SUPPLEMENTAL MATERIAL FOR:

Microbiome variability across the native and invasive range of the ascidian *Clavelina oblonga*

Millie Goddard-Dwyer[1,2], Susanna López-Legentil[1], Patrick M. Erwin[1*]

[1]*Department of Biology & Marine Biology, and Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington NC 28409, United States of America*

[2]*University of Southampton, European Way, Southampton SO14 3ZH, United Kingdom*

Running Title: Microbiome variability in an invasive ascidian

*Corresponding author: Patrick M. Erwin; email: erwinp@uncw.edu; Telephone: +1(910) 962-2326; Fax: +1 (910) 962-2410

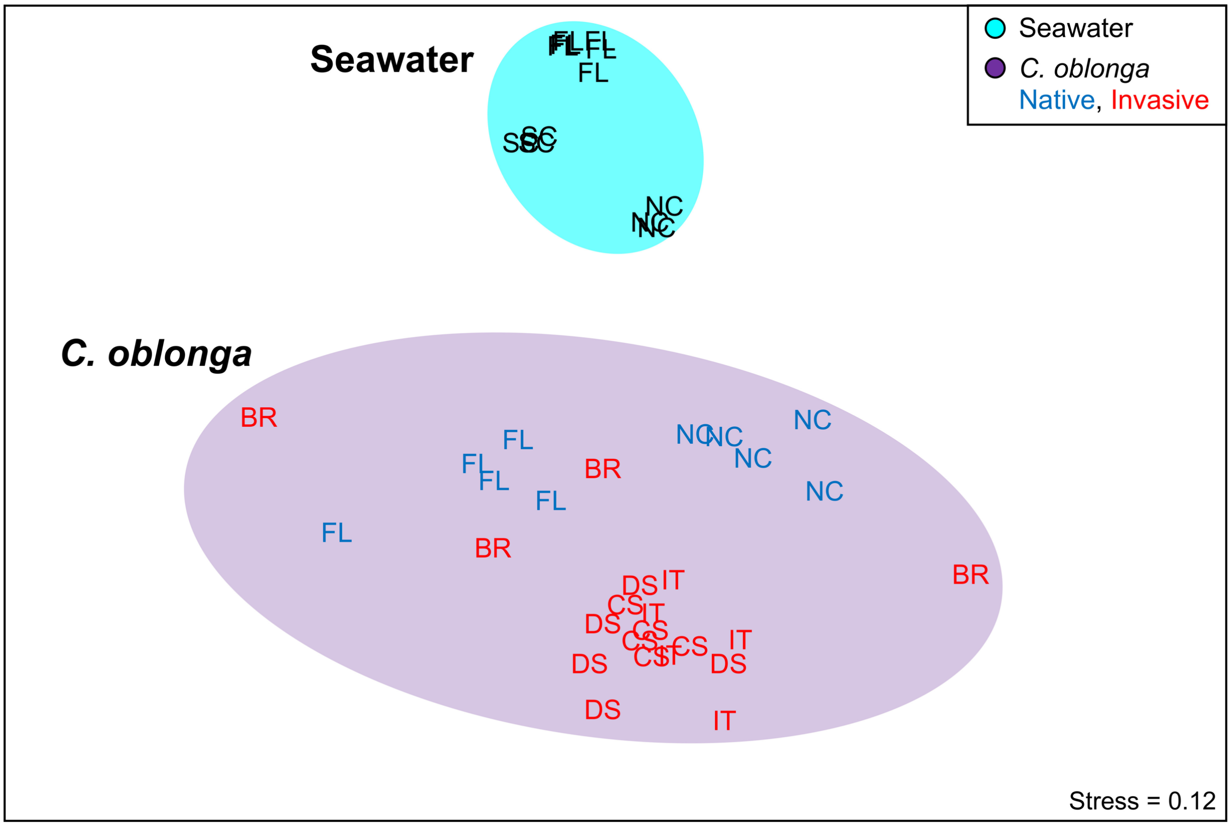

Figure S1. Non-metric multi-dimensional scaling (nMDS) plot visualizing the similarity of microbial communities in *Clavelina oblonga* (purple oval) and ambient seawater (blue oval). Letters denote different collection locations: Florida (FL), North Carolina (NC), South Carolina (SC), Brazil (BR), Ebro Delta, Spain (DS), Cadis, Spain (CS) and Italy (IT). Colored letters indicate *C. oblonga* colonies from invasive (red) and native (blue) locations.

