

## RESEARCH ARTICLE

# Comparative whole-body kinematics of closely related insect species with different body morphology

Leslie M. Theunissen<sup>1,2</sup>, Holger H. Bekemeier<sup>1,2</sup> and Volker Dürr<sup>1,2,\*</sup>

## ABSTRACT

Legged locomotion through natural environments is very complex and variable. For example, leg kinematics may differ strongly between species, but even within the same species it is adaptive and context-dependent. Inter-species differences in locomotion are often difficult to interpret, because both morphological and ecological differences among species may be strong and, as a consequence, confound each other's effects. In order to understand better how body morphology affects legged locomotion, we compare unrestrained whole-body kinematics of three stick insect species with different body proportions, but similar feeding ecology: *Carausius morosus*, *Aretaon asperimus* and *Medauroidea extradentata* (= *Cuniculina impigra*). In order to co-vary locomotory context, we introduced a gradually increasing demand for climbing by varying the height of stairs in the setup. The species were similar in many aspects, for example in using distinct classes of steps, with minor differences concerning the spread of corrective short steps. Major differences were related to antenna length, segment lengths of thorax and head, and the ratio of leg length to body length. Whereas all species continuously moved their antennae, only *Medauroidea* executed high swing movements with its front legs to search for obstacles in the near-range environment. Although all species adjusted their body inclination, the range in which body segments moved differed considerably, with longer thorax segments tending to be moved more. Finally, leg posture, time courses of leg joint angles and intra-leg coordination differed most strongly in long-legged *Medauroidea*.

**KEY WORDS:** Leg movement, Motion capture, Whole-body coordination, Walking, Climbing, Phasmatodea

## INTRODUCTION

Despite the fact that insects have become important model animals for the study of adaptive locomotion (Ritzmann and Büschges, 2007) and a wide range of behavioural (Dürr et al., 2004; Cruse et al., 2009), biomechanical (Full et al., 1991) and neurophysiological (Burrows, 1996; Büschges and Gruhn, 2007; Büschges, 2012) studies on insect locomotion have contributed to a detailed understanding of multi-legged locomotion in general, there are very few studies on comparative kinematics of insect walking or climbing. Of course, there is a host of information on species belonging to various taxa, most notably on cockroaches (Blattodea), crickets and locusts (Orthoptera) and stick insects (Phasmatodea), and several reviews have collated some of the material (e.g. Wilson,

1966; Graham, 1985; Burrows, 1996; Büschges and Gruhn, 2007; Cruse et al., 2009). However, comparative analyses are difficult to interpret if data have been collected in different settings or with different methods.

With regard to the overall aspects of locomotion, such as certain gait characteristics (Wilson, 1966), the movement of the centre of mass during running (Blickhan and Full, 1993) or adaptation to inclines (Birn-Jeffery and Higham, 2014), literature reviews have identified commonalities among species, but they fall short of identifying causes for distinct differences between species. In other words, the causes of inter-species variability in legged locomotion are not well understood. In order to change this, comparative experiments on unrestrained locomotion are needed. Up to now, only few such studies have linked comparative kinematics to morphological differences in invertebrates. In climbing and ground-dwelling ant species, postural differences occur when walking on slopes (Weihmann and Blickhan, 2009), but limb kinematics were not compared. In decapod crustaceans, a combination of comparative motion analysis and morphological measures indicated a link between leg segment length and preferred walking direction (Vidal-Gadea et al., 2008). In vertebrates, where comparative analyses of legged locomotion have been more common, it has become clear that both morphological characters as well as ecological differences, such as habitat preferences, may strongly affect limb kinematics. Typically, it is difficult to disentangle these two factors. For example, in lizards, limb kinematics of two species may be differentially affected by slope (Higham and Jayne, 2004) or may vary with habitat (Fuller et al., 2011). In these studies, the species compared exhibit morphological differences that, at least to some extent, reflect specific habitat adaptations. With regard to the interdependence of body morphology and limb kinematics, Gatesy and Pollard (Gatesy and Pollard, 2011) have argued that limb segment proportions necessarily pose important constraints on limb kinematics. Nevertheless, constraints are not equal to determinants. For example, distantly related birds with similar limb proportions but different habitat preferences were shown to have similar limb kinematics in the sagittal plane, but not in the transversal plane (Stoessel and Fischer, 2012). In arboreal and terrestrial rodents with similar leg segment proportions, the same kinematic adjustments to climbing locomotion were found (e.g. reduced clearance), despite differences in speed and gait (Schmidt and Fischer, 2011). In other words, kinematic variables associated with gait differences (e.g. timing in inter-leg coordination) were species dependent, whereas others were not. Generally, small mammals share several important 'overall' kinematic features during legged locomotion, e.g. scapula movement (Fischer et al., 2002). Nevertheless, they may differ considerably with regard to limb proportions and variables of intra-leg and inter-leg coordination.

The objective of the present study was to identify species differences in kinematics of unrestrained walking and climbing insects, and to relate them to differences in morphology and body

<sup>1</sup>Biological Cybernetics, Faculty of Biology, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany. <sup>2</sup>Cognitive Interaction Technology – Center of Excellence, Bielefeld University, Inspiration 1, 33615 Bielefeld, Germany.

\*Author for correspondence (volker.duerr@uni-bielefeld.de)

Received 17 September 2014; Accepted 27 November 2014



**Fig. 1. The three stick insect species used in this study.** From top to bottom: Adult female *Carausius morosus* (de Sinéty 1901), adult female *Medauroidea extradentata* (Brunner von Wattenwyl 1907) and adult male *Aretaon asperimus* (Redtenbacher 1906). The diameter of a 1 € coin is 23.25 mm.

segment proportions in particular. For this, we recorded whole-body kinematics of three species of unrestrained walking and climbing stick insects: *Carausius morosus*, *Aretaon asperimus* and *Medauroidea extradentata* (= *Cuniculina impigra*). We compared step parameters, movement of the head and thorax segments and intra-leg coordination, i.e. joint angle time courses. All three species are herbivores that live in an arboreal habitat. Moreover, all three species have a similar overall body structure and no obvious kinematic specialisations other than differing proportions of thorax and leg segments.

A known morphological difference with regard to Phasmatodean locomotion concerns the smoothness of tarsal attachment pads, e.g. in *Carausius* and *Medauroidea* (Bußhardt et al., 2012). Despite the structural differences, the motor control of at least two leg joints is very similar in both of these species, for example, the muscle structure and activity of the femur–tibia joint control loop (Bässler et al., 1996) or the retractor unguis that moves the tibia–tarsus joint (Bußhardt et al., 2011). The strongest difference between the three species concerns the proportions of the antennae and legs, because *Medauroidea* has much shorter antennae and longer legs than the other two species. Stick insects use their antennae to explore the near-range environment (Dürr et al., 2001; Dürr and Bläsing, 2001), to sample obstacles during climbing (Schütz and Dürr, 2011; Krause and Dürr, 2012) and to transfer spatial information to the front legs (Schütz and Dürr, 2011). However, kinematic properties of the antennae are conserved among the Phasmatodea, irrespective of antenna length (Mujagic et al., 2007).

All three species have been studied with regard to different aspects of multi-legged locomotion, including leg coordination

**Table 1. Comparison of segment sizes and proportions**

Genus	Sex	N	Body						Hind legs			Middle legs			Front legs			Antennae			
			Mass	Abd	T3	T2	T1	Head	cox	tib	cox	fem	tib	cox	fem	tib	scp	ped	fla		
<i>Carausius</i>	♀	N=10	Mean	39.15	11.96	17.11	3.68	4.21	1.54	13.87	14.11	1.42	11.60	11.20	1.32	15.84	15.03	1.54	0.75	30.87	
			s.d.	0.124	0.56	0.43	0.29	0.23	0.16	0.44	0.57	0.13	0.42	0.34	0.16	0.85	0.61	0.08	0.09	0.69	
			x/T3	<b>3.27</b>	1.00	<b>1.43</b>	0.31	0.35	0.13	1.16	1.18	0.12	0.97	0.94	0.11	1.32	1.26	0.13	0.06	2.58	
<i>Aretaon</i>	♂	N=13	Mean	0.818	25.97	8.49	9.74	4.25	4.81	2.06	12.43	13.00	1.86	9.79	9.67	1.91	10.56	10.65	1.22	0.89	29.85
			s.d.	0.068	0.98	0.83	0.82	0.37	0.28	0.53	1.37	1.49	0.37	0.91	1.01	0.27	1.14	0.99	0.19	0.13	1.40
			x/T3	3.06	1.00	1.15	<b>0.50</b>	<b>0.57</b>	<b>0.24</b>	1.46	1.53	1.14	<b>0.22</b>	1.15	1.14	<b>0.22</b>	1.24	1.25	<b>0.14</b>	<b>0.11</b>	<b>3.51</b>
<i>Medauroidea</i>	♀	N=6	Mean	1.673	42.42	14.86	18.73	4.83	5.46	2.07	27.48	33.87	2.07	21.42	24.00	2.11	32.54	39.07	2.22	0.81	11.94
			s.d.	0.243	1.64	0.42	0.30	0.16	0.42	0.26	1.81	1.65	0.26	1.14	1.19	1.50	2.41	2.41	0.25	0.11	2.28
			x/T3	2.85	1.00	1.00	1.26	0.32	0.37	0.14	<b>1.85</b>	<b>2.28</b>	0.14	<b>1.44</b>	<b>1.61</b>	0.14	<b>2.19</b>	<b>2.63</b>	0.15	0.05	0.80
<i>Medauroidea</i>	♂	N=3	Mean	36.01	13.55	15.55	3.01	4.01	1.57	28.30	34.05	1.67	21.70	25.74	1.46	34.29	41.39	1.26	0.88	20.72	
			s.d.	1.92	1.31	0.90	0.90	0.17	0.66	0.17	1.83	7.44	0.17	1.43	16.32	10.71	0.28	0.18	1.42		
			x/T3	2.66	1.00	1.15	0.22	0.30	0.12	<b>2.09</b>	<b>2.51</b>	0.11	<b>2.53</b>	<b>3.05</b>	0.11	<b>2.53</b>	<b>3.05</b>	0.09	0.06	1.53	

Body mass, absolute and normalised segment lengths of main body axis, the legs and the antennae. Abd, abdomen; T1, prothorax; T2, mesothorax; T3, metathorax; cox, coxa; fem, femur; tib, tibia; scp, scapus; ped, pedicellus; fla, flagellum. All lengths and s.d. are given in mm; proportions (x/T3), are given as the ratio of the respective mean of parameter x and the length of the metathorax (T3). Bold font highlights the species with the largest proportions. The mean mass and s.d. are given in g and were measured from 10 animals per species.

