



# Nutrient stress arrests tentacle growth in the coral model *Aiptasia*

Nils Rådecker<sup>1</sup> · Jit Ern Chen<sup>1</sup> · Claudia Pogoreutz<sup>1</sup> · Marcela Herrera<sup>1</sup> · Manuel Aranda<sup>1</sup> · Christian R. Voolstra<sup>1</sup> 

Received: 3 July 2018 / Accepted: 21 January 2019 / Published online: 4 February 2019  
© The Author(s) 2019

## Abstract

The symbiosis between cnidarians and dinoflagellate algae of the family Symbiodiniaceae builds the foundation of coral reef ecosystems. The sea anemone *Aiptasia* is an emerging model organism promising to advance our functional understanding of this symbiotic association. Here, we report the observation of a novel phenotype of symbiotic *Aiptasia* likely induced by severe nutrient starvation. Under these conditions, developing *Aiptasia* no longer grow any tentacles. At the same time, fully developed *Aiptasia* do not lose their tentacles, yet produce asexual offspring lacking tentacles. This phenotype, termed ‘Wurst’ *Aiptasia*, can be easily induced and reverted by nutrient starvation and addition, respectively. Thereby, this observation may offer a new experimental framework to study mechanisms underlying phenotypic plasticity as well as nutrient cycling within the Cnidaria – Symbiodiniaceae symbiosis.

**Keywords** *Exaiptasia pallida* · Model organism · Nutrient starvation · Stoichiometry · Stress phenotype

## 1 Introduction

Coral reefs are hot spots of biodiversity surrounded by oligotrophic oceans (Reaka-Kudla 1997). The key to understanding the vast diversity and productivity of coral reefs despite these nutrient-limited conditions lies in the ecosystem engineers of these reefs, scleractinian corals (Muscatine and Porter 1977). A mutualistic nutrient-exchange symbiosis with microalgae of the family Symbiodiniaceae (LaJeunesse et al. 2018) allows corals to access autotrophic and heterotrophic energy sources (Rådecker et al. 2015). Indeed, this relationship allows for a highly efficient utilization and recycling of nutrients within the symbiosis, sustaining growth and productivity of corals and forming the

foundation of entire ecosystems, i.e. coral reefs (Muscatine and Porter 1977). Yet, despite their ecological success over evolutionary time frames, corals are in rapid decline. Local and global anthropogenic disturbances are undermining the integrity of the coral - algal symbiosis and lead to the degradation of the entire reef ecosystem (Hughes et al. 2017). Slowing or even reverting this development requires in-depth knowledge of the biology of corals and their symbionts (van Oppen et al. 2015). However, given the diversity of corals and their associated microbial symbionts, understanding the intricacies and the functioning of the coral-alga symbiosis has proven challenging.

The sea anemone *Aiptasia* (sensu *Exaiptasia pallida*) promises to be a powerful new model organism for the study of the coral - alga symbiosis (Rådecker et al. 2018; Voolstra 2013). Just like coral, *Aiptasia* form an endosymbiotic relationship with Symbiodiniaceae. However, *Aiptasia* offer distinct advantages for the study of the cnidarian - Symbiodiniaceae symbiosis: (1.) it can be easily propagated under controlled laboratory conditions; (2.) it can be maintained in a symbiont-free stage and is easily re-infected with a diversity of symbionts; (3.) a diversity of genetic and molecular tools are available for the in-depth study of the symbiosis (Voolstra 2013; Baumgarten et al. 2015). As such, understanding the biology and the functioning of this emerging model organism is critical for the progress of coral reef research.

Here, we report the observation of an environmentally-induced altered phenotype of *Aiptasia*, which may offer

---

Nils Rådecker and Jit Ern Chen contributed equally to this work.

Presented at the 9th International Symbiosis Society (ISS) Congress, July 15-20 2018, Oregon State University, Corvallis, OR, USA

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s13199-019-00603-9>) contains supplementary material, which is available to authorized users.

✉ Manuel Aranda  
manuel.aranda@kaust.edu.sa

✉ Christian R. Voolstra  
christian.voolstra@kaust.edu.sa

<sup>1</sup> Red Sea Research Center, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

interesting new insight into cnidarian biology and symbiosis. We have termed this phenotype ‘Wurst’ (German for sausage) in reference to the altered and distinct morphology of *Aiptasia* anemones.