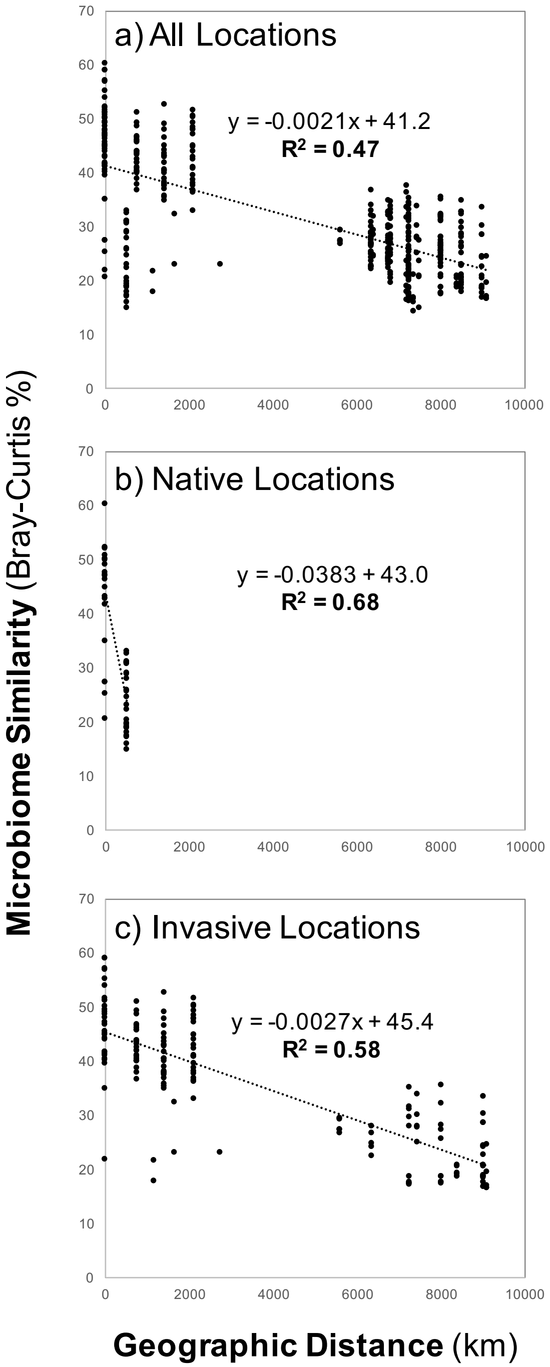

Figure S2. Distance-decay relationships in the microbiome of *Clavelina oblonga* across native and invasive locations.

