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1 **Description of the larval and adult hindgut tract of the common spider crab *Maja***  
2 ***brachydactyla* Balss, 1922 (Brachyura, Decapoda, Malacostraca).**

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15 **Short title:** Brachyuran larval and adult hindgut tract

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19 **Abstract**

20           Arthropods are the most diversified animals on Earth. The morphology of the  
21 digestive system has been widely studied in insects; however, crustaceans have received  
22 comparatively little attention. This study describes the hindgut tract of the common  
23 spider crab *Maja brachydactyla* Balss, 1922 in larvae and adults using dissection, light  
24 and electron microscopical analyses. The hindgut tract maintains a similar general shape  
25 in larvae and adults. Major differences among stages are found in the morphology of  
26 epithelial cells and microspines, the thickness of the cuticle and connective-like tissue,  
27 and the presence of rosette glands (only in adults). Here we provide the description of  
28 the sub-cellular structure of the folds, epithelium (conformed by tendon cells),  
29 musculature, and microspines of the hindgut of larvae and adults of *M. brachydactyla*.  
30 The morphological features of the hindgut of *M. brachydactyla* is compared with those  
31 of other arthropods (Insecta, Myriapoda and Arachnida). Our results suggest that the  
32 morphology of the hindgut is associated mainly with transport of faeces. In adults, the  
33 hindgut may also exert an osmoregulatory function, as described in other arthropods. At  
34 difference from holometabolous insets, the hindgut of *M. brachydactyla* (Decapoda)  
35 does not undergo a true metamorphic change during development, but major changes  
36 observed between larval and adult stages might respond to the different body size  
37 between life stages.

38 **Keywords:** Arthropoda; larval development; rosette glands; cuticular microspines;  
39 tendon cells

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## 1. Introduction

Arthropods are the most diversified animals on Earth, with an estimated global richness of 6–8 million species (ØDegaard 2000; Stork et al. 2015). The alimentary tract of all arthropods shares a number of common traits, mainly subdivision into foregut, midgut and hindgut (Yoshikoshi 1975; Schultz and Kennedy 1976; Terra 1990; Felgenhauer 1992; Klann and Alberti 2010; Klowden 2013; Davie et al. 2015; Nardi et al. 2016). Since arthropod diversity is concomitant with diverse feeding regimes, the morphology of their alimentary tract is equally diverse responding to the phylogeny and the feeding strategies (Wigglesworth 1972; Klowden 2013; Watling 2013; Terra and Ferreira 2020). For example, the foregut of carabid beetles varies from a grinding gizzard when intake consists of insect pieces, to a muscular pump in semi-fluid feeders with pre-oral digestion (Forsythe 1982), the phasmids ("stick" and "leaf insects") have an alimentary tract which midgut caeca are absent in related orders, probably reflecting the evolutionary and dietary constraints of the group (Shelomi et al. 2015), and in hemipterans ("stink" and "assassin bugs") the ultrastructure of the epithelial midgut cells is more correlated with the phylogeny than with the feeding habits (Santos et al. 2017). The digestive system of the crustaceans is also constrained by phylogeny and feeding. Considering the example of the foregut, the shape of the gastric mill of land crabs varies between carnivorous and herbivorous species (Allardyce and Linton 2010), while in mysids ("opossum shrimps") it is dominated by spines and setae which type and distribution is apparently correlated with the diet (Metillo and Ritz 1994), and in amphipods ("beach hoppers" and "sand fleas"), despite being a "simple" tube with channels and setae screens, it showed to be highly divergent when comparing species with different food preferences (Coleman 1991; Coleman 1992; Coleman 1994).

65           The hindgut is the terminal section of the digestive system of the arthropods. In  
66   Decapoda (crabs, lobsters, prawns and related taxa) it is probably one of the digestive  
67   system organs that received less attention, as reflected by several reviews (Ceccaldi  
68   1989; Felgenhauer 1992; Icely and Nott 1992; Watling 2013; Davie et al. 2015). This  
69   organ is generally described as a simple tube lined internally by a cuticle, involved in  
70   the transport of waste material, osmoregulation, and reabsorption of water and ions  
71   (Phillips et al. 1987; Ceccaldi 1989; Felgenhauer 1992; Icely and Nott 1992; Watling  
72   2013; Davie et al. 2015). More than a "simple" tube, in decapods the hindgut shows  
73   several interesting features: 1) the hindgut is longitudinally folded (Barker and Gibson  
74   1977; Barker and Gibson 1978; Harris 1993b); 2) the cuticle projects microspines  
75   pointed backward, probably to protect the cuticle and to help the faecal movement  
76   (Elzinga 1998; Chisaka et al. 1999); 3) a layer of connective-like tissue surrounds the  
77   epithelium, musculature, and glands (Barker and Gibson 1977; Barker and Gibson  
78   1978); 4) it has two main types of musculature: inner longitudinal muscles placed inside  
79   the folds, and outer circular muscles in the periphery, their role is to generate the wave  
80   movements to excrete the waste material (Chisaka et al. 1999); and 5) rosette or  
81   tegumental glands located below the epithelium along the entire hindgut length, its role  
82   is unclear (Barker and Gibson 1977; Barker and Gibson 1978). Little information is  
83   available regarding the sub-cellular structure of the hindgut, even if higher taxa are  
84   considered. In this sense, the cuticle and epithelial cells have been described in crabs  
85   and lobsters (Mykles 1979), woodlouses (Bogataj et al. 2018), and the strange  
86   mystacocarids (Herrera-Alvarez et al. 2000); and the rosette glands were described in  
87   the ghost shrimps (Felder and Felgenhauer 1993).

