Biomechanics of pleon attachment in the European shore crab *Carcinus maenas* (Linnaeus, 1758) (Brachyura: Portunoidea: Carcinidae)

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ABSTRACT

Brachyuran crabs have a distinct outer morphology with a highly reduced pleon, which is folded underneath a usually broad cephalothorax. The pleon is secured in this position with a specialised holding mechanism, which consists of a sternal knob, or press-button, and, in most eubrachyurans, a corresponding pit or socket on the pleon. The effectiveness of this mechanism has not been described so far. In this study a method to investigate the force needed to move the pleon from its folded position was measured in male and female crabs under different experimental conditions is presented. In male specimens a median pull-off force of 167.20 mN was measured in air. The measured force decreased slightly underwater. When the press-buttons were removed artificially, no pleon attachment was feasible underwater. The variation observed in the pull-off forces measured is most likely a result of different levels of wear or damage of the holding structures. In mature females, which lack these structures, no pleon attachment was possible underwater. In air, residual moisture and the morphology of the pleon resulted in attachment via capillary interactions. The method presented in this study can be used in further investigations to evaluate the effectiveness of pleon-holding structures in various brachyuran crabs.

Key Words: force measurements, functional morphology, pleon-holding mechanism, true crabs, abrasive wear

INTRODUCTION

Brachyuran crabs have a rather distinct outer morphology. In contrast to other decapod crustaceans, they have undergone massive morphological changes in the course of their evolution. Due to the so-called carcinization (*sensu* Martin & Abele, 1986), the pleon is reduced to a thin shield and folded underneath the broad cephalothorax. Unlike other decapods with a crab-like habitus, such as the anomurans of families Paguridae Latreille, 1802, Aeglidae Dana, 1852, Galatheidae Samouelle, 1819, and Porcellanidae Haworth, 1825 (e.g., Borradaile, 1916; Martin & Abele, 1986; Keiler *et al.*, 2015), the pleon is secured in this folded position by a so-called pleon-holding mechanism (e.g., Duvernoy, 1850; Pérez, 1928a; Guinot 1979). Guinot & Bouchard, (1998) described different attachment systems, with the most common within eubrachyurans being a press-button.

The press-button mechanism consists of a knob or protrusion on the fifth thoracic sternite, the so-called press-button, and a socket or pit on the sixth somite of the pleon. Both structures come into contact when the pleon is folded and engage closely, thus the name "press-button". This press-button is found in adult males and juveniles of both sexes in most eubrachyurans, but there are some cases where adult males lack the mechanism as in some species of sesarmids and ocypodids (Duvernoy, 1853; Pérez 1928b; Guinot & Bouchard, 1998). If reported, adult females seem to be lacking the mechanism, whereas it is still present in some families considered more basal, such as Dorippidae MacLeay, 1838 and Calappidae De Haan, 1833 (Guinot, 1979; Guinot & Bouchard, 1998; Guinot *et al.*, 2013; Davie *et al.*, 2015), and some less basal taxa, such as Hexapodidae Miers, 1886, Palicoidea Bouvier, 1898, Parthenopoidea MacLeay, 1838, Retroplumidae Gill, 1894, Sotoplacidae Castro, Guinot & Ng, 2010, and Oregoniidae Garth, 1958 (Guinot & Bouchard, 1998; Guinot & Quenette, 2005; Guinot *et al.*, 2010; Castro *et al.*, 2010; Guinot *et al.*, 2013). The reasons for this variation remain unclear.

The European green crab *Carcinus maenas* (Linnaeus, 1758) (family Carcinidae MacLeay, 1838) is a bottom dweller, scavenging on mussels, crustaceans, and benthic detritus (e.g., Crothers, 1968). It is also capable of burying itself in the substrate (Cumberlidge & Uglow, 1978; Bellwood, 2002). Adult males of *C. maenas* feature the press-button mechanism, whereas the structures are often highly reduced or even lacking in adult females (Pérez, 1928a). Males have a narrow triangular pleon with only the first two pairs of pleopods present and modified into gonopods (Crothers, 1967). The pleon can be firmly inserted into a depression in the middle of the sternal plates, the so-called sterno-abdominal cavity (e.g., Guinot & Bouchard, 1998). Due to allometric growth, adult female *C. maenas* have a wider pleon, which exhibits a row of marginal setae (Demeusy, 1958; Crothers, 1967; Kristensen *et al.*, 2012). Furthermore, in females, all pleopods are present except the first pair, and the pleopods are biramous and highly setose to allow egg carrying (Demeusy, 1958; Crothers, 1968; Guinot, 1979). The sterno-abdominal cavity is distinctly less deep and much wider than in males (Guinot 1979; Guinot & Bouchard, 1998).

Carcinus maenas exhibits a range of carapace colours. Juveniles show various camouflaging patterns (e.g., Brian *et al.*, 2006; Todd *et al.*, 2006), whereas adults are most often referred as belonging to the green or red colour form (McGaw *et al.*, 1992). These adult colour forms show differences in distribution, epibiont loads, salinity preference, intermoult duration, and carapace thickness (McGaw & Naylor, 1992; McGaw *et al.*, 1992; Reid *et al.*, 1997). McGaw *et al.* (1992) suggested that the red colouration in adult *C. maenas* is a result of prolonged intermoult duration. All individuals are green immediately after moult and turn red over the intermoult interval due to photo-denaturation of carapace pigments (Reid *et al.*, 1997).

