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2 **Exploring chemical stimuli guiding the pea crab *Dissodactylus primitivus***
3 **towards its echinoid host *Meoma ventricosa***

4 Guillaume Caulier ^(1,2), Lucas Terrana ^(1,3), Quentin Jossart ^(4,6), Igor Eeckhaut ^(1,2), Alain Préat
5 ⁽⁵⁾, Sébastien Motreuil ⁽⁶⁾, Bruno David ^(6,7) and Chantal De Ridder ⁽⁴⁾

6 (1) Biology of Marine Organisms and Biomimetics unit, Research Institute for Biosciences, University
7 of Mons-UMONS, 6, Avenue du Champ de Mars, B-7000 Mons, Belgium.

8 (2) Belaza Marine Station (IH.SM-UMONS-ULIEGE), Toliara, Madagascar.

9 (3) Natural history museum and vivarium of Tournai, 52, Rue Saint-Martin, B-7500 Tournai, Belgium.

10 (4) Laboratoire de Biologie marine (C.P. 160/15), Université Libre de Bruxelles (ULB), 50 av. F.D.
11 Roosevelt, B-1050 Bruxelles, Belgium.

12 (5) Earth & Environmental Sciences (DSTE), Sedimentology & Basin Analysis (CP160/02),
13 Université Libre de Bruxelles, 50, avenue F. D. Roosevelt, CP 160/02. B-1050 Brussels, Belgium.

14 (6) Laboratoire Biogéosciences UMR 6282 , Université de Bourgogne(uB), 6 boulevard Gabriel,
15 21000 Dijon, France.

16 (7) Muséum national d'Histoire naturelle (MNHN), CP 24, 57, Rue Cuvier 75005 Paris, France.

17

18 **Corresponding author:** guillaume.caulier@umons.ac.be

19 Lucas.terrana@tournai.be; qjossart@gmail.com; igor.eeckhaut@umons.ac.be; alain.preat@ulb.be;
20 sebastien.motreuil@u-bourgogne.fr; bruno.david@mnhn.fr; chantal.de.ridder@ulb.be

21 **Abstract**

22 Symbiotic associations require a specific communication between the host and the symbiont.
23 In marine environments, host selection is essentially driven by chemical communication using
24 specific metabolites also known as ecomones or semiochemicals. However, the chemical
25 nature of these stimuli remains mostly unknown in aquatic environments. This paper explores
26 the chemical identification of the signals allowing the pea crab *Dissodactylus primitivus* to
27 recognise/find its host, the sea urchin *Meoma ventricosa*. We used a combination of
28 behavioral tests aiming to monitor the chemotaxis reaction of crabs under water conditioned
29 by different chemical cues. A classical Y-tube olfactometer was used to test pure industrial
30 chemicals closely related to classes of molecules reported in echinoid tissues (*i.e.*, quinonic
31 and carotenoid pigments). An innovative petri-dish device was also developed to test
32 biological fluids – mucus and perivisceral liquid- collected from the host *M. ventricosa*.
33 Results show that 5,8-dihydroxy-1,4- naphthoquinone; 9-10 anthraquinones and beta carotene
34 allow a positive chemotaxis of the crab *D. primitivus*. On the other hand, the perivisceral fluid
35 of *M. ventricosa* was repellent to the crabs, a behavior that may lead the crabs to select a
36 healthy host. Based on other echinoderm-crustacean associations where the ecomones
37 allowing host selection were identified, these results suggest that *D. primitivus* may benefit
38 from several semiochemicals to locate and select its host *M. ventricosa*.

39

40 **Keywords:** Marine chemical ecology – Parasitism – Host selection – Kairomones –

41 Crustacean – Echinoderm

42

43

44 **1. Introduction**

45 Organisms live in a world of odors and flavors where each living or inert entity
46 releases specific molecules in the environment that constitute a chemical signature.
47 Transported in fluids (air or water), most chemical cues are messengers (*i.e.* semiochemicals
48 or ecomones) supporting and orientating biological interactions, a process called chemical
49 mediation (Atema, 2012; Bagnères and Hossaert-McKey, 2017), that is fundamental in
50 biodiversity structuration (Hay, 2009). For instance, marine organisms are ‘guided’ by
51 environmental chemical cues during their entire life, from early developmental to adult stages.
52 These cues act on duration of larval stages, settlement on adequate substrate, displacement
53 and migration, food detection, partner/kin recognition, predator detection and defense
54 mechanisms (Bagnères and Hossaert-McKey, 2017; Brönmark and Hansson, 2012; Hay,
55 2009; Von Elert, 2012;). Semiochemicals encompass pheromones that mediate intraspecific
56 interactions (*e.g.* kin recognition during mating) and allelochemicals that act interspecifically
57 (*e.g.* predator-prey interactions). More particularly, allelochemicals participate to a complex
58 network of information since they can be beneficial for the sender (allomones), the receiver
59 (kairomones) or both (synomones) (Brönmark and Hansson, 2012). Moreover, the effect of a
60 given ecomone may radically change according to the interacting species as a particular
61 chemical cue can be perceived as a repellent or an attractant depending on the organism
62 detecting the cue (*e.g.* a predator or a symbiont) (Caulier et al., 2013, 2022).

