Metagenomic analysis of orange-colored protrusions from the muscle of Queen Conch *Lobatus gigas* (Linnaeus, 1758)

Jaison H. Cuartas [1], **Juan F. Alzate** [2], **Claudia X. Moreno-Herrera** [1], **Edna J. Marquez** Corresp. [1]

[1] Facultad de Ciencias, Universidad Nacional de Colombia, Medellín, Antioquia, Colombia

[2] Facultad de Medicina, Centro Nacional de Secuenciación Genómica, Universidad de Antioquia, Medellín, Antioquia, Colombia

Corresponding Author: Edna J. Marquez
Email address: ejmarque@unal.edu.co

The endangered marine gastropod, *Lobatus gigas,* is an important fishery resource in the Caribbean region. Microbiological and Parasitological research of this species have been poorly addressed despite their role in its fitness, conservation status and prevention of potential pathogenic infections. This study identified taxonomic groups associated with orange-colored protrusions in the muscle of queen conchs using histological analysis, 454 pyrosequencing, and a combination of PCR amplification and automated Sanger sequencing. The molecular approaches indicate that the etiological agent of the muscle protrusions is a parasite belonging to the subclass Digenea. Additionally, the scope of the molecular technique allowed the detection of bacterial and fungi clades in the assignment analysis. This is the first evidence of a digenean infection in the muscle of this valuable Caribbean resource.

Metagenomic analysis of orange-colored protrusions from the muscle of Queen Conch *Lobatus gigas* (Linnaeus, 1758)

Jaison H. Cuartas [1], Juan F. Alzate [2], Claudia X. Moreno-Herrera[1], Edna J. Márquez[1]

[1]Facultad de Ciencias, Universidad Nacional de Colombia, sede Medellín, Colombia. [2] Facultad de Medicina, Centro Nacional de Secuenciación Genómica, Universidad de Antioquia, Medellín, Antioquia, Colombia.

Abstract

The endangered marine gastropod, *Lobatus gigas,* is an important fishery resource in the Caribbean region. Microbiological and Parasitological research of this species have been poorly addressed despite their role in its fitness, conservation status and prevention of potential pathogenic infections. This study identified taxonomic groups associated with orange-colored protrusions in the muscle of queen conchs using histological analysis, 454 pyrosequencing, and a combination of PCR amplification and automated Sanger sequencing. The molecular approaches indicate that the etiological agent of the muscle protrusions is a parasite belonging to the subclass Digenea. Additionally, the scope of the molecular technique allowed the detection of bacterial and fungi clades in the assignment analysis. This is the first evidence of a digenean infection in the muscle of this valuable Caribbean resource.

39

40

41

42 1. Introduction

43 The queen conch, *Lobatus gigas,* is an endangered marine gastropod of great socioeconomic,
44 cultural and ecological importance in the Caribbean region. This species was included in
45 Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and
46 Flora (CITES) in 1992 and in the Red List of the International Union for Conservation of Nature
47 (IUCN) in 1994. Despite these regulations, the natural stocks of this species continued to decline
48 (Theile, 2001; Aldana, 2003), likely by the loss of breeding habitats and detrimental human
49 activities such as overfishing (Glazer & Quintero, 1998; Aldana, 2003).

50 Compared with studies in basic biology (Randall, 1964), fisheries (Brownell & Stevely, 1981;
51 Theile, 2001; Prada et al., 2009), and genetics (Mitton, Berg Jr. & Orr, 1989; Tello-Cetina,
52 Rodríguez-Gil & Rodríguez-Romero, 2005; Zamora-Bustillos et al., 2011; Márquez et al., 2013),
53 parasitological and microbial studies of *L. gigas* are less explored (Acosta et al., 2009; Aldana et
54 al., 2011; Rodriguez, Hariharan & Nimrod, 2011; Pérez et al., 2014). So far, only one parasitic
55 infection with *Apicomplexa* coccidian protozoon has been reported in *L. gigas* (Baqueiro et al.,
56 2007; Aldana et al., 2009, 2011; Gros, Frenkiel & Aldana, 2009; Volland et al., 2010). Similarly,
57 only three published studies report the association of bacteria of the family Vibrionaceae (Acosta
58 et al., 2009), the phyla Firmicutes, Proteobacteria, Actinobacteria (Pérez et al., 2014) as well as
59 potential bacterial pathogens (Rodriguez, Hariharan & Nimrod, 2011). Two recent researches

have also studied the symbiotic association of *L. gigas* with dinoflagellates of genus *Symbiodinium* (Banaszak, García Ramos & Goulet, 2013; García Ramos & Banaszak, 2014).

Moreover, sporadically, an unknown etiological agent produces orange-colored protrusions in the muscle of *L. gigas* from the Colombian San Andres archipelago. However, it remains to elucidate whether such lesions result from different agents and posteriorly colonized by pigment-producing microorganisms or digenean infections as found in other marine gastropods. Specifically, the infections of *Cercaria parvicaudata* and *Renicola roscovita* have been reported to produce orange/lemon-colored sporocysts in different tissues of *Littorina* snails (Stunkard, 1950; Galaktionov & Skirnisson, 2000), whereas *Renicola thaidus* has been found infecting *Nucella lapillus* (Galaktionov & Skirnisson, 2000). These trematodes, *C. parvicaudata*, *R. roscovita* and *R. thaidus* are considered synonymous based on morphological similarities and the cercariae size parameters (Werding, 1969). Similarly, lemon-cream to orange colored sporocysts are produced by the congeners *Renicola* sp. “polychaetophila” and *Renicola* sp. “martini” in infections of gonad and digestive glands in *Cerithidea californica* (Hechinger & Miura, 2014).

This work studied the presence of parasites, bacteria and fungi in orange-colored protrusions in the muscle of Colombian Caribbean queen conchs. This was achieved by using histological analysis and molecular approaches based on 454 FLX and capillary automated sequencing using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). This 454 FLX next-generation platform (Roche) permits the high-throughput identification of hundreds of samples at reasonable cost and time consumption (Mardis, 2008). This approach has allowed the functional analysis of sequencing data sets for comparative analysis of microbiome diversity of orange-colored protrusions found in the muscle of *L. gigas* by using metagenomic taxonomical

classifiers (Huson et al., 2007, 2011). This information is required for queen conch conservation and management strategies of potential pathogenic infections for human beings.

2. Materials and methods

Orange-colored protrusions were taken from three pieces of frozen muscle of *L. gigas* processed for food trading in the Colombian Caribbean, San Andres archipelago (12° -16° N and between 78° - 82° W). These samples were provided by the Gobernación del Archipiélago de San Andrés, Providencia y Santa Catalina, through the scientific cooperation agreement # 083/2012.

Since the etiological agent of these orange-colored protrusions was unknown, we used three approaches to elucidate the origin of these lesions: (1) histological analysis, (2) 454 pyrosequencing of one whole genome shotgun library and (3) automated capillary sequencing (Sanger) of PCR amplified products to confirm the results provided by the metagenomic analysis. For histological analysis, the samples from orange-colored muscle were fixed in 10% neutral phosphate-buffered formalin. The samples were prepared for histological examination by paraffin wax techniques and stained with hematoxylin- eosin following standard protocols (García del Moral, 1993; Prophet et al., 1995).

Due to scarce sample, the orange protrusions were pooled and ground with liquid nitrogen to extract the genomic DNA using the commercial kit DNAeasy Blood & Tissue Kit (Qiagen, Germany), according to manufacturer recommendations. The sample pooling was performed to obtain high-quantity and high-quality DNA required for the generation of the genomic library. Purified DNA from the pooled sample was sequenced using the 454 Whole Genome Shotgun strategy according to standard protocols recommended by 454 GS FLX platform (Roche) at the

Centro Nacional de Secuenciación Genómica – CNSG, Universidad de Antioquia (Margulies et al., 2005). Obtained raw reads were end polished of low-quality regions with the toolkit PRINSEQ lite (Schmieder & Edwards, 2011) and assembled using the MIRA3 v3.4 software (Chevreux, Wetter & Suhai, 1999).

