The following supplement accompanies the article

Infestation biology of *Phallusia nigra* (Tunicata, Phlebobranchia) on hard corals in a subtropical bay

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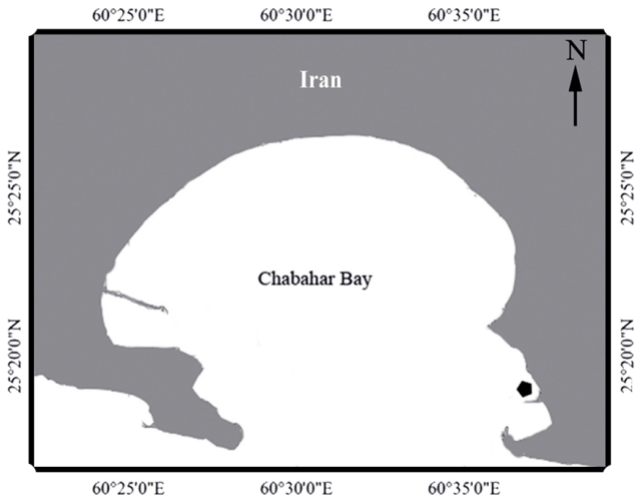

Fig. S1. Map of sampling location (marked by a polygon) in Chabahar Bay

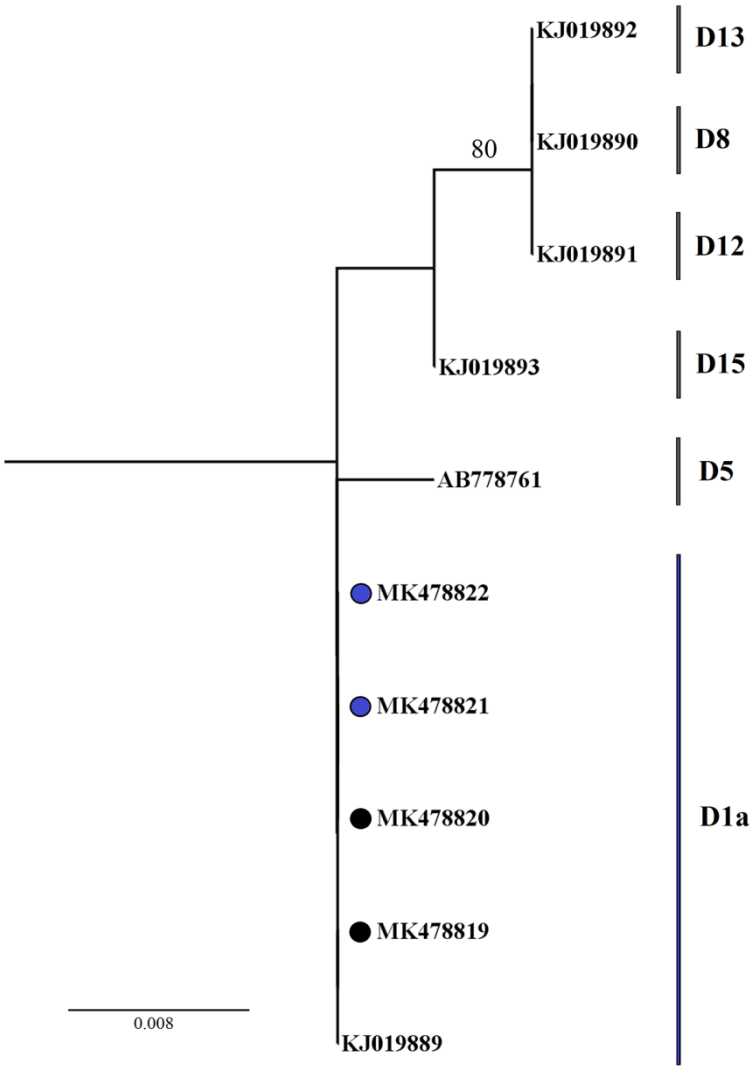

Fig. S2. Maximum Likelihood (ML) tree for genus *Durusdinium* based on ITS2 rDNA. The taxa used to construct the tree are marked with their GenBank ITS2-type designations. Values above branches are ML bootstrap values (support values below 80 are not presented). The tree was rooted with *Cladocolpium thermophilum* (KY358760) which is not shown here. Samples from this study are marked with solid circles (blue: uninfested; black: infested), all of which were placed within D1a ITS2-type (i.e., *Durusdinium trenchii*). The scale shows substitutions per site.

Comparative mineralogy of *Acropora* and *Pocillopora* corals

Methods

Baseline *Acropora* and *Pocillopora* mineralogy was characterized by crystallography for uninfested samples and elemental analyses for powdered samples/ segments of branches (Fig. S3). The powdered samples were obtained from the 3-cm tips of the coral samples (n=3 for each coral genus), which were cleaned in double distilled water (DDW), dried in a desiccator and powdered using a mortar and pestle (Mitsuguchi & Kawakami 2012). Crystalline structures of the products were characterized by X-ray diffraction (XRD) (Bruker AXS, Germany) using a Cu-Kα source (λ=1.54178 Å). Surface and whole-body analytical approaches were applied for the elemental analyses of the samples.