63

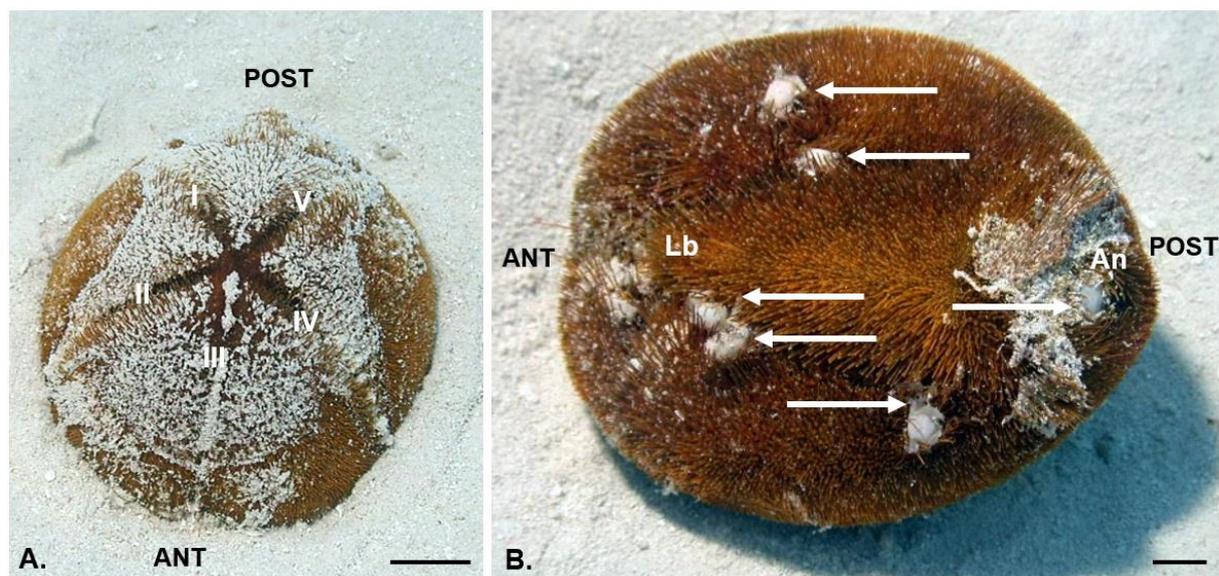
64 Symbioses are, by definition, interspecific interactions between individuals displaying
65 intimate contacts for a relatively long time compared to their life span (*e.g.* Combes, 2001; De
66 Bary, 1879; Parmentier and Michel, 2013; Smith and Douglas, 1987). Here, we do not use
67 symbiotic associations as a synonym of mutualism, but in a broader sense, also including
68 commensalism and parasitism. Among other stimuli, kairomones (in parasitism and

69 commensalism) and synomones (in mutualism) lead to host – symbionts recognition, allowing
70 the establishment and perpetuation of the symbiosis (Smith and Douglas, 1987). Echinoderms
71 can be hosts in all types of symbioses and their symbionts belong to a wide set of taxa, from
72 protozoans to chordates (Davenport, 1950; De Bruyn et al., 2009; Miyazaki et al., 2014).
73 Dozens of studies using behavioral experiments confirmed the importance of chemical
74 communication in host selection (e.g. Davenport, 1950, 1951; De Bruyn et al., 2016;
75 Eeckhaut et al., 2000; Fourgon et al., 2007; Gage, 1966; Van Meter and Ache,
76 1974). However, the exact chemical nature of the ecomones allowing symbiont positive
77 chemotaxy in marine environment has only been highlighted in a limited number of studies
78 (e.g. Brasseur et al., 2018; Caulier et al., 2013, 2022 ;Murata et al., 1986; Myazaki et al.,
79 2014;).

80

81 The pea crab *Dissodactylus primitivus* Bouvier, 1917 (Figure 1B) is an obligatory ectoparasite
82 of two spatangoids (irregular echinoids) species belonging to the Brissidae family, *Meoma*
83 *ventricosa* (Lamarck, 1816) (Figure 1A) and *Plagiobrissus grandis* (Gmelin, 1791). The two
84 echinoid hosts are asymmetrically parasitized by the crab, *M. ventricosa* being the main host
85 for adults throughout the crab geographic distribution (De Bruyn et al., 2009, 2010, 2011;
86 Jossart et al., 2017). The crabs, whatever their genders, are highly mobile, regularly switching
87 host when in search for a mate or to avoid competition with conspecifics (Baeza and Thiel,
88 2007; De Bruyn et al., 2016; Jossart et al., 2013). This behavior implies that the crabs
89 regularly need to find another individual host. Host location and recognition by the crab relies
90 on chemical cues rather than on visual stimuli (De Bruyn et al., 2011; Jossart et al., 2020). De
91 Bruyn et al. (2011) also showed that the crab can chemically discriminate its hosts from non-
92 host echinoid species. However, the nature of the kairomones emitted by *M. ventricosa*
93 allowing host selection by *D. primitivus* remains unknown.

94



95

96 Fig.1. *In situ* pictures of *Meoma ventricosa*. A. Aboral face of a partially buried individual, showing the
 97 adapical portions of the ambulacra (I to V); I, II, IV and V form sunken respiratory petals. B. Oral face of
 98 an excavated individual hosting six *Dissodactylus primitivus* among the spines (see arrows). ANT and
 99 POST: anterior and posterior ends, respectively. An: Anus, Lb: Labrum (the mouth is hidden by the
 100 labrum). The scale bars represent 3.5 cm in A and 0.8 cm in B.

101

102 The current study investigates potential kairomones involved in host selection between the
 103 echinoid host *M. ventricosa* and its crustacean symbiont *D. primitivus*. The kairomone
 104 candidates were chosen based on field observations and on previous literature highlighting
 105 secondary metabolites extracted from sea urchins (*e.g.* Borisova et al., 2022). Particularly,
 106 two quinones, the echinochrome A (red-brown) and spinochrome E (orange-yellow), were
 107 isolated from the test of *M. ventricosa* (Anderson et al., 1969). Another category of isoprenoid
 108 pigment (the carotenoids) is known to occur widely in marine organisms and notably in sea
 109 urchins (Galasko et al., 1969; Tsushima, 2007). Although there is no data on the occurrence
 110 of carotenoids in *M. ventricosa*, a xanthophyllic carotenoid (echinenone) has been isolated
 111 from the integument of a related species, *Brissus agassizii* (Matsumo and Tsushima, 2001).
 112 Taking these observations as a guideline, we investigate the potential role of quinones and