## 2 Material & Methods

### 2.1 Aquaria maintenance

We unintentionally induced the ‘Wurst’ phenotype while developing a low-maintenance, long-term culture system for *Aiptasia*. For this, we set up a 10 L mesocosm containing freshly collected live rock and autoclaved seawater from the Red Sea (Fig. S1). The aquarium was stocked with *Aiptasia* of the clonal line RS1 (collected from the Red Sea in 2015 and maintained in culture for >2 years (for details see Cziesielski et al. 2018)). Animals were propagated over a three-month period via weekly feeding with *Artemia* nauplii and water exchange with autoclaved seawater (rearing at 30  $\mu\text{mol}$  quanta  $\text{m}^{-2} \text{s}^{-1}$ , 12 h:12 h light/dark cycle, 25 °C, salinity 30–35). Once the *Aiptasia* had sufficiently proliferated (>300 animals), all feeding and water exchange ceased. Maintenance was minimized to removing filamentous algal growth in the aquarium and regularly topping up the aquarium with reverse osmosis water to adjust salinity. The aquarium was maintained under these conditions for six months until the distinct ‘Wurst’ phenotype was initially observed in developing *Aiptasia*.

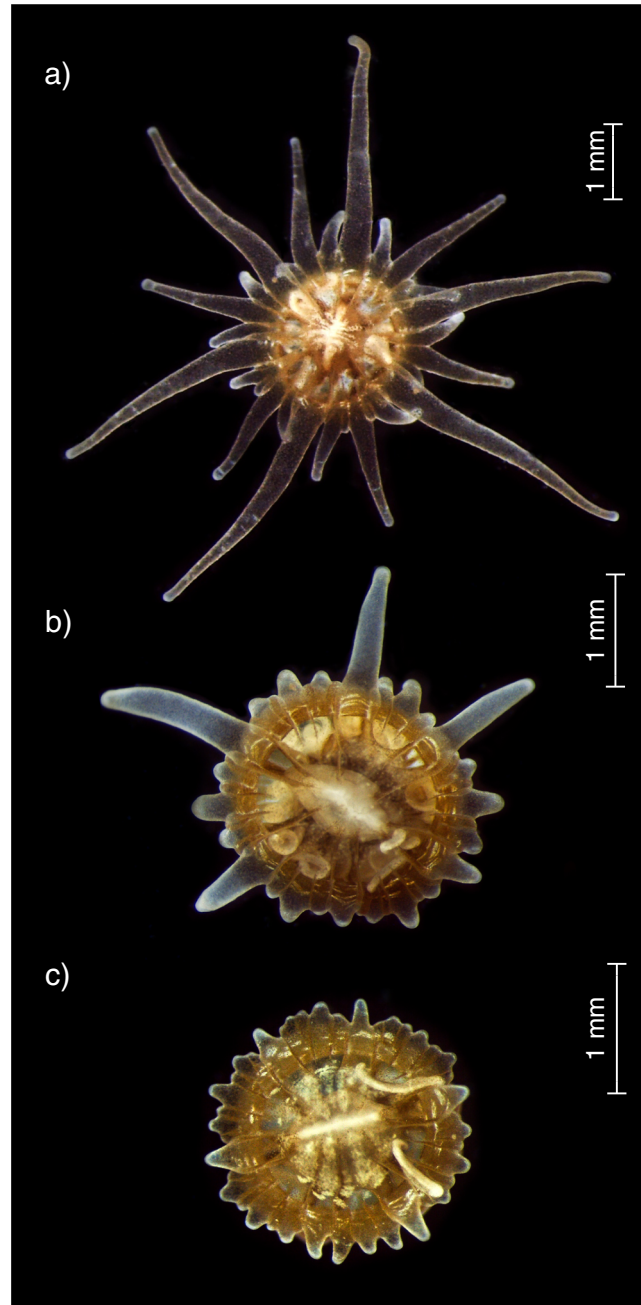
### 2.2 Characterization of *Aiptasia* phenotypes

To provide an empirical measure of phenotypic changes in *Aiptasia*, we measured the ratio of column length to oral disk diameter for ten fully relaxed polyps of the wildtype as well as the ‘Wurst’ phenotype of *Aiptasia*. Symbiont densities as well as total protein content of the host tissue were assessed for five individual animals of each phenotype. Animals were homogenized in 500  $\mu\text{L}$  sterile saline water for 30 s using a Micro DisTec Homogenizer 125 (Kinematica, Switzerland). Algal symbiont cells were separated from host tissue homogenate by centrifugation (3000 g, 5 min). Pelleted cells were resuspended in 200  $\mu\text{L}$  sterile saline water and concentrations were assessed using a hemocytometer. 150  $\mu\text{L}$  of 15-fold diluted host tissue homogenate was used for protein quantification using the Micro BCA Protein Assay kit (Thermo Scientific, USA) according to manufacturer instructions.

