

Skeletal adaptations for forwards and sideways walking in three species of decapod crustaceans

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Abstract

Crustaceans have been successfully employed to study legged locomotion for decades. Most studies have focused on either forwards-walking macrurans, or sideways-walking brachyurans. *Libinia emarginata* is a Majoid crab (Brachyura) and as such belongs to the earliest group to have evolved the crab form from homoloid ancestors. Unlike most brachyurans, *Libinia* walks forwards 80% of the time. We employed standard anatomical techniques and motion analysis to compare the skeleton, stance, and the range of motion of the legs of *Libinia* to the sideways-walking green shore crab (*Carcinus maenas*), and to the forwards-walking crayfish (*Procambarus clarkii*). We found animals tended to have greater ranges of motion for joints articulating in the preferred direction of locomotion. Leg segments proximal to such joints were comparatively longer. Thorax elongation, leg length and placement at rest also reflected walking preference. Comparative studies of walking in *Libinia* and other brachyurans may shed light on the neuroethology of legged locomotion, and on the anatomical and physiological changes necessary for sideways-walking in crustaceans.

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1. Introduction

Most decapod crustaceans can walk in any direction they please. Even so, crayfish and lobsters walk forwards most of the time, whereas crabs are remarkable in that many species walk primarily sideways (Blake, 1985; Martinez, 2001). From an evolutionary standpoint, crabs are believed to have first evolved from homoloid (lobster like) ancestors some 320 MYA (Morrison et al., 2002). Molecular phylogenies suggest that the crab form arose independently no fewer than five times during evolution (Morrison et al., 2002). Whatever the advantage the crab form affords its owner, it appears to be a one-way avenue, as no reversions to elongated bodies with extended abdomens are known.

Sideways and forwards walking are the two main categories of progression in decapods, although intermediate (diagonal)

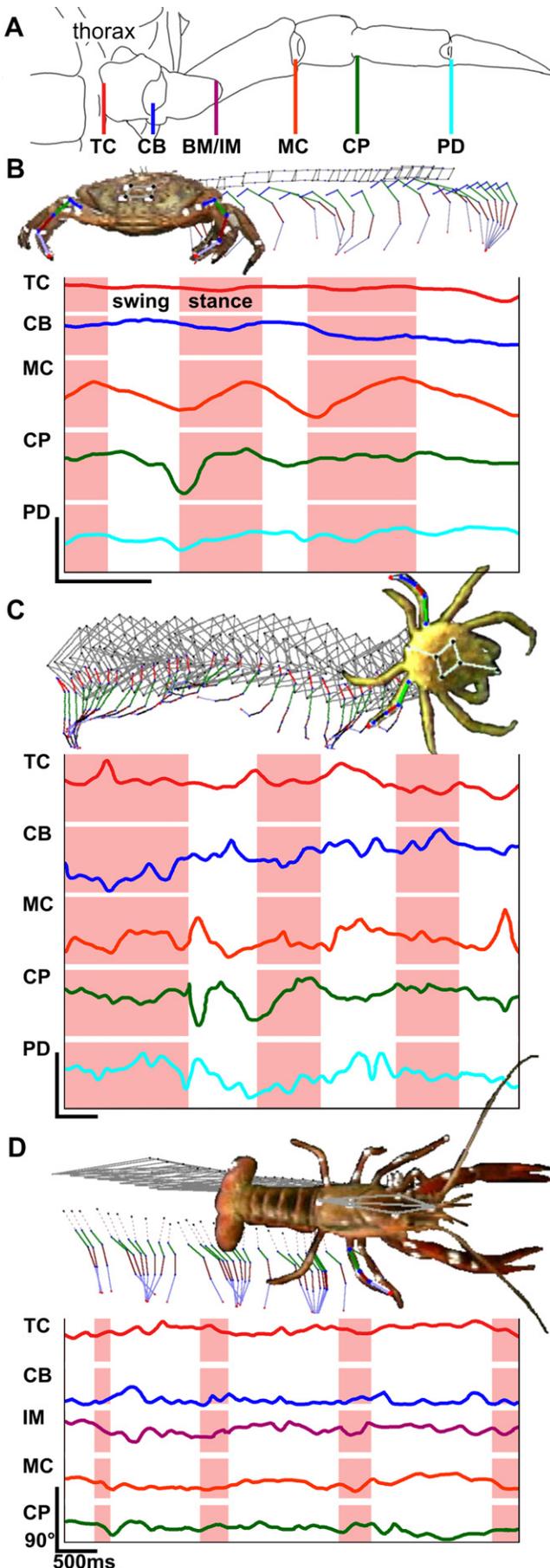
walks have been observed to occur in at least *Mictyris* (Sleinis and Silvey, 1980), *Callinectes* (Weissburg et al., 2003), and *Libinia* crabs (personal observation).

Forwards-walking crustaceans usually employ metachronal gaits where waves of steps travel anteriorly (Macmillan, 1975; Jamon and Clarac, 1995). In sideways walking, the legs on one side of the animal lead the walk while the contralateral side trails them (Barnes, 1975). Although capable of walking in any direction, most homoloid crustaceans walk primarily forwards, and most crabs sideways. There are examples of crabs that walk primarily forwards. The Australian soldier crab (*Mictyris longicarpus*) walks forwards and uses metachronal gaits similar to those of macrurans (Sleinis and Silvey, 1980).

Libinia emarginata is a brachyuran crab that walks forwards 80% of the time (Schreiner, 2004, and Fig. 1), and sideways or diagonally about 20%. *L. emarginata* is a large crab that lends itself to *in vivo* physiological recordings. In addition, it belongs to the family Majidae, proposed by Rice (1983) as one of the first to have diverged from lobster-like ancestors. *Libinia* is

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an interesting system to study the neuroethology of walking in crabs, and also possibly represents a transitional stage between forwards-walking homoloids and sideways-walking crabs.

Whether an animal walks forwards or sideways, it will have to overcome several forces acting upon it. While some of these forces are independent of the walking preference (weight, lift, and buoyancy), other forces are not (drag, acceleration reaction), and need to be actively countered by a walking or even stationary animal (Martinez, 1996, 2001; Martinez et al., 1998). Crabs walking forwards will experience drag forces and acceleration reactions parallel to their longitudinal axis, while crabs walking sideways will experience drag forces that are perpendicular to their longitudinal axis. Furthermore, these forces are always present in a crab's life, and therefore the energy spent to overcome them must have an effect on an animal's fitness. We hypothesize that there will be skeletal adaptations of the morphology of a crustacean that act to minimize the energy required to operate under these circumstances. We propose that forwards-walking crabs and sideways-walking crabs will have adaptations that reflect the preferred direction of locomotion.

The present study is the first of a series looking at the anatomy and physiology of *Libinia* as they pertain to its peculiar locomotor strategy. We looked for skeletal adaptations that might reflect *Libinia's* preferred walking direction and compared them with the skeletal structures of the sideways-walking crab, *Carcinus maenas*, and the forwards-walking crayfish, *Procambarus clarkii*. We chose these species based on the amount of research already conducted on the neuroethology of their walking behaviors (Bévençut et al., 1983; Brante and Hughes, 2001; Bush, 1962; Huxley, 1880; Jamon and Clarac, 1997; Martinez et al., 1998; Parsons, 2005; Pond, 1975).

2. Materials and methods

2.1. Species used

Libinia emarginata and *Carcinus maenas* crabs were obtained from the Marine Resources Center of the Marine Biological Laboratories in Woods Hole, Massachusetts. Crabs were kept at 20 °C in artificial seawater until used. *Procambarus clarkii* were obtained from local suppliers and kept in fresh water until used.

Fig. 1. Walking behavior of *Carcinus*, *Libinia*, and *Procambarus*. Plots showing the trajectories and angular excursion of the segments of the fourth leg during the typical walking behaviors of each species. The open blocks denote the swing phase of a step (no ground contact), while the pink blocks denote the stance phase (ground contact). For each animal the top trace shows the change in position over time of the points labeled in the legs and thorax during a walk. The bottom trace shows the angular excursion of the joints of the leg during the behavior shown (as calculated by Peak Motus). The joints are presented from proximal (top) to distal (bottom), and the scale bars are 90° and 500 ms. (A) Ventral view of a crustacean leg showing the different joints. Anterior is up. (B) *Carcinus*: typical angular excursions of the fourth leading leg (right, in this case) during sideways-walking. (C) *Libinia* walking forwards with a metachronal gait, which is slower and has more irregular angular excursions. (D) *Procambarus* employing a metachronal gait to walk forwards.

