area of overlap (‰²)	$\text{SEA}_B\text{H}$	$\text{SEA}_B\text{S}$
<i>T. gigas</i>	1.19	1.13	0.43	0.41	0.40	0.29	0.24	0.90
<i>T. derasa</i>	0.60	0.59	0.34	0.32	0.10	0.00	0.00	0.00
<i>H. porcellanus</i>	0.14	0.14	0.39	0.37	0.00	0.00	0.00	0.00
<i>T. maxima</i>	0.51	0.41	0.73	0.60	0.00	0.00	0.00	0.00
<i>H. hippopus</i>	0.70	0.65	0.93	0.88	0.00	0.00	0.00	0.00
<i>T. squamosa</i>	0.95	0.87	0.89	0.86	0.00	0.00	0.00	0.00

**Supplementary Table 3.** Comparison of the placement and overlap in isotopic space of six giant clam hosts and their associated algal symbionts. Host and symbiont placements were assessed by measuring the distance between the ellipse centroids of the two groups (DEC) and running a residual permutation procedure (RPP). Host and symbionts occupied distinct space on the isotopic biplot when  $p < 0.05$ . Overlap between the two groups was assessed using a Bayesian analysis to generate a posterior distribution of ellipses fit to encompass 95 % of the variation of each group. The mode of the last 100 posterior ellipses and their overlap was used as the Bayesian ellipse area of the host group (Host  $\text{MEA}_B$ ), the symbiont group (Symbiont  $\text{MEA}_B$ ), and the area of overlap between the two. The proportion of host and symbiont  $\text{MEA}_B$  overlapping that of the other was also calculated (respectively,  $\text{MEA}_B\text{H}$  and  $\text{MEA}_B\text{S}$ ).

Species	DEC (‰)	RPP p-value	Host $\text{MEA}_B$ (‰²)	Symbiont $\text{MEA}_B$ (‰²)	Area of overlap (‰²)	$\text{MEA}_B\text{H}$	$\text{MEA}_B\text{S}$
<i>T. gigas</i>	0.50	0.116	6.97	2.61	2.31	0.32	0.98
<i>T. derasa</i>	0.93	<0.001	3.65	2.02	1.63	0.41	0.82
<i>H. porcellanus</i>	1.14	<0.001	0.78	2.15	0.41	0.49	0.17
<i>T. maxima</i>	1.27	<0.001	2.37	3.97	0.82	0.24	0.20
<i>H. hippopus</i>	1.57	<0.001	4.05	5.28	2.01	0.43	0.34
<i>T. squamosa</i>	1.89	<0.001	5.56	5.26	1.42	0.28	0.31

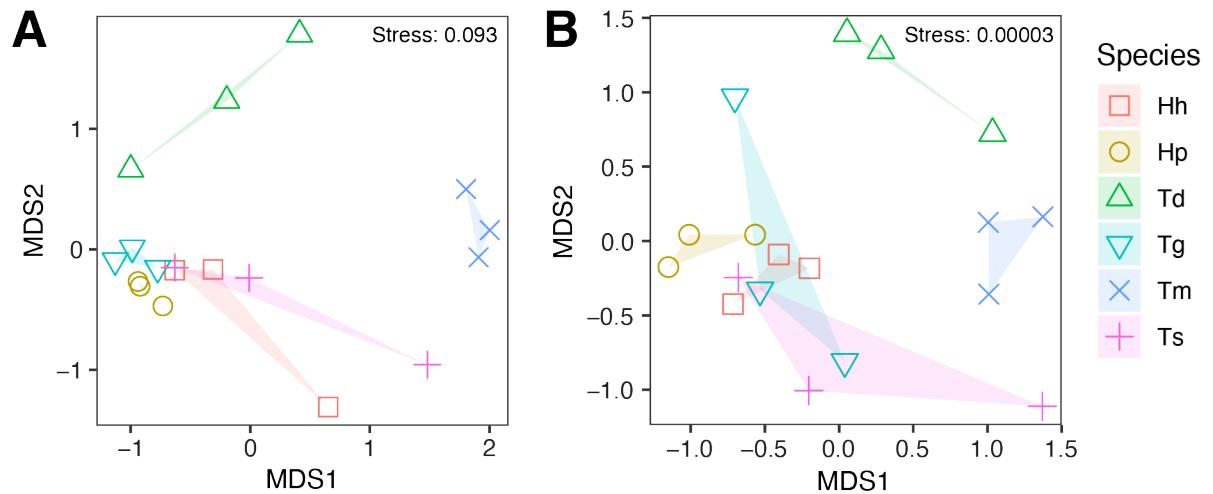
**Supplementary Table 4.** Results of post-hoc pairwise comparisons between giant clam species using either a Games-Howell Post-hoc test for non-normal, heteroscedastic  $\delta^{13}\text{C}$  values or a Tukey's HSD test for normal, homoscedastic  $\delta^{15}\text{N}$  values.