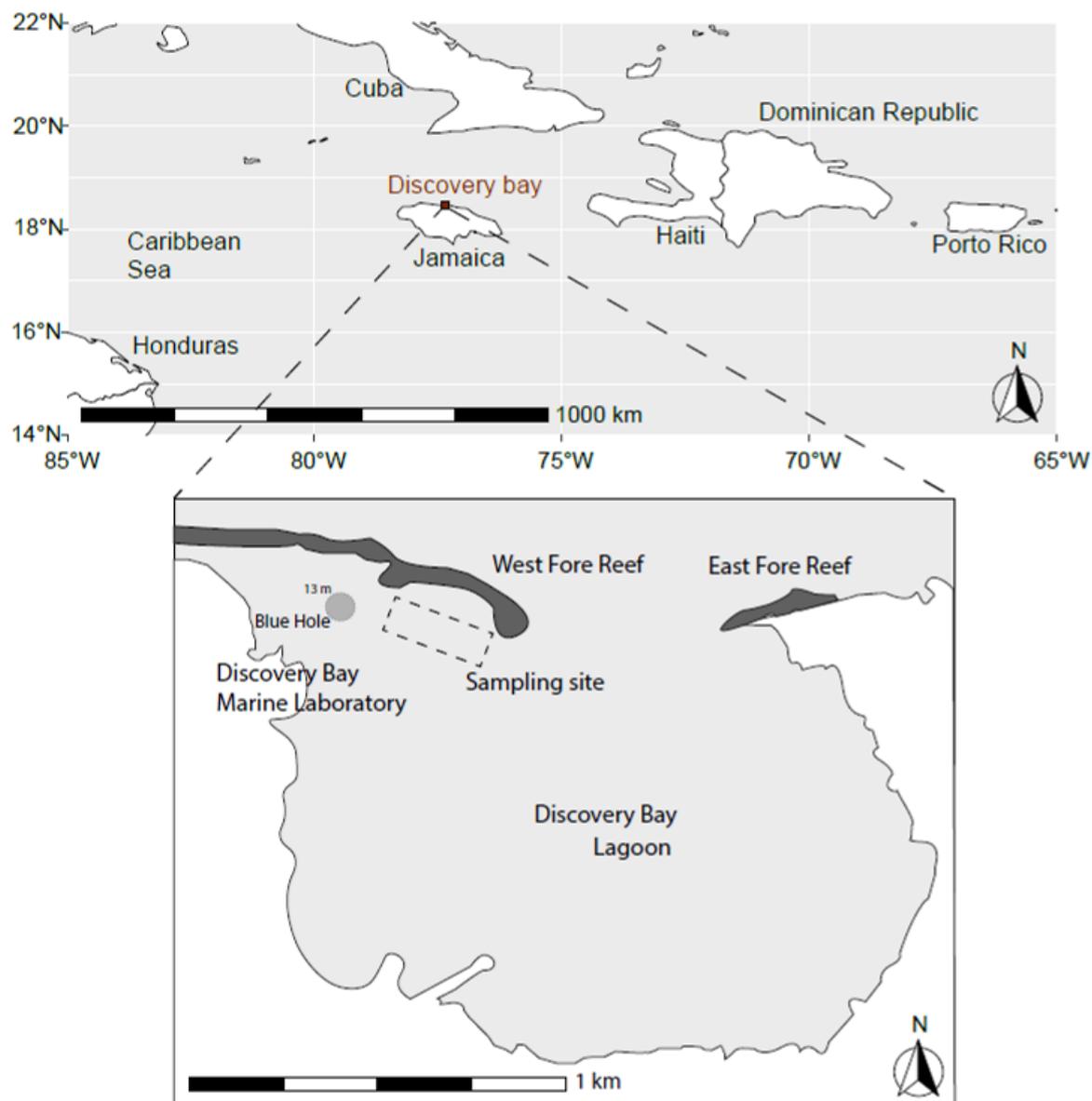
113 carotenoids as potential kairomones in the symbiotic couple *M. ventricosa* - *D. primitivus*.
114 Two other substances produced by *M. ventricosa* and susceptible to play a role in chemical
115 communication were also tested: (1) the mucus abundantly secreted in the surrounding water
116 by tiny glandular spines (the clavulae) and (2) the perivisceral fluid released from the
117 wounded epidermal tissues, such injuries being notably performed by the parasitic crab (De
118 Bruyn et al., 2009).

119 **2. Material and methods**

120 **2.1. Sampling**

121 The echinoids *Meoma ventricosa* and their adult ectoparasite crabs *Dissodactylus*
122 *primitivus* were collected in the lagoon of Discovery Bay (north coast of Jamaica, 18° 28'
123 12.13" N and 77° 24' 46.77" W). The experiments were conducted at the Discovery Bay
124 Marine Laboratory (University of the West Indies) during two scientific missions in March-
125 April 2015 and 2018 at depths from 2 to 5 m by snorkeling and scuba diving (Figure 2).
126 Initially buried in the sediment and only showing the apical portion of their ambulacra (Figure
127 1A), sea urchins were excavated by hand. The sea urchins usually harbored symbiotic crabs
128 on their oral face (Figure 1B). Individual crabs were carefully picked up with forceps during
129 dives from all encountered hosts and placed in 50-ml vials. They were then transferred to the
130 laboratory on *M. ventricosa* individuals within 30 minutes after capture. Individuals were kept
131 in open seawater tanks (28°C; salinity of 35 psu; natural circadian light). Six sea urchins were
132 simultaneously used as hosts (harboring a maximum of 15 crabs to avoid competition between
133 symbionts) and were replaced every 2-3 days by new individuals (a total of 40 sea urchins
134 was utilized for that purpose). Crabs were renewed for each experiment/trial (*i.e.*, one
135 individual was only tested once), requiring daily samplings. After being sexed, the crabs were
136 acclimated during 3 hours before starting the behavioral tests. All echinoids and crabs were

137 returned to their original place in the lagoon after the experiments.



138

139 **Figure 2:** Map indicating sampling and experimental sites in Discovery Bay (Jamaica), a dashed rectangle
 140 locating the sampling area. Behavioral experiments took place in the Discovery Bay Marine Laboratory
 141 (University of the West Indies).