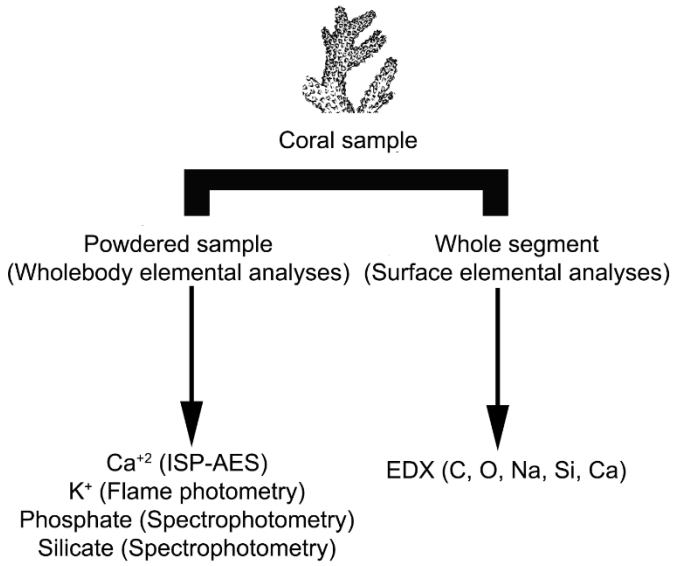

Fig. S3. Schematic of the workflow for the baseline elemental analyses of the coral genera.

For the whole-body assessments, a 100-mg aliquot of each powdered sample was transferred to a 50-mL volumetric flask, dissolved in 2 mL of 4 mol L^{-1} HNO_3 and diluted to 1:7 in DDW (Mitsuguchi & Kawakami 2012). These solutions were centrifuged and filtered through 0.450-µm syringe filters. The concentrations of Ca^+ and K^+ cations in the solutions were determined by inductively coupled plasma atomic emission spectrometry with simultaneous CCD detection (Varian Vista Pro ICP-AES, Australia) and flame photometry, respectively. A UV-VIS spectrophotometer (Rayleigh, USA) was used to determine the concentrations of silicate (molybdate colorimetry, absorbance wavelength of 810 nm) and phosphate (molybdate colorimetry, absorbance wavelength of 882 nm) anions. Surface elements of the coral fragments were semi-quantified by energy dispersive analyses of X-rays (EDX) using field emission SEM (VEGA3 TESCAN, Czech Republic). Differences in ion concentrations between genera were assessed using independent one-way ANOVAs. Data were assessed for assumptions of normality and homoscedasticity using Kolmogorov-Smirnov and Levene's tests, respectively.

