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1 **Comparative study of chemosensory organs of shrimp from hydrothermal vent and**
2 **coastal environments**

3

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18

19 **Abstract**

20 The detection of chemical signals is involved in a variety of crustacean behaviours, such as
21 social interactions, search and evaluation of food and navigation in the environment. At
22 hydrothermal vents, endemic shrimp may use the chemical signature of vent fluids to locate
23 active edifices, however little is known on their sensory perception in these remote deep-sea
24 habitats. Here, we present the first comparative description of the sensilla on the antennules
25 and antennae of four hydrothermal vent shrimp (*Rimicaris exoculata*, *Mirocaris fortunata*,

26 *Chorocaris chacei* and *Alvinocaris markensis*) and of a closely related coastal shrimp
27 (*Palaemon elegans*). These observations revealed no specific adaptation regarding the size or
28 number of aesthetascs (specialized unimodal olfactory sensilla) between hydrothermal and
29 coastal species. We also identified partial sequences of the ionotropic receptor IR25a, a co-
30 receptor putatively involved in olfaction, in 3 coastal and 4 hydrothermal shrimp species, and
31 showed that it is mainly expressed in the lateral flagella of the antennules that bear the
32 unimodal chemosensilla aesthetascs.

33

34 **Key words:** Aesthetascs, Decapod, Hydrothermal shrimp, IR25a, Olfaction

35

36

37 **Introduction**

38 Chemical senses are crucial in mediating important behavioural patterns for most animals. In
39 crustaceans, chemical senses have been shown to play a role in various social interactions,
40 search and evaluation of food, as well as in evaluation and navigation in the habitat (Steullet
41 et al. 2001; Derby and Weissburg 2014). Chemoreception in decapod crustaceans is mediated
42 by chemosensory sensilla that are mainly localized on the first antennae (antennules),
43 pereopod dactyls and mouthparts (Ache 1982; Derby et al. 2016). Chemoreception has been
44 proposed to be differentiated into two different modes (Schmidt and Mellon 2011; Mellon
45 2014; Derby et al. 2016): 1) "olfaction" mediated by olfactory receptor neurons (ORNs)
46 housed in specialized unimodal olfactory sensilla (the aesthetascs), restricted to the lateral
47 flagella of the antennules (Laverack 1964; Grünert and Ache 1988; Cate and Derby 2001) and
48 projecting to the olfactory lobe of the brain (Schmidt and Ache 1996b) and 2) "distributed
49 chemoreception" mediated by numerous bimodal sensilla (containing mecano- and chemo-
50 receptor neurons) occurring on all appendages, projecting to the second antenna and lateral

51 antennular neuropils and the leg neuromeres (Schmidt and Ache 1996a). While the molecular
52 mechanisms of olfaction have been well studied in insects, they remain largely unknown in
53 crustaceans, and the existing knowledge is restricted to a few number of model organisms
54 (lobsters, crayfish and the water flea *Daphnia pulex*; review in Derby et al. 2016). In
55 particular, the nature of crustacean odorant receptors has remained elusive until recently,
56 since searches for the traditional insect olfactory receptors have been unsuccessful. A new
57 family of receptors involved in odorant detection, named the Ionotropic Receptors (IRs), was
58 recently described in *Drosophila melanogaster*, and was subsequently shown to be conserved
59 in Protostomia, including the crustacean *Daphnia pulex* (Benton et al. 2009; review in Croset
60 et al. 2010). Lately, several IRs were identified in other crustaceans, the spiny lobster
61 *Panulirus argus* (Corey et al. 2013), the American lobster *Homarus americanus* (Hollins et
62 al. 2003), the hermit crabs *Pagurus bernhardus* (Groh et al. 2014) and *Coenobita clypeatus*
63 (Groh-Lunow et al. 2015), and were proposed to mediate the odorant detection in the
64 antennules. In the lobster, the authors propose that IRs function as heteromeric receptors, with
65 IR25a and IR93a being common subunits that associate with other IR subunits to determine
66 the odor sensitivity of ORNs.

67 Chemoreception in crustaceans has been largely studied in large decapods like lobsters
68 (Devine and Atema 1982; Cowan et al. 1991; Moore et al. 1991; Derby et al. 2001; Shabani et
69 al. 2008; and see review in Derby et al. 2016). However this research theme remains poorly
70 investigated in shrimp, especially in deep-sea species. Deep-sea hydrothermal vent shrimp
71 inhabit patchy and ephemeral environments along the mid-oceanic ridges. Inhabiting such
72 sparsely distributed habitats presents challenges for the detection of active emissions by
73 endemic fauna, especially in the absence of light. In the early developmental stages, after
74 release and dispersal in the water column, sometimes tens or hundreds of kilometers from
75 their starting point, larvae need to locate a vent site to settle and begin their adult life (Herring

76 and Dixon 1998; Pond et al. 1997). Later as adults, mobile vent fauna may need to evaluate
77 their environment, to find hydrothermal fluid either to feed their symbiotic bacteria or just to
78 be able to detect the appropriate habitat, in an environment characterised by steep
79 physicochemical gradients (Sarrazin et al. 1999, Sarradin et al. 1999, Le Bris et al. 2006).
80 Chemical compounds like sulfide, temperature and dim light emitted by vents have been
81 proposed to be potential attractants for detection of hydrothermal emissions (Van Dover et al.
82 1989; Renninger et al. 1995; Gaten et al. 1998).

83 Only a few studies on olfaction in the hydrothermal shrimp *Rimicaris exoculata* have been
84 published (Renninger et al. 1995; Chamberlain et al. 1996; Jinks et al. 1998), providing the
85 first, brief, description of the sensilla on the antennules and antennae of this species. These
86 authors also reported preliminary behavioural observations, suggesting an attraction to
87 sulfide, and registered electrophysiological responses to sulfide in antennal filaments (but
88 surprisingly not in the antennular lateral ones bearing aesthetascs).

89 Here, we present a comparative morphological description of antennae and antennules of four
90 hydrothermal vent shrimp (*Rimicaris exoculata*, *Mirocaris fortunata*, *Chorocaris chacei* and
91 *Alvinocaris markensis*). We also identified partial sequences of the candidate co-receptor
92 IR25a and studied its expression pattern in the different species. All the approaches were
93 conducted in parallel on a closely related coastal shrimp (*Palaemon elegans*), to give insights
94 in the potential adaptations of sensory organs in deep-sea species. Comparisons within
95 hydrothermal species were also conducted to examine possible specific adaptations related to
96 their different environments and lifestyles, as previous studies showed that chemical senses of
97 crustaceans rapidly evolve and present specialized adaptations according to phylogeny,
98 lifestyle and habitat, as well as to trophic levels (Beltz et al. 2003; Derby and Weissburg
99 2014). Knowledge of the sensory capabilities of hydrothermal species is especially relevant
100 with the growing interest of mining companies for extraction of seafloor massive sulfides

101 hydrothermal deposits (Hoagland et al. 2010). Possible impacts of sulfide exploitation on vent
102 species encompass habitat destruction, increase of suspended particles and the presence of
103 higher levels of toxic elements, leading to physiological disturbances and to potential
104 alteration of their ability to perceive their environment (Lahman and Moore 2015) and detect
105 hydrothermal emissions.

106

107 **Materials and methods**

108 Choice of models

109 Shrimp are one of the dominant macrofaunal taxa of hydrothermal sites in the Mid-Atlantic
110 Ridge (Desbruyères et al. 2000, 2001). They are highly motile, and according to species,
111 occupy different habitats, exhibit different food diets, and show various degrees of association
112 with bacteria. Therefore they provide good models for studying olfactory capabilities since
113 individuals belonging to different species are potentially not sensitive to the same attractants.
114 *Rimicaris exoculata* (Williams and Rona 1986) lives in dense swarms (up to 2500 ind. m⁻²,
115 Desbruyères et al. 2001) on the chimney walls, at around 20-30°C, near the fluid emissions in
116 order to feed their dense symbiotic chemoautotrophic bacterial community (Van Dover et al.
117 1988; Zbinden et al. 2004, 2008). *Chorocaris chacei* (Williams and Rona 1986) is much less
118 abundant (locally 2-3 ind.dm⁻²) than *Rimicaris exoculata*, but may live close to it. It is also
119 found as on sulfide blocks, in areas of weak fluid emissions (Desbruyères et al. 2006, Husson
120 et al. 2016). *Chorocaris* also harbors a bacterial symbiotic community, though less
121 developed than in *Rimicaris* (Segonzac 1992). *Mirocaris fortunata* (Martin and Christiansen
122 1995) lives at lower temperature (4.8- 6.1°C, Husson et al. 2016), in diffuse flow habitats and
123 among *Bathymodiolus* mussel assemblages (Sarrazin et al. 2015). *Mirocaris* is opportunistic
124 and feeds on mussel tissue, shrimp and other invertebrates, being reported as predators and/or
125 scavengers (Gebruk et al. 2000; De Busserolles et al. 2009). *Alvinocaris markensis* (Williams

126 1988) occurs as solitary individuals, at the base of and on the walls of active edifices, close to
127 *Rimicaris exoculata* aggregates, and also on mussel assemblages. It has been reported as
128 necrophagous (Desbruyères et al. 2006), but also as a predator (Segonzac 1992).
129 In order to identify potential adaptations of hydrothermal shrimp sensory faculties,
130 comparisons were made with the related shallow-water palaemonid species *Palaemon elegans*
131 (Rathke 1837). The description of palaemonid antennal structures is also interesting *per se*
132 since olfaction is poorly analyzed in shrimp in general. Two additional palaemonid species,
133 *Palaemon serratus* (Pennant 1777) and *Palaemonetes varians* (Leach 1813), were used for
134 identifying the IR25a sequence.

135

136 Animal collection, conditioning and maintenance

137 Specimens of Alvinocarididae *Mirocaris fortunata*, *Rimicaris exoculata*, *Chorocaris chacei*
138 and *Alvinocaris markensis* were collected during the Momarsat 2011 and 2012, Biobaz 2013
139 and Bicose 2014 cruises, on the Mid-Atlantic Ridge (see Table 1 for cruises and sites).

140 Shrimp were collected with the suction sampler of the ROV ‘Victor 6000’ operating from the
141 RV ‘Pourquoi Pas?’. Immediately after retrieval, living specimens were dissected and tissues
142 of interest (see below) were fixed in a 2.5% glutaraldehyde/seawater solution for
143 morphological observations or frozen in liquid nitrogen for molecular biology experiments.

144 Specimens of Palaemonidae *Palaemon elegans*, *Palaemon serratus*, and *Palaemonetes*
145 *variens* were collected from Saint-Malo region (France ; 48°64’N, -2°00’W), between October
146 2011 and January 2015, using a shrimp hand net. They were transported to the laboratory and
147 transferred to aerated aquaria with a 12 h:12 h light:dark cycle, a salinity of 35 g.l⁻¹, and a
148 water temperature of 18°C. The shrimp were regularly fed with granules (JBL Novo Prawn).
149 Tissues of interest were also fixed in a 2.5% glutaraldehyde/seawater solution for
150 morphological observations or frozen in liquid nitrogen for molecular biology experiments.