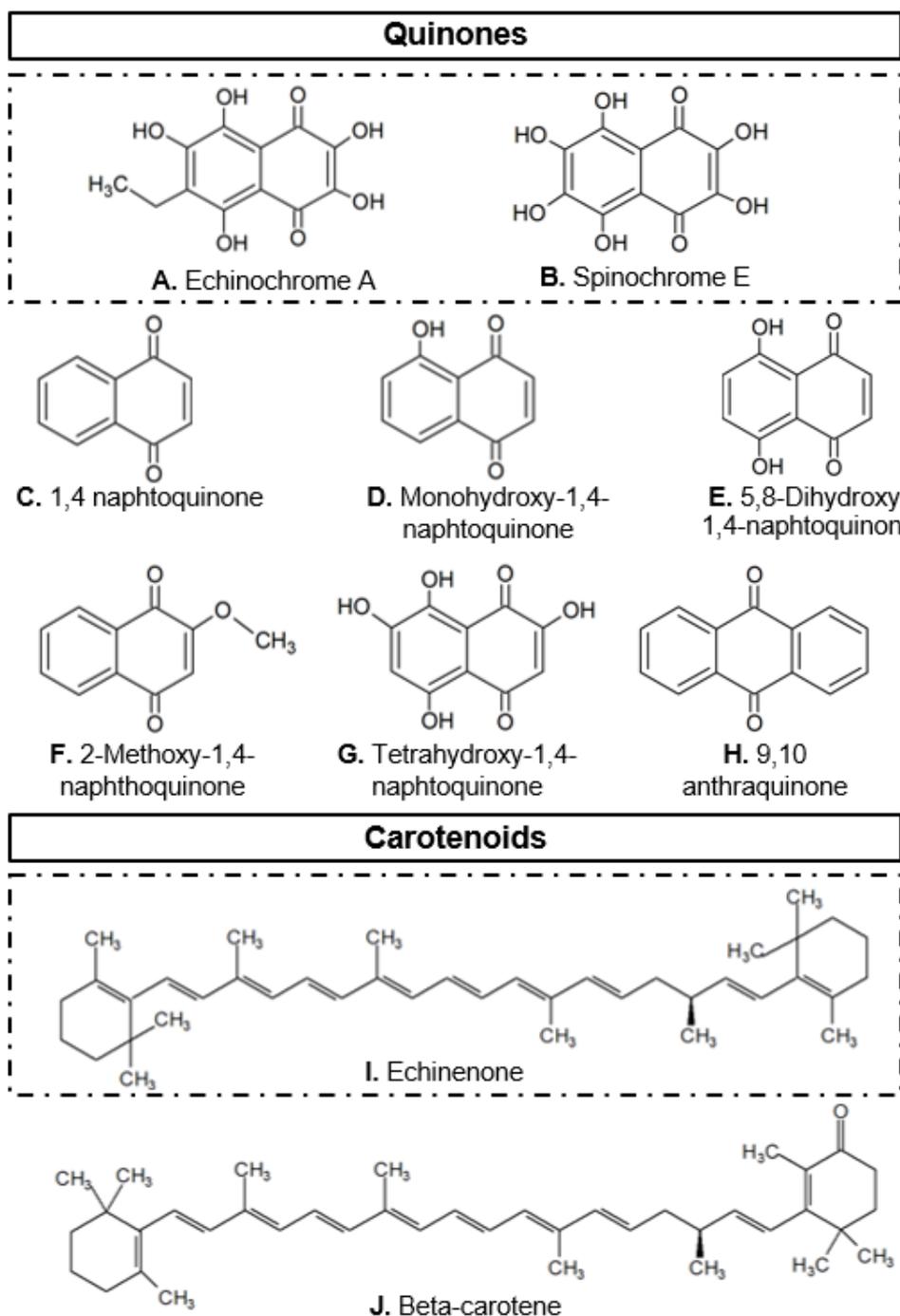
142 2.2. Ecomone candidates

143 Nine different chemical cues were tested as potential attractive kairomones in two
 144 different olfactometers (see below). Six different pure quinones (Figure 3C-H) and one pure
 145 carotenoid (Figure 3J) were chosen for their similarities with chemicals previously detected in

146 sea urchins (Figure 3A, B, I) (Anderson et al., 1969; Brasseur et al., 2018; Tsushima, 2007).
147 In addition, two biological fluids of *M. ventricosa* (*i.e.*, the mucus and the perivisceral fluid)
148 were chosen as they are discharged in the surrounding water by wounded or stressed *M.*
149 *ventricosa*, notably when injured by the parasites *D. primitivus* (De Bruyn et al., 2009).

150 2.2.1. Pure chemicals

151 The quinones echinochrome A (Figure 3A), spinochrome E (Figure 3B) and the
152 carotenoid echinenone (Figure 3I) described in previous literature (Anderson et al., 1969;
153 Brasseur et al., 2017; Tsushima, 2007,) were not commercially available and could not be
154 tested. The alternative was to consider them as references to select other pure chemicals
155 structurally related to the former ones. The selected pure quinones were the 1,4-
156 naphthoquinone (Figure 3C), the monohydroxy-1,4-naphthoquinone (Figure 3D), the 5,8-
157 dihydroxy-1,4-naphthoquinone (Figure 3E), the 2-methoxy-1,4-naphthoquinone (Figure 3F),
158 the tetrahydroxy-1,4-naphthoquinone (Figure 3G) and the 9,10-anthraquinone (Figure 3H). The
159 selected pure carotenoid was the beta-carotene (Figure 3J). Two concentrations were tested
160 depending on the solubility of these chemicals in seawater and previous literature (Brasseur et
161 al., 2018; Caulier et al., 2013, 2022): 0.1g/L and 0.001g/L. Considering that the *Beta-*
162 *carotene*, the 1,4-naphthoquinone and the 9,10-anthraquinone are particularly hydrophobic,
163 only the highest concentration was used for those three candidates to ensure a saturation of
164 water was saturated with those chemicals. On the contrary, only the lowest concentration was
165 used for the tetrahydroxy-1,4 naphthoquinone due to its limited availability at the time of the
166 experiments/fieldwork.



167 **Figure 3:** Chemical
 168 structures of the pure chemicals that were selected as ecomone candidates to allow host selection of the sea
 169 urchin *Meoma ventricosa* by its symbiont *Dissodactylus primitivus*. Dashed rectangles correspond to the
 170 reference molecules that were highlighted to be produced by sea urchins in previous literature.

171 **2.2.2. Biological fluids**

172 The perivisceral fluid (25 ml) of *M. ventricosa* was collected from five individuals
 173 with a 50 ml syringe mounted on a G21 needle that was introduced in the perivisceral coelom

174 (*i.e.* general cavity) through the peristomial membrane. The mucus secreted by *M. ventricosa*
175 was directly collected from 24 individuals with a Pasteur pipette (100 ml) as it forms vertical
176 flocs in the aquaria by extending upward from the sea urchin body. The biological fluids were
177 tested directly after collection.

