Supplementary Data Sheet 1

Symbiont chloroplasts remain active during bleaching-like response induced by thermal stress in *Collozoum pelagicum* (Collodaria, Retaria)

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1 Supplementary Text

1.1 Supplementary Text 1: Detailed methods used for the phylogenetic reconstruction

Quality filtered RNA reads, obtained from Illumina HiSeq2500 sequencing of 30 Collodaria specimens, were trimmed using SortMeRna v 2.1 to retrieve ribosomal sequences. The reads were appaired using trimmomatic 0.36 and assembled using Trinity 2.4.0 with the following parameters: SLIDINGWINDOW:4:5 LEADING:5 TRAILING:5 MINLEN:25. Contigs were blasted against the 16S-18S SILVA database version 132 (Quast et al., 2013), and the best blast hits with Collodaria and Dinophyceae sequences were aligned using MAFFT (Katoh et al., 2005) to build a consensus sequence for each of the 30 samples. Out of the 30 sequenced specimens, we were able to identify 28 sequences of 18S rRNA related to the host and 26 related to the symbiont. As sequences were 99% identical, the longer consensus for the host and the symbiont were deposited in the repository databases under the accession: MG907123 (host) and MG905637 (symbiont).

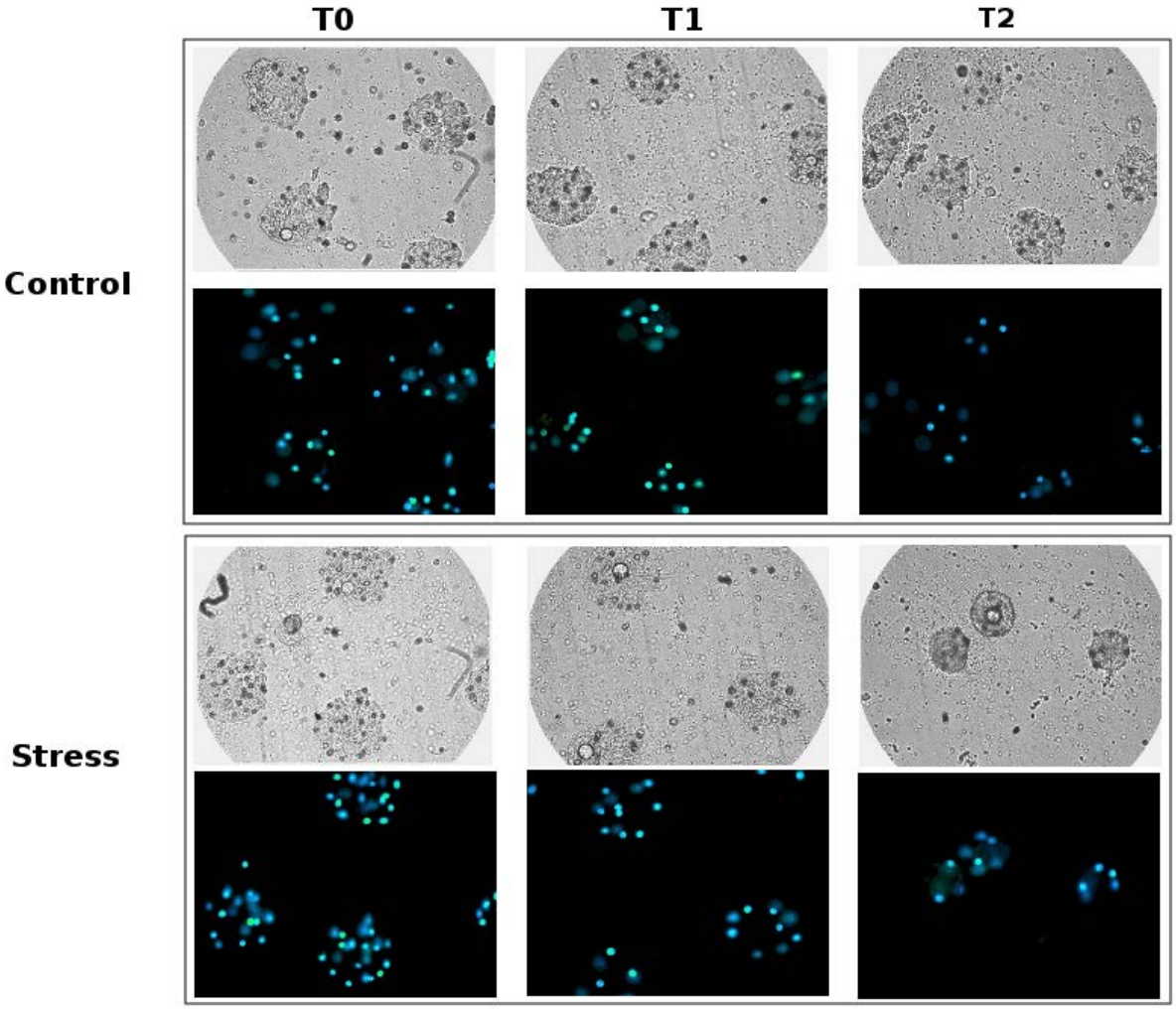
For 18S rRNA tree reconstruction, we retrieved sequences assigned to Collodaria from the PR^2 ribosomal gene database (Guillou et al., 2012). Sequences (>1,000 bp) from described specimens representing the taxonomic diversity of the three current families of collodarians were kept (Ishitani et al., 2012; Biard et al., 2015). For Collodaria phylogenetic identification, 14 Nasselarian sequences were used as outgroup and 2 Apicomplexa sequences were used as outgroup for the Dinophyceae phylogeny. Sequences were aligned using MAFFT (Katoh et al., 2005). We considered the unaligned positions at the extremities of the contig sequence as missing data. Positions having ≥ 5% gaps for Collodaria and ≥ 25% gaps for Dinophyceae were removed from the alignment using Geneious (Kearse et al., 2012). The alignments comprising 736 nucleotide positions for Collodaria and 961 nucleotide positions for Dinophyceae were analyzed using Maximum Likelihood (ML) methods (under the GTR+Γ model and 4 rate categories) in RaxML (Stamatakis., 2014). Node support was computed with 1000 bootstrap replicates.