The classification of assembled contigs was carried out using the BLAST algorithm against the nucleotide and protein non-redundant databases of the NCBI and further computation of the taxonomic position of the assembled dataset with MEGAN software v5.5.3 (Huson et al., 2011). This metagenomic software uses a Lowest Common Ancestor-based algorithm that assigns each contig to taxa such the taxonomical level of the assigned taxon reflects the level of conservation of the sequence (Huson et al., 2007). Then, species-specific and widely conserved sequences were assigned to particular taxa as described by Huson et al. (2007). The contigs were classified using a bit-score threshold of 50 and retaining only those hits that are within 10% of the best hit for a contig. Additionally, the E-value confidence criterion was set at 1E-15, even though a threshold value of 1E-04 is considered a good match (De Wit et al., 2012). Only contig alignment lengths above 100 nucleotides or 100 amino acids were included in the assignment analysis.

Furthermore, in the protein analysis, the assignments were classified to the proper taxonomic level according to Monzoorul Haque et al. (2009), who empirically proposes identity thresholds for restricting the assignments. Alignments having identities in ranges of 61-65%, 56-60%, 51-55%, and 41-50% were conservatively restricted in the level of family, order, class and phylum, respectively. The identity threshold of 66-100% was used for restricting the assignment of contigs to either species or genus or family levels. Additionally, the taxonomic level within this

identity range was distinguished on the basis of the difference between the two alignment parameters, the percentage of identities and positives.

Moreover, a 1000 bp fragment of the mitochondrial *cytochrome c oxidase I* gene was amplified by PCR following conditions reported by Leung et al. (2009) and primers described by Bowles et al. (1993) (JB3: 5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and Králová-Hromadová et al. (2008) (trem.cox1.rrnl: 5'AATCATGATGCAAAAGGTA-3'). This sequence was used instead ribosomal genes since the bioinformatics analysis indicated a high enrichment of molluscan and some fungi ribosomal sequences, which might restrict the amplification of helminth sequences. The amplicon was sequenced by automated Sanger method using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) and compared by BLASTn against the NCBI nucleotide database to look for sequence matches of reported organisms.

Finally, a Bayesian tree was constructed using the sequence obtained from orange-colored protrusions and published sequences of Platyhelminthes. The Bayesian tree construction was performed using MrBayes (MB) V3.2 (Ronquist et al., 2012) setting the GTR+I+G4 substitution model estimated by the software IQ-TREE, 1000000 generations sampled every 1000 generations and the other analysis parameters as default values. The convergence of the Markov Chain Monte Carlo iterations was assessed with the Potential Scale Reduction Factor (PSRF = 1; Gelman & Rubin, 1992) and the standard deviation of split frequencies (0.001).

3. Results

3.1 Assembly and metagenomic approach

147 The massive shotgun sequencing generated 515,368 reads with an average length of 279 bp that
148 were cleaned and then assembled using MIRA software into 5,180 contigs. The taxonomic
149 classification of the contigs was carried out using the software MEGAN. For this analysis, all the
150 contigs were compared with the NCBI's non-redundant protein database using the software
151 BLASTX. With this strategy, 1,588 (30.7%) contigs were assigned to taxa (Bacteria: 412;
152 Eukaryota: 1,157; others: 19), 866 (16.7%) were unassigned, and 2,726 (52.6%) presented no
153 hits. As expected, the Eukaryota group was the dominant due to the origin of the sample.
154 Furthermore, the group Gastropoda was frequently found in this analysis (186 hits), although
155 many sequences remained unclassified due to the poor representation of these organisms in the
156 public databases. Many bacterial sequences were also identified, 19 were assigned to the fungi
157 group and 22 sequences were assigned to the Trematoda category. No viral neither protozoa
158 sequences were detected.

159 Following the MEGAN pipeline, but with nucleotide comparisons using BLASTN and the nt/nr
160 database, displayed very poor classification results. One contig was assigned to the root, 462
161 (8.9%) to particular taxa (Bacteria: 267; Eukaryota: 191; others: 4), 32 (0.6%) were unassigned
162 and 4,685 (90.5%) had no hits. At the nucleotide level, most of the sequences were left
163 unclassified. This reflects the lack of sequences at the databases of closely related organisms to
164 the ones reported in this research.

165

166 ***3.2*** **Bacteria and fungi associated with orange-colored protrusions**

167 The ranges for the confidence criterion represented by the E-value, similarity, and identity for
168 protein comparisons are shown in Table 1. Bacteria assignments included the class

169 *Gammaproteobacteria* and the phylum *Firmicutes,* which includes the orders *Bacillales* and
170 *Lactobacillales* (Fig. 1). Specifically, the class *Gammaproteobacteria* showed 322 assignments
171 for *Psychrobacter*, exhibiting hits to several types of proteins with strains of *Psychrobacter* sp.
172 (Fig. 1, Table 1). The identity criterion for *Psychrobacter* sp. ranged from 67% to 100% (Table
173 1). Similarly, nucleotide sequences showed hits for different genomic regions of *Psychrobacter*
174 sp. strains and congeners, displaying identities ranging from 72% to 99% and alignment lengths
175 from 104 bp to 1061 bp (Table 2).

176 On the other hand, 18 assignments for the order *Lactobacillales* (Fig. 1) showed hits for diverse
177 proteins exhibiting similarities and identities up to 100% for *Carnobacterium jeotgali* and
178 *Carnobacterium* sp. (Table 1). We also found hits for proteins of *Lactobacillus jensenii* and
179 *Enterococcus faecalis* displaying identities above 68% and 44%, respectively (Table 1).
180 Similarly, the nucleotide analysis showed matches for genome regions and plasmids of
181 *Carnobacterium sp*. displaying identities above 82% (Table 2). Additionally, the single hits for a
182 plasmid and a genome fragment of *Enterococcus casseliflavus* and *Enterococcus faecalis*
183 exhibited identities of 81% and 73%, respectively (Table 2).

184 A total of 28 contigs were assigned to different *Bacillales* bacteria within the phylum *Firmicutes*
185 (Fig. 1), specifically *Brochothrix thermosphacta* shows hits for several proteins exhibiting
186 identities up to 100% (Table 1). *Planococcus antarcticus*, *Bacillus cytotoxicus*, *Lactococcus*
187 *lactis* subsp. Lactis and *Staphylococcus aureus* showed identity ranges from 62% to 80% (Table
188 1). Furthermore, the nucleotide analysis showed hits for diverse bacteria belonging to genus
189 *Listeria*, *Bacillus* and *Paenibacillus* (Table 2).

Only 3 out of 19 assignments to Fungi clade satisfied the selection parameters, two hits supported the taxonomical levels of phylum and genus for *Fusarium oxysporum* and one hit classified to the genus taxonomical level for *Neurospora tetrasperma* (Table 1). In addition, the nucleotide analysis (Table 2) showed three assignments for *Mrakia frigida* (rRNA genes, 2 hits) and *Togninia minima* (putative polyubiquitin protein mRNA, 1 hit).

3.3 Parasite associated with orange-colored protrusions

The histological approach showed a tissue lesion characterized by the aggregation of haemocytes (cells endowed with phagocytic and immune-related functions) inside isolated foci surrounded by smooth muscle fibers and a bashophilic tissue contiguous to a lamellated membrane (Fig. 2A). Additionally, some lesions exhibit interstitial immunocytes inclusions whose morphology are similar to a granulation process (Fig. 2B). Although histological approach did not allow to detect key features for identification, the microscopic images showed structures around 0.55 mm in diameter, which are compatible with trematode life cycle stages (likely, sporocysts).

Furthermore, the metagenomic analysis assigned 22 contigs to trematode parasites clade. Specifically, these contigs had hits to an endonuclease-reverse transcriptase of *Schistosoma mansoni* (17) and *Schistosoma japonicum* (4) , showing identities above 46% and 42%, respectively. Similarly, in the nucleotide analysis, seven contigs showed identities above 71% for different regions of two chromosomes of *S. mansoni* (Table 3).