<b>Comparison</b>	<b><math>\delta^{13}\text{C}</math> P-values (Games-Howel post hoc)</b>	<b><math>\delta^{15}\text{N}</math> P-values (Tukey's HSD)</b>
<i>T. gigas</i> x <i>T. derasa</i>	0.253	0.0071009
<i>T. gigas</i> x <i>H. porcellanus</i>	0.0000887	0.0144326
<i>T. gigas</i> x <i>T. maxima</i>	0.378	0.0128298
<i>T. gigas</i> x <i>H. hippopus</i>	0.00000456	0.0000000
<i>T. gigas</i> x <i>T. squamosa</i>	0.00000266	0.0000000
<i>T. derasa</i> x <i>H. porcellanus</i>	0.663	1.0000000
<i>T. derasa</i> x <i>T. maxima</i>	0.918	0.9506383
<i>T. derasa</i> x <i>H. hippopus</i>	0.067	0.0003557
<i>T. derasa</i> x <i>T. squamosa</i>	0.001	0.0028053
<i>H. porcellanus</i> x <i>T. maxima</i>	1.000	0.9479829
<i>H. porcellanus</i> x <i>H. hippopus</i>	0.486	0.0006674
<i>H. porcellanus</i> x <i>T. squamosa</i>	0.015	0.0045442
<i>T. maxima</i> x <i>H. hippopus</i>	0.994	0.2335922
<i>T. maxima</i> x <i>T. squamosa</i>	0.599	0.4599928
<i>H. hippopus</i> x <i>T. squamosa</i>	0.436	0.9920773