So far only information on the presence or absence of the press-button structures has been published for various species of brachyuran crabs (e.g., Guinot, 1979; Guinot & Bouchard, 1998; Bellwood, 1996; Guinot *et al.*, 2013). The mechanism is hypothesized to optimize the use of energy resources for locomotion and reproduction (Guinot & Bouchard, 1998). It is so far unknown how effective the holding mechanism is. To provide insight into this question, pull-off force was applied to the pleon of freshly dead specimens belonging to both colour forms of *C. maenas* to evaluate the effectiveness of the mechanism. We tested how much pull-off force could be applied before the attachment mechanism fails without the activity of the pleonal musculature and thus provide a method to objectively and reproducibly assess its functionality. Male and female crabs were tested under different environmental conditions and after different treatments.

MATERIAL AND METHODS

Specimens

A total 53 adult specimens of *C. maenas* (30 males, 23 females) where collected between March and November 2015 on Sylt, northern Germany (North Sea) in cooperation with the AWI Wattenmeer Station Sylt, and in the Kiel Fjord, northern Germany (Baltic Sea) in cooperation with the Aquarium Geomar, Kiel. Crabs where maintained in aerated seawater (32 ppt for specimens from Sylt, 19 ppt for specimens from Kiel Fjord) at the Zoological Museum Kiel prior to testing. All crabs appeared to be in good condition, with all appendages intact and no excessive load of ectoparasites (< 5). No moulting was observed in the laboratory prior to testing and all crabs showed a hard exoskeleton. The pleon of one specimen was damaged on both sides of the sixth somite. One side was completely torn off, the other only partially. Specimens were individually euthanised using chloroform (Carl Roth, Karlsruhe, Germany) prior to testing. For further

details see Table 1 as well as Table S1 in the supplementary material available at *Journal of Crustacean Biology* online.

Measurements of force

Freshly euthanised specimens were secured on their dorsal side so that only the pleon was able to move, when a pull-off force was applied to the pleon (Fig. 1).

A small (2 mm) hole was drilled using a Proxxon IB/E drill (Proxxon, Trier, Germany) into the middle portion of the sixth pleonic somite between the two sockets of the holding mechanism. A piece of string was inserted into the hole and firmly fixed in this position with ergo 5925 elastomere "super glue" (Kisling AG, Wetzikon, Switzerland). The string was attached to a horizontal mechanical force transducer (World Precision Instruments, Sarasota, FL, USA) and a MP 100 data acquisition system (BIOPAC Systems Inc., Goleta, CA, USA), which was connected to a PC and controlled with the software AcqKnowledge 3.7. The system was first equipped with a 1000 g force transducer to test the maximum force that the holding mechanism could resist. If the forces measured with this transducer were below 100 g, a more precise 100 g transducer was used. This was the case in all but two specimens from the North Sea. Data acquisition rate was set to 200 Hz. For calibration of the force transducer with the string attached to it, a weight of 20 g for the 100 g force transducer and a weight 100 g for the 1000 g transducer were used, respectively. The force transducer was attached to a vertical metal pole, positioned perpendicularly above the specimen and a pull-off force was applied by manually moving the force transducer along the metal pole until attachment of pleon to the body was broken, i.e. until the pleon could be moved from its folded position. The pull-off force at the time of disconnection was recorded (Fig. 2).

After each trial, the pleon was reattached by pushing it back to the closed position. Every measurement was performed ten times to reveal possible fatigue of the material (Boulding & Labarbera, 1986; Zum Gahr, 1987; Zioupos *et al.*, 1996; Suresh, 2004; Vincent & Wegst, 2004) in multiple attachment-detachment cycles for each specimen. If no decline could be detected in a specimen, the mean force was calculated prior to further statistical analysis in order to estimate the force range of individual press-buttons. The experiments were performed under different environmental conditions and different treatments (see flowchart in Fig. 3).

All 30 male specimens were measured in air in their naturally wet condition. The sternal press-buttons were subsequently removed in seven specimens by polishing them off with a polishing attachment for the MicroMOT 50/E. Cuticular debris from the polishing process was removed. The sternal plates and the pleon were rewetted after the polishing process to exclude artefacts caused by drying. Ten specimens were submerged completely (Fig. 1) and tested underwater, first with intact press-buttons, subsequently with these removed. Eleven specimens were stored in 75% ethanol (Carl Roth GmbH, Karlsruhe, Germany) directly after the measurements in air to be processed for scanning electron microscopy (SEM). Three specimens were also taken for SEM analysis after underwater tests (Fig. 3).

All 23 female specimens were similarly measured in air under naturally wet conditions. The sterno-abdominal cavity and the pleon of 12 specimens were subsequently dried with filter paper.

Table 1. List of the specimens used, sorted by collection site and sex. The ratio of crabs of each colour form per collection site is given in brackets.

