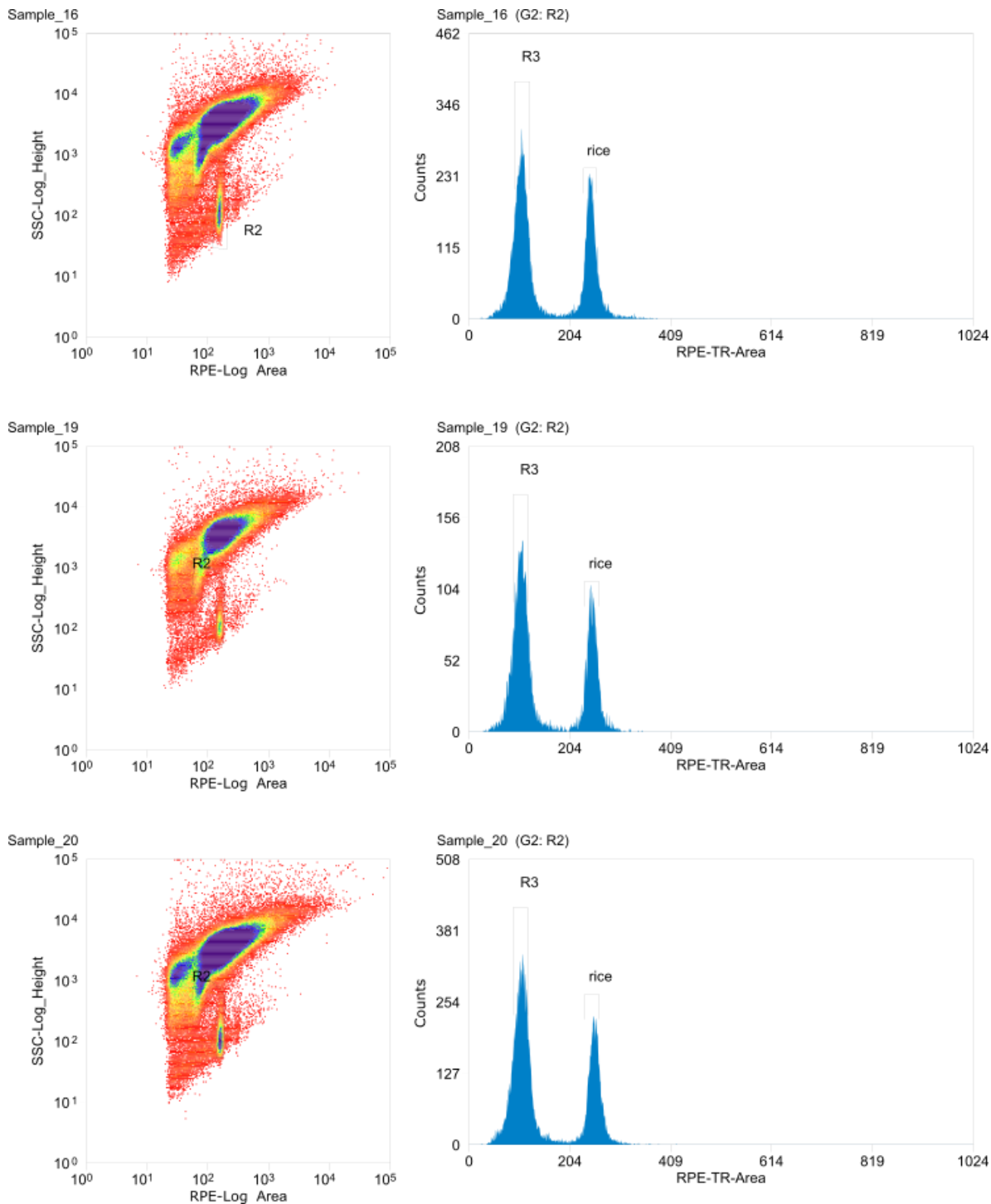
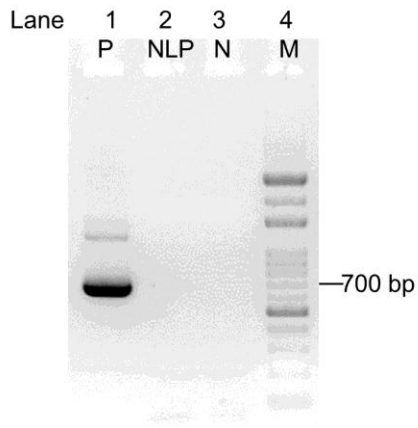


