Warm Temperatures, Cool Sponges: The Effect of Increased Temperatures on the Antarctic Sponge *Isodictya* sp.

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Abstract

Although the cellular and molecular responses to exposure to relatively high temperatures (acute thermal stress or heat shock) have been studied previously, only sparse empirical evidence of how it affects cold-water species is available. As climate change becomes more pronounced in areas such as the Western Antarctic Peninsula, it has become crucial to understand the capacity of these species to respond to thermal stress.

Here we use the Antarctic sponge *Isodictya* sp. to investigate how sessile organisms (particularly Porifera) can adjust to acute short-term heat stress, by exposing this species to 3 and 5 °C for 4 hours, corresponding to predicted temperatures under high-end 2080 IPCC-SRES scenarios. Assembling a *de novo* reference transcriptome (90,188 contigs, >93.7% metazoan BUSCO genes) we have begun to discern the molecular response employed by *Isodictya* to adjust to environmental insult.

Our initial analyses suggest that TGF-β, ubiquitin and hedgehog cascades are involved, alongside other genes. However, the degree and type of response changed little from 3 to 5 °C, suggesting that even moderate rises in temperature could cause stress at the limits of this organism's capacity. Given the importance of sponges to Antarctic ecosystems, our findings are vital for discerning the consequences of increases in Antarctic ocean temperature on these and other species.

Key Words:

Porifera; Thermal stress; Climate change; Transcriptome; Western Antarctic Peninsula

Background

Sponges are vital components of a variety of ecosystems in terms of abundance and ecosystem services[1,2]. This is especially true in the Antarctic, where they are among the most common benthic species, providing habitat and food for a wide range of organisms[3,4,5,6]. Sponges, like most marine species, will be broadly, and perhaps adversely, affected by climate change, but at present studies into their capacity to cope with increases in temperature are limited in scope and number[7,8].

The Western Antarctic Peninsula (WAP) is one of the areas of the planet which has experienced some of the most significant changes in air and water temperature[9,10]. Current estimates of climate change in the Antarctic suggest that the mean temperature of seawater could rise by up to 5 °C by 2080 (IPCC 2001, Table 16.1) although more recent IPCC reports put the best estimate for this at about + 0.6°C (Representative Concentration Pathway 2.6) to 2.0°C (Representative Concentration Pathway 8.5) (IPCC 2014)[11]. This is expected to have major implications for Antarctic organisms as they have evolved in a very stable and cold environment, being highly sensitive to environmental variation[12,13]. For this reason the projected changes in water temperature constitutes a major threat to ecosystem function in these waters. Such changes would modify the prevalence of key functional species, thus affecting associated ecosystem processes[14]. However, we are only beginning to understand the resilience of many species found in the Antarctic to rising temperature conditions [12,15,16,17], and the point to which they will be affected by any changes in mean temperature. The deleterious effects of temperature exposure in sponges from other latitudes include bleaching of symbionts, tissue necrosis and death[18]. It is vital to gain this information in Antarctic sponge species, so that policy decisions can be made with a full understanding of the likely impacts of these changes.

Some sponge species have been suggested to be relatively robust to moderate changes in temperature. Caribbean and Brazilian coral-reef sponges have been investigated and found

77 to survive fluctuations in ambient temperature[19,20]. A recent study, experimentally demonstrated
78 that the boreal deep-sea sponge *Geodia barretti* is able to cope with temperature rises with few
79 ill effects[21]. Other sponge species are however not so resilient [18,22,23], and both reproduction[24]
80 and filtration[25] in sponges has been shown to be affected by increases in temperature. In some
81 cases, this has led to widespread mortality [22,26].

82 We have little current understanding as to what makes some species able to cope with
83 broad temperature ranges, and it is possible that Antarctic-dwelling species may be particularly
84 vulnerable due to their specialisation for extremely cold, relatively stable temperature
85 conditions[27]. Their molecular components in particular may have altered over evolutionary time,
86 as has been observed previously in a variety of species [17,28,29], and warmer temperatures could
87 prove deleterious. The sponge *Isodictya* sp. (Fig 1A) is no exception to this. While this sponge is
88 commonly observed around the WAP and generally lives in waters with temperatures between -
89 1.8 to 2 °C[30], it is rarely found in warmer areas. Increasingly this sponge is exposed to warmer
90 summer temperatures across its natural range, with shallow-water temperatures reaching 3 °C
91 in some places around the WAP[31].

92 Illumina-based sequencing can reveal both subtle and broad changes in expression in
93 studies of model and non-model organisms[32]. The decreasing cost of sequencing and steady
94 increase in affordable computational power has made such approaches increasingly viable,
95 even for small lab teams and consortia[33]. These studies have the additional benefit of also
96 providing a suite of information on the gene sequences of the organism in question, which is
97 particularly useful for non-model species. While genomic-level datasets within the Porifera
98 remain depauperate and limit our understanding of adaptation to particular conditions, a number
99 of investigations into the transcriptomic complements of a variety of sponges have now been
100 successfully performed [34,35,36]. The transcriptional changes in response to temperature shifts
101 that occur within sponges themselves[23,37] and changes in their bacterial symbionts[18,38,39,40.41]
102 have also only recently begun to be unveiled. Some changes in gene expression revealed by

103 these studies, such as those in HSP70 expression[37,42] are easily explained with reference to
104 known metazoan responses to temperature fluctuation. Similarly, responses such as the up-
105 regulation of genes involved in signal transduction, tissue morphogenesis, cell signalling and
106 antioxidant activity are all of clear utility to tissues as they adapt to thermal stresses. What has
107 yet to be tested thoroughly is whether these changes are mirrored in extremophile species. It is
108 possible that gene complements adapted to extremes may be less able to adapt to changes,
109 particularly at rapid time scales.

110 Our study demonstrates that some extremophile species such as *Isodictya* sp. may be
111 able to respond to acute short term stress, but may not have the capacity to adjust further to
112 continued rises in temperature. Using a small number of replicates and two "heat shock"
113 regimes, we have been able to use these findings to investigate the specific pathways used by
114 these organisms, and contrast these with previous findings in species adapted to warmer
115 temperature regimes. The sponge studied here, *Isodictya* sp. is one of the most common
116 species in shallow-water rocky reefs (<25 m depth) around Doumer Island, Palmer Archipelago,
117 WAP (Fig 1B). This knowledge will be vital for understanding the impact of temperature rises on
118 Antarctic species, but also for discrete, targeted investigation of the role of specific pathways in
119 temperature adaptation in the future. In this manuscript we present the first transcriptomic
120 analysis of the effect of acute warming on a cold-adapted sponge.

121

122 **Methods**

123

124 *Animal Collection and Heat Treatment*

125 Adult samples of six individuals of the Antarctic sponge *Isodictya* sp. were collected by
126 SCUBA diving at 10 m depth in Cape Kemp, off Yelcho Research Station, Doumer Island, WAP
127 (64°51′S 63°35′W, Fig. 1B) in January 2017. Sponges were then transferred to the laboratory at
128 Yelcho Station, where they were maintained in 140 L fiberglass tanks with unfiltered, flowing

129 seawater (approximately 0.5 °C, pumped directly from the sea floor near to collection site (5 -10
130 m depth)) for a week to allow acclimation to laboratory conditions. Tanks were covered with two
131 layers of 1 mm shade cloth (fiberglass 50% neutral density screen) to represent light levels
132 occurring *in situ*. Relative light intensity levels and temperature in the experimental tanks were
133 measured (1 measurement every 15 minutes) during the duration of experiment with a HOBO
134 Pendant® temperature/light data logger (Onset, USA). Sponges were then placed into one of
135 three possible treatment conditions - a control tank, where samples were maintained in
136 seawater pumped directly from the sea floor at approximately 0.5 °C, or one of two possible
137 treatment temperatures, 3 and 5 °C, with water warmed in a header tank before pumping into
138 the treatment area, and subsequently maintained at a set temperature using SOBO Aquarium
139 heaters (500 watt). These temperatures were chosen in line with empirical evidence of warming
140 in the WAP[31] and to correspond to the higher possible estimates of warming in the Antarctic
141 (IPCC, 2001 report). Sponge samples were placed in individual treatment tanks, for a total of 3
142 treatments and two replicates (2x control, 2x 3 °C, 2x 5 °C) although one control sample was
143 not used for analysis as described in Results and Discussion.
144
145 *RNA Extraction and Sequencing*
146 Sponges were taken from tanks after 4 hours of exposure to the control/treatment
147 conditions, and a single fragment of each sample, approx. 1 cm^3, was taken immediately and
148 placed in RNAlater (Qiagen) and stored at -20 °C on site, before shipment on dry ice (with an
149 unbroken cold chain) from Yelcho via Escudero Station to a molecular laboratory at the
150 Laboratorio de Biorrecursos at INACH, Punta Arenas. RNA was extracted from sponge samples
151 using E.Z.N.A Total RNA kit II (Omega Bio-Tek Inc.) according to the manufacturer's protocol
152 with a previous step of homogenization with liquid nitrogen. RNA quality and quantity was
153 determined on a 2100 Bioanalyser (Agilent Technologies). Quality control obtained an average
154 RIN value of 8.2. The sole sample with a RIN value less than 7 (a control sample, with RIN 6.2)

155 was not included in this work. 1 µg of RNA from each sample was used to produce RNA
156 libraries for sequencing, with TruSeq Stranded mRNA kit (Illumina), 100bp nominal intra-read
157 fragment size, 15 rounds of amplification and adaptors AGTTCC, ATGTCA, CCGTCC,
158 GTCCGC and CGATGT.

159 Libraries were sequenced on a Hiseq2500 sequencer by the Macrogen provider, using
160 approximately one half of a run in total. The sequencing provider performed initial assessment
161 of read quality and de-multiplexing of reads according to their procedures, and provided us with
162 paired-end reads for download from an external server, with no unpaired orphan reads retained
163 by this process.

164

165 *Quality Control and Assembly*

166 We confirmed the removal of adapter sequences and overall sequence quality using the
167 FastQC program[43]. Low-quality regions of reads were trimmed using Trimmomatic 0.33[44] with
168 the following settings: ILLUMINACLIP:../Adaptors.fa:2:30:10 LEADING:3 TRAILING:3
169 SLIDINGWINDOW:4:20 MINLEN:30 where the Adaptors.fa file consisted of the appropriate
170 indexes for the libraries in question. The resulting trimmed reads were then compressed using
171 gzip and re-analysed with FastQC. Seqtk fqchk[45] was used to determine average sequence
172 quality scores.

173 Trimmed reads from all 5 samples were assembled into a reference transcriptome using
174 Trinity version 2013_08_14[46], with two non-standard settings: a minimum contig length of 200
175 bp and *in silico* read normalisation. DeconSeq standalone version 0.4.3[47] was used to remove
176 contamination, with settings -i 50 -c 50, using pre-prepared bacterial, archean, hsref and viral
177 databases (2,206, 155, 1 and 3,761 complete genomes respectively), resulting in our final
178 reference assembly. This is available from the DOI and URL below as Supplementary File 1.

179

180 *Phylogenetic Analysis*

181 28S and HSP70 sequences were extracted from our assemblies using BLASTN[48] on a
182 local server, using sequences of known orthology from Genbank as search queries. These were
183 aligned to previously published sequences downloaded from the NCBI database using MAFFT
184 [49]. HSP70 gene alignments were cured using Gblocks[50], although this was not necessary for the
185 well-conserved 28S sequence. Phylogenetic analysis was run in RAxML GUI[51] using ML +
186 Rapid Bootstrap, the GTR model (28S)/LG+I+G model (HSP70) and 1,000 bootstrap replicates.
187 Phylogenetic and molecular evolutionary analyses of AIF genes were conducted using MEGA
188 version 7.0[52]. The tree was inferred using the Neighbor-Joining method, based on the alignment
189 of the sequences using ClustalW 2.0[53] (alignment was improved using the Seaview software
190 4.6.5[54]). Resultant tree topologies were evaluated by bootstrap analyses based on 1000
191 replicates.
192
193 *Transcriptome analysis and annotation*
194 To assess the content of the transcriptome BUSCO v1.1b1[55] was run against the
195 reference transcriptome, using the eukaryotic and metazoan Basic Universal Single Copy
196 Orthologue (BUSCO) gene lists to estimate completeness. Annotation of contigs was performed
197 by translating the longest ORF for each contig using the getORF.py python script, taking the
198 longest ORF, then using standalone BLASTP[48] (cutoff *E* value 0.000001) to search against the
199 refseq protein database. This was supplemented using Blast2GO Pro[56], where full InterPro
200 scanning, mapping, annotation (including ANNEX) and enzyme code mapping was performed,
201 the complete annotations for which are available as Supplementary File 2.
202 The KAAS-KEGG automatic annotation server was used to gain an understanding of the
203 recovery of complete pathways in our transcriptome. These were generated using the online
204 tool[57] rather than as integrated into Blast2GO, due to the increased functionality of the
205 standalone server. The bi-directional best hit method was used to identify and annotate the
206 longest orfs from our contigs, with the protein sequences generated earlier used as the basis for

207 these comparisons against a range of eukaryotic species. The maps generated by this were
208 compared directly against extant ones previously generated for the sponge *Amphimedon*
209 *queenslandica,* and the complete annotations are available as Supplementary File 3.
210
211 *Differential Expression Analysis and Over-representation Analysis*
212 Comparative analyses of gene expression were performed using RSEM[58] as packaged
213 within the Trinity[46] module, using Bowtie2[59] for alignment. The three treatments (control, 3 and 5
214 °C) had their replicates (1, 2 and 2 respectively) cross-sample normalised according to Trimmed
215 Mean of M-values. The three treatments were then cross-compared using edgeR[60] within the
216 Trinity[46] perl wrapper script, according to the "classic" pairwise model with a p-value cut off for
217 FDR of 0.0001, a minimum absolute (log2(a/b)) change of 2 (i.e. 4 fold change) and a
218 dispersion setting of 0.1, deliberately over-specified to preclude false positive identification of
219 over-expression. We excluded any differentially expressed contigs where transcription was only
220 detected in a single sample of any of the five used in this analysis, prior to clustering and any
221 further analysis or display, to avoid spurious results caused by transient expression or
222 contamination of single samples. As with the dispersion estimate, the p-value cutoff and
223 minimum absolute change values are stringent, to exclude potentially artifactual changes from
224 our analysis, given the small number of replicates. The remaining differentially-expressed gene
225 lists were then targeted for further analysis. These results are provided as Supplementary File
226 4.
227 GO over-representation analysis was performed in Blast2GO Pro[56] using Fisher's Exact
228 Test, with FDR control for multiple testing, with a p-value cutoff of 0.05[61]. The test sets were the
229 annotated results for the sequences present in each of the over-represented sets derived from
230 the differential expression analysis, analysed in turn, with the reference set the full list of
231 annotated contigs, minus those in the test set.
232

233 **Results and Discussion**

234

235 *Sequencing and Read Cleaning*

236 While 5 individual samples are noted here (a single control, and two replicates for both
237 the 3 °C and 5 °C treatments) another control sample was also initially taken and sequencing
238 performed as described above. Despite being morphologically identical, our initial assays of the
239 sequences from that sample, and particularly the sequence of common molecular markers used
240 in phylogenetic comparison, including *28S rRNA* and *cytochrome c oxidase 1* (*CO1*) markers,
241 revealed that it is in fact a cryptic related species, which will be the subject of description in the
242 future (see Fig. 1C). We have not included this sample in any of the analyses in this work.

243 Basic sequencing metrics can be seen in Table 1, alongside those after read cleaning.
244 Our initial FastQC analysis revealed the presence of low quality nucleotide sequence in the
245 second file of many pairs. Cleaning was therefore stringent, and resulted in markedly fewer, but
246 much better average quality, reads for all samples, which were then used for assembly of the
247 reference transcriptome and for differential expression analysis. GC% (which can be a crude
248 proxy for contamination or changes in expression) was even through all our samples, between
249 44 and 46%, and changed little with cleaning. A small number of over-represented sequences
250 were also initially observed in our reads, as is commonly observed in Illumina-based
251 transcriptomic analysis due to known biases in hexamer binding[62]. The difference in average
252 quality after cleaning was modest, with an average difference of around 2 in Phred score. Our
253 original reads have been uploaded to the NCBI SRA with accession number PRJNA415418.

254

255 *Assembly and Completeness*

256 Reads from all 5 samples were used to construct a reference assembly, for use in
257 further analysis (Supplementary File 1). We checked this for potential contamination using
258 Deconseq, comparing our data to known bacterial, viral, 'archaea' (*sensu* [63], we are aware of

259 archaean paraphyly) and human genomic sequences. With very permissive settings for
260 recognition as foreign sequence (minimum thresholds for removal, 50% similarity across at least
261 50% of the contig length) 797 contigs were tagged as potential contamination. These were
262 removed from our dataset before further analysis. It should be noted, however, that particularly
263 novel bacterial sequences not yet represented in the *nr* database may still be present in our
264 data, despite the use of polyA sequencing, as these could not be represented in our removal
265 database and therefore will not have been removed. While beyond the scope of this manuscript,
266 changes in symbiont content may be vital for long-term adaptation to change.

267 Statistics related to our cleaned reference assembly can be seen in Table 2. A total of
268 90,188 contigs are present, and Trinity, a splice aware assembler, has automatically assigned
269 70,844 as independent "genes". A small amount of heterozygosity or potential splice variation
270 has therefore been recognised by the assembly software, with 19,344 contigs flagged as
271 isoformal variants of other contigs in our assembly. This is not unusual, and is in fact less than
272 that recognised in some other *de novo* assemblies (for instance, [64], where every gene
273 possessed approximately 2 "isoform" variants on average). Our samples may therefore possess
274 little in the way of genetic variability at most loci. Our reference assembly is well-assembled,
275 with a high N50 (1,113 bp) sufficient to span the full length of many protein coding genes.
276 12,997 contigs were longer than this N50 value, and 14,633 longer than 1kb in length. Given
277 that the expected complement of genes in most metazoans is around 20,000 in quantity, a
278 significant number of these are likely to be represented in these well-assembled contigs.

279 To test the completeness of our transcriptomic dataset we used the BUSCO approach[55],
280 which revealed our dataset to be remarkably complete. Of 978 BUSCO orthogroups plausibly
281 expected in any metazoan species, 916 complete BUSCOs were found (654 single copy, 262
282 duplicated, possibly reflecting allelic or isoform differences). 20 were present only as
283 fragmentary sequence, while 42 were missing. Of the 303 eukaryotic BUSCO sequences, 298
284 (98.4%) were present, 213 as single copy, 85 with duplicates and 2 as fragmentary sequence.