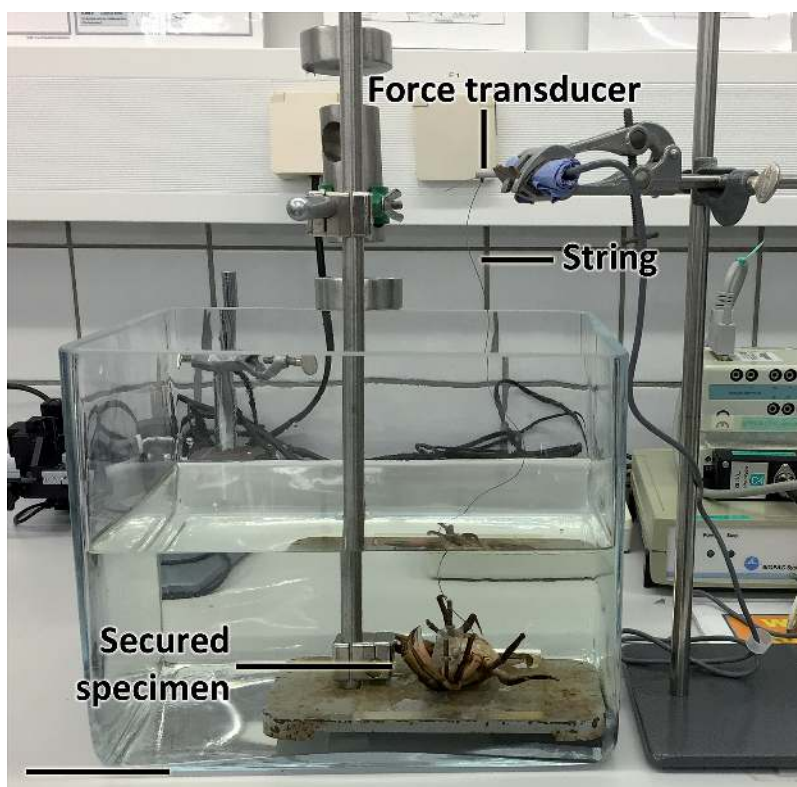| Sex (colour form)	Kiel Fjord, Baltic Sea	Sylt, North Sea
Male (red: green)	22 (20:2)	8 (5:3)
Female (red: green)	17 (8:9)	6 (6:0)

Figure 1. Experimental setup. A freshly euthanised male *C. maenas* was secured on its dorsal surface with a string attached to the pleon. The other side of the string was attached perpendicular to the force transducer. The specimen was submerged in water in this particular experiment. Scale bar = 5 cm.

In another ten specimens, the setae along the edge of the pleon and all pleopods were removed (Fig. 3).

Video and SEM imaging

The movements of the pleons of three male and two female specimens were recorded with a high-speed video camera (Photron Fastcam 1024 PCI, Photron Europe, West Wycombe, Bucks, UK) at 500 frames per second while force measurements were taken in air to document the moment of detachment of the pleon from the sternal plates. Males were filmed both with an intact mechanism and the press-buttons removed.

The pleon-holding structures of 14 male specimens were dissected for a more detailed examination of the surface of the attachment system. The structures were dehydrated in an ascending ethanol series, dried with a Polaron E3000 (Quorum Technologies, Laughton, UK) critical point dryer and subsequently sputter-coated with 15 nm of gold-palladium using a Leica EM SCD500 (Leica Microsystems, Wetzlar, Germany) high-vacuum sputter coater). The samples were viewed with a scanning electron microscope Hitachi TM3000 (Hitachi, Tokyo, Japan) at 15 kV using a backscattered electron (BSE) detector.

Statistical analysis

All statistical analyses were performed using R 3.1.1 (http://www.r-project.org//) and RStudio 0.99.441 (http://www.rstudio.com//) for MacOSX. The force measurement data were imported into R and tested for normal distribution with a Shapiro-Wilks test. Data not fitting a normal distribution were transformed by logarithm for further parametrical tests. Welch Two sample t-test was applied to test for variation due to collection site and colour form. A linear regression analysis was used to test for force variation due to size (carapace width, measured with calipers) and weight. Paired t-test was used to evaluate different experimental

conditions and different animal treatments (e.g., intact against button-less males, wet against dry females).

RESULTS

Attachment forces in males

The median pull-off force that could be applied to the pleon of adult male *C. maenas* before the pleon moved from its folded position was 167.20 mN ± 238.59 mN (N = 30), which was an average of 40.33% of the specimen's body weight (46.93 g ± 12.92 g). It showed a wide variation between the specimens, with the highest force of 1,127.57 mN ± 291.43 mN (73.32 g; 181.63%) in one individual (Fig. 4 and supplementary material video S2) and a minimum of 44.46 mN ± 4.12 mN (59.91 g; 7.56%) in another specimen. No apparent decline was observed over the course of the 10 measurements made on each specimen. A directly observable fatigue of the system (*sensu* Boulding & Labarbera, 1986; Zum Gahr, 1987; Zioupos *et al.*, 1996; Suresh, 2004; Vincent & Wegst, 2004) could be ruled out and the ten measurements were averaged for further statistical analysis.

Five specimens exhibited pull-off forces of below 100 mN. In the majority of 18 of the 30 specimens, however, a force between 100 and 300 mN was measured. Five specimens showed higher forces of 300 to 500 mN, whereas two specimens from the North Sea showed exceptionally high median forces of over 500 mN (1,127.57 mN ± 291.43 mN and 937.20 mN ± 424.94 mN).