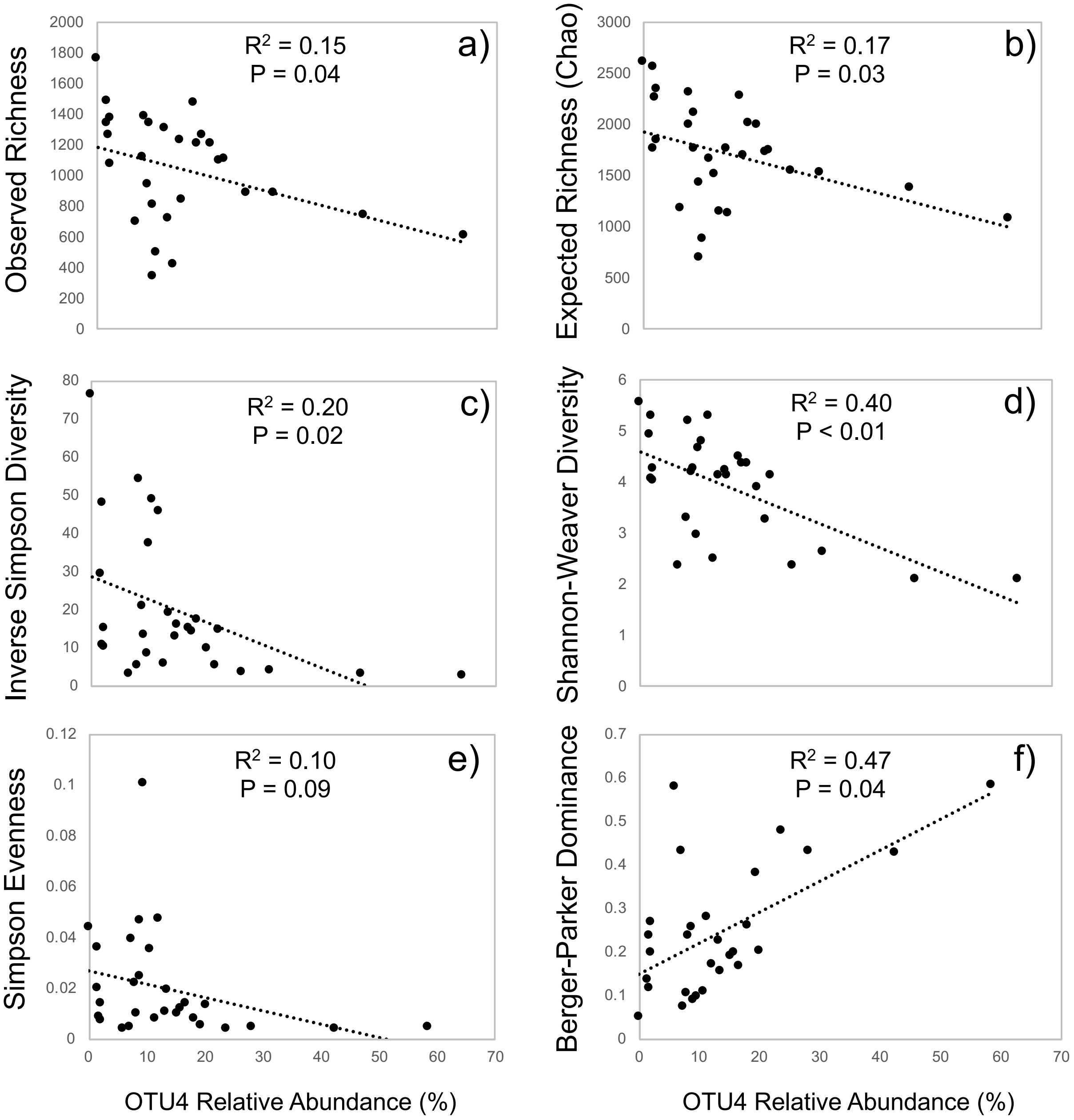

Figure S3. Correlations between the relative abundance of symbiont OTU4 (Saprospirales sp.) and microbiome diversity metrics (a) observed richness, (b) expected (Chao) richness, (c) Inverse Simpson diversity, (d) Shannon-Weaver diversity, (e) Simpson evenness and (f) Berger-Parker dominance.

Table S1. Composition of microbial communities in *Clavelina oblonga* (within native and invasive ranges) and seawater (collected in native range). Asterisks (*) denote significant differences among sites within ranges.

Taxon	**Invasive** (%, ±SE)	**Native** (%, ±SE)	**Seawater** (%, ±SE)	**Invasive vs. Native** (P-value)	***C. oblonga* vs. Seawater** (P-value)
Acidobacteria	0.24 ±0.05	1.17 ±0.69	0.25 ±0.07	0.002*	0.182
Actinobacteria	1.16 ±0.22	3.75 ±1.14	8.99 ±0.54	0.006*	<0.001*
Bacteroidetes	21.91 ±2.97	12.68 ±2.11	11.38 ±0.43	0.037*	0.017*
Crenarchaeota	0.56 ±0.37	2.24 ±1.21	0.11 ±0.03	0.582	0.091
Cyanobacteria	1.94 ±0.83	0.76 ±0.26	1.85 ±0.17	0.836	0.011*
OD1	1.04 ±0.55	0.16 ±0.09	0.00 ±0.00	0.051	<0.001*
Planctomycetes	2.82 ±0.79	2.75 ±0.69	1.71 ±0.35	0.663	0.673
Verrucomicrobia	1.58 ±0.64	2.23 ±1.08	1.71 ±0.41	0.335	0.301
Alphaproteobacteria	26.78 ±2.91	34.65 ±3.99	48.34 ±1.95	0.123	<0.001*
Deltaproteobacteria	17.67 ±4.03	9.57 ±5.45	1.71 ±0.29	0.162	<0.001*
Epsilonproteobacteria	3.04 ±1.67	7.11 ±2.78	0.17 ±0.04	0.731	0.040*
Gammaproteobacteria	18.29 ±1.93	19.4 ±1.31	13.67 ±0.66	0.261	0.006*
Other	3.00 ±0.59	3.54 ±0.97	10.12 ±0.63	0.598	<0.001*

Table S2. Composition of microbial communities in *Clavelina oblonga* across locations with native and invasive ranges. Asterisks (*) denote significant differences among sites within ranges.