285 Only 3 BUSCO groups were missing. By way of comparison, the published draft of the
286 *Amphimedon queenslandica* set is missing 1.6% of the eukaryote set (5 genes), and 4.9% (49)
287 of the metazoan complement[36], and our transcriptome compares favourably with that resource.
288
289 *Annotation and Content*
290 To annotate our data, we used automated methods, including the Blast2GOPro and
291 KEGG platforms. Of 90,188 total contigs, 20,607 were given an initial annotation based on
292 BLAST results *vs*. the Refseq database. Of these contigs, 7,303 had their "best hit" to proteins
293 in the *A. queenslandica* genome. No other species had more than 1,000 contigs with "best hits"
294 to their complements, but the cnidarian *Nematostella vectensis,* a variety of invertebrate
295 deuterostomes (*Branchiostoma floridae, Saccoglossus kowalevskii* and *Strongylocentrotus*
296 *purpuratus)* and lophotrochozoan species (*Crassostrea gigas, Aplysia californica* and *Lottia*
297 *gigantea)* were the next most commonly represented metazoan species in this group. This likely
298 represents the relatively slow rate of molecular evolution in these clades when compared to
299 model species such as *Drosophila melanogaster* and *Caenhorhabditis elegans*. The diatoms
300 *Phaeodactylum tricornutum CCAP 1055/1* and *Thalassiosira pseudonana CCMP1335* were
301 observed as "best hit" species for 798 and 563 contigs respectively, which is not surprising as
302 previous work as described abundant presence of diatoms in other Antarctic sponges [65,66].
303 Annotated diatom data was not included in further analysis. No obvious bacterial, viral, human
304 or archaeal contamination was observed, although as described earlier, 797 contigs were
305 removed from our assembly by Deconseq before any further analysis.
306 More discrete annotation was then performed using Blast2GO Pro. Of the 20,607
307 contigs preliminarily annotated with Blast, 6,267 could not be annotated further. 1,389 were able
308 to be "mapped" to GO terms, but were unable to be annotated further. 12,924 contigs received
309 GO annotations, and were thus fully annotated by this process (Supplementary File 2). 27
310 additional contigs had Interproscan results and were also placed into GO categories. While the

311 expected total gene complement of a metazoan is often up to around twice this figure (e.g.
312 *Drosophila melanogaster* has around 15,500, while humans contain around 20-25,000
313 according to most estimates), our annotations are nonetheless a large proportion of the
314 expected gene count of these sponges, It should be noted that in even the most well-annotated
315 genomes, not all genes can be placed in GO categories.

316 We also performed KEGG annotation on our *de novo* transcriptome using the KAAS-
317 KEGG automatic annotation server, with its default BLAST settings, to understand the
318 representation of key pathways in our transcriptomic datasets. The results of these annotations
319 are provided in full as Supplementary File 3, but in general recovery was excellent with 37,313
320 genes annotated to existing KEGG terms. While sponges do not possess the full canonical
321 complements of other metazoan phyla, as these were not present in the common ancestors of
322 sponges and the broader Metazoa, we generally recover the expected gene complements of the
323 Porifera in our reference transcriptomic assembly, when mapped to the *A, queenslandica* KEGG
324 dataset. Together with BUSCO results and the raw number of annotated genes, this gives us
325 confidence in the depth of our transcriptomic resources.

326

327 *Differential Expression Results*

328 We utilised our samples to perform a differential expression analysis, aimed at
329 discerning the specific genes up- and down- regulated by exposure to acute increased
330 temperatures. It should be noted that due to experimental necessity, these are few in replicate
331 number compared to many experiments, with n=2 at best. The sample correlation matrix and
332 heatmap of relative expression for differentially expressed genes can be seen in Fig 2. While
333 replicate samples at 3 °C correlated almost identically with one another, there was slight
334 fluctuation between our 5°C replicates, perhaps due to inter-individual differential responses to
335 these challenging conditions. The large degree to which the 3 and 5 °C treatments resemble
336 one another can be seen in the matrix in Fig. 2A, where the 4 replicates show little

337 differentiation from one another (black regions of matrix). In Fig 2B, while some discreet inter-
338 sample variation is observed, note the large downregulated quadrant of the matrix at bottom
339 right, relative to high relative expression levels in the control sample at far left. Adding additional
340 replicates would increase confidence in these results, but initial findings are already compelling.

341 In general, we found a large number of genes whose expression was perturbed by heat
342 treatment at both 3 and 5 °C. Between our control and 3 °C treatments, the transcriptional
343 landscape changed significantly (with stringent criteria for significance: cut off for FDR of
344 0.0001, a min abs(log2(a/b)) change of 2), with 1,435 contigs changing significantly in
345 expression (767 upregulated, 668 downregulated at the 3 °C treatment compared to the control
346 sample). This was mirrored in our control *vs.* 5 °C comparison, where 1,230 contigs changed
347 significantly in expression (623 upregulated, 607 downregulated significantly at 5 °C compared
348 to control sample). The complete list of differentially expressed genes and further details are
349 given in Supplementary File 4. There was a large amount of overlap between these sets. In
350 total, 322 genes were downregulated commonly in both the 3 and 5 °C samples when
351 compared to the control. 383 genes were upregulated commonly in both the 3 and 5 °C samples
352 compared to the control. These genes may be particularly interesting for further analysis, given
353 their conserved role in thermal response, and we have provided these in Supplementary File 5.
354 Contrastingly, genes that were not shared could provide specific information about responses at
355 specific temperature points, and are also made available for further study.

356 The 20 most up and down regulated genes in our control vs 3 °C and control vs 5 °C
357 samples are of clear interest in understanding thermal stress response, and can be seen in
358 Table 3. These contigs include those with similarity to heat shock proteins (*heat shock 70 B2-*
359 *like*) and ribosomal proteins (*40S ribosomal S13*, *40S ribosomal S25*), along with a large
360 number of sequences with no clear homologues in the RefSeq database, and some of less clear
361 utility (e.g. the *Plant Cadmium Resistance 3-like* family of eukaryote-conserved genes, although
362 cadmium and these genes are noted to influence the HSP pathway[67]).

363 However, very few genes (5 upregulated in 5 °C sample vs 3 °C, 11 downregulated)
364 changed significantly in expression between the 3 and 5 °C treatment. We speculate that this
365 may be due to the transcriptional machinery of this sponge already operating at maximum
366 capacity to adjust to thermal stress under the 3 °C treatment, with little extra ability to cope with
367 changes brought by further increases in temperature. Despite their small number, these genes
368 are of a variety of annotations, including *allograft inflammatory factor 1*, *ubiquitin carboxyl-*
369 *terminal hydrolase isozyme L3-like* and a *FKBP6-like* sequence, and could still be helping adjust
370 to thermal stress.
371
372 *GO over-representation analysis*
373 To gain a broad understanding of the nature of changes between our samples, we
374 tested differentially expressed genes at given temperatures as our test sets, relative to our
375 reference transcriptomes, and checked for over-representation of any gene categories using
376 Fisher's Exact Test, correcting for false discovery rate and with a cutoff p value of 0.05, as
377 integrated in Blast2GO. Our findings are based on a small number of replicates, but with a high
378 stringency criteria for assigning significance. When we compared our control to our 3 and 5 °C
379 samples a variety of statistically significant differences in contig expression were observed (Fig
380 3A,B). However, there was no difference in significant GO category expression between our 3
381 and 5 °C samples, and we infer that this is due to only minor changes between 3 and 5 °C.
382 These contigs change little in expression, and from their values at 3 °C do not (or can not) alter
383 their levels any further to adjust to the additional stress at 5 °C.
384 In our control vs 3 °C samples (Fig 3A), GO category representation changed
385 significantly in a number of categories. Among the GO categories downregulated at 3 °C,
386 'immune response', 'signal transduction' 'serine type endopeptidase inhibitor activity' and
387 'pantothenate biosynthetic pathway' were significantly more prevalent there than in our overall
388 set. These GO terms indicate that immune responses and normal bodily maintenance may be

389 perturbed by thermal stress. In contrast, GO terms associated with ribosomes and structural
390 molecules are under-represented in this set, and therefore contigs coding for these are not
391 present in the downregulated complement after heat exposure. This is similar to the results
392 observed in Guzman and Conaco (2016)[37], as discussed further below.

393 In the GO categories upregulated at 3 °C, it again seems that normal metabolism is less
394 important for response to thermal stress than cytoskeletal responses and growth factor activity.
395 Many GO terms related to metabolic activity (e.g. 'phosphate-containing compound metabolic
396 process', 'organic substance biosynthetic process') are under-represented in these genes
397 relative to our overall library, while GO terms linked to microtubules and other cytoskeletal
398 components are significantly over-represented. Taken in concert with our GO term distribution
399 findings from downregulated genes, it seems that structural componentry is significantly
400 upregulated in response to a 3 °C heat shock.

401 Many of these findings are mirrored in our control vs 5 °C samples (Fig 3B). Four GO
402 categories, 'intracellular non-membrane-bounded organelle', 'cytoplasmic part', 'pantothenate
403 biosynthetic process' and 'signal transduction', had similar changes in abundance in
404 downregulated contigs from our 3 and 5 °C treatments compared to the control, and 'growth
405 factor activity' was significantly over-represented in contigs upregulated in response to
406 increased temperature in both samples. These have been underlined in the figure. A small
407 number of additional categories were significantly changed in our 5 °C treatment but not
408 observed in our 3 °C treatment, including a number of categories related to the biosynthesis of
409 specific molecular and cellular components. For instance, the category 'cellular macromolecule
410 biosynthetic process', is less prevalent in downregulated genes, (and therefore not itself present
411 in downregulated contigs), while 'tyrosine biosynthetic process' and other categories are over-
412 represented in up-regulated genes, reflecting underlying transcriptional activity in that regard,
413 mirroring our findings in the 3 °C treatment.

414 Previous work [37] observed the upregulation of a number of protective and signalling-
415 related pathways after heat shock. We see a similar pattern in our data, but the exact categories
416 are not seen in *Isodictya* as in their warm-water *Haliclona tubifera.* Protective mechanisms in
417 common with their results include those linked to antioxidant activity and immune response
418 activation, but do not include the toll-like receptor (TLR) signaling pathway. We also observe
419 similar broad representation of cellular ion homeostasis, messenger-mediated pathways,
420 transporter activity and microtubule-based movement-linked categories, but do not see
421 specifically calcium-mediated signaling categories, unlike those seen in their data.

422 While most of the observed categories in our data are quite broad and reflect changes in
423 a number of gene families, we can observe some quite specific changes in expression. For
424 example, the 'Tumor necrosis factor receptor binding' category is over-represented in
425 downregulated contigs at 5 °C, suggesting that this gene family is not utilised as prevalently
426 after heat shock. This finding was born out by our analysis of discrete contig up- and down-
427 regulation, as discussed in detail elsewhere in this manuscript.

428

429 *Identification of Target Signalling Cascades*

430 Given the large number of un-annotatable, novel contigs in the top 20-most differentially
431 expressed complement, we also adopted a target gene approach. From the lists of differentially
432 expressed genes, we have selected genes previously identified as playing a role in thermal
433 response for further study, as well as transcription factors with potential roles as cellular
434 mediators of this process, as can be seen in Table 4. Full details of the exact expression levels
435 of all these genes can be found in Supplementary File 4.

436 Heat shock proteins (HSPs) were prevalent in these complements. We obtained 4
437 contigs encoding a full-length sequence, corresponding to four isoforms of the gene encoding
438 for HSP70 and related proteins. These four sequences were designed as A1, A2, B and HSP-er
439 (contigs: TRINITY_DN27082_c0_g1, TRINITY_DN12545_c0_g2, TRINITY_DN24395_c0_g1,

440 and TRINITY_DN20850_c0_g1_i1, respectively). The alignment of these four putative HSP70
441 sequences from *Isodictya* sp. alongside those previously described in sponges and other
442 marine invertebrates, as well as related species, can be seen in Fig 4A (Full sequences, along
443 with alignment, Supplementary File 6).

444 The full-length sequence of these proteins showed the presence of three classical
445 signature motifs of the HSP70 family (IDLGTTYS; IIDLGGGTFDVSIL; IVLVGGSTRIPKI/VQK)
446 and the ATP/GTP binding site (Fig 4B). These four forms of HSP70 in *Isodictya* possess
447 relatively low sequence similarity (between 41.4% and 68.1% amino acid identity), and
448 individual isoforms are more closely related to some previously described forms, which may
449 indicate that HSP70A and B are ancestrally shared paralogues within the Porifera (Fig 4A). The
450 HSP70A1 and 2 genes are most closely related to the non-inducible or HSP70 cognate (HSC)
451 described for others invertebrates and vertebrates (Fig 4, Supplementary File 7). Their C-
452 terminal region contains five repeats of GGMP motif. The tetrapeptide motif GGMP repeats are
453 thought to be involved in co-chaperone-binding activities of constitutive forms[68]. HSP-A
454 sequences have a terminal motif (EEVD) indicating their cytoplasmatic localisation[69]. We do not
455 observe a clear mitochondrial-located HSP70 homologue, but HSP-er shows clear homology to
456 known endoplasmic reticulum isoforms, and clear sequence similarity to those from both
457 vertebrate and invertebrate species (Fig 4B).

458 In contrast, the isoform HSP70B showed high sequence similarity to the HSP70
459 sequence of *Xestospongia testudinaria* (75.4%) and this sequence does not possess
460 tetrapeptide repeat sequences at the C-terminal (Fig 4B). HSP-B is therefore likely the
461 "inducible" HSP gene in this species. It was up-regulated during thermal stress in this
462 experiment, listed among the most strongly-changed in expression (Table 3), and thus shares
463 characteristics of inducible isoforms previously described in other marine invertebrates such as
464 oysters (see [70]).

465 In other species, the transcription of HSPs is upregulated in response to a variety of
466 stresses[71], preventing the mis-folding and aggregation of proteins, but is notably absent in some
467 species of Antarctic fish where HSPs are constitutively turned on in some cases[67]. HSPs
468 however play a clear role in temperature response in Antarctic invertebrates [17,28,29]. Given the
469 upregulation of isoform B here, it is clear that this role is conserved in sponges, and this
470 collaborates findings previously in this phylum[37,72].

471 A number of contigs with sequence similarity to the *Tumor necrosis factor* gene family
472 were observed in our dataset, in both our upregulated and downregulated complements (n.b.
473 different contigs were present in the two complements). This finding dovetails with the
474 observation of this in the GO term over-representation analysis for contigs downregulated in our
475 5 °C treatment. This cytokine family is responsible for regulating cell death through ubiquitins,
476 but also has other functions, including the regulation of cell survival, proliferation and
477 differentiation[73,74] and the downregulation of some members of this class and upregulation of
478 others likely reflects changing cellular processes within our sponge samples of interest to those
479 studying the molecular processes underlying thermal stress responses. A single gene linked to
480 this has previously been observed to be upregulated after heat shock in sponges (*TNFAIP3*[37])
481 and the additional sequences observed here confirm the likely role of these genes in linking with
482 ubiquitins and mediating cell death and protein degradation in Porifera after heat exposure.

483 The ubiquitin related genes themselves, which play essential roles in protein degradation
484 [75,76], were well recovered in our transcriptome. Fig. 5 shows our annotation of the proteolytic
485 pathway mediated by these genes, as annotated by bi-directional best blast hit. Of genes that
486 are missing, few (12) may be truly absent from our transcriptomic resource- others are also
487 absent from *A. queenslandica* and may be absent ancestrally. Three components of this
488 pathway, TRINITY_DN31115_c0_g1 (*UBE2M; UBC12; ubiquitin-conjugating enzyme E2 M)*,
489 TRINITY_DN8356_c0_g2 (*UBC1; ubiquitin-conjugating enzyme,* aka *HIP2; huntingtin*
490 *interacting protein 2)* and TRINITY_DN25219_c0_g1 (*UBE3A; E6AP; ubiquitin-protein ligase*

491 *E3A*) were significantly upregulated in our dataset in response to heat exposure. These genes,
492 representing E2 and HECT E3 type ubiquitin conjugating genes could play specific roles in the
493 degeneration of misfolded and degenerated peptides after heat exposure, correlates well with
494 previous findings in other sponge species such as *Haliclona tubifera*[37] and the specific genes
495 are worth further consideration as potential mediators of this intracellular protective response.

496 We found a number of notable transcription factor and cell signalling pathways in our up-
497 and down-regulated gene lists, including genes in the Notch, Wnt, TNF, Sox and TGF-β
498 pathways. These genes do not need to be the most up-/down-regulated genes to have a noted
499 effect on transcription, and they represent intriguing targets as possible controllers of wider
500 processes of molecular adaptation.

501 In the most similar study to this work previously performed, in the warm water sponge *H.*
502 *tubifera* from Philippines[37], multiple *G protein coupled receptors* (GPCRs) were noted as
503 differentially expressed. These genes are posited to allow these sponges to monitor and
504 respond to their environment effectively. Several families of GPCR were noted as effected by
505 heat exposure in *H. tubifera,* including *glutamate*, *rhodopsin* and *secretin GPCRs*. None of
506 these were observed here, and therefore the light and contraction responses seen in *H. tubifera*
507 on exposure to heat stress do not seem to be conserved in *Isodictya* sp. Instead, two families of
508 orphan GPCR, *157* and *161*, were noted in our sample. The roles of these specific GPCR in
509 sponges, and in general outside the Chordata, are poorly understood, but their marked up-
510 regulation here, and the absence of the plethora of differentially expressed GPCR seen in *H.*
511 *tubifera,* suggests that these explicit genes may play specific roles in sensing or recovering from
512 heat exposure in this particular species.

513 Other examples of key cell signalling molecules identified in our study include members
514 of the Sox and Wnt gene families, notably *Sox2* and *dishevelled*. These are possibly key in the
515 control of growth response to heat stress - *Sox2* in particular is known for its role in the control
516 of pluripotency in stem cell lineages[77]. Growth will both be promoted by higher temperatures,

517 but also necessary for repairing tissue damage caused by exposure to deleterious conditions.
518 However, by far the most commonly observed family of cell signalling molecules are
519 representatives of the BMP/TGF-β pathway. A variety of contigs with blast similarity to the TGF-
520 β family of signalling molecules were observed in our differentially expressed complements,
521 including a variety of contigs annotated as bone morphogenic proteins and growth differentiation
522 factors, as well as *one eyed pinhead* and the *Tolloid metalloprotease*, which act to modulate
523 TGF-β signalling. These molecules are well known as modulators of cell fates, but any role in
524 controlling response to thermal stress has not before been noted in sponges. It is possible that
525 these molecules are acting downstream of HSP70 (as noted by Lee et al.[78]) or as part of the
526 control of molecular response to physical damage caused by high temperatures [79]. However,
527 the phylogenetic distance between the organisms where this has been studied and sponges is
528 vast, and more targeted analysis is necessary to discern the true roles of these crucial
529 molecules in this Phylum.

530 We noted the presence of the Hedgehog family of genes in our dataset, with both
531 *hedgehog precursor* (TRINITY_DN46355_c0_g1_i1) and *indian hedgehog*
532 (TRINITY_DN16789_c0_g1_i1) automatically annotated – the latter will be a slight error, as
533 *indian hedgehog* is a chordate novelty generated by duplication into paralogues, and the name
534 of this gene's annotation will derive from sequence similarity. Both contigs are therefore likely to
535 represent variants of the *hedgehog* gene itself. These could perform key roles in the regulation
536 of response to thermal stress, as they are instrumental in the fine-tuning of cell differentiation
537 responses [80]. We also note the presence of members of the Notch signalling cascade in the
538 differentially expressed complements following heat stress (e.g.TRINITY_DN30424_c0_g1_i1
539 *delta and Notch-like epidermal growth factor-related receptor-like*), with Notch-related contigs
540 downregulated after exposure to both 3 and 5 °C treatments. This likely reflects changes in how
541 cell differentiation is occurring. *Hedgehog* is often noted as a promoter of stem cell proliferation,

542 and the initial stages of heat stress response may involve this, in concert with genes such as
543 *Sox2* as noted above.