2.2. Labeling segments for filming

Points of interest were marked on each animal using White-out[®]. For each species the carapace was marked at the tip of the rostrum, the widest distinguishable re-occurring points, and the most posterior location on the thorax (on midline). For *Procambarus*, we labeled the posterior most point of the telson. For *Carcinus*, the dorsal-most plane was defined by the four points formed by the intersection of the cervical and urogastric grooves, and the intestinal and the posterior cardiac grooves. In *Libinia* the spines adjacent to the above named points were labeled. In the crayfish, the dorsal midline was marked where it meets the cervical groove. All walking legs (L2–L5) were labeled similarly in all species, with the exception of the ischium in L2 of *Carcinus*, which was visually obstructed from the cameras by the carapace. The remainder of the legs of the two crab species were labeled at the distal end of the segments: basis, merus, carpus, propus, and dactyl. Due to morphological differences at the ischium, *Procambarus* legs were marked at the distal end of the ischium, merus, carpus, and propus. No measurements were made of the crayfish propus-dactyl joint because of the small size of the dactyl segments. The first pair of legs, or the chelae, of *Libinia* and *Procambarus* were marked in the same manner, excluding the carpus. Line-of-sight obstruction by the carapace forced us to mark the chelae of *Carcinus* at the merus and dactyl only.

2.3. Filming

After the animals recovered from the labeling procedure (60 min) they were placed in a two-gallon aquarium on a felt substrate weighted down by a thin layer of sand (<5 mm). Each animal was placed in the filming tank for 1 h before recording, in order to acclimate them to their surroundings. Three cameras (Canon GL-1) were used at different angles to capture video of the animals standing. Six animals of each species were filmed standing between five and eight trials per animal. An individual trial was achieved by inducing the animal to walk, and then allowing it to come to rest of its own accord. All the videos were obtained from animals standing or walking on an unobstructed, flat, and leveled substrate.

2.4. Data analysis

Statistical analyses were performed using SigmaStat 3.5. The angular excursion data were not normally distributed and were analyzed using non-parametric ranked ANOVA tests. Differences were considered significant at $p < 0.05$.

Video clips of each trial were imported into a 3D motion analysis system (Peak Motus) in order to obtain an averaged spatial representation of each animal. The raw coordinates of all the animals in each species were aligned in the same direction, then averaged using Matlab in order to obtain caricatures of each species. Stance width was measured as the distance between dactyls of contralateral legs. Anterior–posterior leg arrangement was described as the longitudinal displacement of

a leg's dactyl from the center of mass (COM, see Table 1 for abbreviations used in this study). All distal joint angles were directly measured and recorded. Proximal joint angles (TC and CB) were inferred from the horizontal displacement of the ischium from the insertion point of the leg on the thorax for the TC joint, and the vertical displacement for the CB joint. Insertion points of each leg were measured in the x , y , and z direction with reference to the rostrum. The walking trials were filmed at 30 frames/s. All angles were calculated by Peak Motus.

2.5. Centers of mass

In all three species, the majority of the mass is contained in the thorax. We used the reaction board method (Reynolds and Lovett, 1909) and an Ohaus Explorer digital scale to find the exact location of the COM in the thoraces. The masses for all the thoraces were recorded individually. They were then secured to the reaction board, which had one end resting on a pivoting point with the other end resting on the scale. The mass of the board alone was also measured and the three values were used to calculate COM with the equation adapted from (Reynolds and Lovett, 1909):

$$\sum T_a = 0 = (R) * (l) - (M_t) * (d) - (M_b) * (0.5l)$$

where $\sum T_a$ is the sum of all torques (N*cm); R is the mass measured by the scale (g); l is the board length (cm); M_t is the thorax mass; d is the COM-end of the board distance (cm); M_b is the board mass (g).

Calculated COMs for the thoraces were then double-checked using the knife-edge method with a piece of plastic 1 mm in thickness (McKinnon et al., 2004). The dorsal carapace was marked at the calculated position along the longitudinal axis. The COM for the thorax was then defined as one-half the thickness of the thorax in the dorso-ventral plane measured at that location. The same method was used to find the COM in the abdomen of *Procambarus*. All legs were removed at the thorax and masses were again recorded. The chelae were dissected into two parts: the distal end comprising the carpus, propus, and dactyl, and the proximal end containing the coxa, basis, ischium, and merus. The segments were then balanced on the lever to find the centers of mass. The remainder of the legs in *Carcinus* and *Libinia* were removed,

Table 1
Abbreviations used

BI	basis-ischium joint in <i>Procambarus</i>
BM	basisischium-merus joint in crabs
CB	coxa-basis joint
COM	center of mass
CP	carpus-propus joint
L2-5	legs two through five
MC	merus-carpus joint
PD	propus-dactyl joint
ROM	range of motion
T1-8	thoracic somites 1 through 8
TC	thorax-coxa joint

and centers of mass were found in a similar manner. The only difference in the procedure was an additional dissection at the propus-dactyl joint. Peak Motus was used to combine segmental centers of mass to find the global COM for each individual. The legs of *Procambarus* only contribute $6.11 \pm 0.28\%$ of the total mass of an individual animal (compared to $19.61 \pm 1.24\%$ in *Libinia*, and $17.53 \pm 3.15\%$ in *Carcinus*). Because of the minimal contribution to the COM by each individual leg, we assumed that the local COM for that leg was located at 50% of the distance between insertion point and dactyl.

2.6. Ranges of motion of leg and leg segment

2.6.1. Whole leg horizontal ranges

Animals were anesthetized by cooling. The horizontal range of the entire leg was found by immobilizing the thorax in its resting position above graph paper. The legs were moved along the horizontal plane to the limit of their range and their path traced on the paper. The angle between the insertion point of the legs on the thorax and the points at either extreme of the range was measured.

2.6.2. Individual segment ranges

After anesthetizing an animal, each segment was moved along its range of motion and the range recorded on the plane of motion using protractors. In order to compare animals of different species, we defined thorax size as:

$$s = \sqrt{(t_1 * t_w)}$$

where t_1 is thorax length (cm) and t_w , thorax width (cm).

2.7. Segment lengths

We measured leg segment lengths ventrally using dial calipers. This was done using the distance between hinge points in consecutive segments.

2.8. Endophragmal structure

Animals were euthanized by cooling, and their dorsal carapace and organs were removed. The skeletons were immersed in 10% KOH in dH₂O and incubated at 50°C for 14 days for *Libinia*, 7 days for *Carcinus*, and 3 days for *Procambarus*. Because of the large amount of calcium in its skeleton, after KOH treatment the endophragm of *Libinia* was rinsed in dH₂O and further treated with 10% HCl for 10 min to render the skeleton translucent. After incubation the skeletons were placed in 5% Formalin and Janus green applied to facilitate viewing.

Pictures of the processed skeletons were taken using a digital camera mounted on a dissecting microscope. Montage photographs were traced using Corel Photo-Paint®, and shaded by hand.

3. Results

3.1. Walking behavior

We refer to the legs by numbers one through five with leg one (L1) corresponding to thoracic somite four (T4), and leg five (L5) referring to thoracic somite eight (T8). Previous work on *Procambarus* suggested that the fourth pair of legs plays a prominent role during locomotion (Jamon and Clarac, 1995). We measured the excursions of the segments comprising the fourth legs (Fig. 1A) during a typical walking bout to determine which joints could be of particular interest for our study (Fig. 1). *Libinia* walks more slowly than both *Carcinus* and *Procambarus* (Fig. 1C). During its forwards walk, *Libinia* made more use of the TC, and CP joints than *Carcinus* while the MC angular excursion was smaller. *Libinia* also walks more jerkily than *Carcinus*. We should mention that although we recorded the kinematics of the fourth leg, the different legs of *Libinia* did not perform the same behavior during forwards walking. The study of how each of the legs of *Libinia* contributes to forwards locomotion is beyond the scope of the present work and will be the focus of a future study.

3.2. Thorax morphology

For each species, the thorax is longer in the preferred direction of locomotion (Fig. 2A). *Procambarus* has the highest thorax length to width ratio at 1.77 ($r^2 = 0.88$), followed by *Libinia* with a 1.09 ratio ($r^2 = 0.95$). *Carcinus* is wider than long, with a thorax length to width ratio of 0.82 ($r^2 = 0.85$). The thorax size (s) vs. body mass ratio has similar slope for the three species 0.33 ($r^2 = 0.88$), 0.28 ($r^2 = 0.92$), and 0.29 ($r^2 = 0.96$) for *Procambarus*, *Libinia*, and *Carcinus*, respectively (Fig. 2B). Therefore, we made interspecies comparisons between animals of similar thorax size.

Of the three species studied, *Libinia* has the heaviest and most calcified thorax (it took twice as long to dissolve the skeleton of *Libinia* as that of *Carcinus*). The thorax to body mass ratio was 0.75 ± 0.01 for *Libinia*, 0.65 ± 0.06 for *Carcinus*, and 0.55 ± 0.02 for *Procambarus*. The thorax of *Procambarus* is laterally compressed (Fig. 3C). Compared to the dorso-ventral compression of *Carcinus* (Fig. 3A), *Libinia*'s thorax is intermediate in form in that it is neither laterally nor dorsoventrally compressed (Fig. 3B). While *Carcinus* has a thorax that is widest dorsally, the thorax of *Libinia* is widest ventrally. The thorax of *Procambarus* does not vary greatly in width between the dorsal and ventral surfaces.