88           The morphology of the alimentary tract, including the hindgut, during larval  
89   stages has been widely documented in numerous insect species (Maxwell 1955; Areekul

90 1957; Jones 1960; Judy and Gilbert 1969; Mall 1980; Rowland and Goodman 2016). In  
91 decapods, the morphology of the alimentary tract of larval stages has been studied in  
92 clawed lobsters and crayfishes (Hinton and Corey 1979; Factor 1981), hermit and king  
93 crabs (Williams 1944; Abrunhosa and Kittaka 1997), true crabs (Schlegel 1911;  
94 Jantrarotai and Sawanyatiputi 2005), spiny lobsters (Mikami et al. 1994), and prawns  
95 and shrimps (Lovett and Felder 1989; Tziouveli et al. 2011). However, these studies  
96 devoted little to none attention to the morphology of the hindgut tract and its ontogeny.  
97 Thus far, the majority of those studies are light microscopy descriptions in which the  
98 hindgut is reduced to a simple tube with cuticle. In holometabolous insects, the hindgut  
99 have been more detailed described showing radical transformations during the  
100 metamorphosis, e.g. in wasps and bees it elongates, convolutes, and differentiates into  
101 ileum and rectum (Green 1933; Gonçalves et al. 2017), while in moths the hindgut  
102 becomes an enlarged and coiled tube with a rectal sac (Judy and Gilbert 1969; Rowland  
103 and Goodman 2016); such changes are associated with drastic changes in lifestyle and  
104 diet (Rowland and Goodman 2016). Since several decapods also undergo a drastic  
105 metamorphosis (Martin et al. 2014), could a detailed description of the larval hindgut  
106 reveal some degree of transformative change?

107         The common spider crab *Maja brachydactyla* Balss, 1922 is a true crab  
108 (Brachyura) native from the coastal waters of the Atlantic Europe (Abelló et al. 2014). It  
109 has a high economic and ecological significance, supporting fisheries along the NE  
110 Atlantic coasts (Spain, Portugal, France, Ireland and UK). The high fishing pressure  
111 tolerated by populations of this crab (Freire et al. 2002), together with its growth and  
112 reproductive characteristics (González-Gurriarán et al. 1995; Andrés et al. 2007; Andrés  
113 et al. 2008; Andrés et al. 2010; Guerao and Rotllant 2010; Simeó et al. 2015) define the  
114 species as potentially interesting for aquaculture. This species also shows interesting

115 particularities as a model species to study the larval development of marine decapods,  
116 including easy adult culture and spawning, high fecundity and a larval development that  
117 requires around two weeks at 21 °C to be completed without special requirements  
118 (Castejón et al. 2018b; Castejón et al. 2019b). The larval development consists in two  
119 planktonic zoeal stages (zoea I and zoea II), and a single transitional planktonic-benthic  
120 megalopa stage that metamorphoses to benthic juvenile (Guerao et al. 2008). Several  
121 digestive organs of *M. brachydactyla* during larval and adult stages have been described  
122 in previous studies, e.g. the general digestive tract anatomy (Castejón et al. 2018a),  
123 oesophagus (Castejón et al. 2018c), stomach (Castejón et al. 2015; Castejón et al.  
124 2019c), and midgut gland or hepatopancreas (Castejón et al. 2019a).

125         Following the previous studies realized on this species, here we describe in  
126 detail the hindgut tract in the common spider crab *Maja brachydactyla* Balss, 1922 in  
127 larval and adult stages, excluding the rectum, combining different techniques:  
128 dissection, and light and electron microscopical analyses. The hindgut morphology was  
129 compared between larval stages and adults and discussed with information available for  
130 other arthropod taxa.