[*Carausius* (Cruse, 1990; Dürr, 2005; Grabowska et al., 2012); *Aretaon* (Jeck and Cruse, 2007)], climbing and/or leg searching-movements [*Carausius* (Cruse, 1976a; Dürr et al., 2001); *Medauroidea* (Karg et al., 1991; Berg et al., 2013); *Aretaon* (Bläsing and Cruse, 2004a)], catalepsy [*Carausius* (Godden, 1974); *Medauroidea* (Bässler and Foth, 1982)] and single-joint kinematics [*Carausius* (Cruse and Bartling, 1995); *Aretaon* (Frantsevich and Cruse, 1997; Jeck and Cruse, 2007)].

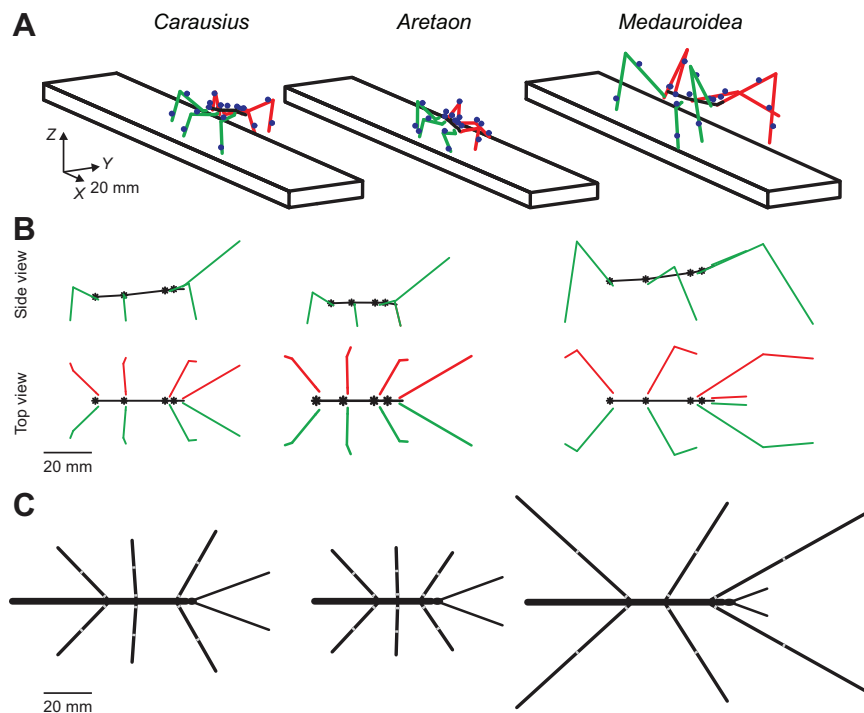
Because none of these data have been acquired using the same combination of setup and method, and many of them concern tethered, planar walking only, direct comparison of the results is not justified without simplifying assumptions. Moreover, none of the above studies systematically varied the setup structure to include both walking and climbing trials. Finally, the movement of all thorax segments during climbing has never been measured before in stick insects. However, this is important, because prothorax inclination has been shown to affect climbing performance in cockroaches (Ritzmann et al., 2005). To the best of our knowledge, we provide the first comparative study of whole-body kinematics of unrestrained insect locomotion. A representative subset of the data has been published (Theunissen et al., 2014a) as part of an initiative to establish an open-access natural locomotion database (Theunissen et al., 2014b).

## RESULTS

With regard to our main objective, we selected three species of stick insects (Phasmatodea) that differ in size and body proportions (Fig. 1), and readily engage in locomotion under experimental conditions: *Carausius morosus*, *Medauroidea extradentata* and *Aretaon asperimus* (subsequently referred to by the genus only). Although *Carausius* and *Medauroidea* are more closely related to each other systematically than *Aretaon* is to either of them (Bradley and Galil, 1977), the overall body proportions are more similar between *Carausius* and *Aretaon*, with *Medauroidea* standing out with particularly long legs and short antennae (Figs 1, 2). Of the three species, *Medauroidea* is also the largest in size and the heaviest (Table 1). In all three species, the centre of mass (CoM) is

located close to the thorax–coxa joints of the hind legs. Because the exact location of the CoM may vary with physiological state, abdominal posture and, in females, number of eggs, we did not measure its location quantitatively. However, qualitatively, there is a clear difference between the three species: in *Carausius*, the CoM is located slightly posterior to the hind leg coxae [corroborating published measurements (Cruse, 1976b)], in *Aretaon* it is slightly anterior, whereas in *Medauroidea* it is located almost between the hind leg coxae, intermediate to the CoM locations of the other two species.

Assuming that the absolute size differences of the body segments are of minor importance to the control of locomotion, we focussed on differences in proportions. Accordingly, we normalised all body segment lengths to the length of the metathorax (Table 1, T3). With regard to the main body, the abdomen and mesothorax are (proportionally) longest in *Carausius*, whereas the prothorax (T1) and head are longest in *Aretaon*. With regard to the legs, the segments femur (fem) and tibia (tib) are longest in *Medauroidea*, whereas the proximal coxa (cox) is longest in *Aretaon*. The antennae of *Carausius* and *Aretaon* reach at least as far as the front leg tarsi (see also Fig. 2B,C). In contrast, *Medauroidea* has very short antennae, particularly in females. Assuming that these differences in proportions can affect the static stability, leg coordination, and the functional use of the legs in natural locomotion, we will test the following hypotheses: (1) Owing to its long and, therefore, relatively heavy abdomen, the hind legs of *Carausius* should stay more retracted than in the other species: this could contribute to the static stability of the body. (2) The different proportions of thorax segments should be reflected in different movement ranges of the corresponding intersegmental joints during climbing, being larger for longer segments. This would indicate the use of the leverage of long segments. (3) Owing to its long legs and short antennae, *Medauroidea* should be able to compensate for spatial disturbances more easily by adjusting leg posture, resulting in less movement of the body axis. Moreover, the antennae should be less suited for tactile exploration, simply because the front leg feet should lead the antennal tips and not the other way round, as described for



**Fig. 2. *Carausius*, *Aretaon* and *Medauroidea* have different body proportions and postures.** (A) Stick figures of each species walking on the flat setup with markers (blue) attached to the body (black) and the right (green) and left (red) legs. (B) Side and top views of median posture during stance. The sex of the illustrated insects are female in *Carausius* and *Medauroidea*, and male in *Aretaon*. (C) Schematics of body proportions with outstretched limbs. Relative to the thorax, *Medauroidea* has the longest legs and the shortest antennae. Its leg posture differs from that of the other species. Segment lengths, but not widths were scaled in B and C.

*Carausius* (Dürr et al., 2001) and *Aretaon* (Bläsing and Cruse, 2004a). As a consequence, the legs of *Medauroidea* should account for tactile searching behaviour during climbing.

In order to test these hypotheses, four major aspects were analysed and compared between the three species: (1) the overall walking and climbing behaviour, (2) the change in intersegmental angles of the thorax and neck during climbing, (3) overall step parameter distributions, i.e. step length, height and direction, and (4) leg kinematics at the single-joint level.

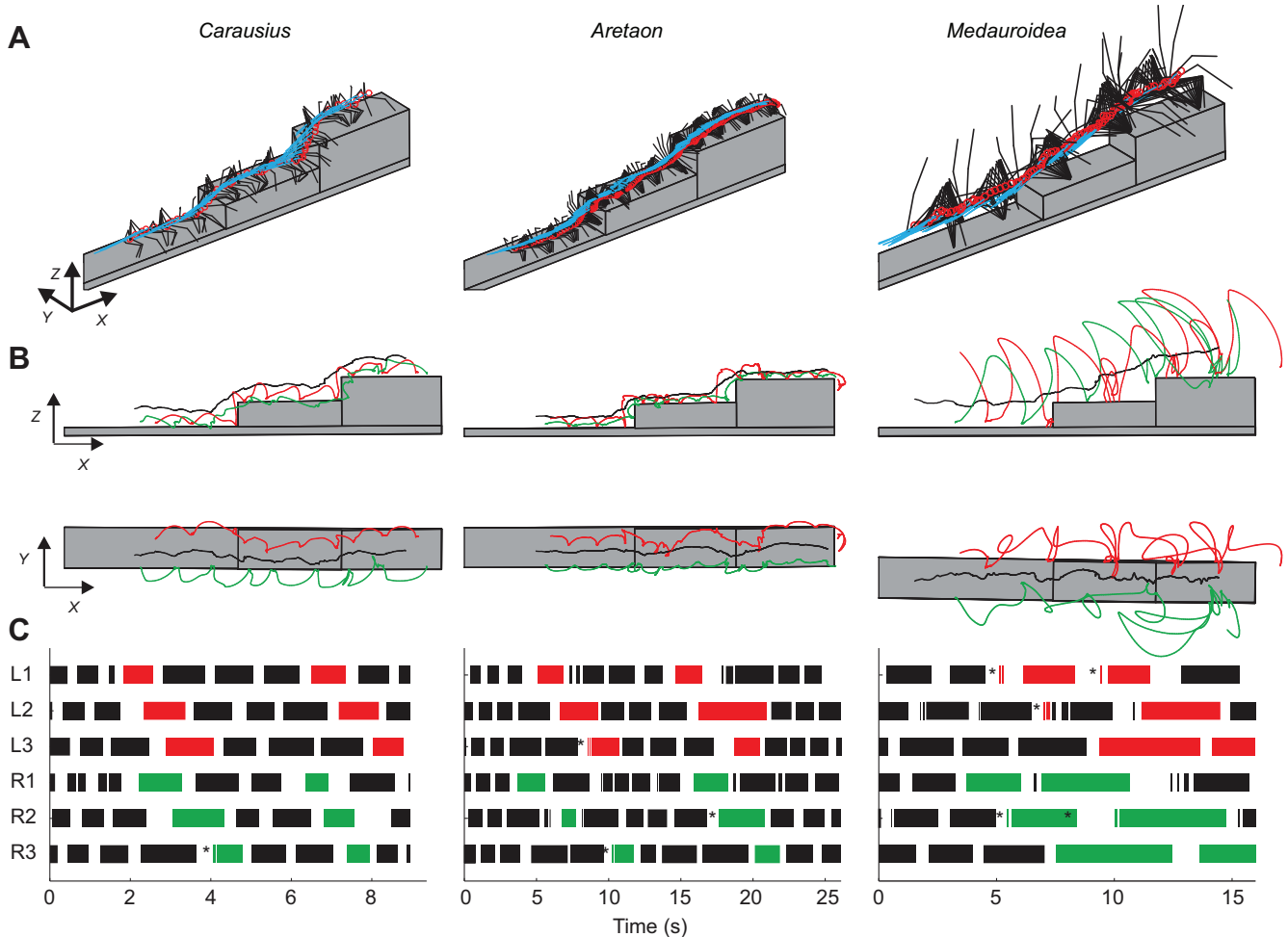
### Differences in posture and overall climbing behaviour

Overall walking and climbing behaviour differed with regard to forward velocity (supplementary material Fig. S1). Clearly, body size did not correlate with speed, because *Carausius* walked significantly faster than *Aretaon* and *Medauroidea* (ANOVA, d.f.=2;  $F=19.53$ ,  $P<0.001$ , multiple comparison). Generally, forward velocity was significantly reduced during climbing (ANOVA, d.f.=3,  $F=6.3$ ,  $P<0.001$ ). Differences in body proportions, as described above and listed in Table 1, were reflected in postural differences. This is shown by the stick figures in Fig. 2B, where the median body postures are drawn to scale. For each leg, the median posture was determined for stance movements only. Whereas the body postures

of *Carausius* and *Aretaon* are fairly similar, that of *Medauroidea* clearly stands out by its short, adducted, less levated antennae and by the knees being held much more above the body axis, i.e. the femora are more levated.