151

152 Tissue collection

153 For morphological observations, antennae and antennules (both medial and lateral flagella)

154 were used. For molecular biology experiments, the following organs were dissected for *P.*

155 *elegans*: the antennular medial and lateral flagella (internal and external ramus separated), the

156 antennae, the mouthparts (mandibles and two pairs of maxillae), the first and second walking

157 legs and the eyestalks. For the hydrothermal shrimp, the dissection included the following

158 organs: the antennular medial and lateral flagella, the antennae, and abdominal muscles.

159

160 Scanning Electron Microscopy (SEM)

161 Samples were post-fixed in osmium tetroxide 1% once in the lab and dehydrated through an

162 ethanol series. They were then critical-point-dried (CPD7501, Quorum Technologies,

163 Laughton, UK) and platinum-coated in a Scancoat six Edwards sputter-unit prior to

164 observation in a scanning electron microscope (Cambridge Stereoscan 260), operating at 20

165 kV.

166

167 RNA extraction and reverse transcription

168 Frozen shrimp tissues were ground in TRIzol™ Reagent (Thermo Fisher Scientific, Waltham,

169 MA, USA) with a Minilys® homogenizer (Bertin Corp., Rockville, MD, USA). Total RNA

170 was isolated according to the manufacturer's protocol, and quantified by spectrophotometry

171 and electrophoresis in a 1.2% agarose gel under denaturing conditions. RNA (500 ng) was

172 DNase treated to remove contamination using the TURBO™ DNase kit (Thermo Fisher

173 Scientific) and then reverse transcribed to cDNA with the Superscript II reverse transcriptase

174 kit (Thermo Fisher Scientific) using a oligo(dT)₁₈ primer according to the manufacturer's

175 instructions.

176

177 IR25a sequencing and mRNA expression (RT- PCR)

178 The cDNA fragments encoding IR25a were amplified by two rounds of PCR. Oligonucleotide

179 primers were designed from a multiple-sequence alignment of IR25a sequences of

180 crustaceans (*Daphnia pulex*, Croset et al. 2010; *Homarus americanus* AY098942, Hollins et

181 al. 2003, *Lepeophtheirus salmonis* PRJNA280127 genome sequencing project), insects

182 (*Acyrtosiphon pisum*, *Aedes aegypti*, *Anopheles gambiae*, *Apis mellifera*, *Bombyx mori*,

183 *Culex quinquefasciatus*, *Drosophila melanogaster*, *Nasonia vitripennis*, *Pediculus humanus*,

184 *Tribolium castaneum*, Croset et al. 2010), gastropod molluscs (*Aplysia californica*, *Lottia*

185 *gigantea*, Croset et al. 2010), nematods (*Caenorhabditis briggsae* XM_002643827, Stein et

186 al. 2003, *Caenorhabditis elegans* NM_076040, The *C. elegans* Sequencing Consortium) and

187 an annelid (*Capitella capitata*, Croset et al. 2010) (primer sequences are listed in Table S1).

188 PCR amplification reactions were performed in a 20 μ l volume containing 1 μ l of cDNA

189 template, 2 μ l of each primer [10 μ M], 11.7 μ l of H₂O, 2 μ l of PCR buffer [10x], 0.8 μ l of

190 MgCl₂ [50 mM], 0.4 μ l of dNTP [10 mM] and 0.1 μ l of BIOTAQ™ polymerase [5 U/ μ l]

191 (Eurobio AbCys, Les Ulis, France). The thermal profile consisted of an initial denaturation

192 (94 °C, 3 min), followed by 35 cycles of denaturation (94°C, 30 s), annealing (45 to 55°C, 45

193 s) and extension (72°C, 2 min), and a final extension (72°C, 10 min) step. The PCR products

194 were separated on a 1.5% agarose gel, purified with the GeneClean® kit (MP Biomedicals,

195 Illkirch, France), and cloned into a pBluescript KS plasmid vector using the T4 DNA ligase

196 (Thermo Fisher Scientific). The ligation product was introduced in competent *E. coli* cells

197 (DH5alpha) that were cultured at 37°C overnight. The clone screening was performed through

198 PstI/HindIII (Thermo Fisher Scientific) digestion of plasmid DNA after plasmid extraction.

199 Positive clones were sequenced on both strands (GATC Biotech, Konstanz, Germany). The

200 resulting nucleotide sequences were deposited in the GenBank database under the accession

201 numbers KU726988 (*M. fortunata* IR25a; consensus sequence from 6 clones), KU726987 (*R.*
202 *exoculata* IR25a; consensus sequence from 3 clones), KU726989 (*C. chacei* IR25a; consensus
203 sequence from 4 clones), KU726990 (*A. markensis* IR25a; consensus sequence from 4
204 clones), KU726984 (*P. elegans* IR25a; consensus sequence from 11 clones), KU726985 (*P.*
205 *varians* IR25a; consensus sequence from 12 clones) and KU726986 (*P. serratus* IR25a;
206 consensus sequence from 3 clones). Specific primers were further designed to amplify IR25a
207 sequences in diverse tissues of the four alvinocaridid species and the palaemonid *P. elegans*
208 (Table S1). PCR amplifications were performed using BIOTAQ™ polymerase (Eurobio,
209 AbCys) in a thermocycler (Eppendorf, Hamburg, Germany) with the following program:
210 94°C for 3 min, 35 cycles of (94°C for 30 s, 55°C for 45 s, 72°C for 2 min), and 72°C for 10
211 min, with minor modifications of annealing temperature for different primer pairs.

212

213 Sequence analyses

214 A dataset of IR amino acid sequences was created, including the IR25a sequences identified
215 in shrimp (present study), in other decapods (*Panulirus argus*, Corey et al. 2013; *Coenobita*
216 *clypeatus*, Groh-Lunow et al. 2015; *Homarus americanus* AY098942, Hollins et al. 2003) and
217 in other crustaceans (*Daphnia pulex*, Croset et al. 2010; *Lepeophtheirus salmonis*
218 PRJNA280127) together with IR sequences from the insects *Bombyx mori*, *Drosophila*
219 *melanogaster*, *Apis mellifera* and *Tribolium castaneum* (Croset et al. 2010). *D. melanogaster*
220 ionotropic glutamate receptor sequences were also included to serve as an out-group, and the
221 final data set contained 173 sequences. These amino acid sequences were aligned with
222 MAFFT v.6 (Kato and Toh 2010) using the FFT-NS-2 algorithm and default parameters.
223 The alignment was then manually curated to remove highly divergent regions (500 amino
224 acid positions conserved in the final dataset). The phylogenetic reconstruction was carried out
225 using maximum-likelihood. The LG+I+G+F substitution model (Le and Gascuel 2008) was

226 determined as the best-fit model of protein evolution by ProtTest 1.3 (Abascal et al. 2005)
227 following Akaike information criterion. Rate heterogeneity was set at four categories, and the
228 gamma distribution parameter was estimated from the data set. Tree reconstruction was
229 performed using PhyML 3.0 (Guindon et al. 2010), with both SPR (Subtree Pruning and
230 Regrafting) and NNI (Nearest Neighbour Interchange) methods for tree topology
231 improvement. Branch support was estimated by approximate likelihood-ratio test (aLRT)
232 (Anisimova et al. 2006). Images were created using the iTOL web server (Letunic and Bork
233 2011).

234

235 **Results**

236

237 **Morphology of the chemosensory organs: description and distribution of setal types on** 238 **the antennae and antennules**

239 In the five shrimp species studied for morphology (*Palaemon elegans*, *Mirocaris fortunata*,
240 *Rimicaris exoculata*, *Chorocaris chacei* and *Alvinocaris markensis*), antennae and antennules
241 both consist of a peduncle and segmented flagella (one for the antennae and two for the
242 antennules: an outer or lateral, and an inner or medial). In the three flagella, the diameter and
243 length of the annuli vary, being large and short at the base and becoming thinner and longer
244 towards the apex. The aesthetasc dimensions vary also along the flagella, being thinner and
245 shorter at the base and growing toward the apex. The set of values (maximum, minimum,
246 mean and standard deviation of diameter and length) for aesthetasc, as well as for non-
247 aesthetasc sensilla, are given in Table S2.

248

249 *Palaemon elegans*

250 The antennules are made of 3 basal annuli and two distal flagella. The lateral flagella are
251 divided in two rami after a short fused basal part: a long external one and a shorter internal
252 one (1/3 of the long one, $n = 12$, $s.d. = 0.61$) (Figure 1A). The aesthetascs are localized
253 ventrally, in a furrow on the shorter ramus (Figure 1B). They are present from the basal fused
254 part of the antennules to the apex of the short ramus (except from the last two annuli, Figure
255 1C). Two rows of 5 to 6 aesthetascs occur on each annulus (one row at the distal part of the
256 annulus and the other at the middle part) (Figure 1B). The 2 or 3 basal and apical annuli have
257 a smaller number of aesthetascs, giving a total number of approximately 140 aesthetascs per
258 ramus (Table 2). Aesthetascs are up to $20.3 \mu\text{m}$ in diameter ($n = 14$) and $393 \mu\text{m}$ in length (n
259 $= 10$) (Table S2). They bear annulation throughout their length (short at the base and longer
260 towards the apex), and lack a terminal pore.

261 Non-aesthetasc setae are also present on all the annuli of the three flagella (antennae and
262 antennules), where they are distributed (up to 8) around the distal part of each annulus (Figure
263 1D). Five setal types are observed on the flagella, named after their morphology (dimensions
264 are given in Table 2): 1) short simple seta (Figure 1E), 2) long simple seta (Figure 1D), 3)
265 beaked scaly seta (Figure 1F) 4) twisted flat seta (Figure 1G) and 5) bifid seta (Figure 1H).
266 All these 5 types appear to have a terminal pore. Short simple, beaked scaly and twisted flat
267 setae are present on the antennae, the medial flagella of the antennules and the long ramus of
268 the lateral flagella of the antennules. They occur as tufts of 5 setae, containing 3 simple short,
269 one twisted flat and one beaked scaly seta (Figure 1E). These tufts are present on each
270 annulus near the base but are spaced further apart towards the apex. The bifid setae are found
271 only on the 2 flagella of the antennules, whereas the long simple are only found on medial
272 flagella of the antennules (two every 5 annuli, on each side of the flagellum). Small round
273 cuticular depressions ($5,5$ to $6,7 \mu\text{m}$ in diameter) are observed on the medial side of the short
274 ramus of the lateral flagella of the antennules, as well as on the antennae (insert in Figure 1C).

275

276 *Mirocaris fortunata*

277 In *M. fortunata*, as well as in the 3 other hydrothermal species, the antennules are also made
278 of 3 basal annuli and two distal flagella (lateral and medial) (Figure 2A). In *M. fortunata*, the
279 aesthetascs are localized latero-ventrally on the inner side of the lateral flagella, from the base
280 to 2/3 of the flagella. One row of 3 to 4 aesthetascs occurs on the distal part of each annulus
281 (Figure 2B), leading to a total number of approximately 60 aesthetascs per ramus (Table 2).
282 Aesthetascs are up to 18.3 μm in diameter ($n = 21$) and 290.3 μm in length ($n = 46$) (Table
283 S2). They bear annulation on the apical half, and lack a terminal pore.