Results

The XRD analysis identified aragonite crystals in both *Acropora* and *Pocillopora* (prominent peaks at 20°-26.4 °, Fig. S4).

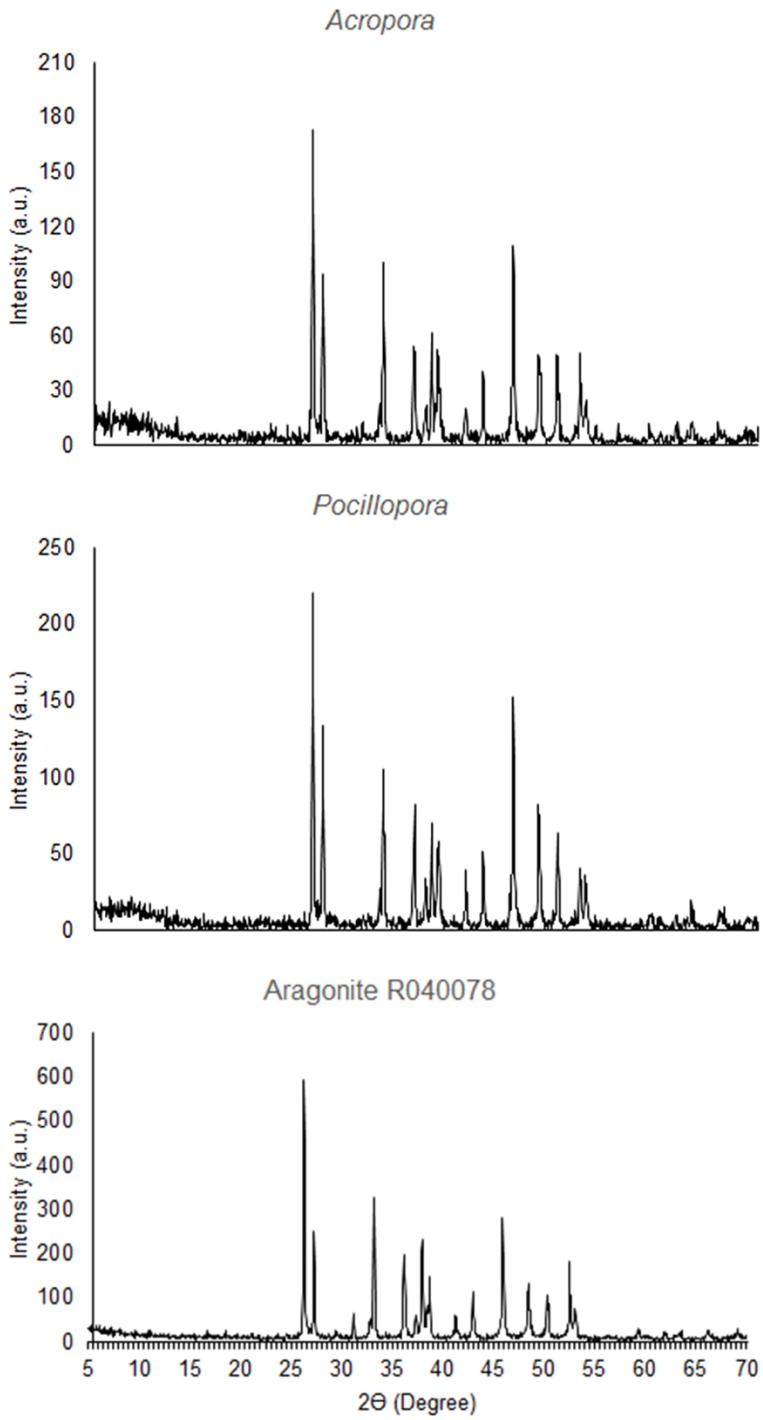

Fig. S4. XRD patterns of *Acropora* and *Pocillopora* corals compared to the standard aragonite pattern.