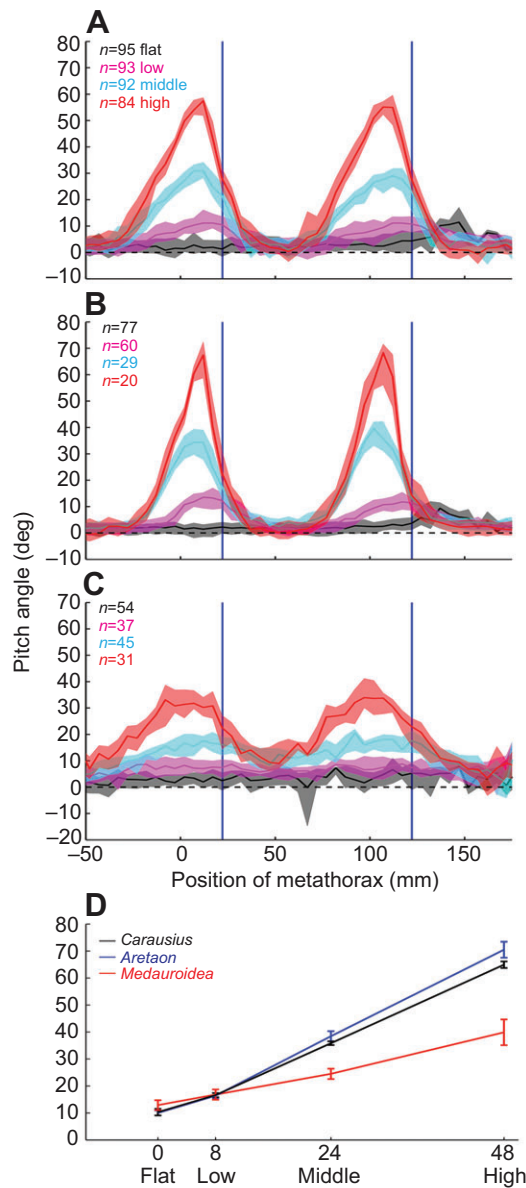
Because *Medauroidea* has considerably longer legs than the other species (Fig. 2B), it took fewer steps to climb the stairs. To illustrate this, Fig. 3 shows representative stepping patterns of trials with high stairs. The first stance phases with a foot standing on the next stair are coloured in red and green for left and right legs, respectively. Whereas *Aretaon* usually took three steps between the stairs and *Carausius* took two, *Medauroidea* was able to step directly from one stair to the next, sometimes with an intermitted short step (e.g. see foot trajectory of R1 in Fig. 3B). The stepping patterns of all species were highly variable, with frequent intermitted short steps (Theunissen and Dürr, 2013) (see below for further analysis) and steady fluctuations in inter-leg coordination, i.e. gait. This corroborates earlier findings that stick insects typically do not show persistent gaits (Dürr, 2005; Grabowska et al., 2012). Accordingly, we did not analyse gait parameters and focused on kinematics of the limbs and body segments instead.

The most obvious difference in overall leg kinematics concerned the foot trajectories of the front legs (Fig. 3A,B). *Medauroidea* lifted



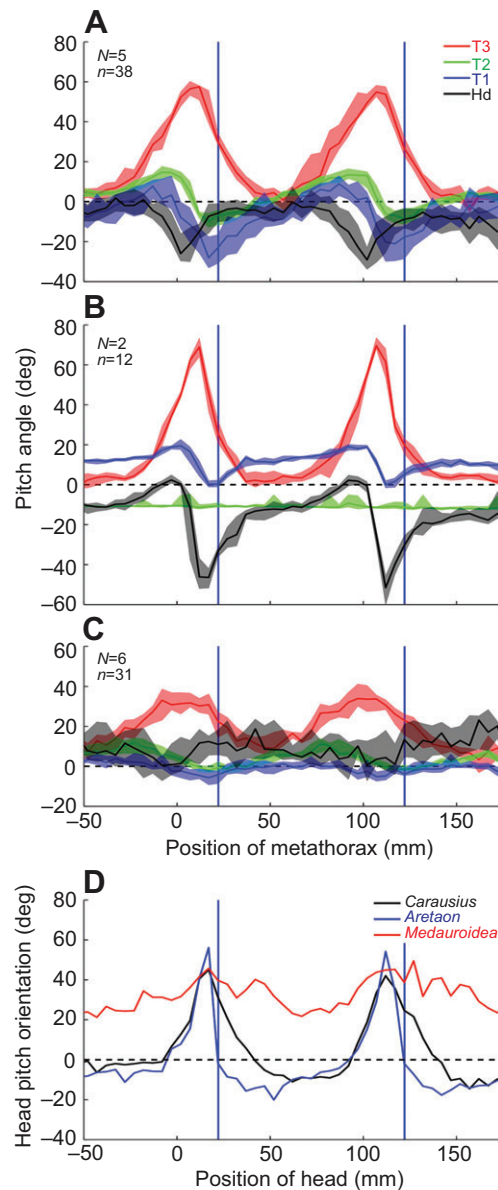
**Fig. 3. Representative trials of unrestrained walking and climbing behaviour.** Columns show representative single trials of the species *Carausius* (♂), *Aretaon* (♂) and *Medauroidea* (♀). (A) Movement of the body axis (blue lines), head (red circles) and front legs (black lines), illustrated by superimposed stick figures every 100 ms. (B) Trajectories of the tibia-tarsus joint of left (red) and right (green) front legs, and of the metathorax (black line) superimposed on the setup in side and top view. Note that foot trajectories regularly reach above the body axis in *Medauroidea*. (C) Podograms of the gait patterns, i.e. time sequences of the alternating swing-stance phases of all six walking legs, where each black line depicts the duration of a stance phase of one of the legs. Red and green lines mark the first stance phases on the next stair in left and right legs, respectively. L1 to L3: left front, middle and hind leg; R1 to R3: corresponding right legs.





**Fig. 4. Pitch angle of the metathorax increases even when climbing low stairs.** (A–C) The median and the quartiles of the metathorax pitch angle plotted against the caudal end of the metathorax for each condition and for the three species of stick insects: *Carausius* (A), *Aretaon* (B) and *Medauroidea* (C). Colours indicate the experimental condition, i.e. the height of the stairs. Grey: flat (no stairs); magenta: low 8 mm stairs; blue: middle, 24 mm stairs; red: high, 48 mm stairs. The vertical blue lines indicate the positions of the stairs. The number of trials is given in each plot. The apparent disturbances in the grey traces beyond 130 mm in A and B are caused by protruding pins on the setup, used to fix the stairs in other conditions. (D) Mean and s.e. of the maximum pitch angle per trial.

its front legs high above the body axis, whereas the other two species lifted their front feet about as high as the body axis (Fig. 3, side view; red and green lines hardly cross the black lines). Since the height of the body axis corresponds to the animal's clearance over ground, lifting the feet higher than the body axis exceeds the maximum height of obstacles beneath the body (see below for further analysis). Therefore, we suggest that *Medauroidea* used the high front leg swing movements to effectively search the space ahead for obstacles and footholds that would require lifting or inclining the body axis. This species difference could not be explained as an adaptive response to



**Fig. 5. Pitch angles of the thorax segments and head are coordinated during climbing.** The median and the quartiles of the pitch angles of the three thorax segments (T3: metathorax; T2: mesothorax; T1: prothorax) and the head (Hd) of *Carausius* (A), *Aretaon* (B) and *Medauroidea* (C) are plotted against the rear end position of the metathorax. Positive/negative angles indicate levation/depression. Only trials using the climbing condition (48 mm stairs) were considered. The vertical blue lines indicate the position of the stairs. Numbers of animals ( $N$ ) and trials ( $n$ ) are indicated on top left of A–C. Note that the binning (5 mm) is different from that in Fig. 4 (1 mm). (D) Pitch angle of the head plotted against the position of the neck. Head pitch changes nearly as much as body axis pitch (T3), but with a time shift.

the stairs on the setup, simply because the clearance of *Medauroidea* was largest (compare side views of the average posture in Fig. 2B). Thus, if swing height had only reached body clearance in *Medauroidea*, its front legs would have easily stepped on top of all but the highest stairs immediately, i.e. without requiring any adaptation of the foot trajectory (see below).

**Stick insects always incline their body axis during climbing**  
Obviously, obstacles higher than the animal's clearance can be climbed only if the body is either lifted in total (as in a push-up) or

inclined. Since different strategies of climbing have been reported for cockroaches (Watson et al., 2002), we were interested in how differences in body size, leg length and obstacle height affect the pitch angle of the body axis. Moreover, because the insect thorax contains two intersegmental joints, we wanted to know how these joints were moved during climbing.