1.2 Supplementary Text 2: Preliminary experiment of thermal stress on *Brandtodinium nutricula* cultures

Cultures of *Brandtodinium nutricula* strain RCC3468, from the Roscoff Culture Collection (http://roscoff-culture-collection.org/), were acclimated to 20°C during 2 weeks under continuous white light irradiance of 100 µmol photons m^{-2} s^{-1}. Cultures were grown in *f*/2 medium (Ref G9903, Sigma, Guillard and Ryther 1962) in 50 mL vented flasks (Starlab) in thermostatic chambers (Lovibond Water testing). Everyday, the temperature in the thermostatic chamber was then increased by 1.5°C, and the following analyses were performed.
Culture aliquots were analyzed using a FACSCanto II flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with 488 and 633 nm lasers and standard filter setup. To allow comparison between samples, 3 µm fluorescent beads (Polysciences, Warrington, PA, USA) were added to each sample as internal reference. Samples were run for 1 min at a flow rate of 100 µL min^{-1}. Measurements were computed with the Cytowin software (Vaulot, 1989) to measure the cellular density and the chlorophyll red fluorescence signal .

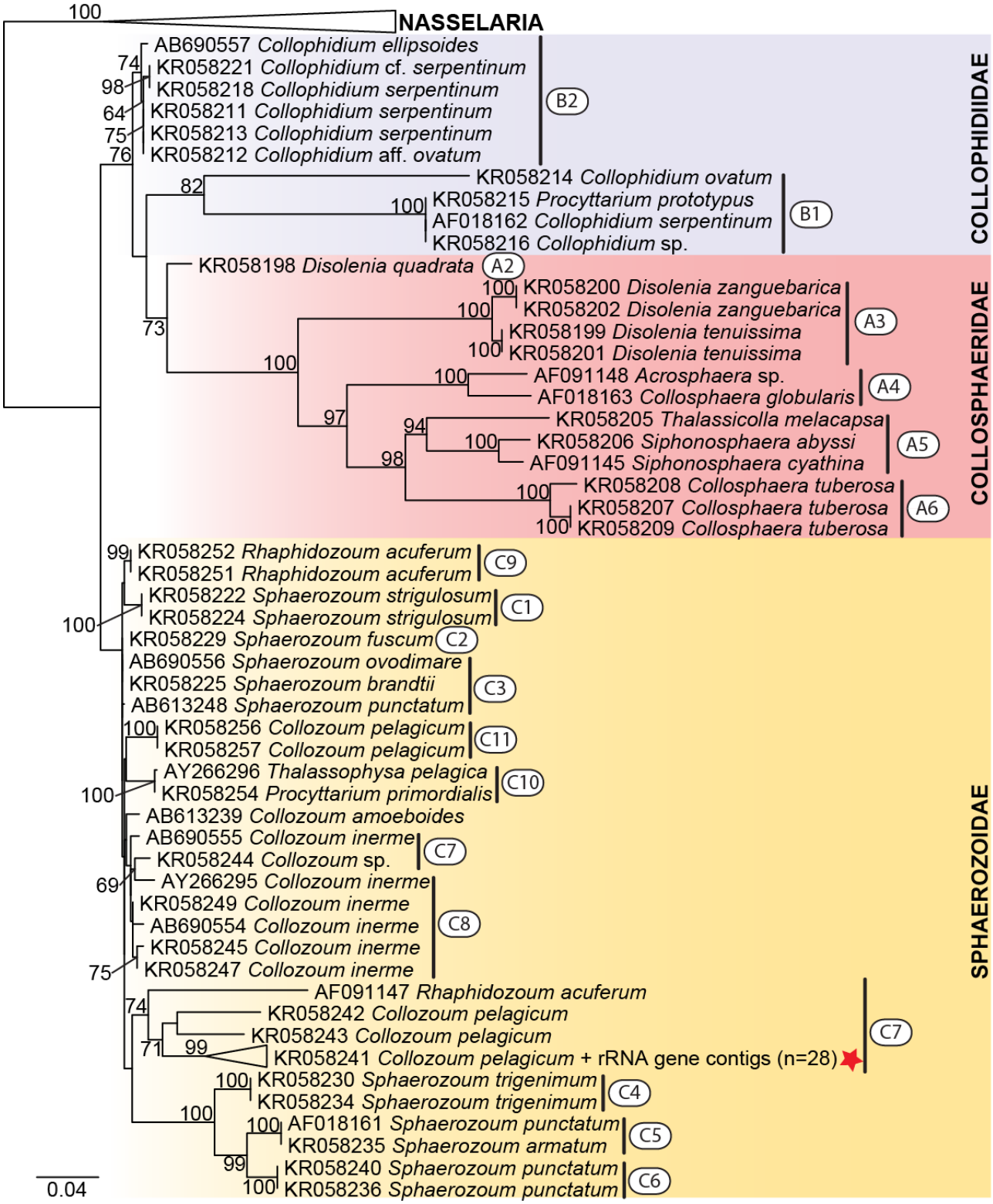
The quantum yield of photosystem II of *Brandtodinium* cells was determined using a pulse amplitude modulated fluorometer (PHYTO-PAM I, Walz, Germany) by daily sampling of 2 mL of cultures. After 5 min relaxation in darkness, the non-actinic modulated light (650 nm) was turned on in order to measure the fluorescence basal level, F_0. A saturating red light pulse (655 nm, 2000 μmol photons m^{-2} s^{-1}, 400 ms) was applied to determine the maximum fluorescence level in the dark-adapted sample, F_M. The maximal photosystem II fluorescence quantum yield of photochemical energy conversion, F_V/F_M, was calculated using the following formula: $F_V/F_M = (F_M-F_0)/F_M$

2 Supplementary Figures



Supplementary Figure 1: Microscope fields analyzed with Pulse Amplitude Modulated fluorometer