544 Marine polar organisms are interesting models for studies on the noxious effects of
545 reactive oxygen species (ROS) in cold waters. In order to cope with the formation of deleterious
546 ROS, some Antarctic marine invertebrates express enzymes and low-molecular weight
547 'scavengers' [81]. The accumulation of ROS during heat stress has been shown in previous
548 studies on Antarctic marine species [82,83], and are important to these species given the higher
549 levels of dissolved oxygen seen in cooler Antarctic waters. In our study, we have obtained a
550 good sequence coverage for several enzymes implicated in antioxidant defense
551 (Supplementary File 8). Two kinds of *superoxide dismutase* (SOD), each with different isoformal
552 variants, were identified in *Isodictya* sp. which are differentiated by the type of metal associated
553 with its active site of the enzyme: two contigs for *manganese SOD*
554 (TRINITY_DN32241_c0_g1_i1 and TRINITY_DN3280_c0_g2_i1) and four contigs for a
555 *copper/zinc SOD* (TRINITY_DN22531_ c0_g1_i1; TRINITY_DN17486_c0_g1_i1;
556 TRINITY_DN_65_c0_g1_i1; TRINITY_DN35772 _c0_g1_i1). The SOD enzyme catalyze the
557 first reaction in the dismutation of superoxide anions to water and hydrogen peroxide, before the
558 catalase enzyme transforms the hydrogen peroxide into water. This enzyme is represented by
559 three contigs that share high sequence identities with marine invertebrates such as molluscs
560 (TRINITY_DN_6504; 28381; 29099). Another transcript well represented in our dataset was the
561 *glutathione S-transferase* (*GST*) which plays a key role in the detoxification of ROS and the
562 regulation of redox balance[84]. In *Isodictya* sp. seven contigs were identified with high sequence
563 similarity to *GST*s. Previous studies have highlighted the upregulation of oxidative response and
564 antioxidant genes in response to thermal challenge in sponges, as also observed in corals[85]. In
565 our data, we observe the upregulation of several contigs with strong similarity to genes with
566 antioxidant activity, *glutaredoxin* (TRINITY_DN32811_c0_g1_i1) and *thioredoxin*
567 (TRINITY_DN32811_c0_g1_i3) [37]. We also see a contig upregulated with similarity to *oxidative*

568 *stress-induced growth inhibitor 2-like* (TRINITY_DN30012_c0_g1_i1), which could play a role in
569 downstream organisation after the detection of an oxidative stress state. It seems likely that
570 these sponges are under severe oxidative stress, and the expression of these genes reflects
571 this.

572 The presence of *allograft inflammatory factor* (TRINITY DN_12683_c0_g1_i1) in our
573 datasets was noted as these have been previously studied in Antarctic species such as the sea
574 urchin *Sterechinus neumayeri*[86]. The *Isodictya* AIF-1 sequence has a high sequence similarity
575 with that known from the sponge *Suberites domuncula,* and has two conserved calcium binding
576 motifs known as EF hands (Fig 6). AIF-1 sequences from several invertebrate and vertebrate
577 species were aligned and showed relatively high levels of conservation of the protein with both
578 groups. Phylogenetic analyses generated a congruent tree positioning the AIF from sponges in
579 a cluster with corals, distinct from vertebrate sequences. In general, *allograft inflammatory factor*
580 is known for its role in recovery from injury, rather than any specific role in protecting against
581 heat shock[87]. It has been studied in sponges previously[88], where it has been shown to act to
582 activate immunocyte-like activity, as well as in protection immediately after trauma. In the
583 Japanese oyster *Crassostrea gigas* AIF-1 stimulates hemocyte immune activation by enhancing
584 phagocytosis and expression of inflammatory cytokines[89]. Its role in the thermal stress response
585 might therefore be in protection against infection, rather than directly in tissue repair, but both
586 these processes are undoubtedly necessary under prolonged periods of acute thermal stress.

587 That Antarctic species of sponge utilize many of the same pathways to respond to
588 thermal stress as warmer water species is a useful if unsurprising finding. Their efficacy at
589 moderating response to different levels of stress does however seem to be diminished, with
590 many of these genes expressed at both 3 and 5 °C, with no significant difference in their
591 expression levels between these temperatures, as noted elsewhere in this work.

592

593 *Poriferan Responses to Temperature Stress*

594 Our results, when considered alongside previous findings in sponges from other
595 latitudes, corroborate previous conclusions regarding their transcriptomic response to acute
596 thermal stress, while suggesting specifically that cold-adapted sponges may have a limited
597 range of tolerance to increased temperatures. The changes in transcription which occurred in
598 our samples after acute short-term thermal exposure confirmed that a poriferan heat-response
599 is activated by increased temperatures, and is present in this species. The complements of all 4
600 replicates in both conditions were very similar in both overall pattern and individual composition,
601 with differentially regulated contigs often perturbed in common at both temperatures. Previous
602 work on sponges from other latitudes[37,54] showed that HSP70 and ubiquitin-related genes were
603 specifically upregulated in response to thermal stress. Our work echoes this finding, observing
604 the activation of similar genes, such as oxidation-stress related genes. The nature of the
605 thermal stress response therefore is broadly similar to that seen in other sponge species. What
606 may differ in Antarctic species of sponge is the limits of tolerance.

607 In other sponges, the degree and type of transcriptional response is temperature-
608 dependent, with specific genes activated at different temperatures[39], where the holobiont
609 changes, or [37], where the sponge itself adapts its transcription). In contrast, our findings showed
610 few significant differences in the transcriptional response of sponges at 3 and 5 °C. We also do
611 not see changes in quite the same genes, with the absence of differentially expressed GPCR
612 and toll-like receptor (TLR) signaling genes in our data a notable difference. While previous
613 research has demonstrated that some Antarctic species are not able to respond to additional
614 increases in seawater temperature, others, such as *Nacella concinna* or *Laternula elliptica*
615 showed a classic thermal stress response with over-expression of heat stress chaperones.

616 A number of differences are noted in the response of cold water sponges here, when
617 contrasted with those of warm-water species[37]. Particularly, there does not seem to be the
618 degree of incremental change that can be seen in the transcriptional profile of warm water
619 species to increasing temperatures, and an “all or nothing” response is observed instead. Since

our experiment exposed sponges to acute stress, our results did not test whether cold-adapted sponges could, in time, adapt to sequentially higher temperatures, and this would be a useful follow-up experiment to those described here. It may be possible that with a period of acclimation, sequential rises in temperature can be met by successive rounds of molecular adaptation. However, at present we cannot speculate as to whether that is possible in this species.

Conclusions

Climate change is a pressing issue globally, and will have a range of effects on organisms worldwide. While some of these effects are more obvious or better studied than others, it is some of the least-studied species which will bear the brunt of these conditions, and these will have a variety of knock-on effects. Here we have studied one such species, a locally abundant sponge, specialised to life in Antarctic conditions. We have generated an excellent reference transcriptome from a small number of replicate samples exposed to acute thermal stress, and begun to identify the molecular responses that these species will use to adjust to warmer ambient temperatures. Further, we have demonstrated the clear stress that even a modest increase in temperature over a short time frame (in this case, 4 hours) will place on such a specialist species.

Isodictya already exercises a full measure of transcriptomic response to ambient temperatures of 3 °C, and further acute stress at 5 °C leaves it little further “wriggle room”. Whether this stress results, in the wild, in the death of these sponges, or whether, given time, they will adapt to increased temperatures, is at present uncertain. However, in the interim the results presented here will allow us to begin to understand the impact of increased temperatures on these still under-investigated, but nonetheless vital, species.

Data Availability

All data are available either as Supplementary Files published on Figshare for direct download at https://figshare.com/s/0c90ae2e0b2a0524b036 and with DOI: 10.6084/m9.figshare.7048727, or from the NCBI SRA with accession number PRJNA415418.

Animal Ethics

No animal ethics approval procedure exists for Porifera, and no permission was required to perform the experiments described in this manuscript.

Permission to Carry Out Fieldwork

The study was conducted under the permit 806/2015 granted by the Chilean Antarctic Institute (INACH).

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Competing Interests

The authors declare they have no competing interests.

Author Contributions

MGA, MO, AF and CAC performed wet lab experiments. NJK, MGA, AR and CAC performed computational analyses. NJK, MGA and CAC wrote the main manuscript text. All authors gave final approval for publication.

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References

1. Bell JJ. The functional roles of marine sponges. Estuar Coast Shelf Sci. 2008 ;79 :341-353.

2. De Goeij JM, Van Oevelen D, Vermeij MJ, Osinga R, Middelburg JJ, de Goeij AF & Admiraal W. Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science. 2013 ;342 :108-110.

3. McClintock JB, Amsler CD, Baker BJ & van Soest RWM. Ecology of Antarctic marine sponges: an overview. Int Comp Biol. 2005;45, 359-368.

4. Cárdenas CA et al. Sponge richness on algae-dominated rocky reefs in the Western Antarctic Peninsula and the Magellan Strait. Polar Res. 2016 ;35, 30532.

5. Cárdenas CA & Montiel A. Coexistence in Cold Waters: Animal Forests in Seaweed-Dominated Habitats in Southern High Latitudes in Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots (ed. Rossi S., Bramanti L., Gori A. & Orejas C.) 1-20 (Springer, 2017).

695 6. Gutt J et al. Antarctic marine animal forests: three-dimensional communities in Southern
696 Ocean ecosystems. Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots (ed
697 Rossi S, Bramanti L, Gori A & Orejas C.) 1-29 (Springer, 2017).
698 7. Bell JJ et al. Global conservation status of sponges. Cons Biol. 2015;29,42–53.
699 8. Carballo JL & Bell JJ. Climate Change and Sponges: An Introduction in Climate Change,
700 Ocean Acidification and Sponges (ed. Carballo JL & Bell JJ) 1-10 (Springer, 2017)
701 9. Meredith MP & King JC. Rapid climate change in the ocean west of the Antarctic Peninsula
702 during the second half of the 20th century. Geophys Res Lett. 2005;32, 19604–19609.
703 10. Stenni B, et al. Antarctic climate variability on regional and continental scales over the last
704 2000 years. Climate of the Past. 2017;13, 1609-1634.
705 11. IPCC Climate Change 2014: Impacts, Adaptation, and Vulnerability in Intergovernmental
706 Panel on Climate Change. Working Group II Report (IPCC, 2014).
707 12. Peck, L. 2018 Antarctic marine biodiversity: adaptations, environments and responses to
708 change. In: Hawkins SJ, Evans AJ, Dale AC, Firth LB and Smith IP, (eds.) Oceanography and
709 Marine Biology: An Annual Review, Volume 56. Taylor and Francis, 105-236.
710 13. Ingels J et al. Possible effects of global environmental changes on Antarctic benthos: a
711 synthesis across five major taxa. Ecol Evol. 2012;2, 453-485.
712 14. Berg MP et al. Adapt or disperse: understanding species persistence in a changing world.
713 Glob Change Biol. 2010;16, 587-98.
714 15. Peck L, Morley S, Richard J & Clark M. Acclimation and thermal tolerance in Antarctic
715 marine ectotherms. J Exp Biol. 2014;217, 16-22.
716 16. Suckling CC et al. Adult acclimation to combined temperature and pH stressors significantly
717 enhances reproductive outcomes compared to short-term exposures. J Anim Ecol. 2015;84,
718 773-784.
719 17. Clark MS et al. Biodiversity in marine invertebrate responses to acute warming revealed by
720 a comparative multi-omics approach. Glob Change Biol. 2017;23: 318-330.

721 18. Ramsby BD, Hoogenboom MO, Smith H A, Whalan S & Webster NS. The bioeroding
722 sponge *Cliona orientalis* will not tolerate future projected ocean warming. Sci Rep. 2018;8,
723 8302.
724 19. Duckworth AR, West L, Vansach T, Stubler A & Hardt M. Effects of water temperature and
725 pH on growth and metabolite biosynthesis of coral reef sponges. Mar Ecol Prog Ser. 2012;462,
726 67–77.
727 20. Kelmo F, Bell JJ & Attrill MJ. Tolerance of sponge assemblages to temperature anomalies:
728 resilience and proliferation of sponges following the 1997–8 El-Nino southern oscillation.
729 2013;PLoS ONE. 8, e76441.
730 21. Strand R et al. The response of a boreal deep-sea sponge holobiont to acute thermal stress.
731 Sci Rep. 2017;7, 1660.
732 22. Cebrian E, Uriz MJ, Garrabou J & Ballesteros E. Sponge mass mortalities in a warming
733 Mediterranean Sea: are cyanobacteria-harboring species worse off?. PLoS One. 2011;6, 20211.
734 23. Webster N et al. A complex life cycle in a warming planet: gene expression in thermally
735 stressed sponges. Mol Ecol. 2013;22, 1854–1868.
736 24. Ettinger-Epstein P, Whalan S, Battershill CN & de Nys R. Temperature cues gametogenesis
737 and larval release in a tropical sponge. Mar Biol. 2007;153,171–178.
738 25. Massaro AJ, Weisz JB, Hill MS & Webster NS. Behavioral and morphological changes
739 caused by thermal stress in the great barrier reef sponge *Rhopaloeides odorabile*. J Exp Mar
740 Biol Ecol. 2012;417, 55–60.
741 26. Cerrano C et al. A catastrophic mass-mortality episode of gorgonians and other organisms
742 in the Ligurian Sea (North-western Mediterranean), summer 1999. Ecol Lett. 2000;3, 284-293.
743 27. Peck LS. Prospects for survival in the Southern Ocean: vulnerability of benthic species to
744 temperature change. Antarct Sci. 2005;17, 497-507.
745 28. Clark MS et al. Insights into shell deposition in the Antarctic bivalve *Laternula elliptica*: gene
746 discovery in the mantle transcriptome using 454 pyrosequencing. BMC Genom. 2010;11, 362.

747 29. Johnson KM & Hofmann GE. A transcriptome resource for the Antarctic pteropod *Limacina*
748 *helicina antarctica*. Mar Genom. 2016;28, 25-28.
749 30. Klinck JM, Hofmann EE, Beardsley RC, Salihoglu B & Howard S. Water-mass properties
750 and circulation on the west Antarctic Peninsula Continental Shelf in Austral Fall and Winter
751 2001. Deep Sea Res II: Topical Studies in Oceanography, 51, 1925-1946 (2004).
752 31. Cárdenas CA, González-Aravena M & Santibañez PA. The importance of local settings:
753 within-year variability in seawater temperature at South Bay, Western Antarctic Peninsula.
754 PeerJ. 2018;6, e4289.
755 32. Cahais V et al. Reference-free transcriptome assembly in non-model animals from next-
756 generation sequencing data. Mol Ecol Resources. 2012;12, 834-845.
757 33. Goodwin S, McPherson JD & McCombie WR. Coming of age: ten years of next-generation
758 sequencing technologies. Nat Rev Genet. 2016;17, 333-351.
759 34. Pérez-Porro AR, Navarro-Gómez D, Uriz MJ & Giribet, G. A NGS approach to the
760 encrusting Mediterranean sponge *Crella elegans* (Porifera, Demospongiae, Poecilosclerida):
761 transcriptome sequencing, characterization and overview of the gene expression along three life
762 cycle stages. Mol Ecol Res. 2013;13, 494-509.
763 35. Riesgo A, Farrar N, Windsor PJ, Giribet G & Leys SP. The analysis of eight transcriptomes
764 from all poriferan classes reveals surprising genetic complexity in sponges. Mol Biol Evol.
765 2014;31, 1102-1120.
766 36. Fernandez-Valverde SL, Calcino AD & Degnan BM. Deep developmental transcriptome
767 sequencing uncovers numerous new genes and enhances gene annotation in the sponge
768 Amphimedon queenslandica. BMC Genom.2015;16, 387.
769 37. Guzman C & Conaco C. Gene expression dynamics accompanying the sponge thermal
770 stress response. PLoS ONE. 2016;11, e0165368.

771 38. Erwin PM, Pita L, Lopez-Legentil S & Turon X. Stability of sponge-associated bacteria over
772 large seasonal shifts in temperature and irradiance. Appl Environ Microbiol. 2012;78, 7358–
773 7368.
774 39. Fan L, Liu M, Simister R, Webster NS & Thomas T. Marine microbial symbiosis heats up:
775 the phylogenetic and functional response of a sponge holobiont to thermal stress. ISME J.
776 2013;7, 991–1002.
777 40. Pita L, Turon X, Lopez-Legentil S & Erwin PM. Host rules: spatial stability of bacterial
778 communities associated with marine sponges (Ircinia spp.) in the Western Mediterranean Sea.
779 FEMS Microbiol Ecol. 2013;86, 268–276.
780 41. Simister R, Taylor MW, Tsai P & Webster N. Sponge-microbe associations survive high
781 nutrients and temperatures. PLoS ONE. 2012;7, e52220.
782 42. Lopez-Legentil S, Song B, McMurray SE & Pawlik JR. Bleaching and stress in coral reef
783 ecosystems: hsp70 expression by the giant barrel sponge *Xestospongia muta*. Mol Ecol.
784 2008;17, 1840–1849.
785 43. Andrews S. FastQC: A quality control tool for high throughput sequence data. Babraham
786 Bioinformatics. 2010; http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
787 44. Bolger AM, Lohse M & Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence
788 data. Bioinformatics. 2014;30, 2114–2120.
789 45. Li H. Seqtk: a fast and lightweight tool for processing FASTA or FASTQ sequences, Github.
790 2013; https://github.com/lh3/seqtk.
791 46. Grabherr MG et al. Full-length transcriptome assembly from RNA-Seq data without a
792 reference genome. Nat Biotechnol. 2011;29, 644–652.
793 47. Schmieder R & Edwards R. Fast identification and removal of sequence contamination from
794 genomic and metagenomic datasets. PLoS One. 2011;6, e17288.
795 48. Altschul SF et al. Basic local alignment search tool. J. Mol Biol. 1990;215, 403-10.

796 49. Katoh K & Standley DM. MAFFT multiple sequence alignment software version 7:
797 improvements in performance and usability. Mol Biol Evol. 2013;30, 772-780.
798 50. Castresana J. Selection of conserved blocks from multiple alignments for their use in
799 phylogenetic analysis. Mol Biol Evol. 2000;17, 540-552.
800 51. Silvestro, M. raxmlGUI: a graphical front-end for RAxML. Organis Div Evol. 2012;12, 335-
801 337.
802 52. Kumar S, Stecher G & Tamura K. MEGA7: molecular evolutionary genetics analysis version
803 7.0 for bigger datasets. Mol Biol Evol. 2016;33, 1870-1874.
804 53. Larkin MA et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23, 2947-2948.
805 54. Gouy M, Guindon S & Gascuel, O. SeaView version 4: a multiplatform graphical user
806 interface for sequence alignment and phylogenetic tree building. Mol Biol Evol. 2009;27, 221-
807 224.
808 55. Simao FA, Waterhouse RM, Ioannidis P, Kriventseva EV & Zdobnov EM. BUSCO:
809 assessing genome assembly and annotation completeness with single-copy orthologs.
810 Bioinformatics. 2015;31, 3210–3212.
811 56. Conesa A et al. Blast2GO: a universal tool for annotation, visualization and analysis in
812 functional genomics research. Bioinformatics.2005;15, 3674-3676.
813 57. Moriya Y, Itoh M, Okuda S, Yoshizawa AC & Kanehisa M. KAAS: an automatic genome
814 annotation and pathway reconstruction server. Nucleic Acids Res. 2007;35, 182-185.
815 58. Li B & Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or
816 without a reference genome. BMC Bioinformat. 2011;12, 323.
817 59. Langmead B & Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods.
818 2012;9, 357-359.
819 60. Robinson MD, McCarthy DJ & Smyth GK. edgeR: a Bioconductor package for differential
820 expression analysis of digital gene expression data. Bioinformatics. 2010;1,139-140.