284 The rows of aesthetascs are flanked on the inner side by non-aesthetasc setae, organised as
285 follows: one intermediate seta (thinner and shorter than the aesthetascs) and 2 or 3 short thin
286 setae (thinner and shorter than the former) (Figure 2B). The intermediate setae have a peculiar
287 apex shape with no obviously visible pore (Figure 2D), whereas the short setae are simple
288 with a clearly visible pore at the apex (Figure 2E).

289 Intermediate and short simple setae also occur along with a sparse third type of non-
290 aesthetasc setae (Figure 2F) on the 2 other flagella (medial flagella of the antennules and the
291 antennae), distributed around the distal part of each annulus (about 10 over the entire
292 circumference by extrapolation of what is seen on one face). Small round cuticular
293 depressions (7 to 10 μm in diameter) are observed on the lateral flagella of the antennules, on
294 the medial side of the aesthetascs (Figure 2B). Flagella are often densely covered by a thick
295 bacterial layer of filamentous and rod-shaped bacteria (Figure 2C), which was never observed
296 on *Palaemon elegans*. Rod-shaped bacteria also sometimes covered the entire aesthetasc
297 surface (not shown).

298

299 *Rimicaris exoculata*

300 The aesthetascs are localized laterally on the medial side of the lateral flagella, from the base
301 (except the 2 or 3 first annuli) up to the apex (except for the 4 last annuli). One row of 3 to 4
302 aesthetascs occurs on the distal part of each annulus (Figure 3A), leading to a total number of
303 approximately 108 aesthetascs per ramus. Aesthetascs are up to 22 μm in diameter ($n = 22$)
304 and 191 μm in length ($n = 26$) (Table S2). They bear annulation on the apical half, and lack a
305 terminal pore.

306 The arrangement pattern of the non-aesthetasc setae around the aesthetascs is quite similar to
307 that observed in *M. fortunata*, but with different setal types: one long thick beaked seta, one
308 intermediate beaked seta and 6 or 7 short thin beaked setae (Figure 3B). All these setae have a
309 pore at the apex (Figure 3C), but they are devoid of scales unlike the beaked setae observed in
310 *Palaemon elegans*.

311 Long thick, intermediate and short thin beaked setae also occur on the outer side of the lateral
312 flagella, on the medial flagella of the antennules, and on the antennae, distributed over the
313 circumference (20-25 over the entire circumference by extrapolation of setae seen on one
314 face, or counted on the periphery of the apex), with a tight tuft of 6-8 setae on the inner side.

315 Small round cuticular depressions were (rarely) observed (6 to 8 μm in diameter) in *R.*
316 *exoculata*, but they are barely observable due to a dense rod-shaped bacterial coverage.

317 Indeed, for this species too, we have observed that the flagella (even the aesthetascs) can be
318 covered by layer of filamentous and rod-shaped bacteria (not shown).

319

320 *Chorocaris chacei*

321 The aesthetascs are localized laterally on the medial side of the lateral flagella, from the base
322 (except the 4 or 5 first annuli) to 2/3 of the flagella. One row of 2 to 4 aesthetascs occurs on
323 the distal part of each annulus (Figure 3D), leading to a total number of approximately 113

324 aesthetascs per ramus. Aesthetascs are up to 23.2 μm in diameter ($n = 50$) and 339.5 μm in
325 length ($n = 58$) (Table S2). They bear annulation on the apical half, and lack a terminal pore.
326 The arrangement pattern of the non-aesthetasc setae around the aesthetascs is also quite
327 similar to that observed in *M. fortunata* with one intermediate beaked seta, and 1 to 3 short
328 simple or beaked thin setae on both the medial and lateral sides (Figure 3E-F).
329 Intermediate beaked and short setae (either simple or beaked shaped) also occur on the medial
330 flagella of the antennules, and on the antennae, distributed over the circumference, roughly
331 equidistant (around 15 over the entire circumference by extrapolation of setae seen on one
332 face, or counted on the periphery of the apex), with a tight tuft of 8 to 10 setae on the inner
333 side.
334 Small cuticular depressions (5 to 5.5 μm in diameter) are observed on the lateral flagella of
335 the antennules, on the medial side of the aesthetascs but are difficult to observe as they are
336 covered by rod-shaped bacteria. For this species again, the flagella (and even the aesthetascs)
337 can be covered by filamentous and rod-shaped bacteria (not shown).

338

339 *Alvinocaris markensis*

340 The aesthetascs are localized laterally on the medial side of the lateral flagella, from the base
341 (except the 3 or 4 first annuli) up to half of the flagella. One row of 3 to 4 aesthetascs (rarely
342 5) occurs on the distal part of each annulus (Figure 3G), leading to a total number of
343 approximately 110 aesthetascs per ramus. Aesthetascs are up to 25.2 μm in diameter ($n = 39$)
344 and 879.1 μm in length ($n = 49$) (Table S2). They bear annulation almost throughout their
345 length (short at the base and longer towards the apex), and lack a terminal pore.
346 The arrangement pattern of the non-aesthetasc setae around the aesthetascs is quite similar to
347 that observed in *M. fortunata* with one intermediate seta and 1 short thin seta (Figure 3H).
348 Two (sometimes 3 or 4) short setae occur at mid-length of each annulus. Intermediate and

349 short thin setae all seem to all be simple, with a pore (Figure 3I). They also occur on the
350 medial flagella of the antennules and on the antennae, in fewer numbers than observed in the
351 other species (4-6 over the entire circumference, mostly on the medial side). Long simple
352 setae also occur on few basal annuli on the medial flagella of the antennules and of the
353 antennae.

354 Small cuticular depressions (4.5 to 7.5 μm diameter) were also observed in *A. markensis*, on
355 the lateral flagella of the antennules, on the distal part of the annuli, occurring by one, 2 or
356 sometimes 3, which had not been observed in other species (not shown). They are also
357 observed on the antennae. Only a few rod-shaped bacteria occurred on the two specimens
358 observed.

359

360 **Identification and expression of the putative olfactory co-receptor IR25a in** 361 **hydrothermal vent and coastal shrimp**

362 In order to identify the regions of antennules and antennae putatively involved in olfaction,
363 we studied the expression pattern of the ionotropic receptor IR25a, which belongs to a
364 conserved family of olfactory receptors amongst Protostomia (review in Croset et al. 2010),
365 involved in olfaction, taste, thermosensation and hygrosensation. Recently the homologue of
366 IR25a was identified in the lobster, and had been associated with olfactory sensilla (Corey et
367 al. 2013). Using homology-based PCR with primers designed from the alignment of IR25a
368 sequences from diverse organisms, we obtained partial sequences for seven species of shrimp:
369 903 bp for *R. exoculata*, *P. elegans* and *P. varians*, 763 bp for *M. fortunata*, *C. chacei* and *A.*
370 *markensis*, and 881 bp for *P. serratus* (Figure 4A, B). A phylogenetic analysis confirmed that
371 these sequences are IR25a orthologs (Figure 5). All shrimp sequences grouped with IR25a
372 sequences from other arthropods, and were closely related to IR25a sequences from the
373 decapod crustaceans *Panulirus argus* (Corey et al. 2013), *Homarus americanus* (Hollins et al.

374 2003) and *Coenobita clypeatus* (Groh-Lunow et al. 2015). The Palaemonidae and
375 Alvinocarididae sequences formed distinct clusters within the shrimp sequences, therefore
376 being congruent with the phylogeny of these groups (Figure 6). The IR25a partial amino acid
377 sequences obtained in this study are about 250 to 300 amino acids in length, which represents
378 25 to 30% of the total length expected for such sequences (Figure 4). They include the ligand-
379 gated ion channel and the ligand-binding S2 domain, localized in the C-terminal part of the
380 protein. When considering the ligand-binding S2 domain, the threonine and aspartate, which
381 are characteristic glutamate binding residues, are conserved among shrimp sequences.
382 Then, we studied the expression pattern of IR25a in antennules, antennae, mouthparts and
383 walking legs, as well as in non-chemosensory tissues (abdominal muscles, eye), from the four
384 hydrothermal vent shrimp and the coastal shrimp *P. elegans* (Figure 7). IR25a was
385 predominantly expressed in the lateral antennular flagella (A1 lateral) for all shrimp. In *P.*
386 *elegans*, a weaker expression was observed in the external ramus (A1 lateral R2) than in the
387 internal ramus of the lateral antennular flagella (A1 lateral R1), which bear the aesthetascs. A
388 weak expression was also detected in the medial antennular flagella of *R. exoculata* and *C.*
389 *chacei* (A1 medial), and in the antennae (A2) of *R. exoculata*. IR25a transcripts were
390 undetectable in other tissues.

391

392 **Discussion**

393 **Comparative morphology of sensilla of antennae and antennules among decapods, and** 394 **in coastal palaemonid vs. hydrothermal alvinocarid shrimp**

395 Setae are outgrowths of the arthropod integument presenting a multitude of sizes and shapes.
396 These ubiquitous features of crustacean integuments are involved in a variety of vital
397 functions including locomotion, feeding, sensory perception and grooming (Felgenhauer
398 1992). Sensilla (setae innervated by sensory cells) were shown to present a great inter- and

399 intra-specific diversity in crustaceans (see references in the paragraphs below).

400 In the most studied « large » decapods like lobsters and crayfish, the aesthetascs are localized
401 in tufts on the distal half or two-thirds of the ventral side of each lateral antennular flagellum
402 (*Panulirus argus*, Cate and Derby 2001; *Homarus americanus*, Guenther and Atema 1998;
403 *Orconectes sanborni*, MacCall and Mead 2008; *O. propinquus*, Tierney et al. 1986;
404 *Procambarus clarkii*, Mellon 2012). The localisation at the tip of the antennules may increase
405 the spatial resolution of the chemical environment, but could also increase their chance of
406 damage during encounters with the environment or other animals. On the contrary, in shrimp
407 (the 4 alvinocaridid species and *P. elegans* (this study), as well as other palaemonid species
408 like *P. serratus* and *Macrobrachium rosenbergii* (Hallberg et al. 1992)), the aesthetascs are
409 localized on the basal half or two-thirds of the lateral flagella (for the alvinocarididae) or on
410 the basal part of the short ramus of the lateral flagella (for the palaemonidae). The aesthetascs
411 are thus less likely to be lost or damaged, but this arrangement may decrease spatial
412 resolution.