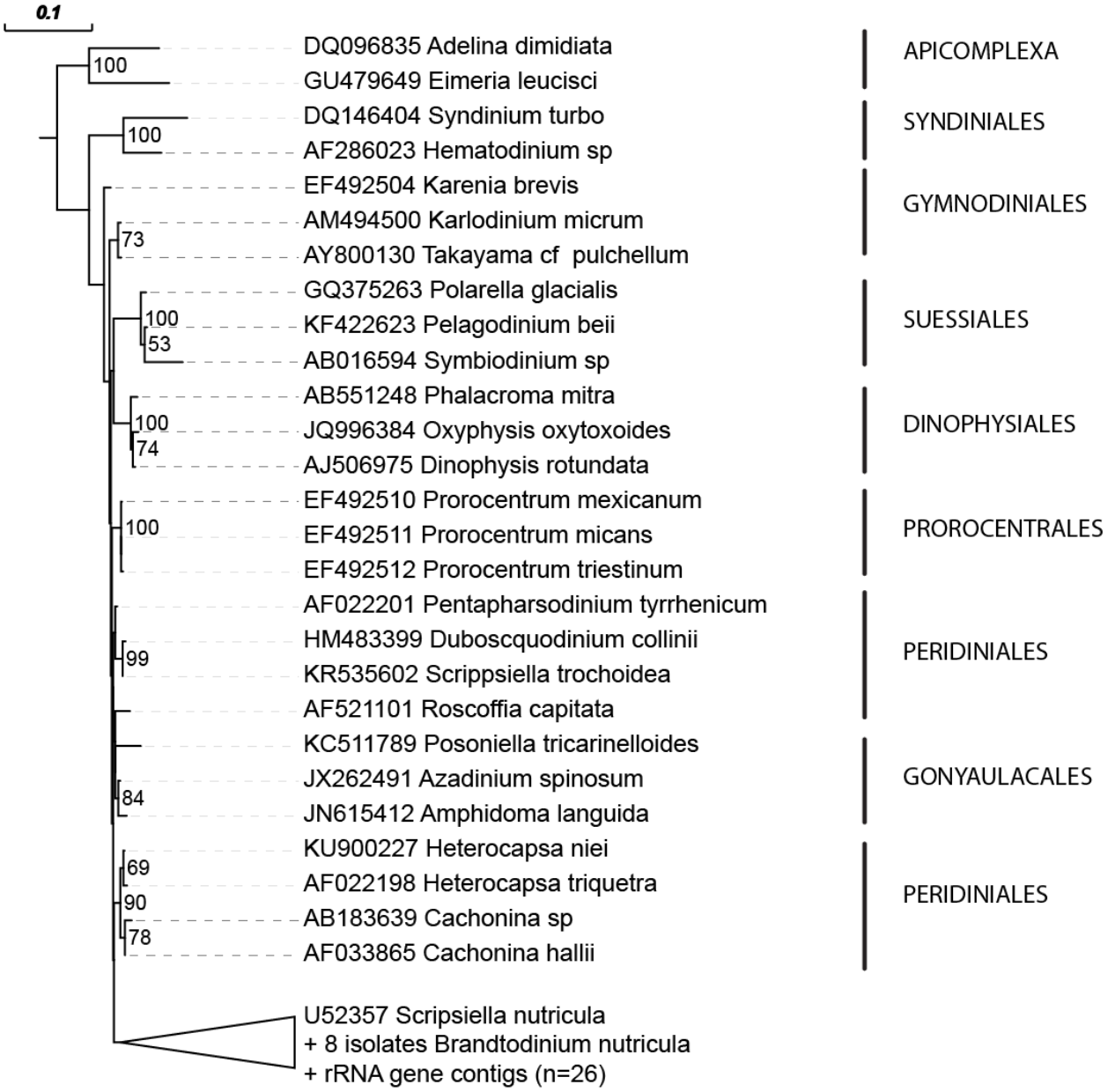
Stressed and control *Collozoum pelagicum* colonies were compared at 3 sampling times. While control colonies remained at 21°C during the experiment, stressed colonies were at 21°C at T0, 25°C at T1 and 28°C at T2. For each condition, the white light image is represented at the top, and the image captured by the PAM-microscope is at the bottom. Blue dots represent pixels with positive quantum yield of photosystem II.