Videos of representative climbing trials may be accessed through an open access data publication (<http://movement.cit-ec.de/stick-insect-locomotion/data.php>) (Theunissen et al., 2014a). Concerning the dependence of body axis inclination on obstacle height, Fig. 4 shows the median pitch angle of the metathorax and its inter-quartile range with respect to the position of the metathorax. During flat walking, the median pitch angles of all species were positive, indicating that clearance was larger beneath the front legs than beneath the hind legs. Whenever stairs were present, the pitch angle increased in front of a stair and decreased again as the animals mounted the stair. The increase was proportional to the height of the stairs (Fig. 4D). This was the case in all three species, although it differed between species, with *Aretaon* showing the largest pitch angles (two-way ANOVA, species: d.f.=2,  $F=32.55$ ,  $P<0.001$ ; obstacle height: d.f.=3,  $F=346.98$ ,  $P<0.001$ ). The significant interaction of the factors species and obstacle height (d.f.=6,  $F=17.47$ ,  $P<0.001$ ) revealed that the slope of the increase in pitch angle with obstacle height differed among species. The slope was steeper in *Carausius* and *Aretaon* than in *Medauroidea* (Fig. 4D). Interestingly, in *Carausius* and *Aretaon* the pitch angles increased significantly even for low stairs (8 mm; *t*-test: *Carausius*  $P<0.001$ , d.f.=8,  $t=10$ ; *Aretaon*  $P<0.001$ , d.f.=7,  $t=9.5$ ), although this would not have been necessary for mechanical reasons. In contrast, *Medauroidea* did not significantly increase its body pitch angle for the low stairs (*t*-test:  $P=0.0853$ , d.f.=5,  $t=2.14$ ).

During climbing, the pitch angles between the three thorax segments and of the neck varied in a coordinated manner (Fig. 5; examples for high stairs). In *Carausius*, the upward inclination of the body segments started with an upward movement by the prothorax (Fig. 5A, T1, blue) followed by the mesothorax (T2, green) and the metathorax (T3, red). The inclination of the metathorax was accompanied by a sequence of downward

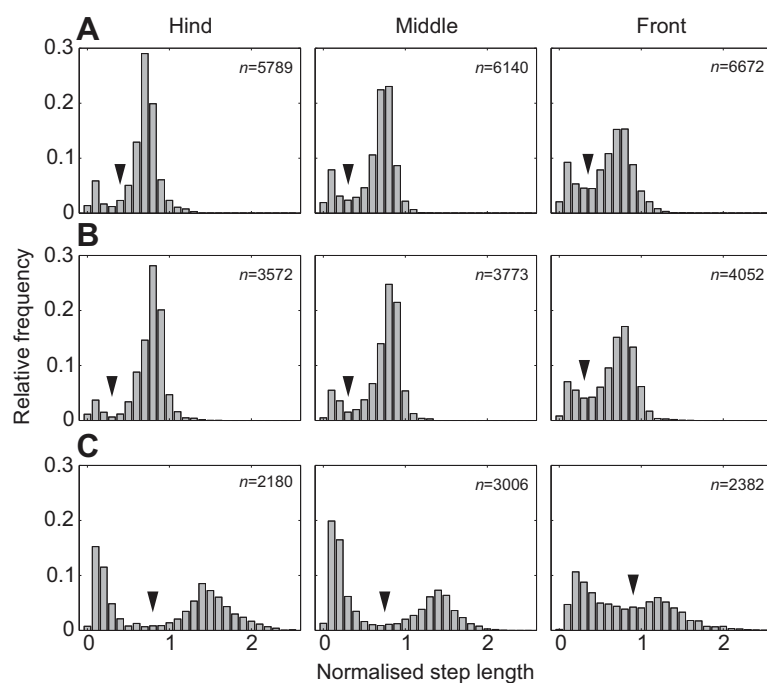
movements (depression) of the head (Hd, black) and the two anterior thorax segments (T1, T2). As soon as the pitch angle of the metathorax reached the peak and reverted to its resting value, the pitch angles of head, prothorax and mesothorax also reverted back to the values typical for flat walking. When climbing the second stair, this sequence was repeated in the same way as on the first stair, indicating that the animals did not adapt their strategy in any obvious way. Note that all pitch angles were plotted against the position of the metathorax, which means that the head had already passed the stair, when its angle decreased to negative values.

Although all species revealed such coordinated, sequential changes in pitch angles, they made different use of the joints. For example, in *Aretaon* the mesothorax was held at a constant angle with respect to the metathorax (Fig. 5B). Instead, *Aretaon* strongly moved the head, reaching about twice the depression of *Carausius*. In *Medauroidea*, the prothorax and the head were moved less than by the other two species (Fig. 5C). Nevertheless, the sequence in which the pitch angles of the head and thorax segments increased and decreased followed the same order as in the other two species: the head leading the prothorax, the prothorax leading the mesothorax.

Overall, the observed sequence of pitch angle variation along the body axis was reminiscent of gaze stabilisation reflexes that are commonly observed in insect locomotion. In order to measure the effect of these movements on the inclination of the head (and thus gaze), we plotted the sum of the four angles against the position of the head on the setup (Fig. 5D). The results show that head orientation was not stabilised. In fact, head orientation varied almost as much as the pitch angle of the metathorax, though it peaked at a different time. We conclude that the actions of the neck and thorax joints do not stabilise gaze in space but appear to adapt head pitch to the forthcoming substrate surface.

### Stick insects use two classes of steps

Following the overall description of climbing behaviour and the analysis of the movements of the body axis, the remaining sections will focus on the movements of the legs. At first, we wondered



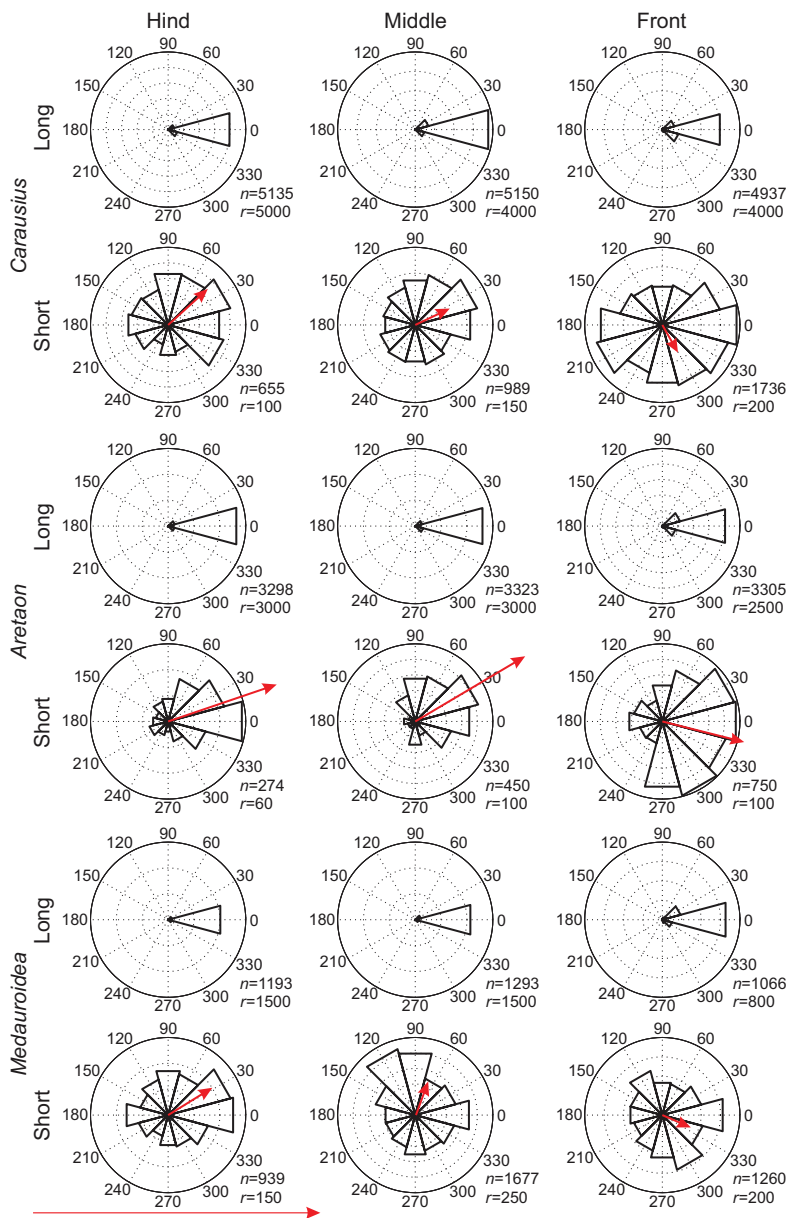
**Fig. 6. Step-length distributions are always bimodal.** The relative frequency of normalised step length is shown for all leg types of *Carausius* (A), *Aretaon* (B) and *Medauroidea* (C). Step length, which was defined as the Euclidian distance in body-fixed coordinates covered by a swing movement, was normalised to the length of the thorax of each individual. Arrowheads indicate the class boundary between short and long steps. The number of steps is given in the top right corner of each panel.

whether the two classes of steps that were recently described for *Carausius* (Theunissen and Dürr, 2013) also occur in *Aretoan* and *Medauroidea*. Indeed, the step length distributions of *Aretoan* and *Medauroidea* were also bimodal. Accordingly, we applied the same method as used for *Carausius* and used the local minimum between the two peaks to divide the step distributions into two distinct classes: short steps and long steps (Fig. 6B,C). After normalisation to the thorax length, i.e. accounting for size differences, there were no obvious differences between the step length distributions of *Carausius* and *Aretoan*. In both species, long steps were used most often (Fig. 6A,B). Also, the two modes were located at similar step lengths. This was different in *Medauroidea*, which used short steps more often. Furthermore, *Medauroidea* took steps that were much longer than the thorax length and, in all three leg types, the entire distributions were broader than in the other species.

