

**Temporal and spatial infection patterns of the rhizocephalan parasite *Parasacculina leptodiae* (Guérin-Ganivet, 1911) in the crab *Leptodius exaratus* along the shores of Kuwait**

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1 **Temporal and spatial infection patterns of the rhizocephalan parasite *Parasacculina***  
2 ***leptodiae* (Guérin-Ganivet, 1911) in the crab *Leptodius exaratus* along the shores of**  
3 **Kuwait**

4  
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22

23

24 **Abstract**

25 Rhizocephalan cirripedes are a very unique group of parasites infecting decapod crustaceans  
26 but apart from a few well-studied species little is known on their ecology and impact on hosts.  
27 Here we report on the results of a 14 month study of infestations of the rhizocephalan  
28 *Parasacculina leptodiae* in the rocky shore crab *Leptodius exaratus* along the shores of  
29 Kuwait in the Persian (Arabian) Gulf. Monthly samples along an intertidal gradient revealed a  
30 slightly higher prevalence of *P. leptodiae* in female (18%) compared to male crabs (11%) and  
31 marked differences in prevalence among the sampling sites. Crabs from more sheltered  
32 locations in Kuwait Bay showed lower prevalence of *P. leptodiae* compared to crabs from  
33 more exposed sites. Seasonal patterns were largely absent but prevalence in female crabs  
34 showed some monthly variation depending on the site. Rhizocephalan prevalence was  
35 generally highest in both crab sexes at the lower shores. This possibly resulted from lower  
36 exposure of crabs to infective stages in the higher intertidal and movements of infected crabs  
37 to lower parts of the shore. Prevalence of ovigerous females significantly declined with  
38 increasing local parasite prevalence. This suggests that the well-known castrating effects of  
39 rhizocephalans on individual hosts can also affect local crab reproduction at the population  
40 level which has not been shown before. Our results indicate that the rhizocephalan *P.*  
41 *leptodiae* is a common parasite of the rocky shore crab *L. exaratus* along the shores of  
42 Kuwait, with potential effects on the crab's population dynamics which warrants further  
43 study.

44

45 **Keywords**

46 Brachyura, distribution, host, parasitic barnacle, Persian/ Arabian Gulf

47

48 Introduction

49 Among the more unusual groups of parasites in marine systems are the rhizocephalans. They  
50 belong to the Cirripedia, but their adult morphology does not resemble their acorn and  
51 gooseneck barnacle relatives, which are omnipresent on marine hard surfaces worldwide.  
52 Rhizocephalans infect decapods and other crustaceans and are recognizable by a conspicuous  
53 sack-like structure, the externa, underneath the pleon of their hosts (Fig. 1). The non-visible  
54 internal part of a rhizocephalan, the interna, is an extensive net-like structure branching  
55 throughout the host's body (Noever et al. 2016). Only the free-living larval stages, which are  
56 released from the externa, resemble their barnacle relatives. In one group of rhizocephalans,  
57 the "akontrogonid-type", the cypris larva is the only free-swimming larval type and acts both  
58 as a spreading, searching, settling and host injection stage. In another group, "the  
59 kontrogonid-type", which includes *P. leptodiae*, female larvae develop via a series of naupliar  
60 stages into a final settlement stage, the cypris. The female cypris larva settles on the  
61 integument of a suitable host and metamorphoses into a kentrogon, an infective stage that via  
62 a syringe-like device, the stylet, penetrates the integument of the host and injects the parasitic  
63 material into its haemolymph. The injected parasitic material develops a root-like trophic  
64 organ, the interna, in the host's blood system. Later, a reproductive organ, the externa, emerge  
65 through the host's cuticle on the ventral side of the abdomen. Here, exposed to the ambient  
66 water, the virgin externa is fertilized by 1 – 2 male cypris larvae and can now start the  
67 reproductive cycle (Høeg et al. 2005). In kentrogonid-type rhizocephalans belonging to the  
68 genus *Parasacculina*, infection usually leads to single externa, although it is not known  
69 whether there may be more individually different internas that just do not develop an externa.  
70 Prevalence of infections in crustacean hosts can vary widely even over relatively small spatial  
71 scales (Werner 2001; Pernet et al. 2010) and abiotic drivers such as water depth and salinity  
72 have been discussed to underlie such spatial heterogeneities in infection levels (Reisser and

73 Forward 1991; Walker and Lester 1998; Waser et al. 2016). Infection prevalence can also  
74 vary temporarily and seasonality in water temperature or other factors may be the underlying  
75 cause (Alvarez et al. 2001; Sloan et al. 2010; Mouritsen et al. 2018).