## Supplementary information



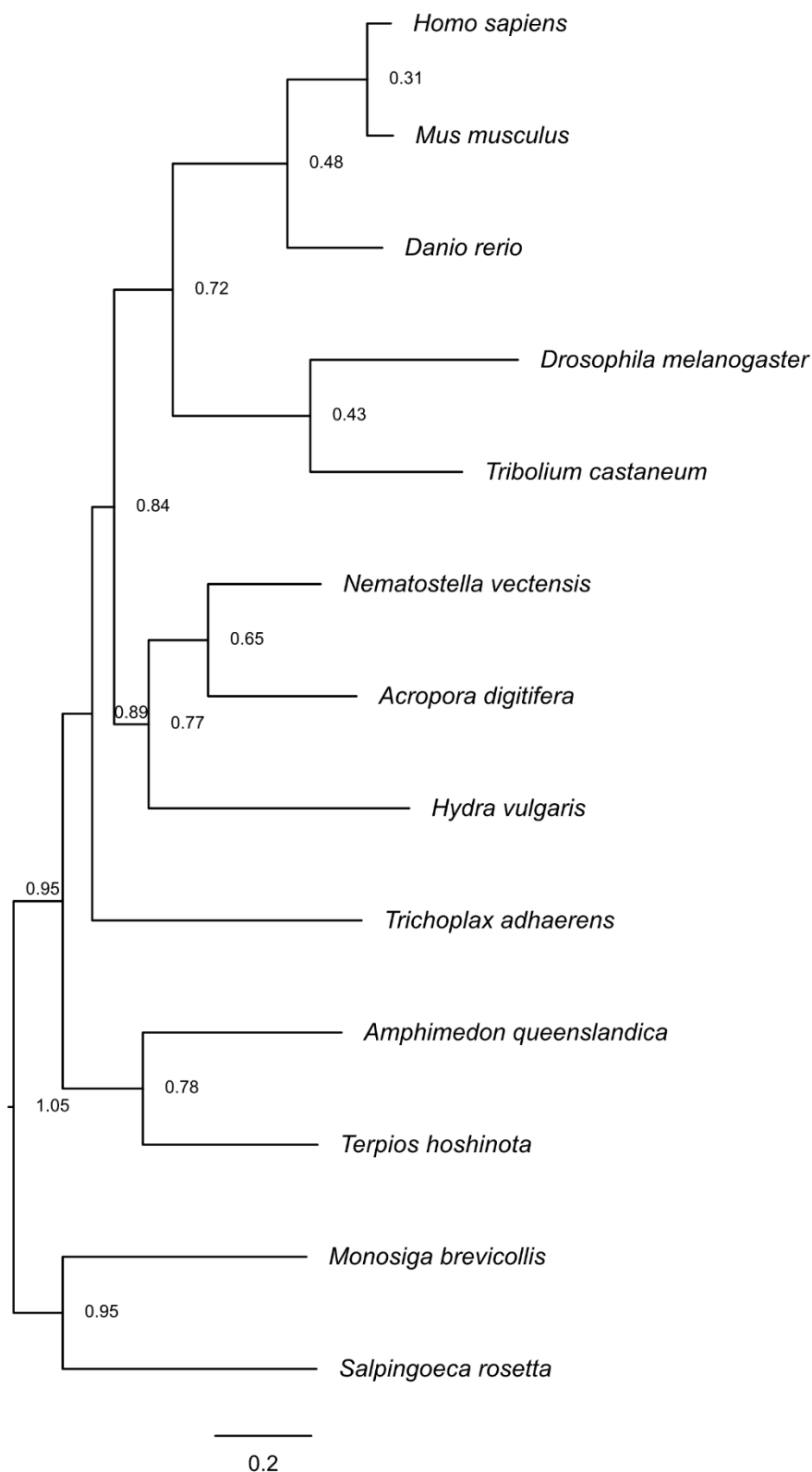
### Supplementary Figure S1. Genome size of *Terpios hoshinota*, estimated by flow cytometry.

Using rice (*Oryza sativa*) as a reference standard, the genome size of *T. hoshinota* was analyzed with a 488-nm laser under conditions of cell counts exceeding 100,000. An analysis of nucleic acid content, based on three independent measurements, determined the genome size to be  $186.48 \pm 4.30$  Mbp. Region R2 was gated for analysis, with R3 representing the cumulative fluorescence signal of *T. hoshinota*.



1X TAE buffer, 1.5% agarose gel.  
100V run 50 min. Stain with HealthView.