Supplementary Figure 2: Phylogenetic tree allowing the Collodarian specimen identification

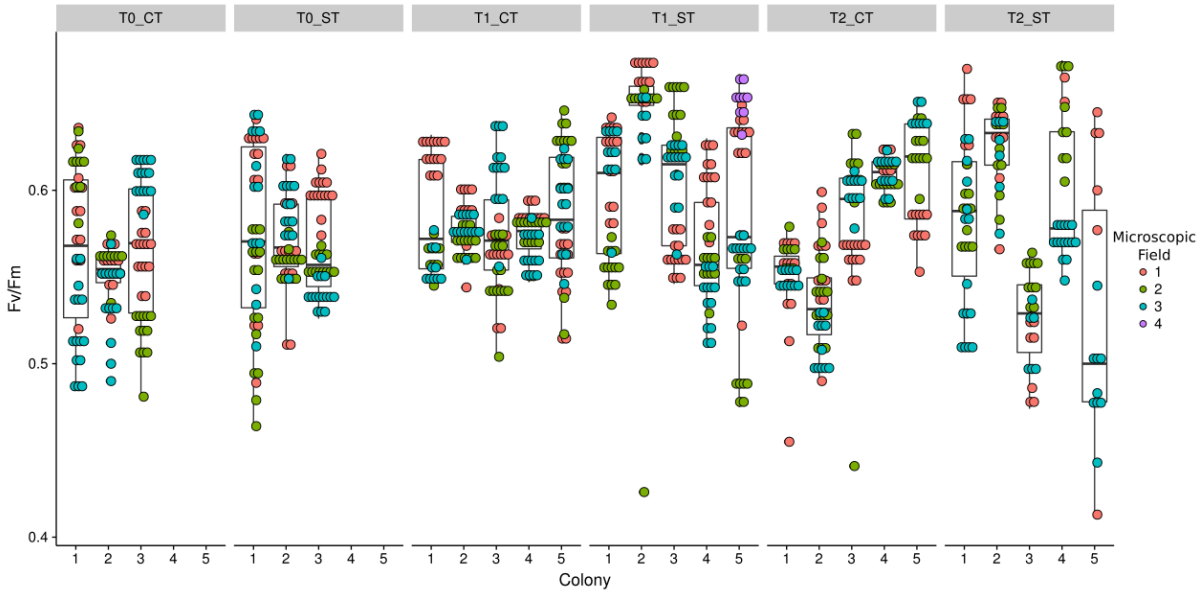
The molecular phylogeny of Collodaria was inferred from the 18S rRNA gene, with 95 sequences and 736 aligned nucleotidic positions. The tree was obtained by a Maximum Likelihood reconstruction method implemented using the GTR + Γ model of sequence evolution. RAxML bootstrap values (1,000 replicates) are shown at nodes when supports are higher than 50%. The red star highlights the phylogenetic position of the consensus sequences extracted from the transcriptome data. The clade and sub-clade naming and coloring follow the nomenclature system as described in Biard and colleagues (2015). The tree has been rooted using nasselarian sequences

for display purpose only. Note that the *Rhaphidozoum acuferum* sequence (accession number #AF091147) has potentially been mislabeled in the original publication because the presence of spicules has not been fully confirmed (Amaral Zettler *et al.*, 1999).