413 The aesthetascs are usually organised in two successive rows (in the different lobsters and
414 crayfishes cited above and also in *Lysmata* shrimp, Zhang et al. 2008) or in two juxtaposed
415 rows in the short antennules of the crab *Carcinus maenas* (Fontaine et al. 1982). Surprisingly,
416 there is only one row of aesthetascs on each annulus in the 4 hydrothermal species (an
417 exception also occurs in the crayfish *Cherax destructor*, see Table 2). Nevertheless,
418 comparisons of the total number of aesthetascs in diverse decapod species (Table 2) revealed
419 that this number is relatively similar among shrimp group and other decapods of comparable
420 size (the crayfish *Orconectes propinquus* or the crab *Carcinus maenas*) (Table 2 and see Beltz
421 et al. 2003 for more comprehensive data). Hydrothermal shrimp do not seem to present any
422 specific adaptation regarding this character. The total number, as well as the size of
423 aesthetascs seems related to the size of the animal rather than to its environment. Indeed,

424 based on a study of 17 Reptentia decapods, Beltz et al. (2003) found a strong linear
425 relationship between the number of aesthetascs and carapace length, which was also reported
426 earlier for the crayfish *Cherax destructor* by Sandeman and Sandeman (1996). Among
427 hydrothermal species, it can however be noted that the aesthetascs of *Alvinocaris markensis*
428 are longer than those of the three other species, with the maximum length being 2 to 4 times
429 higher than for the 3 other species (see Table S2). The adult hydrothermal shrimp lack the
430 usual externally differentiated eye (eye-stalked), having instead a pair of large, highly
431 reflective, dorsal organs (Van Dover et al. 1989). These modifications have been reported to
432 be an adaptation for the detection of extremely faint sources of light emitted by the vents
433 (Pelli and Chamberlain 1989). These eyes are unusual in having no image-forming optics, but
434 a solid wall of light-sensitive rhabdom containing rhodopsin, with the exception of *A.*
435 *markensis*, which also lacks this photoreceptor and is completely blind (Wharton et al. 1997;
436 Gaten et al. 1998). The longer olfactory sensilla observed in this species may possibly be
437 interpreted as a development of the olfactory capacity to compensate for the lack of vision.
438 Zhang et al. (2008) showed for *Lysmata* species that shrimp living in aggregations (*L.*
439 *boggessi* and *L. wurdemanni*, 460 aesthetascs) possess a significantly higher number of
440 aesthetascs than pair-living species (*L. amboinensis* and *L. debelius*, 210 aesthetascs),
441 suggesting a possible correlation between the number of aesthetascs and the social behaviour.
442 Our results do not support this hypothesis, since no significant differences were observed
443 between vent species living in dense swarms (*R. exoculata*) and the others.
444 Most studies on olfaction in crustaceans have focused on aesthetascs. Several lines of
445 evidence however suggest that non-aesthetasc bimodal chemosensilla (innervated by mecano-
446 and chemo-receptive cells, also called distributed chemosensilla (Schmidt and Mellon 2011)
447 or non-olfactory sensilla (Derby and Weissburg 2014), distributed over both flagella of the
448 antennules, as well as on the antennae, also play a role in the detection of water-borne

449 chemicals (Cate and Derby 2001; Guenther and Atema 1998). Non-aesthetasc setae exhibit a
450 wide variety of sizes and morphologies. These setae are named in the literature according to
451 their morphology, size or location on the flagellum. For example, there are 9 setal types in
452 *Panulirus argus* (hooded, plumose, short setuled, long simple, medium simple, short simple,
453 guard, companion, and asymmetric: Cate and Derby 2001), but only 1 type in the shrimp *Thor*
454 *manningi* (curved simple: Bauer and Caskey 2006). The role of these setae is still poorly
455 known and whether their diversity corresponds to a multiplicity of perceived stimuli remains
456 an open question (Derby and Steullet 2001; Cate and Derby 2001). Among the shrimp studied
457 here, the coastal shrimp *P. elegans* showed the highest diversity in non-aesthetasc setal types
458 (5 setal types: short simple, long simple, beaked scaly, twisted flat, bifid) when compared
459 with the 4 hydrothermal species (2 or 3 types). Among hydrothermal species, the setal types
460 vary essentially by their size (long, intermediate or short) and less by their morphology (all
461 simple in *Alvinocaris*, all beaked in *Rimicaris*, a mix of the two in *Chorocaris*, while
462 *Mirocaris* exhibit more original morphologies (see Figures 2D and 2F)). At this point of our
463 knowledge, it is difficult to explain the observed differences and even more to speculate on
464 the functions of these different setae.

465 Surprisingly, dense bacterial populations were often observed on the antennae and antennules
466 of the 4 hydrothermal shrimp (see for example *Mirocaris*, Figure 2C), sometimes even
467 covering the whole surface of aesthetacs (not shown), whereas no bacterial coverage was ever
468 observed in the coastal *P. elegans* specimens. The type of bacteria present on the antennae of
469 hydrothermal shrimp, as well as their potential impact on olfaction or other role for the
470 shrimp should be investigated in future studies.

471

472 **Comparative expression of the putative olfactory co-receptor IR25a in hydrothermal**
473 **vent and coastal shrimp**

474 We identified, in the four alvinocaridid hydrothermal shrimp and in three palaemonid
475 species (*P. elegans*, *P. varians* and *P. serratus*), a member of the Ionotropic Receptor (IR)
476 family, which was recently proposed to be involved in the odorant detection in crustaceans:
477 the common IR25a subunit (Corey et al. 2013). In the five shrimp species tested, IR25a was
478 predominantly expressed in the lateral antennular flagella that bear the aesthetascs olfactory
479 sensilla (Figure 7), consistent with the expression pattern of this IR subunit in *Homarus*
480 *americanus* (iGluR1, Stepanyan et al. 2004), *Panulirus argus* (Corey et al. 2013) and
481 *Coenobita clypeatus* (Groh-Lunow et al. 2015). IR25a expression in other chemosensory
482 tissues than the lateral antennular flagella varies amongst decapod crustacean species, with
483 either no detection (for *M. fortunata*, *A. markensis*, *P. elegans*: this study; for *H. americanus*:
484 Stepanyan et al. 2004), or detection in different organs (medial antennular flagella in *R.*
485 *exoculata* and *C. chacei*: this study; mouth and two first walking legs in *P. argus*: Corey et al.
486 2013). Taken together, these results raise the question of whether IR25a may play a more
487 general role in decapod crustacean chemosensation beyond just mediating odor detection
488 (Corey et al. 2013), or if organs other than the aesthetascs bearing flagella can also have an
489 olfactory role, as Keller et al. (2003) suggested for the antennae and walking legs of the blue
490 crab *Callinectes sapidus*. According to several recent studies and reviews (Schmidt and
491 Mellon 2011, Mellon 2014; Derby and Weissburg 2014, Derby et al. 2016), only the
492 aesthetascs are considered as olfactory sensilla, which rather plead for the first hypothesis.

493 Among hydrothermal species, the different patterns of IR25a expression obtained for *R.*
494 *exoculata* and *C. chacei* on one hand and for *M. fortunata* and *A. markensis* on the other hand,
495 would suggest different chemosensory mechanisms in these two shrimp groups. This may be
496 related to their diet and thus to their direct dependence to the hydrothermal fluid. Indeed,
497 *Rimicaris* and *Chorocaris* to a lesser extent live in symbiosis with chemoautotrophic bacteria
498 from which they derive all or part of their food (Segonzac et al. 1993; Ponsard et al. 2014),

499 forcing them to stay permanently close to hydrothermal emissions to supply their bacteria in
500 reduced compounds necessary for chemosynthesis. These two species are also
501 phylogenetically closely related, which recently led Vereshchaka et al. (2015) to propose to
502 synonymize all the genus *Chorocaris* with *Rimicaris*. On the other hand, *Mirocaris* and
503 *Alvinocaris* are secondary consumers, scavenging on local organic matter and living at greater
504 distances from the vent emissions. Regarding the IR25a expression pattern, the coastal shrimp
505 *P. elegans* has a profile similar to hydrothermal secondary consumers *Mirocaris* and
506 *Alvinocaris*, itself having an opportunistic omnivorous diet of invertebrate tissues.

507

508 In future studies, we will attempt to identify, and subsequently localize, other receptors of the
509 IR family that could be involved in olfaction, and in particular the members generally found
510 associated with IR25a (like IR93a and IR8a). We recently developed an electrophysiological
511 method that allows the recording of shrimp olfactory receptor neurons (ORNs) activity
512 (Machon et al., 2016). This method will be used to conduct a comparative study of the global
513 antennule activity upon exposure to environmental stimuli, in the hydrothermal species *M.*
514 *fortunata* and the coastal species *P. elegans*. An ultrastructural approach could help to refine
515 the morphological comparison between hydrothermal and coastal species, by analyzing other
516 characteristics like the number of ORNs per aesthetascs, the number of outer dendritic
517 segments per ORNs or the aesthetasc cuticle thickness. This combined morphological and
518 functional approach will provide insights into deep-sea vent shrimp olfaction, and ultimately
519 in the potential adaptations of the sensory organs to their peculiar environment.

520

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524

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530

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720

721

722 **Figure legends**

723 **Fig. 1: Morphology of antennules and setal types of *Palaemon elegans*.** (A) Antennules are

724 made of 3 basal annuli (bs) and two flagella: a medial (mf) and a lateral one (lf), which is
725 divided in two rami: a long (outer) and a short (inner), bearing the aesthetascs (as). (B) Close-
726 up on the ventral side of the furrow on the shorter ramus of the lateral flagellum bearing the
727 aesthetascs. (C) Apex of the shorter ramus, showing the absence of aesthetascs on the last two
728 annuli and the occurrence of small cuticular depressions (d), enlarged in insert. (D) Medial
729 antennular flagellum showing the long simple seta (ls). (E) Tuft of 3 simple short (ss), one
730 twisted flat (tf) and one beaked scaly (b) setae. (F) Beaked scaly seta. (G) Twisted flat seta.
731 (H) Bifid seta. Scale bars : A = 1 mm ; B, C, D = 100 μm ; E = 10 μm ; F, G, H = 2 μm . Scale
732 bar in insert in C = 5 μm .

733

734 **Fig. 2: Morphology of antennule and setal types of *Mirocaris fortunata*.** (A) Antennules
735 are made of 3 basal annuli (bs) and two flagella: a medial (mf) and a lateral one (lf), bearing
736 the aesthetascs (as). Box: area enlarged in B. (B) Close-up on the lateral flagellum bearing the
737 aesthetascs, and intermediate (i) and short thin setae (st). (C) Lateral flagellum covered by
738 dense filamentous and rod-shaped bacteria. Some setae are visible, protruding from the layer
739 of bacteria (arrows). (D) Apex of the intermediate simple setae. (E) Short setae are simple
740 with a clear pore at the apex. (F) Third setal type. Scale bars: A = 1 mm ; B = 50 μm ; C =
741 100 μm ; D, E, F = 1 μm

742

743 **Fig. 3: Morphology of lateral flagella and setal types of *Rimicaris exoculata* (A, B, C),**
744 ***Chorocaris chacei* (D, E, F) and *Alvinocaris markensis* (G, H, I).** as: aesthetascs, lt: long
745 thick seta, i: intermediate seta, st: short thin seta, Scale bars: A, D, G = 500 μm ; B, E, H =
746 100 μm ; C, F, I = 2 μm

747

748 **Fig. 4: IR25a partial sequences obtained for hydrothermal and coastal shrimp.** (A)
749 IR25a protein domain organization (modified from Croset et al. 2010) showing the position of
750 the shrimp partial sequences obtained in the present study. The ligand-binding domains are
751 named S1 and S2. (B) Alignment of shrimp IR25a sequences. The ligand-binding S2 domain
752 is underlined, and putative ligand-binding residues are indicated by an asterisk.