In *Carausius*, short steps were shown to have a different function than long steps, suggesting that they serve as correction steps rather than for propulsion. One of the main characteristics of

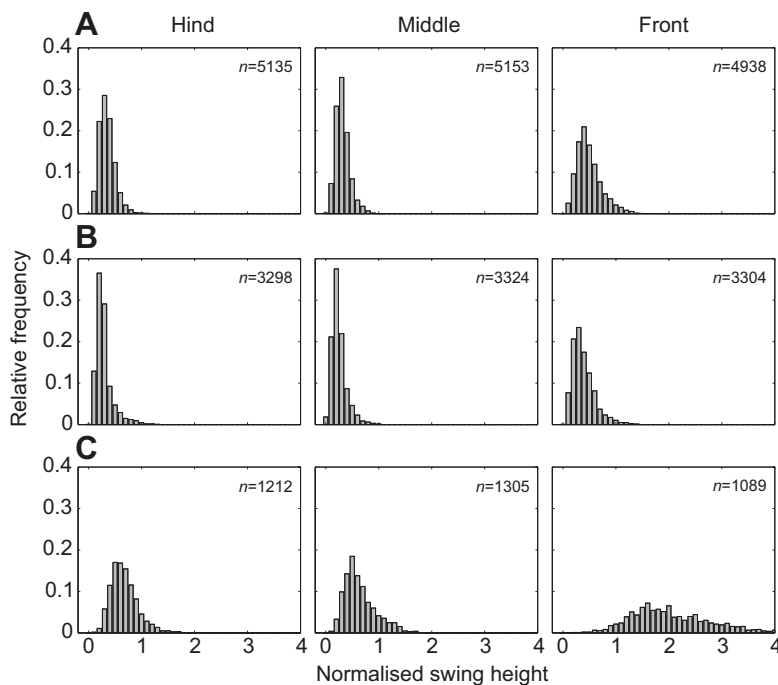
short steps found in *Carausius* was that their swing directions varied much more than that of long steps (Theunissen and Dürr, 2013). In order to compare this property of short steps among species, Fig. 7 shows polar plots of swing directions for both short and long steps.

In all species, the swing movements of long steps were almost exclusively directed to the front. In contrast, short steps showed much broader distributions of their swing directions. Middle and hind legs swung preferably to a medial-frontal direction, whereas front legs swung preferably to lateral-frontal directions (Fig. 7, red arrows). This was consistent among species, and all mean directions were statistically significant (supplementary material Table S1). In *Aretoan*, short steps showed less variability in their swing direction (supplementary material Table S1, SD). In summary, the swing phases of long steps were directed frontally in all species, whereas short steps could point in any direction, even though there was a statistically significant preferred direction. The similarities between species suggest that short steps share the same function in *Aretoan* and *Medauroidea*, as it was postulated for *Carausius*. As yet, the



**Fig. 7. Swing direction differs between long and short steps.**

Circular histograms of long steps and short steps are shown for *Carausius* (top), *Aretoan* (middle) and *Medauroidea* (bottom). Step direction was calculated from lift-off to touch-down in the horizontal plane of body-fixed coordinates. Because of the bin width of 30 deg, step directions of  $0 \pm 15$  deg are considered as anteriorly directed steps. Medial steps are directed towards 90 deg, backward steps to 180 deg and lateral steps to 270 deg. The number of steps ( $n$ ) and the radius ( $r$ ) of the outer circle are given to the lower right of each plot. The statistics for the mean direction vectors (red) are given in supplementary material Table S1. The length of the red arrow on lower left indicates  $r=1$ .



**Fig. 8. *Medauroidea* executes high swing movements with its front legs.** Swing height distributions are shown for all leg types in *Carausius* (A), *Aretaon* (B) and *Medauroidea* (C). Swing height was defined as the vertical range in body-centred coordinates covered during a swing phase. Only long steps were considered. Swing heights were normalised to the length of the thorax of each individual. The number of steps is given in the top right of each panel.

lower variability of short step direction in *Aretaon* indicates the presence of an additional or stronger constraint on swing direction than in the other species.

#### ***Medauroidea* performs front leg searching movements**

As mentioned already with regard to Fig. 3, long steps appeared to differ in swing height among species: the front leg foot trajectories of *Medauroidea* reached high above the body axis. For a more detailed analysis of this point, we defined swing height as the vertical range covered by the foot during a swing movement and normalised it to the thorax length (Fig. 8). Note that this vertical range was measured in the body coordinate system (CS) and that only long steps were considered. The distributions of swing heights differed between species and they were broader for the front legs than for the other legs (two-way ANOVA, species: d.f.=2,  $F=55.91$ ,  $P<0.001$ ; leg: d.f.=2,  $F=50.77$ ,  $P<0.001$ ). Multiple comparison revealed that the distributions were similar in *Carausius* and *Aretaon* (Fig. 8A,B), but swing heights were significantly higher in *Medauroidea* (Fig. 8C). Furthermore, the front legs made significantly higher swing movements than the middle and hind legs. Also, the interaction of the factors ‘species’ and ‘leg’ was significant (d.f.=4,  $F=26.96$ ,  $P<0.001$ ), indicating that differences among leg types depended on the species. In summary, *Medauroidea* made higher swing movements (higher than one thorax length) with all legs, but the front legs made particularly high swing movements, reaching up to four thorax lengths.

#### **Leg kinematics differ in the long-legged species**

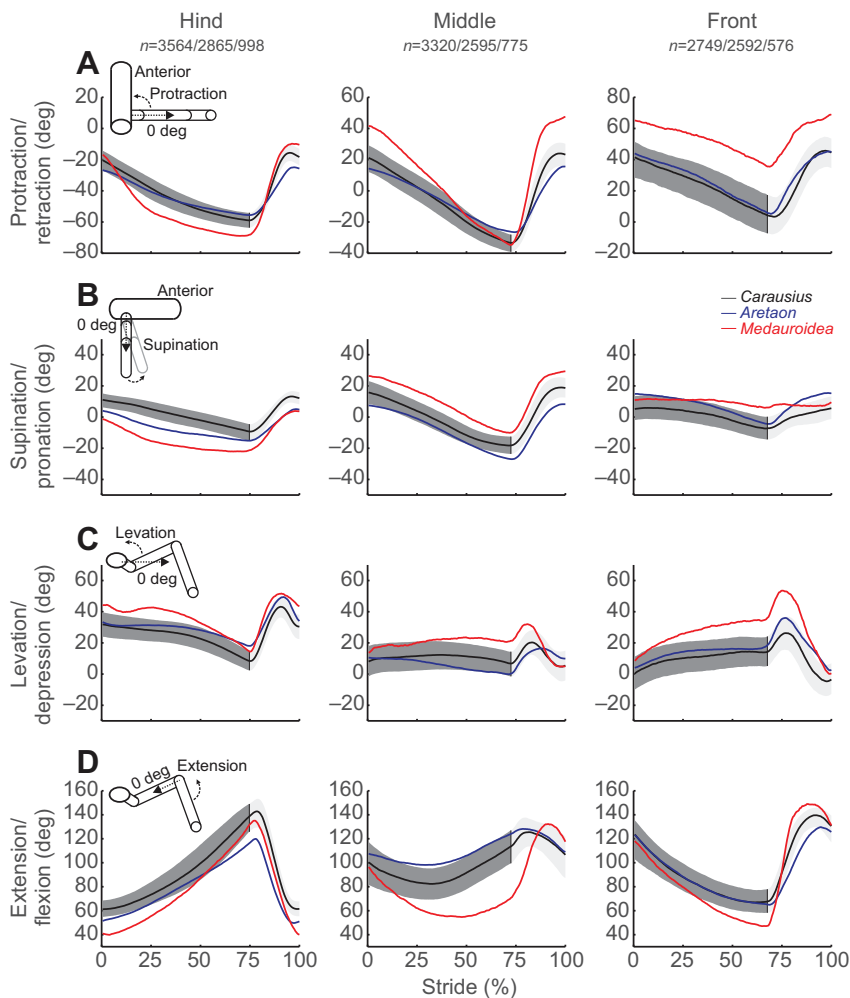
Fig. 9 shows the average time courses of the leg joint angles within one stride of a long step. Protraction/retraction of the thorax–coxa joint was very similar for *Carausius* and *Aretaon*, but in *Medauroidea* only the hind legs were protracted in a similar way (Fig. 9A). The front and the middle legs were more protracted in *Medauroidea* than in the other two species. At the same time, the amplitude of the protraction/retraction movement was smaller in the front legs of *Medauroidea*. Also, the amplitude of pronation and supination was smallest in the front legs of *Medauroidea*.

Irrespective of the species, the largest amplitudes of protraction/retraction and pronation/supination were observed in the middle legs (Fig. 9A,B).

The levation angles were generally similar among species and all legs were levated in the first half of the swing movement and depressed in the second half (Fig. 9C). During stance, the levation angles of the middle legs were nearly constant in *Carausius* and *Medauroidea* and slightly decreased in *Aretaon* (indicating depression). In all species, the hind leg femora were depressed during stance, whereas front leg femora were levated. The high swing movements of the front legs of *Medauroidea* (Fig. 8C) were reflected by the levation of the femur at the coxa–trochanter joint, and by the extension of the tibia at the femur–tibia joint, both of which strongly increased at the beginning of the swing phase (Fig. 9C,D). For the flexion/extension angle of the femur–tibia joint (Fig. 9D), the hind legs showed the opposite behaviour of the front legs in all species. Hind legs were extended in the stance phase and flexed in the swing phase, whereas the front legs were flexed in the stance phase and extended in the swing phase. The former indicated pushing of the hind legs, whereas the latter indicated pulling of the front legs. The middle legs were flexed in the first third of the stance phase and extended at the end of the stance phase. During swing, they were first extended and then flexed. In the middle leg, the extension during swing was much stronger in *Medauroidea* than in *Aretaon* and *Carausius*.

To analyse the intra-leg coordination of the joints, we plotted the protraction angle as a function of the other joint angles (Fig. 10). The resulting curves for *Carausius* and *Aretaon* were of reasonably similar shape for all joint angles. In contrast, the curves for *Medauroidea* often differed in shape and location, particularly in the front and middle legs. Both of these leg types were protracted more strongly in *Medauroidea* than in the other species, leading to displaced curves. Protraction was always correlated with supination, but the curves differed for swing and stance movements, indicating slack of the rotation axis in the thorax–coxa joint, which is commonly considered fixed in stick insects (Cruse and Bartling, 1995; Dürr et al., 2004).





**Fig. 9. Leg kinematics differs among leg types and among species.** The median leg angles of *Aretaon* (blue) and *Medauroidea* (red) are superimposed on the median leg angles of *Carausius* (black; grey shaded areas show the quantiles). The stride period was normalised to the mean stance (dark grey) and swing (light grey) durations of each leg. (A) Protraction/retraction angle of the thorax-coxa joint. At 0 deg, the leg is perpendicular to the corresponding thorax segment. Positive angles indicate forward movement, i.e. protraction. (B) Pronation/supination angle with positive angles representing supination. (C) Levation/depression of the thorax-coxa joint and the coxa-trochanter joint. Note that the femur is fused with the trochanter in these species, without a movable joint in between. Positive angles indicate levation and negative angles, depression of the leg. (D) Flexion/extension of the femur-tibia joint.