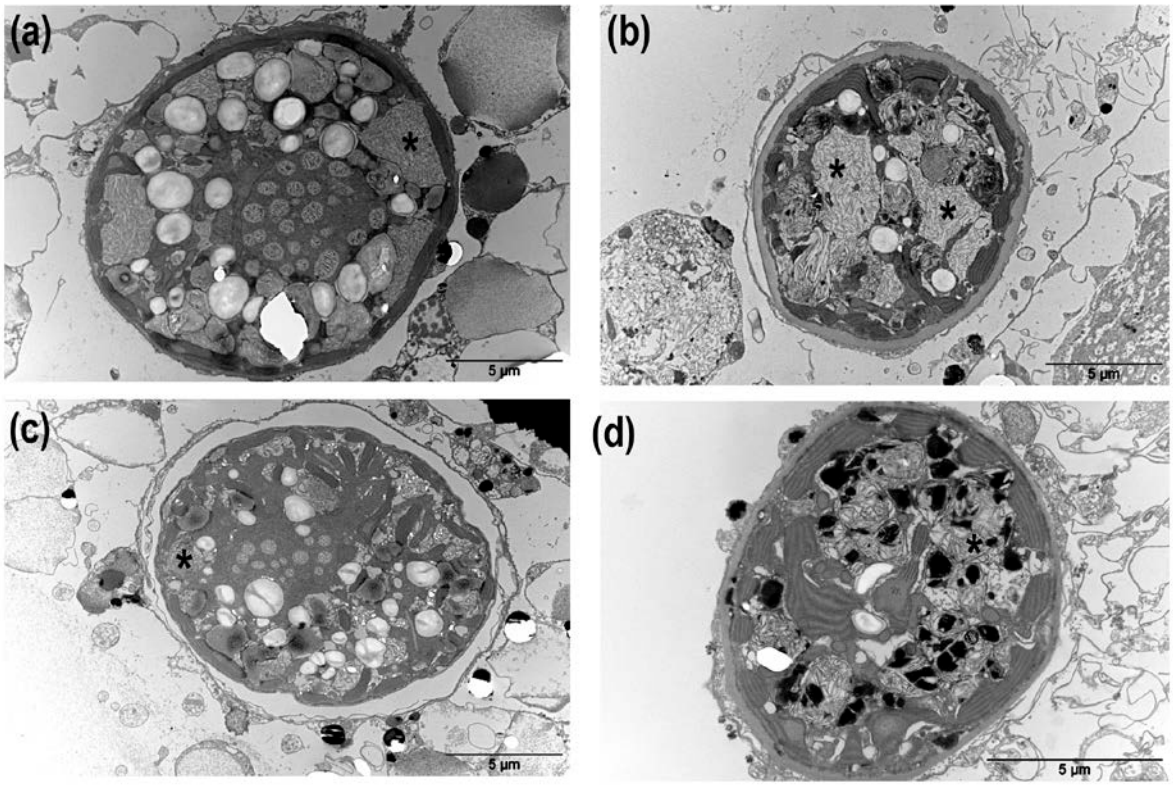
Supplementary Figure 3: Phylogenetic tree allowing the Dinophyceae specimen identification

The molecular phylogeny of Dinophyceae was inferred from the 18S rRNA genes, with 62 sequences and 961 aligned nucleotidic positions. The tree was obtained by Maximum Likelihood reconstruction method implemented using the GTR + Γ model of sequence evolution. RaxML bootstrap values (1,000 replicates) are shown at nodes when supports are higher than 50%. The tree has been rooted using Apicomplexa sequences for display purpose only. The contigs extracted from the transcriptome data were all clustered with *Brandtodinium nutricula* sequences (accession numbers: U52357, KF557491, KF557497, KF557498, KF557502, KF557522, KF557523, KF557524, KF557525).