A preliminary semi-quantitative analysis of the main elements indicated higher surface silica, sodium and oxygen contents in *Pocillopora* than *Acropora*, but calcium and carbon contents were higher in *Acropora* (Fig. S5).

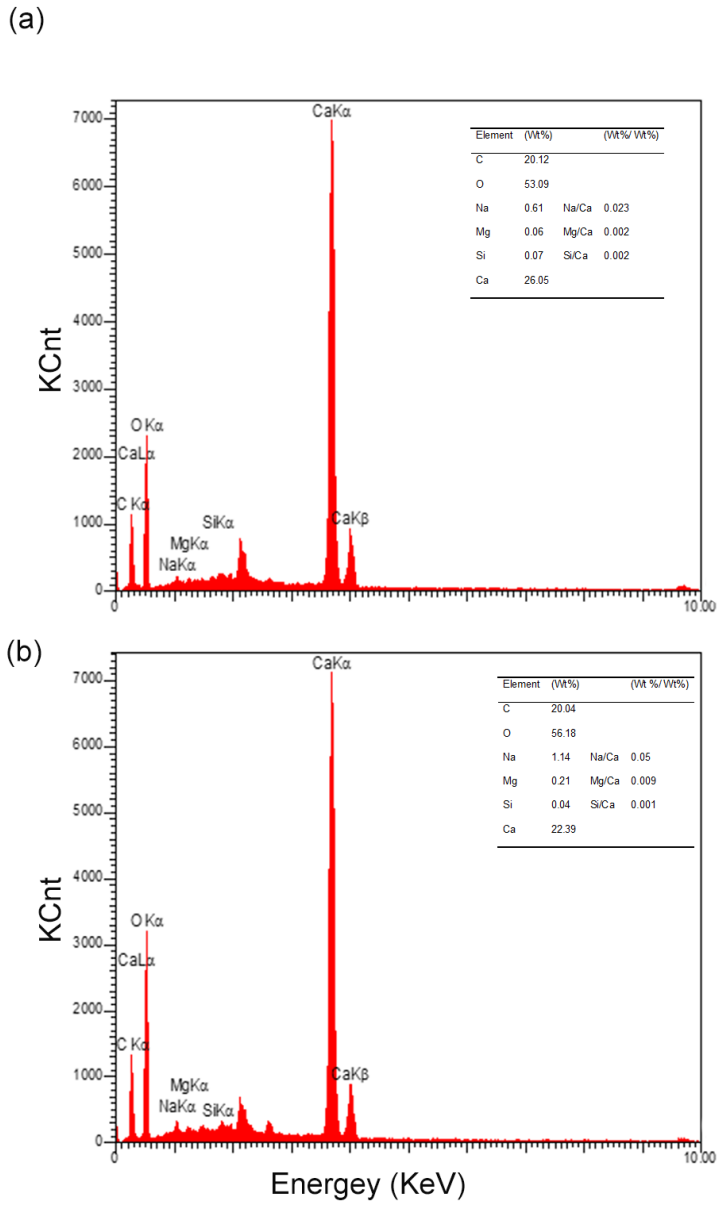

Fig. S5. EDX spectrum from the elemental analysis of (a) *Acropora* and (b) *Pocillopora* corals at Chabahar Bay.

Whole-body K/Ca, PO_4/Ca and SiO_4/Ca ratios also varied between the coral genera, with higher ratios in *Pocillopora* (Fig. S6).