76  
77 Hosts, which are infected by rhizocephalans, usually undergo various morphological,  
78 physiological and behavioural changes induced by the parasite (for review see (Høeg 1995;  
79 Høeg and Lützen 1995; Waiho et al. 2020, 2021). Among the induced changes that have a  
80 particularly strong effect on host populations is functional host castration (Fazhan et al.  
81 2020). This effect does not only remove a part of the reproductive adult host population, but  
82 it may also affect mating success of uninfected conspecifics as male crabs have been observed  
83 to mate with sterilized infected conspecifics (Shields and Wood 1993). While general  
84 distribution patterns and effects on hosts are relatively well studied for a few rhizocephalan  
85 species, very little is known about the ecology of the majority of the approximately 250  
86 rhizocephalan species (see reviews by (Høeg 1995; Høeg and Lützen 1995; Høeg et al. 2005).

87  
88 In this study, we investigated infections of the rhizocephalan *Parasacculina leptodiae* in the  
89 rocky shore crab *Leptodius exaratus* along the shores of Kuwait in the Persian Gulf (also  
90 known as Arabian Gulf (Fig. 1). This rhizocephalan species has first been described on *L.*  
91 *exaratus* from the Gulf of Aden (Guérin-Ganivet 1911) and since then the species has been  
92 recorded at other locations in the Pacific Ocean (Boschma 1948, 1955; Moazzam and  
93 Moazzam 2004). In the Persian Gulf, it has been recorded on *L. exaratus* in Kuwait (Al-  
94 Wazzan 2012) and at a few localities along the Iranian coast, although the species was not  
95 identified by the authors (Afkhani et al. 2016). Apart from these limited local observation  
96 records, not much is known about the ecology of the species. In this paper, a 14-month  
97 sampling along the shores of Kuwait (6 sites with 8 sampling stations along a tidal gradient at

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98 each site) was conducted to determine spatial and temporal patterns of infection of *P.*  
99 *leptodiae*, and 2) to identify its potential effects of infections on host populations. The results  
100 of the study will contribute with new knowledge about the biology of this unique group of  
101 parasites in marine systems.

102

### 103 **Materials and Methods**

#### 104 *Field sampling*

105 Monthly surveys were carried out at six sites dominated by rocky substrate along the shores  
106 of Kuwait between November 2013 and December 2014 (Fig. 2). Three of these sites were  
107 relatively sheltered shores located inside Kuwait Bay and the other three were more exposed  
108 shores located along the open coast to the south of the Bay. At each site, three line transects  
109 were established with eight sampling stations in each transect covering the intertidal zone  
110 (stations 1 and 2: high intertidal; stations 3 to 5: mid intertidal; stations 6 to 8: low intertidal),  
111 resulting in a total of 24 sampling plots per location (see Al-Wazzan et al. 2020 for more  
112 details). At each sampling station, a 1 m<sup>2</sup> quadrat was used to collect crabs, and they were  
113 subsequently sexed and their carapace width (CW) measured to the nearest 0.1 mm using a  
114 dial Vernier calliper. All crabs were checked for the presence of rhizocephalan parasites  
115 (clearly visible externa on the ventral abdomen, see Fig. 1) and all female crabs were also  
116 checked for the presence of eggs.