## 131         **2. Material and methods**

### 132             *2.1 Adult and larval culture system*

133         Local enterprises (CADEMAR S.COOP.R.L., Tarragona, Spain; FUNDACIÓN  
134 LONXANET, A Coruña, Spain) provided the adult specimens. They were transported  
135 to the Institut de Recerca i Tecnologia Agroalimentàries (IRTA, Sant Carles de la  
136 Ràpita, Tarragona, Spain). The broodstock was maintained in 2,000 L cylindrical tanks  
137 connected to a recirculation unit system: 3.5 m<sup>3</sup> h<sup>-1</sup> renewal rate, 18 ± 1 °C, 35 ± 1 psu,  
138 12 light h:12 dark h photoperiod, and fed with fresh and frozen mussels (genus *Mytilus*).

139 The broodstock tanks were connected to collector units in which the larvae were  
140 recovered ca. 12 hours after hatching. The larvae were maintained in 600 mL glass  
141 beakers placed inside 360 L tanks (96 x 96 x 40 cm) used as incubation chambers with  
142 the following conditions:  $21 \pm 1$  °C,  $35 \pm 1$  psu, 12 light h:12 dark h photoperiod. The  
143 larvae were fed with *Artemia* sp. nauplii and metanauplii (INVE Aquaculture Nutrition,  
144 Salt Lake UT, USA). The larvae were sampled daily. The larvae reached the zoea II  
145 stage in 3 days, the megalopa stage in 6 days, and the first juvenile in 11-12 days.

## 146 *2.2 Gross morphology*

147 An adult female was placed for 45 min in ice for sedation before dissection to  
148 show the alimentary tract. The alimentary tract was fixed in formaldehyde 4% and  
149 photographed using a digital camera (Panasonic DMC-TZ3, Kadoma, Japan). Around  
150 80 larvae were fixed in formaldehyde 4% and dissected to show the midgut-hindgut  
151 junction as starting point to measure the hindgut length. Then, a Nikon SMZ800  
152 stereomicroscope was used to show by transparency the hindgut through the pleon. The  
153 total length of the hindgut was measured as the distance from the midgut-hindgut  
154 junction to the anus employing AnalySIS® software tools (Soft Imaging System,  
155 Münster, Germany). The total length of the hindgut was measured in four to six larvae  
156 *per* day of development.

## 157 *2.3 Light microscopical analysis*

158 The whole larvae and portions of the hindgut tract of the adults were fixed with  
159 Davidson's fixative (ethanol absolute: seawater: formaldehyde 37%: glycerol: glacial  
160 acetic acid in proportion 3: 3: 2: 1: 1) during 24 h. The material was dehydrated in an  
161 increasing graded ethanol series and embedded in paraffin using a paraffin processor  
162 (AP208, Myr, Spain). The paraffin blocks were cut into 2 µm slices (microtome Leica  
163 RM2155, Wetzlar, Germany). The slices were stained using: 1) Hematoxylin and Eosin



164 (H-E) to show the general morphology; 2) Periodic Acid–Schiff (PAS) with Methylene  
165 Blue to reveal substances with affinity to neutral polysaccharides and  
166 mucopolysaccharides; 3) Periodic acid–Schiff (PAS) combined with Alcian Blue (pH  
167 2.5) and contrasted with Hematoxylin to reveal the presence of acid  
168 mucopolysaccharides; and 4) Mallory's Trichrome stain (Acid Fuchsin, Orange G and  
169 Aniline Blue stains) to visualize the structure of the muscular and connective tissues.  
170 The observations were realized on the zoea I, zoea II, megalopa, and adult stages, using  
171 an optical microscope (Leica LB30T 111/97, Wetzlar, Germany) with a camera  
172 (Olympus DP70 1.45 Mpx) and an image analyzing system (DP Controller 2.1.1.83 and  
173 DP Manager 2.1.1.163; Olympus).

#### 174 *2.4 Electron microscopical analysis*

175 The whole larvae and portions of the adult hindgut tract were fixed in a solution  
176 of cacodylate buffer (0.1 mol L<sup>-1</sup> pH 7.4) with 2% paraformaldehyde and 2.5%  
177 glutaraldehyde; the samples were maintained in total darkness at 4 °C for 12 h. Then,  
178 they were rinsed twice with cacodylate buffer and post-fixed in 1% osmium tetroxide  
179 solution in cacodylate buffer. After the post-fixation the samples were dehydrated in an  
180 increasing graded series of acetone. The transmission electron microscopy required the  
181 embedding of the post-fixed samples in Spurr's resin and cut into semi-thin (0.5 µm)  
182 and ultrathin (50-70 nm) sections with an ultramicrotome (Leica UCT, Wetzlar,  
183 Germany). Before observation, grids were contrasted with uranyl acetate and lead  
184 citrate. The observations were realized on the megalopa and adult stages using a JEOL  
185 EM-1010 electron microscope at 80 kV equipped with an image analysis system  
186 (AnalySIS, SIS, Münster, Germany). The scanning electron microscopy required the  
187 critical-point-drying of the post-fixed samples, then they were mounted on SEM stubs  
188 with self-adhesive stickers and coated with carbon. The observations were realized on

189 the zoea I and adult stages using a JEOL JSM-7001F scanning electron microscope. The  
190 post-fixative treatment and TEM and SEM observations were realized at CCiTUB  
191 (Hospital Clinic, University of Barcelona, Spain).