All force measurements were not distributed normally and therefore were transformed by logarithm for further statistical analysis. No direct correlation between the pull-off force measured in air and the collection site (P = 0.115) or the colour form (P = 0.766) could be detected with a Welch Two sample t-test. Additionally, no correlation was found between the force necessary in air and the carapace width (P = 0.102) or the weight
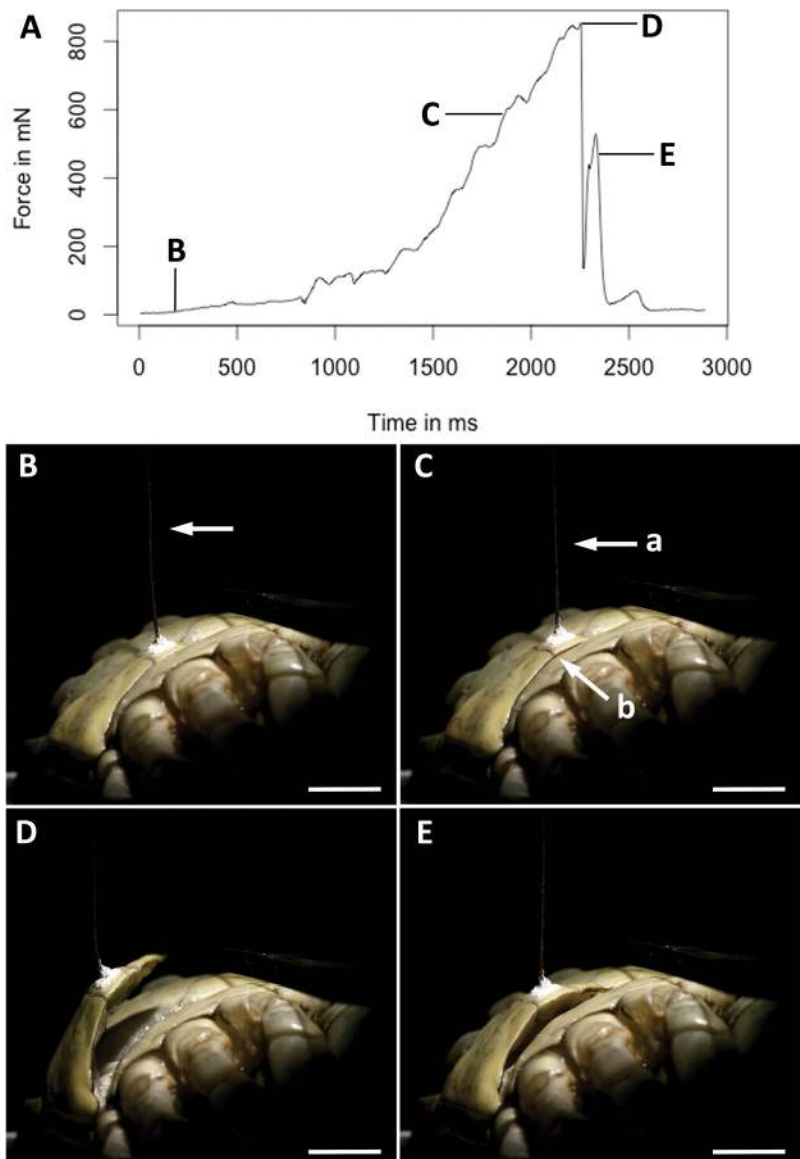
Figure 2. Representative experimental trial. (**A**) Typical force-time curve. Specific force values of important phases are indicated by letters (**B–E**) referring to the still frames below. The complete video sequence is available at *Journal of Crustacean Biology* online as Supplementary Material S2 Video. (**B**) Pleon closed in folded position, no pull-off force applied, string in relaxed position (arrow). (**C**) Application of force has started, the string is in tension (**a**) and a small gap forms between the pleon and the sternal plates (**b**) while the pleon is still attached. (**D**) Attachment press-button fails, pleon snaps out of the fixed position: this causes a sharp drop in the force. (E) Relaxation period, freely movable pleon is pulled upwards, then lowered back onto sternal plates. Scale bar = 1 cm.

of the individuals ($P = 0.118$) via linear regression. A median force of 84.74 mN ± 90.59 mN ($N = 10$) was recorded underwater. When comparing the force in air (102.74 mN ± 70.53 mN) to the force underwater in the same specimens via paired t-test, the force decreased slightly, but significantly, underwater ($P = 0.037$).

When the press-buttons were removed, the median pull-off force decreased significantly ($P < 0.001$) to 29.50 mN ±19.62 mN ($N = 7$) in comparison to the same individuals with intact structures (228.91 mN ± 112.37mN; Fig. 4 and supplementary material video S3). Only in one specimen a force of over 100 mN (105.50 mN ± 16.21 mN) was necessary after removal of the press-buttons. In the other six specimens, a force of less than 60 mN was measured, the lowest being 23.50 mN ± 8.28 mN. When the crabs without the press-buttons were submerged, the pleon did not stay in the folded position and tended to open.