821 61. Benjamini Y & Hochberg Y. Controlling the false discovery rate: a practical and powerful
822 approach to multiple testing. J Royal Stat Soc B. 1995;57, 289-300.
823 62. Hansen KD, Brenner SE & Dudoit S. Biases in Illumina transcriptome sequencing caused by
824 random hexamer priming. Nucleic Acids Res. 2010;38, e131.
825 63. Woese CR, Kandler O & Wheelis ML. Towards a natural system of organisms: proposal for
826 the domains Archaea, Bacteria, and Eucarya. PNAS USA. 1990;87, 4576-4579.
827 64. Kenny NJ et al. Towards the identification of ancestrally shared regenerative mechanisms
828 across the Metazoa: a transcriptomic case study in the demosponge *Halisarca caerulea*. Mar
829 Genom. 2018;37, 135-147.
830 65. Cerrano C et al. Diatom invasion in the antarctic hexactinellid sponge *Scolymastra joubini*.
831 Polar Biol. 2000;23, 441-444.
832 66. Cerrano C, Cucchiari E, Di Camillo C, Totti C & Bavestrello G. The diversity of relationships
833 between Antarctic sponges and diatoms the case of *Mycale acerata* Kirkpatrick, 1907 (Porifera,
834 Demospongiae). Polar Biol. 2004;27, 231-237.
835 67. Hofmann GE, Buckley BA, Airaksinen S, Keen JE & Somero GN. Heat-shock protein
836 expression is absent in the antarctic fish *Trematomus bernacchii* (family Nototheniidae). J Exp
837 Biol. 2000;203, 2331-2339.
838 68. Cascella K et al. Diversification, Evolution and Sub-Functionalization of 70kDa Heat- Shock
839 Proteins in Two Sister Species of Antarctic Krill: Differences in Thermal Habitats, Responses
840 and Implications under Climate Change. PLoS ONE. 2015;10, 0121642.
841 69. Shonhai A, Boshoff A & Blatch GL. The structural and functional diversity of Hsp70 proteins
842 from *Plasmodium falciparum*. Prot Sci, 2007;16(9), 1803–1818.
843 70. Piano A, Franzellitti S, Tinti F & Fabbri E. Sequencing and expression pattern of inducible
844 heat shock gene products in the European flat oyster, *Ostrea edulis*. Gene. 2005;361, 119–126.
845 71. Craig EA & Gross CA. Is hsp70 the cellular thermometer?. Trends Biochem Sci.
846 1991;16,135-140.

847 72. Krasko A et al. Diagnosis of sublethal stress in the marine sponge *Geodia cydonium*:
848 application of the 70 kDa heat-shock protein and a novel biomarker, the Rab GDP dissociation
849 inhibitor, as probes. Aquat Toxicol. 1997;37,157-168.
850 73. Vilcek J & Lee TH. Tumor necrosis factor. New insights into the molecular mechanisms of its
851 multiple actions. J Biol Chem. 1991;266, .7313-7316.
852 74. Wang X & Lin Y. Tumor necrosis factor and cancer, buddies or foes?. Acta Pharmacologica
853 Sinica. 2008;29, 1275-1288.
854 75. Glickman MH & Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction
855 for the sake of construction. Physiol Rev. 2002;82, 373-428.
856 76. Mukhopadhyay D & Riezman H. Proteasome-independent functions of ubiquitin in
857 endocytosis and signaling. Science. 2007;315, 201-205.
858 77. Rizzino A. Sox2 and Oct 3/4: a versatile pair of master regulators that orchestrate the self-
859 renewal and pluripotency of embryonic stem cells. WIREs Syst Biol Med. 2009;1, 228-236.
860 78. Yao Y, Watson AD, Ji S & Boström KI. Heat shock protein 70 enhances vascular bone
861 morphogenetic protein-4 signaling by binding matrix Gla protein. Circulation Res. 2009;105,
862 575-584.
863 79. Lee SB, Lim AR, Rah DK, Kim KS & Min HJ. Modulation of heat shock protein 90 affects
864 TGF-β-induced collagen synthesis in human dermal fibroblast cells. Tiss Cell. 2016;48, 616-
865 623.
866 80. Ingham PW & McMahon AP. Hedgehog signaling in animal development: paradigms and
867 principles. Genes Dev. 2001;15, 3059-3087.
868 81. Camus L, Gulliksen B, Depledge M H & Jones M B. Polar bivalves are characterized by high
869 antioxidant defences. Polar Res. 2005;24,1-2.
870 82. Abele D, Burlando B, Viarengo A & Pörtner HO. Exposure to elevated temperatures and
871 hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal
872 limpet *Nacella concinna*. Comp Biochem Physiol B. 1998;120, 425-435.

873 83. Heise K, Puntarulo S, Portner HO & Abele D. Production of reactive oxygen species by
874 isolated mitochondria of the Antarctic bivalve *Laternula elliptica* (King and Broderip) under heat
875 stress. Comp Biochem Physiol C. 2003;134, 79-90.
876 84. Kim M, Ahn IY, Cheon J & Park H. Molecular cloning and thermal stress-induced expression
877 of a pi-class glutathione S-transferase (GST) in the Antarctic bivalve *Laternula elliptica*. Comp
878 Biochem. Physiol A Mol Integr Physiol, 2009;152, 207-213.
879 85. DeSalvo MK et al. Differential gene expression during thermal stress and bleaching in the
880 Caribbean coral *Montastraea faveolata*. Mol Ecol. 2008;17, 3952–3971.
881 86. Ovando F et al. Cloning and expression analysis of allograft inflammatory factor type 1 in
882 coelomocytes of Antarctic sea urchin (*Sterechinus neumayeri*). J Shellfish Res. 2012;31. 875-
883 883.
884 87. Utans U, Arceci RJ, Yamashita Y & Russell ME. Cloning and characterization of allograft
885 inflammatory factor-1: a novel macrophage factor identified in rat cardiac allografts with chronic
886 rejection. J Clinic Invest. 1995;95 2954.
887 88. Kruse M, Steffen R, Batel R, Muller IM & Muller WE. Differential expression of allograft
888 inflammatory factor 1 and of glutathione peroxidase during auto-and allograft response in
889 marine sponges. J Cell Sci. 1999;112, 4305-4313.
890 89. Zhang Y, Li J, Yu F, He X & Yu Z. Allograft inflammatory factor-1 stimulates hemocyte
891 immune activation by enhancing phagocytosis and expression of inflammatory cytokines in
892 *Crassostrea gigas*. Fish Shellfish Immunol. 2013;34, 1071-1077.
893 90. Peck LS. A Cold Limit to Adaptation in the Sea. Trends Ecol Evol. 2016;31, 13-26.

894 **Tables**

895

896 *Table 1:* Raw Read Data, Before and After Cleaning

897

Metric	Control (209)	3 °C Replicate A (117)	3 °C Replicate B (123)	5 °C Replicate A (135)	5 °C Replicate B (141)
Total Read Pairs (Initial)	25,988,569	28,513,364	26,960,296	25,793,570	26,289,401
Total Read Pairs (After Cleaning)	18,784,882	20,653,864	19,553,789	18,122,106	18,763,103
Total Bases in All Reads (Initial)	5,249,690,938	5,759,699,528	5,445,979,792	5,210,301,140	5,310,459,002
Total Bases in All Reads (After Cleaning)	3,591,119,834	3,940,343,972	3,742,325,651	3,441,581,533	3,575,958,067
GC% (Initial)	45	46	45	46	45
GC% (After Cleaning)	45	46	44	46	44
Average Q (Initial)	35.75	35.75	35.8	35.55	35.75
Average Q (After Cleaning)	37.45	37.4	37.45	37.4	37.4
% less than Q20 (Initial)	4.55	4.55	4.55	5.05	4.7
% less than Q20 (After Cleaning)	0.2	0.2	0.2	0.25	0.25

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901 *Table 2:* Statistics, Reference Transcriptome Assembly

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Number of transcripts	90,188
Number of Trinity 'genes'	70,844
Total bp in assembly	59,274,448
Max contig length (bp)	19,068
Mean contig length (bp)	657.23
Median contig length (bp)	338
% GC	43.10 %
N20 Contig Length	2,921
N50 Contig Length	1,113
# contigs in N50	12,997
Number of transcripts over 1000 bp	14,633
Transcripts w/ blast hit	20,607
Transcripts w/ GO term	12,924

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907 *Table 3:* The 20 most up/downregulated contigs in each sample cross-comparison
908

Control vs 3 degrees, Downregulated	**Annotation**	**Control vs 5 degrees, downregulated**	**Annotation**	**3 vs 5 degrees, downregulated**	**Annotation**
TRINITY_DN20177_c1_g1	---NA---	TRINITY_DN24223_c2_g2	PLANT CADMIUM RESISTANCE 3-like	TRINITY_DN23764_c0_g2	polyadenylate-binding -interacting 1-like
TRINITY_DN33508_c0_g1	---NA---	TRINITY_DN20177_c1_g1	---NA---	TRINITY_DN24223_c2_g2	PLANT CADMIUM RESISTANCE 3-like
TRINITY_DN45390_c0_g1	---NA---	TRINITY_DN8570_c0_g1	hypothetical protein	TRINITY_DN12683_c0_g1	allograft inflammatory factor 1
TRINITY_DN33508_c0_g4	---NA---	TRINITY_DN32712_c4_g3	---NA---	TRINITY_DN6163_c0_g2	ubiquitin carboxyl-terminal hydrolase isozyme L3-like
TRINITY_DN35469_c0_g1	soluble calcium-activated nucleotidase 1 isoform X1	TRINITY_DN24027_c0_g1	---NA---	TRINITY_DN23190_c0_g1	---NA---
TRINITY_DN35123_c0_g3	---NA---	TRINITY_DN40223_c0_g1	---NA---	TRINITY_DN24468_c0_g2	---NA---
TRINITY_DN19331_c1_g1	hypothetical protein	TRINITY_DN8786_c0_g1	oxysterol-binding 1-like	TRINITY_DN30615_c2_g9	---NA---
TRINITY_DN24815_c0_g1	---NA---	TRINITY_DN35469_c0_g1	soluble calcium-activated nucleotidase 1 isoform X1	TRINITY_DN8356_c0_g2	ubiquitin-conjugating enzyme E2 K-like
TRINITY_DN8271_c0_g1	---NA---	TRINITY_DN45390_c0_g1	---NA---	TRINITY_DN26078_c0_g2	COMM domain-containing 8-like
TRINITY_DN31187_c0_g1	---NA---	TRINITY_DN33246_c0_g3	---NA---	TRINITY_DN58418_c0_g1	nucleoporin GLE1-like
TRINITY_DN49200_c0_g1	---NA---	TRINITY_DN33508_c0_g4	---NA---	TRINITY_DN35203_c0_g2	---NA---
TRINITY_DN33462_c0_g1	---NA---	TRINITY_DN11747_c0_g1	hypothetical protein, partial		
TRINITY_DN4804_c0_g2	hypothetical protein TRIADDRAFT_59764	TRINITY_DN24815_c0_g1	---NA---		
TRINITY_DN23863_c0_g1	---NA---	TRINITY_DN24481_c0_g1	---NA---		
TRINITY_DN33246_c0_g3	---NA---	TRINITY_DN30570_c0_g1	replicase poly		
TRINITY_DN30570_c0_g1	---NA---	TRINITY_DN35123_c0_g3	---NA---		
TRINITY_DN26078_c0_g1	COMM domain-containing 8-like	TRINITY_DN59119_c0_g1	---NA---		
TRINITY_DN12307_c0_g1	PREDICTED: uncharacterized protein	TRINITY_DN13825_c0_g1	endoplasmic reticulum-Golgi intermediate compartment 3-like		
TRINITY_DN36675_c0_g1	---NA---	TRINITY_DN26662_c0_g1	hypothetical protein crov191		
Control vs 3 degrees, Upregulated	**Annotation**	**Control vs 5 degrees, Upregulated**	**Annotation**	**3 vs 5 degrees, upregulated**	**Annotation**
TRINITY_DN24284_c0_	hypothetical protein	TRINITY_DN24284_c0_	hypothetical protein	TRINITY_DN33462_c0_	---NA---

g3	BRAFLDRAFT_114823	g3	BRAFLDRAFT_114823	g1	
TRINITY_DN44573_c0_g1	hypothetical protein	TRINITY_DN24223_c2_g1	PLANT CADMIUM RESISTANCE 3-like	TRINITY_DN53922_c0_g3	inactive peptidyl-prolyl cis-trans isomerase FKBP6-like
TRINITY_DN18944_c0_g2	40S ribosomal S13	TRINITY_DN44573_c0_g1	hypothetical protein	TRINITY_DN23764_c0_g1	polyadenylate-binding - interacting 1-like
TRINITY_DN33508_c0_g2	---NA---	TRINITY_DN22854_c0_g2	PREDICTED: tetraspanin-7-like	TRINITY_DN58083_c0_g1	---NA---
TRINITY_DN24223_c2_g1	PLANT CADMIUM RESISTANCE 3-like	TRINITY_DN8786_c0_g2	oxysterol-binding 1-like	TRINITY_DN23863_c0_g1	---NA---
TRINITY_DN34845_c4_g12	---NA---	TRINITY_DN29050_c0_g1	hypothetical protein		
TRINITY_DN34845_c4_g3	---NA---	TRINITY_DN18944_c0_g2	40S ribosomal S13		
TRINITY_DN22854_c0_g2	PREDICTED: tetraspanin-7-like	TRINITY_DN53805_c0_g1	PREDICTED: uncharacterized protein		
TRINITY_DN34845_c4_g9	---NA---	TRINITY_DN30712_c0_g1	---NA---		
TRINITY_DN22240_c0_g4	---NA---	TRINITY_DN58083_c0_g1	---NA---		
TRINITY_DN34845_c4_g10	---NA---	TRINITY_DN26231_c0_g4	dolichyl-diphosphooligosaccharide-- glycosyltransferase 48 kDa subunit-like		
TRINITY_DN44814_c0_g1	---NA---	TRINITY_DN27017_c0_g1	---NA---		
TRINITY_DN26511_c0_g1	hypothetical protein	TRINITY_DN34845_c4_g10	---NA---		
TRINITY_DN34845_c4_g4	---NA---	TRINITY_DN24395_c0_g1	heat shock 70 B2-like		
TRINITY_DN1002_c0_g1	predicted protein	TRINITY_DN34845_c4_g4	---NA---		
TRINITY_DN23682_c0_g2	---NA---	TRINITY_DN34845_c4_g12	---NA---		
TRINITY_DN26231_c0_g4	dolichyl-diphosphooligosaccharide-- glycosyltransferase 48 kDa subunit-like	TRINITY_DN34845_c4_g3	---NA---		
TRINITY_DN2258_c0_g1	40S ribosomal S25	TRINITY_DN22240_c0_g4	---NA---		
TRINITY_DN30873_c1_g2	---NA---	TRINITY_DN26511_c0_g1	---NA---		
TRINITY_DN20209_c0_g1	---NA---	TRINITY_DN34845_c4_g9	---NA---		

911 *Table 4:* Manually identified target genes

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Target gene approach					
Control vs 3 degrees, Downregulated	**Annotation**	**Control vs 5 degrees, downregulated**	**Annotation**	**3 vs 5 degrees, downregulated**	**Annotation**
TRINITY_DN49841_c0_g3_i1	tumor necrosis factor ligand superfamily member 15	TRINITY_DN49841_c0_g3_i1	tumor necrosis factor ligand superfamily member 15	TRINITY_DN23764_c0_g2	polyadenylate-binding -interacting 1-like
TRINITY_DN49577_c0_g1_i1	mesoderm development candidate 1-like	TRINITY_DN49577_c0_g1_i1	mesoderm development candidate 1-like	TRINITY_DN24223_c2_g2	PLANT CADMIUM RESISTANCE 3-like
TRINITY_DN34885_c0_g2_i2	neurogenic locus notch homolog 1-like	TRINITY_DN34885_c0_g2_i2	neurogenic locus notch homolog 1-like	TRINITY_DN12683_c0_g1	allograft inflammatory factor 1
TRINITY_DN28743_c0_g1_i1	E3 ubiquitin- ligase TRIM71-like	TRINITY_DN28743_c0_g1_i1	E3 ubiquitin- ligase TRIM71-like	TRINITY_DN6163_c0_g2	ubiquitin carboxyl-terminal hydrolase isozyme L3-like
TRINITY_DN28248_c0_g1_i1	ribosome-binding 1-like	TRINITY_DN33363_c1_g1_i1	G2 M phase-specific E3 ubiquitin- ligase- partial	TRINITY_DN8356_c0_g2	ubiquitin-conjugating enzyme E2 K-like
TRINITY_DN30424_c0_g1_i1	delta and Notch-like epidermal growth factor-related receptor-like	TRINITY_DN28248_c0_g1_i1	ribosome-binding 1-like	TRINITY_DN26078_c0_g2	COMM domain-containing 8-like
TRINITY_DN31499_c1_g3_i1	calmodulin isoform X1	TRINITY_DN4806_c0_g1_i1	polycomb group RING finger 1-like	TRINITY_DN58418_c0_g1	nucleoporin GLE1-like
TRINITY_DN29988_c0_g1_i1	TGF-beta receptor type-1-like	TRINITY_DN30424_c0_g1_i1	delta and Notch-like epidermal growth factor-related receptor-like		
TRINITY_DN35391_c8_g6_i2	TNF receptor-associated factor 5-like	TRINITY_DN31499_c1_g3_i1	calmodulin isoform X1		
TRINITY_DN29288_c0_g1_i1	G- coupled receptor 161-like	TRINITY_DN35391_c8_g6_i2	TNF receptor-associated factor 5-like		
TRINITY_DN54189_c0_g2_i1	probable G- coupled receptor 157	TRINITY_DN29288_c0_g1_i1	G- coupled receptor 161-like		
		TRINITY_DN54189_c0_g2_i1	probable G- coupled receptor 157		
Control vs 3 degrees, Upregulated	**Annotation**	**Control vs 5 degrees, Upregulated**	**Annotation**	**3 vs 5 degrees, upregulated**	**Annotation**
TRINITY_DN12683_c0_g1_i1	allograft inflammatory factor 1	TRINITY_DN25219_c0_g1_i1	ubiquitin- ligase E3A-like	TRINITY_DN53922_c0_g3	inactive peptidyl-prolyl cis-trans isomerase FKBP6-like
TRINITY_DN27456_c0_g1_i1	tolloid 1	TRINITY_DN40009_c0_g1_i1	transcription factor Sox-2-like	TRINITY_DN23764_c0_g1	polyadenylate-binding -interacting 1-like
TRINITY_DN25219_c0_g1_i1	ubiquitin- ligase E3A-like	TRINITY_DN24395_c0_g1_i1	heat shock 70 B2-like		
TRINITY_DN40009_c0_g1_i1	transcription factor Sox-2-like	TRINITY_DN33837_c0_g3_i1	TNF receptor-associated factor 5-like		
TRINITY_DN24395_c0_g1_i1	heat shock 70 B2-like	TRINITY_DN34897_c0_g1_i1	tolloid 2		
TRINITY_DN16789_c0_g1_i1	indian hedgehog	TRINITY_DN29751_c0_g1_i1	growth differentiation factor 7		
TRINITY_DN33837_c0_g3_i1	TNF receptor-associated factor 5-like	TRINITY_DN29790_c0_g3_i1	ubiquitin-60S ribosomal L40		
TRINITY_DN34897_c0_g1_i1	tolloid 2	TRINITY_DN35733_c0_g1_i1	probable E3 ubiquitin-ligase partial		