### 192 **3. Results**

193 *Gross morphology.* The hindgut tract of larvae and adults is a large tube that  
194 runs along the length of the animal from the midgut-hindgut junction (located in the  
195 middle of the cephalothorax length) to the rectum (Fig. 1A-B). During the larval  
196 development (zoea I to megalopa), the hindgut tract maintains a similar morphology,  
197 cellular organisation and total length (mean length =  $1.7 \pm 0.1$  mm; Fig. 1C). The gross  
198 morphology of the hindgut tract shares certain features between larvae and adults: 1) the  
199 hindgut lumen has a stellate shape with radial symmetry caused by the presence of five  
200 main longitudinal folds (Figs. 2C-D; 3C); 2) the lumen is lined by a simple epithelium  
201 covered by a cuticle (Figs. 2-3) with microspines projected backward (Fig. 2D; 4-5; 7);  
202 and 3) the inner longitudinal muscles are located inside the folds, while the outer  
203 circular muscles surround the hindgut perimeter (Figs. 2D; 3C-E).

204 *Epithelial cells.* The epithelial cells of larvae are generally squamous and  
205 surround the inner longitudinal muscle cells located in the centre of the fold (Figs. 4B;  
206 5). The apical membrane forms short microvilli-like extensions projected toward the  
207 cuticle (Fig. 5B-D); the lateral membranes show electron-dense epithelial-to-epithelial  
208 cell junctions located near to the cell apex (Fig. 5A-C); while the basal membrane  
209 shows highly electron-dense epithelial cell-to-muscle cell hemiadherens junctions (Fig.  
210 5). The cytoplasm contains mitochondria, short globular cisternae of the rough  
211 endoplasmic reticulum, and bundles of fibres structures (Figs. 4B; 5B-C). The epithelial  
212 cells of adults differ considerably from those of larvae. In this regard, they are tall  
213 columnar cells (ca. 30–40  $\mu\text{m}$  height, Figs. 3; 6). The apical membrane shows electron-

214 dense infolds, we denominated them as "apical complexes" (Fig. 6E). The lateral  
215 membranes have epithelial-to-epithelial cell junctions near the cell apex (Fig. 6D), as  
216 well numerous interdigitations, whose number, size and complexity increases toward  
217 the base of the cell (Fig. 6A-C). The basal membrane is highly infolded (Fig. 6C). The  
218 cytoplasm is rich in PAS-positive granules. The cytoplasm contains mitochondria (ca.  
219 2–3  $\mu\text{m}$  length and 200–300 nm width) concentrated in the supra-nuclear region (Fig.  
220 6B). The cells are crossed by well-developed bundles of fibres structures (Fig. 6F)  
221 extended from the base (Fig. 6C) to the apex of the cell, where they anchor to the  
222 "apical complexes" (Fig. 6E).

223         *Cuticle and microspines.* The epithelium of the hindgut tract is covered by a  
224 cuticle. The cuticle of larvae is very thin (Figs. 2; 4-5). Electron-microscopy reveals that  
225 the larval cuticle has an outer electron-dense epicuticle and an inner less electron-dense  
226 procuticle (Figs. 5C). The cuticle of adults accounts for approximately a third of the  
227 epithelial cell height (ca. 12  $\mu\text{m}$  height) and shows an outer epicuticle and an underlying  
228 procuticle (Fig. 3B). The cuticular surface is rich in microspines (Figs. 2D; 4-5; 7), in  
229 which some bacillus-shaped bacteria are occasionally present (Fig. 7C-D). The  
230 microspines of the larval stages are very short (ca. 0.5–1  $\mu\text{m}$  length). The larval  
231 microspines are simple fang-like structures projected backward into the hindgut (Fig.  
232 7B). In contrast, adults show two types of microspines: elongated and sharp microspines  
233 (ca. 4–6  $\mu\text{m}$ ; Fig. 7D), and midget and blunt microspines (less than 1  $\mu\text{m}$ ; Fig. 7D-E).  
234 Microspines can be classified into two types of aggregations: type I, composed of 1–4  
235 elongated microspines (occasionally higher numbers can be observed) and up to 200  
236 midget microspines surrounding the elongated microspines (Fig. 7C-D, F); and type II,  
237 composed of more than 200 midget microspines (Fig. 5E-F). Type I aggregations form

238 longitudinal bands in the tip and lateral sides of the hindgut folds, while those belonging  
239 to type II are intercalated between two parallel type I aggregations (Fig. 7A, F).