SEM of males

The pleonal socket of the holding mechanism of males has a prominent, thickened edge along the anterior and lateral margins (Fig. 5). Some cuticular folds are visible on the inner wall of the socket (Fig. 5A–C, E). The sternal press-button of *C. maenas* is slightly asymmetrical, in accordance with Bouchard (2000), with a steeper inclination on the anterior side (Fig. 6). As illustrated by the SEM images (Figs. 5A, 6A), the pleon-holding structures of *C. maenas* do not show any distinct additional microstructures such as an apical hook comparable to the bythograeid crab *Austinograea alayseae* Guinot, 1990 or other regular structures like those in the plagusiid *Plagusia immaculata* Lamarck, 1818 (Guinot & Bouchard, 1998). Some folds are visible on the sternal projection, if intact, in *C. maenas* (Fig. 6A).

SEM images of the pleon-holding mechanism also revealed a wide variety of surface conditions. The surfaces of the structures
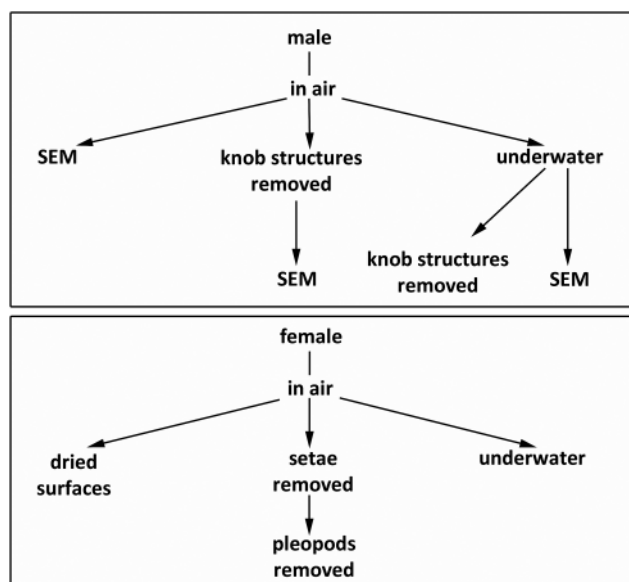
Figure 3. Flowchart showing different experimental conditions and animal treatments used in the study.
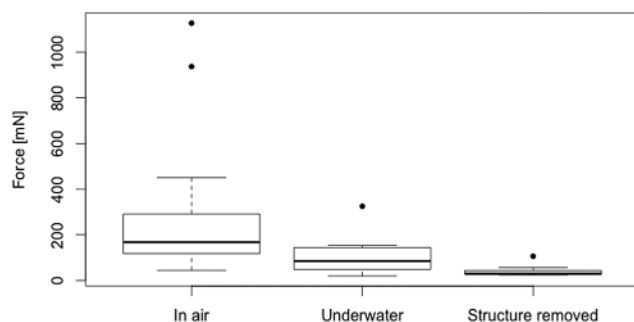

Figure 4. Results of the force measurements on male *Carcinus maenas* under different conditions: *In air*, intact wet specimens ($N = 30$); *Underwater*, intact specimens completely submerged ($N = 10$); *Structure removed*, sternal press-button structures are removed by polishing, specimen is in the wet condition ($N = 7$).

were in an entirely clean state without any foreign material (Figs. 5A, 6B) to instances where the surfaces were nearly completely covered with debris (Figs. 5C, 6C). Additionally, the cuticle was either intact (Figs. 5A, 6A), slightly flattened and indented to various degrees (Figs. 5B–D, 6B–D), or even severely torn off (Figs. 5E–F, 6E–F).

Attachment forces in females

The median pull-off force in adult female *C. maenas* in wet condition was 240.16 mN ± 167.25 mN ($N = 23$) which was 189.41% of the body weight of individuals (14.94 g ± 4.60 g).

There was nevertheless a wide variation among the specimens (Fig. 7 and supplementary material video S4). Only in one specimen a force below 100 mN was measured (92.76 mN ± 9.79 mN; 11.56 g; 63.45%). Forces between 100 and 300 mN were measured in 14 specimens. Seven specimens showed forces between 300 and 600 mN. A force of over 600 mN was measured (629.09 mN ± 40.57 mN; 18.94 g; 377.58%) in only one specimen.

When the sternal plate and the pleon were artificially dried with filter paper, the pull-off force decreased significantly to a median of 53.75 mN ± 16.98 mN ($N = 12$). The same effect was found when the setae on the edge of the pleon were removed (81.29 mN ± 37.09 mN; $N = 10$) or when both the setae and the pleopods

were removed (41.03 mN ± 24.57 mN; $N = 10$). The pleon did not stay in the folded position in specimens underwater regardless of the presence or absence of setae and pleopods.

DISCUSSION

Males

The firm pleon attachment known from male *C. maenas* (Duvernoy, 1850; Kollmann, 1937; Demeusy, 1958) could be clearly documented with the method presented here. The pleon attachment is solely maintainable by the press-button mechanism in males. The results support the idea that the male pleon-holding mechanism is a method to save muscle energy in *C. maenas* (Guinot & Bouchard, 1998).

The pull-off force needed to move the pleon from a folded to the unfolded position varied greatly between specimens (Fig. 4), but the variation could not be attributed to the collection site, size, and weight or colour form of the specimen. The lack of a detectable difference between the collection sites suggests, that there are no regional differences in the calcification of the cuticle.