3.3. Endophragmal structure

For the description of the endophragmal skeleton, we use the terminology of Pilgrim and Wiersma (1963). Removal of the dorsal carapace exposes the endophragmal skeleton housing the proximal leg musculature (Fig. 3ii). The epimerites are the endophragmal structures that lay directly above the leg openings. They are segmentally arranged and have grooves between them from which the vertically invaginating

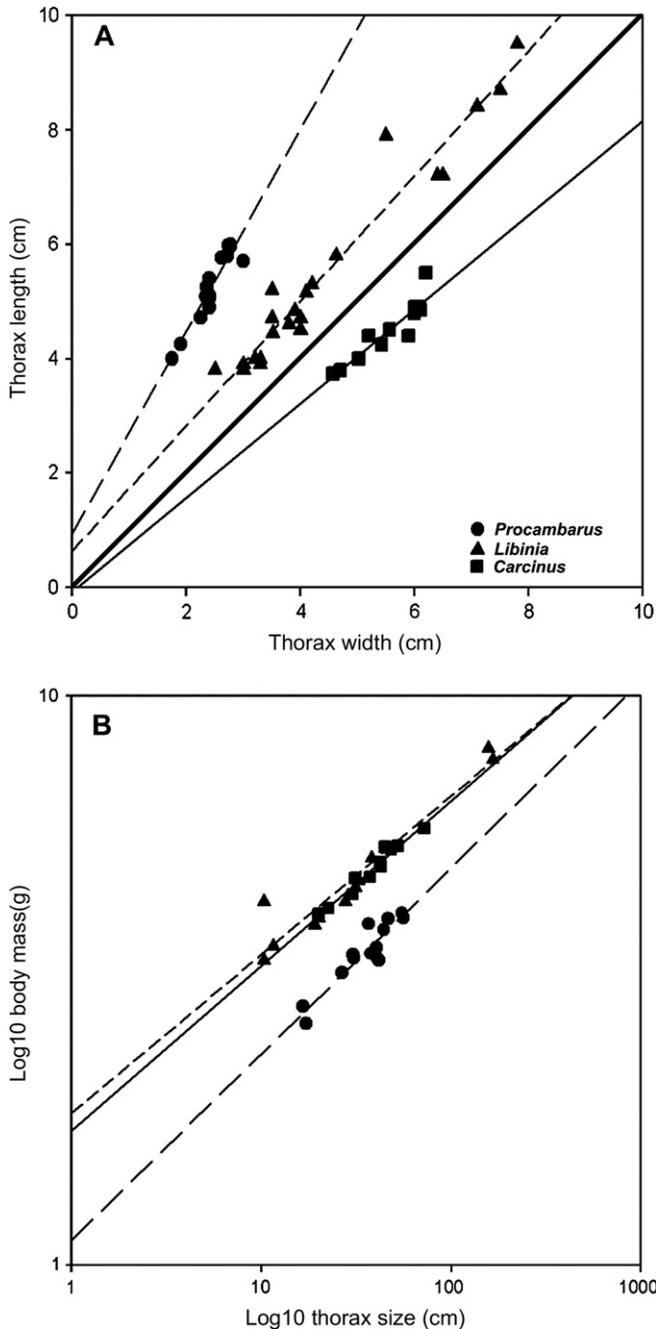


Fig. 2. (A) The thorax of each species is elongated in the direction of preferred locomotion. *Procamburus* has the most elongated thorax in the anterior–posterior direction, *Libinia* has a slightly longer than wide thorax, while *Carcinus* is wider than it is long. (B) The ratio of thorax size to body mass is similar for *Carcinus* and *Libinia* and larger than for *Procamburus*.

endopleurites arise (Fig. 3iii v). Each endopleurite gives rise to an anterior and a posterior epimeral ridge (Fig. 3iii d). These structures divide each endophragmal compartment into several cavities. While the ventral compartments of each segment do not overlap with neighboring segments, the dorsal compartments overlap with posterior segments (Fig. 3iii). Paralleling the radial arrangement of the legs around the thorax, the dorsal endophragmal units in *Libinia* lay less perpendicular to the

longitudinal axis of the body than those in *Carcinus* (Fig. 3Aii, iii, Bii, iii), increasing the possible length for the muscle they house. In both crabs the hinge points for the coxa are actually dorsal anterior, and ventral posterior so that the axis of the legs are rotated. This rotation is most pronounced in *Libinia*. *Carcinus* and *Procamburus* differ from *Libinia* in having the dorsal endophragmal subunits more vertically arranged (Fig. 3iv, v). This allows them to accommodate the muscles despite the relatively narrow space that is available ventrally.

3.4. Leg morphology

Libinia's legs are arranged radially around the thorax (Fig. 3B). The legs of *Libinia* are all morphologically similar (Fig. 4A), and decrease in size from anterior to posterior in a similar fashion to *Procamburus* legs (Fig. 4D). The leg segments are cylindrical in shape and heavily calcified. Sensory hairs are distributed along the entire surface of the legs of both *Procamburus* and *Libinia* with higher concentrations at the joints. In addition to the sensilla covering the entire leg surface, *Procamburus* has areas with a higher density of sensory hairs at the base of the fourth and fifth dactyl (Fig. 4B), and the ventral edge of the propus of the second and fourth pairs of legs. The second legs of *Procamburus* have chelae and are more flattened and sclerotized than the more posterior legs. The third legs of *Procamburus* also have chelae. The legs of *Carcinus* are similar in morphology (slightly increasing in length posteriorly) with the exception of the fifth leg, which is shorter and wider (specialized for swimming). In contrast to the legs of *Libinia* and *Procamburus*, the legs of *Carcinus* have sensory hairs only along the dorsal and ventral margin of their leg segments (Fig. 4C). *Carcinus* and *Procamburus* have laterally compressed legs. If the highly specialized second legs of *Procamburus* and fifth legs of *Carcinus* are excluded, the length of the walking legs in all the three species is closely related to the size of the thorax (Fig. 4D).

3.5. Combined range of motion for all legs in the anterior–posterior direction

While the range of motion of the legs in the forward direction (horizontal plane) varies minimally within species, in *Libinia* it is more influenced by thorax size than in the other two species (Table 2). The combined horizontal range of motion for all legs is significantly larger for *Procamburus* ($312 \pm 11^\circ$, $p < 0.001$) than *Libinia* ($250 \pm 17^\circ$), and is significantly smaller for *Carcinus* ($223 \pm 5^\circ$, $p < 0.005$) than *Libinia* (Fig. 5B).

3.6. Ranges of motion for individual leg segments

The joints of the legs of the crabs are uniplanar (with the exception of BM and CP in *Libinia*) and can be divided into those articulating forwards to backwards (in the frontal plane) and those moving side to side (in the transverse plane). The joints that move in the frontal plane are TC and CP. The joints that