178 **2.3. Experimental devices**

179 The attracting effects of the kairomone candidates were tested on the parasite *D.*
180 *primitivus* using two types of olfactometers depending on the viscosity/surface-interacting
181 properties of the cues. By using these devices, chemotaxis of the crabs in response to specific
182 chemical stimuli can be visualized and recorded (chemotaxis is the movement in space of an
183 organism in response to chemoreception).

184 Pure chemicals were tested in a Y-tube design (Figure 4 and SM1) identical to the one used in
185 previous works demonstrating the specific chemical attraction exerted by the echinoid on its
186 parasite crab (see De Bruyn et al., 2011). The Y-tube device is detailed in De Bryun et al.
187 (2011) being adapted from Davenport (1950) and Johnson (1952). The chemical signal is
188 transported along a set of four interconnected modules by a flux of seawater (Figure 4). The
189 flux is generated by gravity and regulated by taps. The first module is made of two large (5 l)
190 aquaria regularly filled with seawater. One of these aquaria (module 1) also contains the
191 signal (Figure 4, green color). In order to limit dispersion, the pure chemicals were canalized
192 to the basal opening of the' aquarium through a narrow plastic (2 mm diameter) connected to
193 a tank containing the tested solution. A continuous flux was ensured and regulated by a
194 peristaltic pump (30 ml/minute). Flow laminarity inside the olfactometer was controlled using
195 fluorescein salt (Figure SM1) before the experiments and the water flux was regulated to a
196 speed of 2–3 cm s⁻¹. Each outflow from one 'module 1' aquarium accesses one smaller (1l)
197 aquarium ('module 2') that acts as a flow regulator. In turn, each of the two 'module 2'

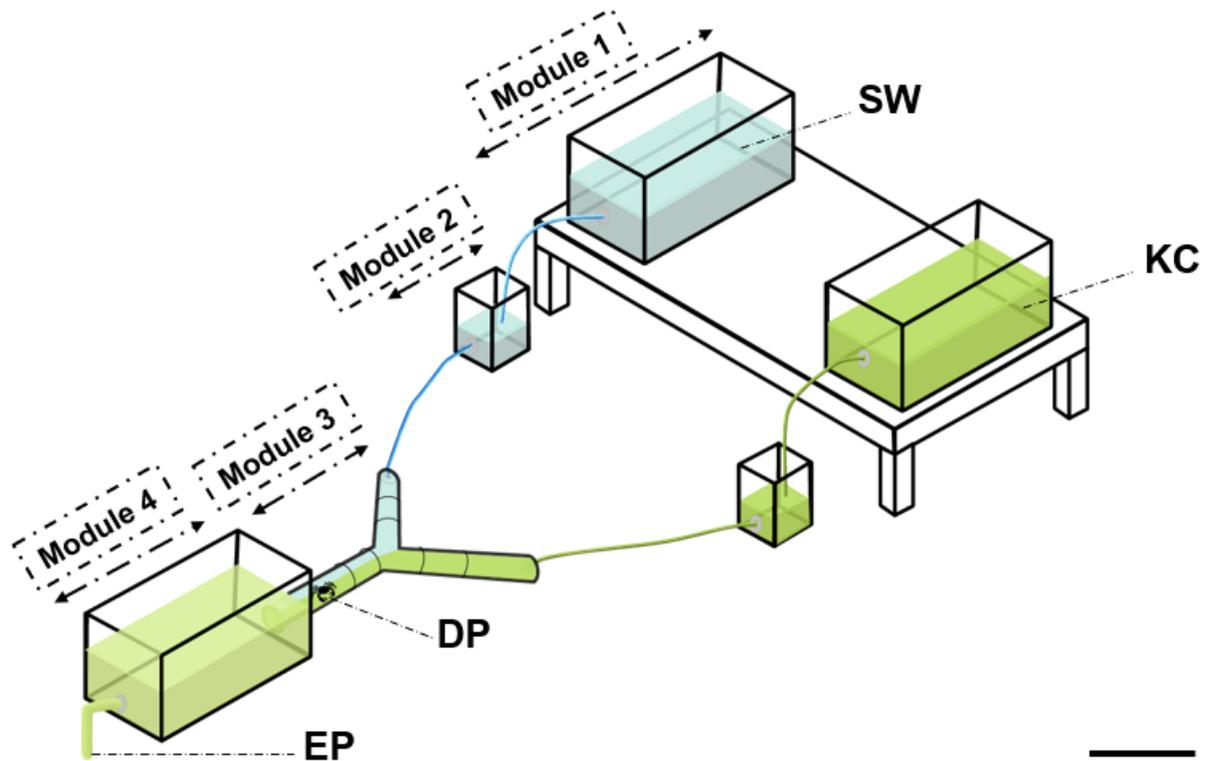
198 aquaria was connected to one of the Left/Right (L/R) distal branches of the Y tube ('module
199 3'). The Y-tube was in glass (tube diameter 30 mm) and kept in horizontal position. At the
200 beginning of each experiment, the crab was placed in a removable tube that has one end
201 closed by a grid; this tube is inserted in the Y proximal branch allowing the crab to move
202 upstream towards the distal branches. The device ends in 'module 4' aquarium that was
203 connected to a sink. The seawater flux was kept at 300 mL/min. Lighting was kept uniform
204 and non-directional throughout the experiments.

205

206 During each trial, one crab was introduced at the base of the unpaired branch of the Y tube. If
207 it was not stimulated, the crab remained at the base of the branch without moving and the run
208 was aborted and considered as null after 10 min. If the crab was stimulated, it moved into the
209 unpaired branch up to the junction of the two-paired branches where it usually stopped
210 moving for a few seconds, before entering one of the paired branches. The orientation
211 behavior was recorded as positive when crabs entered one of the two paired branches and
212 reached at least half of the distance in it. Except for the Monohydroxy-1,4-naphtoquinone that
213 quickly appeared not be attractant, the number of replicates for each condition was minimum
214 30 different trials. Tanks in which the stimuli were introduced were exchanged every five
215 runs. The entire device was washed after each crab.