The red colour form prevails in larger specimens (McGaw *et al.*, 1992). Different cuticle properties are most likely related to these colour forms (Brian *et al.*, 2006). No direct correlation between the effectiveness of the pleon-holding mechanism and the colour form and its associated differences in cuticle properties could be detected in our study.

The time intervals between moulting events is assumed to be longer in red individuals (McGaw *et al.*, 1992). Available energy is most likely redirected from growth into fecundity after reaching a certain size, thus lengthening the intermoult intervals (Aldrich, 1983). This could lead to an accumulation of wear events over time. Furthermore, the thickness of the cuticle and its properties change greatly during their moulting cycle of crustaceans (Welinder, 1975; McGaw *et al.*, 1992). At the time of moulting, the epicuticle and exocuticle are present but remain unmineralised. In the postmoult period, epi- and exocuticle are mineralized and the first part of the endocuticle is deposited. The endocuticle is fully deposited and mineralized in the following intermoult period until the last intermoult stage, when growth stops and only organic reserves are accumulated (Welinder, 1975). The different stages of mineralization and thickness most certainly possess different cuticle properties such as hardness and brittleness. This would mean that the cuticle is susceptible to different degrees of abrasive wear during different stages of the moulting cycle. Possible damage of the surface during postmoult and early intermoult might be more severe than during the late intermoult period when the cuticle is greatly mineralized. Damage during the beginning of a moult cycle, might therefore result in more extensive changes than damage acquired later in the cycle. It is also possible that different conditions of surfaces (Figs. 5, 6) are observed as the result of injuries obtained during different stages in the moulting phase. Further investigations are needed to demonstrate this.

Other factors, like the degree of contamination of the surface due to habitat (i.e. silt bottoms, rocky shores), also have to be taken into consideration. Filling of the pleonal socket (Fig. 5C) could interfere with proper attachment. The cuticular folds on the inside of the socket could provide additional resistance to the slightly anteriorly-oriented structure of the press-button. Any filling of these folds might thus influence the effectiveness of the mechanism.

The effects of abrasive wear (Figs. 5, 6) represent the most likely explanation for the variation in measured pull-off force, but further quantitative investigations of the area and depth of cuticle changes are needed. Even a small functional part of the socket remaining is sufficient to securely attach the pleon. In one specimen, one side of the pleon was completely torn off whereas on the other side only a small part of the edge surrounding the pleonal
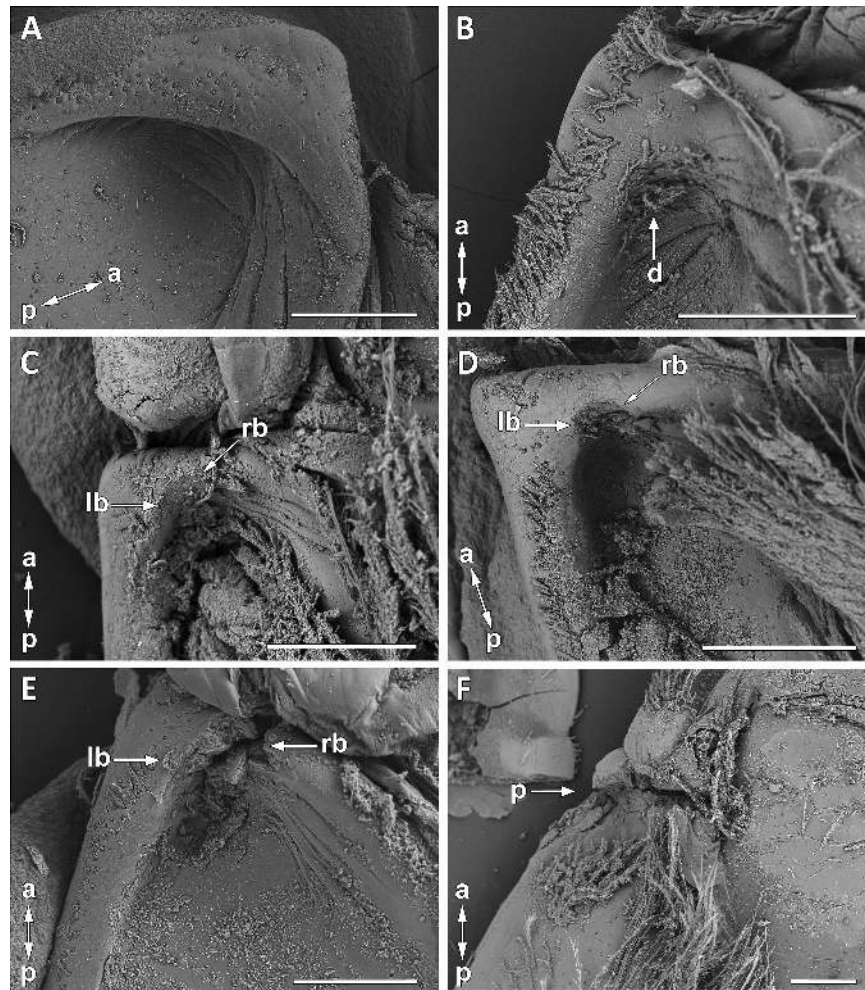
Figure 5. SEM images of the left pit on the sixth pleonal somite of six individual males of *Carcinus maenas*. (**A**) Cuticle is clean and intact. (**B**) Cuticle is slightly covered with foreign material, but still intact. (**C**) Cuticle is heavily covered with foreign material filling the pleonal pit. Indentation is visible on the anterior edge of the pit. Arrows indicate left and right borders of the altered surface area. (**D**) Cuticle partially covered with debris. Indentation is clearly visible on anterior edge of the pit. Arrows indicate the left and right borders of the altered surface area. (**E**) Cuticle is slightly covered with debris and torn off on the anterior edge of the pit. Arrows indicate the borders of the tear. (**F**) Cuticle is slightly covered with debris and heavily damaged. Only a small portion of the edge of the pit is still present on the anterior side, the outer wall is completely missing (arrow). a: anterior; d: debris, foreign material on the surface. lb, left border of altered cuticle; p, posterior; rb, right border of altered cuticle. Scale bar = 500 μm.