240         *Connective-like tissue and musculature.* The presence of a connective-like tissue  
241 is unclear in the larvae. It may be the single cell layer that surrounds perimeter of the  
242 outer circular musculature (Fig. 2D). In adults, a wide layer of connective-like tissue  
243 surrounds the epithelium, the rosette glands, the inner longitudinal muscles located  
244 inside the folds, and the periphery of the outer circular musculature (Fig. 3C-E).  
245 Occasional haemolymph have been identified in the outer connective-like layer located  
246 on the periphery of the outer circular musculature, probably corresponding to the blood  
247 sinuses denominated by Wirkner and Richter (2013) (Fig. 3E). The inner longitudinal  
248 musculature has a characteristic organisation in larvae (Figs. 2; 4-5). In a transversal  
249 section, the inner longitudinal muscle cells are columnar and occupy the centre of the  
250 folds, being surrounded by a single layer of epithelial cells. The epithelial and inner  
251 longitudinal muscle cells are connected by electron-dense junctions similar to the  
252 hemiadherens junctions described by Bitsch and Bitsch (2002) (Fig. 5). The packs of  
253 myofibrils are located on the apex of the inner longitudinal muscle cells (Figs. 2; 4-5).  
254 In adults, the inner longitudinal musculature comprises numerous bundles located inside  
255 the folds (Fig. 3). The outer circular musculature of larvae and adults forms a thin band  
256 on the periphery of the organ (Figs. 2-4).

257         *Rosette glands.* Rosette glands are absent during larval stages (Fig. 2B-D) but  
258 are found during adulthood (Fig. 8A,C). The distribution pattern of the rosette glands is  
259 still unclear (Fig. 3A). Albeit observed along the entire hindgut length, in some  
260 histological sections the rosette glands are very scarce or absent (Fig. 3C-E) and in  
261 others they are very abundant (Fig 8C). These glands are globular clusters of cells  
262 composed of gland cells surrounding a slender central duct, which is formed by duct

263 cells (Fig. 8A-B, D-E). The gland cells are pyramidal (Fig. 8A-B, D-E). The cytoplasm  
264 has a foamy appearance due to the abundance of vesicles, whose content has variable  
265 staining affinity (Fig. 8A). The vesicles have a variable degree of fusion among them  
266 and show a variable electron-density (Fig. 8E-F). The gland cells are rich in Golgi  
267 complexes composed of numerous densely packed cisternae (Fig. 8E, G). The central  
268 duct of the rosette glands is formed by cells which cytoplasm does not contain vesicles  
269 (Fig. 8E, H). The central duct is lined by a very thin cuticle (Fig. 8H).

#### 270 **4. Discussion**

271 The hindgut tract of *M. brachydactyla* is a large, straight tube without  
272 differentiated regions, excluding the rectum. This morphology has been observed in  
273 other crustacean species (Reddy 1937; Pugh 1962; Holdich and Ratcliffe 1970;  
274 Yoshikoshi 1975; McLaughlin 1983; Schmitz and Scherrey 1983; Günzl 1991), and it  
275 differs from that of insects, in which it is subdivided into highly specialised regions, e.g.  
276 pylorus and ileum (Richins 1938; Areekul 1957; Klowden 2013; Rowland and  
277 Goodman 2016). In Myriapoda, the hindgut is a large tube resembling that described in  
278 the present study (Nardi et al. 2016), while in some Arachnida it is reduced to a short  
279 anal atrium (Mathieson and Lehane 2002; Talarico et al. 2011). The independent  
280 evolution of the digestive system might explain the divergent morphological differences  
281 reported among the above mentioned arthropods.

282 Our findings reveal that the hindgut of *M. brachydactyla* larvae is formed by  
283 five folds composed of inner longitudinal muscle cells occupying the centre of each fold  
284 and attached to the epithelial cells, the periphery is surrounded by an outer circular  
285 musculature separated by a thin basal lamina. This morphology has not been reported to  
286 date. Previous studies of decapod larvae did not describe the morphology of the hindgut

287 folds (Schlegel 1911; Factor 1981; Mikami et al. 1994; Abrunhosa and Kittaka 1997).  
288 The hindgut morphology of other arthropods differs greatly from that of *M.*  
289 *brachydactyla* described herein: 1) in many insects (including larval stages), no muscles  
290 are present inside the folds (Woods 1918; Mathur 1973; Diaz et al. 1998), and  
291 longitudinal and circular muscles occasionally transpose positions between the ileum  
292 and colon (Woods 1918; Potts 1927); 2) in isopods, the musculature forms a square  
293 mesh network (Holdich and Ratcliffe 1970; Holdich and Mayes 1975); and 3) in  
294 tardigrades, the musculature consists of two pairs of muscles (Dewel and Dewel 1979).  
295 However, if musculature and epithelium are separated by a wide connective-like layer,  
296 then an adult-like morphology is described, which is similar to the reported in several  
297 adult decapods such as crabs (Barker and Gibson 1978; Erri Babu et al. 1982; Heeren  
298 and Mitchell 1997), clawed lobsters (Barker and Gibson 1977), and crayfishes (Chisaka  
299 et al. 1999). The hindgut of *M. brachydactyla* shows inner longitudinal muscles more  
300 developed than the outer circular muscles. Given these muscular features, we propose  
301 that the main movement of the hindgut involves longitudinal contraction waves. These  
302 waves, helped by folds and microspines, may allow the transport of waste materials, as  
303 discussed below.