753

754 **Fig. 5: Phylogeny of insect and crustacean ionotropic receptors (IRs).** This tree is based
755 on a maximum-likelihood analysis of an amino acid dataset. *D. melanogaster* ionotropic
756 glutamate receptor sequences were used as an out-group. Branch support was estimated by
757 approximate likelihood-ratio test (aLRT) (circles: >0.9). The scale bar corresponds to the
758 expected number of amino acid substitutions per site. Crustacean IRs are in bold and the new
759 IRs identified in this study are in larger font size, and highlighted with an asterisk. Amar,
760 *Alvinocaris markensis* ; Amel, *Apis mellifera*; Bmor, *Bombyx mori*; Ccha, *Chorocaris chacei* ;
761 Ccly, *Coenobitus clypeatus*; Dmel, *Drosophila melanogaster*; Dpul, *Daphnia pulex* ; Hame,
762 *Homarus americanus* ; Lsal, *Lepeophtheirus salmonis* ; Mfor, *Mirocaris fortunata* ; Parg,
763 *Panulirus argus* ; Pele, *Palaemon elegans* ; Pser, *Palaemon serratus* ; Pvari, *Palaemon*
764 *varians* ; Rexo, *Rimicaris exoculata* ; Tcas, *Tribolium castaneum*.

765

766 **Fig. 6: Detail of the IR25a clade of the IR phylogeny.** This sub-tree is a zoom of the IR25a
767 clade from the tree depicted in Figure 5.

768

769 **Fig. 7: IR25a gene expression in hydrothermal vent shrimp *R. exoculata*, *M. fortunata*, *A.*
770 *markensis*, *C. chacei*, and in the coastal shrimp *P. elegans*.** Control RT-PCR products for
771 comparative analysis of gene expression correspond to the glycolysis enzyme GAPDH for
772 hydrothermal vent shrimp, and to the ribosomal protein gene RPL8 for *P. elegans*. No

773 amplification was detected in the absence of template (data not shown). A1, antennules; R1,
774 internal ramus of the lateral antennular flagella; R2, external ramus of the lateral antennular
775 flagella; A2, second antennae; Md, mandibles; Mx1-2, maxillae; p1 and p2, first and second
776 walking legs.

777

778 **Table 1:** Cruises, locations and depths of the different sampling sites of the faunal samples
779 used in this study.

780 **Table 2:** Comparative table of aesthetasc setae characteristics in different species of
781 decapods. Rough animal lengths are given for comparison. Total length is given for lobster,
782 crayfish and shrimp, carapace width for crabs.

783

784 **Supplementary Figures :**

785 **Table S1.** Nucleotide sequences of primers used in polymerase chain reaction (R=A/G,
786 Y=C/T, N= A/T/G/C, S= G/C; Fw, forward ; Rv, reverse).

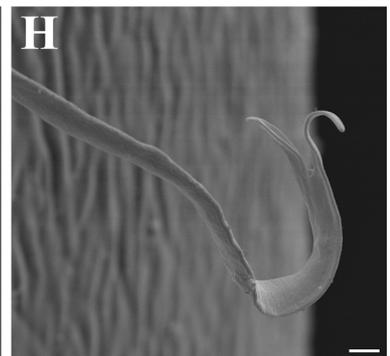
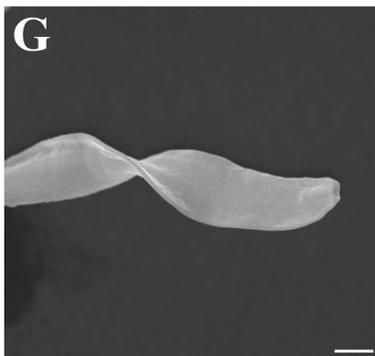
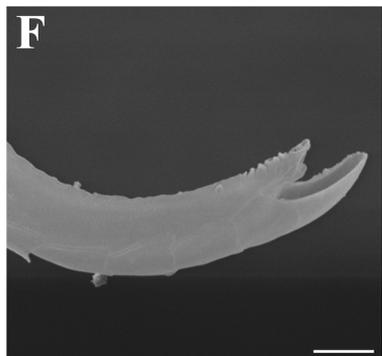
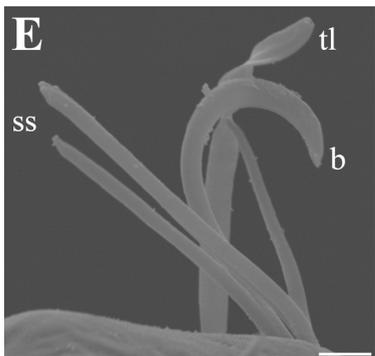
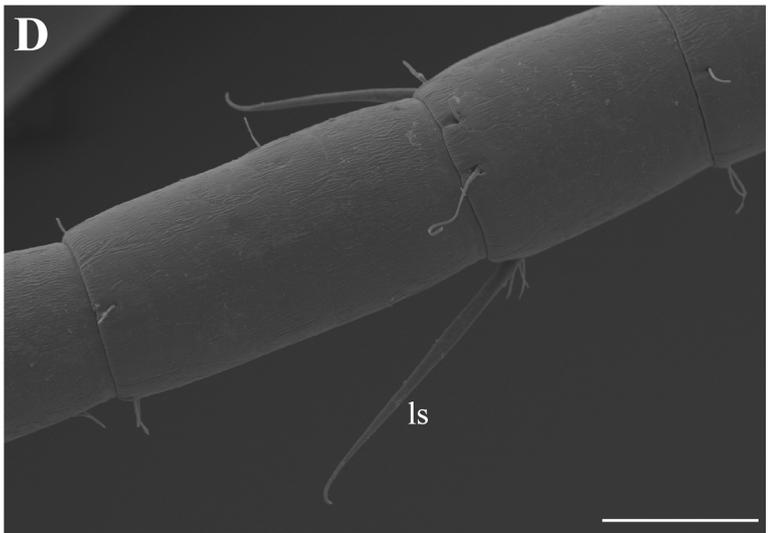
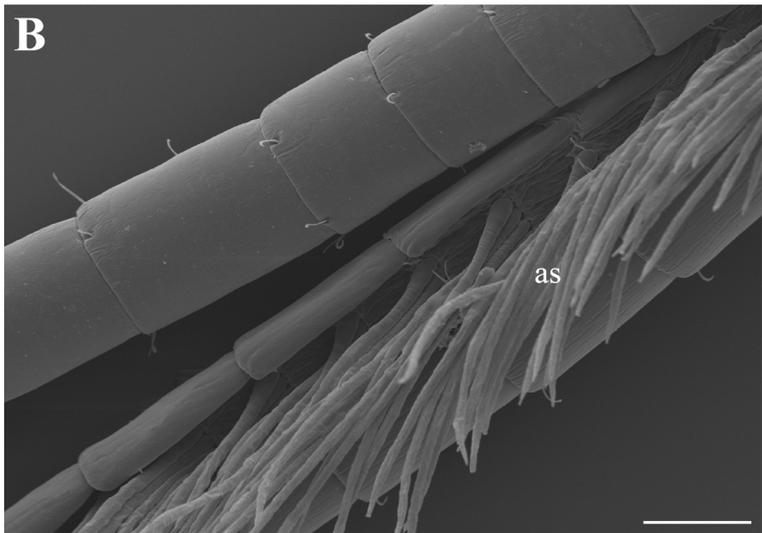
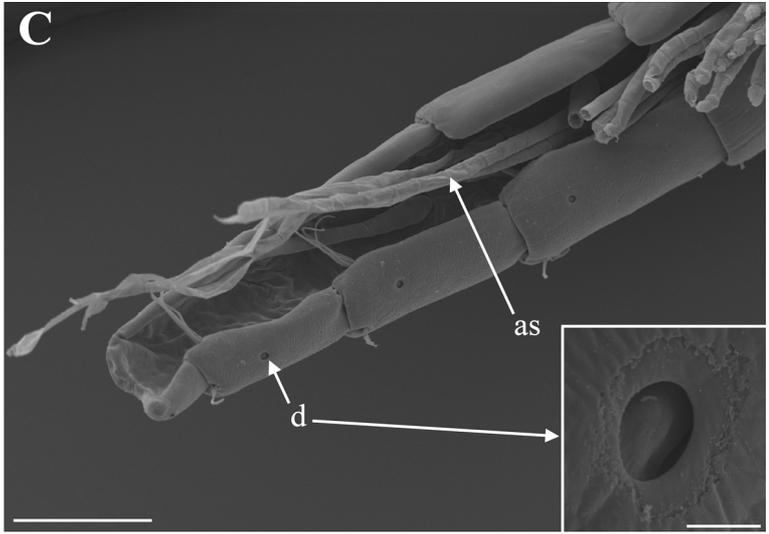
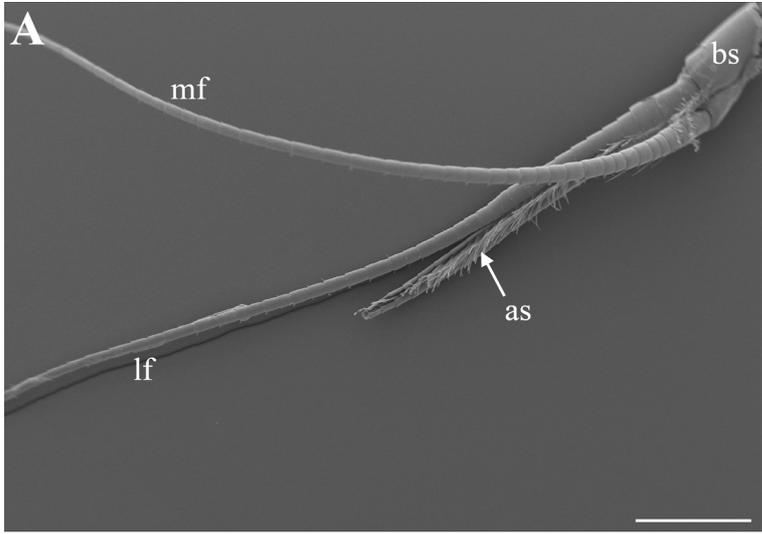
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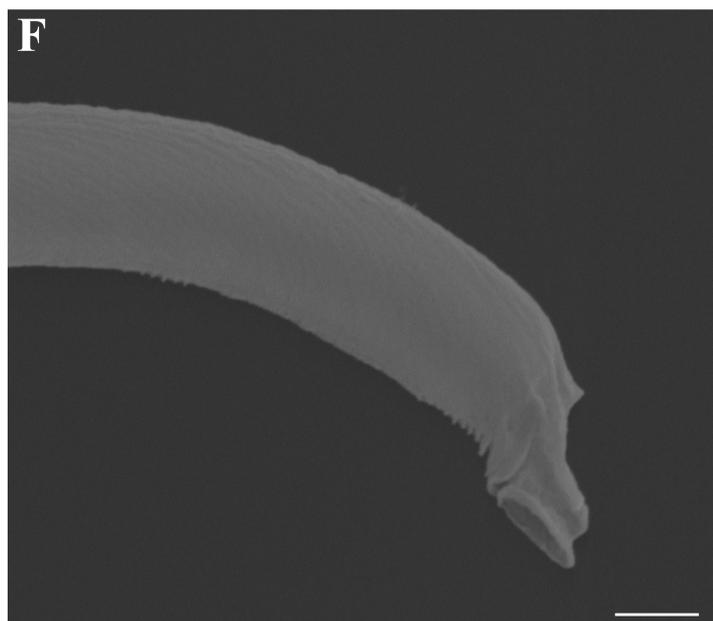
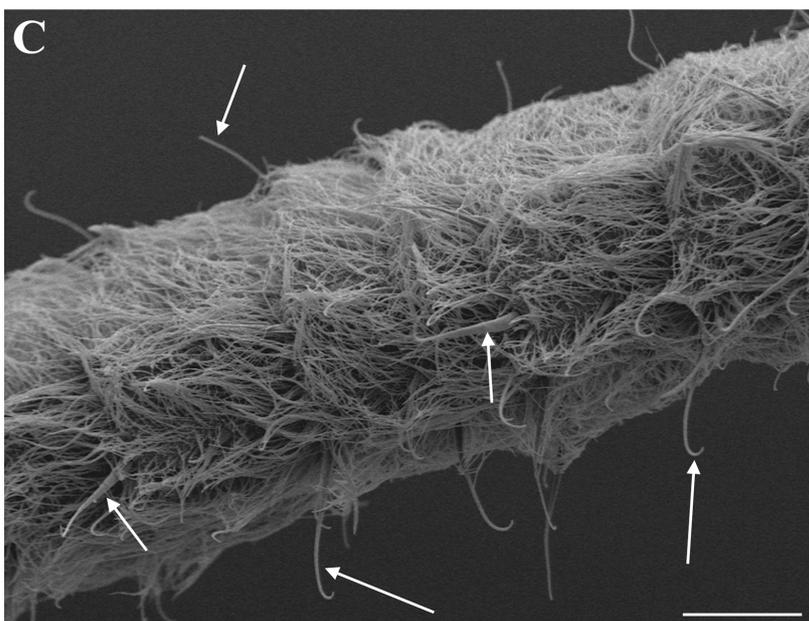
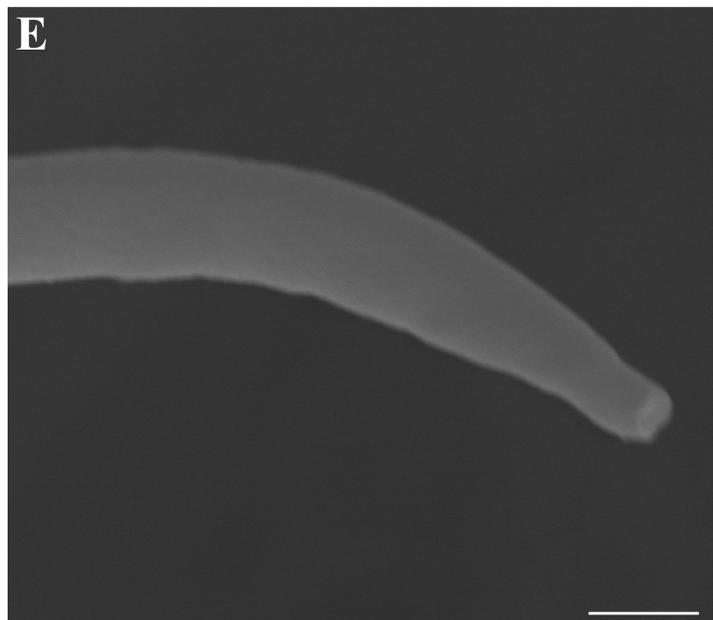
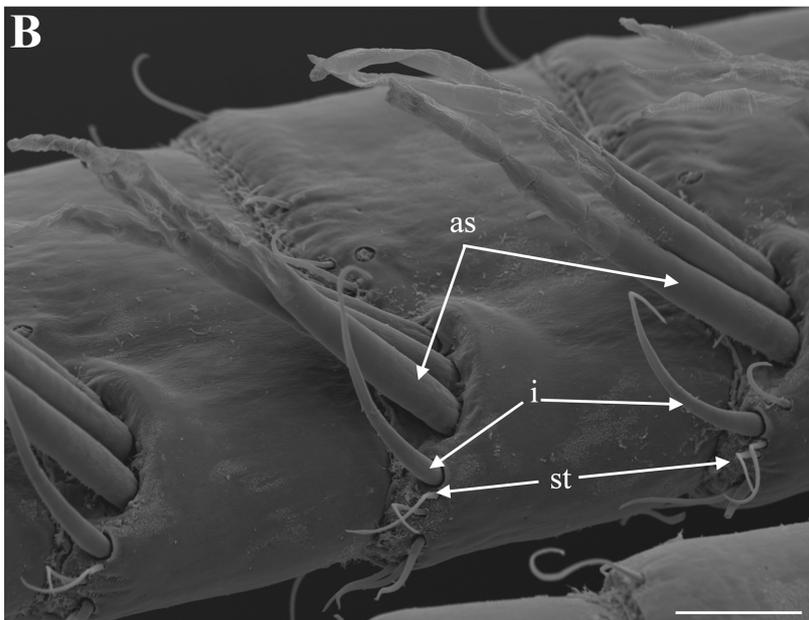
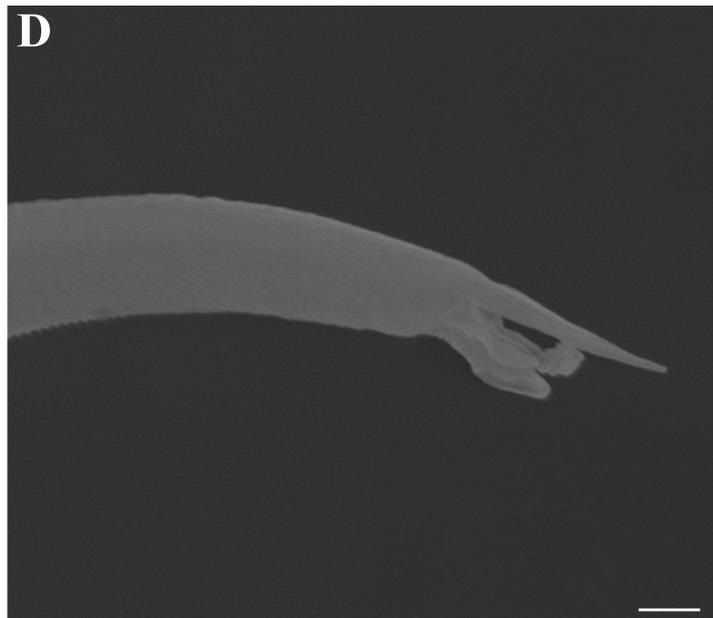
788 **Table S2.** Morphometrics of the different antennal and antennular setal types of one coastal
789 (*P. elegans*) and four hydrothermal (*M. fortunata*, *R. exoculata*, *C. chacei* and *A. markensis*)
790 shrimp species. Values are given in μm .