socket remained (Fig. 5F). The pleon attachment was functional in the air and underwater, but artificial removal of both press-buttons prevented the pleon from staying in place. Only a relatively small force of 44.46 mN was required to open the pleon in this case, in comparison to 100–300 mN or higher in the majority of specimens with an intact mechanism. Overall, the presence of even distinctly damaged structures allowed for pleon attachment via mechanical interaction. This probably represents an energy-saving mechanism, because the crab does not need to exert muscular power to hold the pleon in its position as predicted by Kollmann (1937) and Guinot & Bouchard (1998). The measured attachment force provides the first objective and reproducible experimental documentation of successful attachment by mechanical interaction only.

Measurements in air tended to yield a slightly higher maximum pull-off force in comparison to the natural condition underwater. There are two possible explanations for this. The lower force measured underwater could be due to a lubrication effect of the fluid, or a higher pull-off force in air could be a result of the residual moisture on the surface of the crab when taken out of the water, which creates an adhesion effect via capillary interactions (Colbeck, 1996; Qian & Gao, 2006) between the pleon and the closely fitting sterno-abdominal cavity. The second effect could provide an explanation

for the loss of an effective press-button mechanism in most adult males of the thoracotreme families Sesarmidae Dana, 1851 and Ocypodidae Rafinesque, 1815 (Duvernoy, 1853; Pérez 1928b; Guinot & Bouchard, 1998). The loss often coincides with a terrestrial or semiterrestrial lifestyle (Guinot, 1979; Guinot & Bouchard, 1998), quite often in connection with burrowing behaviour. The burrowing crabs are found in close proximity to the sea or freshwater systems (e. g., Ng, 1988), with the entrance to the burrow above the waterline but its end being filled with water. When emerging from the burrow to forage terrestrially, the pleon of the crab could be held in its position by the same adhesion effect described above, whereas inside the confined space of its burrow, no particularly tight locking of the pleon could be necessary. A connection between the lifestyle and the observed loss of the press-buttons was predicted, and a possible explanation for an alternative attachment method was envisaged by Guinot & Bouchard (1998).

Females

Pleons of female individuals were difficult to move in air when specimens were wet (Fig. 7). The pull-off force decreased significantly when the surface of the specimen was either dried, the
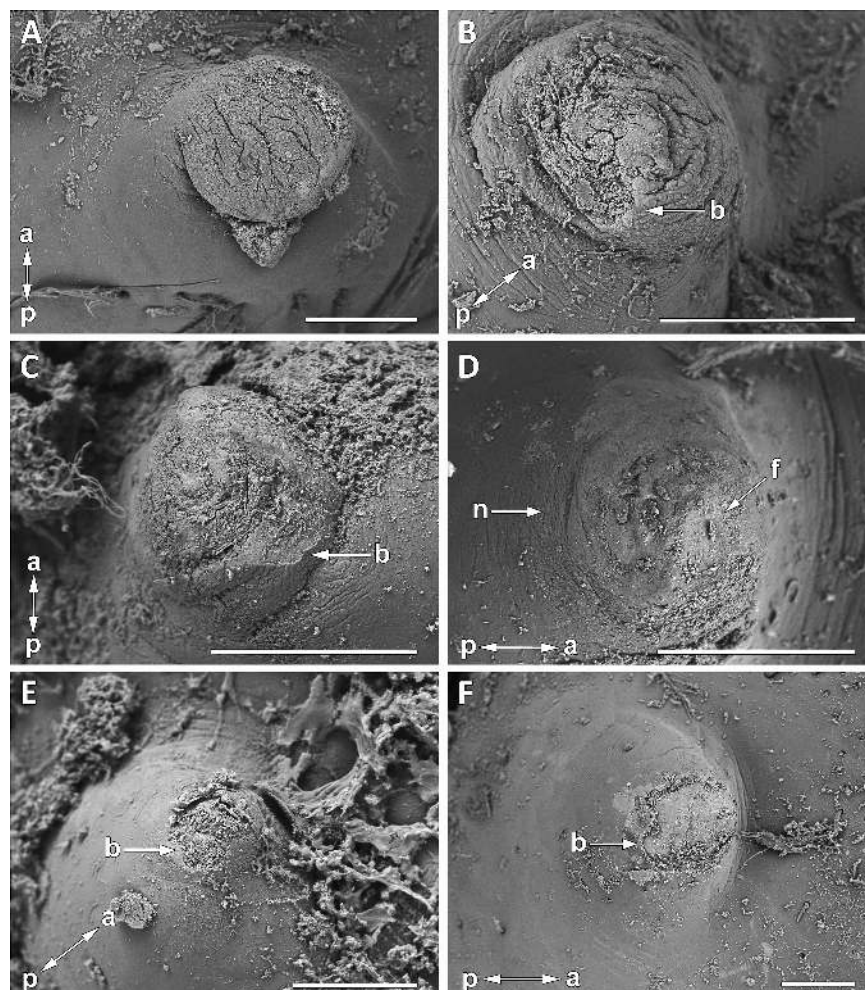
Figure 6. Scanning electron (SEM) images of the left press-button, or knob, on the fifth thoracic sternite of six individual males of *Carcinus maenas*. (**A**) Cuticle is mostly clean and intact. Multiple folds are visible on the surface of the knob. (**B**) Cuticle is slightly covered with foreign material, folds are visible but slightly flattened. (**C**) Cuticle is slightly covered with foreign material adjacent to the knob. Folds are nearly entirely flattened. (**D**) Cuticle is mostly clean. Folds on the knob are only visible in a small area on the left side in an area of non-altered cuticle, while other parts are severely flattened. (**E**) Cuticle is covered with foreign material next to knob. Part of the cuticle is torn off, border is clearly visible. (**F**) Cuticle is mostly clean and heavily damaged. Part of the cuticle is torn off, border is clearly visible. b: border between altered and non-altered cuticle; f: flattened surface; n: non-altered cuticle. Scale bar = 200 µm.
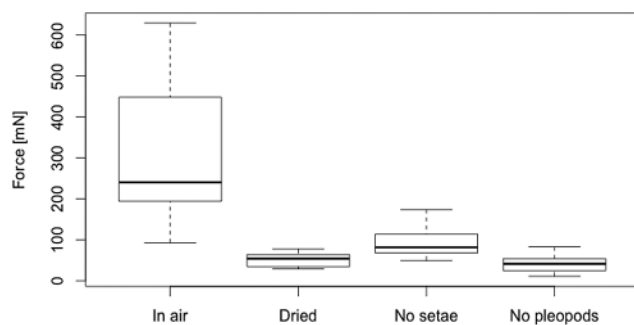