**Supplementary Figure S2. PCR analysis of cyanobacterial contamination in the nucleus-like particle (NLP) working solution.** Gel electrophoresis results indicate low levels of cyanobacterial contamination in the NLP solution, as detected using the cyanobacteria-specific 16S rRNA gene primer pair, CYA106F and CYA781R. M: DNA marker (100 bp ladder); P: Positive control (*Escherichia coli* DNA); NLP: Nucleus-like particle working solution; N: Negative control.



**Supplementary Figure S3. Species tree of various metazoans, inferred by OrthoFinder using 196 orthogroups.** This phylogeny was reconstructed based on concatenated alignments of conserved orthologs, representing major metazoan lineages. Node support values were derived using OrthoFinder's default STAG algorithm. This tree provides an evolutionary framework for comparative genomic analyses among metazoan taxa.

Table S1 Top 10 GO slims of *molecular function* (MF), *biological process* (BP), and *cellular component* (CC) of *Terpios hoshinota* annotated genes

| GO ontology | GO Term                                                     | Count |
|-------------|-------------------------------------------------------------|-------|
| MF          | GO:0003824 catalytic activity                               | 1105  |
| MF          | GO:0016740 transferase activity                             | 413   |
| MF          | GO:0005488 binding                                          | 397   |
| MF          | GO:0016787 hydrolase activity                               | 295   |
| MF          | GO:0016491 oxidoreductase activity                          | 197   |
| MF          | GO:0005215 transporter activity                             | 165   |
| MF          | GO:0005515 protein binding                                  | 161   |
| MF          | GO:0003676 nucleic acid binding                             | 95    |
| MF          | GO:0016829 lyase activity                                   | 67    |
| MF          | GO:0016874 ligase activity                                  | 66    |
| BP          | GO:0008152 metabolic process                                | 1233  |
| BP          | GO:0044238 primary metabolic process                        | 1011  |
| BP          | GO:0043170 macromolecule metabolic process                  | 691   |
| BP          | GO:0065007 biological regulation                            | 663   |
| BP          | GO:0050789 regulation of biological process                 | 581   |
| BP          | GO:0006139 nucleobase-containing compound metabolic process | 495   |
| BP          | GO:0016043 cellular component organization                  | 429   |
| BP          | GO:0051179 localization                                     | 379   |
| BP          | GO:0050896 response to stimulus                             | 312   |
| BP          | GO:0051234 establishment of localization                    | 310   |
| CC          | GO:0110165 cellular anatomical entity                       | 727   |
| CC          | GO:0032991 protein-containing complex                       | 527   |
| CC          | GO:0043226 organelle                                        | 512   |
| CC          | GO:0043229 intracellular organelle                          | 483   |
| CC          | GO:0043231 intracellular membrane-bounded organelle         | 371   |
| CC          | GO:0005737 cytoplasm                                        | 274   |
| CC          | GO:0005634 nucleus                                          | 199   |
| CC          | GO:0016020 membrane                                         | 184   |
| CC          | GO:0043232 intracellular non-membrane-bounded organelle     | 168   |
| CC          | GO:0005856 cytoskeleton                                     | 67    |

Table S2. Silicatein protein subtypes identified in *T. hoshinota*.

| Gene ID   | Classification | KEGG Orthology | Prot                             | Pfam                                         | Signature description | E-value   | Subject description                                     |
|-----------|----------------|----------------|----------------------------------|----------------------------------------------|-----------------------|-----------|---------------------------------------------------------|
| THG008206 | silicatein     | K01365         | CTSL; cathepsin L [EC:3.4.22.15] | Peptidase_C1_Inhibitor_I29<br>Peptidase_C1_2 | Cysteine proteinases  | 5.63E-101 | AAF21819.1 silicatein beta [Tethya aurantium]           |
| THG008208 | silicatein     | K01365         | CTSL; cathepsin L [EC:3.4.22.15] | Peptidase_C1_Inhibitor_I29<br>Peptidase_C1_2 | Cysteine protease     | 1.80E-111 | ABC94586.1 silicatein alpha [Hymeniacidon perlevis]     |
| THG008209 | silicatein     | K01365         | CTSL; cathepsin L [EC:3.4.22.15] | Peptidase_C1_Inhibitor_I29<br>Peptidase_C1_2 | Cathepsin S           | 7.30E-98  | CBY80149.1 silicatein yellow variant [Tethya aurantium] |
| THG015260 | silicatein     | blank          | NA_value                         | Peptidase_C1_Inhibitor_I29<br>Peptidase_C1_2 | Cathepsin S           | 1.65E-113 | CAD67990.1 silicatein beta [Suberites domuncula]        |