117

#### 118 *Species ID*

119 A random sample of 15 infected crabs was collected and fixed in 70% ethanol for parasite  
120 identification. The morphology of the external part (the externa) of the parasites from Kuwait  
121 in the present study corresponds to the original description of *Sacculina leptodiae* by Guérin-  
122 Ganivet in 1911 from the Gulf of Aden and the extended description in Boschma (1955). In  
123 both cases the rhizocephalans were found on the host *Leptodius exaratus* (H. Milne Edwards,

124 1834). Based on this the rhizocephalans of the present study are identified as *Parasacculina*  
125 *leptodiae* (Guérin-Ganivet, 1911). To further confirm the identification of the morphological  
126 species, a molecular identification was performed (details in the subsequent Molecular  
127 techniques sequence), which unequivocally showed that the DNA sequences from the present  
128 Kuwait study differ from the reference sequences of *P. leptodiae* deposited in Genbank.

129

130 Molecular techniques

131 DNA extraction and gene amplification

132 Genomic DNA was extracted from ~ 1mm<sup>3</sup> of tissue from the mantle of individual externae  
133 of *Parasacculina leptodiae* and the distal (dactylus) segment of walking leg number 4 (left)  
134 using the QiagenDNeasy Blood & Tissue Kit following the QiagenDNeasy Protocol for  
135 Animal Tissues 07/2006.

136 Mitochondrial COI amplification was performed using standard DNA Barcoding protocols  
137 with Folmer primers (LCO1490 and HCO2198). The nuclear 18s gene were amplified and  
138 sequenced using the primers published in (Korn et al. 2020). The concatenated sequences  
139 produced 702 nucleotides of the mitochondrial COI gene and 2136 of the ribosomal nuclear  
140 18s gene.

141 All PCR reactions were carried out using a Bio-Rad C1000 Thermal Cycler in 25 ml volumes  
142 containing 1 ml of DNA extract, 2.5 ml 10\_PCR buffer, 1.2 ml of dNTP mixture (2.5 mM  
143 each), 1 ml of each 10 mM primer and 0.75U of Takara polymerase. Conditions for all  
144 amplifications were as follows: initial denaturation at 94 \_C for 5 min then 35 cycles of 30s  
145 denaturation at 94 \_C, 1 min primer annealing at 52 \_C and 1 min extension at 72 \_C, with a  
146 final 7 min 72 \_C extension. All PCR products were visualized on 1% agarose gels and stored  
147 at 4 \_C prior to purification and sequencing. PCR products were cleaned by the addition of

148 0.1 ml (1U) Exonuclease I, 1 ml (1U) of Shrimp Alkaline Phosphatase and 0.9 ml of ddH<sub>2</sub>O  
149 to 8ul of PCR product. This was followed by incubation at 37 °C for 30 min and deactivation  
150 of the enzymes at 85 °C for 15 min. Sequence reactions were performed using the BigDye  
151 v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Norwalk, CT, USA) with the same  
152 primers used for initial PCR amplification. Both strands of all PCR products  
153 were sequenced using an ABI 3730 capillary sequencer.

154  
155 *Evolutionary analysis*

156 **P distances**

157 Estimates of Evolutionary Divergence between CO1 Sequences (SI 1): The number of base  
158 differences per site from between sequences are shown. This analysis involved 15 nucleotide  
159 sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions  
160 were removed for each sequence pair (pairwise deletion option). There was a total of 702  
161 positions in the final dataset. P-distance analyses were conducted in MEGA X (Kumar et al.,  
162 2018)

163

164 **Phylogenetic analysis using Maximum Likelihood**

165 The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-  
166 Nei model (Tamura and Nei, 1993). The concatenated sequences of 702 nucleotides of the  
167 mitochondrial CO1 gene and 2136 of the ribosomal nuclear 18s gene were used for the  
168 phylogenetic analysis. The tree with the highest log likelihood (-18022.09) is shown (SM 2).  
169 The percentage of trees in which the associated taxa clustered together is shown next to the  
170 branches. Initial tree(s) for the heuristic search were obtained automatically by applying  
171 Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the  
172 Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree  
173 is drawn to scale, with branch lengths measured in the number of substitutions per site. This

174 analysis involved 23 nucleotide sequences. There were a total of 2838 positions in the final  
175 dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018; Stecher et al.,  
176 2020)