We successfully amplified and sequenced a 740 bp region that confirmed the presence of trematode DNA in the *L. gigas* tissue (GenBank accession KR092371). Moreover, this sequence clustered in a basal position to the suborder Xiphidiata (Trematoda: Digenea), which encompasses *Renicola* and *Helicometrina* genera (posterior probability: 0.98; Fig. 3).

212

213 **4. Discussion**

214 In this study three approaches, including histological analysis, 454 pyrosequencing and
215 automated Sanger amplification of *cytochrome c oxidase I* gene were used to explore the
216 potential causal agent of orange-colored protrusions in the muscle of *L. gigas*. The identification
217 by the histological approach was limited since no characteristic structures were detected in the
218 sample. In addition, several contigs had no hits for proteins (~52%) and nucleotide sequences
219 (~90%), indicating a lack of information on such sequences in reference databases. This
220 explanation is plausible since the current protein sequence reference databases cover only a small
221 fraction of the biodiversity believed to be present in the environment (Wu et al., 2009). Despite
222 these limitations, the contigs alignment lengths (≥100 nucleotides or amino acids) and the bit-
223 scores (50) used in this research ensure a reasonable level of confidence in the taxonomic
224 assignments (Huson et al., 2007).

225

226 **Bacteria and fungi associated with orange-colored protrusions**

227 The scope of the massive sequencing approach allowed the detection of some bacteria previously
228 reported as microbiota associated with *L. gigas* (Acosta et al., 2009; Pérez et al., 2014) as well as
229 new reports. For instance, *Psychrobacter* sp. was found in the *L. gigas* muscle in both nucleotide
230 and protein analyses (Tables 1 and 2). This outcome corroborates previous studies that found
231 *Psychrobacter* sp. in environmental (Acosta et al., 2009; Pérez et al., 2014) and tissue (Pérez et
232 al., 2014) samples from *L. gigas*.

However, this study also found bacteria and fungi that have not been reported so far in *L. gigas*. Specifically, homologous protein and nucleotide sequences of species (e.g. *Carnobacterium jeotgali*), family or genus of *Carnobacterium sp.* were detected in the *L. gigas* muscle (Tables 1 and 2). The *Carnobacterium* strains have been reported to inhabit live fish and a variety of seafood, dairy, and meat (Leisner et al., 2007).

In addition, this research found homologous protein and nucleotide sequences of genus *Bacillus* and *Enterococcus* in the affected tissue of *L. gigas*. *Bacillus* species have a ubiquitous distribution, inhabiting different environments such as soils, rocks, vegetation, foods and waters (Nicholson, 2002). Similarly, the ubiquitous nature of enterococci determines their frequent finding in foods as contaminants, although their predominant habitat is human and animal gastrointestinal tract (Giraffa, 2002). However, they also occur in soil, surface waters, vegetables, and fermented foods such as sausages, meat and cheese (Giraffa, 2002; Foulquié et al., 2006).

Furthermore, another bacteria present in the sample is *Brochothrix thermosphacta* since it was assigned to species or genus taxonomical levels according to Monzoorul Haque et al. (2009). This bacterium closely related to *Listeria* is a non-proteolytic food spoilage organism in prepacked meats and fish products (Gardner, 1981; Lannelongue et al., 1982; Pin, García de Fernando & Ordóñez, 2002). In addition, some *Listeria* hits were detected in the nucleotide analysis, although the identity values did not allow to identify the species. This result is concordant with studies that have isolated *Listeria* members from freshwater and marine environments (Colburn et al., 1990; El Marrakchi, Boum'handi & Hamama, 2005).

Metagenomic analysis also showed some fungi assignments related to *Fusarium, Neurospora, Togninia* and *Mrakia*. Both *Fusarium* and *Neurospora* exhibit wide distribution including humid tropical and subtropical marine environments (Steele, 1967; Turner, Perkins & Fairfield, 2001; Babu et al., 2010; Summerell et al., 2010; Jebaraj et al., 2012; Saravanan & Sivakumar, 2013; Kumar, Gousia & Latha, 2015). Specifically, some *Fusarium* species are associated with infections in crustacean and cultivated fishes (Hatai, 2012), whereas other species are endosymbionts of some seaweeds (Suryanarayanan, 2012), corals (Raghukumar & Ravindran, 2012) and some sea sponges (Höller et al., 2000; Wang, Li & Zhu, 2008; Liu et al., 2010; Paz et al., 2010).

In contrast, *Togninia* and *Mrakia* show more restricted distributions. For instance, *Togninia* comprises pathogenic fungi responsible for the development of wood diseases and some strains have been isolated from submerged wood from streams, lakes, ponds, reservoirs and ditches (Hu, Cai & Hyde, 2012). Likewise, several *Mrakia* species have been isolated from icy environments, including melt waters from glaciers and permafrost in Antarctica (Hua et al., 2010; Pathan et al., 2010; Carrasco et al., 2012; Zhang et al., 2012; Tsuji et al., 2013a,b), Argentina (Brizzio et al., 2007; de Garcia, Brizzio & van Broock, 2012), the Qinghai-Tibet Plateau (Su et al., 2016), Italy (Turchetti et al., 2008; Branda et al., 2010; Thomas-Hall et al., 2010) and Arctic (Pathan et al., 2010).

Considering that several of the new bacteria reports are related to food microorganisms, we hypothesized that they might grow under environmental or freezing conditions instead of being native microbiota. Fungi findings suggest an environmental source; however, since some species of *Fusarium* and *Neurospora* produce orange spores (Davis & Perkins, 2002; Hatai, 2012), they may explain the colored protrusions found in *L. gigas* due to an opportunistic or primary

277 infection. Thus, it remains to explore the role of bacteria and fungi in the muscle of *L. gigas* and
278 their relationship with the lesion, native microbiota or the environment.

279

280 **Parasite associated with orange-colored protrusions**

281 The histological approach showed evidence of a membrane that is consistent with a syncytium
282 enclosing a multicellular parasite, a mollusk inflammatory response elicited by haemocytes (De
283 Vico & Carella, 2012). Moreover, such membrane is also compatible with the wall layers of life
284 cycle stage of Platyhelminthes, suggesting a possible infection by trematodes that infect other
285 mollusks (Cake, 1976; Sorensen & Minchella, 2001). This finding was supported by the
286 metagenomics analysis that showed sequences homologous to an endonuclease-reverse
287 transcriptase of some species of trematodes like *Schistosoma* (Table 3). This result is expected
288 since highly repetitive sequences, such non-LTR retrotransposons, which estimated copy number
289 goes up to 24000, are more likely to be detected in the whole genome shotgun amplification
290 (DeMarco et al., 2005). Following Monzoorul Haque et al. (2009), these sequences can be
291 assigned to the taxonomic levels of phylum, class, order and even family. Although this outcome
292 is biased by the nucleotide and protein sequences available in the NCBI databases, it supports the
293 histological finding that the protrusions may be caused by a parasite.

294 Bayesian tree supported the last result due to the clustering of the sample in a basal position to
295 the suborder Xiphidiata (Trematoda: Digenea), which includes *Renicola* species that produce
296 colored pigments (Stunkard, 1950; Galaktionov & Skirnisson, 2000). This outcome reveals a
297 phylogenetic relationship between the sequence found in this study and *Renicola,* althought it
298 remains to determinate its genetic distance with *Cercaria parvicaudata*, *R. roscovita* and *R.*

299 *thaidus* due to the lack of information of *cytochrome c oxidase I* and endonuclease-reverse
300 transcriptase sequences of these taxa.

301 In conclusion, this study found evidence of a trematode infection as well the presence of fungi
302 and bacteria in the protruded muscle of *L. gigas*, which provides novel information for the
303 parasitology and microbiology of this species. This first insight of a trematode infection in *L.*
304 *gigas* is a baseline to study the identification at the species level, trematode life cycle,
305 environmental conditions that trigger its appearance and epidemiological aspects regarding the
306 host and possible effects on human health.