216

217 The negative test consisted of only seawater filling the device and the positive test consisted
218 of water conditioned by the live host *M. ventricosa* that was introduced in one of the 'module
219 1' aquaria for 30 minutes (*i.e.* without any crab on the sea urchin to avoid any potential
220 pheromonal communication between crabs). The attractiveness of seawater (conditioned by
221 *M. ventricosa*) towards its symbiont *D. primitivus* was already demonstrated by De Bruyn et
222 al. (2011).



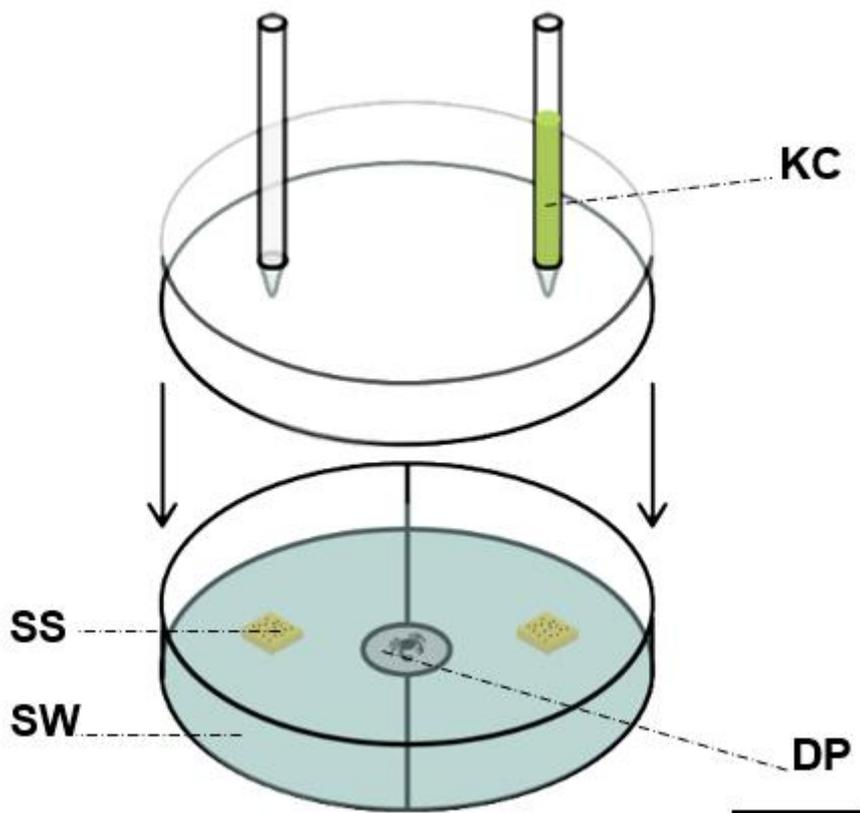
223

224 **Figure 4:** Scheme of the Y-tube olfactometer. Blue and green fluids respectively correspond to seawater and
 225 seawater with tested kairomone candidate. The flow circulates from Module 1 to Module 4 and is regulated by
 226 gravity and by a tap on the evacuation pipe. Diffusion pattern of the tested chemical was beforehand tested with
 227 fluorescein (see Figure SM1). The crab *Dissodactylus primitivus* is introduced at the beginning of the Y-tube
 228 (Module 3). DP: *Dissodactylus primitivus*; EP: Evacuation pipe; KC: Kairomone candidate; SW: Seawater.

229

230 Because of a higher viscosity (mucus) or due to the presence of surface-adhering
 231 coelomocytes (perivisceral fluid, see Smith et al., 2018 for review), biological fluids required
 232 another experimental device. An original device olfactometer was built in a Petri-dish (Figure
 233 5 and SM2) where the tested stimuli were passively diffusing in stagnant seawater without
 234 flux. The Petri-dishes were plastic (polystyrene) and transparent with a dimension of 150 mm
 235 x 20 mm (diameter x height). The bottom of the dish was divided into two halves along a
 236 diameter by a line drawn on the outside. The lid was pierced by three aligned holes: a central
 237 hole (diam 2.5 cm) and two lateral holes (diam 0.5 cm) each located at 2 cm from the edge.
 238 The central hole was crossed by a tube which allows to position the tested crab in the center

239 of the device. The lateral orifices were each crossed by the narrow end of a micropipette tip
240 that was applied against a fragment of synthetic sponge (1 cm², 0.3 cm thick). This fragment
241 was rinsed several times with distilled water, then wrung out and used as a support for the test
242 substance (stimulus). These fragments were renewed for each new substance tested. Similar
243 olfactometers were already used in previous terrestrial studies (Ikawati et al., 2017; Ruther et
244 al., 2019) but never for marine organisms. At least sixteen different crabs were tested for each
245 experiment and Petri-dish left and right parts were exchanged every five runs. Again, each
246 crab individual was only tested once to avoid any potential habituation effect. The entire
247 device was washed after each crab. In order to validate this new device, negative control trials
248 (n=6) using fluorescein salt in one of the tips was performed to visualize the diffusion pattern
249 of a chemical cue in the Petri-dish device and allow us to estimate the time needed for the
250 chemical to spread in the device (Figure SM2). In average, the green dye was present in \approx
251 90% of the corresponding half side of the Petri-dish after 3 minutes (Figure SM2E). The
252 negative control corresponded to the device only filled with normal seawater. The positive
253 test involved one pure chemical (9,10-anthraquinone) that proved to be attractant in the Y-
254 tube olfactometer (see results Table 1). Biological fluids were tested at natural concentration
255 as they were produced by the urchins, and the pure chemical was tested at 0.1 g/L. For each
256 run, the device was filled with a layer of 1 cm of seawater so that the crab was completely
257 submerged during the test. The two micropipette tips were filled with the same volume of
258 either seawater, or chemical stimulus. The crab was then introduced in the center of the device
259 and let for one minute of habituation time before the cue was deposited on the synthetic
260 sponges.



261

262 **Figure 5:** Scheme of the Petri-dish olfactometer at T_0 . Blue and green fluids respectively correspond to seawater
 263 and seawater with tested kairomone candidate. A 1cm² fragment of synthetic sponge was soaked with the tested
 264 chemical allowing its subsequent diffusion in the Petri-dish seawater. Diffusion pattern of the tested chemical
 265 was beforehand tested with fluorescein salt (see Figure SM2). DP: *Dissodactylus primitivus*; KC: Kairomone
 266 candidate; SS: Synthetic sponge; SW: Seawater.