## DISCUSSION

We used a step-climbing paradigm to highlight the differences of the walking and climbing behaviour between three species of stick insects. The three species differed in size and body proportions (Fig. 2; Tables 1, 2). Whereas *Carausius* and *Aretaon* had long antennae, *Medauroidea* was the largest of the three species and had the largest leg-to-body length ratio (Fig. 2; Table 2). All three species used two distinct classes of steps (Figs 6, 7), but the differences in body proportion led to different use of the thorax segments (Figs 4, 5) and to differences in intra-leg coordination of joints (Figs 9, 10). Furthermore, *Medauroidea* used its front legs for searching movements in every step (Figs 3, 8).

### Use of thorax angles

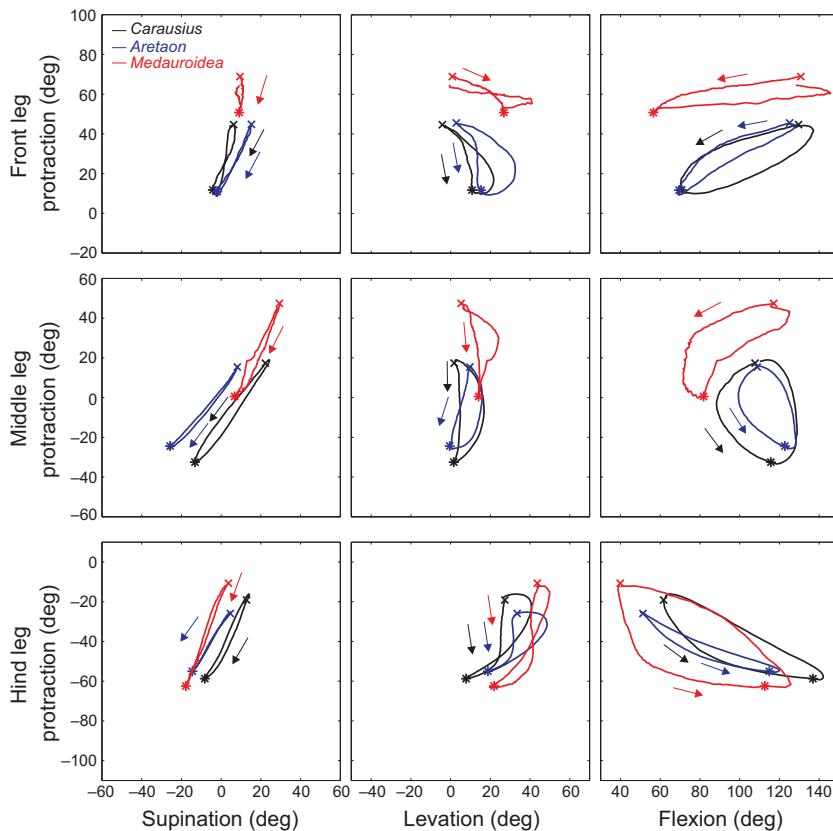
One of the main functions of the legs is to maintain a sufficient clearance between the body and the ground (Graham, 1985), which is usually kept constant in stick insects (Cruse, 1976a). When cockroaches climb over obstacles of different height, the adaptation of their walking behaviour ranges from 'small disturbances' when running across low obstacles, to more specialised and complex movement sequences when climbing across high obstacles (Watson et al., 2002). Before climbing, they raise up the body to place their feet on top of the obstacle (Watson et al., 2002). In our experiment, all three species of stick insects raised the body similar to the cockroach. But, although the height of the low stairs was chosen such that the animals could keep the clearance constant without increasing the body pitch angle, *Carausius* and *Aretaon* also

increased the pitch angle significantly when climbing the low stairs (Fig. 4). This was not the case for *Medauroidea*, who climbed over the low stairs without changing the body pitch angle. Generally, the change in pitch angle depended on body size and leg length. *Aretaon*, the shortest of the species under study, reached larger pitch angles than *Carausius* and *Carausius* reached larger angles than *Medauroidea* (Table 2).

With regard to our second hypothesis (segment length proportions are reflected in movement ranges), we can confirm that the species in which a given segment was largest in proportion to the metathorax tends to move this segment more than the other two. Indeed, *Carausius* most strongly moved the mesothorax, whereas *Aretaon* most strongly moved the head (Table 2). With regard to the prothorax, the hypothesis would have predicted that *Aretaon* moved this segment most strongly. This was not the case. However, *Aretaon* did move this segment very strongly, and more in synchrony with the head than this was the case in *Carausius*. With regard to our third hypothesis (long legs compensate for short antennae), we can confirm that the most long-legged species showed the least movement of the thoracic joints, which is in line with the observations that *Medauroidea* did not significantly incline the body axis when climbing the low stairs (8 mm; see Fig. 4D) and that its legs could step from one stair directly to the next (see Fig. 3C; 24 mm stairs).

### Step parameters

Stick insects do not adjust their step length to increase walking velocity (*Carausius*: Wendler, 1964; *Aretaon*: Jeck and Cruse,



**Fig. 10. Joint angle movements are similar for *Carausius* and *Aretaon*, but different for *Medauroidea*.** Protraction angles of *Carausius* (black), *Aretaon* (blue) and *Medauroidea* (red) plotted against supination, levation and flexion for front, middle and hind legs. Crosses and asterisks show the beginning and end of the stance phase, respectively. The direction of the stance movement is indicated by the arrow next to it. Note that protraction of the front legs of *Medauroidea* (red) range less than 20 deg, whereas flexion ranges over around 80 deg. Sample sizes are the same as in Fig. 9.

2007). However, step length varies during turning (e.g. Dürr and Ebeling, 2005) and climbing. We recently showed that *Carausius* takes two distinct classes of steps: short steps and long steps (Theunissen and Dürr, 2013). Whereas long steps support propulsion, short steps serve to correct for inappropriate foothold. Additional step types were also identified in *Aretaon*, when describing climbing sequences across large gaps (Bläsing and Cruse, 2004b). In our study, step length distributions were always bimodal, indicating the occurrence of short and long steps in *Aretaon* and *Medauroidea* too (Fig. 6). The most characteristic feature of short steps is the omnidirectionality of the swing movement (Theunissen and Dürr, 2013). Our results confirm this feature in *Aretaon* and *Medauroidea* with significant mean swing directions to medial directions in middle and hind legs and lateral directions in front legs (Fig. 7). The variable swing directions of short steps strongly suggest a corrective function in all three species. However, the proportion of short to long steps differed between species. In *Medauroidea*, the proportion of short steps was higher than in the other two species. This might be explained by the function of short steps and the width of the walkway (40 mm), which was adjusted to the lateral distance between contralateral legs of *Carausius* and *Aretaon*. In *Medauroidea*, the distance between contralateral legs is larger, resulting in more frequent foot placement on the sides of the setup. Possibly, this was one reason for *Medauroidea* taking more corrective short steps than the other species.

Swing height of long steps was largest in *Medauroidea*. This was particularly pronounced in the front legs, where the tarsi frequently reached high above body clearance. Such high swing movements are only sensible when exploring the near range environment, allowing for efficient climbing of obstacles that require the adaptation of body pitch. By doing so, the front legs essentially take on the functional role of tactile feelers, which in other species is

done by the antennae (Dürr et al., 2001; Krause and Dürr, 2012). In line with this interpretation, the trajectories of front leg swing movements in *Medauroidea* were similar to the trajectories of the searching movements at the end of the setup (not shown), as described previously for *Carausius* (Dürr, 2001). All this suggests that *Medauroidea* actively uses its front legs to search for obstacles in their path, thus confirming hypothesis 3: *Medauroidea* appears to compensate for insufficient tactile sampling of its antennae by actively involving the legs in near-range searching.

A combination of the parameters step length and step direction reflects the spatial coordination of legs. In a recent study, we have shown that a ‘targeting mechanism’ is part of the spatial coordination of legs in three-dimensional space in *Carausius* (Theunissen et al., 2014c). A preliminary look at the accuracy and precision of hind leg targeting towards the position of the middle leg in other species suggests that this mechanism works the same in climbing *Aretaon* and *Medauroidea* (Theunissen et al., 2014b). This may also explain the reduced proportion of short steps in hind legs, because improved targeting towards an appropriate foothold – as experienced and signalled by a leading leg – should reduce the necessity of corrective short steps.

### Leg kinematics

The analysis of the leg joint angles was focused on long steps, because short steps occurred irregularly and joint angles do not change much in short steps (Theunissen and Dürr, 2013). Our kinematic analysis differed from several earlier studies in that we did not assume a fixed, single DoF joint axis of the thorax–coxa joint, but measured both the protraction/retraction and the pronation/supination of the leg plane independently. This was important for two reasons: first, the measurement of the slanted thorax–coxa joint axis is complicated, as reflected by the different

**Table 2. Comparative locomotion summary**

		<i>Carausius morosus</i>	<i>Medauroidea extradentata</i>	<i>Aretaon asperimus</i>
Systematics (Bradley and Galil 1977)		Anareolatae, Heteronemiidae, Lonchodinae, Lonchodini	Anareolatae, Phasmatidae, Phasmatinae, Baculini	Areolatae, Bacillidae, Heteropteryginae, Obrimi
Size and proportions	Body length	72 mm (♀)	81 mm (♀), 68 mm (♂)	49 mm (♂)
	Ratio T2:T3	1.43	1.26 (♀), 1.15 (♂)	1.15
	Ratio T1:T3	0.31	0.32 (♀), 0.22 (♂)	0.50
	Ratio FL:T3	2.69	4.96 (♀), 5.69 (♂)	2.71
	Ratio Ant:FL	1:1	1:5 (♀), 1:3.4 (♂)	1.4:1
Leg	Leg posture	Knees at body height	Knees >> body height	Knees at body height
	Swing height	≈ Clearance	> Clearance	≈ Clearance
Steps	Predominant step type	Long	Short	Long
	Long step length	<1.2×T3	>2×T3	<1.2×T3
	Short step s.d. (HL/ML/FL)	105/115/120 deg	106/117/121 deg	78/73/89 deg
Leg joints	FL protraction range	45 deg	20 deg	40 deg
	ML protraction mean	0 deg	25 deg	0 deg
	HL flexion mean	100 deg	80 deg	85 deg
Thorax joints	Thorax inclination	1.2 deg mm <sup>-1</sup>	0.6 deg mm <sup>-1</sup>	1.1 deg mm <sup>-1</sup>
	T2 levation range	25 deg	10 deg	0 deg
	T1 levation range	35 deg	5 deg	20 deg
	Head levation range	30 deg	15 deg	45 deg

Data from *Carausius* concern only females, those of *Aretaon* concern only males. Where the sex of *Medauroidea* is not specified, data have been pooled for both sexes. Ant, antenna; FL, front leg; HL, hind leg; ML, middle leg; T1, prothorax; T2, mesothorax; T3, metathorax. Steps: long step length is given as multiples of T3 length; Short step s.d. gives the angular dispersion of short step direction. Leg joints: range gives the approximate joint angle range shown in Fig. 10; mean gives the approximate central angle, midway between minimum and maximum angle in Fig. 10. Thorax joints: thorax inclination gives the slope of Fig. 5D; ranges give the approximate joint angle ranges shown in Fig. 5A–C.

published values for *Carausius* (Cruse, 1976b; Pfeiffer and Cruse, 1994; Cruse and Bartling, 1995). Second, it is unclear how much slack is in this joint, and how much the joint axis orientation may vary during unrestrained walking and climbing. Finally, the direct measurement of the two angles that specify the orientation of the leg plane greatly simplifies the comparison between insects, particularly when including non-phasmatodean species. As yet, the different kinds of analyses somewhat complicate the comparison with results of earlier studies, although the overall differences from earlier results on *Carausius* (Cruse and Bartling, 1995) and *Aretaon* (Jeck and Cruse, 2007) are fairly minor.