307

308 **5. Acknowledgements**

309 The authors thank to the Laboratorio de Patología Animal and Centro de Secuenciación
310 Genómica, Universidad de Antioquia, for assistance and services provided. The authors also
311 thank to the anonymous reviewers for their comments, which substantially improved the final
312 version of this article.

313

314

315

316

317

318 **6. References**

319

320 Acosta EA., Gómez E., Romero M., Cadavid GE., Moreno CX. 2009. Molecular identification of
321 bacterial populations associated to Queen Conch (*Strombus gigas*) from Colombian

Caribbe. *Acta Biologica Colombiana* 14:83–96.

Aldana D. 2003. *El caracol* Strombus gigas *: Conocimiento integral para su manejo sustentable en el Caribe*. Yucatán, México: CYTED. Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo.

Aldana D., Frenkiel L., Brulé T., Montero J., Baqueiro E. 2011. Occurrence of Apicomplexa-like structures in the digestive gland of *Strombus gigas* throughout the Caribbean. *Journal of Invertebrate Pathology* 106:174–178. DOI: 10.1016/j.jip.2010.09.004.

Aldana D., Glazer R., Delgado G., Baqueiro E., Montero J. 2009. Occurance of Apicomplexa infecting Queen Conch, *Strombus gigas*, from offshore and nearshore in Florida. *Proceedings of the Gulf and Caribbean Fisheries Institute* 61:491–497.

Banaszak AT., García Ramos M., Goulet TL. 2013. The symbiosis between the gastropod *Strombus gigas* and the dinoflagellate *Symbiodinium*: An ontogenic journey from mutualism to parasitism. *Journal of Experimental Marine Biology and Ecology* 449:358–365. DOI: 10.1016/j.jembe.2013.10.027.

Baqueiro E., Frenkiel L., Zetina A., Aldana D. 2007. Coccidian (Apicomplexa) parasite infecting *Strombus gigas* Linné, 1758 digestive gland. *Journal of Shellfish Research* 26:319–321.

Bowles J., Hope M., Tiu WU., Liu X., McManus DP. 1993. Nuclear and mitochondrial genetic markers highly conserved between Chinese and Philippine *Schistosoma japonicum*. *Acta Tropica* 55:217–229.

Branda E., Turchetti B., Diolaiuti G., Pecci M., Smiraglia C., Buzzini P. 2010. Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). *FEMS Microbiology Ecology* 72:354–369. DOI: 10.1111/j.1574-6941.2010.00864.x.

Brizzio S., Turchetti B., de García V., Libkind D., Buzzini P., van Broock M. 2007. Extracellular enzymatic activities of basidiomycetous yeasts isolated from glacial and subglacial waters of northwest Patagonia (Argentina). *Canadian Journal of Microbiology* 53:519–525. DOI: 10.1139/W07-010.

Brownell WN., Stevely JM. 1981. The biology, fisheries, and management of the Queen Conch, *Strombus gigas*. *Marine Fisheries Review*.

Cake EW. 1976. A key to larval cestodes of shallow-water, benthic mollusks of the northern Gulf of Mexico. *Proceedings of the Helminthological Society of Washington* 43:160–171.

Carrasco M., Rozas JM., Barahona S., Alcaíno J., Cifuentes V., Baeza M. 2012. Diversity and extracellular enzymatic activities of yeasts isolated from King George Island, the sub-Antarctic region. *BMC Microbiology* 12:251–260. DOI: 10.1186/1471-2180-12-251.

Chevreux B., Wetter T., Suhai S. 1999. Genome sequence assembly using trace signals and additional sequence information. In: *Computer Science and Biology: Proceedings of the German Conference on Bioinformatics (GCB)*. 45–56.

Colburn KG., Kaysner CA., Abeyta Jr. C., Wekell MM. 1990. *Listeria* species in a California

359 coast estuarine environment. *Applied and Environmental Microbiology* 56:2007–2011.

360 Davis RH., Perkins DD. 2002. *Neurospora*: a model of model microbes. *Nature reviews.*
361 *Genetics* 3:397–403. DOI: 10.1038/nrg797.

362 DeMarco R., Machado AA., Bisson-Filho AW., Verjovski-Almeida S. 2005. Identification of 18
363 new transcribed retrotransposons in *Schistosoma mansoni*. *Biochemical and Biophysical*
364 *Research Communications* 333:230–240. DOI: 10.1016/j.bbrc.2005.05.080.

365 Foulquié MR., Sarantinopoulos P., Tsakalidou E., De Vuyst L. 2006. The role and application of
366 enterococci in food and health. *International Journal of Food Microbiology* 106:1–24. DOI:
367 10.1016/j.ijfoodmicro.2005.06.026.

368 Galaktionov K V., Skirnisson K. 2000. Digeneans from intertidal molluscs of SW Iceland.
369 *Systematic Parasitology* 47:87–101. DOI: 10.1023/A:1006426117264.

370 de Garcia V., Brizzio S., van Broock MR. 2012. Yeasts from glacial ice of Patagonian Andes,
371 Argentina. *FEMS Microbiology Ecology* 82:540–550. DOI: 10.1111/j.1574-
372 6941.2012.01470.x.

373 García del Moral R. 1993. *Laboratorio de Anatomía Patológica*. Madrid: McGraw-Hill,
374 Interamericana de España.

375 García Ramos M., Banaszak AT. 2014. Symbiotic association between symbiodinium and the
376 gastropod *Strombus gigas*: larval acquisition of symbionts. *Marine biotechnology (New*
377 *York, N.Y.)* 16:193–201. DOI: 10.1007/s10126-013-9536-x.

378 Gardner GA. 1981. *Brochothrix thermosphacta* (*Microbacterium thermosphactum*) in the
379 spoilage of meats: a review. In: Roberts TA, Hobbs GA, Christian JHB, Skovgaard N eds.
380 *Psychrotrophic microorganisms in spoilage and pathogenicity*. London, England:
381 Academic Press, 139–173.

382 Gelman A., Rubin DB. 1992. Inference from iterative simulation using multiple sequences.
383 *Statistical Science* 7:457–472.

384 Giraffa G. 2002. Enterococci from foods. *FEMS Microbiology Reviews* 26:163–171. DOI:
385 10.1016/S0168-6445(02)00094-3.

386 Glazer R., Quintero I. 1998. Observations on the sensitivity of Queen Conch to water quality:
387 implications for coastal development. In: *Proceedings of the Gulf and Caribbean Fisheries*
388 *Institute*. 78–93.

389 Gros O., Frenkiel L., Aldana D. 2009. Structural analysis of the digestive gland of the Queen
390 Conch *Strombus gigas* Linnaeus, 1758 and its intracellular parasites. *Journal of Molluscan*
391 *Studies* 75:59–68. DOI: 10.1093/mollus/eyn041.

392 Hatai K. 2012. Diseases of Fish and Shellfish Caused by Marine Fungi. In: Raghukumar C ed.
393 *Biology of Marine Fungi*. Berlin, Germany: Springer-Verlag Berlin Heidelberg, 15–52.
394 DOI: 10.1007/978-3-642-23342-5_14.

Hechinger RF., Miura O. 2014. Two “new” renicolid trematodes (Trematoda: Digenea: Renicolidae) from the California horn snail, *Cerithidea californica* (Haldeman, 1840) (Gastropoda: Potamididae). *Zootaxa* 3784:559–574. DOI: 10.11646/zootaxa.3784.5.5.

Höller U., Wright AD., Matthée GF., Konig GM., Draeger S., Aust H-J., Schulz B. 2000. Fungi from marine sponges: diversity, biological activity and secondary metabolites. *Mycological Research* 104:1354–1365. DOI: 10.1017/S0953756200003117.

Hua MX., Chi Z., Liu GL., Buzdar MA., Chi ZM. 2010. Production of a novel and cold-active killer toxin by *Mrakia frigida* 2E00797 isolated from sea sediment in Antarctica. *Extremophiles* 14:515–521. DOI: 10.1007/s00792-010-0331-6.