### 2.3 Characterization of seawater conditions

We characterized nutrient conditions in the long-term aquaria culture in comparison to freshly collected autoclaved Red Sea seawater to identify potential causes of the altered *Aiptasia* phenotype. For inorganic nutrient analysis (ammonium

( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), silicate ( $\text{SiO}_2$ )), three 50 mL replicate samples were filtered (0.45  $\mu\text{m}$ ), collected in 50 mL centrifuge tubes, and analyzed using the AA3 Continuous Segmented Flow Analyzer (SEAL Analytical, UK). For quantification of dissolved organic carbon (DOC) concentrations, three 20 mL samples were filtered



**Fig. 1** Phenotype alteration in the coral model anemone *Aiptasia* induced by nutrient starvation. Long-term aquaria culture in the absence of nutrient input from feeding or water exchange arrests tentacle growth in polyps. While fully grown *Aiptasia* do not lose their elongated tentacles (a), their asexually produced offspring show partially (b) or completely (c) inhibited tentacle growth. We have termed this new phenotype ‘Wurst’ (German for sausage) *Aiptasia* in reference to their elongated, tentacle-free appearance

(0.22  $\mu\text{m}$ ), collected in acid-washed 30 ml HDPE bottles, acidified with concentrated phosphoric acid ( $\text{pH} < 2$ ), and analyzed using the TOC-L TOC Analyzer (Shimadzu, Japan).

## 2.4 Experimental manipulation of aquaria conditions

To confirm that the observed phenotypic changes were the result of altered nutrient availability, we performed a series of consecutive manipulations of culture conditions in the aquarium. We tested three modifications of the culture conditions outlined above: (1.) weekly feeding and water exchange, (2.) weekly feeding and no water exchange, (3.) no feeding and weekly water exchange. These experimental manipulations were performed consecutively using the same aquarium, animals, and procedures. For each of the modifications, animals were first exposed to the altered culturing conditions until all animals had reverted to the wildtype phenotype. Following the recovery of the wildtype, conditions were reverted to the above-mentioned culture conditions with no feeding or water exchange for at least three months until the ‘Wurst’ phenotype was observed again.

## 3 Results & Discussion

After *Aiptasia* were maintained in the absence of feeding or water exchange, the appearance of the new ‘Wurst’ phenotype was noticed. Curiously, all asexually produced offspring no longer developed the wildtype *Aiptasia* phenotype. Instead, the new *Aiptasia* phenotype exhibited partial (some tentacles  $< 1$  mm) or complete inhibition of tentacle growth (all tentacles  $< 1$  mm; Fig. 1). The loss of tentacles was accompanied by an elongated body shape. Accordingly, ‘Wurst’ *Aiptasia* had a significantly higher mean column length to oral disk diameter ratio of  $5.98 \pm 0.78$  compared to  $1.87 \pm 0.20$  in wildtype *Aiptasia* (mean  $\pm$  SE;  $t_{(18)} = 4.78$ ,  $p < 0.001$ ). The ‘Wurst’ phenotype was detectable within days of polyp development and remained stable over time (at least three months). At the same time, already fully developed *Aiptasia* showed no immediate signs of tentacle-loss or reduction, suggesting that the ‘Wurst’ phenotype was indeed the result of arrested tentacle growth

during development rather than an actual loss of tentacles. Of note, ‘Wurst’ *Aiptasia* remained fully symbiotic with symbiont densities comparable to those of regular *Aiptasia* ( $t_{(8)} = 0.15$ ,  $p = 0.887$ ; Supplementary Table S1). However, ‘Wurst’ *Aiptasia* showed a significant reduction in host protein content per dry weight by more than 60% compared to regular *Aiptasia* ( $t_{(8)} = 2.98$ ,  $p = 0.017$ ; Supplementary Table S1). Apart from this observation, ‘Wurst’ *Aiptasia* showed no obvious symptoms of impaired health or fitness.

Notably, as the initial tank was not intended for experimental comparisons, the observation of this new phenotype is restricted to one mesocosm for now. However, upon further investigation we found a similar report in an aquarist forum describing the observation of the ‘Wurst’ phenotype under identical conditions, i.e. long-term culture without feeding and water exchange (<http://reefcentral.com/forums/showthread.php?t=2559009>, accessed: May 26th, 2018). Hence, we are confident that the observed phenotype is indeed caused by the described long-term culturing conditions.