Taxon	**Brazil** (%, ±SE)	**Cadis, Spain** (%, ±SE)	**Ebro Delta, Spain** (%, ±SE)	**Florida** (%, ±SE)	**Italy** (%, ±SE)	**North Carolina** (%, ±SE)	**P-value**	**Pairwise Comparisons**
Acidobacteria	0.35 ±0.16	0.17 ±0.02	0.23 ±0.09	0.33 ±0.05	0.22 ±0.09	2.02 ±1.33	0.470	
Actinobacteria	1.01 ±0.36	0.19 ±0.28	1.19 ±0.47	2.53 ±0.9	1.22 ±0.64	4.96 ±2.07	0.138	
Bacteroidetes	18.4 ±3.36	22.31 ±2.49	14.39 ±2.12	18.03 ±2.03	31.83 ±9.58	7.32 ±1.25	0.029*	IT > NC
Crenarchaeota	0.29 ±0.05	0.19 ±0.08	0.18 ±0.15	4.42 ±2.04	1.52 ±1.4	0.06 ±0.02	0.025*	FL > NC
Cyanobacteria	0.98 ±0.87	1.84 ±1.45	4.63 ±2.52	0.91 ±0.46	0.12 ±0.02	0.62 ±0.3	0.122	
OD1	0.14 ±0.08	0.58 ±0.29	3.15 ±1.9	0.3 ±0.17	0.11 ±0.04	0.03 ±0.01	0.036*	
Planctomycetes	4.36 ±3.58	2.04 ±0.66	3.46 ±1.05	3.26 ±0.81	1.7 ±0.71	2.24 ±1.16	0.712	
Verrucomicrobia	4.7 ±2.71	0.47 ±0.15	0.77 ±0.18	3.83 ±1.99	1 ±0.36	0.64 ±0.22	0.067	
Alphaproteobacteria	29.1 ±4.55	13.53 ±2.55	37.71 ±3.79	39.03 ±3.9	27.24 ±6.07	30.27 ±6.83	0.024*	FL > CS, DS > CS
Deltaproteobacteria	10.57 ±4.21	39.83 ±4.88	7.58 ±2.63	4 ±0.62	11.26 ±8.21	15.13 ±10.85	0.228	
Epsilonproteobacteria	5.71 ±3.87	0.15 ±0.01	0.16 ±0.02	0.05 ±0.02	6.66 ±5.47	14.16 ±3.13	0.002*	NC > FL, IT > FL
Gammaproteobacteria	21.94 ±6.23	15.22 ±0.83	21.84 ±3.64	20.15 ±1.43	14.89 ±3.93	18.65 ±2.33	0.528	
Other	2.46 ±1.16	2.47 ±0.48	4.71 ±1.92	3.16 ±0.93	2.23 ±0.48	3.92 ±1.81	0.782	

Table S3. Analyses of variance (ANOVA) results comparing diversity indices of symbiont communities in *Clavelina oblonga* within native and invasive ranges. Asterisks (*) denote significant differences among sites within ranges.

Range	**Diversity index**	**d.f.**	***F*-ratio**	**p**
Native	Observed richness	1, 8	0.238	0.638
	Expected richness, Chao	1, 8	1.345	0.280
	Simpson evenness	1, 8	3.029	0.120
	Berger Parker	1, 8	1.297	0.288
	Inverse Simpson	1, 8	0.370	0.560
	Shannon-Weaver	1, 8	1.104	0.324
Invasive	Observed richness	3, 15	1.535	0.246
	Expected richness, Chao	3, 15	3.295	0.050*
	Simpson evenness	3, 15	2.396	0.109
	Berger Parker	3, 15	3.737	0.035*
	Inverse Simpson	3, 15	1.981	0.160
	Shannon-Weaver	3, 15	1.257	0.324

Table S4. Symbiont OTUs that contributed to 23% of the dissimilarity between symbiotic communities in *Clavelina oblonga* and ambient seawater (SIMPER analysis), showing phylum and lowest level taxonomy and characteristic traits (grey boxes denote OTUs with taxonomic resolution too low to assign functionality). Average relative abundance of each OTU in *C. oblonga* (CO) and ambient seawater (SW) is shown, with asterisks (*) signifying significant differences between ranges (*P*<0.05, Metastats). BLAST matches include the source and pairwise identity (%) of the closest known relative for each OTU, with crosses (†) indicating that the BLAST result is an example chosen from a list of equally matched pairwise identity sequences.