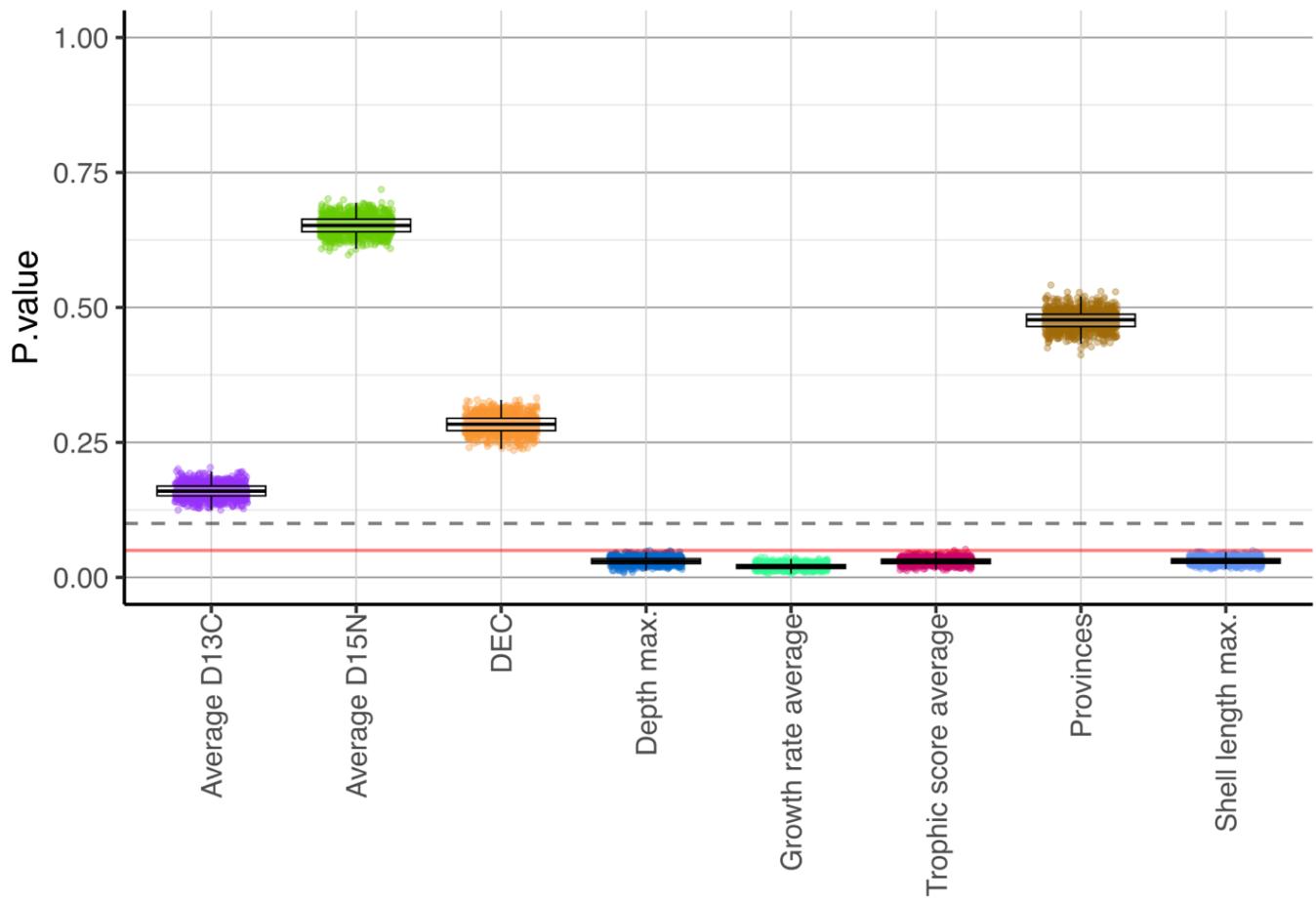
**Supplementary Figure 3.**  $\Delta^{13}\text{C}$  ( $\delta^{13}\text{C}_{\text{Host}} - \delta^{13}\text{C}_{\text{Symbiont}}$ ; A) and  $\Delta^{15}\text{N}$  ( $\delta^{15}\text{N}_{\text{Host}} - \delta^{15}\text{N}_{\text{Symbiont}}$ ; B) of each giant clam species. Boxplots show the median and interquartile range; individual data are shown with grey points. Species labeled with one or more of the same letters are not significantly different (e.g. “a” and “ab” share the letter “a” and are thus not significantly different from one another). In contrast, species with labels that do not share any common letters are significantly different from one another (e.g. “a” is significantly different from “b”) as determined through pairwise comparisons ( $\Delta^{13}\text{C}$ : Games-Howell post hoc tests;  $\Delta^{15}\text{N}$ : Tukey’s HSD tests). P-values for each pairwise comparison are provided in Supplementary Table 4.