We hypothesized that the ‘Wurst’ phenotype may be caused by a stress response of the animal to either the over-accumulation or the depletion of critical nutrients during the long-term culture. Indeed, nutrient analysis of seawater from the rearing aquarium revealed a substantial deviation of nutrient concentrations when compared to regular autoclaved Red Sea seawater used for the initial setup of the tank as well as for water exchanges (Table 1). Notably, DOC,  $\text{NO}_3^-$ , and  $\text{PO}_4^{3-}$  concentrations were about 6-, 8-, and 4-fold higher than in regular seawater, respectively. In contrast,  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{SiO}_2$  were depleted up to 80% in the aquarium rearing water compared to regular seawater. The observed accumulation of some nutrients and depletion of other nutrients are likely not only restricted to the macronutrients reported here, but are probably also reflected in patterns of availability of micronutrients or other compounds (e.g., toxins) in the system as well. While the nutrient data do not allow the identification of the specific nutrient(s) causing the ‘Wurst’ phenotype, they strongly support the notion that this phenotype represents a stress response to the depletion or over-accumulation of nutrients in the aquarium. On that note, it is important to mention that the *Aiptasia* host (in contrast to its endosymbiotic

**Table 1** Overview of nutrient availability in regular Red Sea and ‘Wurst’ tank seawater

	DOC $\mu\text{mol L}^{-1}$	$\text{NH}_4^+$ $\mu\text{mol L}^{-1}$	$\text{NO}_2^-$ $\mu\text{mol L}^{-1}$	$\text{NO}_3^-$ $\mu\text{mol L}^{-1}$	$\text{PO}_4^{3-}$ $\mu\text{mol L}^{-1}$	$\text{SiO}_2$ $\mu\text{mol L}^{-1}$
Regular seawater	206.00 $\pm 12.72$	0.17 $\pm 0.00$	0.25 $\pm 0.11$	0.40 $\pm 0.09$	0.02 $\pm 0.00$	0.50 $\pm 0.09$
‘Wurst’ seawater	1298.84 $\pm 13.88$	0.03 $\pm 0.01$	0.10 $\pm 0.04$	3.29 $\pm 1.08$	0.08 $\pm 0.00$	0.12 $\pm 0.01$

DOC = dissolved organic carbon;  $\text{NH}_4^+$  = ammonium;  $\text{NO}_2^-$  = nitrite;  $\text{NO}_3^-$  = nitrate;  $\text{PO}_4^{3-}$  = phosphate;  $\text{SiO}_2$  = silica. Note that the ‘Wurst’ aquarium water showed enriched levels of DOC,  $\text{NO}_3^-$ , and  $\text{PO}_4^{3-}$ , whilst being depleted of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , and  $\text{SiO}_2$  compared to regular seawater.

Symbiodiniaceae) lack the cellular machinery for the assimilation of  $\text{NO}_3^-$  (Lehnert et al. 2014; Rädecker et al. 2015). The strong depletion of  $\text{NH}_4^+$  levels in the aquarium coupled with the lack of heterotrophic nitrogen sources, therefore, likely causes strong nitrogen limitation of the host. In this context, the reduced protein content in the tissue of ‘Wurst’ *Aiptasia* may point to a disruption of protein biosynthesis as a consequence of nitrogen starvation (Oakley et al. 2017). The observation that the phenotype was only present in developing *Aiptasia* but not in fully grown *Aiptasia* further supports the notion of a state of nutrient starvation. Grown animals may be able to fulfill most of their nitrogen demand by efficient recycling of nutrients. In contrast, developing animals rely on environmental nutrient supply in order to support their growth. As such, low environmental nutrient availability is more likely to affect growing rather than fully developed animals.

To confirm that the observed phenotype was the result of nutrient stress, we tested the reversibility of the ‘Wurst’ phenotype. Indeed, feeding and/or water exchange caused rapid tentacle growth in all ‘Wurst’ *Aiptasia* (< 7 days, > 50 animals) resulting in the recovery to the regular phenotype. Notably, the ‘Wurst’ *Aiptasia* phenotype re-emerged approximately three months after any further feeding and water exchanges were suspended again.