Table S3. Distribution of various protein domains and families associated with selected aspects of eukaryotic cell physiology in *T. hoshinota* and representative animal genomes.

| Process/Domain                 | Description                                              | InterPro ID | Th  | Aq  | Am  | Sp  | Dm  | Hs   |
|--------------------------------|----------------------------------------------------------|-------------|-----|-----|-----|-----|-----|------|
| <b>Development</b>             |                                                          |             |     |     |     |     |     |      |
| Wnt                            | Wnt                                                      | IPR005817   | 4   | 5   | 56  | 17  | 11  | 65   |
| HD                             | Homeodomain                                              | IPR001356   | 57  | 42  | 242 | 178 | 260 | 953  |
| <b>Cell Adhesion Molecules</b> |                                                          |             |     |     |     |     |     |      |
| Ig_I-set                       | Immunoglobulin I-set                                     | IPR013098   | 138 | 109 | 320 | 182 | 329 | 1467 |
| Integrin_alpha                 | Integrin alpha chain                                     | IPR000413   | 4   | 10  | 6   | 12  | 14  | 115  |
| Integrin_bsu                   | Integrin beta subunit                                    | IPR015812   | 14  | 14  | 2   | 6   | 6   | 76   |
| Cadherin-like_dom              | Cadherin-like                                            | IPR002126   | 44  | 29  | 67  | 40  | 49  | 422  |
| Cadherin                       | Cadherin                                                 | IPR039808   | 18  | 14  | 31  | 18  | 22  | 167  |
| Selectin_CTLD                  | Selectin, C-type lectin-like domain                      | IPR033991   | 0   | 0   | 0   | 0   | 0   | 9    |
| Selectin_superfamily           | Selectin superfamily                                     | IPR002396   | 0   | 0   | 0   | 3   | 0   | 9    |
| <b>Innate immunity</b>         |                                                          |             |     |     |     |     |     |      |
| TIR_dom                        | Toll/interleukin-1 receptor homology (TIR) domain        | IPR000157   | 49  | 5   | 111 | 271 | 28  | 169  |
| NLRP_innate_immun_reg          | NLRP family, innate immunity and inflammation regulators | IPR050637   | 127 | 10  | 70  | 0   | 0   | 140  |
| SRCR                           | SRCR domain                                              | IPR001190   | 356 | 375 | 95  | 639 | 10  | 132  |

Th: *Terpios hoshinota*, Aq: *Amphimedon queenslandica*, Am: *Acropora millepora*, Sp: *Strongylocentrotus purpuratus*, Dm: *Drosophila melanogaster*, Hs: *Homo sapiens*

Table S4. Distribution of various subclasses of protein domains associated with selected aspects of eukaryotic cell physiology in *T. hoshinota* and representative animal genomes, based on KEGG Orthology.

| Process                        | Description                | Th | Hp | Aq | Da | Cc | Ol | Sc | Am | Sp | Dm | Hs  |
|--------------------------------|----------------------------|----|----|----|----|----|----|----|----|----|----|-----|
| <b>Development</b>             |                            |    |    |    |    |    |    |    |    |    |    |     |
| Wnt                            | WNT4                       | 2  | 1  | 1  | 3  | 3  | 3  | 12 | 4  | 2  | 0  | 1   |
|                                | WNT6                       | 3  | 0  | 0  | 2  | 0  | 0  | 0  | 1  | 1  | 1  | 1   |
|                                | WNT7                       | 1  | 1  | 3  | 1  | 0  | 6  | 4  | 3  | 1  | 0  | 2   |
|                                | WNT11                      | 1  | 1  | 1  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 1   |
| Homeodomain                    | ANTP: HOXL                 | 0  | 0  | 0  | 0  | 0  | 0  | 1  | 14 | 16 | 17 | 52  |
|                                | ANTP: NKL                  | 6  | 5  | 5  | 11 | 7  | 7  | 9  | 32 | 24 | 18 | 48  |
|                                | Paired-related             | 6  | 6  | 5  | 6  | 15 | 5  | 6  | 29 | 30 | 15 | 61  |
|                                | LIM                        | 4  | 5  | 4  | 6  | 12 | 9  | 9  | 7  | 8  | 8  | 15  |
|                                | POU                        | 3  | 4  | 9  | 3  | 2  | 2  | 12 | 5  | 6  | 4  | 19  |
|                                | SINE                       | 2  | 2  | 2  | 1  | 2  | 2  | 3  | 3  | 3  | 3  | 6   |
|                                | ZF                         | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 2  | 2  | 12  |
|                                | CUT                        | 2  | 1  | 1  | 1  | 3  | 2  | 4  | 2  | 4  | 2  | 7   |
|                                | PROS                       | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 2  | 1  | 2   |
|                                | TALE                       | 3  | 3  | 0  | 1  | 6  | 7  | 15 | 4  | 7  | 8  | 20  |
| <b>Cell Adhesion Molecules</b> |                            |    |    |    |    |    |    |    |    |    |    |     |
| Immunoglobulin                 | Immunoglobulin superfamily | 10 | 19 | 9  | 56 | 64 | 84 | 59 | 31 | 22 | 6  | 55  |
| Integrins                      | Integrins                  | 12 | 7  | 5  | 4  | 21 | 13 | 26 | 5  | 11 | 1  | 26  |
| Cadherins                      | Cadherins                  | 39 | 36 | 15 | 53 | 63 | 71 | 53 | 10 | 12 | 9  | 113 |
| Selectins                      | Selectins                  | 26 | 27 | 0  | 30 | 5  | 6  | 60 | 0  | 4  | 0  | 3   |