The front leg searching movements of *Medauroidea* were characterised by single high swing movements and they seemed to be little influenced by the thorax–coxa joint. Instead, the movements were dominated by high levation of the leg and large extension of the femur–tibia joint. These characteristics also occur when *Medauroidea* performs a sequence of searching movements to find objects (Karg et al., 1991; Berg et al., 2013). The searching movements also affected the coordination of the other legs, leading to more protracted middle legs: whenever the front legs perform high swing movements without ground contact, the other legs must account for balance and stability.

Since several joint angle time courses were shown to vary strongly among leg types (Fig. 9, see above), inter-joint coordination also differed strongly between leg types (Fig. 10). With regard to hypothesis 1 (abdomen length is paralleled by amount of hind leg retraction), our results are not conclusive. Judged from the retraction angles, it is *Medauroidea*, the species with the shortest abdomen (in proportion), that lifted off its hind legs most posteriorly (e.g. see red asterisks in bottom panels of Fig. 10). This contradicts hypothesis 1. However, *Carausius*, the species with the longest abdomen (in proportion), showed the strongest hind leg extension at lift-off (see black asterisk in lower right panel of Fig. 10). This supports hypothesis 1, because in a retracted hind leg, extension essentially

pushes the foot rearwards, thus shifting the contact point away from the CoM and expanding the stability polygon. An assumption underlying hypothesis 1 is that the location of the CoM is directly related to the length of the abdomen. However, the relationship between the CoM location (most posterior in *Carausius*, most anterior in *Aretaon*) and abdomen length (longest in *Carausius*, shortest in *Medauroidea*) proved to be less straightforward than expected. Moreover, the use of claws and attachment pads during locomotion might differ among species, further confounding the dependence of hind leg posture at lift-off, CoM location and stability. As a consequence, there may be no clear-cut relationship between abdomen length and leg posture at lift-off, because the former does imply location of CoM in a simple manner, and the latter is not the only means by which the animal can maintain stability.

## Conclusions

We conclude that climbing behaviour as well as single leg movements are adjusted to morphological demands (see also Table 2). *Carausius* and *Aretaon* raised their body to reach the top of the stairs more than *Medauroidea* and the use of the thorax segments reflects their segment length proportions. In contrast to the other species, *Medauroidea* compensated for the low disturbance with their leg angles and showed less movement of the head. Instead of the antennae, *Medauroidea* used its long front legs to search for obstacles in the near-range environment. All three species use long steps for propulsion and short steps for correction of inappropriate foothold. Finally, the leg joint angles reflect the functionality of the legs, showing similar time courses in *Carausius* and *Aretaon* and a high levation and a large extension for the front legs of *Medauroidea*.

## MATERIALS AND METHODS

### Animals

We compared adult individuals of three species of stick and leaf insects (order: Phasmatodea, Fig. 1), each one from a different taxonomic family

(following the taxonomy of Bradley and Galil (Bradley and Galil, 1977), including suborder, family, subfamily and tribe): *Carausius morosus* (De Sinéty 1901), Anareolatae, Heteronemiidae, Lonchodinae, Lonchodini; *Medauroidea extradentata* (Brunner von Wattenwyl 1907), Anareolatae, Phasmatidae, Phasmatinae, Baculini; and *Aretaon asperrimus* (Redtenbacher 1906), Areolatae, Bacillidae, Heteropteryginae, Obrimi. Among neurobiologists, *M. extradentata* is commonly called *Cuniculina impigra*. All three species come from south-east Asia: *C. morosus* from southern India, *A. asperrimus* from Borneo and *M. extradentata* from Vietnam.

All animals were bred in colonies at the Department for Biological Cybernetics at Bielefeld University, where they were kept at 24°C and a 12 h:12 h light:dark cycle. We used female *Carausius* only ( $N=9$ ), because the colony was predominantly parthenogenetic and males occur only rarely. Of the other two species, the colonies were bisexual. From *Aretaon*, we used males only ( $N=10$ ) because the females are reluctant to walk through several trials. From *Medauroidea*, we used both sexes (females,  $N=4$ ; males,  $N=2$ ). Additional animals were included for morphological measurements.

### Behavioural experiments

The setup, recording technique and kinematic calculations were the same as described by Theunissen and Dürr (Theunissen and Dürr, 2013). The essential information will be reiterated here. The animals walked along a walkway (490×40 mm) and climbed over a staircase placed at the end of it. The height of the stairs was varied pseudo-randomly trial by trial between 0 (no stairs), 8, 24 and 48 mm, resulting in four different conditions: flat, low, middle and high. The flat condition was used as the reference condition without spatial disturbances. The low condition introduced two spatial disturbances, both of which were lower than the animals' clearance. In the middle condition, the height of the stairs required a change in body inclination but the top of the stairs could be reached by all species with a single high swing movement. The high condition required climbing, and at least *Carausius* and *Aretaon* had to place a foot on the vertical surface of the stairs. The four conditions were tested in at least 10 trials per animal. When the animal stopped walking in front of the first stair or when it climbed onto the sides of the setup, the same condition was repeated. The raw data set on *Carausius* was the same as used by Theunissen and Dürr (Theunissen and Dürr, 2013). With the exception of Fig. 7A, which was included for reasons of comparison, all aspects analysed in the present study have not been published previously. The data underlying Fig. 6A and Fig. 8A have been used by Theunissen and Dürr (Theunissen and Dürr, 2013) as well, but without normalisation to thorax length.

In conjunction with the publication of a concept for an open access database comprising experimental data on natural motion in animals and humans (Theunissen et al., 2014b), a representative sample data set (comprising 36 trials, i.e. 12 per species) has been made available to the public on <http://movement.cit-ec.de> (Theunissen et al., 2014a).

### Animal recording and reconstruction

Unrestrained climbing animals were recorded by use of a motion capture system (Vicon MX10, Oxford, UK). This system consisted of eight Vicon T10 cameras, recording marker positions at 200 frames per second (fps) with a spatial resolution of ~0.1 mm. An additional digital video camera (Basler A602fc, Ahrensburg, Germany) equipped with a near-range zoom lens (Edmund Optics, Barrington, NJ, USA) was used to record a complementary side view for visual validation of the kinematic analysis (50 fps, synchronised with the Vicon system, spatial resolution ~0.14 mm per pixel).

Up to 20 markers with a diameter of 1.5 mm were attached to the thorax, the head, the legs and the antennae, using nail polish. For the reconstruction of the real animal, segment dimensions and positions of all markers on their respective body segment were measured from high-resolution photographs (0.02 mm per pixel) taken under a stereo lens (Olympus SZ61T, equipped with a Pixelink PL-B681CU digital camera). Together, the positions of the markers and the body measurements were used to reconstruct the real moving animal in MATLAB (MathWorks Inc., Natick, MA, USA). Kinematic calculations were done with the help of a main kinematic chain (thorax and head) and six kinematic side chains for the legs (coxa, trochantero–femur and tibia). Three markers attached to the metathorax were

used to define the right-handed, body-fixed Cartesian CS, with its origin in the centre of the segment border between the 1st and 2nd abdominal segment (in stick insects, the 1st abdominal segment is fused to the metathorax). The  $x$ -axis of the resulting body CS pointed from posterior to anterior, the  $y$ -axis to the left and the  $z$ -axis upward. Local, segment-fixed CS axes were calculated for each thorax segment and the head. The roll angle described a right-handed rotation around the  $x$ -axis, the pitch angle rotation around the  $y$ -axis and the yaw angle rotation around the  $z$ -axis. Pitch angles were inverted such that positive angles described upward rotations and negative angles downward rotations.

### Leg kinematics

The angles of the leg joints were calculated with the help of markers labelling the distal femur and the distal tibia. First, the reference of the respective thorax CS was translated into the thorax–coxa joint and the orientation of the leg plane, i.e. the plane containing both the femur and the tibia, was calculated with respect to this CS. This resulted in the protraction/retraction and the supination/pronation angle of the thorax–coxa joint. Protraction was indicated by positive angles, retraction by negative angles, with zero degrees indicating that the leg plane was perpendicular to the body axis. The supination/pronation angle was positive in the case of supination and negative in the case of pronation. For this, the CS of right legs was right-handed, that for left legs, left-handed. The marker on the femur served to calculate the levation/depression angle of the leg (levation positive, depression negative). The flexion/extension of the femur–tibia joint was defined by the inner angle between femur and tibia, with zero indicating that the leg was fully flexed.

### Foot contacts and step length

The position of the tibia–tarsus joint was assumed as foot position, because the tarsi could not be labelled with markers and, thus, not be reconstructed. Ground contact was determined based on the velocity of the tibia–tarsus joints and the distance to the surface (see also, Theunissen et al., 2014c). The velocity had to decrease below a certain threshold for at least eight consecutive frames (40 ms). The threshold was set to 25 mm s<sup>-1</sup> for *Carausius* and *Aretaon*, and to 50 mm s<sup>-1</sup> for *Medauroidea*. This threshold was set higher for *Medauroidea* because the tibia–tarsus joint position moved considerably during the stance phase. Additionally, a plausibility criterion was introduced, where we calculated the distance between the foot and the surface. If the velocity criterion was fulfilled and the distance between the foot and the surface was below 10 mm, the leg was assumed to have ground contact. Note that this seemingly large margin was only necessary to catch false positives detected by the velocity criterion, e.g. extremely slow episodes in swing movements. False negatives were a lot more unlikely because they would have required very brief stance phases and/or pronounced tibia movement (marker displacement) during stance. A margin of approximately twice the tarsus length proved to be useful, partly to account for the fact that our kinematics calculations estimated the location of the tibia–tarsus joint and not the location of the distal tarsomeres, partly to account for inaccuracies in the setup position estimates.