267

268 2.4 Statistical analyses

269 In the Y-tube olfactometer, the orientation behavior was assessed using a binomial test by
 270 comparing the percentage of crabs that entered aquarium A to a random distribution
 271 (Probability of success = 0.5). All statistical tests were performed with the R software (R
 272 project, 2022).

273 In the Petri-dish olfactometer, the choice behavior was assessed using a binomial test by
 274 comparing the percentage of the position of the crabs in the Petri-dish (*i.e.*, left or right)

275 after 3 minutes to a random distribution (Probability of success = 0.5). All statistical tests
276 were performed with the R software (R project, .64 3.2.3; R Core Team (2015)).

277

278 **2. Results**

279 **3.1. Behavioral results using the Y-tube olfactometer**

280 Overall, a total of 418 trials were made in the Y-tube olfactometer considering the 11
281 different conditions (molecules and concentrations). Over all the crabs tested, no sex-related
282 difference was observed during the experimentations. The results are summarized in Table 1
283 and Table SM1 for raw data. The negative control test confirmed that the crabs *D. primitivus*
284 did not significantly preferred one side of the Y-tube (Table 1-1; 52% right and 48% left)
285 indicating the absence of both chemical attractant in unconditioned seawater and external bias
286 (*e.g.* lighting or temperature of the room). Over the 77 crabs that were tested with host-
287 conditioned seawater (Table 1, line 2), each individual showed a typical chemoreception
288 behavior pattern (Harzsch and Krieger, 2018) and 52 (80%) oriented correctly in terms of host
289 selection confirming the results of De Bruyn et al. (2011). Three molecules were found
290 significantly attractant (p-value < 0.05) for the pea crabs at various concentrations (Table 1,
291 lines 5, 6, 10, 11). Indeed, 68% and 70% of the crabs moved towards the 5,8-dihydroxy-1,4-
292 naphtoquinones (at 0.001 and 0.1 g/L, respectively); 76% of the crabs displayed a positive
293 chemotaxis towards the 9,10-anthraquinone (0.1 g/L) and 69% were attracted towards *Beta*-
294 carotene (0.1 g/L) (table 1-11). On the contrary, four molecules had no effect on the crabs
295 regardless of the concentration (p-value > 0.05): 1,4-naphtoquinone (0.1 g/L) with 56% of
296 choice towards the cue (table 1-3); Monohydroxy-1,4-naphtoquinone (0.001 g/L) with 26% of
297 choice towards the cue (table 1-4); 2-methoxy-1,4-naphtoquinone (0.001 and 0.1 g/L) with

298 41% and 56% of choice towards the cue, respectively (table 1-7 and 8); and tetrahydroxy-1,4-
 299 naphtoquinone (0.001 g/L) with 56% of choice towards the cue (table 1-9).

300

301 **Table 1:** Y-tube olfactometer results showing the effect of different chemical cues on the chemotaxis of the crab
 302 *Dissodactylus primitivus*. Significant results ($p < 0.05$) are highlighted in bold. Corresponding chemical
 303 structures of the cues can be found in figure 3 and raw results can be consulted in supplementary material (Table
 304 SM1).

#	Attractant versus seawater	p-value	Total runs	Conc (g/L)	Result
Negative control					
1.	Seawater	1	30	/	No effect
Positive control					
2.	<i>Meoma ventricosa</i> live sea urchin	$1.2 \cdot 10^{-6}$	77	/	Attractant
Quinone-related molecules					
3.	1,4-naphtoquinone	0.8	29	0.1	No effect
4.	Monohydroxy-1,4-naphtoquinone	0.3	17	0.001	No effect
5.	5,8-Dihydroxy-1,4-naphtoquinone	$3.3 \cdot 10^{-2}$	42	0.001	Attractant
6.	5,8-Dihydroxy-1,4-naphtoquinone	$2.4 \cdot 10^{-2}$	35	0.1	Attractant
7.	2-methoxy-1,4-naphtoquinone	0.5	30	0.001	No effect
8.	2-methoxy-1,4-naphtoquinone	0.8	30	0.1	No effect
9.	Tetrahydroxy-1,4-naphtoquinone	0.7	30	0.001	No effect
10.	9,10-anthraquinone	$4.5 \cdot 10^{-3}$	39	0.1	Attractant
Carotenoids					
11.	<i>Beta</i> carotene	$6.4 \cdot 10^{-3}$	59	0.1	Attractant

305 3.2. Behavioral results using the Petri-dish olfactometer

306 Overall, a total of 136 runs were made in the Petri-dish olfactometer considering the 4

307 different conditions. The results are summarized in Table 2 and Table SM2 for raw results

308 (displaying the details of the choice results). The negative control test confirmed that the crabs
 309 *M. ventricosa* did not significantly prefer one side of the Petri-dish olfactometer (Table 2-1
 310 56% right and 44% left) confirming the absence of external bias that may influence the taxis
 311 of the crab. When testing the 9,10-anthraquinones 0.1g/L (Table 2, line 2), 72% of the crabs
 312 significantly chose to remain in the corresponding side of the olfactometer. This confirmed
 313 the attractive effect of the 9,10-anthraquinones on the positive chemotaxis of the crab and
 314 justifies the use of the Petri-dish olfactometer for further experiments with marine organisms.
 315 Neither the mucus nor the perivisceral fluid of *M. ventricosa* were attractive to *D. primitivus*
 316 (Table 2, line 3, 4) even if chemodetection behaviors (*i.e.* flicking/grooming) could be
 317 observed on most tested crabs. The perivisceral fluid even had a significant negative
 318 chemotaxis effect: the crab was avoiding this chemical cue, being monitored preferentially
 319 (75% of the tested crabs) on the Petri-dish side that only contains seawater (Table 2, line 4).

320

321 **Table 2:** Petri-dish olfactometer results showing the impact of different chemical cues on the chemotaxis of the
 322 crab *Dissodactylus primitivus*. Significant results ($p < 0.05$) are highlighted in bold. Corresponding chemical
 323 structures of the cues can be found in figure 3 and raw results can be consulted in supplementary material (Table
 324 SM2).

