



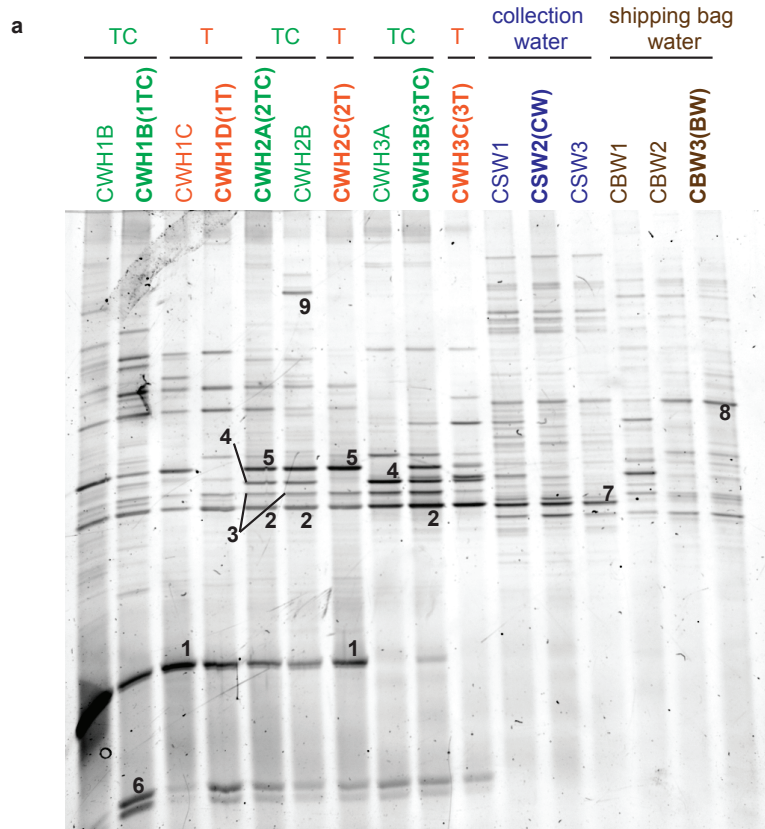
**Supplementary Table S3.** Richness and diversity of 3% OTUs. Calculations were performed in Mothur by averaging 1000 random subsamples of 2500 sequences per sample.

Sequencing Sample Name	DNA Sample	Good's coverage % (sd)	Observed Richness 3% OTUs (sd)	Non-parametric Shannon diversity (sd)	Chao (lower-upper 95% CI)
1TC	CWH1B	97.4 (0.2)	147 (4)	2.98 (0.02)	224 (188-294)
1T	CWH1D	99.89 (0.05)	15 (1)	1.64 (0.02)	18 (16-31)
2TC	CWH2A	98.5 (0.2)	72 (4)	2.00 (0.02)	126 (95-198)
2T	CWH2C	99.67 (0.09)	22 (2)	1.34 (0.02)	35 (26-76)
3TC	CWH3B	99.64 (0.04)	29 (1)	2.06 (0.01)	38 (31-68)
3T	CWH3C	99.82 (0.03)	22 (1)	2.06 (0.01)	28 (23-56)
BW	CBW3	88.0 (0.5)	526 (11)	4.51 (0.04)	973 (853-1136)
CW	CCW2	94.2 (0.4)	278 (9)	3.70 (0.04)	528 (437-671)