177

178

### 179 *Field data analysis*

180 As a first step, the total number of crabs caught during all sampling events and at all sites was  
181 used to derive size frequency distributions of infected and uninfected crabs (both for males  
182 and females separately) as well as that of ovigerous females. The minimum size of crabs  
183 showing a parasite externa and females bearing eggs was then used as a size cut-off for  
184 further analyses, enabling us to focus on crab sizes that are a) potential hosts for  
185 rhizocephalan parasites, and b) equal to sizes of mature female crabs. Following this, the  
186 proportions of infected crabs (larger than the cut-off size) were calculated for each sex  
187 separately based on either the total number of males or females. Differences in prevalence  
188 between sexes were tested with likelihood-ratio tests (G-tests). A potential relationship  
189 between local prevalence of infection and crab density was investigated with Pearson  
190 correlations and using crab density data from Al-Wazzan et al. (2020). As there was no  
191 significant relationship, we excluded crab density from further analyses.

192

193 As prevalence generally differed between sexes (see Result section below), we separated  
194 females and males in further analyses. Statistical differences in prevalence of rhizocephalan  
195 infections in crabs among different sampling months as well as between different tidal heights  
196 were tested using two-factorial ANOVAs with logit transformed prevalence values. When  
197 testing for temporal patterns, we lumped data from all tidal height stations and when testing  
198 for spatial patterns, we lumped seasonal data. For this lumping, we considered the three line

199 transects at each site as replicates, resulting in 3 replicate values of prevalence per tidal height  
200 station and month per site. Sampling site served as a second (fixed) factor in both analyses,  
201 and we tested for factor effects as well as interactions. At some tidal height-site and month-  
202 site combinations, there were no crabs of a specific sex present, resulting in slightly  
203 unbalanced data sets. To cope with the unbalance ANOVAs with sums of squares Type III  
204 were applied.

205

206 The relationship between the prevalence of ovigerous females and the prevalence of infected  
207 female *L. exaratus* was tested with a logistic regression (quasi- binomial error fix, because of  
208 overdispersion). As ovigerous females were only observed in the summer period (Al-Wazzan  
209 2017), we confined the data for this analysis to the period May 2014 to September 2014,  
210 when a relatively high portion of females was bearing eggs. All analyses were performed  
211 using the R platform.

212

## 213 **Results**

### 214 *Species identification.*

215 The morphological study of the rhizocephalic parasites from Kuwait in the present study  
216 indicates that the species is *Parasacculina leptodiae*. DNA sequence data from the ribosomal  
217 core gene 18s and the mitochondrial protein coding gene CO1 differ greatly from the  
218 *Parasacculina leptodiae* sequences deposited in the gene bank for the species (see SM 2).  
219 Genetic p-distance between the CO1 sequences of *Parasacculina leptodiae* ORIG from  
220 Kuwait and the other Parasacculinid species in the analysis including *P. leptodiae* NEW sp.  
221 obtained from Genbank were extremely large (see SM 1). Since the Genbank data probably  
222 are derived from a parasite of the south east Asian crab (*Leptodius affinis*) and not from the  
223 type-host (*Leptodius exaratus*), from which *P. leptodiae* was originally described (Djibuti,  
224 Gulf of Aden), we assume that the sequence in Genbank belongs to another presumable

225 undescribed Parasacculinid species. A study of this relationship will be the subject of a future  
226 study. A phylogenetic maximum likelihood analysis of the new DNA sequences including 23  
227 rhizocephalan species (SM 1. in the online resources) places the original *P. leptodiae* (ORIG)  
228 from Kuwait basally in a monophyletic *Parasacculina* clade, while the species from Genbank  
229 (*P. leptodiae* NEW SP.) is positioned distally in the clade.

230

### 231 *Field results*

232 Of the 4749 crabs investigated at the 6 sites between November 2013 and December 2014  
233 along the shores of Kuwait, 664 crabs carried an externa of *Parasacculina leptodiae*. The size  
234 of infected crabs ranged from 9 to 32 and 10 to 28 mm CW in male and female crabs,  
235 respectively (Fig. 3a, b), and were similar in size to ovigerous females (10-30 mm CW; Fig.  
236 3c). 255 crabs were juveniles and smaller than 9 mm CW, and were thus smaller than the  
237 minimum detection limit of 9 mm for parasites (i.e. crabs with an externa). These juveniles  
238 were excluded from further analysis, resulting in 4494 crabs that were considered for more  
239 detailed analysis. Out of these 4494 crabs, the prevalence of *P. leptodiae* was significantly  
240 different between sexes (G-test,  $G = 48.96$ ,  $p < 0.001$ ), with a prevalence in female crabs of  
241 18% compared to a prevalence of 11% in male crabs. For more detailed general information  
242 on sex ratios and crab densities depending on site and season see AL-Wazzan (2017) and AL-  
243 Wazzan et al. (2020).