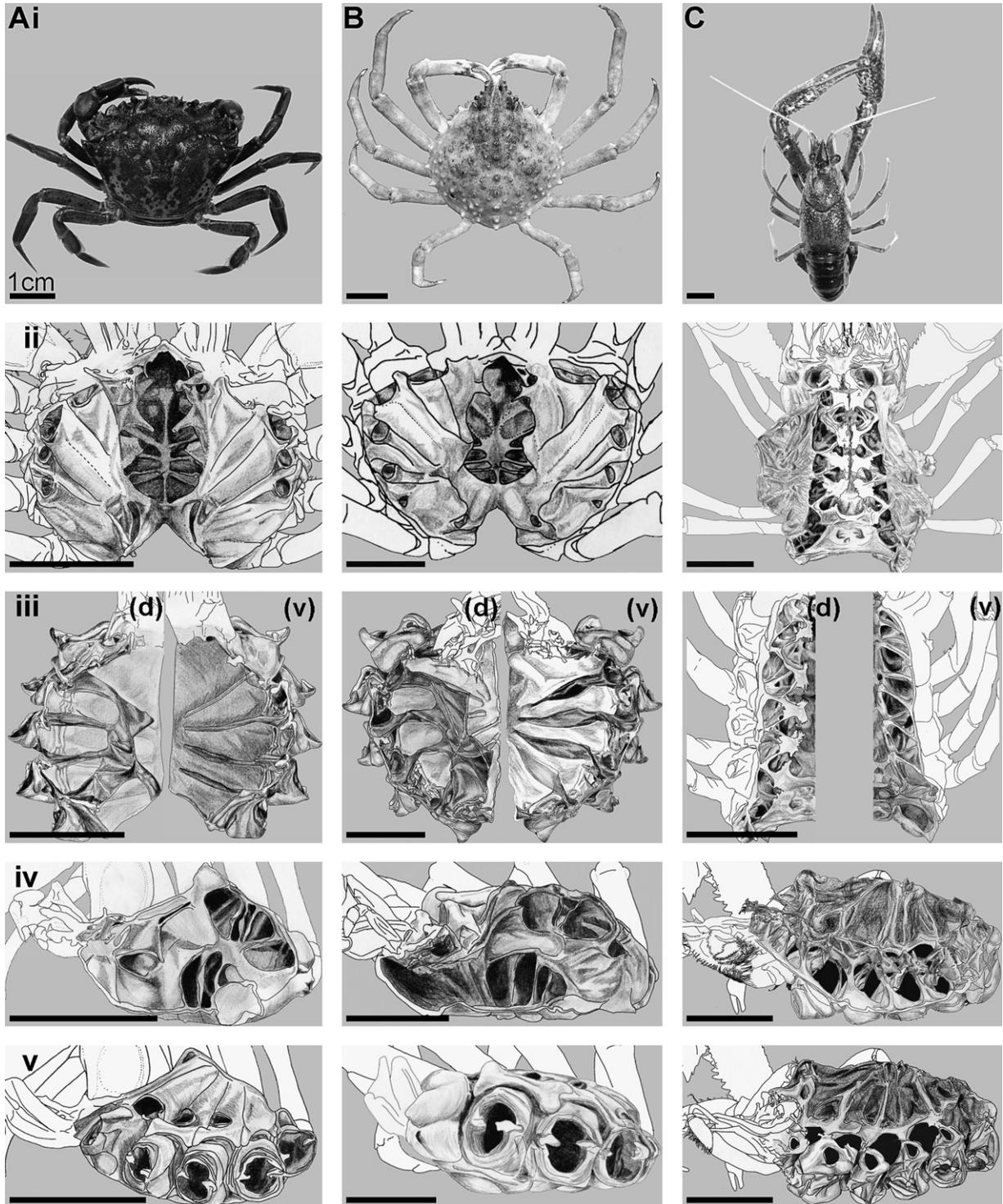


Fig. 3. Endophragmal structure. (A) Dorsal view of *Carcinus*, (B) *Libinia*, and (C) *Procambarus*. (i) For the three species, the thorax is longest in the preferred direction of locomotion. (ii) Removal of carapace and digestion of soft tissue exposes the underlying endophragmal skeleton, dorsal view. (iii) Dorsal view of endophragmal structure after dissection of successive overlying layers. In each case the left panel (d) shows the dorsal compartment, and the right panel (v) shows the ventral compartment of the endophragm. The proximal muscles for each leg are separated by endophragm into four groups by a horizontal dorsal rib, and a medial vertical rib. While the ventral ribs are aligned with the long axis of the legs, the dorsal ribs are oriented more posteriorly (iii d). In *Libinia* the dorsal endophragm is more oblique with respect to the midline than in *Carcinus* or *Procambarus*. (iv) Sagittal sections through the thoracic midline (left is anterior). The endophragm is taller in *Carcinus* (A iv) than in *Libinia* (B iv), but shorter than in *Procambarus* (C iv). The convex nature of the ventral thorax of *Carcinus* (A iv) is apparent when compared with *Libinia* (B iv) and *Procambarus* (C iv). (v) Lateral view of endophragm after removal of ipsilateral legs. The legs of *Carcinus* (A v) are arranged less radially than the legs of *Libinia* (B v) but are not as parallel as those of *Procambarus* (C v).

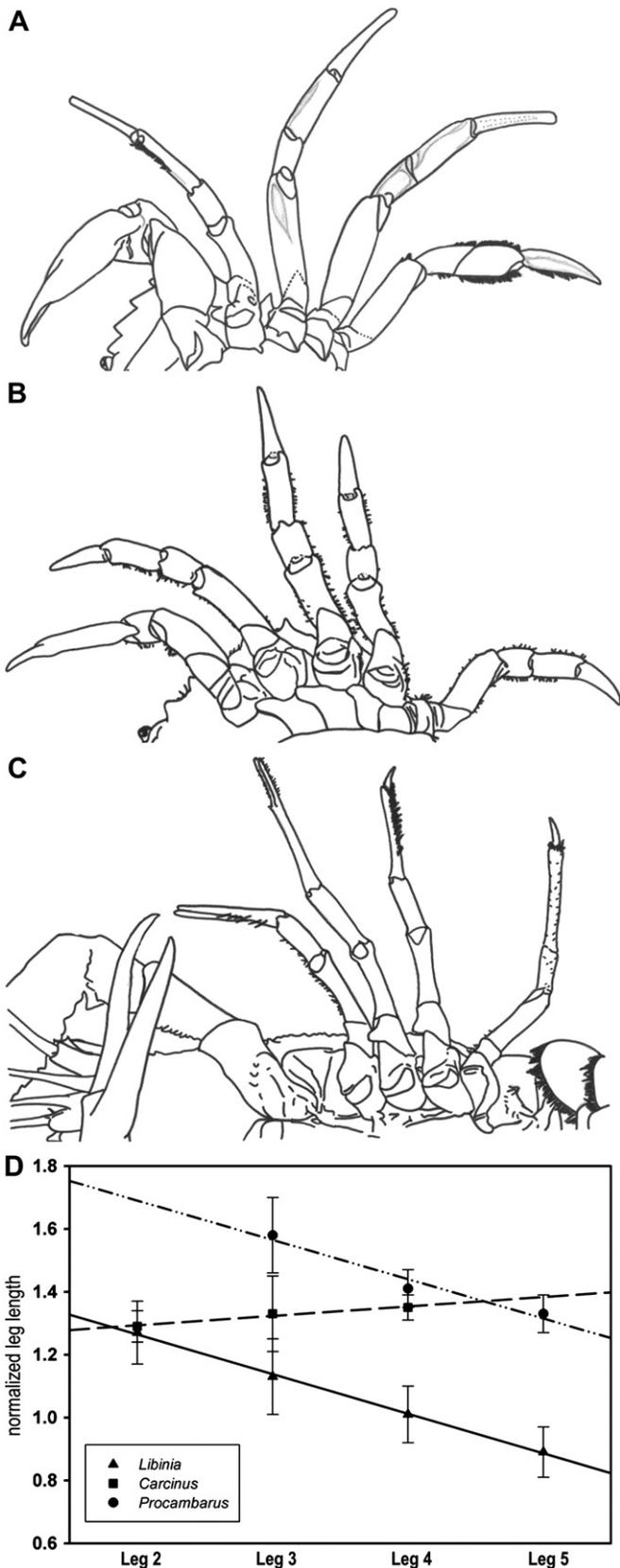


Fig. 4. Morphology of walking legs. (A) Ventral view of the walking legs of *Carcinus*, (B) *Libinia*, and (C) *Procambarus*. (D) Relationship between thorax size and leg length for the three species, not counting legs specialized for tasks other than walking (*Procambarus* legs 1 and 2, *Carcinus* legs 1 and 5, and *Libinia* leg 1).

Table 2
Relationship between horizontal range of motion and thorax size

Species	Adj. r^2 for horizontal ROM vs. thorax size			
	Leg 2	Leg 3	Leg 4	Leg 5
<i>Libinia</i>	0.214	0.370	0.455	0.396
<i>Carcinus</i>	0.000	0.000	0.000	0.161
<i>Procambarus</i>	0.023	0.025	0.000	0.000

move the leg in the transverse plane are CB, MC, and PD. The plane of motion of BM in *Libinia* (and to a lesser extent in *Carcinus*) is diagonal to the transverse and frontal planes of the crab so that its movement results in a rotation of the longitudinal axis of the leg in the anterior direction. Joints used to propel the animal forwards tended to have a larger mean range in *Libinia* and *Procambarus* than in *Carcinus* (Table 3 and Fig. 5). The converse was true for joints moving the animals in the sideways direction. This relationship increased posteriorly as shown by the two extremes of legs 2, and legs 5 (Fig. 5C vs. D). The combined range of motion in the horizontal plane for all legs is greatest for *Procambarus*, and smallest for *Carcinus*.

3.6.1. Joints moving in the horizontal plane

3.6.1.1. Thorax-coxa joint. The range of motion of the TC joint for all legs combined was not significantly different between *Libinia* and *Carcinus* ($n = 46$ each, see Table 3). The larger range of motion in the TC joint in *Libinia* than in *Carcinus* became more pronounced (and significant) for each posterior leg (Fig. 5C,D). Both *Libinia* and *Carcinus* had a greater ($p < 0.001$, and $p < 0.05$, respectively) TC range of motion than *Procambarus*.

3.6.1.2. Basis-merus joint. *Libinia* and *Carcinus* had similar BM ranges. While in the crabs the BM joint rotates the axis of the leg forwards, in *Procambarus* the joint with equivalent action is the IM, which rotates around a vertical axis and contributes considerably to forwards locomotion. *Procambarus* had a larger range of motion than *Libinia* or *Carcinus* ($p < 0.001$).