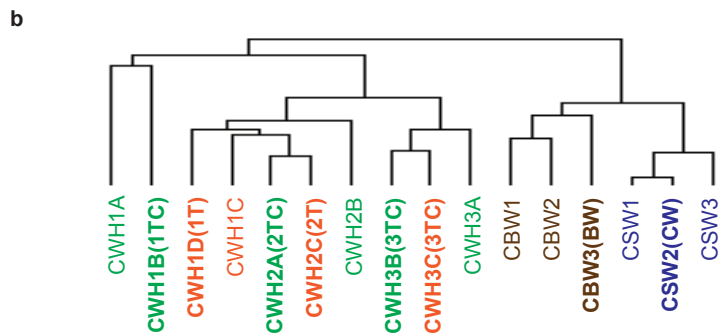
**Supplementary Figure S1.** Phylogenetic tree of tunicate COI genes. The COI gene from two Woods Hole *C. intestinalis* individuals was amplified and sequenced using primers Ci-CoIF (5'-CCTAGATTTAGAATAATTAGTCATG-3') and Ci-CoIR (5'-CGAGGTATTCTACTTAATCT-3') as described in (Caputi *et al.*, 2007). Amino acid sequences of tunicate COI genes were aligned in ClustalX and a neighbor-joining tree with 1000x bootstraps was calculated in ClustalX. Nodes with bootstrap support >90% are indicated by filled circles.



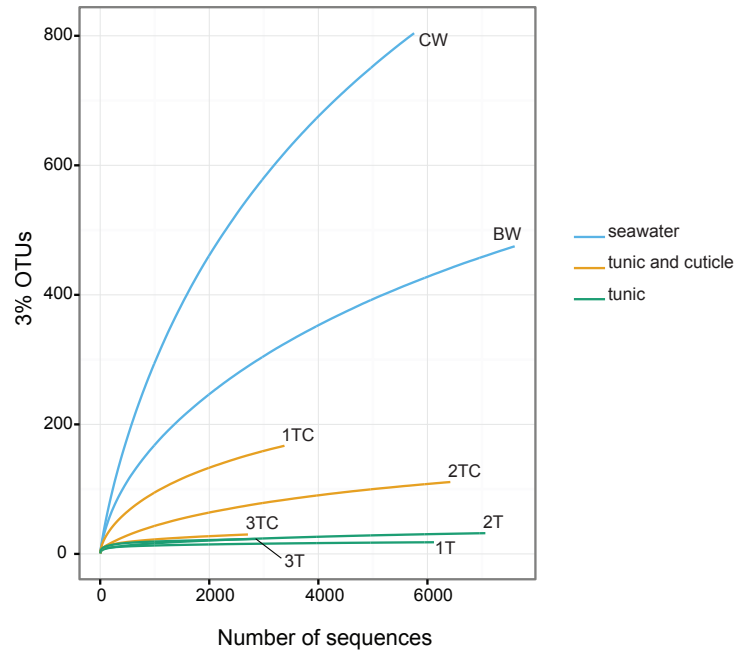
**Supplementary Figure S2.** 16S rRNA gene DGGE analysis. **(a)** Samples containing both tunic and cuticle (TC) are shown in green, tunic only (T) in orange, collection site seawater (CSW1-3) in blue, and shipping bag seawater (CBW1-3) in brown. Samples that were selected for 454 sequencing are shown in bold, and their 454 sequencing ID is indicated in parentheses. Numbers indicate sequenced bands and RDP classification at a 50% bootstrap cutoff is shown. **(b)** Clustering of DGGE banding patterns by similarity.



- 1 Flavobacteria\_unclassified (100% ID to isolate CWH016)
- 2 Alphaproteobacteria\_unclassified (100% isolate CWH001)
- 3 Alphaproteobacteria\_unclassified
- 4 Alphaproteobacteria\_Terasakiella (100% ID to isolate CWH003)
- 5 Alphaproteobacteria\_unclassified
- 6 Alphaproteobacteria\_unclassified
- 7 Alphaproteobacteria\_unclassified
- 8 Cyanobacteria\_GpIIa
- 9 Verrucomicrobia\_Rubritalea



**Supplementary Figure S3.** Rarefaction curves of 454 amplicon sequencing samples.



### Supplementary References

Caputi L, Andreakis N, Mastrototaro F, Cirino P, Vassillo M, Sordino P (2007). Cryptic speciation in a model invertebrate chordate. *Proc Natl Acad Sci USA* **104**: 9364-9369.

Muyzer G, de Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**: 695-700.