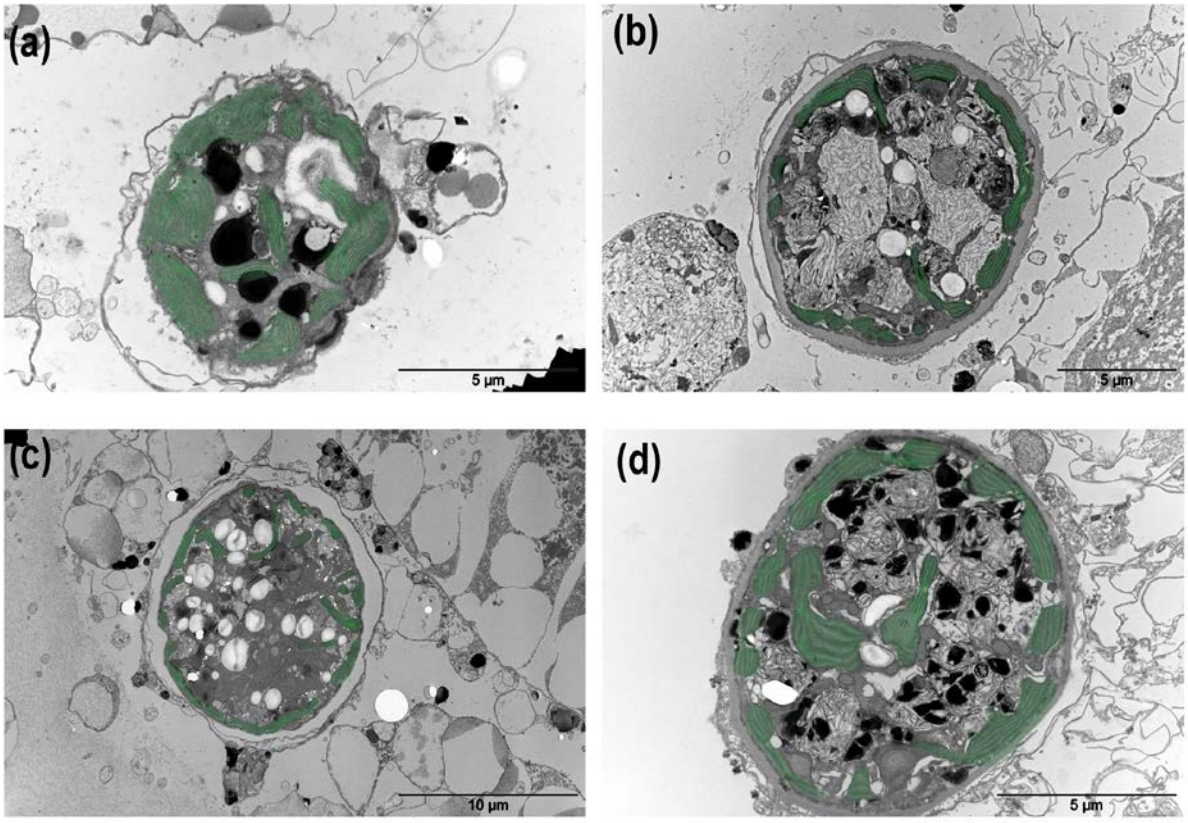
Supplementary Figure 4: PAM fluorometry data variability

Stressed and control *Collozoum pelagicum* colonies were compared at 3 sampling times. While control colonies remained at 21°C during the experiment, stressed colonies were at 21°C at T0, 25°C at T1 and 28°C at T2. Plot of F_V/F_M values measured for each symbiont in 3 microscopic fields (4 microscopic fields for T1_ST_5) from the 5 replicates of each studied condition.