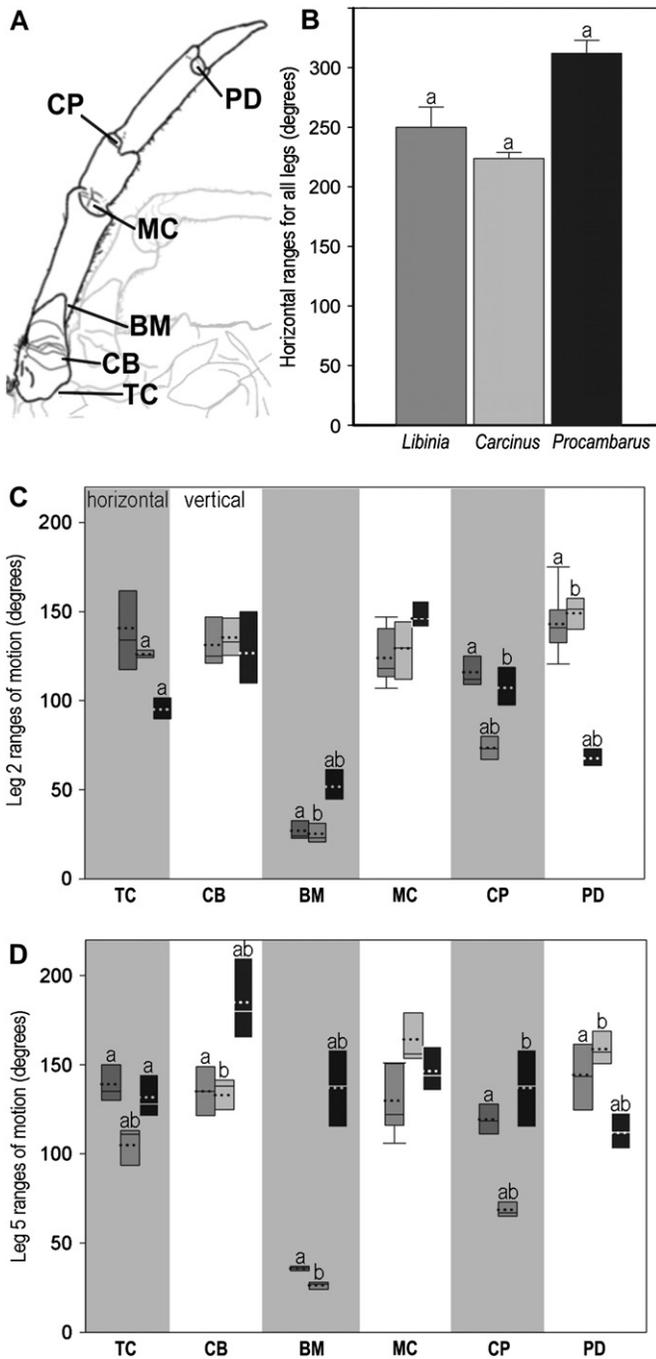
3.6.1.3. Carpus-propus joint. Although *Procambarus* had the largest CP range of motion, in *Libinia* the CP range of motion was significantly larger ($p < 0.0001$) than in *Carcinus*.

3.6.2. Joints moving in the vertical plane

3.6.2.1. Coxa-basis joint. Both *Libinia* and *Carcinus* ($n = 24$ each) had similar CB ranges of motion. *Procambarus* ($n = 32$) had a larger CB range than either crab ($p = 0.001$, and $p = 0.02$ respectively).

3.6.2.2. Merus-carpus joint. *Procambarus* ($n = 32$) and *Carcinus* ($n = 23$) had larger ($p < 0.001$) ranges of motion for the MC joint than *Libinia* crabs ($n = 37$).

3.6.2.3. Propus-dactyl joint. *Libinia* ($n = 36$) and *Carcinus* ($n = 22$) had similar ranges of PD motion. The dactyl of



Procambarus is greatly reduced and serves as a claw in the first two walking legs. Consequently, the range of motion was smaller for PD in *Procambarus* than in the crabs.

3.7. Length of leg segments

There was a positive correlation between joints having increased ranges of motion, and the length of the segments proximal to these joints (Fig. 6). In the three species, the leg segments housing the musculature for a particular joint were longer for joints with larger ranges of motion (Fig. 6). Paralleling the ranges of motion, the effect was smallest for the second legs (Fig. 6B), and largest for the fifth legs (Fig. 6C). Joints that had larger ranges of motion were accompanied by longer proximal segments. In order to compare segment lengths across species we normalized the length of the segments to the thorax size. In general, segments that housed the musculature for a joint articulating in the frontal plane (basis and carpus) were longer in *Libinia* (0.14 ± 0.02 and 0.12 ± 0.2 respectively for L5) than in *Carcinus* (0.11 ± 0.01 and 0.09 ± 0.01). The converse was also true of segments proximal to joints articulating in the transverse plane (merus and propus), where the sideways-walking *Carcinus* had longer (0.34 ± 0.03 and 0.23 ± 0.02 respectively for L5) normalized segments than *Libinia* (0.31 ± 0.02 and 0.18 ± 0.03 respectively). Because the CB joint musculature is largely contained within the thorax, and because the dactyl does not house any musculature, the coxa and the dactyl were not used in this comparison.

3.8. Leg arrangement and stance

After comparing leg morphologies and ranges of motion, we looked for differences in joint use at rest. We videotaped animals standing motionless and generated average limb configurations, body postures, and calculated centers of mass (Fig. 7A). The insertion points of the legs onto the thorax were determined and are shown in dorsal and lateral aspects (Fig. 7B) and show both brachyurans standing with their COM between dactyls 3 and 4, while in *Procambarus* it lays between dactyls 4 and 5. *Carcinus* and *Procambarus* stood with their limbs in an elliptical pattern elongated in the preferred direction of movement (Fig. 7C). *Libinia* maintained a more circular arrangement of the legs. Each animal stood

Fig. 5. Leg ranges of motion for all of the legs of the three species. (A) The leg of each crab is composed of six joints, but *Procambarus* has seven joints because its basis and ischium are not fused (not shown here). Each of the segments comprising the legs was measured. (B) The combined range of motion in the horizontal plane for all legs of *Procambarus*, *Libinia* and *Carcinus*. (C) Range of motion for each joint of leg 2. (D) Range of motion for each joint of leg 5. (C, D) Range of motion of each joint in leg 2 (C) and leg 5 (D). The boxes show the 10th, 25th, 75th, and 90th percentiles with the solid lines indicating the median and the dotted lines indicating the mean ($n = 10$). Bars with the same letter are significantly different. The data were analyzed using non-parametric ranked ANOVAs. Grey columns denote joints articulating in the horizontal plane, while white columns for joints moving in the vertical plane.

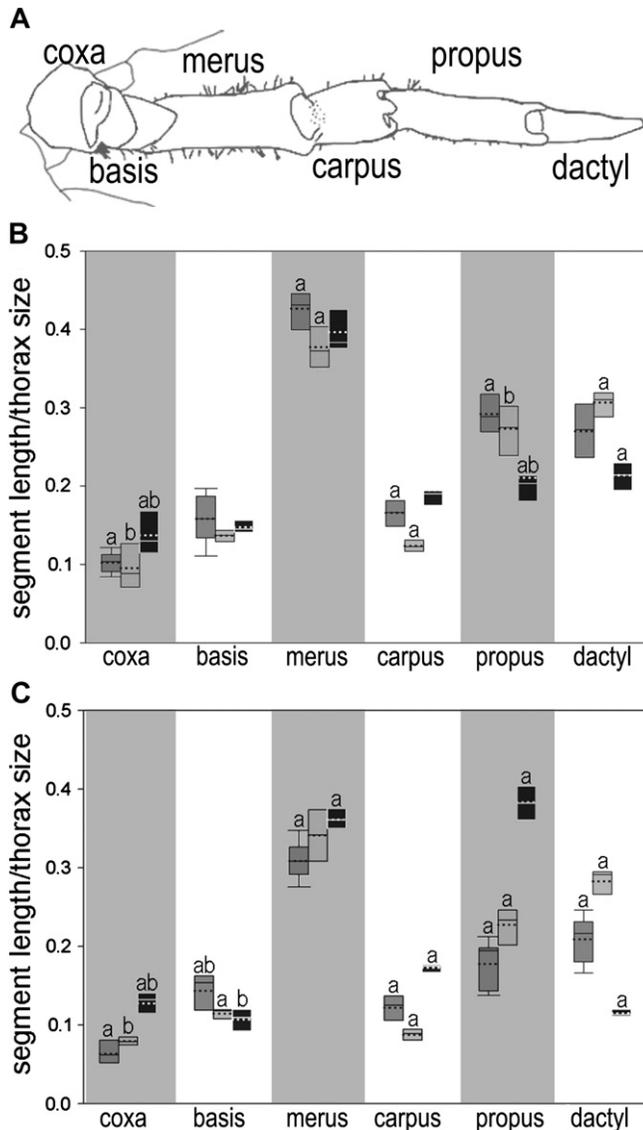


Fig. 6. (A) The legs of the crabs are composed of six segments (since the ischium and basis are fused), while in *Procambarus* there are seven segments. (B, C) Box-whisker plots showing mean segment length, normalized to thorax size (s), for the second leg (B) and the fifth leg (C) of each of the three species ($n = 8$ for each). Boxes with the same letter are significantly different. These data were analyzed using two-way ANOVA. The boxes show the 10th, 25th, 75th, and 90th percentiles with the solid lines indicating the median and the dotted lines indicating the mean. Legend as in Fig. 5.

with the legs arranged in an arc so that L3 and L4 had the largest interleg width compared to L2 and L5 (Fig. 7D). *Carcinus* adopted a wider stance where interleg width increased with leg length (compare Figs. 4D and 7D). In *Libinia* the longest (L2) and shortest legs (L5) were similarly spaced (Fig. 7D). *Procambarus* adopts a wider stance in the fifth leg and a much shorter interleg distance between the specialized second legs. *Procambarus* further differs from the other two species in having the COM between L4 and L5, and as a result only L5 is behind the COM (Fig. 7E). The third leg pair of *Procambarus* is positioned much closer to the second legs, suggesting that each pair of legs provides similar stability in the direction of

the longitudinal axis (Fig. 7Ciii). In the crabs the COM is between the third and fourth legs. Spacing between L3 and L4 is similar in both *Libinia* and *Carcinus*; however, both leg pairs are shifted anteriorly in *Carcinus* resulting in the COM being closer to L4.