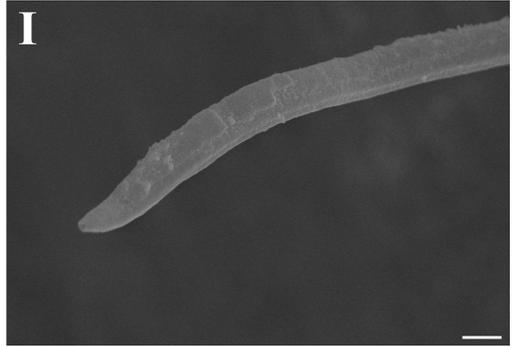
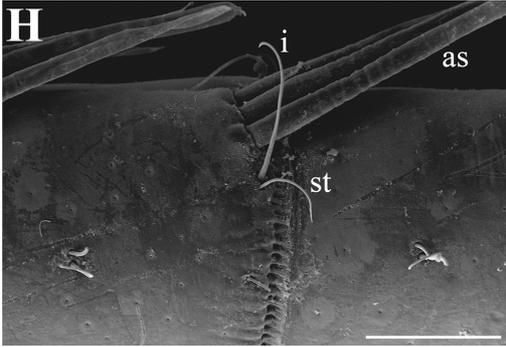
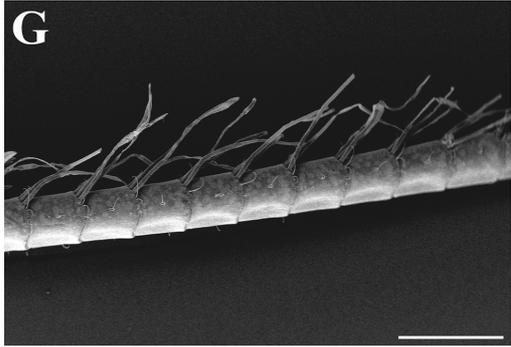
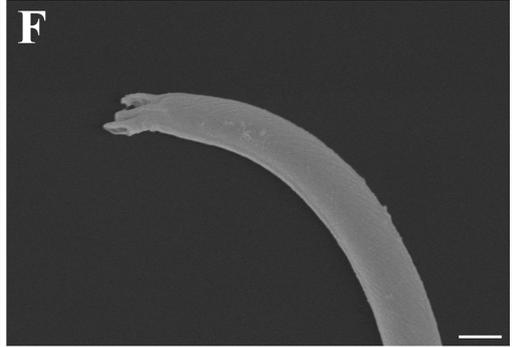
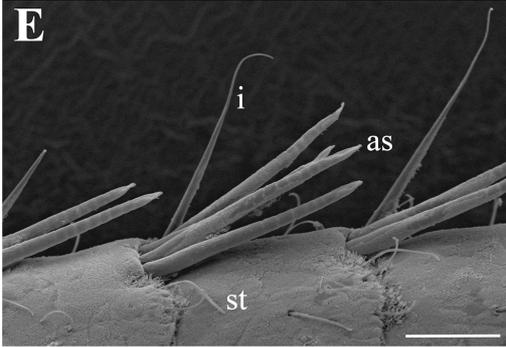
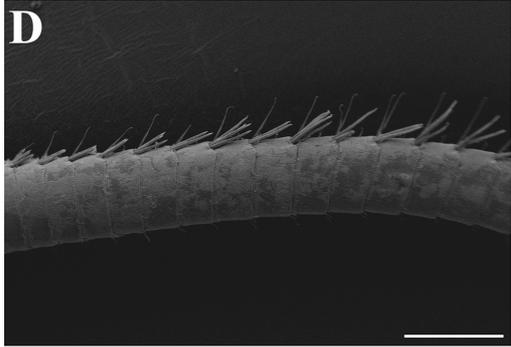
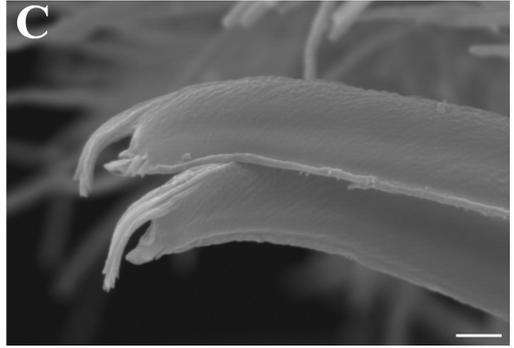
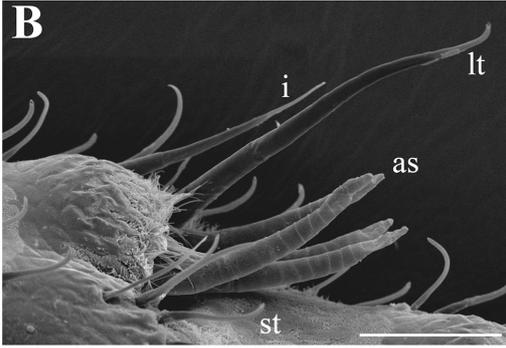
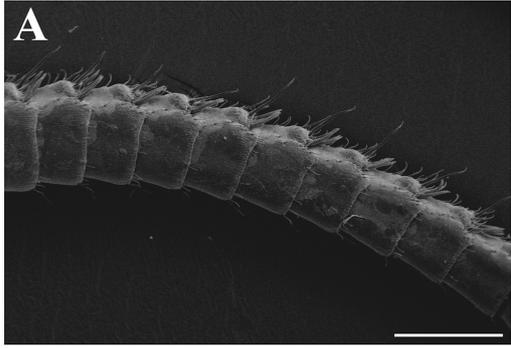
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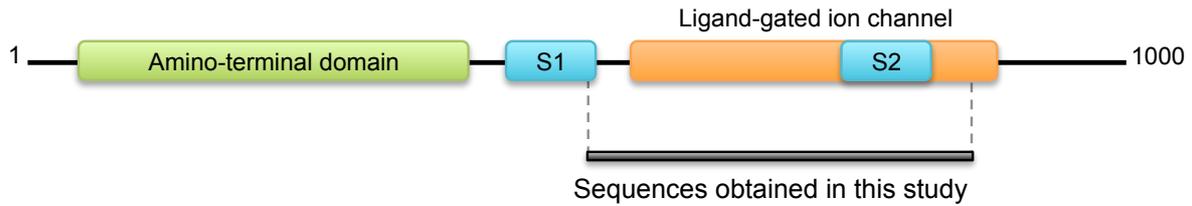
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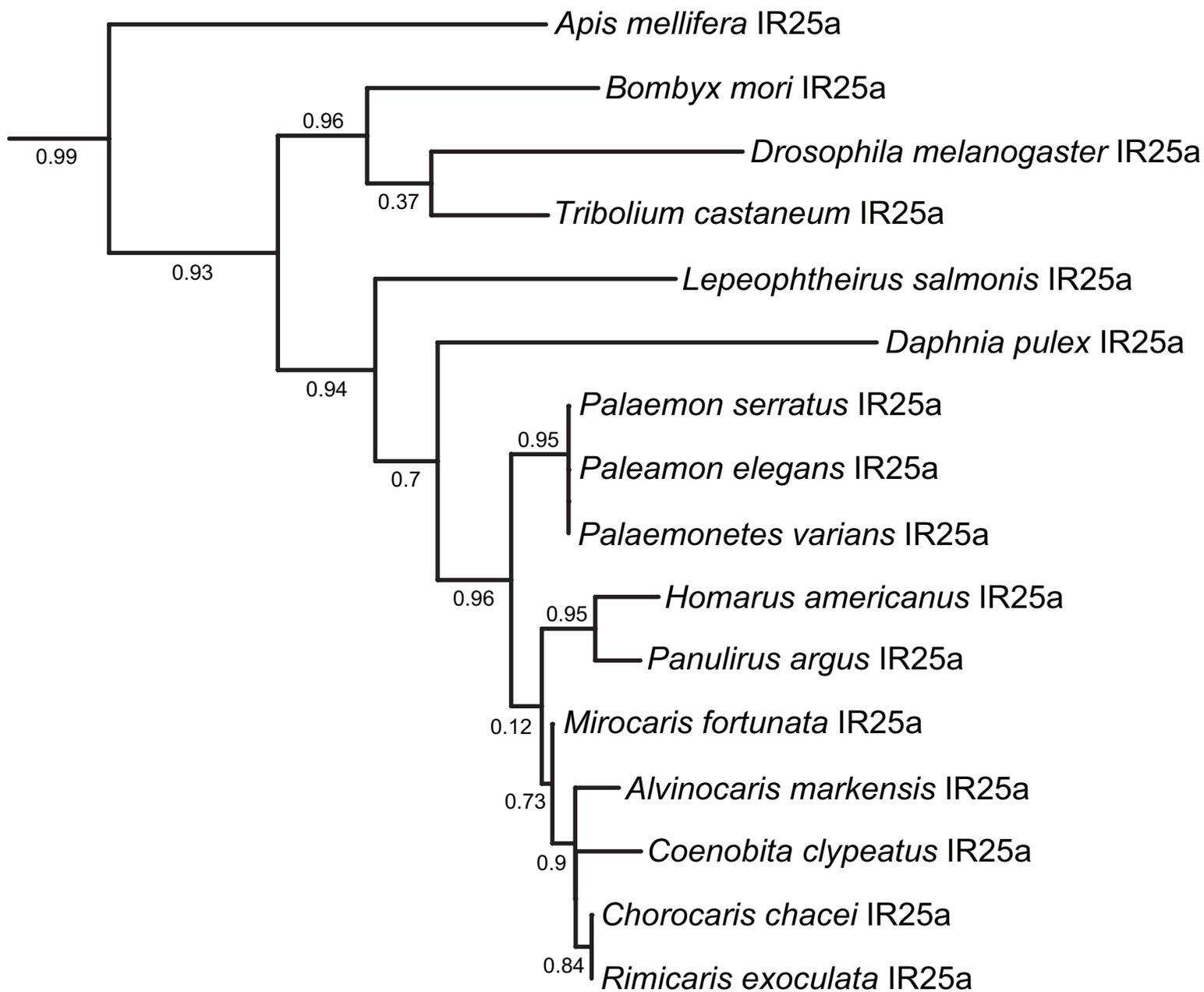