Sponge species—Th: *Terpios hoshinota*, Hp: *Halichondria panicea*, Aq: *Amphimedon queenslandica*, Da: *Dysidea avara*, Cc: *Corticium candelabrum*, Ol: *Oscarella lobularis*, Sc: *Sycon ciliatum*. Other species—Am: *Acropora millepora*, Sp: *Strongylocentrotus purpuratus*, Dm: *Drosophila melanogaster*, Hs: *Homo sapiens*

Table S5. Comparison of motility-related genes (GO terms) among poriferan reference genomes.

| GO ID      | GO.term.name                          | Th  | Hp  | Aq | Dy  | Cc | Ol | Sc  |
|------------|---------------------------------------|-----|-----|----|-----|----|----|-----|
| GO:0016477 | cell migration                        | 155 | 151 | 83 | 120 | 74 | 65 | 107 |
| GO:0048870 | cell motility                         | 8   | 8   | 7  | 11  | 18 | 14 | 19  |
| GO:0006935 | chemotaxis                            | -   | -   | 1  | 8   | -  | 1  | -   |
| GO:0001764 | neuron migration                      | 3   | 5   | 6  | 28  | 16 | 19 | 11  |
| GO:0043542 | endothelial cell migration            | 1   | 1   | 1  | 3   | 6  | 7  | 2   |
| GO:0030335 | positive regulation of cell migration | 5   | 11  | 5  | 6   | 8  | 9  | 1   |
| GO:0030336 | negative regulation of cell migration | 3   | 3   | 7  | 5   | 3  | 5  | 5   |
| GO:0040011 | locomotion                            | 2   | 2   | 1  | 1   | 1  | 1  | 1   |

Th: *Terpios\_hoshinota*, Hp: *Halichondria\_panicea*, Aq: *Amphimedon queenslandica*, Da: *Dysidea\_avara*, Cc: *Corticium candelabrum*, Ol: *Oscarella\_lobularis*, Sc: *Sycon\_ciliatum*.



Table S6. Biosynthesis potential of essential nutrients (amino acids and vitamin B12) compared among Porifera reference and *Ca. P. terpiosi* LD50 (*T. hoshinota*'s symbiotic bacteria) genomes.