304         The similarities of the hindgut of *M. brachydactyla* between larval and adult  
305 stages might be a reason to not consider a truly metamorphic change. However, four  
306 major features of the hindgut of *M. brachydactyla* suffer a certain transformation during  
307 the development: 1) the morphology and ultrastructure of the epithelium, 2) the  
308 morphology of the microspines, 3) the formation of a wide layer of connective-like  
309 tissue, and 4) the apparition of the rosette glands. The role of each one these structures  
310 is discussed below, then a hypothesis to explain such transformation will be proposed.

311           The larval epithelial cells have squamous shape and abundant microvilli-like  
312 extensions, the latter are associated with the formation of new cuticle (Dillaman et al.  
313 2013), an expected role considering the short intermoult intervals during the larval  
314 development (Guerao et al. 2010; Pazos et al. 2018). The larval epithelial cells show  
315 two characteristic features: epithelial-to-muscle cell hemiadherens junctions and  
316 bundles of fibres structures; both are associated with an arthropod cell type called  
317 "tendon cells", i.e. specialized epithelial cells that connect the cuticle with the  
318 underlying muscular cells (Nakazawa et al. 1992; Bitsch and Bitsch 2002; Žnidaršič et  
319 al. 2012), which have been identified in insects (Smit and Akster 1974; Reedy and Beall  
320 1993), crustaceans (Nakazawa et al. 1992; Žnidaršič et al. 2012), and arachnids (Smith  
321 et al. 1969; Beadle 1973). The adult epithelial cells show abundant mitochondria in the  
322 apical region, and infolded membranes with lateral junctions. Such features are  
323 associated with the reabsorption of water and ions in several arthropods, including  
324 insects, crustaceans and millipedes (Mykles 1979; Phillips et al. 1987; Nardi et al. 2006;  
325 Nardi et al. 2009; Nardi et al. 2016; Bogataj et al. 2018). Moreover, the fibres structures  
326 of the epithelial cells are more developed in adults than in larvae, and have also been  
327 proposed to be involved in the intracellular transport of water and ions in different  
328 crustacean groups (Komuro and Yamamoto 1968; Witkus et al. 1969; Vernon et al.  
329 1974). Adult cells also have features of tendon cells: fibres structures crossing the cell  
330 height and anchored to conical invaginations of the apical membrane, which are  
331 described in this study as "apical complexes" (Smith et al. 1969; Beadle 1973;  
332 Nakazawa et al. 1992; Reedy and Beall 1993; Žnidaršič et al. 2012). Since the fibres  
333 structures have two potential roles (intracellular transport and mechanical connection),  
334 it raises one question: are both roles inclusive or mutually exclusive? Bogataj et al.  
335 (2018) suggested both roles for the hindgut epithelial cells of the woodlouse. In our

336 opinion, the hindgut epithelial cells of the larvae and adults probably are tendon cells,  
337 connecting the muscular action with the cuticle, but in adults the same cells also  
338 develop a sophisticated transport system for osmoregulation.

339 *M. brachydactyla* larvae show separate individual microspines, while two types  
340 of aggregations are observed in adults. The presence of microspines in the hindgut is  
341 widely observed among arthropod groups (Tables 1-3). The morphology of microspines  
342 in *M. brachydactyla* varies spatially, as occurs in other adult malacostracans (Chisaka et  
343 al. 1999; Moon and Kim 1999), insects (Byers and Bond 1971; Elzinga and Hopkins  
344 1995) and millipedes (Miyoshi et al. 2005). The role of microspines is unknown;  
345 however, it has been proposed that they: 1) attach the peritrophic membrane, thus  
346 promoting the backward movement of faeces (Hopkin and Nott 1980; Felder and  
347 Felgenhauer 1993) and preventing their forward movement under conditions of anal  
348 water intake (Felder and Felgenhauer 1993); 2) shred the peritrophic membrane, thereby  
349 facilitating water and ion absorption (Byers and Bond 1971); and 3) serve as binding  
350 sites for microbial communities (Harris 1993a; Cazemier et al. 1997; Elzinga 1998;  
351 Nardi et al. 2016). We agree with these roles for microspines in *M. brachydactyla*,  
352 excepting the shredding of the peritrophic membrane, since it is excreted undamaged.