Figure 7. Results of the force measurements on female *Carcinus maenas* under different conditions: *In air*, intact and wet specimens ($N = 23$); *Dried*, surface of the sternal plates and that of the pleon dried with filter paper ($N = 12$); *No setae*, setae at the edge of the pleon removed, wet condition ($N = 10$); *No pleopods*, setae at the edge of the pleon and pleopods removed, wet condition ($N = 10$).

setae along the edge of the pleon were removed, or the setae as well as the highly setose pleopods were removed. This indicates a case of wet adhesion based on the capillary interactions (Colbeck, 1996; Qian & Gao, 2006). Contact between the setae, pleopods, and the surface of the sternal plates together with the residual water on the surface results in adhesion of the pleon and thus the higher pull-off force. All parts together most likely create an enclosed space between the pleon and the sternal plates, resulting in an additional suction effect. The effect can be removed by either drying off the surface of the specimen, or removing the setae and pleopods. Drying off the surface is presumably reversible, and two specimens rewetted by pipetting water inside and around the sterno-abdominal cavity showed a tendency for an increase in the pull-off force.

The pleon of females did not stay in its folded position underwater, as in the case of males without a sternal press-button. Any pleon attachment in females in their natural underwater habitat is therefore solely achievable by an active use of muscles and not by any mechanical interactions. Females carry fertilized eggs attached to the pleopods (Crothers, 1967). In these ovigerous females, no close contact between the pleon region normally having a socket structure and the sternal plate of the fifth pereiopod is possible (Guinot & Bouchard, 1998). Such a situation rendered the locking structures obsolete for a significant amount of the adult life span of females, and in combination with other morphological changes of the pleon due to allometric growth (e.g., Demeusy,

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1958; Guinot, 1979) led to an eventual loss of the locking mechanism in adult females. The investigations of species in which locking is possible even in egg-bearing females due to the presence of a brood chamber (Dorippidae, Leucosiidae Samouelle, 1819), or species with a less derived pleon (Calappidae) (Guinot, 1979; Guinot & Bouchard, 1998) should provide further insights into the functionality of the mechanism and the evolutionary tendencies to maintain the attachment system among eubrachyurans.

Juvenile females present a notable case because their pleons are particularly narrow and closely resemble those of males, especially in the early stages. Over subsequent moults, the pleon enlarges and the pleopods become more setose until the adult condition is reached (Demeusy, 1958). Pleon-holding structures are also present in juvenile females and because they are locked, they are fully functional and most likely allow for effective attachment at this juvenile stage. The testing of functionality of the press-button in juvenile specimens of both sexes would be a noteworthy topic for further studies.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. List of specimens detailing colour form, collection site, size, weight, and experimental conditions.

S2 Video. High speed video of a male specimen, intact mechanism, pleon detachment using vertical pull-off force in air.

S3 Video. High speed video of a male specimen, knob structures removed, pleon detachment using vertical pull-off force in air.

S4 Video. High speed video of a female specimen, setae and pleopods intact and in wet condition in air, pleon detachment using vertical pull-off force.

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