Hu D-M., Cai L., Hyde KD. 2012. Three new ascomycetes from freshwater in China. *Mycologia* 104:1478–1489. DOI: 10.3852/11-430.

Huson DH., Auch AF., Qi J., Schuster SC. 2007. MEGAN analysis of metagenomic data. *Genome Research* 17:377–386. DOI: 10.1101/gr.5969107.

Huson DH., Mitra S., Ruscheweyh HJ., Weber N., Schuster SC. 2011. Integrative analysis of environmental sequences using MEGAN4. *Genome Research* 21:1552–1560. DOI: 10.1101/gr.120618.111.

Králová-Hromadová I., Spakulová M., Horácková E., Turceková L., Novobilský A., Beck R., Koudela B., Marinculić A., Rajský D., Pybus M. 2008. Sequence analysis of ribosomal and mitochondrial genes of the giant liver fluke *Fascioloides magna* (Trematoda: Fasciolidae): Intraspecific variation and differentiation from *Fasciola hepatica*. *Journal of Parasitology* 94:58–67. DOI: 10.1645/GE-1324.1.

Lannelongue M., Hanna MO., Finne G., Nickelson II R., Vanderzant C. 1982. Storage characteristics of finfish fillets (*Archosargus probatocephalus*) packaged in modified gas atmospheres containing carbon dioxide. *Journal of Food Protection* 45:440–444.

Leisner JJ., Laursen BG., Prévost H., Drider D., Dalgaard P. 2007. *Carnobacterium*: Positive and negative effects in the environment and in foods. *FEMS Microbiology Reviews* 31:592–613. DOI: 10.1111/j.1574-6976.2007.00080.x.

Leung TLF., Donald KM., Keeney DB., Koehler A V., Peoples RC., Poulin R. 2009. Trematode parasites of Otago Harbour (New Zealand) soft-sediment intertidal ecosystems: Life cycles, ecological roles and DNA barcodes. *New Zealand Journal of Marine and Freshwater Research* 43:857–865. DOI: 10.1080/00288330909510044.

Liu WC., Li CQ., Zhu P., Yang JL., Cheng KD. 2010. Phylogenetic diversity of culturable fungi associated with two marine sponges: *Haliclona simulans* and *Gelliodes carnosa*, collected from the Hainan Island coastal waters of the South China Sea. *Fungal Diversity* 42:1–15. DOI: 10.1007/s13225-010-0022-8.

Mardis ER. 2008. Next-generation DNA sequencing methods. *Annual Review of Genomics and Human Genetics* 9:387–402. DOI: 10.1146/annurev.genom.9.081307.164359.

Margulies M., Egholm M., Altman WE., Attiya S., Bader JS., Bemben LA., Berka J., Braverman

MS., Chen Y-J., Chen Z., Dewell SB., Du L., Fierro JM., Gomes X V., Godwin BC., He W., Helgesen S., Ho CH., Irzyk GP., Jando SC., Alenquer MLI., Jarvie TP., Jirage KB., Kim J-B., Knight JR., Lanza JR., Leamon JH., Lefkowitz SM., Lei M., Li J., Lohman KL., Lu H., Makhijani VB., McDade KE., McKenna MP., Myers EW., Nickerson E., Nobile JR., Plant R., Puc BP., Ronan MT., Roth GT., Sarkis GJ., Simons JF., Simpson JW., Srinivasan M., Tartaro KR., Tomasz A., Vogt KA., Volkmer GA., Wang SH., Wang Y., Weiner MP., Yu P., Begley RF., Rothberg JM. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380. DOI: 10.1038/nature04726.

Márquez E., Landínez-García RM., Ospina-Guerrero SP., Segura JA., Prada M., Castro E., Correa JL., Borda C. 2013. Genetic analysis of Queen Conch *Strombus gigas* from the Southwest Caribbean. In: *Gulf and Caribbean Fisheries Institute*. 410–416.

El Marrakchi A., Boum'handi N., Hamama A. 2005. Performance of a new chromogenic plating medium for the isolation of *Listeria monocytogenes* from marine environments. *Letters in Applied Microbiology* 40:87–91. DOI: 10.1111/j.1472-765X.2004.01651.x.

Mitton JB., Berg Jr. CJ., Orr KS. 1989. Population structure, larval dispersal, and gene flow in the Queen Conch, *Strombus gigas*, of the Caribbean. *Biological Bulletin* 177:356–362.

Monzoorul Haque M., Ghosh TS., Komanduri D., Mande SS. 2009. SOrt-ITEMS: Sequence orthology based approach for improved taxonomic estimation of metagenomic sequences. *Bioinformatics* 25:1722–1730. DOI: 10.1093/bioinformatics/btp317.

Nicholson WL. 2002. Roles of *Bacillus* endospores in the environment. *Cellular and Molecular Life Sciences* 59:410–416. DOI: 10.1007/s00018-002-8433-7.

Pathan AAK., Bhadra B., Begum Z., Shivaji S. 2010. Diversity of Yeasts from Puddles in the Vicinity of Midre Lovénbreen Glacier, Arctic and Bioprospecting for Enzymes and Fatty Acids. *Current Microbiology* 60:307–314. DOI: 10.1007/s00284-009-9543-3.

Paz Z., Komon-Zelazowska M., Druzhinina IS., Aveskamp MM., Shnaiderman A., Aluma Y., Carmeli S., Ilan M., Yarden O. 2010. Diversity and potential antifungal properties of fungi associated with a Mediterranean sponge. *Fungal Diversity* 42:17–26. DOI: 10.1007/s13225-010-0020-x.

Pérez OM., Posada M., Cadavid GE., Moreno CX. 2014. Assessment of the bacterial community diversity associated with the queen conch *Strombus gigas* (Linnaeus, 1758) from the Caribbean coast of Colombia using denaturing gradient gel electrophoresis and culturing. *Aquaculture Research* 45:773–786. DOI: 10.1111/are.12016.

Pin C., García de Fernando GD., Ordóñez JA. 2002. Effect of modified atmosphere composition on the metabolism of glucose by *Brochothrix thermosphacta*. *Applied and Environmental Microbiology* 68:4441–4447. DOI: 10.1128/AEM.68.9.4441.

Prada M., Castro E., Taylor E., Puentes V., Appeldoorn R., Daves N. 2009. Non-detrimental findings for the Queen Conch (*Strombus gigas*) in Colombia. *NDF Workshop Case Studies*:34.

Prophet E., Mills B., Arrington J., Sobón L. 1995. Métodos histotecnológicos. Washington D.C.: Instituto de Patología de las Fuerzas Armadas de los Estados Unidos de América (AFIP), Registro de Patología de los Estados Unidos de América (ARP). :280.

Raghukumar C., Ravindran J. 2012. Fungi and Their Role in Corals and Coral Reef Ecosystems. In: Raghukumar C ed. *Biology of Marine Fungi*. Berlin, Germany: Springer-Verlag Berlin Heidelberg, 89–113. DOI: 10.1007/978-3-642-23342-5_14.

Randall JE. 1964. Contributions to the biology of the Queen Conch, *Strombus gigas*. *Bulletin of Marine Science of the Gulf and Caribbean* 14:246–295.

Rodriguez AI., Hariharan H., Nimrod S. 2011. Occurrence and antimicrobial drug resistance of potential bacterial pathogens from shellfish, including Queen Conchs (*Strombus gigas*) and Whelks (*Cittarium pica*) in Grenada. *WebmedCentral Microbiology* 2:1–11.

Ronquist F., Teslenko M., Van der Mark P., Ayres DL., Darling A., Höhna S., Larget B., Liu L., Suchard MA., Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61:539–542. DOI: 10.1093/sysbio/sys029.

Schmieder R., Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27:863–864. DOI: 10.1093/bioinformatics/btr026.

Sorensen RE., Minchella DJ. 2001. Snail-trematode life history interactions: past trends and future directions. *Parasitology* 123 Suppl:S3–S18. DOI: 10.1017/S0031182001007843.