| Amino acid                       | Th | Hp | Aq | Dy | Cc | Ol | Sc | LD05 |
|----------------------------------|----|----|----|----|----|----|----|------|
| Ala                              | +  | +  | +  | +  | +  | +  | +  | -    |
| Arg                              | -  | -  | -  | -  | +  | +  | +  | +    |
| Asn                              | -  | -  | -  | +  | +  | +  | +  | +    |
| Asp                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Cys                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Glu                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Gln                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Gly                              | +  | +  | +  | +  | +  | +  | +  | +    |
| His                              | -  | -  | -  | -  | +  | +  | +  | +    |
| Ile                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Leu                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Lys                              | -  | -  | -  | -  | -  | -  | -  | +    |
| Met                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Phe                              | -  | -  | -  | -  | -  | -  | -  | -    |
| Pro                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Ser                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Thr                              | +  | +  | +  | +  | -  | -  | +  | +    |
| Trp                              | -  | -  | -  | +  | -  | -  | -  | +    |
| Tyr                              | +  | +  | +  | +  | +  | +  | +  | -    |
| Val                              | +  | +  | +  | +  | +  | +  | +  | +    |
| Anaerobic Cobalamin biosynthesis | Th | Hp | Aq | Dy | Cc | Ol | Sc | LD05 |
| cysG                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cobA                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cobA-hemD                        | -  | -  | -  | -  | -  | -  | -  | +    |
| MET8                             | -  | -  | -  | -  | -  | -  | -  | -    |
| sirC                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cbiK                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cbiX                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cfbA                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cobI-cbiL                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cobJ                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cbiGH-cobJ                       | -  | -  | -  | -  | -  | -  | -  | +    |
| cbiH60                           | -  | -  | -  | -  | -  | -  | -  | -    |
| cobM                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cbiG                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cbiGH-cobJ                       | -  | -  | -  | -  | -  | -  | -  | +    |
| cbiD                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cobK-cbiJ                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cbiT                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cbiE                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cobL-cbiET                       | -  | -  | -  | -  | -  | -  | -  | +    |
| cobH-cbiC                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cobB-cbiA                        | -  | -  | -  | -  | -  | -  | -  | +    |
| Aerobic Cobalamin biosynthesis   | Th | Hp | Aq | Dy | Cc | Ol | Sc | LD05 |
| cobA                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cobA-hemD                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cobI-cbiL                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cobI                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cobG                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cobJ                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cobI                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cbiGH-cobJ                       | -  | -  | -  | -  | -  | -  | -  | +    |
| cobM                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cobF                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cobK-cbiJ                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cobL-cbiET                       | -  | -  | -  | -  | -  | -  | -  | +    |
| cobH-cbiC                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cobB-cbiA                        | -  | -  | -  | -  | -  | -  | -  | +    |
| cobN                             | -  | -  | -  | -  | -  | -  | -  | +    |
| cobS                             | -  | -  | -  | -  | -  | -  | -  | -    |
| cobT                             | -  | -  | -  | -  | -  | -  | -  | -    |

Th: *Terpios\_hoshinota*, Hp: *Halichondria\_panicea*, Aq: *Amphimedon queenslandica*, Da: *Dysidea\_avara*, Cc: *Corticium candelabrum*, Ol: *Oscarella\_Tobularis*, Sc: *Sycon\_ciliatum*. LD05: *Candidatus Paraprochloron terpios* LD05.

Table S7. Significant Biological Process [BP] GO terms of differentially expressed genes in *T. hoshinota* under heat and acidification stress.