TRINITY_DN29790_c0_g3_i1	ubiquitin-60S ribosomal L40	TRINITY_DN16075_c0_g1_i1	bone morphogenetic 7		
TRINITY_DN28521_c0_g1_i1	bone morphogenetic 6-like	TRINITY_DN27508_c0_g1_i1	growth differentiation factor 8-like		
TRINITY_DN8356_c0_g2_i1	ubiquitin-conjugating enzyme E2 K-like	TRINITY_DN27456_c0_g1_i1	tolloid 1		
TRINITY_DN16075_c0_g1_i1	bone morphogenetic 7	TRINITY_DN34419_c1_g1_i1	pinhead precursor		
TRINITY_DN27508_c0_g1_i1	growth differentiation factor 8-like	TRINITY_DN30012_c0_g1_i1	oxidative stress-induced growth inhibitor 2-like		
TRINITY_DN31167_c0_g1_i2	segment polarity dishevelled homolog	TRINITY_DN32735_c0_g1_i2	G- coupled receptor 161-like		
TRINITY_DN46355_c0_g1_i1	hedgehog precursor				
TRINITY_DN34419_c1_g1_i1	pinhead precursor				
TRINITY_DN19433_c0_g1_i1	MULTISPECIES: cold-shock				
TRINITY_DN32811_c0_g1_i1	glutaredoxin				
TRINITY_DN32811_c0_g1_i3	thioredoxin				
TRINITY_DN33675_c0_g2_i1	alkyl hydroperoxide reductase				

Figure Legends

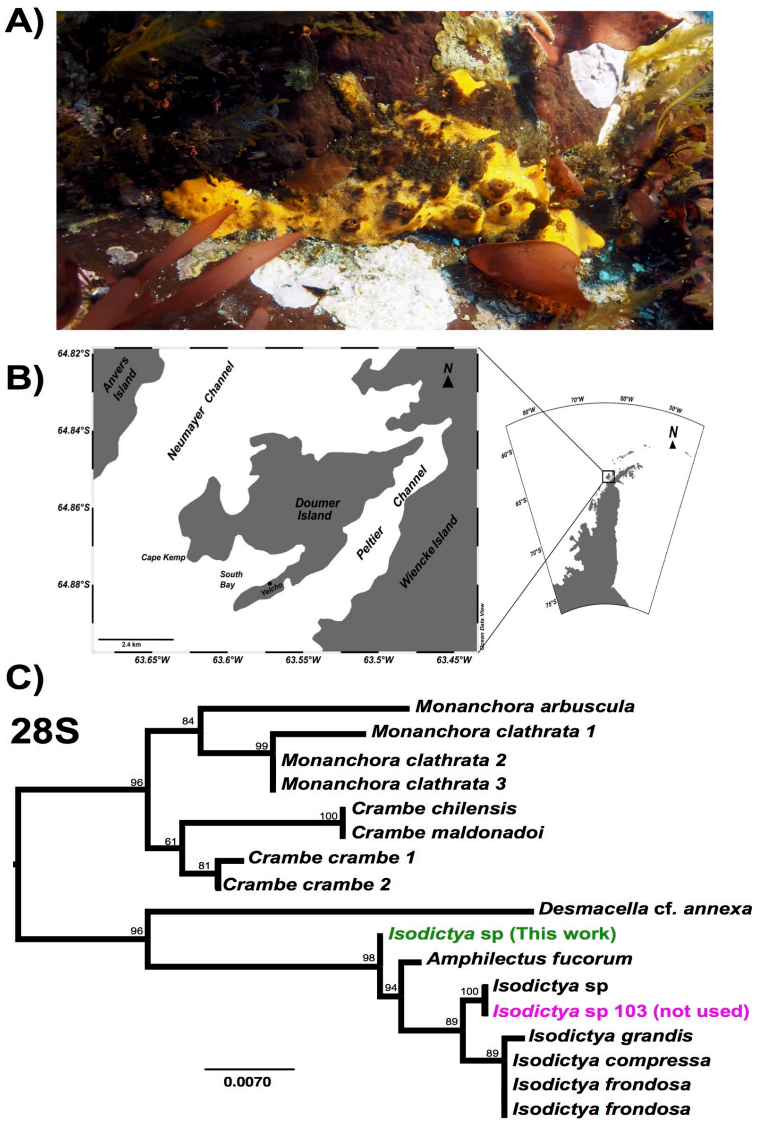

Figure 1: A) Image of *Isodictya* sp. collected at South Bay, WAP (10 m depth). B) Sample collection location, Doumer Island, WAP. C) RAxML trees of 28S rRNA under the GTR model from the *Isodictya* species mentioned in this manuscript, along with those of related species. Note the difference in placement of the samples used in the present manuscript (in green, *Isodictya* sp., this work) to that of one of the samples sequenced for this work, *Isodictya* sp. 103 (in magenta), which was excluded due to its being identity as a cryptic, but separate, species.

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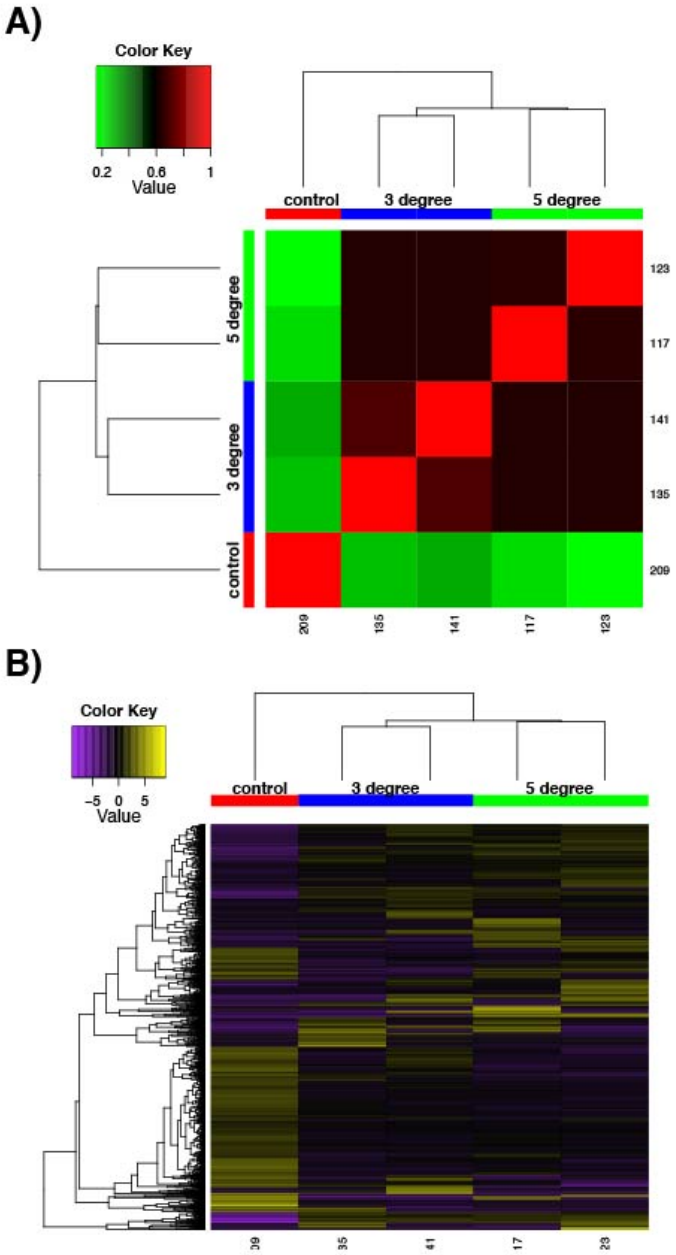

930

931 **Figure 2:** Differential expression analysis results performed by RSEM within the Trinity
932 framework, with “as gene” results shown. A) is the sample correlation matrix for the five samples
933 used in the final cross-comparison. B) shows the relative expression of each differentially
934 expressed contig across all the samples. Note that contigs occurring uniquely in these samples
935 are not included, as detailed in Methods. For both A and B, note the areas of the matrix shown
936 in black hues, indicating few measurable differences between-samples.

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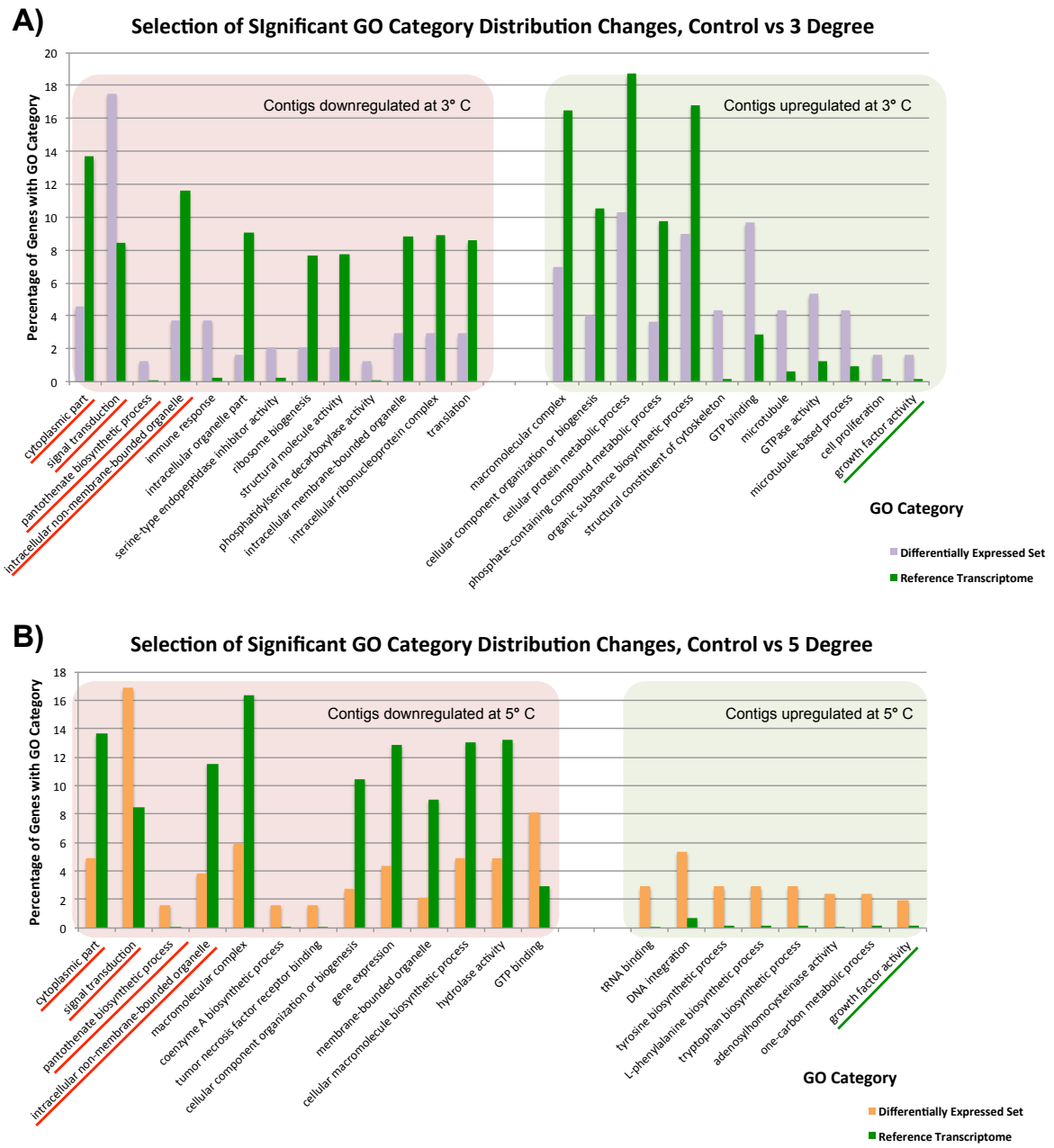


Figure 3: Significantly differentially represented GO categories from contigs up/down regulated at 3 and 5 °C compared to control sample. A) shows categories from our control vs 3 °C comparison, while B) shows those from the control vs 5 °C comparison. Those categories represented in both comparisons are underlined.

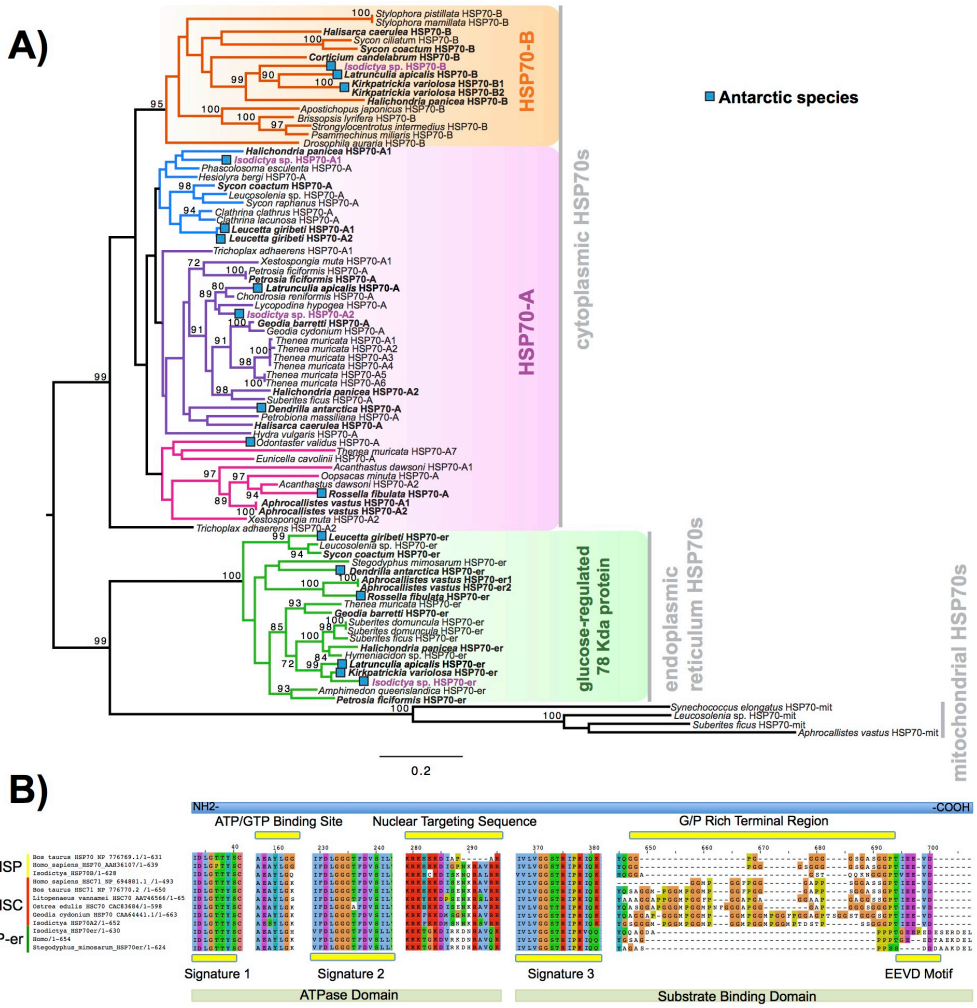


Figure 4: A) Maximum likelihood-derived phylogeny (RAxML, LG+I+G) of HSP70 sequences of known homology, together with novel *Isodictya* sequences (Names in purple). HSP70 sequences of Antarctic-resident species are bolded. Boxed as indicated on the figure are the HSP70-B, HSP70-A (HSC) and glucose-regulated 78 kDa HSP70 clades. Numbers at base of nodes indicate bootstrap support (as %ge of 1000 replicates). 5B: Multiple alignment of different HSP70 isoforms found in *Isodictya* sp. with (inducible) HSP70-B, sequence at top, HSP70-A (non-inducible heat-shock cognate) central, and below, HSP-mt sequences. These are shown alongside sequences from other species, showing the differences in domain content between isoforms. All HSPs have two main domains: the ATPase domain involved in ATP hydrolysis, and the substrate binding domain, which binds extended polypeptides, as shown at the bottom of the figure. Yellow boxes represent signature domains within HSP70 proteins. The G/P rich terminal region is prominent in HSPC (HSP-B). The EEVD sequence motif allows the cytoplasmic localisation of HSP and HSC isoforms. Accession numbers as provided in Figure, all sequences are provided, along with alignments, in Supplementary File 6.

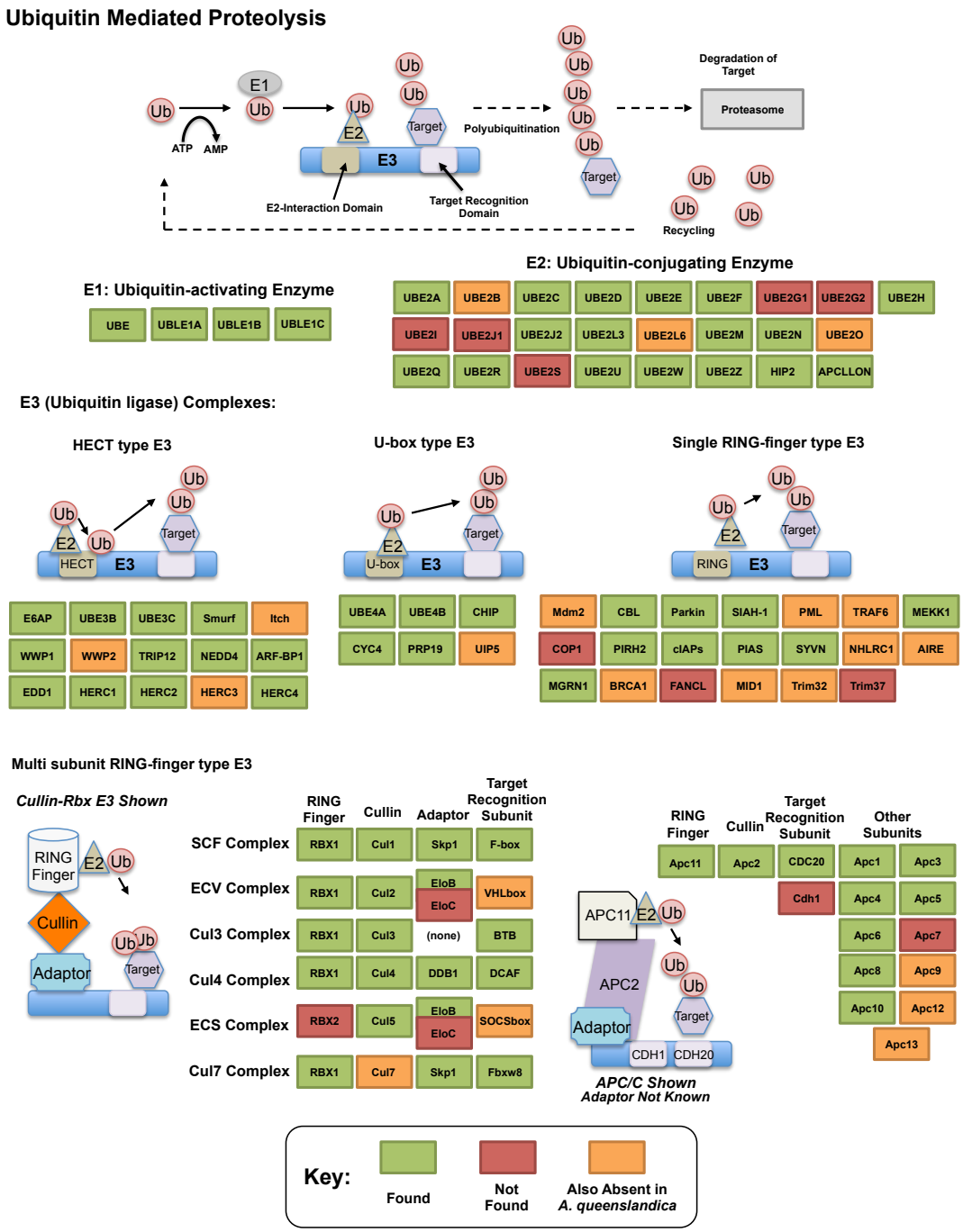
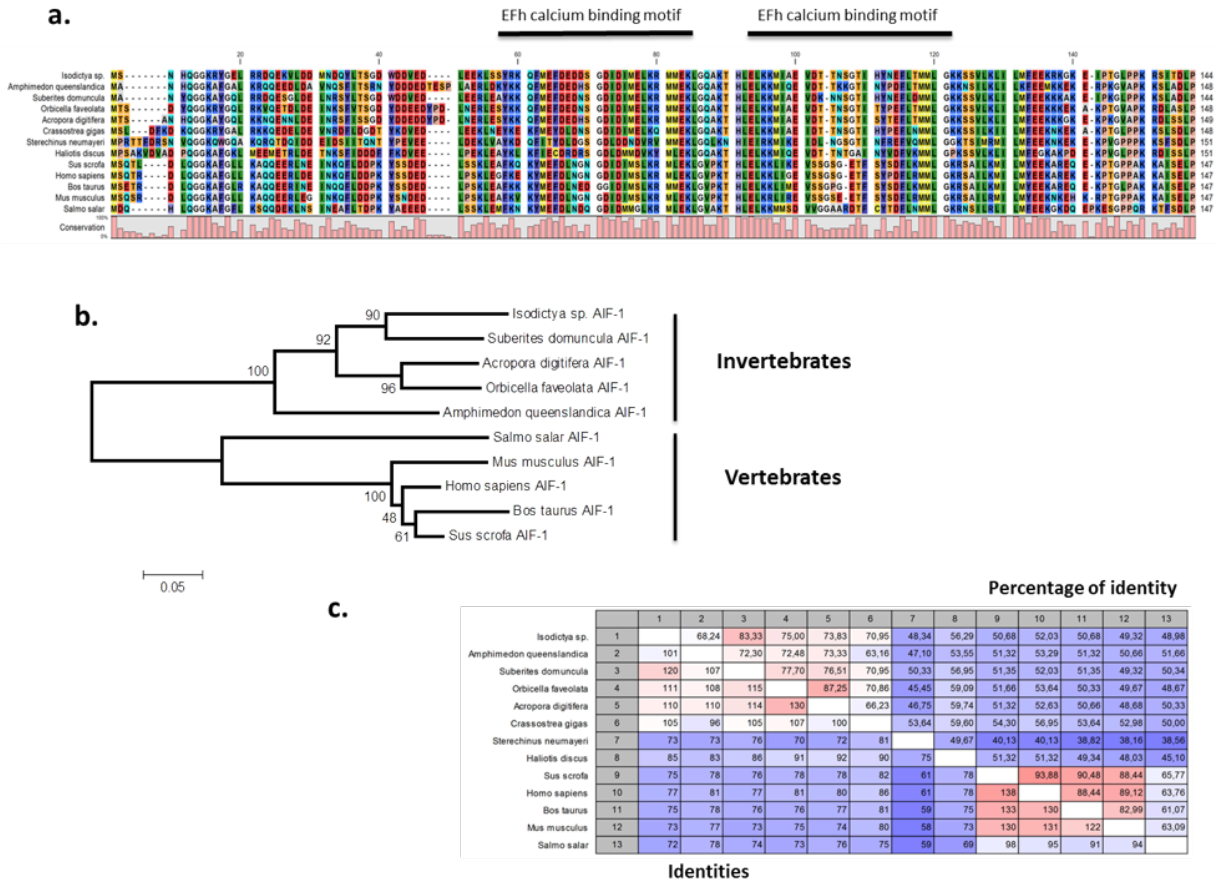