A.



B.

R.exoculata	-----DLVGI TILMKK PDVPT SLFKFL TVLEPEVWVCILFAYAF TSVLLYIFDR FS SPY 53
C.chacei	-----VCILFAYAF TSVLLYIFDR FS SPY 23
A.markensis	-----VCILFAYAF TSVLLYISDR FS SPY 23
M.fortunata	-----VCILFAYAF TSVLLYIFDR FS SPY 23
P.elegans	DFTVPDYDLVGI TILMKK PEVPTSLFKFL TVLEPEVWVCILFAY GFTSVLLYIFDR FS SPY 60
P.varians	DFTVPYYDLVGI TILMKK PEVPTSLFKFL TVLEPEVWVCILFAY GFTSVLLYIFDR FS SPY 60
P.serratus	-----DLVGI TILMKK PEVPT SLFKFL TVLEPEVWVCILFAY GFTSVLLYIFDR FS SPY 53
R.exoculata	SYQNNKERYKDDDEKREFTFKE CLWFCMTSLTPQGGGEAP KNLSGRLVAATWWLFGFIII 113
C.chacei	SYQNNKERYKDDDEKREFTFKE CLWFCMTSLTPQGGGEAP KNLSGRLVAATWWLFGFIII 83
A.markensis	SYQNNKERYKDDDEKREFTFKE CLWFCMTSLTPQGGGEAP KNLSGRLVAATWWLFGFIII 83
M.fortunata	SYQNNKERYKDDDEKREFTFKE CLWFCMTSLTPQGGGEAP KNLSGRLVAATWWLFGFIII 83
P.elegans	SYQNNKEKYKDDDEKREFTFKE CLWFCMTSLTPQGGGEAP KNLSGRLVAATWWLFGFIII 120
P.varians	SYQNNKEKYKDDDEKREFTFKE CLWFCMTSLTPQGGGEAP KNLSGRLVAATWWLFGFIII 120
P.serratus	SYQNNKEKYKDDDEKREFTFKE CLWFCMTSLTPQGGGEAP KNLSGRLVAATWWLFGFIII 113
R.exoculata	ASYTANLAAFLTVSRLDTPIE SLDDLSN QYKVQYAPMNGTSTMTYFERMAYIEKKFYEIW 173
C.chacei	ASYTANLAAFLTVSRLDTPIE SLDDLSN QYKVQYAPMNGTSTMTYFERMAYIEKKFYEIW 143
A.markensis	ASYTANLAAFLTVSRLDTPIE SLDDLSN QYKVQYAPMNGTSTMTYFERMAYIEKKFYEIW 143
M.fortunata	ASYTANLAAFLTVSRLDTPIE SLDDLSN QYKVQYAPMNGTSTMTYFERMAYIEKKFYEIW 143
P.elegans	ASYTANLAAFLTVSRLDTPIE SLDDLSN QYKVQYAPVNGTSTMTYFERMAYIENKFYEIW 180
P.varians	ASYTANLAAFLTVSRLDTPIE SLDDLSN QYKVQYAPVNGTSTMTYFERMAYIENKFYEIW 180
P.serratus	ASYTANLAAFLTVSRLDTPIE SLDDLSN QYKVQYAPVNGTSTMTYFERMAYIENKFYEIW 173
	-----*
R.exoculata	KDMSLNDSMSDVERAKLAVWDYPVSDKYTKMWQSMQ EAGLPP DFDKALERVKSTSSSEG 233
C.chacei	KDMSLNDSMSDVERAKLAVWDYPVSDKYTKMWQSMQ EAGLPP DFDKALERVKSTSSSEG 203
A.markensis	KDMSLNDSMSDVERAKLAVWDYPVSDKYTKMWQSMQ EAGL INFDKALDRVKSTSSSEG 203
M.fortunata	KDMSLNDSMSDVERAKLAVWDYPVSDKYTKMWQSMQ EAGL NTFEKALERVKSTSSSEG 203
P.elegans	KDMSLNDSMSEVERAKLAVWDYPVSDKYTKMWQSMQ EAGL PRTMESAVERVRKSTSSSEG 240
P.varians	KDMSLNDSMSEVERAKLAVWDYPVSDKYTKMWQSMQ EAGL PRTMESAVERVRKSTSSSEG 240
P.serratus	KDMSLNDSMSEVERAKLAVWDYPVSDKYTKMWQSMQ EAGL PRTMESAVERVRKSTSSSEG 233
R.exoculata	FAYIGDATDIRYLVL TNCDLQ IVGEEFSRKP YAVAVQ QGSPLKDQFNDAILKLLNQRKLE 293
C.chacei	FAYIGDATDIRYLVL TNCDLQ IVGEEFSRKP YAVAVQ QGSPLKDQFNDAIL ----- 254
A.markensis	FAYIGDATDIRYLVL TNCDLQ IVGEEFSK KPYAVAVQ QGSPLKDQFNDAIL ----- 254
M.fortunata	FAYIGDATDIRYLVL TNCDLQ IVGEEFSRKP YAVAVQ QGSPLKDQFNDAIL ----- 254
P.elegans	FAFIGDATDI -YLVL TNCDLQMVGEEFSRKP YAVAVQ QGSPLKDQFNDAILKLLNQRKLE 299
P.varians	FAFIGDATDIRYLVL TNCDLQ QMVGEEFSRKP YAVAVQ QGSPLK-QFNDAILKLLNQRKLE 299
P.serratus	FAFIGDATDIRYLVL TNCDLQ QMVGEEFSRKP YAVAVQ QGSPLKDQFNDAILKLLNQRKLE 293
	-----*
R.exoculata	TLKERWWK 301
C.chacei	-----
A.markensis	-----
M.fortunata	-----
P.elegans	T ----- 300
P.varians	K ----- 300
P.serratus	K ----- 294