3.9. Joint contribution to stance

We next wanted to investigate how each of the joints contributed to the position of each of the legs. In the brachyurans there was a marked difference between all angles across all legs that contribute to displacement in the transverse (Fig. 8, hatched bars) and frontal planes (Fig. 8, solid bars). The majority of the joints articulating in the frontal plane (TC, BM, CP) were much straighter (closer to 180°) than the joints that contribute to motion in the transverse plane (CB, MC, PD). In *Procambarus*, the two vertically articulating joints measured (CB and MC) were very different. The CB joint was near 180° in all legs while the MC joint was closer to 90° (Fig. 8C,D).

The use of the TC joint was similar between *Procambarus* and *Libinia*. In *Carcinus*, all of the TC joint angles were $>159^\circ$ (L2 = $159 \pm 9^\circ$; L3 = $174 \pm 4^\circ$; L4 = $175 \pm 3^\circ$; L5 = $169 \pm 6^\circ$), which means that the legs were close to perpendicular to the longitudinal axis of the crabs. *Libinia*, however, had a wider range of TC angles. Legs 2 and 4 were $159 \pm 9^\circ$ and $162 \pm 11^\circ$, while leg 3 exited the thorax with a large (straight) angle ($172 \pm 5^\circ$). Conversely, leg 5 TC joint had an average angle of $145 \pm 11^\circ$ directed posteriorly. In *Procambarus*, all four TC joints had an angle of $<162^\circ$ (L2 = $141 \pm 8^\circ$; L3 = $151 \pm 8^\circ$; L4 = $162 \pm 10^\circ$; L5 = $162 \pm 9^\circ$). This suggests that in forwards-walking species the TC joint contributes more to the observed leg spread in the anterior–posterior direction (Fig. 8).

A closer look at the distal joints articulating in the frontal plane (BM and CP) across species revealed additional differences. In *Carcinus* these joints were (at rest) bent away from the COM, providing spread in the anterior–posterior direction. In *Libinia* the same joints were always bent forwards. In the posterior two legs of *Procambarus*, most of the forwards to backwards placement of the dactyls was due primarily to the IM joint.

4. Discussion

4.1. Walking preference

Libinia crabs walk preferentially in the forward direction (Schreiner, 2004) employing primarily metachronal gaits similar to those used by macrurans (Fig. 1). Walking in the forward direction means that each leg is committed to either pull, or push the center of mass, which is not the case for sideways-walking crabs. This distinction means the limbs of *Libinia* could be specialized with front and hind limbs performing different functions (see Ritzmann et al., 2004). Our preliminary observations of musculature and the kinematics of walking in *Libinia* further support this view.

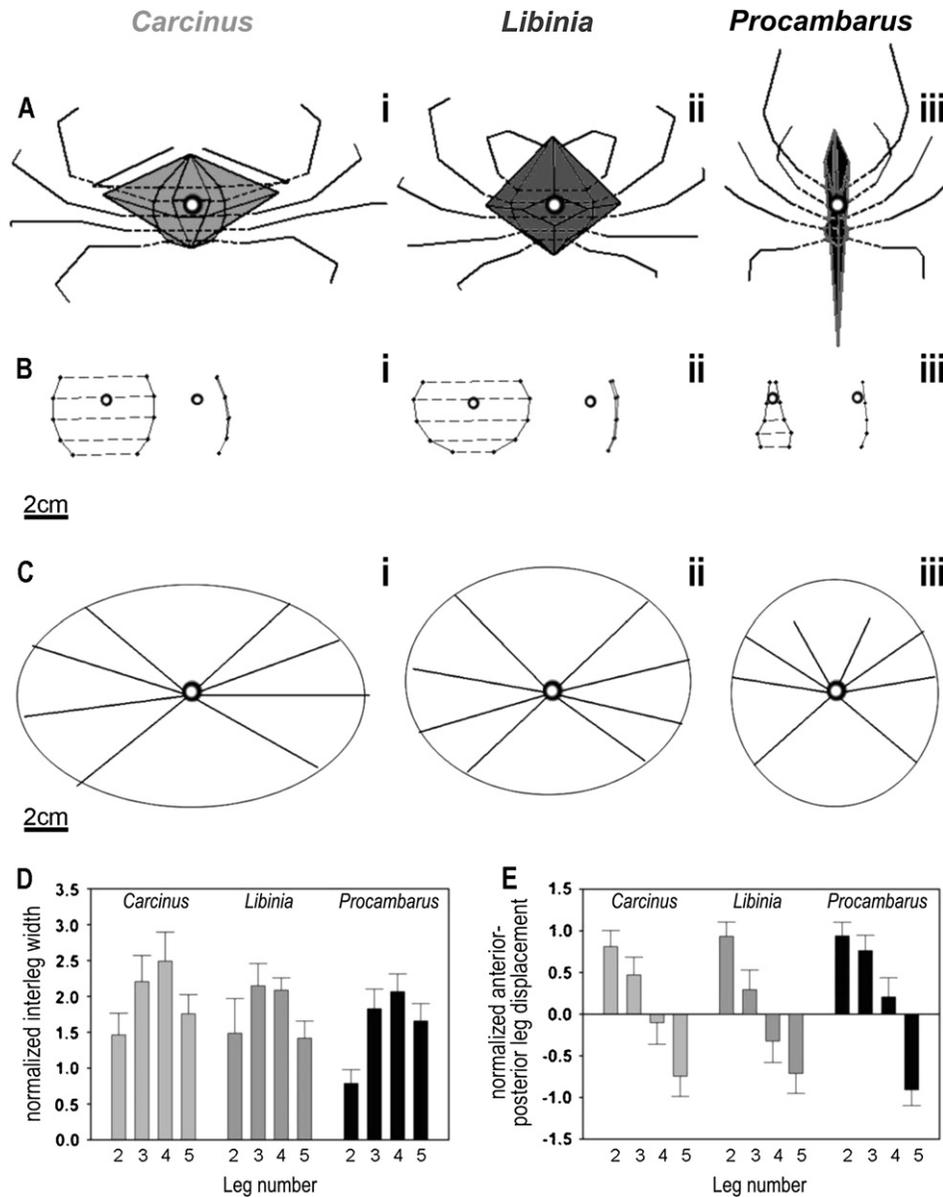


Fig. 7. (A) Three-dimensional caricatures of *Carcinus*, *Libinia*, and *Procambarus* (dorsal views) based on averaged coordinates of five animals. The leg insertion points in the carapace are shown (B) in dorsal (left) and lateral (right) views (anterior is up). (C*i*–iii) Placement of the dactyls fall along an ellipse around the centers of mass (denoted by black and white circles). (D) Stance width normalized to thorax size, *s*. (E) graph of the overall leg displacement from the COM in the anterior–posterior direction.

4.2. Thoracic structure

Although *Libinia* and *Carcinus* differ in walking strategies, skeletal adaptations, and degree of calcification, their thoracic size to mass ratios are similar. They are, therefore, exposed to similar forces along the vertical axis (Fig. 2B).

All three species exhibited elongation in the preferred direction of locomotion. Previous studies on shell hydrodynamics have revealed the optimal streamline of crab shells to be in the transverse direction for blue crabs (Blake, 1985). The commitment to a particular direction of locomotion seems to be reflected in the degree of elongation exhibited (Fig. 2A). *Procambarus* walks mostly in the forward direction (Pond, 1975) and has the largest elongation ratio (1.77 length/width).

Libinia walks primarily forwards, but also sideways about 20% of the time (Schreiner, 2004), and it has a 1.09 length to width ratio. *Carcinus* however rarely walks forwards and has a 0.82 ratio. This finding is supported by the only other forwards-walking crab found in the literature (*Mictyris longicarpus*) where the thorax is also longer than it is wider (Sleinis and Silvey, 1980). For forwards-walking crustaceans, a longer thorax translates into a decrease in overlap between the ranges of motion of neighboring legs in the forwards direction. A thin (narrow) profile could help these animals reduce drag when walking forwards. For sideways-walking crustaceans a short thorax may reduce drag while walking sideways. Blake (1985) studied a non-brachyuran king crab and concluded that its carapace was not adapted to reduce drag; however,