353 The rosette glands of the hindgut of *M. brachydactyla* were observed only in  
354 adults. Similar glands appear in the hindgut of other adult decapods (Barker and Gibson  
355 1977; Barker and Gibson 1978; To et al. 2004), as well associated to other body  
356 structures, e.g. shrimp gills (Doughtie and Rao 1982) and mouthparts (Alexander 1989),  
357 crab oesophagus (Castejón et al. 2018c), ghost shrimp pereopods (Dworschak 1998),  
358 and clawed lobster pleopods (Talbot et al. 1991). Despite being distributed widespread  
359 within the decapods, to our knowledge the presence of those glands in non-crustacean  
360 arthropods is non-described. We did not find evidence of duct openings on the



361 epithelium or cuticle, coinciding with previous authors (Barker and Gibson 1977;  
362 Barker and Gibson 1978; To et al. 2004). The fact that rosette glands are associated to  
363 different body structures in other decapods might suggests that they are different glands  
364 sharing morphology and staining affinity, but looks unlikely. The staining affinity of  
365 rosette glands coincides with that observed in previous studies done in other crabs,  
366 which reported a content comprising neutral and acid mucopolysaccharides, including  
367 sulphated mucopolysaccharides, sulphated sialomucins and hyaluronic acid (Erri Babu  
368 et al. 1979; Trinadha Babu et al. 1989). Considering their location and the type of  
369 secretion, the rosette glands located in the hindgut could produce lubricant barriers  
370 against abrasive and toxic agents and against pathogens. *M. brachydactyla* presents a  
371 terminal moult after maturation (González-Gurriarán et al. 1993; Corgos et al. 2011),  
372 while larvae moult frequently (Guerao et al. 2010). Therefore, adults will benefit from  
373 the protection of this secretion, while the secretion and rosette glands are redundant in  
374 larvae as their cuticle is renewed regularly. Further studies are necessary to elucidate the  
375 role of the rosette glands.

376 In our opinion, the hindgut of *M. brachydactyla* does not realize a true  
377 metamorphic change during the development. Still, a few major changes were observed.  
378 In our opinion, these changes can be explained by the animal size, i.e. the carapace  
379 length increases from around 1.1 mm in the the zoea I (Guerao et al. 2008), to more than  
380 150 mm in late juveniles and adults (Guerao and Rotllant 2010). The small size during  
381 the larval stages could facilitate the movement of water and ions through the epithelium  
382 without requiring a sophisticated transport system like the adults. Moreover, the smaller  
383 hindgut of the larvae precise thinner musculature to generate the peristalsis required for  
384 excretion, while the adult hindgut requires a more developed musculature to move  
385 larger masses, which in turn requires a wide connective-like tissue and blood irrigation

386 for accommodation and maintenance. If the rosette glands participate in the cuticle  
387 maintenance, then their absence during the larval development can be easily explained  
388 because the larvae moult in short time periods, on the contrary moulting and cuticle  
389 restoration ceased when adulthood is reached (González-Gurriarán et al. 1993; Corgos  
390 et al. 2011). The crustacean literature support this hypothesis. Small sized crustaceans  
391 (hindgut diameter 150 µm or less) have a hindgut without connective-like tissue that  
392 resembles the larval hindgut described in this study (Wägele et al. 1981; Schmitz and  
393 Scherrey 1983; Herrera-Alvarez et al. 2000); while big sized crustaceans (hindgut  
394 diameter 800 µm or higher) have a wide hindgut with a developed connective-like tissue  
395 resembling the adult hindgut described in this study (Barker and Gibson 1977; Barker  
396 and Gibson 1978; Erri Babu et al. 1982; Heeren and Mitchell 1997; Chisaka et al.  
397 1999). We suggest two complementary methods to test the proposed hypothesis. The  
398 first method will consists in analysing the hindgut morphology in the entire  
399 development of a big sized species, with special attention to juvenile stages to establish  
400 the start of the formation of the connective-like tissue. Alternatively, the hindgut of taxa  
401 with significant size differences can be compared. In both cases, a size disparity around  
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702 **Figure 1.** *Maja brachydactyla*. Digestive tract, fixed in 4 % formaldehyde. Adult, the  
703 midgut gland ("hepatopancreas") has been removed (A). Megalopa, the midgut gland  
704 has been removed (B). Average length of the hindgut tract on each day during larval  
705 development (C). Abbreviations: Jv, juvenile; Mg, megalopa; ZI, zoea I; ZII, zoea II.