Stunkard HW. 1950. Further observations on *Cercaria parvicaudata* Stunkard and Shaw, 1931. *Biological Bulletin* 99:136–142.

Su Y., Jiang X., Wu W., Wang M., Hamid MI., Xiang M., Liu X. 2016. Genomic, Transcriptomic and Proteomic Analysis Provide Insights into the Cold Adaptation Mechanism of the Obligate Psychrophilic Fungus *Mrakia psychrophila*. *G3: Genes|Genomes|Genetics* 6:3603–3613. DOI: 10.1534/g3.116.033308.

Suryanarayanan TS. 2012. Fungal Endosymbionts of Seaweeds. In: Raghukumar C ed. *Biology of Marine Fungi*. Berlin, Germany: Springer-Verlag Berlin Heidelberg, 53–70. DOI: 10.1007/978-3-642-23342-5_14.

Tello-Cetina JA., Rodríguez-Gil LA., Rodríguez-Romero F. 2005. Genética poblacional del caracol rosado *Strombus gigas* en la Península de Yucatán: Implicaciones para su manejo y pesquería. *Ciencias Marinas* 31:379–386.

Theile S. 2001. *Queen Conch fisheries and their management in the Caribbean*. Brussels, Belgium.

Thomas-Hall SR., Turchetti B., Buzzini P., Branda E., Boekhout T., Theelen B., Watson K. 2010. Cold-adapted yeasts from Antarctica and the Italian Alps-description of three novel species: *Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. *Extremophiles* 14:47–59. DOI: 10.1007/s00792-009-0286-7.

508 Tsuji M., Fujiu S., Xiao N., Hanada Y., Kudoh S., Kondo H., Tsuda S., Hoshino T. 2013a. Cold
509 adaptation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area,
510 Antarctica. *FEMS Microbiology Letters* 346:121–130. DOI: 10.1111/1574-6968.12217.

511 Tsuji M., Singh SM., Yokota Y., Kudoh S., Hoshino T. 2013b. Influence of Initial pH on Ethanol
512 Production by the Antarctic Basidiomycetous yeast *Mrakia blollopis*. *Bioscience,*
513 *Biotechnology and Biochemistry* 77:2483–2485. DOI: 10.1271/bbb.130497.

514 Turchetti B., Buzzini P., Goretti M., Branda E., Diolaiuti G., D'Agata C., Smiraglia C.,
515 Vaughan-Martini A. 2008. Psychrophilic yeasts in glacial environments of Alpine glaciers.
516 *FEMS Microbiology Ecology* 63:73–83. DOI: 10.1111/j.1574-6941.2007.00409.x.

517 De Vico G., Carella F. 2012. Morphological features of the inflammatory response in molluscs.
518 *Research in Veterinary Science* 93:1109–1115. DOI: 10.1016/j.rvsc.2012.03.014.

519 Volland JM., Gros O., Frenkiel L., Aldana D. 2010. Apicomplexan parasite in the digestive
520 gland of various species of the family Strombidae: *Strombus costatus*, *S. gigas*, and *S.*
521 *pugilis*. In: *Gulf and Caribbean Fisheries Institute*. 430–432.

522 Wang G., Li Q., Zhu P. 2008. Phylogenetic diversity of culturable fungi associated with the
523 Hawaiian Sponges *Suberites zeteki* and *Gelliodes fibrosa*. *Antonie van Leeuwenhoek*
524 93:163–174. DOI: 10.1007/s10482-007-9190-2.

525 Werding B. 1969. Morphologie, Entwicklung und Ökologie digener Trematoden-Larven der
526 Strandschnecke *Littorina littorea*. *Marine Biology* 3:306–333.

527 De Wit P., Pespeni MH., Ladner JT., Barshis DJ., Seneca F., Jaris H., Therkildsen NO.,
528 Morikawa M., Palumbi SR. 2012. The simple fool's guide to population genomics via
529 RNA-Seq: An introduction to high-throughput sequencing data analysis. *Molecular Ecology*
530 *Resources* 12:1058–1067. DOI: 10.1111/1755-0998.12003.

531 Wu D., Hugenholtz P., Mavromatis K., Pukall R., Dalin E., Ivanova NN., Kunin V., Goodwin L.,
532 Wu M., Tindall BJ., Hooper SD., Pati A., Lykidis A., Spring S., Anderson IJ., D'haeseleer
533 P., Zemla A., Singer M., Lapidus A., Nolan M., Copeland A., Han C., Chen F., Cheng J-F.,
534 Lucas S., Kerfeld C., Lang E., Gronow S., Chain P., Bruce D., Rubin EM., Kyrpides NC.,
535 Klenk H-P., Eisen JA. 2009. A phylogeny-driven genomic encyclopaedia of Bacteria and
536 Archaea. *Nature* 462:1056–1060. DOI: 10.1038/nature08656.

537 Zamora-Bustillos R., Rodríguez-Canul R., García de León FJ., Tello-Cetina J. 2011. Diversidad
538 genética de dos poblaciones del caracol *Strombus gigas* (Gastropoda: Strombidae) en
539 Yucatán, México, con microsatélite. *Revista de Biologia Tropical* 59:1127–1134.

540 Zhang X., Hua M., Song C., Chi Z. 2012. Occurrence and Diversity of Marine Yeasts in
541 Antarctica Environments. *Journal of Ocean University of China* 11:70–74. DOI:
542 10.1007/s11802-012-1820-2.

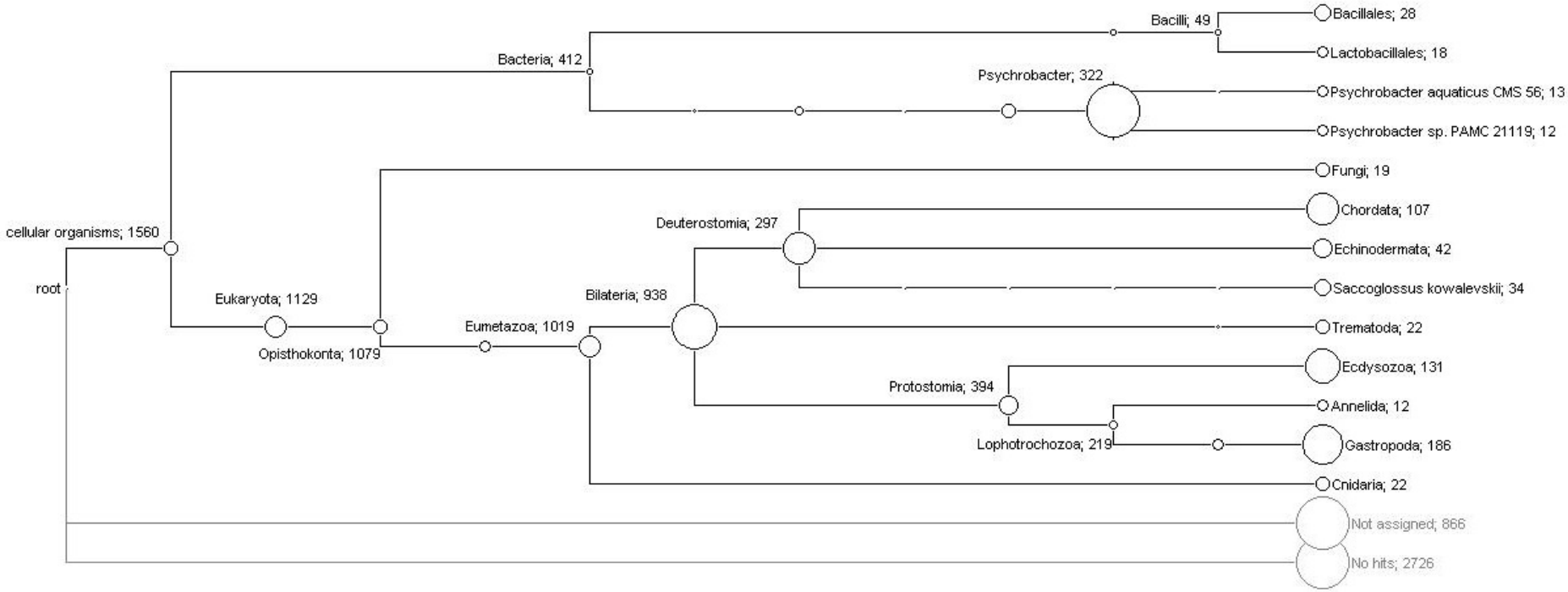
543

544

545

546 **Figure 1.** Phylogenetic diversity of translated contigs from orange-colored protrusions of *Lobatus gigas* computed by MEGAN. The
547 nodes of the cladogram represent the assigned taxa and the numbers indicate the relative abundance of assigned contigs.