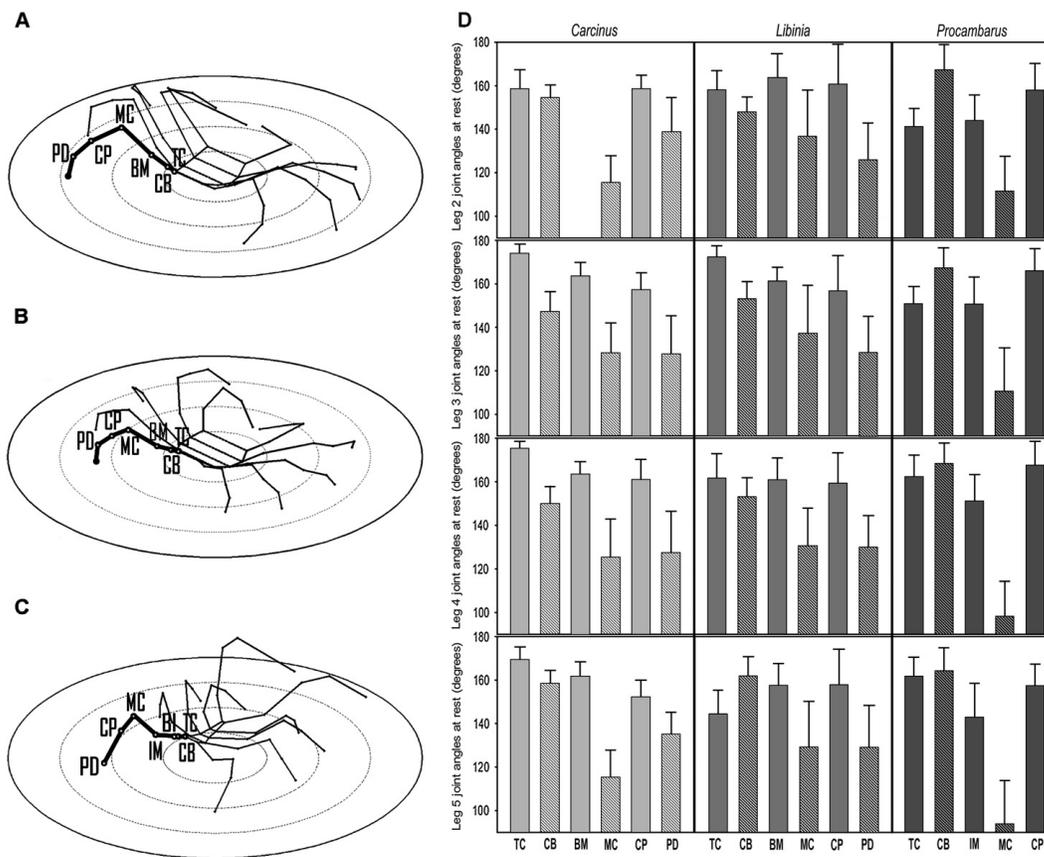


Fig. 8. Comparison of the joint angles of the legs at rest. A three-dimensional caricature of (A) *Carcinus*, *Libinia* (B), and *Procambarus* (C) showing the averaged contribution of each joint during stance. (D) Average joint angles for each species and leg during stance. Etched bars indicate vertically moving joints, while solid bars indicate joints articulating in the horizontal plane. Joints are displayed from proximal to distal (from left to right).

he assumed sideways locomotion and did not test drag past 20° from the sideways direction, thereby potentially missing the preferred walking direction of this animal. Drag is likely a major factor in determining thorax morphology, since animals have to spend energy to overcome it every time they move. Work on chemotaxis has shown that blue crabs will deviate from the most hydrodynamic posture only when food odorants and slow flows co-occur (Weissburg et al., 2003).

4.3. Endophragmal differences

While the thorax of *Carcinus* is widest dorsally, the thorax of *Libinia* is widest ventrally and can accommodate longer proximal musculature (levators, depressors, promoters, removers) than *Carcinus*. This is correlated with the large range of motion of its TC joint (Fig. 1). In contrast to *Libinia*, both *Carcinus* and *Procambarus* have their endophragmal compartments arranged more vertically (Fig. 3) like those of *Callinectes* crabs (Cochran, 1935). This could be related to these animals' ability to walk on land, since the increase in experienced weight would require stronger forces to support the weight of the animal on land, and thus the animals would benefit from having shorter muscles with more fibers in parallel. Because (unlike the other two species) *Libinia* does not venture on land, its proximal musculature does not have to deal with increased weight, and it can afford to have the longer muscles required for the increased TC range observed during forwards locomotion. The posterior arrangement of the epimerites in *Libinia* further increases the potential length that the muscles can reach and lends support to the importance of the TC and CB joints in this behavior (Fig. 3Bii). The ventral endophragm was also different in *Libinia* and *Carcinus*, with the posterior segments (for L4 and L5) laying less perpendicular to the midline in *Libinia*. This is a reflection of the radial arrangement of the legs of *Libinia* and increases the length of the compartment available for the ventral musculature (Fig. 3iii v).

4.4. Leg specializations

The specialization of the legs of decapod crustaceans is not always as obvious as a pair of chelipeds. The three species we studied had additional specializations in their walking legs. *Carcinus* legs are laterally flattened and mostly devoid of sensilla except for the ventral and dorsal edges of L2 and L5 (sometimes also L3 and L4). The sensilla in these areas have been shown to be involved in chemotaxis in blue crabs (Keller et al., 2003). The last pair of legs of *Carcinus* is shorter, wider, and rotated more horizontally to perform their swimming behavior (Fraser, 1974). *Procambarus* legs are flattened and have sensilla throughout but (like those of *Carcinus*) have areas of greater sensilla population in the second and fourth legs. Keller et al. (2003) suggested that because of boundary layers, the distal segments are exposed to slower, more viscous water that facilitates the task of tracking odorants. *Procambarus* differs from both crabs in not having the basipodite and ischiopodite segments fused, so it has an

additional joint. In *Procambarus*, the plane of the basisischium joint rotates the longitudinal axis of the leg, while the ischiopodite-meropodite moves in the anterior–posterior direction.

Libinia exhibits the least morphological differences between legs. Sensilla cover the legs (and thorax) homogeneously without clearly specialized sensory areas besides the long sensilla around their proximal joints. The biggest difference between the legs of *Libinia* is in length and the angle between the longitudinal axis of the legs and of the thorax. This difference in insertion angles means the legs are more evenly spaced around the perimeter of the thorax and therefore have a wide horizontal range of movement without contacting adjacent legs. This also implies that the action performed by analogous muscles in different legs will not be equivalent.

4.5. Ranges of motion

When we compared the combined angular displacement in the horizontal plane (forward direction), we observed that *Procambarus* had the largest range of motion followed by *Libinia*, and lastly *Carcinus* (Fig. 5B). Thus, the animals have a maximal range of motion in the preferred walking orientation. We next wanted to find out if this was correlated with the radial arrangement of the legs in *Libinia* (since the legs of *Procambarus* are the least radial of the three species) or with increased ranges of motion in the segments that move the leg in the anterior–posterior direction. We found that joints moving the legs in the forward direction had a larger range of motion in *Procambarus* and *Libinia* than in *Carcinus*, the opposite was true for joints moving the leg in the sideways direction (Fig. 5C,D). The difference increased for posterior legs (Fig. 5). This is probably due to the fact that posterior legs are less perpendicular to the longitudinal axis of the body, and therefore a bigger proportion of the range of motion translates into lateral displacement instead of forward propulsion. From our observations, *Libinia* appear to make minimal use of their second and third legs during forwards-walking (unpublished observation). The forwards-walking soldier crab *Mictyris* has also been reported to have larger ranges of motion for joints in the horizontal plane than for these joints in sideways-walking species (Sleinis and Silvey, 1980).

4.6. Segment lengths

We found a positive correlation between length of segments proximal to a joint and that joint's range of motion. This suggests that coupled with a bigger range of motion is a longer cavity housing the musculature responsible for the movement. This is similar to the increased length of the endophragmal compartments in *Libinia*.

4.7. Stance width and length

Of the three species studied, *Carcinus* shows the most lateral placement of the dactyls (Fig. 7Ai, D). This leg arrangement is perhaps a reflection of *Carcinus* walking preference,

since it increases its ability to counteract drag forces in the sideways direction. A wider stance in the direction of drag forces implies that the center of mass would rest further inside the polygon of support and therefore be more stable (Alexander, 2002). This idea is further supported by *Procambarus* whose dactyl placement formed an ellipse elongated in the anterior–posterior axis. *Libinia* had the most circular dactyl arrangement around the COM. This reflects *Libinia* being an intermediate between the other two species, since it engages in both walking behaviors (forwards and sideways) more than either of the other two species (Fig. 7Aii,D,E).

4.8. Contribution of joints to stance

Our goal was to find whether there are morphological and functional differences between a forwards-walking brachyuran crab (*Libinia*), a forwards-walking macruran (*Procambarus*), and a more typical brachyuran that walks sideways (*Carcinus*). At rest we found some similarities between the two brachyurans but also other similarities shared between the forwards-walkers. Individual joint angles at rest in the two crab species were similar. All the vertically moving joints were partially flexed, which resulted in each leg adopting an arc-shape. Joints bending in the horizontal plane were close to 180° contributing to an increased sprawl of the dactyls, and a larger stance base. This leg use differed in *Procambarus* in that one joint (MC) was flexed more than any of the other vertically moving joints. This gives the crayfish legs an L-shaped appearance. The ensuing reduction in stance width reduces *Procambarus* stability in the sideways direction but increases stability in the anterior–posterior direction by reducing drag. This occurs by having the legs closer to the thorax and by having the legs in line, both of which reduce the animal's frontal area.