706 **Figure 2.** *Maja brachydactyla*. Hindgut tract of larvae. Light and electron microscopical  
707 analyses. General diagram (A). Zoea II, PAS contrasted with Methylene Blue (B-C):  
708 longitudinal (B) and transversal sections (C). Megalopa, transversal section, TEM (D).  
709 Abbreviations: C, cuticle; CM, outer circular muscles; CT, connective-like tissue; EC,  
710 epithelial cells; LM, inner longitudinal muscles; Ms, microspines.

711 **Figure 3.** *Maja brachydactyla*. Hindgut tract of adults. Light microscopical analyses.  
712 Mallory's trichrome stain. General diagram (A). Detailed view of the epithelium (B).  
713 Transversal (C) and longitudinal sections (D). Detailed view of the connective-like  
714 tissue, longitudinal section (E). Abbreviations: BS, blood sinus; C, cuticle; CM, outer  
715 circular muscles; CT, connective-like tissue; E, epithelium; Ep, epicuticle; LM, inner  
716 longitudinal muscles; Pr, procuticle.

717 **Figure 4.** *Maja brachydactyla*. Hindgut tract of megalopa larvae. Ultrastructure (TEM)  
718 of the hindgut fold. General diagram (A). General view, transversal section (B). Detail  
719 of the outer circular musculature, high magnification of the square "D" marked in the  
720 picture B (C). Abbreviations: asterisk, epithelial-to-muscle cell junction; BL, basal  
721 lamina; C, cuticle; CM, outer circular muscles; EC, epithelial cell; EEJ, epithelial-to-  
722 epithelial cell junction; LM, inner longitudinal muscle cell; Mt, mitochondria; Ms,  
723 microspines; My, myofibrils; RER, rough endoplasmic reticulum.

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725 **Figure 5.** *Maja brachydactyla*. Hindgut tract of megalopa larvae. Ultrastructure (TEM)  
726 of the epithelial and muscle cells. Transition from the basal lamina to the epithelial-to-  
727 muscle cell junction (A). Detailed view of an epithelial cell (B). Detailed view of an  
728 epithelial-to-epithelial cell junction (C). Detailed view of an epithelial-to-muscle cell  
729 junction (D). Abbreviations: asterisk, epithelial-to-muscle cell junction; BL, basal  
730 lamina; C, cuticle; EC, epithelial cell; EEJ, epithelial-to-epithelial cell junction; Ep;  
731 epicuticle; FS, fibres structures; LM, inner longitudinal muscle cell; Mt, mitochondria;  
732 My, myofibrils of the inner longitudinal muscle cells; Ms, microspines; Mv, microvilli-  
733 like extensions; Pr, procuticle; RER, rough endoplasmic reticulum.

734 **Figure 6.** *Maja brachydactyla*. Hindgut tract of adults. Ultrastructure (TEM) of the  
735 epithelial cells. General diagram (A). General view: apex of the cell, supranuclear  
736 region (B), and base of the cell, infranuclear region (C). Cell apex, detail of the  
737 epithelial-to-epithelial cell junction (D). "Apical complex", high magnification (E).  
738 Mitochondria and fibres structures, high magnification (F). Abbreviations: AC, "apical  
739 complex"; BI, basal invaginations; BL, basal lamina; C, cuticle; EEJ, epithelial-to-  
740 epithelial cell junction; LI, lateral interdigitations; FS, fibres structures; Mt,  
741 mitochondria; N, nucleus; RER, rough endoplasmic reticulum.

742 **Figure 7.** *Maja brachydactyla*. Hindgut tract. Microspines (SEM). Distribution of the  
743 microspines in adults, general diagram (A). Zoea I (B). Adult: aggregation type 1  
744 covered by bacillus-shaped bacteria (C), aggregation type 1 with sparse bacteria (arrow-  
745 heads) (D), aggregation type 2 (E), and transition between aggregation type 1 and type 2  
746 (F). Abbreviations: AT1, aggregation type 1; AT2, aggregation type 2; EM, elongated  
747 microspines; MM, midget microspines.

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749 **Figure 8.** *Maja brachydactyla*. Hindgut tract. Rosette glands. Adult. General view, PAS  
750 and Alcian Blue contrasted with Hematoxylin (A). General diagram of the rosette  
751 glands (B). Glandular masses (bluish) in the hindgut tract, PAS contrasted with  
752 Methylene Blue (C). General diagram of the gland and duct cells (D). General view of  
753 the gland and duct cells, TEM (E). Detailed view of the vesicles (showing differential  
754 electron-density) of the gland cells, TEM (F). Detailed view of the Golgi cisternae of  
755 the gland cells, TEM (G). Detailed view of the cytoplasm and cuticle lining of the duct  
756 cells, TEM (H). Abbreviations: C, cuticle; CD, central ducts; CT, connective-like tissue;  
757 DC, duct cells; E, epithelium; G, Golgi cisternae; GC, gland cells; RG, rosette glands;  
758 V, vesicles.

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