**Upregulated GO under heat stress**

| GO.ID      | Term                                        | Annotated | Significant | Expected | weightFisher | Ontology |
|------------|---------------------------------------------|-----------|-------------|----------|--------------|----------|
| GO:0006589 | octopamine biosynthetic process             | 21        | 6           | 0.94     | 0.00023      | BP       |
| GO:0042420 | dopamine catabolic process                  | 21        | 6           | 0.94     | 0.00023      | BP       |
| GO:0042421 | norepinephrine biosynthetic process         | 21        | 6           | 0.94     | 0.00023      | BP       |
| GO:0007214 | gamma-aminobutyric acid signaling pathwa... | 113       | 14          | 5.04     | 0.00046      | BP       |
| GO:0007186 | G protein-coupled receptor signaling pat... | 277       | 26          | 12.36    | 0.0005       | BP       |
| GO:0030224 | monocyte differentiation                    | 2         | 2           | 0.09     | 0.00198      | BP       |
| GO:0006564 | L-serine biosynthetic process               | 2         | 2           | 0.09     | 0.00198      | BP       |
| GO:2000369 | regulation of clathrin-dependent endocyt... | 3         | 2           | 0.13     | 0.00577      | BP       |
| GO:0045747 | positive regulation of Notch signaling p... | 3         | 2           | 0.13     | 0.00577      | BP       |
| GO:0051046 | regulation of secretion                     | 21        | 4           | 0.94     | 0.01111      | BP       |
| GO:0006470 | protein dephosphorylation                   | 133       | 12          | 5.93     | 0.01521      | BP       |
| GO:0071577 | zinc ion transmembrane transport            | 13        | 3           | 0.58     | 0.01801      | BP       |
| GO:0006468 | protein phosphorylation                     | 222       | 15          | 9.9      | 0.01831      | BP       |
| GO:0046512 | sphingosine biosynthetic process            | 6         | 2           | 0.27     | 0.02641      | BP       |
| GO:0006882 | intracellular zinc ion homeostasis          | 7         | 2           | 0.31     | 0.03589      | BP       |
| GO:0034587 | piRNA processing                            | 7         | 2           | 0.31     | 0.03589      | BP       |
| GO:0006643 | membrane lipid metabolic process            | 72        | 9           | 3.21     | 0.04383      | BP       |
| GO:0051028 | mRNA transport                              | 22        | 2           | 0.98     | 0.0446       | BP       |
| GO:0019243 | methylglyoxal catabolic process to D-lac... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0008298 | intracellular mRNA localization             | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0099044 | vesicle tethering to endoplasmic reticul... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0006616 | SRP-dependent cotranslational protein ta... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0071233 | cellular response to leucine                | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:1990253 | cellular response to leucine starvation     | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0140300 | serine import into mitochondrion            | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0033499 | galactose catabolic process via UDP-gala... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0042989 | sequestering of actin monomers              | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0009098 | leucine biosynthetic process                | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0050728 | negative regulation of inflammatory resp... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:1903232 | melanosome assembly                         | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0010165 | response to X-ray                           | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0140021 | mitochondrial ADP transmembrane transpor... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0010142 | farnesyl diphosphate biosynthetic proces... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0106300 | protein-DNA covalent cross-linking repai... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0035229 | positive regulation of glutamate-cystein... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0006011 | UDP-glucose metabolic process               | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0051694 | pointed-end actin filament capping          | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:1901029 | negative regulation of mitochondrial out... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0015811 | L-cystine transport                         | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0009104 | lipopolysaccharide catabolic process        | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:1990810 | microtubule anchoring at mitotic spindle... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0070301 | cellular response to hydrogen peroxide      | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:1901031 | regulation of response to reactive oxyge... | 1         | 1           | 0.04     | 0.04461      | BP       |
| GO:0016031 | tRNA import into mitochondrion              | 1         | 1           | 0.04     | 0.04461      | BP       |

|            |                                             |   |   |      |            |
|------------|---------------------------------------------|---|---|------|------------|
| GO:0033211 | adiponectin-activated signaling pathway     | 1 | 1 | 0.04 | 0.04461 BP |
| GO:0090160 | Golgi to lysosome transport                 | 1 | 1 | 0.04 | 0.04461 BP |
| GO:0140206 | dipeptide import across plasma membrane     | 1 | 1 | 0.04 | 0.04461 BP |
| GO:0043457 | regulation of cellular respiration          | 1 | 1 | 0.04 | 0.04461 BP |
| GO:1990544 | mitochondrial ATP transmembrane transpor... | 1 | 1 | 0.04 | 0.04461 BP |

#### Downregulated GO under heat stress

| GO.ID      | Term                                        | Annotated | Significant | Expected | weightFisher | Ontology |
|------------|---------------------------------------------|-----------|-------------|----------|--------------|----------|
| GO:0006891 | intra-Golgi vesicle-mediated transport      | 40        | 4           | 0.24     | 9.10E-05     | BP       |
| GO:0032508 | DNA duplex unwinding                        | 55        | 4           | 0.33     | 0.0042       | BP       |
| GO:0085020 | protein K6-linked ubiquitination            | 1         | 1           | 0.01     | 0.0061       | BP       |
| GO:1902103 | negative regulation of metaphase/anaphas... | 2         | 1           | 0.01     | 0.0121       | BP       |
| GO:0061136 | regulation of proteasomal protein catabo... | 32        | 2           | 0.19     | 0.0177       | BP       |
| GO:0055070 | copper ion homeostasis                      | 3         | 1           | 0.02     | 0.0181       | BP       |
| GO:0018022 | peptidyl-lysine methylation                 | 3         | 1           | 0.02     | 0.0181       | BP       |
| GO:0010950 | positive regulation of endopeptidase act... | 3         | 1           | 0.02     | 0.0181       | BP       |
| GO:2000009 | negative regulation of protein localizat... | 3         | 1           | 0.02     | 0.0181       | BP       |
| GO:0006268 | DNA unwinding involved in DNA replicatio... | 38        | 2           | 0.23     | 0.022        | BP       |
| GO:0034085 | establishment of sister chromatid cohesi... | 4         | 1           | 0.02     | 0.0241       | BP       |
| GO:0015014 | heparan sulfate proteoglycan biosyntheti... | 5         | 1           | 0.03     | 0.03         | BP       |
| GO:0070475 | rRNA base methylation                       | 5         | 1           | 0.03     | 0.03         | BP       |
| GO:1902306 | negative regulation of sodium ion transm... | 5         | 1           | 0.03     | 0.03         | BP       |
| GO:0090263 | positive regulation of canonical Wnt sig... | 5         | 1           | 0.03     | 0.03         | BP       |
| GO:2000045 | regulation of G1/S transition of mitotic... | 5         | 1           | 0.03     | 0.03         | BP       |
| GO:0030050 | vesicle transport along actin filament      | 6         | 1           | 0.04     | 0.0359       | BP       |
| GO:0018401 | peptidyl-proline hydroxylation to 4-hydr... | 6         | 1           | 0.04     | 0.0359       | BP       |
| GO:0031398 | positive regulation of protein ubiquitin... | 7         | 1           | 0.04     | 0.0418       | BP       |