OTU	**Phylum (lowest taxonomy)**	**Characteristic Traits**	**Average Rel. Abundance**			**% Contrib.**	**BLAST match**	
			CO	*SW*	*P*		*Source (acc. no.)*	*% ID*
3	Proteobacteria (c. δ-proteobacteria)		**12.0**	0.04	0.001*	6.28	Chiton gill tissue (HE663396)	91.2
4	Bacteroidetes (o. Saprospirales)		**13.5**	0.04	0.001*	7.09	Pu-erh tea (KT360474)	91.2
2	Proteobacteria (*Rhodobacteraceae*)	Chemoheterotroph, hydrolyses urea (49)	**9.48**	0.39	0.001*	4.88	Culture (NR114028)	98.8
8	Proteobacteria (*Arcobacter* sp.)	Chemoheterotroph, reduces nitrate (52)	**2.00**	0.06	0.101	1.06	Marine sediment (JX391310)	100.0
6	Crenarchaeota (g. *Nitrosopumilus*)	Oxidise NH_4^+ and vitamin B_{12} synthesis (51)	**1.08**	0.08	0.009*	0.55	Seawater (KX950758) †	100.0
11	Proteobacteria (*Vibrio shiloi*)	Heavy metal resistance (50), coral pathogen (54)	**1.14**	0.13	0.009*	0.58	*Doclea* sp. host (MG077075)	100.0
15	Proteobacteria (f. Endozoicimonaceae)		**1.47**	0.12	0.002*	0.75	*Styela clava* host (KU648381)	99.6
51	Proteobacteria (f. Rhodobacteraceae)		**0.82**	0.01	0.001*	0.43	Seawater (NR132291)	100.0
24	Proteobacteria (c. δ-proteobacteria)		**1.20**	0.00	0.001*	0.63	Subseafloor sediment (JQ989870)	90.8
25	Proteobacteria (c. γ-proteobacteria)		**1.08**	0.01	0.001*	0.56	*Galaxea fascicularis* host (KU353970)	95.2
35	Proteobacteria (*Arcobacter* sp.)	Chemoheterotroph, reduces nitrate (52)	**0.97**	0.00	0.002*	0.51	Seawater (KX179260) †	100.0

Table S5. Collection location, GPS coordinates and sample codes for *Clavelina oblonga* and seawater samples used in this study.

Source	Location	Coordinates	Sample Code
Clavelina oblonga	Beaufort, NC USA (July 2014)	34°42'55.1"N, 76°40'44.4"W	CO.1T, CO.2T, CO.3T, CO.4T, CO.5T
	Fernandina Beach, FL USA (Mar 2017)	30º40’11.5”N, 81º27’59.8”W	HS34.44, HS34.45, HS34.46, HS34.47, HS34.48
	Cadiz, Spain (Autumn 2014)	36º3º’59”N, 6º18’41”W	COC.1, COC.2, COC.3, COC.4, COC.5
	Ebro Delta, Spain (2011-2012)	40º37’01”N, 0º37’26” E	COD.1, COD.2, COD.3, COD.4, COD.5
	Taranto, Italy (2013)	40º28’36”N, 17º15’5” E	COT.1, COT.2, COT.3, COT.4, COT.5
	Pier Baia Golfinhos, Brazil (Sep 2016)	27º24’16.97”S, 48º33’41.70”W	HS34.04
	Pier Florianópolis, Brazil (Sep 2016)	27º35’11.75”S, 48º33’26.12”W	HS34.05
	Pier Enseada Suá, Vitória, Brazil (May 2016)	20º19’07”S, 40º17’56”W	HS34.06, HS34.07
Seawater	Wrightsville Beach, NC USA	34°13'01.6"N, 77°48'47.4"W	SW.128, SW.115, SW.215, SW.315
	Fernandina Beach, FL USA (Mar 2017)	30º40’11.5”N, 81º27’59.8”W	HS34.14, HS34.15, HS34.16
	Smyrna Beach, FL USA (Mar 2017)	29º01’40.8”N, 80º55’14.1”W	HS34.26, HS34.27, HS34.28
	Sunset Cay, SC USA (Mar 2017)	32º38’53.8”N, 79º57’42.3”W	HS34.08, HS34.09, HS34.10