548



549

550

551

552

553

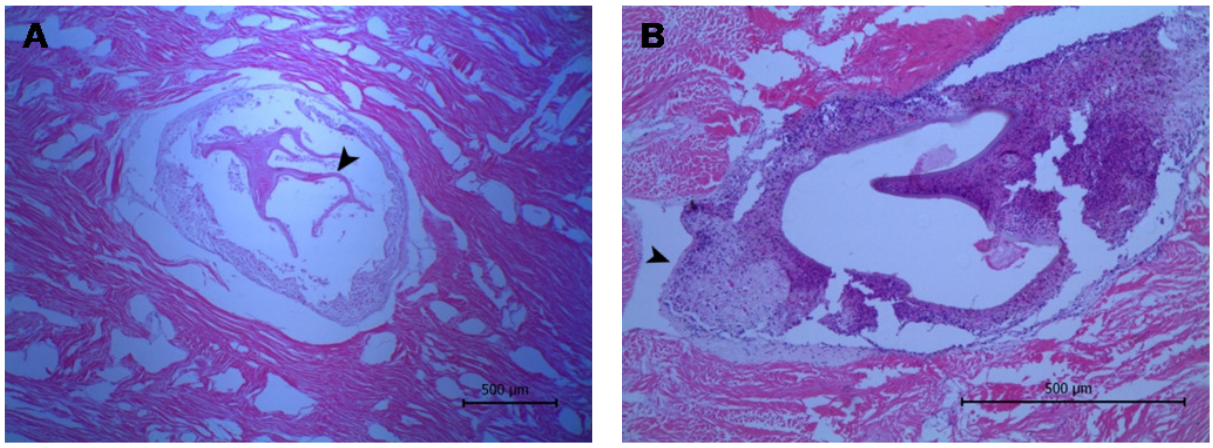
554 **Figure 2.** Histological sections of orange-colored protrusions in the muscle of *Lobatus gigas.*

555 The lesions showed haemocytes stained purplish-blue and the smooth muscle fibers pink-red. **A.**

556 presence of lamellated membrane (arrowhead) (40X). **B.** granulation process (arrowhead)

557 (100X).

558



559

560

561

562

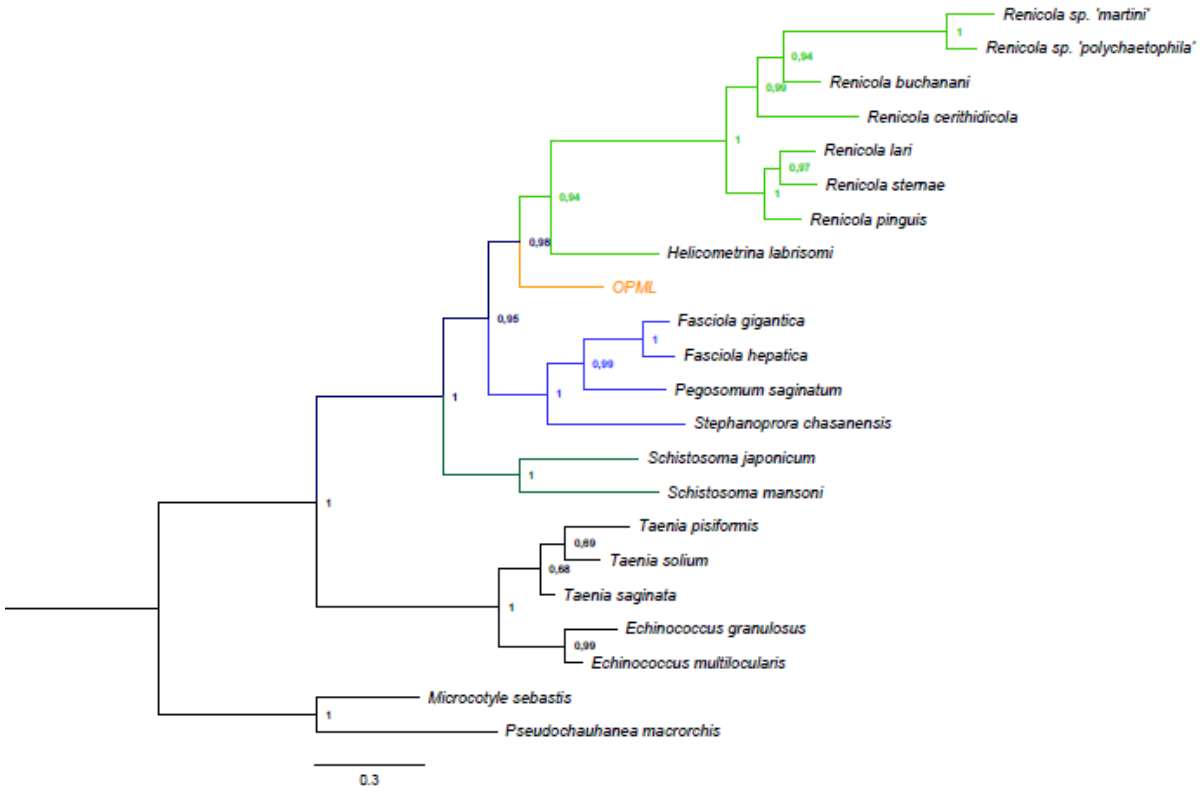
563

564

565

566 **Figure 3.** Bayesian tree obtained from *cytochrome c oxidase I* gene sequences of orange-colored
567 protrusions in the muscle of *L. gigas* (OPML) and GenBank Platyhelminthes sequences. *P.*
568 *macrorchis* (JN592039.1), *M. sebastis* (NC_009055.1), *E. multilocularis* (AB018440.2), *E.*
569 *granulosus* (AF297617.1), *T. saginata* (AY195858.1), *T. solium* (AY211880.1), *T. pisiformis*
570 (GU569096.1), *S. mansoni* (AF216698.1), *S. japonicum* (AF215860.1), *S. chasanensis*
571 (KU757308.1), *P. saginatum* (KX097855.1), *F. hepatica* (AF216697.1), *F. gigantica*
572 (KF543342.1), *H. labrisomi* (KJ996009.1), *R. pinguis* (KU563724.1), *R. sternae* (KU563723.1),
573 *R. lari* (KU563727.1), *R. cerithidicola* (KF512573.1), *R. buchanani* (KF512572.1), *Renicola* sp.
574 'polychaetophila' (KF512551.1), *Renicola* sp. 'martini' (KF512560.1).

575



576

577

578

579

580 **Tables**

581

582

583

584

585

586

587

588 **Table 1.** Diversity content in Bacteria and Fungi clades found in a pooled sample of orange-colored protrusions from *L. gigas* muscle
589 using translated contig sequences and the taxonomic classifier MEGAN. [a] The assignments were classified to the taxonomic level
590 according to Monzoorul Haque *et al*. (2009), [b] *Bacillales*, [l] *Lactobacillales*.