#### Upregulated GO under acidification stress

| GO.ID      | Term                                        | Annotated | Significant | Expected | weightFisher | Ontology |
|------------|---------------------------------------------|-----------|-------------|----------|--------------|----------|
| GO:0106217 | tRNA C3-cytosine methylation                | 1         | 1           | 0.01     | 0.0091       | BP       |
| GO:0070197 | meiotic attachment of telomere to nuclea... | 1         | 1           | 0.01     | 0.0091       | BP       |
| GO:0006513 | protein monoubiquitination                  | 18        | 2           | 0.16     | 0.0114       | BP       |
| GO:0006814 | sodium ion transport                        | 39        | 3           | 0.36     | 0.0123       | BP       |
| GO:0055070 | copper ion homeostasis                      | 3         | 1           | 0.03     | 0.0271       | BP       |
| GO:0090309 | positive regulation of DNA methylation-d... | 3         | 1           | 0.03     | 0.0271       | BP       |
| GO:2000009 | negative regulation of protein localizat... | 3         | 1           | 0.03     | 0.0271       | BP       |
| GO:0006470 | protein dephosphorylation                   | 133       | 4           | 1.21     | 0.0324       | BP       |
| GO:0006122 | mitochondrial electron transport, ubiqui... | 4         | 1           | 0.04     | 0.036        | BP       |
| GO:0051026 | chiasma assembly                            | 4         | 1           | 0.04     | 0.036        | BP       |
| GO:1904491 | protein localization to ciliary transiti... | 4         | 1           | 0.04     | 0.036        | BP       |
| GO:1902306 | negative regulation of sodium ion transm... | 5         | 1           | 0.05     | 0.0448       | BP       |
| GO:0048268 | clathrin coat assembly                      | 5         | 1           | 0.05     | 0.0448       | BP       |

#### Downregulated GO under acidification stress

| GO.ID      | Term                 | Annotated | Significant | Expected | weightFisher | Ontology |
|------------|----------------------|-----------|-------------|----------|--------------|----------|
| GO:0000423 | mitophagy            | 3         | 1           | 0.02     | 0.016        | BP       |
| GO:0006814 | sodium ion transport | 39        | 2           | 0.21     | 0.019        | BP       |

|            |                                             |     |   |      |          |
|------------|---------------------------------------------|-----|---|------|----------|
| GO:0033209 | tumor necrosis factor-mediated signaling... | 42  | 2 | 0.23 | 0.022 BP |
| GO:0070534 | protein K63-linked ubiquitination           | 42  | 2 | 0.23 | 0.022 BP |
| GO:0006122 | mitochondrial electron transport, ubiqui... | 4   | 1 | 0.02 | 0.022 BP |
| GO:0016560 | protein import into peroxisome matrix, d... | 5   | 1 | 0.03 | 0.027 BP |
| GO:0036371 | protein localization to T-tubule            | 5   | 1 | 0.03 | 0.027 BP |
| GO:0055117 | regulation of cardiac muscle contraction    | 5   | 1 | 0.03 | 0.027 BP |
| GO:0048240 | sperm capacitation                          | 5   | 1 | 0.03 | 0.027 BP |
| GO:0030317 | flagellated sperm motility                  | 6   | 1 | 0.03 | 0.032 BP |
| GO:1903566 | positive regulation of protein localizat... | 6   | 1 | 0.03 | 0.032 BP |
| GO:0006470 | protein dephosphorylation                   | 133 | 3 | 0.72 | 0.035 BP |
| GO:0034427 | nuclear-transcribed mRNA catabolic proce... | 7   | 1 | 0.04 | 0.037 BP |

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