244

245 The prevalences for both sexes were generally significantly different between sites (Tables 1  
246 & 2; Figs. 4 & 5). While there was no temporal difference in parasite prevalence for male  
247 crabs, prevalence in female crabs varied significantly between months, depending on the site  
248 as indicated by a significant interaction term (Table 1; Fig. 4). At some sites, prevalence  
249 slightly increased over the sampling period (site 5) while at other it slightly decreased (sites 2

250 & 4). Concerning tidal height, there were significant differences in parasite prevalence for  
251 both male and female crabs, independent of the sampling site (Table 2; Fig. 5). Prevalence  
252 was generally lowest at the uppermost sampling locations (Fig. 5).

253

254 The prevalence of ovigerous females significantly decreased with an increase in the  
255 prevalence of infected female crabs ( $R^2= 0.26, p=0.004$ ; Fig. 6).

256

## 257 **Discussion**

258 Our study is the first to verify the presence of the rhizocephalan *Parasacculina leptodiae* in  
259 the rocky shore crab *Leptodius exaratus* in the Persian Gulf. The species has probably also  
260 been recorded at a few localities along the Iranian coast but the species was not identified by  
261 the authors (Afkhani et al. 2016).

262

263 Our 14-month study at 6 sites along the shores of Kuwait indicated that *P. leptodiae* is a  
264 common parasite of *L. exaratus*, with a slightly higher prevalence in females (18%) than in  
265 males (11%). A higher rhizocephalan prevalence in female compared to male crab hosts has  
266 also been observed in other rhizocephalan species, sometimes also with even more marked  
267 differences (Rasmussen 1973; Høeg and Lützen 1995; Mouritsen et al. 2018). It has been  
268 suggested that this difference in prevalence does not result from a preferred settlement of  
269 infective stages on female crabs but rather stems from a higher survival of parasites in  
270 females as their broader abdomen gives better protection for the parasite and its externa  
271 (Sloan 1984, 1985; Reisser and Forward 1991; Høeg and Lützen 1995; Kristensen et al.  
272 2012). However, further studies are necessary to investigate whether this is also the case in *P.*  
273 *leptodiae*.

274

275 Differences in rhizocephalan prevalence between male and female hosts were also observed  
276 in our study in regard to season, with prevalence in female crabs showing significant  
277 differences among months, depending on the site, while there was no significant effect of  
278 season on prevalence in male crabs. We can only speculate about the underlying mechanisms  
279 but potential differences between males and females in their moulting cycle may play a role.  
280 Host penetration might be easier on soft skinned host crabs and it has been documented that  
281 cypris larvae of several rhizocephalan species prefer to settle on recently moulted crabs  
282 (Veillet 1947; Walker 1992; Glenner and Werner 1998; Tindle et al. 2004). Moulting in *L.*  
283 *exaratus* were reported all year round with higher growth rate during the warmer season  
284 (March and September) with males and females have a similar seasonal growth pattern which  
285 are highly driven by seasonal environmental changes (Al-Wazzan 2017). Thus, rhizocephalan  
286 penetration into hosts could be expected to be higher during growth season. However, it is  
287 hard to predict the seasonal effect without understanding the time needed from infection to the  
288 emergence of the externa. For that, further investigations will be needed to understand the  
289 observed difference in seasonal effects between male and female crabs.

290  
291 In regard to spatial variations in *P. leptodiae* prevalence, both sexes showed similar patterns.  
292 In general, there were significant differences in prevalence among the studied sites. Crabs  
293 from sites in the more sheltered part of Kuwait Bay (sites 1-3) showed lower infection levels  
294 than crabs from more exposed sites in the Persian Gulf (sites 4-6). Similar differences in  
295 prevalence among sites have also been observed in other rhizocephalan species such as in  
296 *Sacculina carcini* infecting shore crabs *Carcinus maenas* (Werner 2001; Waser et al. 2016;  
297 Mouritsen et al. 2018) and are considered to result from differences in abiotic conditions such  
298 as salinity, water depth and exposure (Høeg and Lützen 1995; Werner 2001; Waser et al.  
299 2016). Exposure may play a particularly important role in our system as lowest infections or

300 even a complete absence of *P. leptodiae* were observed at the more sheltered sites inside  
301 Kuwait Bay. This pattern may point to differences in exposure, e.g. infective stages of  
302 rhizocephalans could be more limited in sheltered sites due to the counter clockwise current  
303 pattern which is dominant in most of Kuwait's waters (Alosairi and Pokavanich 2017).  
304 Another possible explanation could relate to the high-water temperature and salinity in  
305 Kuwait Bay waters (Al-Yamani, et al., 2004), which could act as limiting factors for *P.*  
306 *leptodiae* to flourish. However, it is also possible that exposure is similar at the different sites  
307 but that susceptibility of crabs differs due to environmental stress. In the intertidal porcelain  
308 crab *Petrolisthes cabrilloi*, host defences against infections include the removal of infective  
309 parasite stages from the gills by grooming appendices on the limbs. Crabs in high exposure  
310 environments show significant higher damage of the grooming appendages (the modified  
311 fifth pair of thoracic legs walking legs of porcelain crabs), making them more susceptible to  
312 infections while conspecifics from sheltered environments have low infection levels (Høeg et  
313 al. 2005). Similar mechanisms may explain the lower prevalence of *P. leptodiae* at the  
314 sheltered sites in our study.

315  
316 However, a differential susceptibility of crabs depending on exposure cannot explain the  
317 lower infection levels of crabs observed at the higher tidal locations in our survey, as crabs  
318 most likely will experience more wave action at these locations. An alternative explanation  
319 for the lower prevalence at high tidal levels may be a lower exposure of crabs to infective  
320 stages due to shorter immersion times and thus shorter time windows for infection in the high  
321 intertidal. In addition, infected crabs may migrate down the shore to a more protected depth,  
322 as part of a parasite induced behavioural changes of their hosts, which has been observed in  
323 European shore crabs (*Carcinus maenas*) infected with *S. carcini* (Rasmussen 1959; Rainbow  
324 et al. 1979; Lützen 1984; Waser et al. 2016). Alternatively, crabs weakened by rhizocephalan

325 parasites might avoid the competition with healthy conspecifics in unpredictable and energy  
326 demanding habitats as the high intertidal.

327

328 Besides affecting the behaviour of their hosts, rhizocephalans can also affect the physiology  
329 and reproduction of their hosts (Høeg 1995; Høeg and Lützen 1995). Infections with  
330 rhizocephalans usually lead to the castration of their hosts with severe fitness consequences  
331 for individual hosts (Høeg 1995). Such an infection-mediated reduction in individual fitness  
332 may also have carry-over effects on the crab population level, as high infection levels in a  
333 crab population may remove a significant number of crabs from the pool of reproducing  
334 individuals, thus leading to lower production of offspring. Our data suggest that this may  
335 indeed be a relevant scenario for infections with *P. leptodidae*, as the proportion of ovigerous  
336 female crabs declined with increasing infection levels. Whether this decline in ovigerous  
337 females has any consequences for local population dynamics will depend on the level of  
338 recruitment from local stocks. In any case, the observed relationship suggests that the effects  
339 of *P. leptodidae* infections may be significant on the host population level.

340

341 In conclusion, our results indicate that the rhizocephalan *P. leptodidae* is commonly parasitized  
342 by the rocky shore crab *L. exaratus* along the shores of Kuwait and that it may have the  
343 potential to affect the crab's population dynamics. Further studies will be helpful in verifying  
344 the underlying mechanisms and population-level consequences of infections.

345

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357

358

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361 ZW, AW, HG, and DT conducted experiments. AW and HG analyzed data. ZW, AW,  
362 HG, and DT wrote first draft of the manuscript. All authors read, reviewed, revised and  
363 approved the manuscript.

364

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372

373

374

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479 **Table 1:** Results of two-factorial ANOVAs (sums of squares Type III) on the effect of  
 480 months and sites on prevalence (logit transformed) of *Parasacculina leptodiae* in adult female  
 481 and male *Leptodius exaratus* between November 2013 to December 2014. Table is showing  
 482 degrees of freedom (DF), sum of squares (SS), mean squares (MS) and F and *p* values.

483

<b>Source of variation</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b><i>p</i></b>
<b>Female crabs</b>					
Month	13	37.42	2.88	1.879	0.037
Site	5	215.06	43.01	28.075	<0.001
Month × Site	65	146.04	2.25	1.466	0.029
Residuals	153	234.41	1.53		
<b>Male crabs</b>					
Month	13	22.89	1.76	0.868	0.588
Site	5	162.44	32.4982	16.007	<0.001
Month × Site	65	95.83	1.47	0.726	0.928
Residuals	155	314.58	2.03		

484

485

486 **Table 2:** Results of two-factorial ANOVAs (sums of squares Type III) on the effect of tidal  
 487 height and site on prevalence (logit transformed) of *Parasacculina leptodiae* in adult female  
 488 and male *Leptodius exaratus* along different tidal height levels. Table is showing degree of  
 489 freedom (DF), sum of squares (SS), mean squares (MS) and F and *p* values.

490

Source of variation	DF	SS	MS	F	<i>p</i>
<b>Female crabs</b>					
Tidal height	7	24.96	3.57	4.425	<0.001
Site	5	105.57	21.11	26.198	<0.001
Tidal height × Site	35	39.28	1.12	1.392	0.109
Residuals	88	70.92	0.81		
<b>Male crabs</b>					
Tidal height	7	19.27	2.75	2.833	0.010
Site	5	88.01	17.60	18.112	<0.001
Tidal height × Site	35	36.48	1.04	1.072	0.385
Residuals	92	89.41	0.972		

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494 **List of figures:**

495 **Fig. 1:** A rocky shore crab *Leptodius exaratus* infected by the rhizocephalan parasite  
496 *Parasacculina leptodiae*, showing an externa (containing the parasite ovary) below the  
497 abdomen of the host.

498 **Fig. 2:** Sampling sites (1–6) along the shore of Kuwait in the Persian Gulf.

499 **Fig. 3:** Size frequency distributions of crabs *Leptodius exaratus* (on a log-scale) combined for  
500 all sampling sites and dates showing a, male crabs infected with *Parasacculina leptodiae* in  
501 comparison to all male crabs caught; b, female crabs infected with *P. leptodiae* and the total  
502 of female crabs caught; and c, ovigerous female crabs compared to all female crabs caught.

503 **Fig. 4:** Monthly prevalence ( $\pm$  SE) of *Parasacculina leptodiae* in female and male *Leptodius*  
504 *exaratus* between November 2013 and December 2014 at 6 sampling sites along the shores of  
505 Kuwait. For a general overview, also the total monthly prevalence of the crab population  
506 along the Kuwait coast during the entire sampling period is shown.

507 **Fig. 5:** Prevalence ( $\pm$  SE) of *Parasacculina leptodiae* in female and male *Leptodius exaratus*  
508 at each of the 8 sampling stations along a tidal gradient (stations 1 and 2: high intertidal;  
509 stations 3 to 5: mid intertidal; stations 6 to 8: low intertidal) at 6 sampling sites along the  
510 shores of Kuwait. For a general overview, also the total prevalence of the crab population  
511 along the Kuwait coast during the entire sampling period is shown.

512 **Fig. 6:** Relationship between the prevalence of female *Leptodius exaratus* infected with  
513 *Parasacculina leptodiae* and the prevalence of ovigerous females at the 6 sampling sites  
514 during the period May 2014 to September 2014. The plot shows observed values (symbols)  
515 and fitted values of the logistic regression model (solid line) with 95% confidence intervals  
516 (dashed lines).

517