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1 ***De novo* transcriptome assembly of the Qatari pearl oyster *Pinctada imbricata***
2 ***radiata***

3

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12

13 **Abstract**

14 The pearl oyster *Pinctada imbricata radiata* is an iconic species in Qatar, representing an integral part
15 of the nation's cultural heritage and one of the main economic foundations upon which the nation
16 developed. During the early part of the 20th century, nearly half of Qatar population was involved in
17 the pearl oyster industry. However, the fishery has undergone steady decline since the 1930s, and the
18 species is now under threat due to multiple confounding pressures. This manuscript presents the first
19 *de novo* transcriptome of the Qatari pearl oyster assembled into 30,739 non-redundant coding
20 sequences and with a BUSCO completeness score of 98.4%. Analysis of the transcriptome reveals the
21 close evolutionary distance to the conspecific animal *Pinctada imbricata fucata* but also highlights
22 differences in immune genes and the presence of distinctive transposon families, suggesting recent
23 adaptive divergence. This data is made available for all to utilise in future studies on the species.

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30 Author input into the manuscript

31 **TPB** - conceived and designed the study, performed the field sampling and laboratory sample
32 processing, analysed the data, contributed reagents/materials/analysis tools, prepared figures
33 and/or tables, authored and reviewed drafts of the paper, approved the final draft.

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42 approved the final draft.

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47 contributed reagents/materials/analysis tools, authored and reviewed drafts of the paper, approved
48 the final draft.

49 Introduction

50 Qatar is located in the Arabian-Persian Gulf (hereafter referred to as The Gulf), a semi-enclosed sea
51 characterized by a weak hydrodynamic flushing, high evaporation rates and low rates of discharge
52 (Sheppard et al, 2010). Due to its naturally arid conditions, the Gulf is a challenging marine
53 environment (Riegl et al 2012; Camp et al 2018), presenting wide variations in sea temperatures
54 (annual range of 14-36 °C) and high salinities all year round (reaching 70 psu in the Gulf of Salwah)
55 (Riegl et al 2012; Sheppard et al 2010). These conditions have produced several distinctive
56 ecosystems; such as the characteristic Pearl oyster beds generated by the pearl oyster *Pinctada*
57 *imbricata radiata* (Leach, 1814 and e.g. Smyth et al 2016). During the early part of the 20th century,
58 nearly half of Qatar population was involved in the pearl oyster industry, which at present-day prices
59 would have been worth an estimated \$2.5 billion per annum to the nation's economy (Carter 2005).
60 However, as Qatar has prospered and developed there has been a decline in the historical *P. i. radiata*
61 oyster beds (Smyth et al 2016). A survey in 2014 showed that only one out of the five studied sites in
62 Qatar could still be characterised as oyster dominant, even though all five sites previously
63 corresponded to places of highly productive oyster fishery (Smyth et al 2016). These results
64 highlighted the overwhelming likelihood that a combination of anthropogenic effects (such as
65 overfishing, water quality shifts and petrochemical industry operations) have had a negative impact
66 on the traditional Qatari pearl oyster beds (Smyth et al 2016, Al-Maslamani et al 2018).

67 *P. i. radiata* native range goes from the Indian Ocean to the Western Atlantic, including the
68 Arabian Gulf and the Red Sea (Cunha et al. 2011). *P. i. radiata* is found as non-native species in
69 Australasia and Japan and it is currently classified as one of the most successful invasive species in the
70 Mediterranean Sea, likely due to the opening of the Suez Canal in 1869 (Hume, 2009), which links the
71 Red Sea to the Mediterranean Sea. *P. i. radiata* was first reported in the Mediterranean Sea off the
72 Egyptian coast in 1874 (Monterosato, 1878), making it one of the earliest Lessepsian invaders. Its
73 success as an invasive species is in part due to its inherent plasticity, which has been used to cope
74 against the substantial influence of natural and anthropogenic stressors present in the Qatar peninsula
75 (Sheppard et al 2010; Ibrahim et al 2018). However, a recent study suggests that overall health of *P. i.*
76 *radiata* beds is currently at a low ebb and not able to (Smyth et al 2016).