591

592

593

594

595

596

597

			Ranges				
Contig	**Organism**	**Gene**	**E-value**	**Positives (%)**	**Identities (%)**	**Length (aa)**	**Assigment[a]**

8	*Psychrobacter* sp.	binding protein, kinase, transporter, adaptor, hypothetical proteins, membrane protein, glycosylase	0,000E+00; 1,325E-30	96; 100	96; 100	113; 259	Species
11	*Psychrobacter* sp.	dehydrogenase, hypothetical proteins, catalase, cytoplasmic protein, propionase, transferase, chaperone, deaminase, membrane protein	0,000E+00; 4,451E-73	94; 100	90; 99	105; 306	Genus
2	*Psychrobacter* sp.	channel protein, hypothetical protein	1,189E-107; 4,390E-77	77; 83	67; 73	183; 242	Family
2	*Carnobacterium jeotgali* [1]	replication initiator, phosphorylase	5,120E-93; 4,347E-87	99; 100	99; 100	135; 138	Species
2	*Carnobacterium* sp. [1]	hypothetical protein; integrase	2,691E-127; 4,938E-58	100; 100	100; 100	109; 183	Species
1	*Carnobacterium* sp. [1]	integrase	1,94E-61	86	80	129	Genus
1	*Carnobacterium* sp. [1]	hypothetical protein	2,20E-69	94	86	158	Family
2	*Lactobacillus jensenii* [1]	hypothetical protein	2,022E-78; 2,550E-60	73; 85	68; 81	166; 169	Genus
1	*Enterococcus faecalis* [1]	hypothetical protein	3,70E-45	68	44	172	Phylum
7	*Brochothrix thermosphacta* [b]	kinase, transcriptional regulator, transporter, ribosomal protein, reductase, hypothetical proteins	3,514E-162; 9,715E-69	100; 100	100; 100	180; 248	Species
6	*Brochothrix thermosphacta* [b]	dehydrogenase, hypothetical proteins, transposase, transferase	1,918E-148; 1,431E-53	76; 100	71; 99	120; 243	Genus
1	*Planococcus antarcticus* [b]	hypothetical protein	2,65E-55	90	80	106	Family
1	*Bacillus cytotoxicus* [b]	synthetase	7,05E-164	83	71	223	Family
1	*Lactococcus lactis* subsp. Lactis [1]	replication protein	6,02E-91	81	62	164	Family
1	*Staphylococcus aureus* [b]	hypothetical protein	2,05E-32	71	64	104	Family
1	*Fusarium oxysporum*	glutamine-rich protein	1,064E-16	56	41	243	Phylum
1	*Fusarium oxysporum*	glutamine-rich protein	2,9E-16	85	82	243	Genus
1	*Neurospora tetrasperma*	hypothetical protein	1,426E-58	92	90	107	Genus

Table 2. Diversity content in Bacteria and Fungi clades found in a pooled sample of orange-colored protrusions from *S. gigas* muscle using nucleotide contig sequences and the taxonomic classifier MEGAN. [b] *Bacillales*, [l] *Lactobacillales*, gf: genome fragment, p: plasmid, rRNA: ribosomal fragment.

			Ranges		
Contig	**Organism**	**Gene**	**E-value**	**Identities (%)**	**Length (bp)**
1	*Psychrobacter* sp.	pRWF101	0,00E+00	98	993

1	*Psychrobacter* sp.	gf	0,00E+00	99	815
6	*Psychrobacter* sp.	p, gf	0,000E+00; 7,247E-59	90; 96	176; 679
1	*Psychrobacter* sp.	gf	0,00E+00	88	1162
7	*Psychrobacter* sp.	p, gf	0,000E+00; 3,877E-131	80; 85	520; 914
3	*Psychrobacter* sp.	gf	4,648E-143; 2,430E-35	76; 79	288; 748
1	*Psychrobacter cryohalolentis*	gf	0,00E+00	93	1047
3	*Psychrobacter cryohalolentis*	p, gf	5,595E-138; 6,961E-40	92; 95	112; 352
4	*Psychrobacter cryohalolentis*	gf	3,316E-153; 1,041E-103	83; 84	419; 537
3	*Psychrobacter cryohalolentis*	gf	1,626E-92; 3,574E-74	75; 78	524; 700
1	*Psychrobacter arcticus*	gf	0,00E+00	91	646
1	*Psychrobacter arcticus*	gf	5,09E-120	94	307
1	*Psychrobacter arcticus*	gf	8,83E-47	91	148
11	*Psychrobacter arcticus*	gf	0,000E+00; 3,900E-148	81; 88	611; 1061
11	*Psychrobacter arcticus*	gf	0,000E+00; 6,195E-25	80; 89	104; 603
7	*Psychrobacter arcticus*	gf	1,962E-172; 1,181E-24	72; 79	224; 978
1	*Carnobacterium* sp. [l]	gf	0,00E+00	94	2422
1	*Carnobacterium* sp. [l]	pWNCR9	0,00E+00	92	1466
1	*Carnobacterium* sp. [l]	gf	0,00E+00	96	1244
1	*Carnobacterium* sp. [l]	gf	0,00E+00	98	1029
3	*Carnobacterium* sp. [l]	p, gf	0,000E+00; 1,117E-155	95; 98	321; 624
6	*Carnobacterium* sp. [l]	p, gf	0,000E+00; 8,843E-57	82; 88	264; 897
1	*Enterococcus casseliflavus* [l]	pTnpA	4,68E-85	81	464
1	*Enterococcus faecalis* [l]	gf	3,31E-37	73	537
1	*Listeria grayi* [b]	23S rRNA	0,00E+00	90	1263
1	*Listeria welshimeri* [b]	23S rRNA	1,15E-176	88	539
1	*Listeria monocytogenes* [b]	gf	3,85E-103	74	902
1	*Listeria innocua* [b]	gf	7,87E-109	76	754
1	*Bacillus megaterium* [b]	gf	5,41E-170	78	908
1	*Bacillus toyonensis* [b]	gf	1,43E-66	74	622
1	*Bacillus cereus* [b]	gf	2,57E-17	78	174
1	*Paenibacillus larvae* [b]	pPL374	1,11E-170	100	335
1	Uncultured compost bacterium [b]	16S rRNA	0,00E+00	99	436
1	*Mrakia frigida*	25S rRNA	0,000E+00	100	1429
1	*Mrakia frigida*	18S rRNA	0,000E+00	99	1793
1	*Togninia minima*	protein mRNA	3,407E-28	90	3261

625

626

627

628

629 **Table 3.** Diversity content in Trematoda clade found in a pooled sample of orange-colored protrusions from *L. gigas* muscle using
630 contig sequences and the taxonomic classifier MEGAN. [a] The assignments were classified to the taxonomic level according to
631 Monzoorul Haque *et al*. (2009).

632

633

			Ranges				
Contig	**Organism**	**Gene**	**E-value**	**Positives (%)**	**Identities (%)**	**Length**	**Assigment[a]**
Translated contig sequences							
2	*Schistosoma japonicum*	endonuclease-reverse transcriptase	3,637E-61; 6,062E-42	75; 76	64; 64	141; 165	Family
2	*Schistosoma japonicum*	endonuclease-reverse transcriptase	2,102E-122; 1,995E-56	61; 63	42; 43	262; 489	Phylum
5	*Schistosoma mansoni*	endonuclease-reverse transcriptase	3,147E-152; 4,075E-45	77; 81	61; 67	155; 345	Family
5	*Schistosoma mansoni*	endonuclease-reverse transcriptase	2,497E-172; 2,424E-61	71; 74	56; 59	204; 346	Order
4	*Schistosoma mansoni*	endonuclease-reverse transcriptase	0,000E+00; 9,203E-89	69; 70	52; 52	275; 695	Class
3	*Schistosoma mansoni*	endonuclease-reverse transcriptase	1,343E-66; 1,719E-51	65; 68	46; 50	193; 262	Phylum
Nucleotide contig sequences							
1	*Schistosoma mansoni*	chromosome fragment W	1,32E-20	80	80	161	-
5	*Schistosoma mansoni*	chromosome fragments	1,640E-55; 7,006E-27	71; 73	71; 73	649; 763	-
1	*Schistosoma mansoni*	chromosome fragment 4	1,99E-19	77	77	199	-

634

635