It is tempting to interpret the lack of tentacles as an acclimation by the host to the absence of food. Yet, our observations show that either feeding or water exchange can revert this phenotype. Hence, it appears that the ‘Wurst’ *Aiptasia* is the result of severe nutrient limitation caused by the absence of both heterotrophic as well as inorganic nutrient sources. Given that ‘Wurst’ *Aiptasia* remain symbiotic, it is unlikely that carbon limitation alone is the cause of the distinct phenotypic change. Instead, other limiting macro- (e.g., nitrogen) or micronutrients (e.g., copper) may be driving this phenotype.

At this point, it is not possible to disentangle the cellular mechanism underlying the ‘Wurst’ phenotype. Yet, this observation may open new doors to understanding the functioning of the cnidarian - alga symbiosis. The ‘Wurst’ phenotype is illustrating the ecological advantages of the cnidarian – Symbiodiniaceae symbiosis as well as its limitations. On the one hand, the translocation of photosynthates by their algal symbionts likely enables ‘Wurst’ *Aiptasia* to survive even in the absence of heterotrophic food sources. At the same time, the photosynthates provided are ‘junk food’: they are high in carbon yet low in nitrogen content (Wiedenmann and D’Angelo 2018). As such, the cnidarian host relies on external nutrient sources to supplement its diet and avoid nutrient starvation.

Given its distinct characteristics and its controlled inducibility and reversibility, the ‘Wurst’ phenotype provides a promising experimental framework for the study of nutrient cycling in *Aiptasia*. ‘Wurst’ *Aiptasia* may help shed light on the mechanisms underlying phenotypic plasticity in *Aiptasia* as well as environmental controls of nutrient exchange in the Cnidaria – Symbiodiniaceae symbiosis. Future work integrating genetic

and physiological tools will thus not only help to shed light on the cause(s) of the ‘Wurst’ phenotype, but may offer new insights into the biology and functioning of symbiotic cnidarians, in particular reef-building corals, in general.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

- Baumgarten S, Simakov O, Esherick LY, Liew YJ, Lehnert EM, Michell CT, Li Y, Hambleton EA, Guse A, Oates ME, Gough J, Weis VM, Aranda M, Pringle JR, Voolstra CR (2015) The genome of *Aiptasia*, a sea anemone model for coral symbiosis. *Proceeding Natl Acad Sci* 112:11893–11898
- Cziesielski MJ, Liew YJ, Cui G, Schmidt-Roach S, Campana S, Marondedze C, Aranda M (2018) Multi-omics analysis of thermal stress response in a zooxanthellate cnidarian reveals the importance of associating with thermotolerant symbionts. *Proc R Soc B Biol Sci* 285:20172654
- Hughes TP, Barnes ML, Bellwood DR, Cinner JE, Cumming GS, Jackson JBC, Kleypas J, van de Leemput IA, Lough JM, Morrison TH, Palumbi SR, van Nes EH, Scheffer M (2017) Coral reefs in the Anthropocene. *Nature* 546:82–90
- LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr Biol* 28:2570–2580
- Lehnert EM, Mouchka ME, Burriesci MS et al (2014) Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. *G3 Genes Genomes Genet* 4:277–295
- Muscantine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 27:454–460
- Oakley CA, Durand E, Wilkinson SP, Peng L, Weis VM, Grossman AR, Davy SK (2017) Thermal shock induces host proteostasis disruption and endoplasmic reticulum stress in the model symbiotic cnidarian *Aiptasia*. *J Proteome Res* 16:2121–2134
- Rädecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends Microbiol* 23:490–497
- Rädecker N, Raina J-B, Pernice M, Perna G, Guagliardo P, Kilburn MR, Aranda M, Voolstra CR (2018) Using *Aiptasia* as a model to study metabolic interactions in cnidarian-*Symbiodinium* symbioses. *Front Physiol* 9:214
- Reaka-Kudla ML (1997) The global biodiversity of coral reefs: a comparison with rain forests. In: Reaka-Kudler ML, Wilson DE, Wilson EO (eds) *Biodiversity II: understanding and protecting our biological resources*. Joseph Henry Press, Washington, DC, pp 83–108
- van Oppen MJH, Oliver JK, Putnam HM, Gates RD (2015) Building coral reef resilience through assisted evolution. *Proc Natl Acad Sci* 112:2307–2313
- Voolstra CR (2013) A journey into the wild of the cnidarian model system *Aiptasia* and its symbionts. *Mol Ecol* 22:4366–4368
- Wiedenmann J, D’Angelo C (2018) Symbiosis: high-carb diet of reef corals as seen from space. *Curr Biol* 28:1263–1265