Step length was defined as the Euclidian distance between subsequent foot contact positions. We projected the step lengths into body-fixed coordinates, to account for differences in body velocity. Note that a faster-walking animal would take longer steps in external, but not necessarily in body-fixed coordinates.

### Acknowledgements

We would like to thank A. F. Krause for helpful comments on the manuscript, A. Exter for excellent technical assistance and B. Otte-Eusterglering for animal care.

### Competing interests

The authors declare no competing or financial interests.

### Author contributions

L.M.T. carried out experiments on *Carausius morosus* and *Aretaon asperrimus*, analysed data, interpreted the findings and wrote the manuscript. H.H.B. carried out experiments and analysed data on *Aretaon asperrimus* and *Medauroidea extradentata*. V.D. designed experiments, supervised L.M.T. and H.H.B., interpreted findings and edited the manuscript.



## Funding

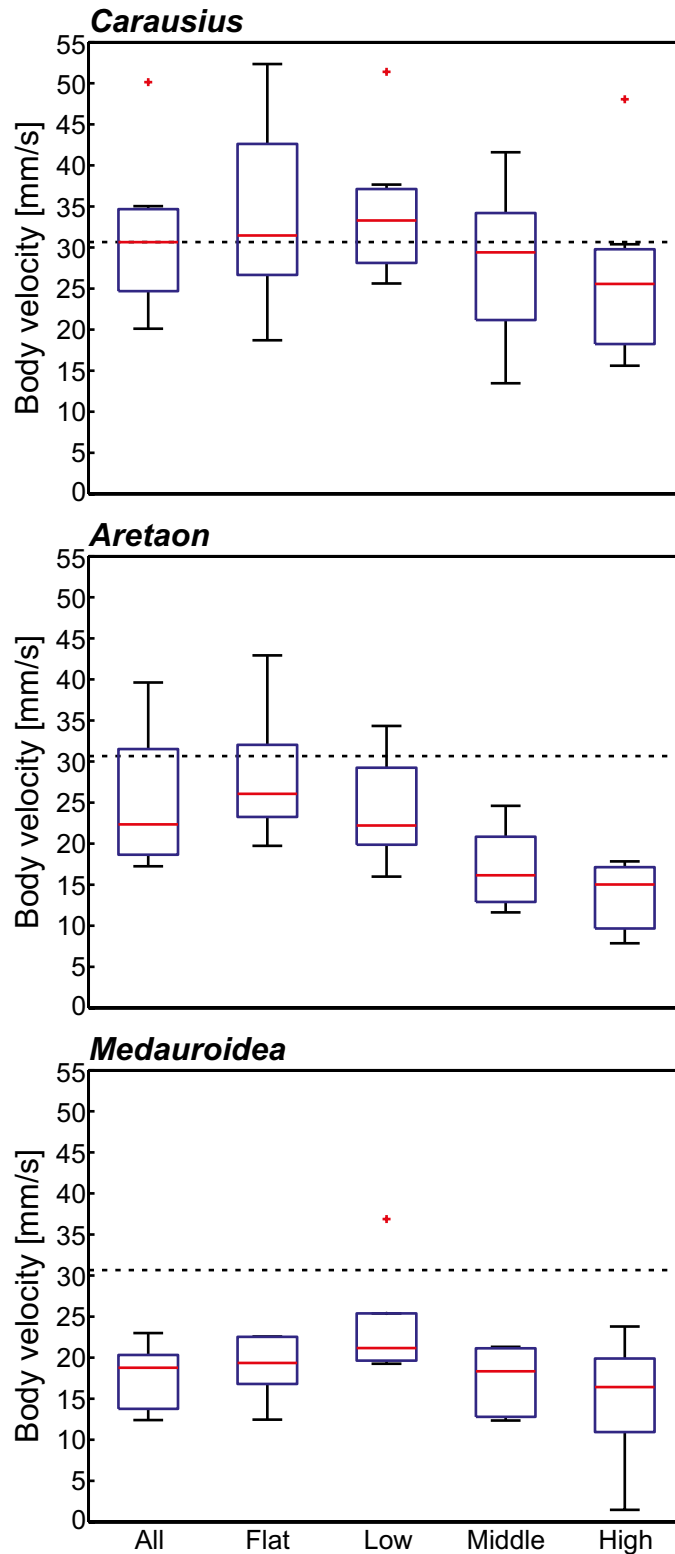
This study was supported by the EU project EMICAB (Embodied Motion Intelligence for Cognitive Autonomous robots; FP7-ICT-270182) to V.D.

## Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.114173/-/DC1>

## References

- Bässler, U. and Foth, E. (1982). The neural basis of catalepsy in the stick insect *Cuniculina impigra*. 1. Catalepsy as a characteristic of the femur-tibia control system. *Biol. Cybern.* **45**, 101-105.
- Bässler, D., Büschges, A., Meditz, S. and Bässler, U. (1996). Correlation between muscle structure and filter characteristics of the muscle-joint system in three orthopteran insect species. *J. Exp. Biol.* **199**, 2169-2183.
- Berg, E., Büschges, A. and Schmidt, J. (2013). Single perturbations cause sustained changes in searching behavior in stick insects. *J. Exp. Biol.* **216**, 1064-1074.
- Birn-Jeffery, A. V. and Higham, T. E. (2014). The scaling of uphill and downhill locomotion in legged animals. *Integr. Comp. Biol.* **54**, 1159-1172.
- Bläsing, B. and Cruse, H. (2004a). Mechanisms of stick insect locomotion in a gap-crossing paradigm. *J. Comp. Physiol. A* **190**, 173-183.
- Bläsing, B. and Cruse, H. (2004b). Stick insect locomotion in a complex environment: climbing over large gaps. *J. Exp. Biol.* **207**, 1273-1286.
- Blickhan, R. and Full, R. J. (1993). Similarity in multilegged locomotion: bouncing like a monopode. *J. Comp. Physiol. A* **173**, 509-517.
- Bradley, J. C. and Galil, B. S. (1977). The taxonomic arrangement of the Phasmatodea with keys to the subfamilies and tribes. *Proc. Entomol. Soc. Wash.* **79**, 176-208.
- Burrows, M. (1996). *The Neurobiology of an Insect Brain*. Oxford: Oxford University Press.
- Büschges, A. (2012). Lessons for circuit function from large insects: towards understanding the neural basis of motor flexibility. *Curr. Opin. Neurobiol.* **22**, 602-608.
- Büschges, A. and Gruhn, M. (2007). Mechanosensory feedback in walking: from joint control to locomotor patterns. *Adv. In Insect Phys.* **34**, 193-230.
- Bußhardt, P., Gorb, S. N. and Wolf, H. (2011). Activity of the claw retractor muscle in stick insects in wall and ceiling situations. *J. Exp. Biol.* **214**, 1676-1684.
- Bußhardt, P., Wolf, H. and Gorb, S. N. (2012). Adhesive and frictional properties of tarsal attachment pads in two species of stick insects (Phasmatodea) with smooth and nubby euplantulae. *Zoology* **115**, 135-141.
- Cruse, H. (1976a). The control of body position in the stick insect (*Carausius morosus*), when walking over uneven surfaces. *Biol. Cybern.* **24**, 25-33.
- Cruse, H. (1976b). The function of the legs in the free walking stick insect *Carausius morosus*. *J. Comp. Physiol.* **112**, 235-262.
- Cruse, H. (1990). What mechanisms coordinate leg movement in walking arthropods? *Trends Neurosci.* **13**, 15-21.
- Cruse, H. and Bartling, C. (1995). Movement of joint angles in the legs of a walking insect, *Carausius morosus*. *J. Insect Physiol.* **41**, 761-771.
- Cruse, H., Dürr, V., Schilling, M. and Schmitz, J. (2009). Principles of insect locomotion. In *Spatial Temporal Patterns for Action-Oriented Perception in Roving Robots* (ed. P. Arena and L. Patané), pp. 43-96. Berlin: Springer.
- Dürr, V. (2001). Stereotypic leg searching movements in the stick insect: kinematic analysis, behavioural context and simulation. *J. Exp. Biol.* **204**, 1589-1604.
- Dürr, V. (2005). Context-dependent changes in strength and efficacy of leg coordination mechanisms. *J. Exp. Biol.* **208**, 2253-2267.
- Dürr, V. and Bläsing, B. (2001). Antennal movements of two stick insect species: spatio-temporal coordination with leg movements. In *Zoology: Analysis of Complex Systems*, Vol. 103, pp. 17. Munich: Urban & Fischer Verlag.
- Dürr, V. and Ebeling, W. (2005). The behavioural transition from straight to curve walking: kinetics of leg movement parameters and the initiation of turning. *J. Exp. Biol.* **208**, 2237-2252.
- Dürr, V., König, Y. and Kittmann, R. (2001). The antennal motor system of the stick insect *Carausius morosus*: anatomy and antennal movement pattern during walking. *J. Comp. Physiol. A* **187**, 131-144.
- Dürr, V., Schmitz, J. and Cruse, H. (2004). Behaviour-based modelling of hexapod locomotion: linking biology and technical application. *Arthropod Struct. Dev.* **33**, 237-250.
- Fischer, M. S., Schilling, N., Schmidt, M., Haarhaus, D. and Witte, H. (2002). Basic limb kinematics of small therian mammals. *J. Exp. Biol.* **205**, 1315-1338.
- Frantsevich, L. and Cruse, H. (1997). The stick insect, *Obrimus asperimus* (Phasmatodea, Bacillidae) walking on different surfaces. *J. Insect Physiol.* **43**, 447-455.
- Full, R. J., Blickhan, R. and Ting, L. H. (1991). Leg design in hexapedal runners. *J. Exp. Biol.* **158**, 369-390.
- Fuller, P. O., Higham, T. E. and Clark, A. J. (2011). Posture, speed, and habitat structure: three-dimensional hindlimb kinematics of two species of padless geckos. *Zoology* **114**, 104-112.
- