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Al-Wazzan, Z.; Waser, A.M.; Glenner, H.; Giménez, L. & Thieltges, D.W. (2021). Temporal and spatial infection patterns of the rhizocephalan parasite *Parasacculina leptodiae* (Guérin-Ganivet, 1911) in the crab *Leptodius exaratus* along the shores of Kuwait. *Mar. Biodiv.* 51:90. DOI: 10.1007/s12526-021-01235-3

Published version: <https://dx.doi.org/10.1007/s12526-021-01235-3>

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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 **Abstract**

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

43

44 **Keywords**

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

46

47 **Introduction**

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

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

75
76 Hosts, which are infected by rhizocephalans, usually undergo various morphological,
77 physiological and behavioural changes induced by the parasite (for review see (Høeg 1995;
78 Høeg and Lützen 1995; Waiho et al. 2020, 2021). Among the induced changes that have a
79 particularly strong effect on host populations is functional host castration (Fazhan et al.
80 2020)). This effect does not only remove a part of the reproductive adult host population, but
81 it may also affect mating success of uninfected conspecifics as male crabs have been observed
82 to mate with sterilized infected conspecifics (Shields and Wood 1993). While general
83 distribution patterns and effects on hosts are relatively well studied for a few rhizocephalan
84 species, very little is known about the ecology of the majority of the approximately 250
85 rhizocephalan species (see reviews by (Høeg 1995; Høeg and Lützen 1995; Høeg et al. 2005).

86
87 In this study, we investigated infections of the rhizocephalan *Parasacculina leptodiae* in the
88 rocky shore crab *Leptodius exaratus* along the shores of Kuwait in the Persian Gulf (also
89 known as Arabian Gulf (Fig. 1). This rhizocephalan species has first been described on *L.*
90 *exaratus* from the Gulf of Aden (Guérin-Ganivet 1911) and since then the species has been
91 recorded at other locations in the Pacific Ocean (Boschma 1948, 1955; Moazzam and
92 Moazzam 2004). In the Persian Gulf, it has been recorded on *L. exaratus* in Kuwait (Al-
93 Wazzan 2012) and at a few localities along the Iranian coast, although the species was not
94 identified by the authors (Afkhami et al. 2016). Apart from these limited local observation
95 records, not much is known about the ecology of the species. In this paper, a 14-month
96 sampling along the shores of Kuwait (6 sites with 8 sampling stations along a tidal gradient at

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

101

102 **Materials and Methods**

103 *Field sampling*

104 Monthly surveys were carried out at six sites dominated by rocky substrate along the shores
105 of Kuwait between November 2013 and December 2014 (Fig. 2). Three of these sites were
106 relatively sheltered shores located inside Kuwait Bay and the other three were more exposed
107 shores located along the open coast to the south of the Bay. At each site, three line transects
108 were established with eight sampling stations in each transect covering the intertidal zone
109 (stations 1 and 2: high intertidal; stations 3 to 5: mid intertidal; stations 6 to 8: low intertidal),
110 resulting in a total of 24 sampling plots per location (see Al-Wazzan et al. 2020 for more
111 details). At each sampling station, a 1 m² quadrat was used to collect crabs, and they were
112 subsequently sexed and their carapace width (CW) measured to the nearest 0.1 mm using a
113 dial Vernier calliper. All crabs were checked for the presence of rhizocephalan parasites
114 (clearly visible externa on the ventral abdomen, see Fig. 1) and all female crabs were also
115 checked for the presence of eggs.

116

117 *Species ID*

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

123 1834). Based on this the rhizocephalans of the present study are identified as *Parasacculina*
124 *leptodiae* (Guérin-Ganivet, 1911). To further confirm the identification of the morphological
125 species, a molecular identification was performed (details in the subsequent Molecular
126 techniques sequence), which unequivocally showed that the DNA sequences from the present
127 Kuwait study differ from the reference sequences of *P. leptodiae* deposited in Genbank.

128

129 Molecular techniques

130 DNA extraction and gene amplification

131 Genomic DNA was extracted from ~ 1mm³ of tissue from the mantle of individual externae
132 of *Parasacculina leptodiae* and the distal (dactylus) segment of walking leg number 4 (left)
133 using the QiagenDNeasy Blood & Tissue Kit following the QiagenDNeasy Protocol for
134 Animal Tissues 07/2006.

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

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

147 0.1 ml (1U) Exonuclease I, 1 ml (1U) of Shrimp Alkaline Phosphatase and 0.9 ml of ddH₂O
148 to 8ul of PCR product. This was followed by incubation at 37 °C for 30 min and deactivation
149 of the enzymes at 85 °C for 15 min. Sequence reactions were performed using the BigDye
150 v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Norwalk, CT, USA) with the same
151 primers used for initial PCR amplification. Both strands of all PCR products
152 were sequenced using an ABI 3730 capillary sequencer.

153

154 *Evolutionary analysis*

155 **P distances**

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

162

163 **Phylogenetic analysis using Maximum Likelihood**

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

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

176

177 *Field data analysis*

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

190

191 As prevalence generally differed between sexes (see Result section below), we separated
192 females and males in further analyses. Statistical differences in prevalence of rhizocephalan
193 infections in crabs among different sampling months as well as between different tidal heights
194 were tested using two-factorial ANOVAs with logit transformed prevalence values. When
195 testing for temporal patterns, we lumped data from all tidal height stations and when testing
196 for spatial patterns, we lumped seasonal data. For this lumping, we considered the three line
197 transects at each site as replicates, resulting in 3 replicate values of prevalence per tidal height

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

203

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

210

211 **Results**

212 *Species identification.*

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

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

228

229 *Field results*

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

242

243 The prevalences for both sexes were generally significantly different between sites (Tables 1
244 & 2; Figs. 4 & 5). While there was no temporal difference in parasite prevalence for male
245 crabs, prevalence in female crabs varied significantly between months, depending on the site
246 as indicated by a significant interaction term (Table 1; Fig. 4). At some sites, prevalence
247 slightly increased over the sampling period (site 5) while at other it slightly decreased (sites 2
248 & 4). Concerning tidal height, there were significant differences in parasite prevalence for

249 both male and female crabs, independent of the sampling site (Table 2; Fig. 5). Prevalence
250 was generally lowest at the uppermost sampling locations (Fig. 5).

251

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

254

255 **Discussion**

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

260

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

272

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

288

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

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

313

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

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

325

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

338

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

343

344 **Acknowledgements**

345 We would like to thank family, friends, and colleagues for their assistance during the
346 fieldwork and Jessica Schop from Wageningen University and Research- Wageningen Marine

347 Research in The Netherlands for her unlimited support. Special thanks to the anonymous
348 reviewers for their valuable comments and suggestions to the original manuscript

349

350 - **Declarations: Funding:** The authors did not receive support from any organization for
351 the submitted work.

352 - **Conflict of Interest:** The authors declare that they have no conflict of interest

353 - **Availability of data and material (data transparency):** Data available on request from
354 the authors.

355

356

357 - **Code availability:** Not applicable.

358 - **Author Contribution Statement:** ZW, LG, and DT conceived and designed research.

359 ZW, AW, HG, and DT conducted experiments. AW and HG analyzed data. ZW, AW,

360 HG, and DT wrote first draft of the manuscript. All authors read, reviewed, revised and

361 approved the manuscript.

362

363 - **Ethical approval:** No animal testing was performed during this study.

364 - **Sampling and field studies:** All necessary permits for sampling and observational field

365 studies have been obtained by the authors from the competent authorities and are

366 mentioned in the acknowledgements, if applicable. The study is compliant with CBD and

367 Nagoya protocols.

368 - **Consent to participate:** Not applicable

369 - **Consent for publication:** Not applicable

370

371

372

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476

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

Source of variation	DF	SS	MS	F	<i>p</i>
Female crabs					
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		
Male crabs					
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		

482

483

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

Source of variation	DF	SS	MS	F	<i>p</i>
Female crabs					
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		
Male crabs					
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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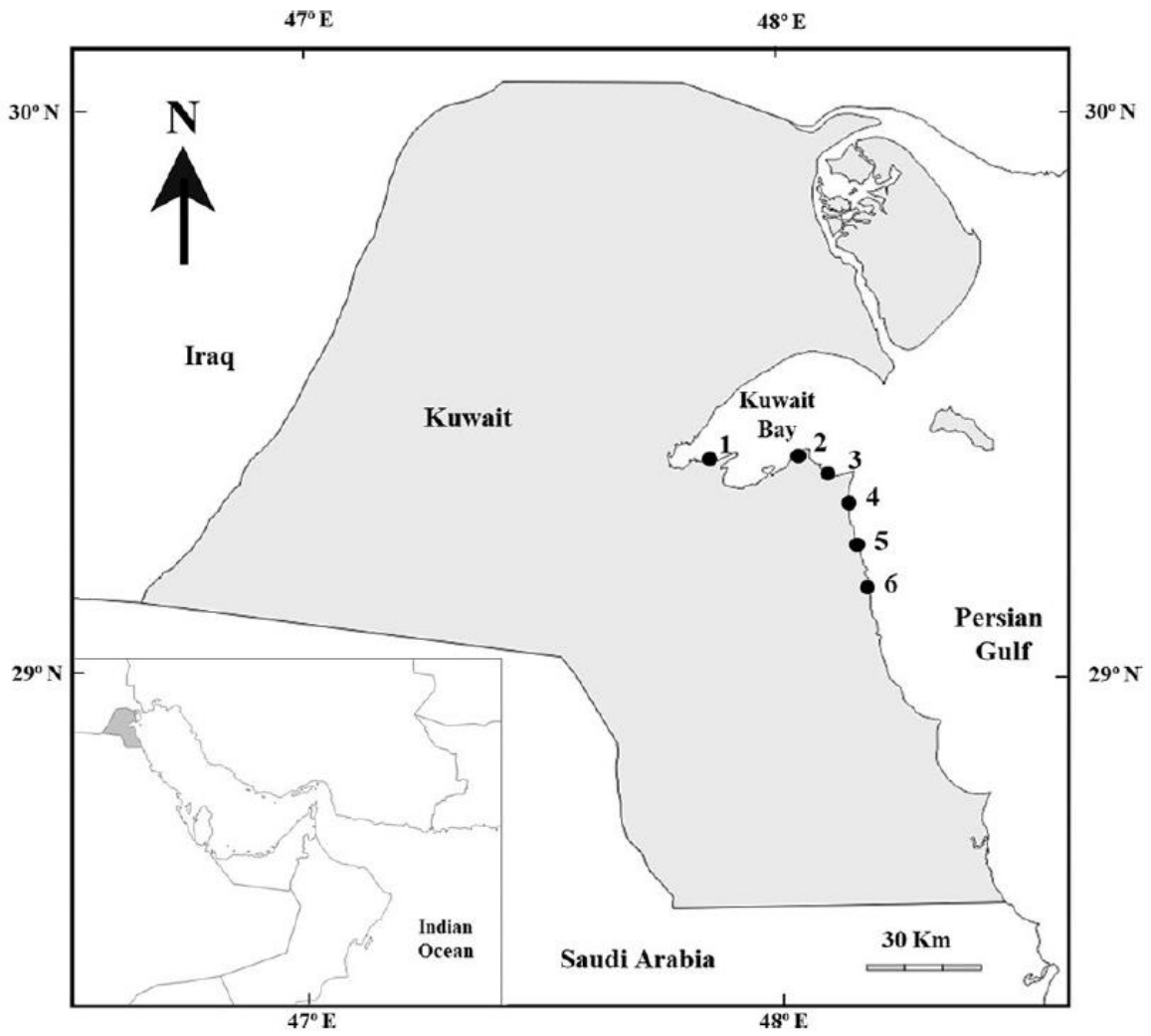
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493 **Fig. 1:** A rocky shore crab *Leptodius exaratus* infected by the rhizocephalan parasite

494 *Parasacculina leptodiae*, showing an externa (containing the parasite ovary) below the

495 abdomen of the host.

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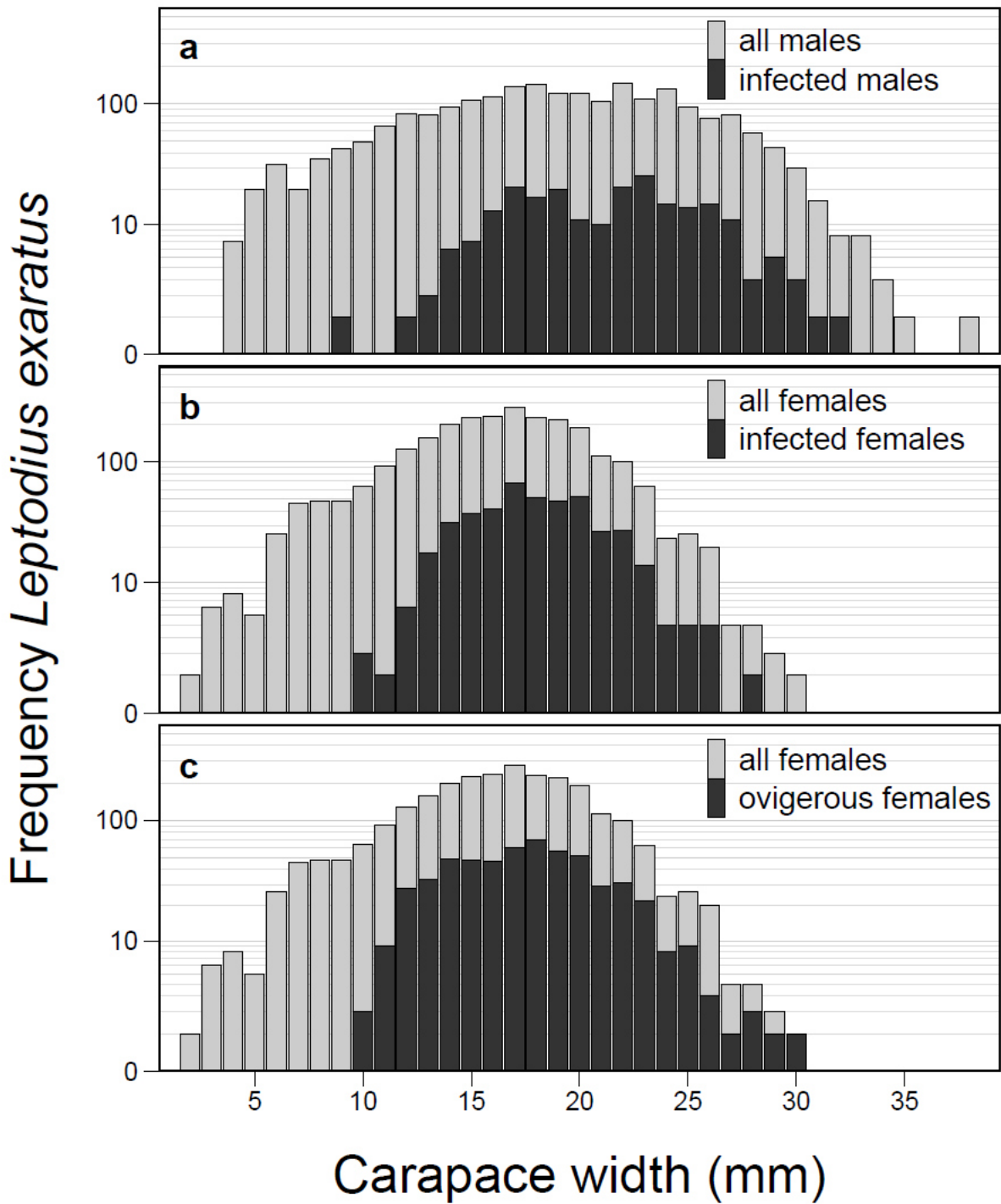


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499 **Fig. 2:** Sampling sites (1–6) along the shore of Kuwait in the Persian Gulf.

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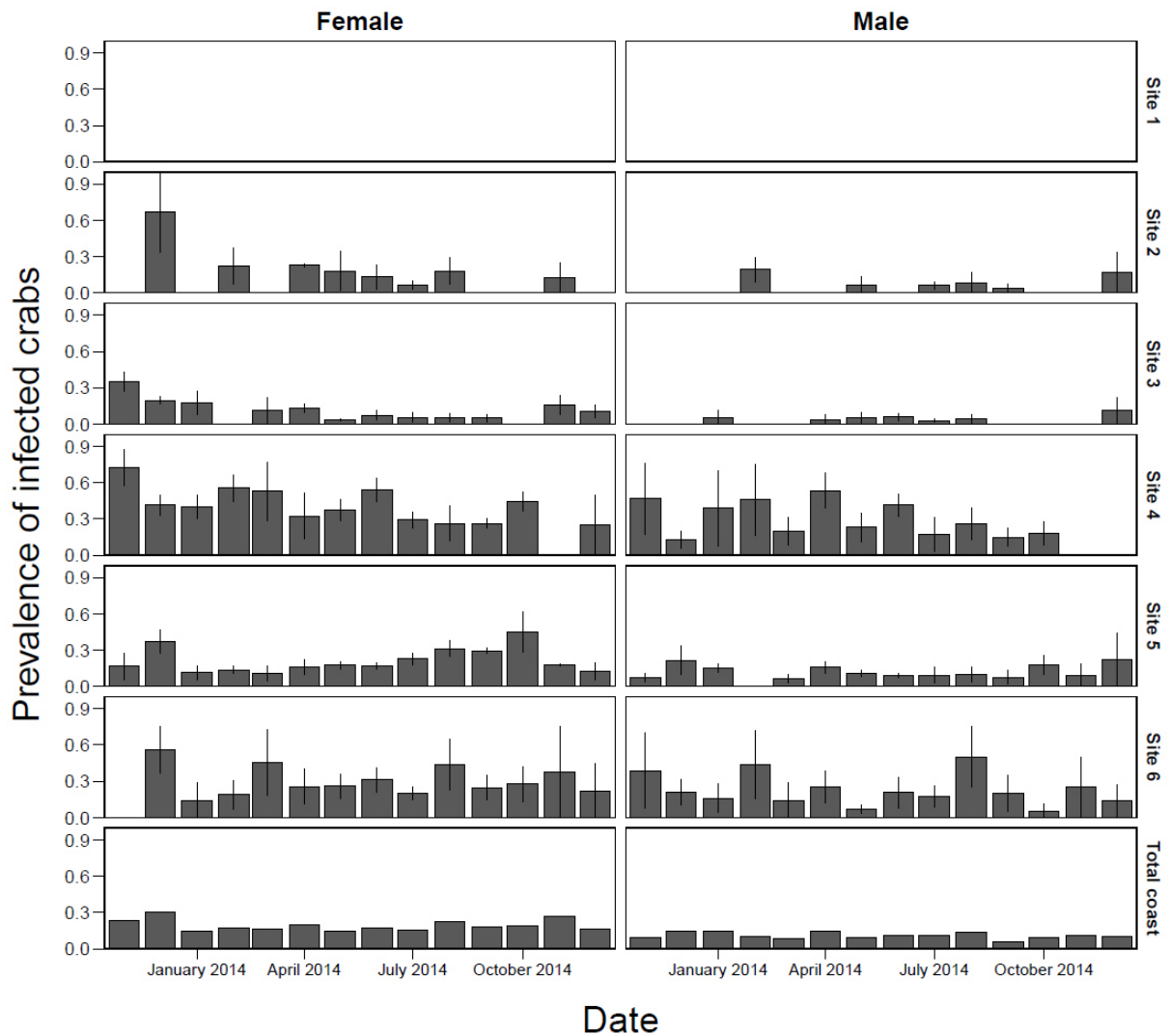
503 **Fig. 3:** Size frequency distributions of crabs *Leptodius exaratus* (on a log-scale) combined for

504 all sampling sites and dates showing a, male crabs infected with *Parasacculina leptodiae* in

505 comparison to all male crabs caught; b, female crabs infected with *P. leptodiae* and the total

506 of female crabs caught; and c, ovigerous female crabs compared to all female crabs caught.

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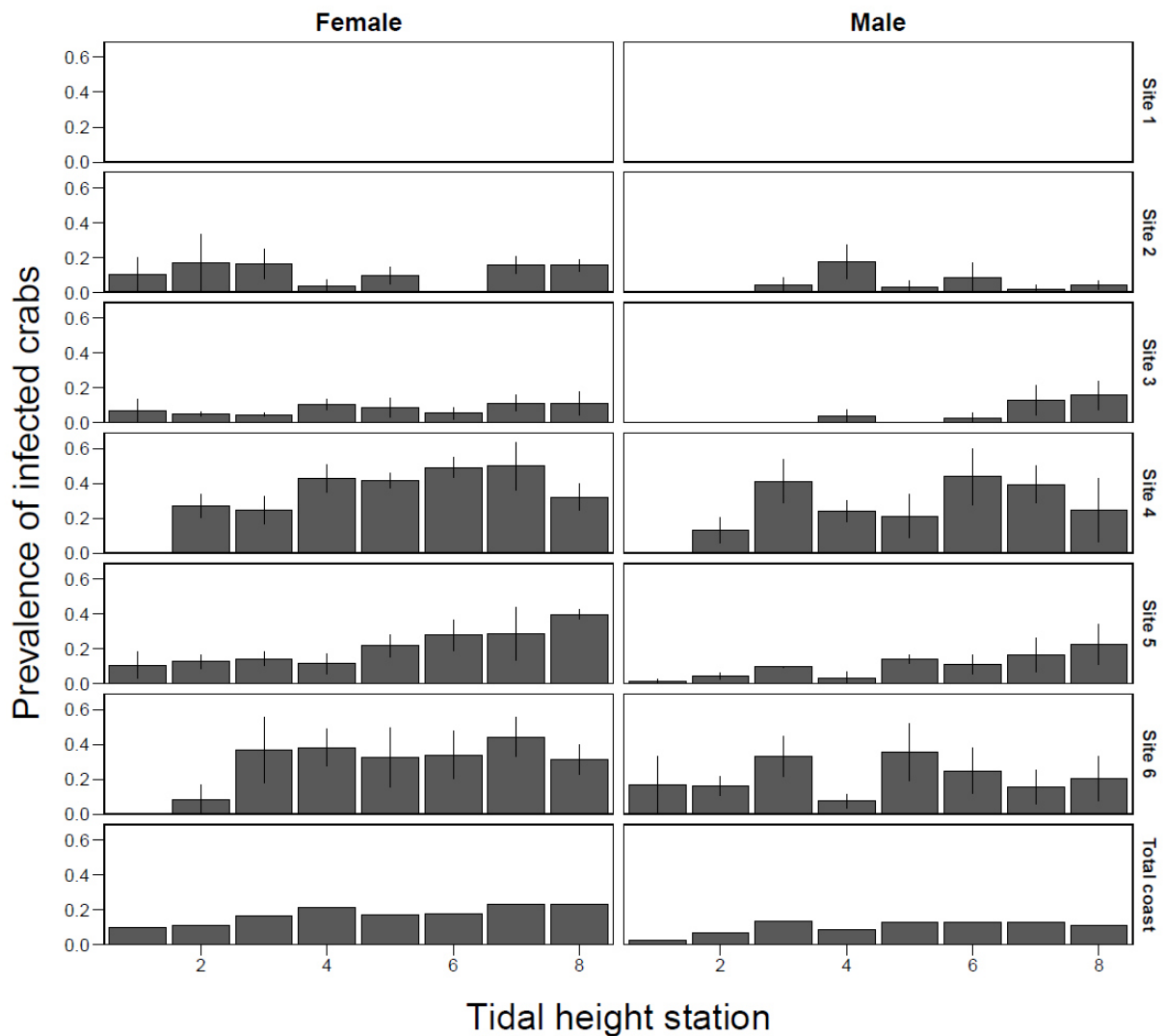
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510 **Fig. 4:** Monthly prevalence (\pm SE) of *Parasacculina leptodiae* in female and male *Leptodius*
 511 *exaratus* between November 2013 and December 2014 at 6 sampling sites along the shores of
 512 Kuwait. For a general overview, also the total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

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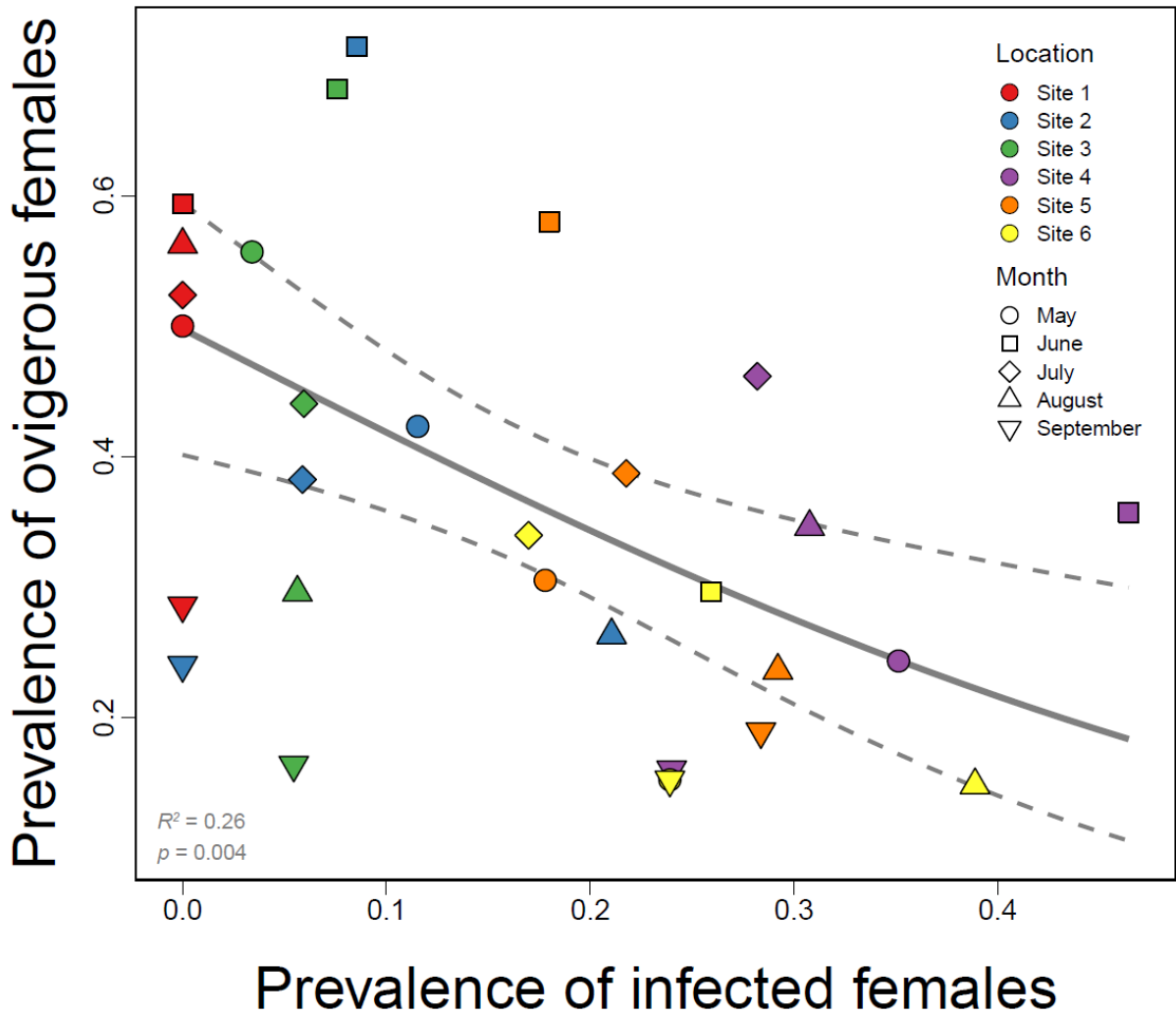
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518 **Fig. 5:** Prevalence (\pm SE) of *Parasacculina leptodiae* in female and male *Leptodius exaratus*
 519 at each of the 8 sampling stations along a tidal gradient (stations 1 and 2: high intertidal;
 520 stations 3 to 5: mid intertidal; stations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

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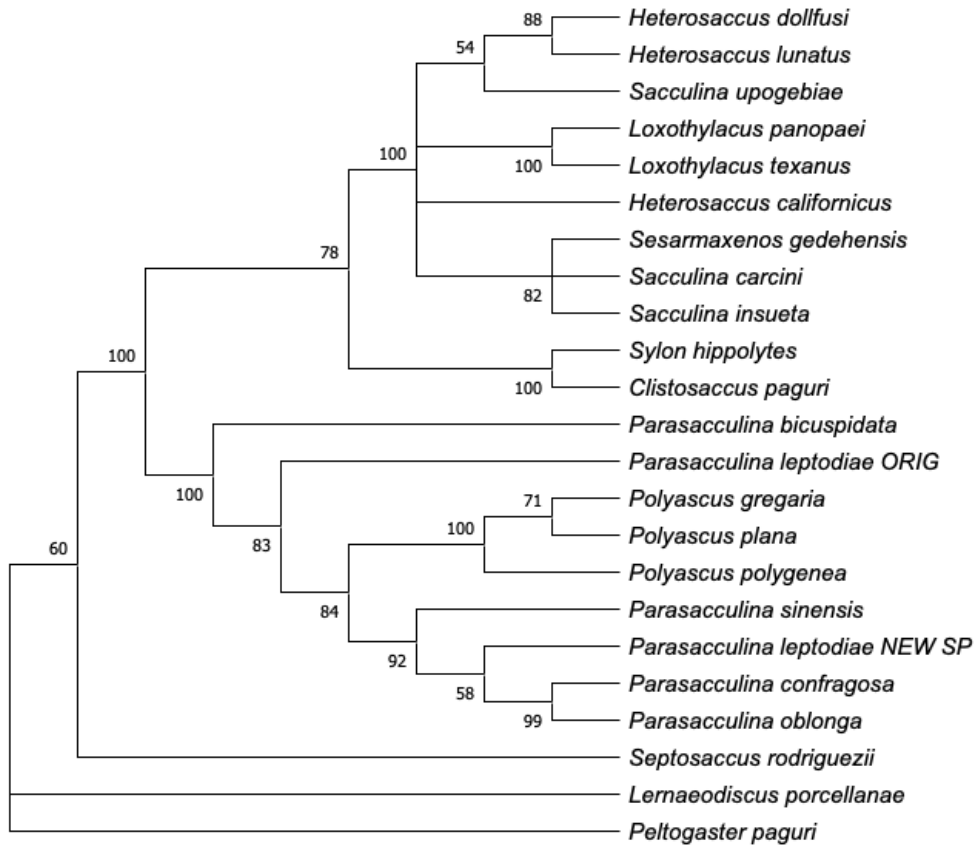
527 **Fig. 6:** Relationship between the prevalence of female *Leptodius exaratus* infected with
 528 *Parasacculina leptodiae* and the prevalence of ovigerous females at the 6 sampling sites
 529 during the period May 2014 to September 2014. The plot shows observed values (symbols)
 530 and fitted values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

532

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

Zainab Al-Wazzan*, Andreas M. Waser, Henrik Glenner, Luis Gimenez, David W. Thieltges

Supplementary Information



SM 1. Maximum likelihood phylogenetic tree for the two-gene concatenated dataset (18S and COI). The phylogeny shows the position of *Parasacculina leptodiae* Orig within a monophyletic clade of Parasacculinids. Nodal support is indicated in the form of maximum likelihood bootstrap values. Note that *Parasacculina leptodiae* Orig (sequenced for the present study), has a different phylogenetic position in the tree than *Parasacculina leptodiae* New sp (which sequences were obtained from genbank) indicating that the Genbank specimen has been misidentified. The rhizocephalan species *Peltogaster paguri*, *Lernaediscus porcellanae*, and *Septosaccus rodriguezii* have been used as out group in the analysis.

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Supplementary Materials

SM 2. Estimates of Evolutionary Divergence between 15 Rhizocephalan CO1 Sequences.

The number of base differences per site from between sequences are shown. This analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 702 positions in the final dataset.

	<i>Sylon hippolytes</i>	<i>Parasacculina sinensis</i>	<i>Parasacculina ablonga</i>	<i>Parasacculina leptodiae NEW SP</i>	<i>Parasacculina confragosa</i>	<i>Sacculina carcini</i>	<i>Polyascus polygenea</i>	<i>Polyascus plana</i>	<i>Polyascus gregaria</i>	<i>Peltogaster paguri</i>	<i>Loxothylacus panopaei</i>	<i>Parasacculina leptodiae ORIG</i>	<i>Heterosaccus lunatus</i>	<i>Heterosaccus dollfusi</i>	<i>Clistosaccus paguri</i>	
<i>Sylon hippolytes</i>																
<i>Parasacculina sinensis</i>	0.6069															
<i>Parasacculina ablonga</i>	0.5992	0.2135														
<i>Parasacculina leptodiae NEW SP</i>	0.6124	0.2204	0.2149													
<i>Parasacculina confragosa</i>	0.5763	0.2097	0.1445	0.2291												
<i>Sacculina carcini</i>	0.3644	0.5717	0.5694	0.5717	0.5569											
<i>Polyascus polygenea</i>	0.5814	0.2572	0.2505	0.253	0.2643	0.5639										
<i>Polyascus plana</i>	0.5789	0.2689	0.243	0.2469	0.2679	0.553	0.0727									
<i>Polyascus gregaria</i>	0.6004	0.2657	0.247	0.2495	0.271	0.5521	0.0568	0.0685								
<i>Peltogaster paguri</i>	0.3951	0.5878	0.5744	0.5884	0.5763	0.3091	0.5717	0.5628	0.5666							
<i>Loxothylacus panopaei</i>	0.3696	0.5734	0.5579	0.563	0.5618	0.2143	0.5412	0.541	0.5473	0.296						
<i>Parasacculina leptodiae ORIG</i>	0.3982	0.6126	0.624	0.6185	0.6267	0.3297	0.5901	0.5911	0.5984	0.3328	0.3006					
<i>Heterosaccus lunatus</i>	0.3467	0.5996	0.5918	0.5926	0.5840	0.2150	0.5774	0.5788	0.5825	0.3003	0.2399	0.3173				
<i>Heterosaccus dollfusi</i>	0.3626	0.6044	0.5988	0.5967	0.5945	0.2102	0.5942	0.5954	0.5908	0.3067	0.2396	0.3155	0.0607			
<i>Clistosaccus paguri</i>	0.2994	0.5821	0.5763	0.5562	0.5630	0.3297	0.5640	0.5506	0.5586	0.3191	0.3328	0.3556	0.3220	0.3403		

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