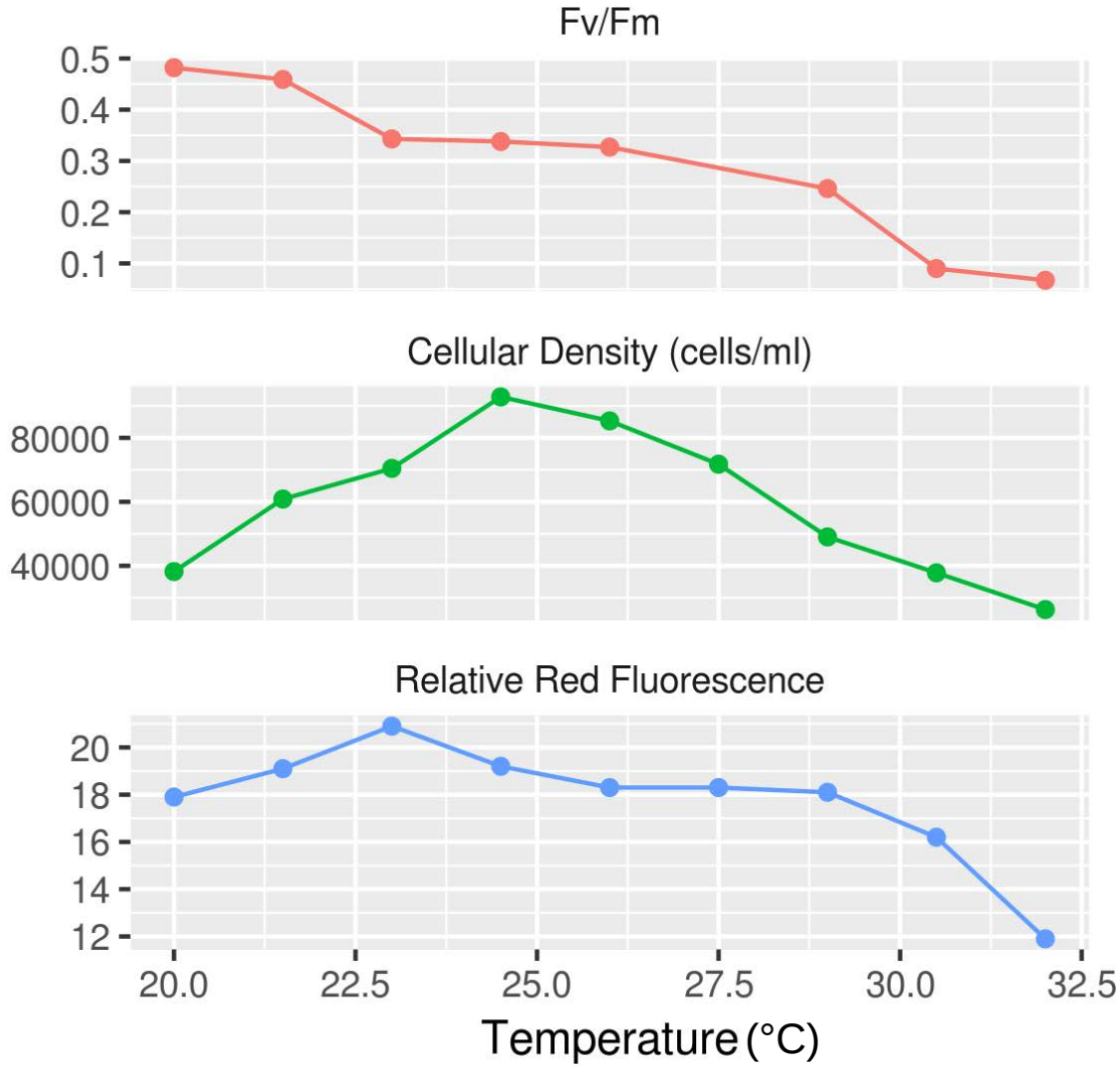
Supplementary Figure 5: Evidencing viral infections by transmission electron microscopy

Stressed and control *Collozoum pelagicum* colonies were compared at 3 sampling times. While control colonies remained at 21°C during the experiment, stressed colonies were at 21°C at T0, 25°C at T1 and 28°C at T2. Virus-like particles were observed in thin sections of *in hospite Brandtodinium* cells on transmission electron micrographs. The particles are indicated by an asterisk and observed as long, thin filaments in control T1 (**a**), control T2 (**b**) at 21°C, and thermal stress T1 at 25°C (**c**) and thermal stress T2 at 28°C (**d**).



Supplementary Figure 6: Chloroplast structure is preserved in decaying symbiotic cells

Stressed and control *Collozoum pelagicum* colonies were compared at 3 sampling times. While control colonies remained at 21°C during the experiment, stressed colonies were at 21°C at T0, 25°C at T1 and 28°C at T2. Presence and distribution of chloroplasts (highlighted in green) in *in hospite Brandtodinium* cells were observed by transmission electron micrographs during the experiment, in both control and stress conditions: in control T1 (**a**), control T2 (**b**) at 21°C, and thermal stress T1 at 25°C (**c**) and thermal stress T2 at 28°C (**d**).



Supplementary Figure 7: Physiology of cultured *Brandtodinium* exposed to thermal stress

The plots show the variations of the maximal photosystem II fluorescence quantum yield (F_V/F_M), the culture cell density and the relative red fluorescence of three replicate cultures subjected to a daily increase of temperature, from 20°C to 31°C. See Supplementary Material Text 1 for methods.

3 References

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