Figure 5: KEGG style map showing recovery of *Ubiquitin* mediated proteolysis genes in our resource as assessed by bi-directional best blast hit, alongside that of the sequenced genome of *Amphimedon queenslandica.* Genes noted as present in this resource indicated in green, with those absent in our transcriptome noted in orange if also missing from *A. queenslandica* (and therefore a possible poriferan absence), or red if absent from our resource only (and therefore a likely true absence).



	1	2	3	4	5	6	7	8	9	10	11	12	13
Isodictya sp. 1		68,24	83,33	75,00	73,83	70,95	48,34	56,29	50,68	52,03	50,68	49,32	48,98
Amphimedon queenslandica 2	101		72,30	72,48	73,33	63,16	47,10	53,55	51,32	53,29	51,32	50,66	51,66
Suberites domuncula 3	120	107		77,70	76,51	70,95	50,33	56,95	51,35	52,03	51,35	49,32	50,34
Orbicella faveolata 4	111	108	115		87,25	70,86	45,45	59,09	51,66	53,64	50,33	49,67	48,67
Acropora digitifera 5	110	110	114	130		66,23	46,75	59,74	51,32	52,63	50,66	48,68	50,33
Crassostrea gigas 6	105	96	105	107	100		53,64	59,60	54,30	56,95	53,64	52,98	50,00
Sterechinus neumayeri 7	73	73	76	70	72	81		49,67	40,13	40,13	38,82	38,16	38,56
Haliotis discus 8	85	83	86	91	92	90	75		51,32	51,32	49,34	48,03	45,10
Sus scrofa 9	75	78	76	78	78	82	61	78		93,88	90,48	88,44	65,77
Homo sapiens 10	77	81	77	81	80	86	61	78	138		88,44	89,12	63,76
Bos taurus 11	75	78	76	76	77	81	59	75	133	130		82,99	61,07
Mus musculus 12	73	77	73	75	74	80	58	73	130	131	122		63,09
Salmo salar 13	72	78	74	73	76	75	59	69	98	95	91	94	

Figure 6: Alignment and phylogenetic analysis of the AIF sequences found in our transcriptome. A) The conserved two EF-hand motifs are indicated in the alignment of several AIF-1 from other invertebrate and vertebrate sequences; b) Phylogenetic tree of the AIF-1 family using the N-J method. Bootstrap values represent the frequency of appearance (expressed as a percentage) of each clade of 1000 bootstrap replicates; c) Percentage amino acid identities between AIF-1 genes from invertebrates and vertebrates. The GenBank accession numbers for the sequences are as follows: *Suberites domuncula* CAC38780; *Acropora digitifera* XP015755194; *Orbicella faveolata* XP020615825; *Amphimedon queenslandica* XP003387413; *Salmo salar* AC169994; *Mus musculus*; *Homo sapiens* P55008; *Bos taurus* NP001071547; *Sus crofa* P81076; *Crassostrea gigas* NP001292275; *Sterechinus neumayeri* ACO40483; *Haliotis discus* ABH10674.

981 **Supplementary Files:** https://figshare.com/s/0c90ae2e0b2a0524b036

982

983 Supplementary File 1: Reference transcriptome assembly (.fasta, zipped)

984 Supplementary File 2: Blast2GO annotation file incorporating Annex and Interproscan results

985 (.annot, .b2g)

986 Supplementary File 3: KEGG KAAS annotation (.txt)

987 Supplementary File 4: Differential gene expression results (.txt, zip)

988 Supplementary File 5: Gene lists, overlapping in treatments (.txt)

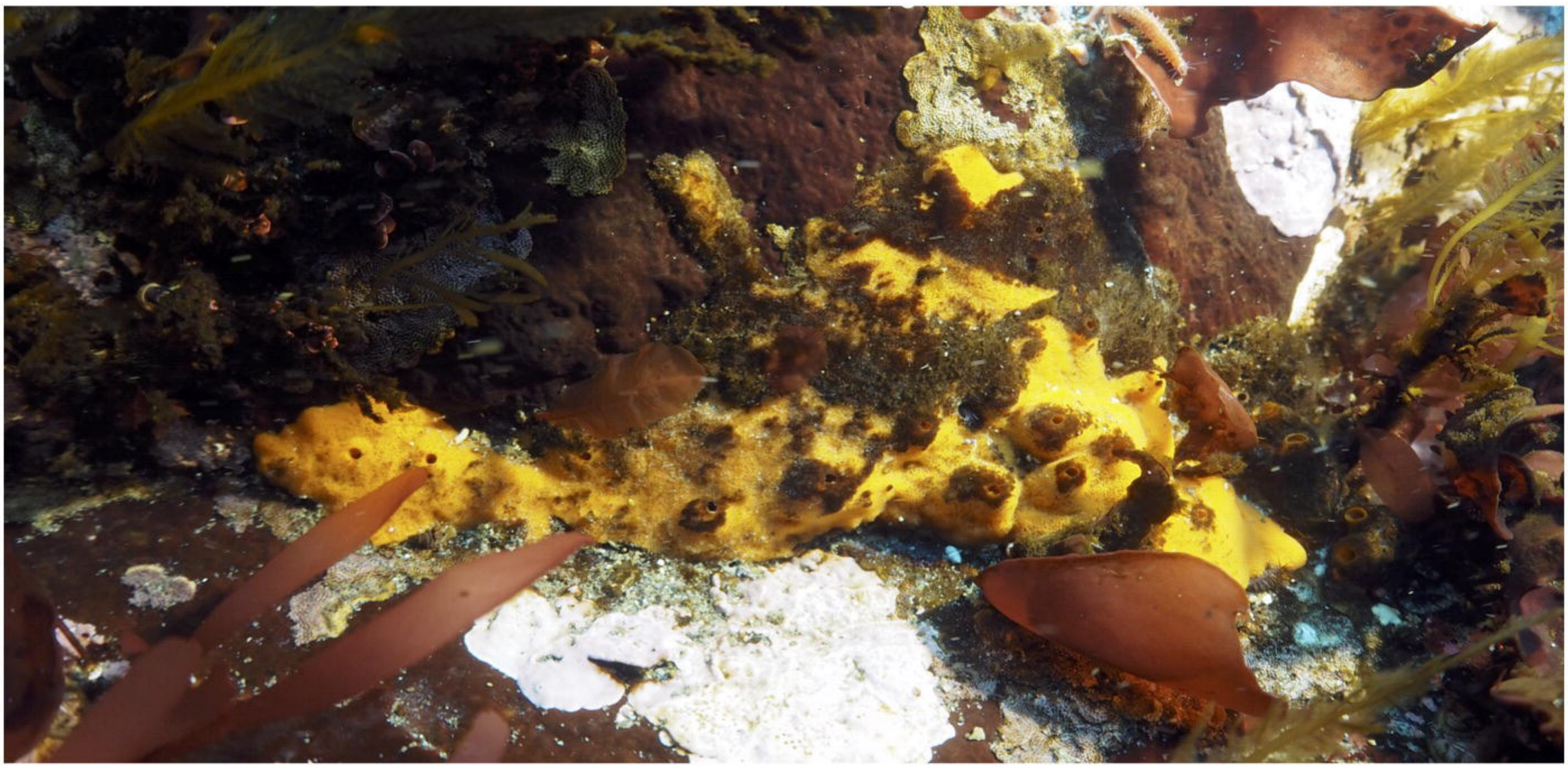
989 Supplementary File 6: HSP70 phylogeny alignment (.phy, .fas, .nxs, zipped)

990 Supplementary File 7: Percentage identity comparison, HSP70 genes (.jpg)

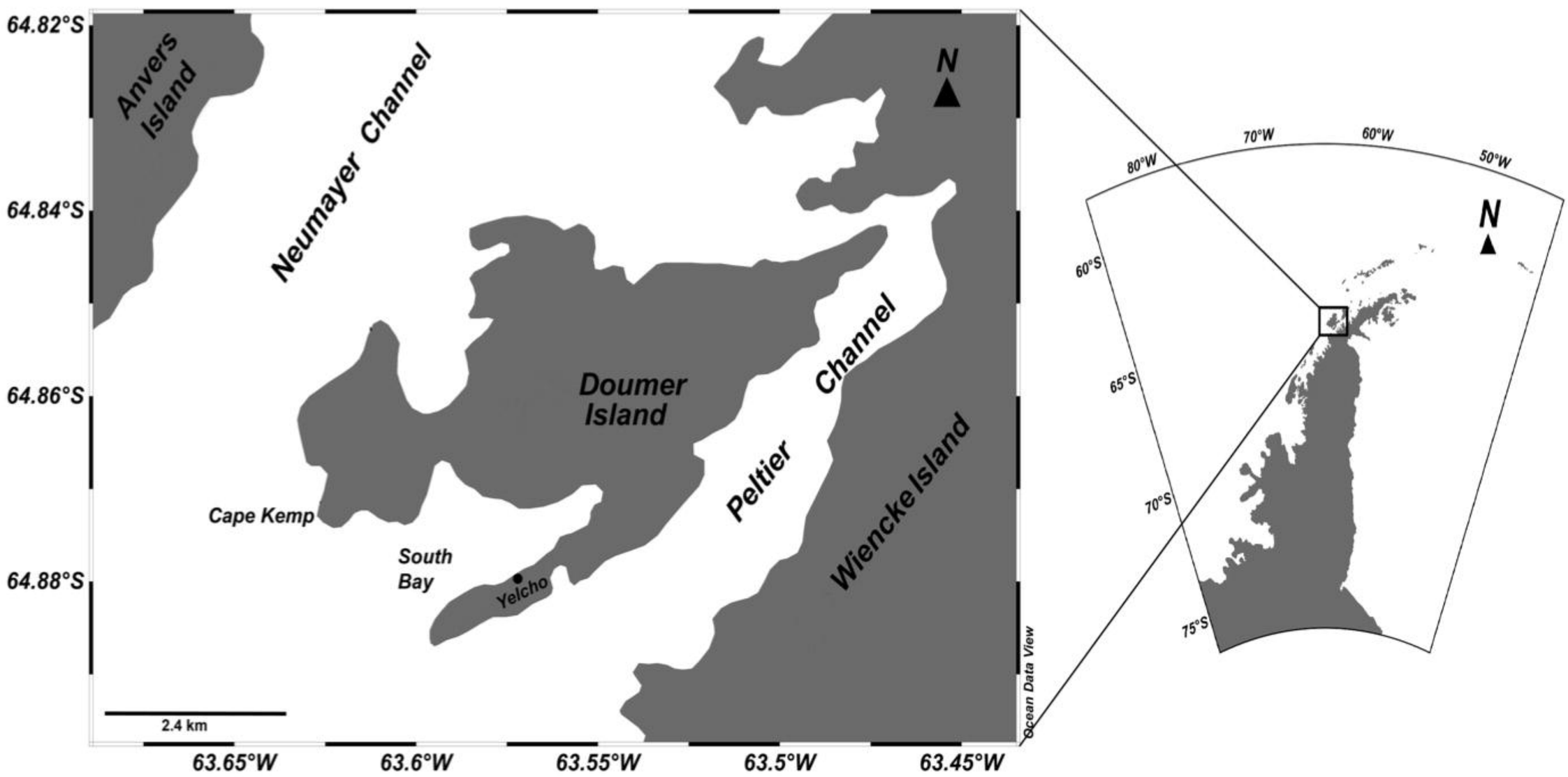
991 Supplementary File 8: Antioxidant sequences

992

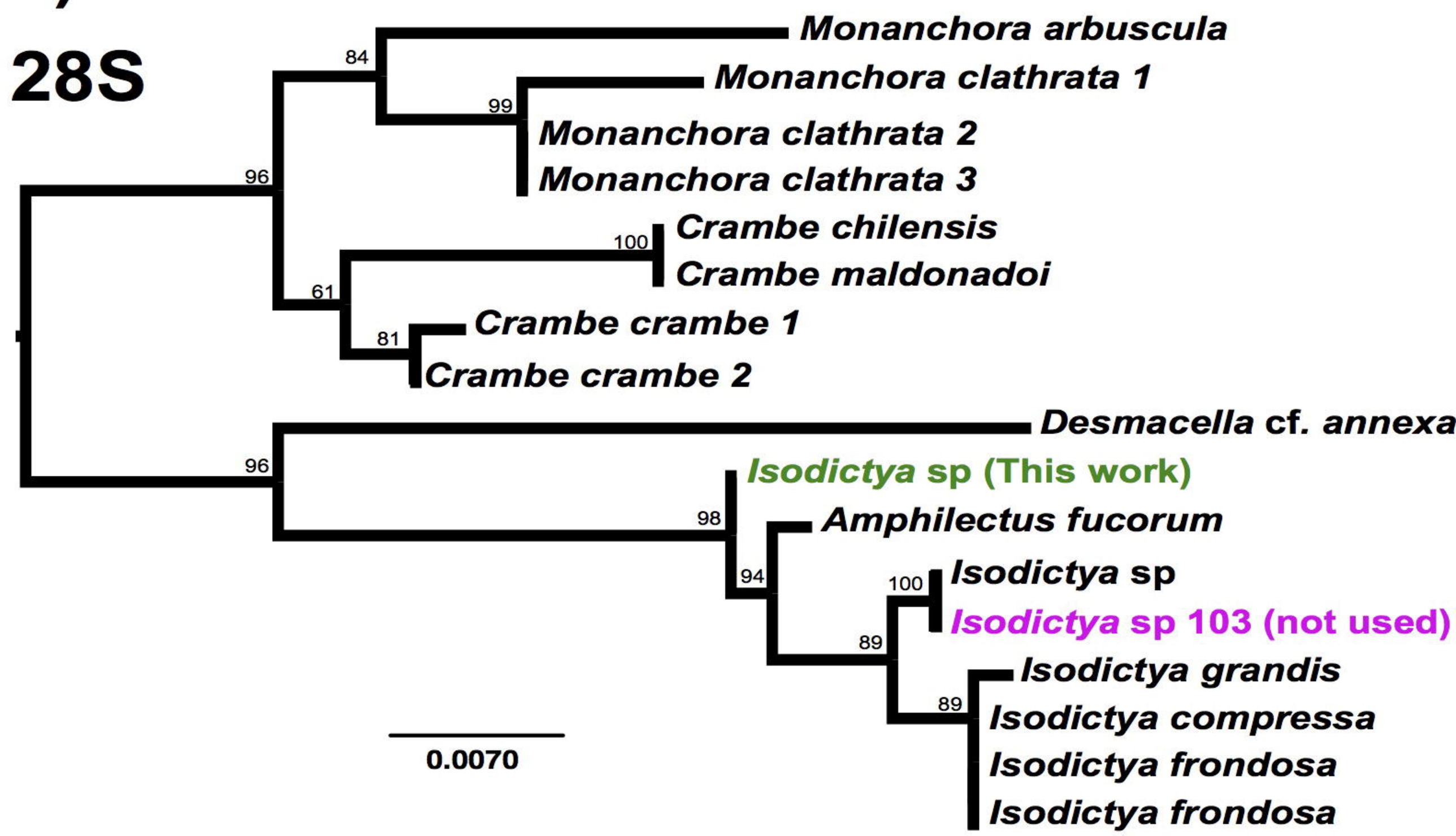
A)