The main differences in leg arrangements during stance between crab species lay in the two posterior limbs. Here we saw fairly similar dactyl placements achieved by different means. In *Carcinus*, all the distal joints of legs four and five maximize the breadth of the stance. This distal displacement is necessary for stability when we examine how the legs insert in the thorax. The insertion points in *Carcinus* were aligned more in parallel to the longitudinal axis. This reduces the maximum ROM possible for the TC joint. In *Libinia*, however, the legs exit the thorax more radially increasing the maximum ROM possible. This also means that the proximal joints and segments are already displaced in the anterior–posterior direction and the distal joints can be primed for walking (bent forwards) without compromising stability.

4.9. *Libinia* and *Mictyris*

The skeletal similarities we found between *Libinia* and *Procambarus* are also present in the other forwards-walking crab described in the literature, *Mictyris longicarpus* (Sleinis and Silvey, 1980). Both *Libinia* and *Mictyris* crabs have orbicular thorax shape and legs arranged radially around the carapace, instead of the parallel arrangement found in most sideways-walking crabs. *Mictyris* also has a bigger range of motion

than sideways-walking crabs for joints propelling the animal forward. These similarities are more striking when one considers the fact that *Libinia* and *Mictyris* are not closely related species. Leg arrangement, thoracic elongation in preferred walking direction, and increased segmental ranges of motion appear to show adaptations in forwards-walking crabs and macrurans. This suggests that these are important skeletal features subject to selective pressures.

4.10. Evolution of the crab form and walking preference

The joints of crustacean legs articulate along consecutively perpendicular planes. This was possibly an exaptation for sideways walking since the initial change in preferred direction would not have required any concomitant structural change. Predatory pressure acting on forwards-walking homoloid ancestors might have produced species that dealt with predators by means of one of several strategies. Tail flips continued to serve homoloids until they became too massive for successfully escaping predators; increasing chelae size might have provided a means of defense for these animals. Crayfish and lobsters are examples of this strategy.

Since the abdomen is by far the most vulnerable part of the crustacean body, animals not relying on tail flips for escape might have reduced its size, and eventually tucked this organ under the thorax giving rise to the crab form. This had the added advantage of allowing animals to walk in any direction without the obstruction of the abdomen. Reduction of the abdomen, however, limited the maximum size of the chelae by bringing the center of mass forwards. Lacking their ancestors' main weakness, but also limiting the size of their primary weapons, newly evolved crabs faced two options to escape predators, strength or speed. The first of these options lead to the use of camouflage and the increase in the toughness of the exoskeleton. *Libinia* and other Majoids have heavily calcified skeletons that are often camouflaged and provide slow but successful protection from predators. The latter option relied on increasing walking speed. The speed at which an animal can walk depends on several factors including the length of the limbs, and potential stride length, which is in turn dependent on the legs' range of motion. Walking sideways allows legs a greater stride length, as individual legs are not impeded by their neighbors. Sideways-walking provided crabs the additional and unique benefit of being equally fast in opposite directions.

5. Concluding remarks

Much of the work done in crustacean locomotion has taken place under the assumption that all legs functioned in a similar fashion. Anatomical and physiological works often neglect to report which leg they have chosen to study and the reason for their choice. The limbs of decapod crustaceans are subject to the same selective pressures that over time have produced the remarkable variety of arthropod forms we see in the world today.

Past assumptions often made on the structural and functional similarities between different legs need to be replaced by more detailed descriptions of each limb and its roles in stance and locomotion. Only by these means will we have the correct context in which to place the ever-increasing wealth of knowledge obtained from these organisms.

Comparison between *Libinia* and closely related sideways-walking crabs might prove useful in understanding the anatomical changes that were necessary for forwards-walking homoloid ancestors to become sideways-walking brachyuran crabs.

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References

- Alexander, R.Mc.N., 2002. Stability and manoeuvrability of terrestrial vertebrates. *Integrative and Comparative Biology* 42, 158–164.
- Barnes, W.J.P., 1975. Leg co-ordination during walking in the crab, *Uca pugnax*. *Journal of Comparative Physiology (A)* 96, 237–256.
- Bévangut, M., Simmers, A.J., Clarac, F., 1983. Central neuronal projections and neuromuscular organization of the basal region of the shore crab leg. *Journal of Comparative Neurology* 221 (2), 185–198.
- Blake, R.W., 1985. Crab carapace hydrodynamics. *Journal of Zoology (A)* 207 (3), 407–423.
- Brante, A., Hughes, R.N., 2001. Effect of hypoxia on the prey-handling behavior of *Carcinus maenas* feeding on *Mytilus edulis*. *Marine Ecology Progress Series* 209, 301–305.
- Bush, B.M.H., 1962. Proprioceptive reflexes in the legs of *Carcinus maenas* (L.). *Journal of Experimental Biology* 39, 89–105.
- Cochran, D.M., 1935. The skeletal musculature of the blue crab, *Callinectes sapidus* Rathbun. *Smithsonian Miscellaneous Collections* 92, 1–76.
- Fraser, P.J., 1974. Interneurons in crab connectives (*Carcinus maenas* (L.)): Directional statocyst fibers. *Journal of Experimental Biology* 61, 615–628.
- Huxley, T.H., 1880. *The Crayfish: An Introduction to the Study of Zoology*. D. Appleton and Co, New York.
- Jamon, M., Clarac, F., 1995. Locomotor patterns in freely moving crayfish (*Procambarus clarkii*). *Journal of Experimental Biology* 198, 683–700.
- Jamon, M., Clarac, F., 1997. Variability of leg kinematics in free-walking crayfish, *Procambarus clarkii*, and related inter-joint coordination. *Journal of Experimental Biology* 200 (8), 1201–1213.
- Keller, T.A., Powell, I., Weissburg, M.J., 2003. Role of olfactory appendages in chemically mediated orientation of blue crabs. *Marine Ecology Progress Series* 261, 217–231.
- Macmillan, D.L., 1975. A physiological analysis of walking in the American lobster (*Homarus americanus*). *Philosophical Transactions of the Royal Society of London B. Biological Sciences* 270 (901), 1–59.
- Martinez, M.M., 1996. Issues for aquatic pedestrian locomotion. *American Zoologist* 36, 619–627.
- Martinez, M.M., Full, R.J., Koehl, M.A., 1998. Underwater punting by an intertidal crab: A novel gait revealed by the kinematics of pedestrian locomotion in air versus water. *Journal of Experimental Biology* 201 (18), 2609–2623.
- Martinez, M.M., 2001. Running in the surf: hydrodynamics of the shore crab *Grapsus tenuicrustatus*. *Journal of Experimental Biology* 204, 3097–3112.
- McKinnon, W., Hartford, C., Di Zio, L., van Schalkwyk, J., Veliotes, D., Hofmeyr, A., Rogers, G., 2004. The agreement between reaction-board measurements and kinematic estimation of adult male human whole body centre of mass location during running. *Physiological Measurements* 25 (6), 1339–1354.
- Morrison, C.L., Harvey, A.W., Lavery, S., Tieu, K., Huang, Y., Cunningham, C.W., 2002. Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like form. *Proceedings of the Royal Society B: Biological Sciences* 269, 345–350.
- Parsons, D.W., 2005. The leg flexor muscle of *Carcinus*. I. Innervation and excitatory neuromuscular physiology. *Journal of Experimental Zoology* 224 (2), 157–168.
- Pilgrim, R.L.C., Wiersma, C.A.G., 1963. Observations on the skeleton and somatic musculature of the abdomen and thorax of *Procambarus clarkii* (Girard), with notes on the thorax of *Panulirus interruptus* (Randall) and *Astacus*. *Journal of Morphology* 113 (3), 453–487.
- Pond, C.M., 1975. The role of the ‘walking legs’ in aquatic and terrestrial locomotion of the crayfish *Austropotamobius pallipes* (Lereboullet). *Journal of Experimental Biology* 62, 447–454.
- Reynolds, E., Lovett, R.W., 1909. A method of determining the position of the center of gravity in relation to certain bony landmarks in the erect position. *American Journal of Physiology* 24 (2), 286–293.
- Rice, A.L., 1983. Zoeal evidence for brachyuran phylogeny. In: Schram, F.R. (Ed.), *Crustacean Phylogeny*. *Crustacean Issues*, Vol. 1. Balkema, Rotterdam, pp. 313–329.
- Ritzmann, R.E., Quinn, R.D., Fischer, M.S., 2004. Convergent evolution through complex terrain by insects, vertebrates and robots. *Arthropod Structure and Development* 33, 361–379.
- Schreiner, J.N., 2004. Adaptations by the locomotor systems of terrestrial and amphibious crabs walking freely on land and underwater. Master’s thesis, Louisiana State University. http://www.etd.lsu.edu/docs/available/etd-06092004-130835/unrestricted/Schreiner_thesis.pdf.
- Sleinis, S., Silvey, G.E., 1980. Locomotion in a forward walking crab. *Journal of Comparative Physiology A* 136 (4), 301–312.
- Weissburg, M.J., James, C.P., Smece, D.L., Webster, D.R., 2003. Fluid mechanics produces conflicting, constraints during olfactory navigation of the blue crabs, *Callinectes sapidus*. *Journal of Experimental Biology* 206, 171–180.