#	Attractant versus seawater	p-value	Total runs	Conc (g/L)	Result
Negative control					
1.	Seawater	0.6	32	/	/
Positive control					
2.	9,10-anthraquinones	$2 \cdot 10^{-2}$	32	0.1	Attractant
Biological fluids					
3.	Mucus	0.870	40	/	No effect
4.	Perivisceral fluid	$7 \cdot 10^{-3}$	32	/	Repellent

325 4. Discussion

326 Despite the fact that chemical communication is considered the most widespread mode of
327 communication in aquatic environments (Hay, 2009), most chemical signals involved in
328 organisms' interactions remain unidentified. Marine organisms live in a complex olfactory
329 world where they have to identify behaviorally relevant odors (Corey and Ache, 2016).
330 Initially considered as uninformative signals, marine secondary metabolites (*i.e.* specialized
331 molecules not directly involved in the primary metabolism) are now considered as key
332 molecules mediating biological interactions (Demain and Fang, 2000; Hay, 1996; Stonik and
333 Eliakov, 1988; Williams et al., 1989). Secondary metabolites count diversified
334 semiochemicals classified into six main groups related to their metabolic pathways
335 (isoprenoids, acetogenins, amino-acids, shikimates, nucleic acids and carbohydrates) and
336 some of them may only be present in precise taxa (Harper et al., 2001). Particularly, the
337 phylum Echinodermata presents specific chemicals that are true chemical signatures for each
338 class (Kornprobst, 2005). Based on this statement, Caulier et al. (2013) identified, for the first
339 time, the chemical signature of a marine kairomone produced by holothuroids to attract their
340 associated Harlequin crab, *Lissocarcinus orbicularis*. In 2018, Brasseur et al. characterized
341 that spinochromes produced by the urchin *Echinometra mathaei* could attract the symbiotic
342 shrimp *Tuleariocaris holthuisi* and Caulier et al. (2022) highlighted the kairomonal role of
343 anthraquinones allowing the snapping shrimp *Synalpheus stimpsonii* to select its host crinoid
344 *Phanogenia distincta*. In the latter, artificial anthraquinones (*i.e.*, not directly extracted from a
345 host tissue) also had an attractive effect on the snapping shrimp *S. stimpsonii* (Caulier et al.,
346 2022). Our present results also suggest that the specific molecular structure of the chemical
347 signature of the host is not always the only one to allow a positive chemotaxis on the
348 symbiont. For instance, three different chemical cues that elicited an attraction for *D.*
349 *primitivus* were also attractive for the symbiotic shrimp in Caulier et al. (2022).

350

351 Considering the association between *M. ventricosa* and *D. primitivus*, three molecules among
352 the nine tested significantly attracted the parasite crabs: a naphthoquinone (at 0.001 and 0.1
353 g/L), an anthraquinone (0.1 g/L) and a carotenoid (0.1 g/L). These positive results suggest at
354 least three possible situations: (1) the three molecules can be *per se* kairomones, (2) the three
355 molecules are part of a more complex mixture including a range of kairomones, (3) the
356 molecules (or one of them) mimic another kairomone through their chemical structure (*cf.* the
357 presence and/or position of a particular group such as the $-C(=O)-$ groups in
358 naphthoquinones,. Considering that, in echinoderms, anthraquinones are specific to crinoids
359 (Kornprobst, 2005) and that only industrially synthesized molecules were tested in the current
360 study, the hypothesis (3) should be preferred. Each of the five tested quinones has a
361 characteristic number of aromatic cycles and harbors hydroxyls that differ in position and
362 number. These structural differences could be indicative of the nature of the kairomone acting
363 on the crab. However, comparison of the molecular structures of the chemicals having a
364 positive chemotaxis effect in this study does not reveal a specific common pattern that would
365 be recognised by an olfactory receptor (*i.e.*, GOLF: Olfactory Neuron Specific-G Protein).
366 More research should be done in the future including the characterization of the odorant
367 receptors of *D. primitivus*, and other crustaceans in general. Characterization of the genes and
368 their associated proteins allowing chemoreception is a future relevant step. More specifically,
369 the 3D conformation of the ligand and of the receptor should allow us to better understand the
370 complex host selection process. Also, appropriate chemical extractions and quantifications of
371 quinones and carotenoids in the tissues of *Meoma ventricosa* and in the conditioned water
372 would be more ecologically relevant. It would also be relevant to compare the attractive
373 chemicals together to investigate a potential preference of the symbiont for quinones or
374 carotenoids.

375 Being repelled by the perivisceral fluid of *M. ventricosa* may help the symbiotic *D. primitivus*
376 to avoid hosts that are harmed/diseased or overloaded with parasites. It is also noteworthy to
377 mention that Chesher (1969) observed a yellow presumably repellent substance secreted by
378 *M. ventricosa* when disturbed/stressed. However, this secretion was demonstrated lacking a
379 repellent effect on *D. primitivus* (De Bruyn et al., 2011). Numerous Pinnotherid crabs are
380 symbiotic and developed various adaptations to their way of life (De Gier and Becker, 2020)
381 and it would therefore not be surprising that they have developed a specific olfactory pathway
382 to select healthy hosts. In crustaceans, it has already been demonstrated that lobsters could
383 chemically recognize and avoid diseased conspecifics (Behringer et al., 2006) but the
384 potential host avoidance by a metazoan symbiont has, to date, never been investigated. The
385 negative chemotaxis shown by the symbiont when detecting the host perivisceral fluid should
386 be investigated other symbiotic associations in the future to evaluate its commonness. In
387 addition, the agent of the repelling activity should be also further explored to see whether it
388 originates from the fluid itself or from the specific cells it contains (i.e. the coelomocytes).

389 **5. Conclusion**

390 In conclusion, this study provides new insights regarding marine chemical communication
391 between symbiotic organisms showing the attractive effect of three artificial chemicals
392 belonging to the groups of specific secondary metabolites produced by echinoderms. This first
393 step suggests the potential role of quinones and carotenoids as kairomones allowing the crab
394 *D. primitivus* to find and select its host *M. ventricosa*. This study also suggests how symbionts
395 may avoid diseased/harmed hosts by sensing chemicals.

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