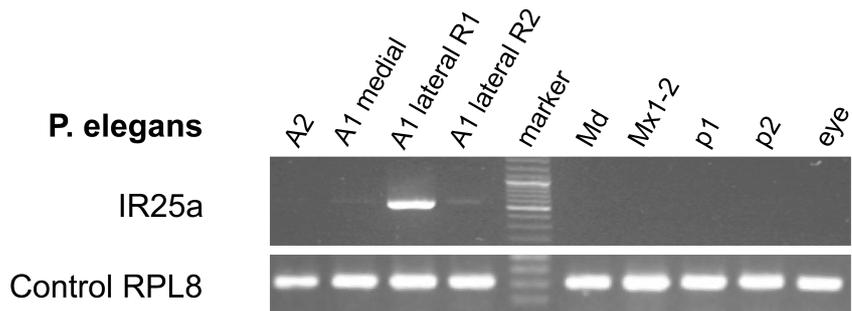
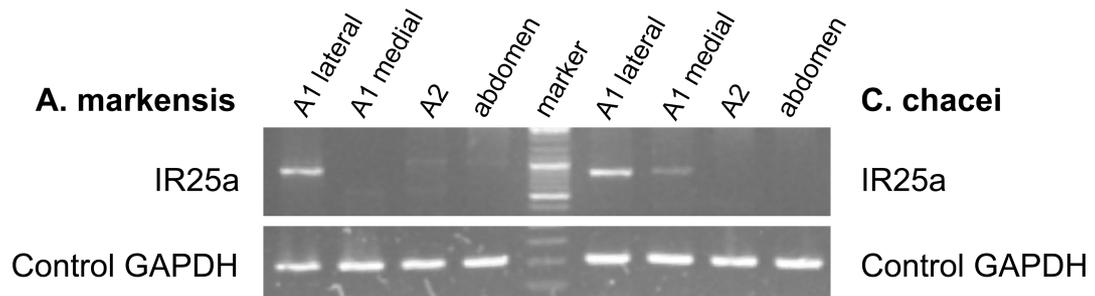
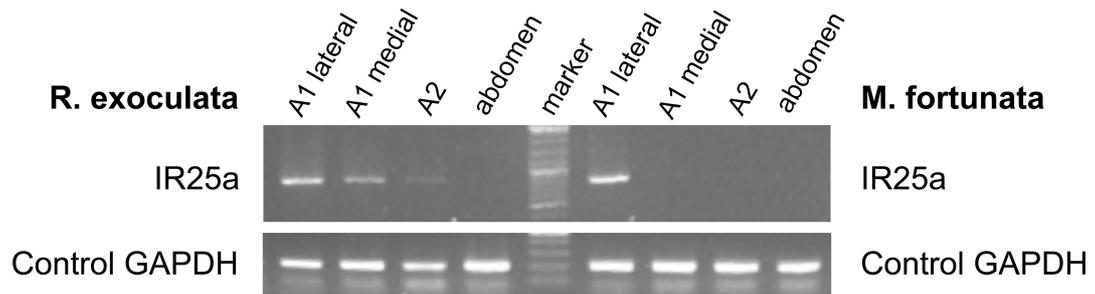


Table 1 : Cruises, locations and depths of the different sampling sites of the samples used in this study.

Sites	Lat.	Long.	Depth (m)	Cruise, year	Ship / Submersible	Chief scientist
Menez Gwen	37°51'N	31°31'W	840	Biobaz, 2013	Pourquoi Pas? / ROV Victor	F. Lallier
Lucky Strike	37°17'N	32°16'W	1700	Biobaz, 2013	Pourquoi Pas? / ROV Victor	F. Lallier
				Momarsat 2011 Momarsat 2012	Pourquoi Pas? / ROV Victor Thalassa / ROV Victor	M. Cannat M. Cannat and PM Sarradin
Rainbow	36°13'N	33°54'W	2260	Biobaz, 2013	Pourquoi Pas? / ROV Victor	F. Lallier
TAG	26°08'N	44°49'W	3600	Bicose, 2014	Pourquoi Pas? / ROV Victor	MA Cambon-Bonavita
Snake Pit	23°23'N	44°58'W	3480	Bicose, 2014	Pourquoi Pas? / ROV Victor	MA Cambon-Bonavita

Table 2 : Comparative table of aesthetascs setae characteristics in different species of decapods. Rough animal lengths are given for comparison. Total length is given for lobster, caryfish and shrimp, carapace width for crabs.

Species	Total number	Number per row	Dimensions (diameter x length in μm)	Reference
Lobster				
<i>Panulirus argus</i> (20-60 cm)	2000 to 4000	9-10	40x1000	Gleeson et al. 1993 Laverack 1964
<i>Homarus americanus</i> (20-60 cm)	2000	10-12	20x600	Guenther and Atema 1998
Crayfish				
<i>Orconectes propinquus</i> (4-10 cm)	160	3-6	12x150	Tierney et al. 1986
<i>Cherax destructor</i> (10-20 cm)	260*	2-5	18x100	Sandeman and Sandeman 1996 Beltz et al. 2003
Crab				
<i>Callinectes sapidus</i> (23 cm)	1400	~ 20	12x795	Gleeson et al. 1996
<i>Carcinus maenas</i> (9 cm)	100-300	8-10	13x750	Fontaine et al. 1982
Shrimp				
<i>Lysmata</i> ¹ (5-7 cm)	210-460	3-5	20x800	Zhang et al. 2008
<i>Palaemon elegans</i> (7 cm)	280	5-6	14x230	This study
<i>Mirocaris fortunata</i> (3 cm)	120*	3-4	16x234	This study
<i>Rimicaris exoculata</i> (5.5 cm)	206*	3-4	20x170	This study
<i>Chorocaris chacei</i> (5.5 cm)	226*	2-4	19x251	This study
<i>Alvinocaris markensis</i> (8.2 cm)	220*	3-4	21x531	This study

* : species with only one row of aesthetascs per annuli ; ¹ : study realised on *Lysmata boggei*, *L. wurdemanni*, *L. amboinensis* and *L. debelius*

Table S1. Nucleotide sequences of primers used in polymerase chain reaction (R=A/G, Y=C/T, N= A/T/G/C, S= G/C ; Fw, forward ; Rv, reverse).

Species	IR25a sequencing	Localisation in tissues by RT-PCR
<i>Mirocaris fortunata</i>	Fw-IR25a-5 / Rv-IR25a-8	Fw-IR25a-5 / Rv-IR25a-8
<i>Rimicaris exoculata</i>	Fw-IR25a-1 / Rv-IR25a-4 Fw-IR25a-2 / Rv-IR25a-3	Fw-IR25a-5 / Rv-IR25a-8
<i>Chorocaris chacei</i>	Fw-IR25a-5 / Rv-IR25a-8	Fw-IR25a-5 / Rv-IR25a-8
<i>Alvinocaris markensis</i>	Fw-IR25a-5 / Rv-IR25a-8	Fw-IR25a-5 / Rv-IR25a-8
<i>Palaemon elegans</i>	Fw-IR25a-1 / Rv-IR25a-4 Fw-IR25a-2 / Rv-IR25a-3	Fw-PE-IR25a-2 / Rv-PE-IR25a-3
<i>Palaemonetes varians</i>	Fw-IR25a-1 / Rv-IR25a-4 Fw-IR25a-2 / Rv-IR25a-3	
<i>Palaemon serratus</i>	Fw-IR25a-1 / Rv-IR25a-4 Fw-IR25a-2 / Rv-IR25a-3	

Primer	Specificity	Sequence
Fw-IR25a-1	generalist	TGGAACGGCATGATYAARSA
Fw-IR25a-2	generalist	GAYTTCACSGTGCCTTACTA
Rv-IR25a-3	generalist	TCCACCATCKCTCYTTSAGCG
Rv-IR25a-4	generalist	ACGATRAASACACCACCGATGT
Fw-PE-IR25a-2	<i>Palaemon elegans</i>	GAATGCCTCTGGTTCTGCATGACA
Rv-PE-IR25a-3	<i>Palaemon elegans</i>	TCGAGAATTCCTCACCTACCATCTGC
Fw-IR25a-5	<i>Rimicaris exoculata</i>	TGACTGTACTAGAGCCTGAGGTGT
Rv-IR25a-8	<i>Rimicaris exoculata</i>	AGCTTCCTCTGGTTCAAGAGCTTC

Table S2: Morphometrics of the different antennal and antennular setal types of one coastal (*P. elegans*) and four hydrothermal (*M. fortunata*, *R. exoculata*, *C. chacei* and *A. markensis*) shrimp species. Values are given in μm .

A. Palaemon elegans

	Aesthetascs		Short simple		Long simple		Beaked		Flat twisted		Bifid		Round depression
	Diameter	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
n	14	10	7	9	7	7	14	13	11	11	6	6	8
Mean	14	230	2	36	12	253	2	28	3	44	4	60	6
Standard deviation	4.8	95	0.3	3.8	1.2	30.7	0.3	4.3	0.4	5.7	0.9	16.8	0.4
Minimum value	5.8	154.6	1.6	29.9	11	215.3	1.7	20	2.1	36.7	2.3	27.3	5.5
Maximum value	20.3	393	2.2	42.6	14.5	298.2	2.7	43.5	3.3	52.5	4.9	71.4	6.7

B. Mirocaris fortunata

	Aesthetascs		Short simple		Intermediate		Round depression
	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
n	21	46	40	40	33	32	26
Mean	16	234	3	49	10	135	8
Standard deviation	1.56	26.28	0.55	13.62	1.7	31.8	0.98
Minimum value	12.8	172.1	2.2	27.6	6	80.2	7
Maximum value	18.3	290.3	4.2	73.8	12.7	215	10.4

C. Rimicaris exoculata

	Aesthetascs		Short thin beaked		Intermediate beaked		Long thick beaked		Round depression
	Diameter	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
n	22	26	47	47	30	30	28	28	3
Mean	20	170	6	77	11	113	21	282	7
Standard deviation	1.7	9.1	1.1	11.3	2.7	26.5	4.1	52.4	1
Minimum value	15.9	154.1	3.1	58.4	5.1	79	15	202	6.2
Maximum value	22	191	7.9	96.8	18.3	174.8	30.3	384.9	8.2

D. Chorocaris chacei

	Aesthetascs		Short simple		Short beaked		Intermediate beaked		Round depression
	Diameter	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
n	50	58	31	31	28	28	16	17	3
Mean	19	251	3	68	4	69	13	222	5
Standard deviation	3.52	74.24	0.49	9.57	0.57	11.44	1.72	46.17	0.26
Minimum value	9.2	84.4	2.4	51.4	2.3	50.1	9.6	137.8	5
Maximum value	23.2	339.5	4.3	81.5	5.1	88.70	16.4	296.1	5.5

E. Alvinocaris markensis

	Aesthetascs		Short simple		Intermediate simple		Long simple		Round depression
	Diameter	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
n	39	49	33	33	14	14	11	11	21
Mean	21	531	3	68	4	115	10	206	6
Standard deviation	3.5	189.9	0.4	15.9	0.7	23.5	0.9	41.9	0.9
Minimum value	11.4	186	1.6	42.2	3	64.1	8.4	174.9	4.7
Maximum value	25.2	879.1	3.8	107.4	5.2	146.8	11.9	307.8	7.7