### Symbiodiniaceae diversity and community structure



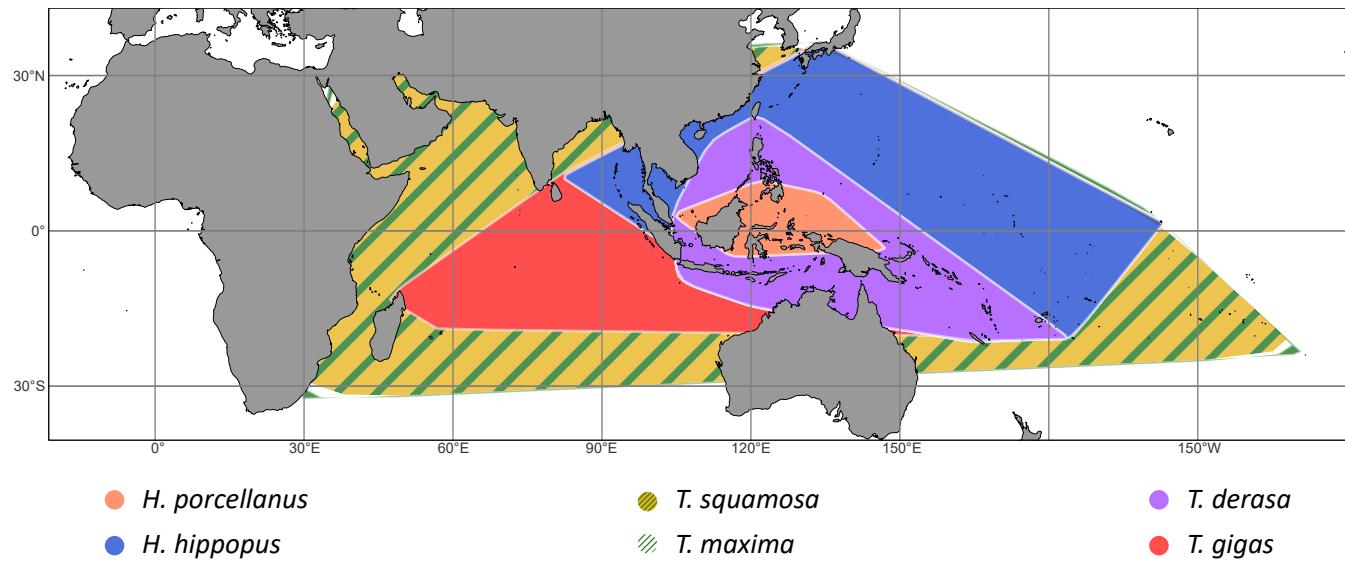
**Supplementary Figure 4.** Non-metric multi-dimensional scaling plots of the Symbiodiniaceae community composition in six giant clams species, based on Bray-Curtis dissimilarities. Ordinations are shown for A) ITS2 sequences and B) ITS2 type profiles. For each species, three replicate samples are represented by distinct symbols and colors, with points connected and enclosed by shaded polygons to illustrate within-species variation. Species are denoted as follows: *Hippopus hippopus* (Hh, red squares), *Hippopus porcellanus* (Hp, brown circles), *Tridacna derasa* (Td, upward-pointing green triangles), *Tridacna gigas* (Tg, downward-pointing turquoise triangles), *Tridacna maxima* (Tm, blue crosses), and *Tridacna squamosa* (Ts, pink upright crosses).

## Phylogenetic signal analysis



**Supplementary Figure 5.** P-values of the eight ecological traits assessed for the phylogenetic signal analysis highlighting four significant traits: maximum depth, growth rate average, trophic niche score, and maximum shell length. Each ecological trait is represented by 1,000 individual p-values, shown as color-coded circles: Average D13C (purple), Average D15N (green), distance between the ellipse centroids DEC (orange), maximum depth (dark blue), growth average rate (light green), trophic niche score average (red), provinces (brown) and maximum shell length (light blue). The boxplot displays the median and the first and third quartiles of the 1,000 results for each trait. Whiskers extend to 1.5 times the interquartile range from the quartiles, and values beyond this range are plotted as outliers.

## **Discussion**



**Supplementary Figure 6.** Geographic distribution of the 6 giant clam species of interest. Orange: *Hippopus porcellanus*, blue: *Hippopus hippopus*, Yellow: *Tridacna squamosa*, Green: *Tridacna maxima*, Purple: *Tridacna derasa*, Red: *Tridacna gigas*. Location data were obtained from Neo et al. 2017 [4].

## **Supplementary References:**

1. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*. 2009;75:7537–41.
2. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. *BMC Bioinformatics* [Internet]. 2009 [cited 2017 Nov 7];10:421. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20003500>
3. Eren AM, Morrison HG, Lescault PJ, Reveillaud J, Vineis JH, Sogin ML. Minimum entropy decomposition: Unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. *ISME Journal* [Internet]. 2015;9:968–79. Available from: <http://dx.doi.org/10.1038/ismej.2014.195>

4. Neo ML, Wabnitz CCC, Braley RD, Heslinga GA, Fauvelot C, Van Wijnsberge S, et al. Giant clams (Bivalvia: Cardiidae: Tridacninae): a comprehensive update of species and their distribution, current threats and conservation status. *Oceanography and Marine Biology: An annual review*. 2017;55:87–387.

**Data S1. (separate file)**

Stable isotopes data, trophic niche scores, phylogenetic traits and growth rates

**Data S2. (separate file)**

Description of the giant clam samples

**Data S3. (separate file)**

Most abundant ITS2 sequences and sequences accession numbers