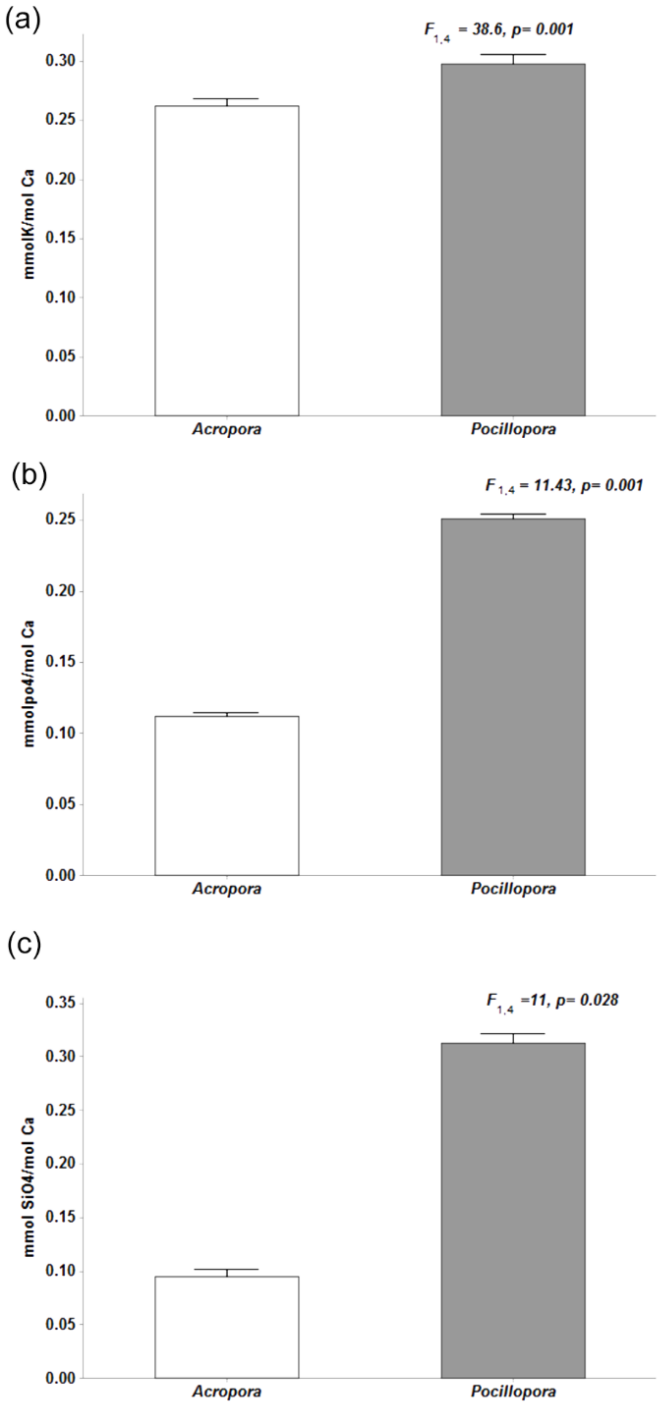

Fig. S6. Differences in mean K, PO_4 and SiO_4 concentrations between *Acropora* and *Pocillopora* corals at Chabahar Bay. Error bars: standard errors. Sample size=3

Discussion

In GLM analyses of the infestation intensity and density we found a significant between-genera differences in tunicate numbers even by including morphological parameters as covariates in the model. As such, it seems that, factors other than morphological aspects of the host colonies (e.g. chemical composition) may also contribute to the observed differences. The higher K^+ concentrations in *Pocillopora* than *Acropora* tissue may lead to the settlement of more tunicates. KCl induces larval settlement in some sessile invertebrates, including *P. nigra*, by the depolarization of receptor cells in the larvae (Zardus et al. 2008). Ions are usually incorporated into coral rocky material (Mitsuguchi & Kawakami 2012) and may not be detected by larvae, but acid producing activity of *P. nigra* and ocean acidification dissolves coral skeletons, leading to the leakage of ions into the surrounding environment. Interestingly, the higher rate of infestation on *Pocillopora* contradicts the higher rates of skeletal dissolution in *Acropora*, highlighting the extensive effects of differences in concentrations between the coral genera on colonization rates of *P. nigra* (Comeau et al. 2014).

Literature cited

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