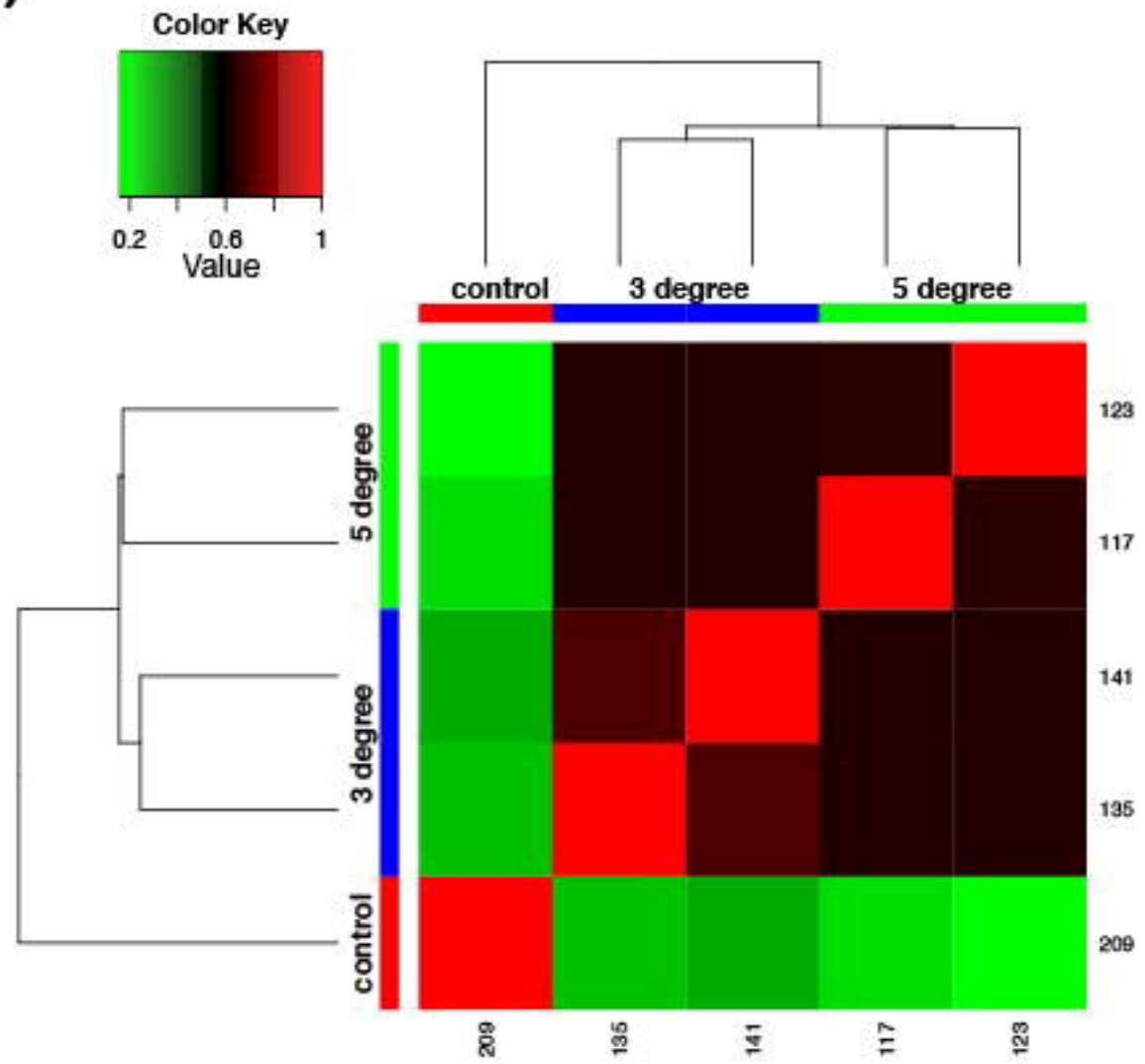
B)


64.82°S
64.84°S
64.86°S
64.88°S
Anvers Island
Neumayer Channel
Doumer Island
Peltier Channel
Wienckle Island
Cape Kemp
South Bay
Yelcho
2.4 km
63.65°W
63.6°W
63.55°W
63.5°W
63.45°W
N
Ocean Data View
80°W
70°W
60°W
50°W
60°S
65°S
70°S
75°S

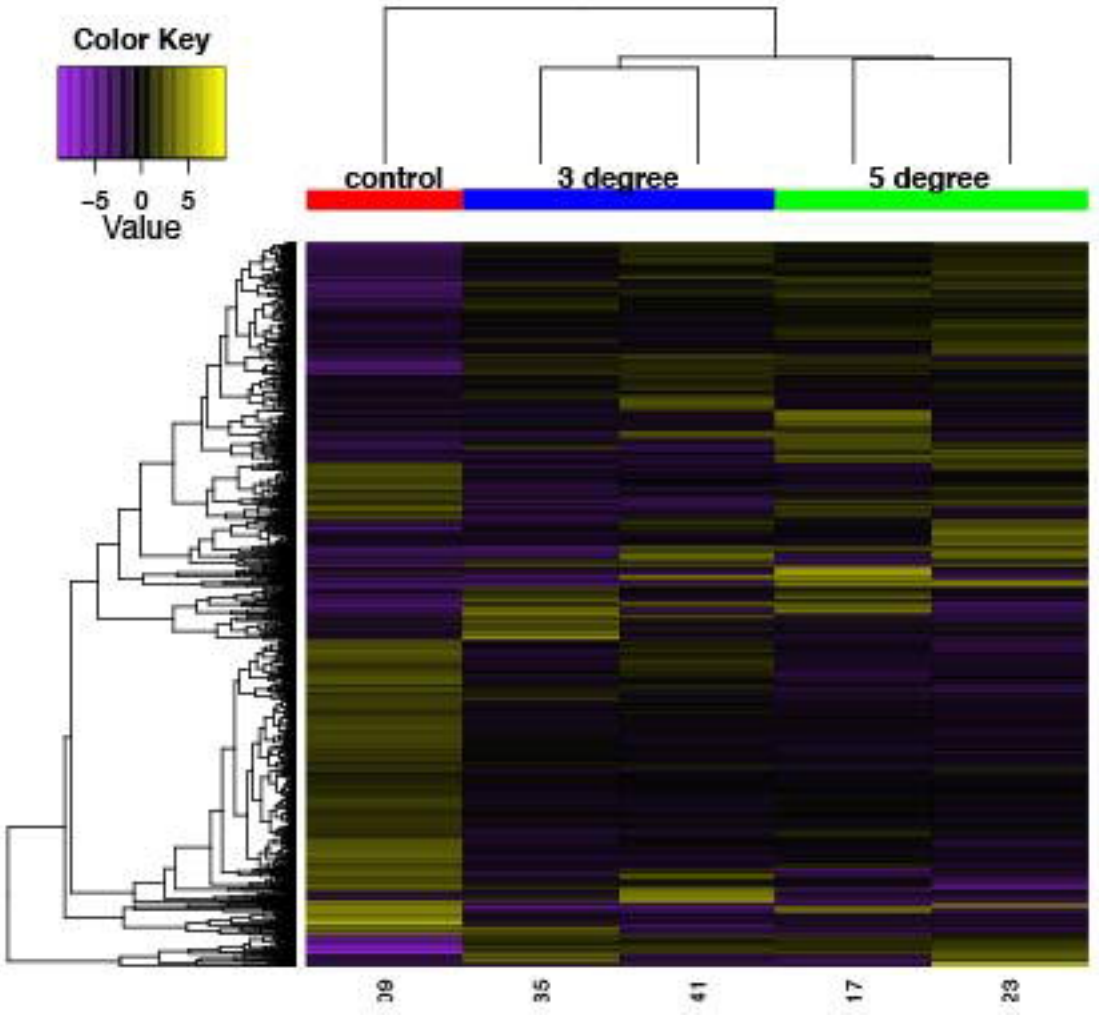
C)

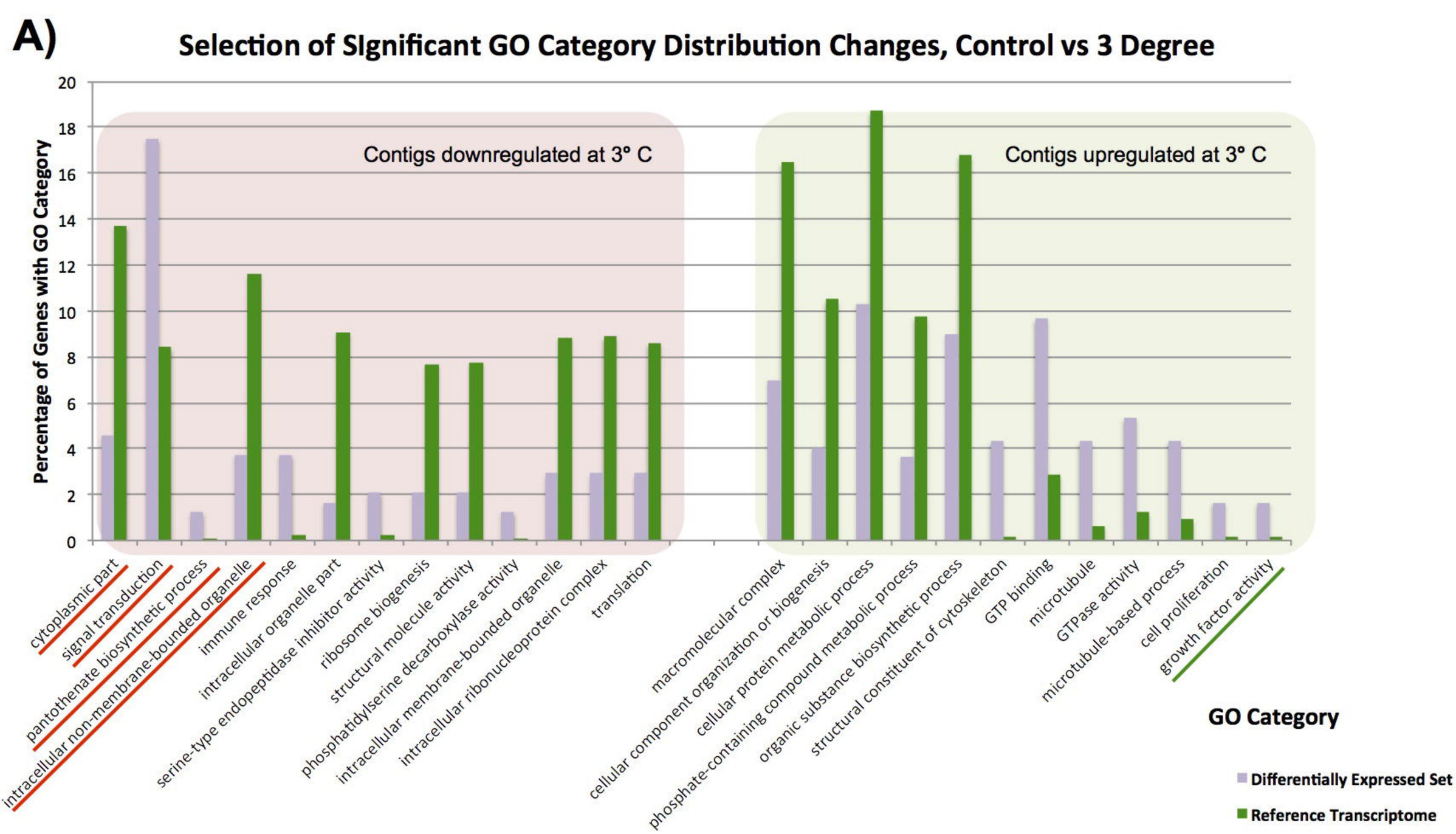

28S
Monanchora arbuscula
Monanchora clathrata 1
Monanchora clathrata 2
Monanchora clathrata 3
Crambe chilensis
Crambe maldonadoi
Crambe crambe 1
Crambe crambe 2
Desmacella cf. annexa
Isodictya sp (This work)
Amphilectus fucorum
Isodictya sp
Isodictya sp 103 (not used)
Isodictya grandis
Isodictya compressa
Isodictya frondosa
Isodictya frondosa
84
99
96
100
61
81
96
98
94
100
89
89
0.0070

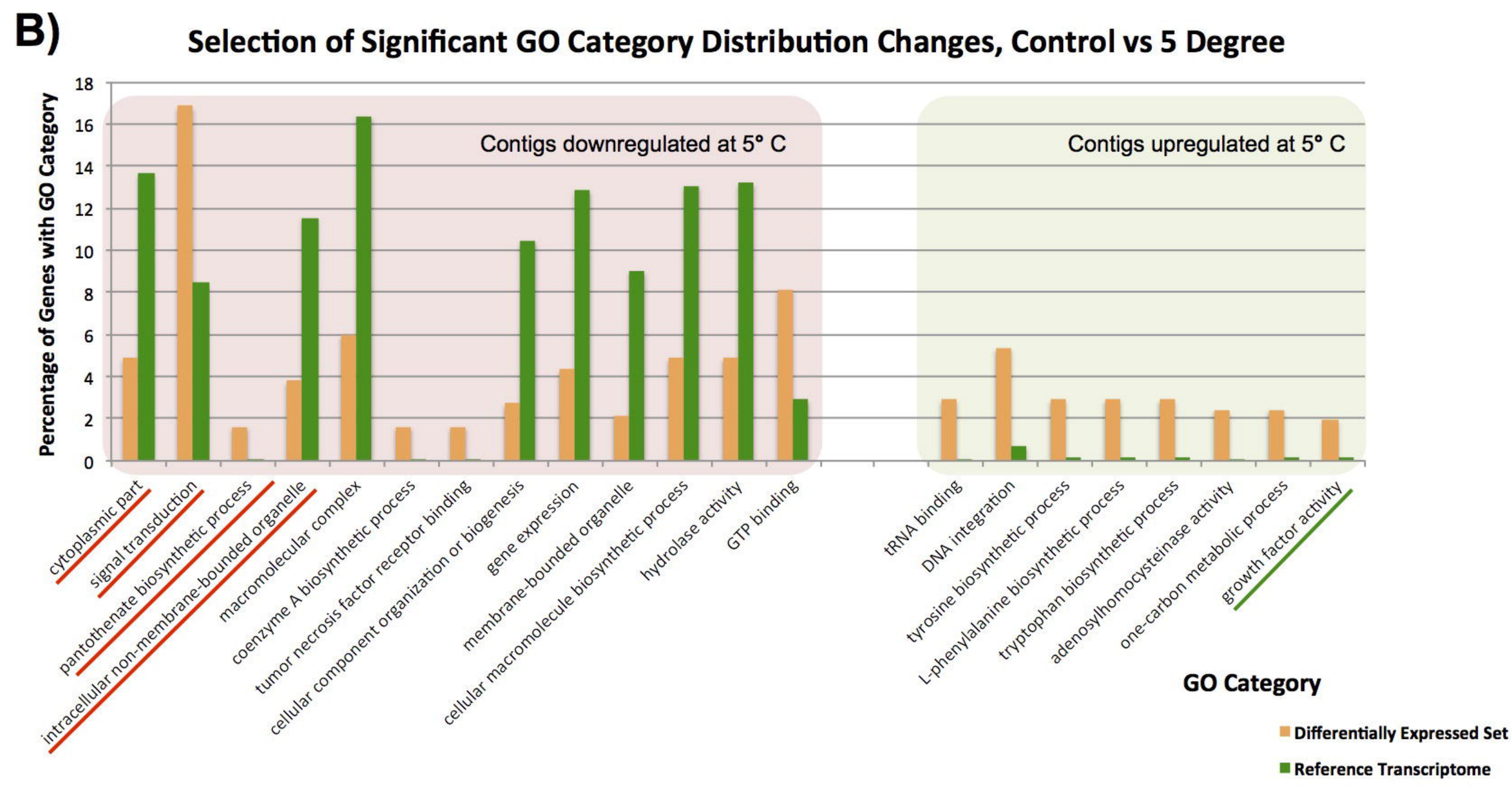
A)

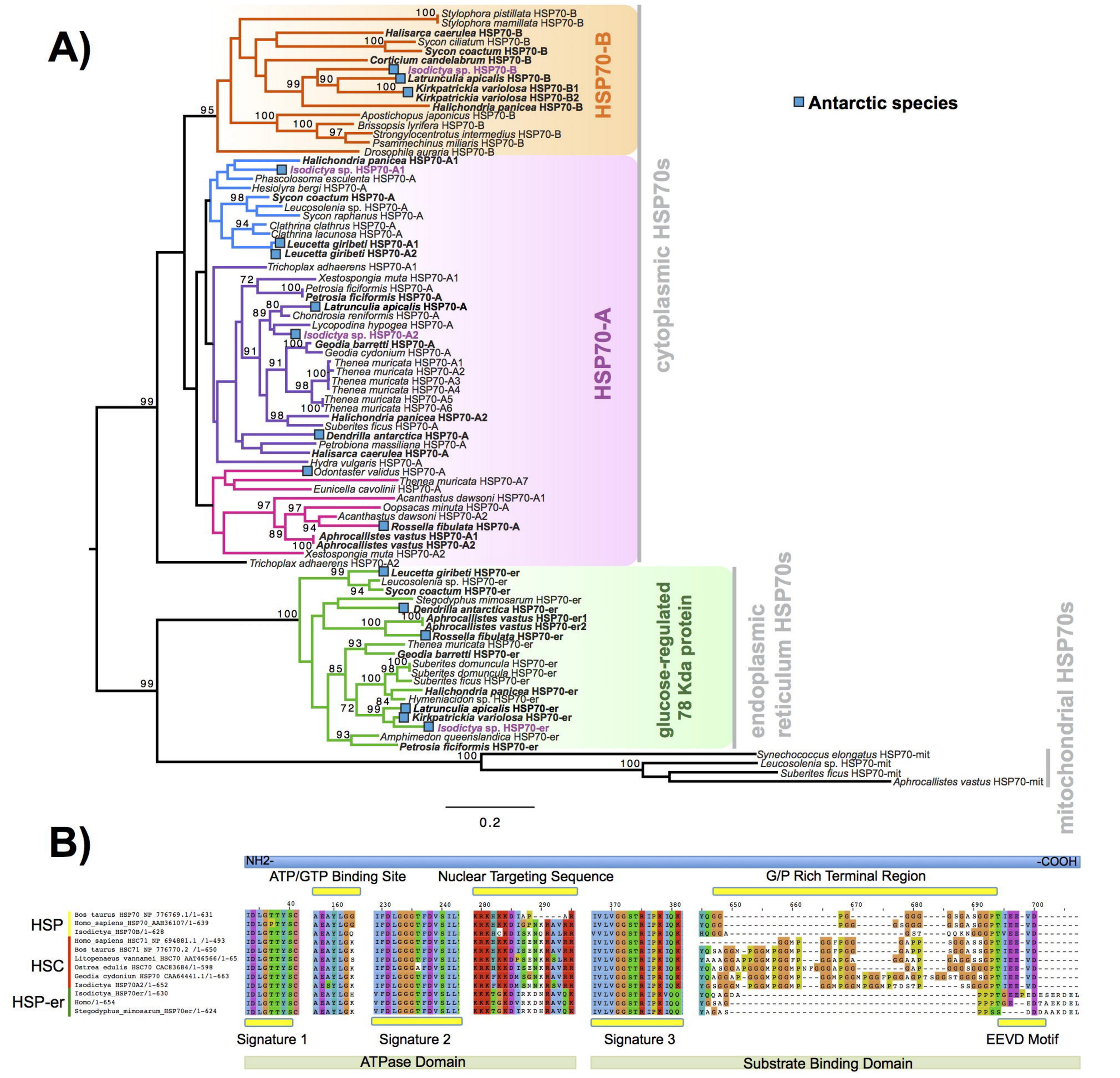

Color Key
0.2
0.6
1
Value
control
3 degree
5 degree
5 degree
3 degree
control
123
117
141
135
209
209
135
141
117
123

B)

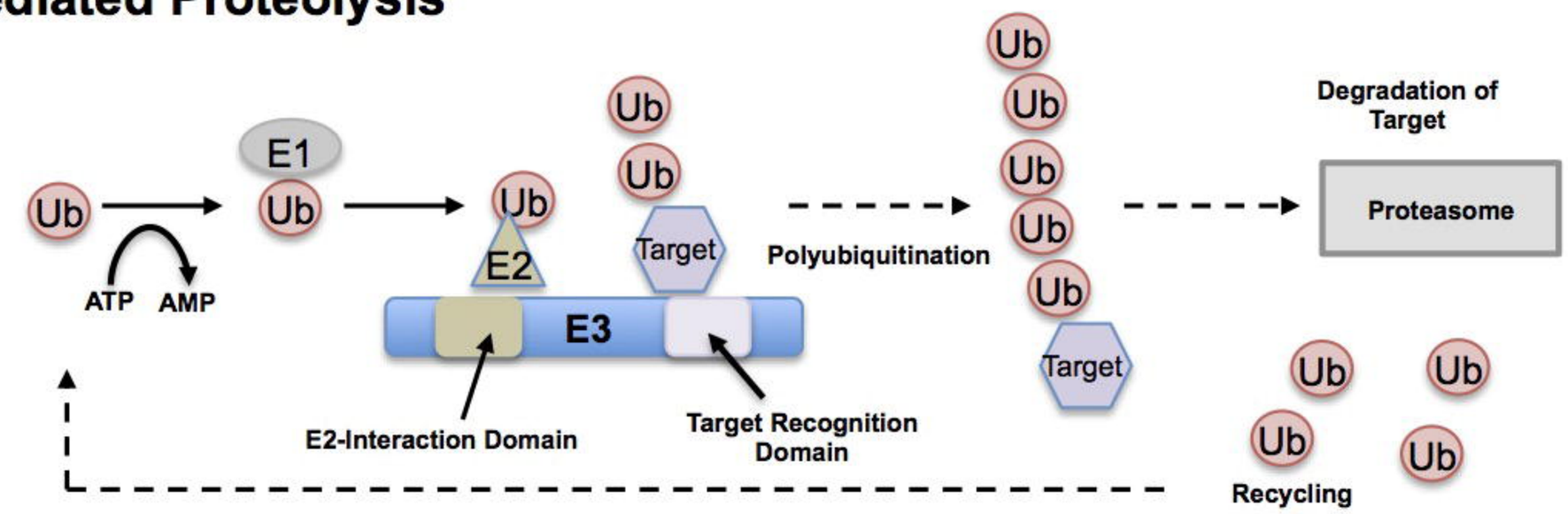

Color Key
-5
0
5
Value
control
3 degree
5 degree


A)
Selection of SIgnificant GO Category Distribution Changes, Control vs 3 Degree
Percentage of Genes with GO Category
Contigs downregulated at 3° C
Contigs upregulated at 3° C
cytoplasmic part
signal transduction
pantothenate biosynthetic process
intracellular non-membrane-bounded organelle
immune response
intracellular organelle part
serine-type endopeptidase inhibitor activity
ribosome biogenesis
structural molecule activity
phosphatidylserine decarboxylase activity
intracellular membrane-bounded organelle
intracellular ribonucleoprotein complex
translation
macromolecular complex
cellular component organization or biogenesis
cellular protein metabolic process
phosphate-containing compound metabolic process
organic substance biosynthetic process
structural constituent of cytoskeleton
GTP binding
microtubule
GTPase activity
microtubule-based process
cell proliferation
growth factor activity
GO Category
Differentially Expressed Set
Reference Transcriptome


B)
Selection of Significant GO Category Distribution Changes, Control vs 5 Degree
Percentage of Genes with GO Category
Contigs downregulated at 5° C
Contigs upregulated at 5° C
cytoplasmic part
signal transduction
pantothenate biosynthetic process
intracellular non-membrane-bounded organelle
macromolecular complex
coenzyme A biosynthetic process
tumor necrosis factor receptor binding
cellular component organization or biogenesis
gene expression
membrane-bounded organelle
cellular macromolecule biosynthetic process
hydrolase activity
GTP binding
tRNA binding
DNA integration
tyrosine biosynthetic process
L-phenylalanine biosynthetic process
tryptophan biosynthetic process
adenosylhomocysteinase activity
one-carbon metabolic process
growth factor activity
GO Category
Differentially Expressed Set
Reference Transcriptome


A)
Antarctic species
HSP70-B
Stylophora pistillata HSP70-B
Stylophora mamillata HSP70-B
Halisarca caerulea HSP70-B
Sycon ciliatum HSP70-B
Sycon coactum HSP70-B
Corticium candelabrum HSP70-B
Isodictya sp. HSP70-B
Latrunculia apicalis HSP70-B
Kirkpatrickia variolosa HSP70-B1
Kirkpatrickia variolosa HSP70-B2
Halichondria panicea HSP70-B
Apostichopus japonicus HSP70-B
Brissopsis lyrifera HSP70-B
Strongylocentrotus intermedius HSP70-B
Psammechinus miliaris HSP70-B
Drosophila auraria HSP70-B
HSP70-A
Halichondria panicea HSP70-A1
Isodictya sp. HSP70-A1
Phascolosoma esculenta HSP70-A
Hesiolyra bergi HSP70-A
Sycon coactum HSP70-A
Leucosolenia sp. HSP70-A
Sycon raphanus HSP70-A
Clathrina clathrus HSP70-A
Clathrina lacunosa HSP70-A
Leucetta giribeti HSP70-A1
Leucetta giribeti HSP70-A2
Trichoplax adhaerens HSP70-A1
Xestospongia muta HSP70-A1
Petrosia ficiformis HSP70-A
Petrosia ficiformis HSP70-A
Latrunculia apicalis HSP70-A
Chondrosia reniformis HSP70-A
Lycopodina hypogea HSP70-A
Isodictya sp. HSP70-A2
Geodia barretti HSP70-A
Geodia cydonium HSP70-A
Thenea muricata HSP70-A1
Thenea muricata HSP70-A2
Thenea muricata HSP70-A3
Thenea muricata HSP70-A4
Thenea muricata HSP70-A5
Thenea muricata HSP70-A6
Halichondria panicea HSP70-A2
Suberites ficus HSP70-A
Dendrilla antarctica HSP70-A
Petrobiona massiliana HSP70-A
Halisarca caerulea HSP70-A
Hydra vulgaris HSP70-A
Odontaster validus HSP70-A
Thenea muricata HSP70-A7
Eunicella cavolinii HSP70-A
Acanthastus dawsoni HSP70-A1
Oopsacas minuta HSP70-A
Acanthastus dawsoni HSP70-A2
Rossella fibulata HSP70-A
Aphrocallistes vastus HSP70-A1
Aphrocallistes vastus HSP70-A2
Xestospongia muta HSP70-A2
Trichoplax adhaerens HSP70-A2
glucose-regulated 78 Kda protein
Leucetta giribeti HSP70-er
Leucosolenia sp. HSP70-er
Sycon coactum HSP70-er
Stegodyphus mimosarum HSP70-er
Dendrilla antarctica HSP70-er
Aphrocallistes vastus HSP70-er1
Aphrocallistes vastus HSP70-er2
Rossella fibulata HSP70-er
Thenea muricata HSP70-er
Geodia barretti HSP70-er
Suberites domuncula HSP70-er
Suberites domuncula HSP70-er
Suberites ficus HSP70-er
Halichondria panicea HSP70-er
Hymeniacidon sp. HSP70-er
Latrunculia apicalis HSP70-er
Kirkpatrickia variolosa HSP70-er
Isodictya sp. HSP70-er
Amphimedon queenslandica HSP70-er
Petrosia ficiformis HSP70-er
Synechococcus elongatus HSP70-mit
Leucosolenia sp. HSP70-mit
Suberites ficus HSP70-mit
Aphrocallistes vastus HSP70-mit
cytoplasmic HSP70s
endoplasmic reticulum HSP70s
mitochondrial HSP70s
0.2
B)
NH2-
-COOH
ATP/GTP Binding Site
Nuclear Targeting Sequence
G/P Rich Terminal Region
HSP
HSC
HSP-er
Bos taurus HSP70 NP 776769.1/1-631
Homo_sapiens_HSP70_AAH36107/1-639
Isodictya_HSP70B/1-628
Homo sapiens HSC71 NP 694881.1 /1-493
Bos taurus HSC71 NP 776770.2 /1-650
Litopenaeus vannamei HSC70 AAT46566/1-65
Ostrea edulis HSC70 CAC83684/1-598
Geodia cydonium HSP70 CAA64441.1/1-663
Isodictya HSP70A2/1-652
Isodictya_HSP70er/1-630
Homo/1-654
Stegodyphus_mimosarum_HSP70er/1-624
Signature 1
Signature 2
Signature 3
EEVD Motif
ATPase Domain
Substrate Binding Domain

Ubiquitin Mediated Proteolysis



E1: Ubiquitin-activating Enzyme

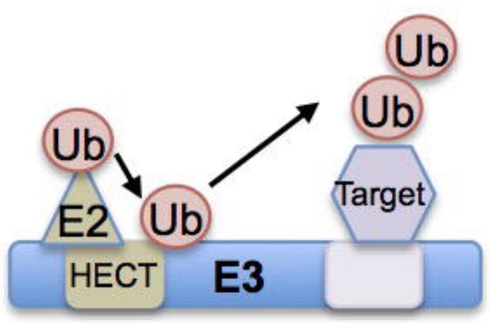
UBE	UBLE1A	UBLE1B	UBLE1C

E2: Ubiquitin-conjugating Enzyme

UBE2A	UBE2B	UBE2C	UBE2D	UBE2E	UBE2F	UBE2G1	UBE2G2	UBE2H
UBE2I	UBE2J1	UBE2J2	UBE2L3	UBE2L6	UBE2M	UBE2N	UBE2O	
UBE2Q	UBE2R	UBE2S	UBE2U	UBE2W	UBE2Z	HIP2	APCLLON	

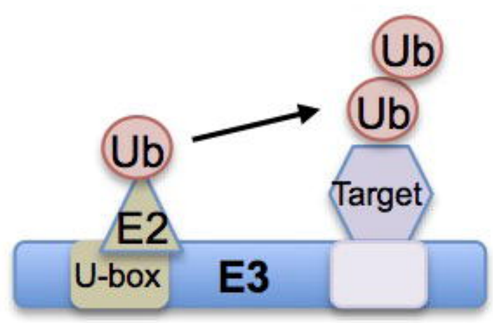
E3 (Ubiquitin ligase) Complexes:

HECT type E3



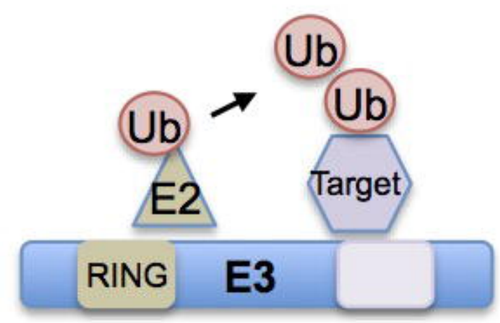
E6AP	UBE3B	UBE3C	Smurf	Itch
WWP1	WWP2	TRIP12	NEDD4	ARF-BP1
EDD1	HERC1	HERC2	HERC3	HERC4

U-box type E3



UBE4A	UBE4B	CHIP
CYC4	PRP19	UIP5

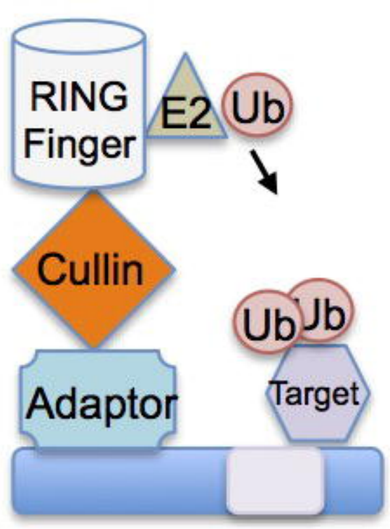
Single RING-finger type E3



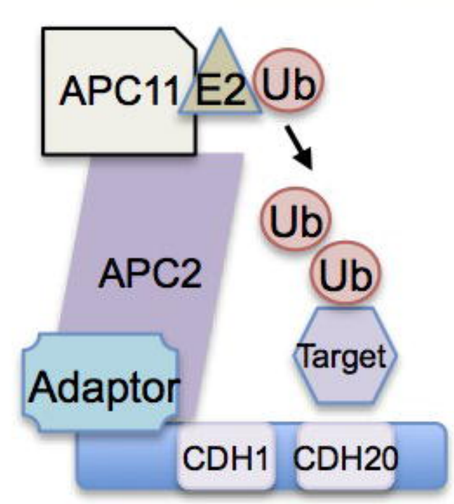
Mdm2	CBL	Parkin	SIAH-1	PML	TRAF6	MEKK1
COP1	PIRH2	cIAPs	PIAS	SYVN	NHLRC1	AIRE
MGRN1	BRCA1	FANCL	MID1	Trim32	Trim37	

Multi subunit RING-finger type E3

Cullin-Rbx E3 Shown



	RING Finger	Cullin	Adaptor	Target Recognition Subunit
SCF Complex	RBX1	Cul1	Skp1	F-box
ECV Complex	RBX1	Cul2	EloB EloC	VHLbox
Cul3 Complex	RBX1	Cul3	(none)	BTB
Cul4 Complex	RBX1	Cul4	DDB1	DCAF
ECS Complex	RBX2	Cul5	EloB EloC	SOCSbox
Cul7 Complex	RBX1	Cul7	Skp1	Fbxw8



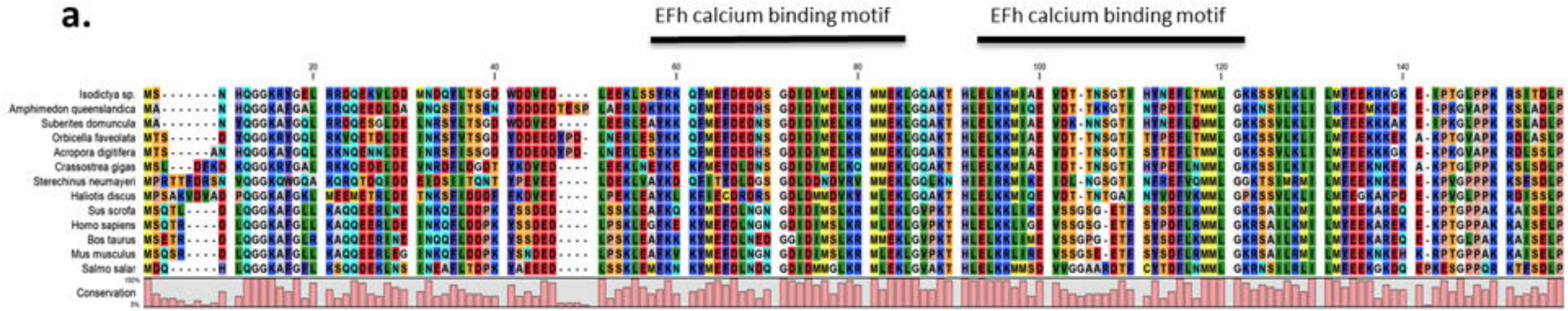
APC/C Shown
Adaptor Not Known

RING Finger	Cullin	Target Recognition Subunit	Other Subunits	
Apc11	Apc2	CDC20	Apc1	Apc3
		Cdh1	Apc4	Apc5
			Apc6	Apc7
			Apc8	Apc9
			Apc10	Apc12
			Apc13	

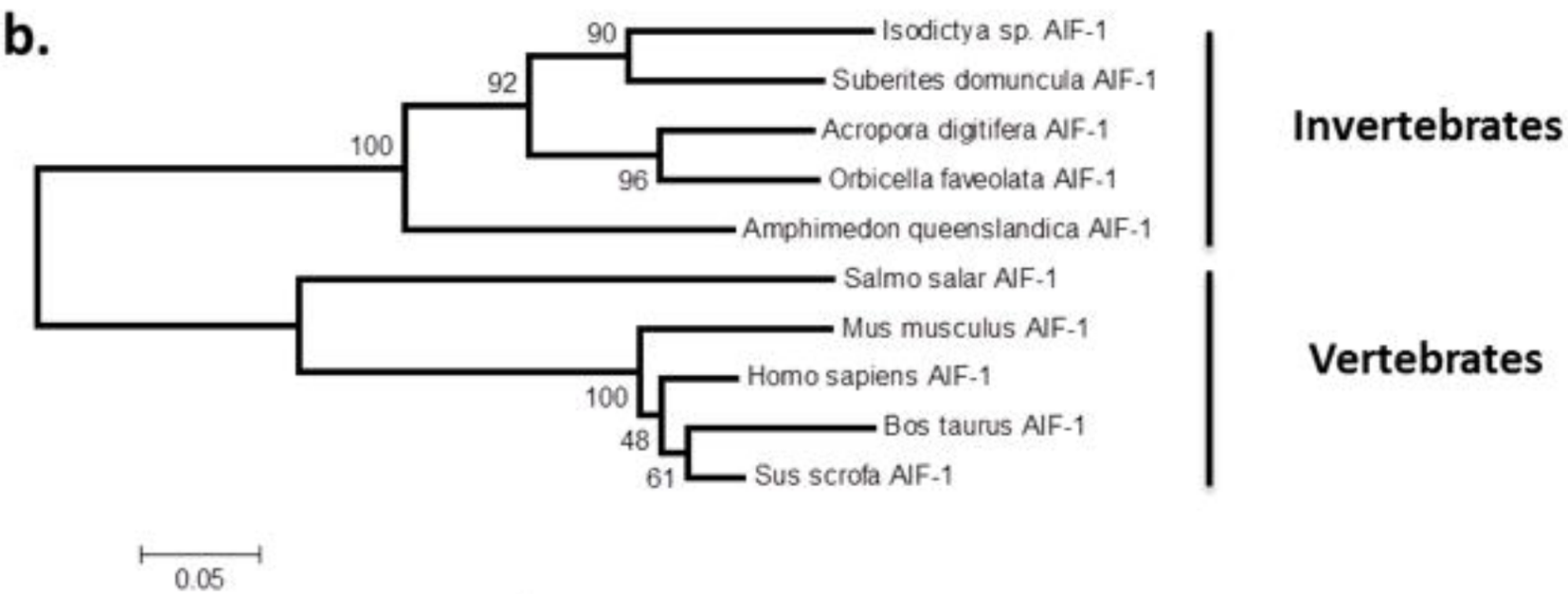
Key:

Found	Not Found	Also Absent in *A. queenslandica*

a.



b.



c.

Percentage of identity

		1	2	3	4	5	6	7	8	9	10	11	12	13
Isodictya sp.	1		68,24	83,33	75,00	73,83	70,95	48,34	56,29	50,68	52,03	50,68	49,32	48,98
Amphimedon queenslandica	2	101		72,30	72,48	73,33	63,16	47,10	53,55	51,32	53,29	51,32	50,66	51,66
Suberites domuncula	3	120	107		77,70	76,51	70,95	50,33	56,95	51,35	52,03	51,35	49,32	50,34
Orbicella faveolata	4	111	108	115		87,25	70,86	45,45	59,09	51,66	53,64	50,33	49,67	48,67
Acropora digitifera	5	110	110	114	130		66,23	46,75	59,74	51,32	52,63	50,66	48,68	50,33
Crassostrea gigas	6	105	96	105	107	100		53,64	59,60	54,30	56,95	53,64	52,98	50,00
Sterechinus neumayeri	7	73	73	76	70	72	81		49,67	40,13	40,13	38,82	38,16	38,56
Haliotis discus	8	85	83	86	91	92	90	75		51,32	51,32	49,34	48,03	45,10
Sus scrofa	9	75	78	76	78	78	82	61	78		93,88	90,48	88,44	65,77
Homo sapiens	10	77	81	77	81	80	86	61	78	138		88,44	89,12	63,76
Bos taurus	11	75	78	76	76	77	81	59	75	133	130		82,99	61,07
Mus musculus	12	73	77	73	75	74	80	58	73	130	131	122		63,09
Salmo salar	13	72	78	74	73	76	75	59	69	98	95	91	94	

Identities