77 The taxonomic status of the three commercially relevant pearl producing species *Pinctada*
78 *imbricata*, *Pinctada fucata*, and *Pinctada radiata* remains unresolved and has become a contentious
79 issue in the literature (Wada and Tëmkin 2008). Recent studies based on DNA sequence analysis have
80 shown very low levels of divergence among the three species and, in pair-wise comparison cases, the
81 levels of divergence were comparable to conspecific individuals of other *Pinctada* species (Tëmkin

2010). Based on these data and the lack of diagnostic morphological features, the three species have been classified as subspecies of the senior synonym *P. imbricata* (*Pinctada imbricata imbricata*, *P. imbricata fucata*, and *P. imbricata radiata*) (Tëmkin 2010). This classification is followed in the present study.

Improvement in next generation sequencing technologies has led to a huge increase in the availability of genomic information, with large scale genomics and transcriptomics projects becoming commonplace. *P. i. radiata* is currently underrepresented in public databases such as NCBI (<https://www.ncbi.nlm.nih.gov>). In the present study we have sequenced, analysed, and publicly released a comprehensive atlas of expressed mRNA from *P. i. radiata*. This is a new resource available for detailed functional or comparative analysis of this species. In addition, as examples of the studies that can be undertaken with this data we have briefly characterised differences in genes expression between tissue types and compared genes found in *P. i. radiata* to other bivalve species.

Methods

Sampling

The Pearl oyster for this study was hand dived from a site at Al Wakra off the Qatar coast on 17/04/2017 (N 25°09.150, E 51°37.072). This is a site with moderate pollution in close proximity to Doha, the most populous city in Qatar. Live sample was transported directly to the laboratory and stored in seawater from the sampling site overnight at ambient temperature to allow depuration of the digestive tract before individual tissues (digestive gland, gill, adductor muscle, gonad, and mantle) were dissected (Figure 1). The oyster was split in two, with roughly half section stored in RNAlater at -20 °C until subsequent RNA extraction, and the remaining half section fixed in Davidson's seawater fixative for 24 hours, before being changed to 70% ethanol and then processed for formalin fixed paraffin embedded (FFPE) histological assessment using haematoxylin and eosin staining.

RNA extraction and sequencing

Total RNA was extracted from five *P. i. radiata* tissues (digestive gland, gills, adductor muscle, gonad, and mantle) with Ribozol (AMRESCO VWR, USA) using an adapted manufacturer protocol: 50 to 100 mg of individual tissues were homogenised in 1 ml of Ribozol in Lysing Matrix A FastPrep® tubes with a Fast Prep cell disrupter (1 min at 5 ms⁻¹) (MPBio, UK); samples were centrifuged at 12,000 g for 10 min at 4 °C, supernatant transferred in fresh tube and incubated at room temperature for 5 min. 200 µl of chloroform was added to each sample, vortexed for 15 sec, incubated at room temperature for 2 min, and centrifuged at 12,000 g for 15 min at 4 °C. Aqueous phase was removed and total RNA precipitated by adding 500 µl of isopropanol, followed by incubation at room temperature for 10 min,

114 and centrifugation 12,000 g for 10 min at 4 °C. Pellet was subsequently washed in 1 ml of 75 % ethanol,
115 centrifuged at 7,500 g for 5 min at 4 °C, air dried for 5 minutes and dissolved in 50 µl RNase free water.
116 Quality was checked by TapeStation (Agilent, USA) with RINs of 7, 9.3, 8.8, 9.3 and 9.2 recorded for
117 digestive gland, gill, adductor muscle, gonad, and mantle respectively (note low value for digestive
118 gland appeared to be due to an elevated concentration of RNA rather than low integrity). Libraries
119 were prepared and sequenced by the University of Exeter Sequencing Service. Briefly, libraries were
120 produced with Illumina Truseq stranded mRNA kit (Illumina, USA), QA checked by TapeStation
121 (Agilent, USA) and Quantus Fluorometer (Promega, UK), pooled in equimolar concentrations, and
122 sequenced as 125 bp paired-end reads on one lane of an Illumina HiSeq 2500 system in standard
123 mode.

124 **Bioinformatics**

125 Sequences were trimmed by trim_galore (version 0.4.0) for paired sequences, but with –
126 retain_unpaired and –fastqc arguments included, and then used for all subsequent analysis. To
127 produce the transcriptome, all sequences were pooled and normalised with bbnorm, including the
128 pre-filter parameter, before being assembled by Trinity (version 2.8.4) with default parameters except
129 for inclusion of the --no_normalize_reads parameter (Haas et al 2013). The transcriptome was run
130 against the NCBI nr protein database (07/09/2018) using the blastx feature in diamond (version 0.9.22)
131 including the –sensitive parameter an e-value cut off of 0.001 and arguments to increase speed on a
132 high memory server (--index-chunks 1 and --block-size 10). Results visualised in MEGAN (version
133 6.5.8). Reads identified as metazoan were used for all further analysis, while non-metazoan reads
134 were discarded. The transcriptome (isoform sequences) was loaded into Blast2GO with associated
135 Blast results for annotation. The gene expression matrix was calculated by RSEM using the dispersion
136 index of 0.4, which was deemed most appropriate for no replicate reads. Differential expression was
137 calculated by edgeR using two different methods. A standard matrix was used for tissue to tissue
138 comparison, whereas for Gene Set Enrichment Analysis (GSEA), each tissue was analysed in
139 comparison to all other tissues as replicates. Differentially expressed genes (FDR > 0.01, fold change
140 >2) were used for GSEA analysis within Blast2GO software. Tissues were furthermore analysed for
141 uniquely expressed genes; any genes in any tissue with FPKM > 0.25 was selected as expressed, and
142 visualised within the package VennDiagram (version 1.6.2.0). Transdecoder (version 5.5.0) was used
143 to identify probable open reading frames and redundancy removed with cd-hit (Version 4.8.1, Li et al
144 2006, Fu et al 2012) using an identity threshold of 0.9 resulting in 30739 non-redundant coding
145 sequences (Supplementary Figure 1). These sequences were run against metazoan databases with
146 benchmarking single-copy orthologs software (BUSCO, version 3.0.2) to check the completeness of
147 the transcriptome (Waterhouse et al., 2017).

148 **Orthology analysis**

149 Orthologous gene groups were assigned to *P. i. radiata* and five other species using OrthoFinder
150 software (Emms et al., 2015 and 2018). Briefly, total non-redundant protein sequence files for *P. i.*
151 *fucata* (Takeuchi et al., 2016), *Crassostrea gigas* (Zhang et al., 2012), *C. virginica*
152 (<https://www.ncbi.nlm.nih.gov>), *M. yesso* (Wang et al., 2017) and *Octopus bimaculoides* (Albertin et
153 al., 2015) were downloaded from publically available databases. These sequences were run through
154 Orthofinder (version 2.3.3), using default parameters, alongside the 30739 non-redundant *P. i. radiata*
155 protein sequences output from Transdecoder (see above and Supplementary data 1).

156 **Results**

157 **Gross morphology and histopathology**

158 The oyster chosen for sequencing had height of 53 mm (anterior to posterior), a width of 17 mm
159 (maximum distance from left to right valve) and a total wet-weight of 62 g. No pearls or notable
160 morphologies were observed. Pathology samples were examined histologically as in previous studies
161 (Ward et al., 2006; Hines et al., 2007). The oyster individual sequenced was a female, with a developed
162 gonad. One instance of an unknown trematode with granuloma was observed. No other notable
163 pathologies were observed.

164 **Sequencing and transcriptome Assembly**

165 After trimming, over 45 Gb of data were available for further analysis (Table 1). The transcriptome
166 assembled into 179,599 contigs with max, min, and average lengths of 16,371 bp, 201 bp, and 1,119
167 bp respectively (Table 2). N50 and N90 were 2,013 bp and 430 bp respectively (Table 2). Trinity
168 assigned these transcripts into 24,676 gene clusters (Table 2). Of the transcripts, 70,114 mapped to a
169 sequence record associated with a cellular organism in the NCBI nr reference database
170 (<https://www.ncbi.nlm.nih.gov>), of these there were sequences for 68,930 Metazoa; 60,285
171 Protostomia; 58,378 Lophotrochozoa; 55,935 Mollusca and 49,835 Bivalvia. On a species level, most
172 of the bivalve sequences mapped to rock oysters, for which two species have whole genome
173 sequences available within the NCBI nr reference database, with just under 2,000 reads mapping to
174 *Pinctada* species. Twenty reads mapped to the common molluscan parasite phylum Platyhelminthes.
175 After removal of redundant ORFs, Transdecoder and cd-hit returned 30,739 non-redundant, expressed
176 sequences, or hypothetical proteins. BUSCO checks on the overall completeness of both redundant
177 and non-redundant set of coding sequences found 957 complete BUSCOs and 5 fragmented BUSCOs
178 in both sequence sets, with an overall completeness score of 98.4%.

179 **Gene expression and gene set enrichment analysis**

180 Gene expression was analysed quantitatively and qualitatively. Expression of 13,657 genes was shared
181 by all tissues. Each tissue had uniquely expressed genes with the digestive gland having the most
182 unique transcripts 730, compared to 471 in the mantle, 401 in the gill, 145 in the gonad, and just 80
183 in the adductor muscle. The pattern of differentially expressed transcripts was distinct, with the
184 digestive gland having 3,972 differentially expressed transcripts (compared to all other tissues),
185 adductor muscle 3,649, gill 2,208, gonad 1,835, and mantle 1,466. Gene Set Enrichment Analysis
186 (GSEA) was performed on these differentially expressed genes from each of the tissue types (assessed
187 against all other tissues). Table 3 shows how each tissue type was enriched for several gene ontology
188 (GO) terms which relate to the function of that particular tissue (for example contractile fiber in the
189 adductor muscle). Digestive gland, which had the highest number of differentially and uniquely
190 expressed genes, also showed the highest number of enrichment terms. Functions, including
191 endopeptidase and peptidase inhibitor activity, were enriched in differentially expressed genes,
192 suggesting function associated with the process of digestion.

193 **OrthoFinder**

194 In brief, 20,870 orthogroups were assigned to the six different species and included 83.5 % of the total
195 number of genes (Supplementary Data 2). Only 1.6 % of the orthogroups were species-specific. As
196 expected, the all-gene phylogeny grouped the *Pinctada* species together and the *Crassostrea* species
197 together, with the scallop *M. yesso* completing the bivalve clade and the cephalopod mollusc *O.*
198 *bimaculoides* being most distance to all other species. The two *Crassostrea* species and the *Pinctada*
199 species each had around 16,000 orthogroups and each genus shared similar numbers of orthogroup
200 overlaps. As a lone and more distant species, *O. bimaculoides* only had around 9,000 orthogroups but
201 shared the majority of these with all other species sequenced. Orthogroups containing at least five
202 genes in *P. i. radiata* and zero genes from *P. i. fucata* (Supplementary data 3) were analysed in
203 Blast2GO. These 24 gene groups included functional groups such as transposable elements,
204 transcription factors and immune system receptors (data not shown).

205 **Discussion**

206 All metrics suggest that the overall quality of *P. i. radiata* transcriptome produced in the present study
207 is very high, with a similar number of non-redundant expressed sequences close to the highly related
208 species *P. i. fucata* (Du et al., 2017), which has had its whole genome sequenced, and with
209 benchmarking single-copy ortholog (BUSCO) analysis giving a score of 98.4% completeness. The N50
210 score of 2,013 bp, which is within the region of most complete bivalve transcriptomes (e.g. Ryu et al

211 2019, Viricel 2018, Patnaik et al 2016). This sequence data is now available online via public databases
212 with relevant details available in Table 4.

213 Orthogroups, representative of groups of homologous genes, are a useful way of inferring and
214 comparing functional biology of multiple species, and also identifying shared genes with which
215 multigene phylogenies can be drawn (Figure 2A) (Emms et al 2015). In order to assess the comparative
216 differences between *P. i. radiata* and other molluscs, non-redundant protein databases of four other
217 bivalves (*P. i. fucata*, *C. virginica*, *C. gigas* and *M. yesso*) and one cephalopod mollusc (*O. bimaculoides*)
218 were analysed with OrthoFinder (Emms et al 2015) (Figure 2). In total circa 20,000 orthogroups were
219 identified across the five different species, 83.5 % of which spanned across more than one species. As
220 expected, *P. i. radiata* groups closely to and shares a high proportion of its orthogroups with *P. i.*
221 *fucata*. Interestingly, the multi-gene phylogeny inferred within OrthoFinder suggests the distance
222 between *P. i. radiata* and *P. i. fucata* is similar to that between *C. gigas* and *C. virginica*. In addition,
223 the two *Pinctada* species compared in this study share roughly the same number of orthogroups as
224 the two *Crassostrea* species (14,801 vs 15,308 respectively). Together, these data suggest a similar
225 level of phylogenetic relationship between the *Pinctada*, rather than a conspecific relationship.
226 However, it should be noted that less than 0.6 % of the *Pinctada* genes reside in species specific
227 orthogroups, compared to over 1 % of the *Crassostrea* genes, suggesting that the two *Crassostrea*
228 species have several more divergent orthogroups, in addition to the large number of shared groups.
229 The cephalopod (*O. bimaculoides*) only had genes assigned to around 9000 orthogroups, but it shared
230 the majority of these with all other species sequenced. This finding includes the orthogroups which
231 are present across mollusca, and likely includes genes with many essential functions, rather than those
232 evolved for lineage specific functions. It will be interesting to continue repeating this analysis with
233 more bivalve transcriptomes and genomes as they continue to become available and identify the
234 groups of genes specific to each class of mollusc, and to further study those genes which allowed such
235 successful adaptive radiation of the molluscs (Seed 1983). In order to elucidate some of the functional
236 differences between *P. i. radiata* and *P. i. fucata* genomes, orthogroups which contained at least five
237 genes from *P. i. radiata* and none from *P. i. fucata* were studied in more detail. Among this set of 24
238 orthogroups were genes with homology to transposable elements, transcription factors and innate
239 immune signalling. The function of these genes suggests they have evolved in relation to specific
240 pressures, which may underlie some of the more recent lineage specific adaptations. In general, the
241 relationship between *P. i. radiata* and *P. i. fucata* has proven to be challenging to resolve both from a
242 morphological and genetic point of view (Tëmkin 2010), with the current taxonomic sub-species
243 designation being somewhat of a compromise. The analysis presented in this study, however, suggests
244 that the current designation could someday be re-visited with a thorough genome-wide analysis.

245 In order to demonstrate the potential of this transcriptome for study of functional properties
246 of the differentially expressed transcripts from each organ, gene set enrichment analysis (GSEA) was
247 utilised (Table 3). Most organs were enriched for categories highly symbolic of the overall function,
248 for example the adductor muscle was enriched for myosin complex, contractile fibers, myofibril,
249 sarcomere and actin cytoskeleton, all of which are associated with muscle contraction. The mantle,
250 perhaps the most bivalve-specific tissue, was enriched for chitin binding and metabolism, glucosamine
251 containing processes, aminoglycan and amino sugar metabolic processes. These findings point
252 towards the key function of shell formation, with chitin metabolism being previously identified as a
253 basic component of nacre in *P. fucata martensii* (Du et al., 2017) and the amino glycan and amino
254 sugar pathways previously identified as enriched protein components in *C. gigas* shell (Wang et al
255 2013), likely to be involved in formation of complex matrices. Otherwise, the digestive gland was
256 enriched for categories associated with peptidase regulation, the gonad, enriched for categories
257 including nucleoplasm, biosynthesis and protein assembly suggesting active biosynthetic processes,
258 such as gonadogenesis. Enrichment categories in the gill suggested the process of post-translational
259 modification via dephosphorylation, which may, for example, play a key role in regulation of ion-
260 transport across the membrane of the gill (Lucena et al., 2017).

261 This species has demonstrated an incredible ability to survive a range of challenging conditions,
262 but it appears that is now reaching the limit of this inherent flexibility. The existence of this high-
263 quality reference transcriptome will now allow for transcriptomic studies into the ability of *P. i. radiata*
264 to survive challenging conditions.

265

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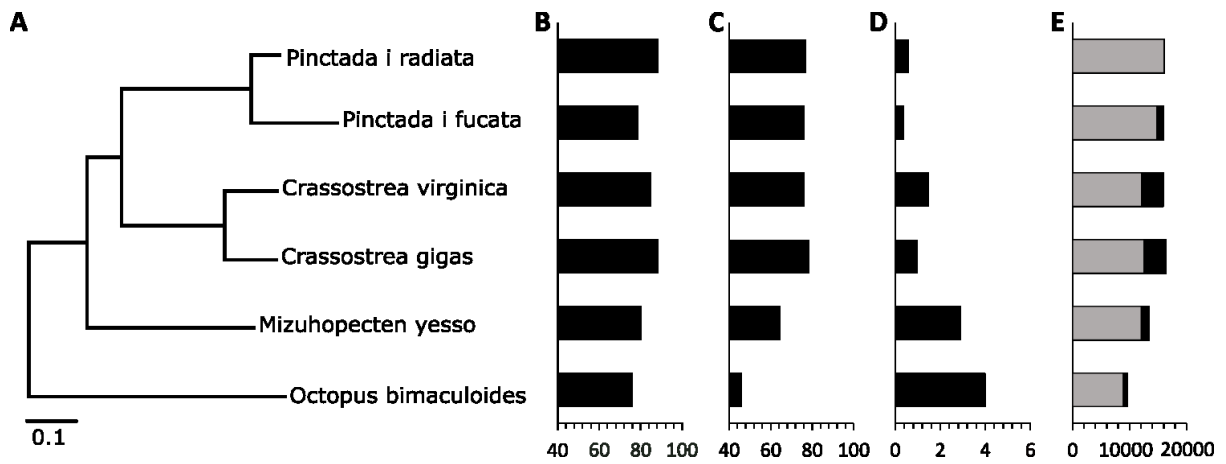


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381 Figure 1. Left valve of the pearl oyster, *Pinctada imbricata radiata*, with tissues utilised in
382 transcriptomic analysis identified.

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387 Figure 2. Results of phylogenomic orthology screens using OrthoFinder. A) Phylogeny of species as
388 defined by all genes. B) Percentage of genes from each species in orthogroups. C) Percentage of
389 orthogroups containing each species. D) Percentage of genes in species-specific orthogroups. E)
390 Total number of orthogroups (black) which included genes from this species, and number of those
391 shared with *Pinctada i radiata* (grey).

392 Table 1. Number and length of reads pre and post trimming via trimmomatic.

Tissue	Number of read pairs	Average length	Number of trimmed pairs	Average length Forward	Reverse
Digestive gland	35,856,234	2x125	35,770,112	114.4	111.6
Gill	38,123,232	2x125	37,997,395	116.9	112.9
Adductor muscle	43,089,935	2x125	43,009,382	116.2	112.7
Gonad	39,136,760	2x125	39,044,852	115.5	112
Mantle	44,816,064	2x125	44,737,749	114.9	111.3

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395 Table 2. Transcriptome statistics

Descriptive Statistic	Summary
Number of transcripts	179,599
Number of genes*	24,676
Total length (bp)	201,029,654
Shortest transcript length (bp)	201
Mean transcript length (bp)	1,119.30
Longest Transcript length (bp)	16,371
N50 (bp)	2,013

*gene cluster as identified with Trinity assembler (see methods)

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400 Table 3. Top five (or all) categories for gene set enrichment analysis from each tissue.

Tissue	GO ID	GO Name	GO Category	Nominal p-val	FDR q-val
Digestive Gland	GO:0004866	endopeptidase inhibitor activity	Molecular Function	0	0
	GO:0061135	endopeptidase regulator activity	Molecular Function	0	0
	GO:0061134	peptidase regulator activity	Molecular Function	0	0
	GO:0004857	enzyme inhibitor activity	Molecular Function	0	0
	GO:0004867	serine-type endopeptidase inhibitor activity	Molecular Function	0	0
Gill	GO:0004721	phosphoprotein phosphatase activity	Molecular Function	5.941E-03	7.582E-02
	GO:0004725	protein tyrosine phosphatase activity	Molecular Function	7.905E-03	2.096E-01
	GO:0016311	dephosphorylation	Biological Process	7.937E-03	1.103E-01
	GO:0006570	tyrosine metabolic process	Biological Process	1.504E-02	8.915E-02
	GO:0006470	protein dephosphorylation	Biological Process	1.590E-02	7.120E-02
Adductor muscle	GO:0015629	actin cytoskeleton	Cellular Component	0	0
	GO:0016459	myosin complex	Cellular Component	0	0
	GO:0043292	contractile fiber	Cellular Component	0	0
	GO:0030016	myofibril	Cellular Component	0	0
	GO:0030017	sarcomere	Cellular Component	0	0
Gonad	GO:0034622	cellular protein-containing complex assembly	Biological Process	0.000E+00	3.651E-02
	GO:0005654	nucleoplasm	Cellular Component	7.937E-03	1.067E-01
	GO:0016053	organic acid biosynthetic process	Biological Process	1.235E-02	1.510E-01
	GO:0046394	carboxylic acid biosynthetic process	Biological Process	1.594E-02	1.517E-01
Mantle	GO:1901071	glucosamine-containing compound metabolic process	Biological Process	0	0
	GO:0006030	chitin metabolic process	Biological Process	0	0
	GO:0008061	chitin binding	Molecular Function	0	0
	GO:0006040	amino sugar metabolic process	Biological Process	0	0
	GO:0006022	aminoglycan metabolic process	Biological Process	0	0

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402 Table 4. MIS specifications of the *P. i. radiata* transcriptome.

Item	Description
Investigation_type	Eukaryote
Project_name	Reference transcriptome of <i>Pinctada imbricata radiata</i>
Organism	<i>Pinctada imbricata radiata</i>
Classification	Metazoa (kingdom); Mollusca (phylum); Bivalvia (class); Pteriida (order); Pteriidae (family); Pinctada (genus)
Lat_lon	25°09.150 N 51°37.072 E
Geo_loc_name	Al Wakrah, Qatar
Collection_date	17/04/2018
Collector	Alexandra Leitão
Environment (biome)	marine benthic biome (ENVO:01000024)
Environment (feature)	sand (ENVO:01000017)
Environment (material)	sea water (ENVO:00002149)
Env_package	Water
Seq_meth	Illumina
Transcriptome_platform	HiSeq 2500
Assembly_method	Trinity v2.8.4
	Bioproject ID: PRJDB8463
Submitted_to_INSDC	Biosample ID: SAMD00178207-SAMD00178211
	Short read archive ID: DRA008674
	Accession: ICPG01000001-ICPG01068930

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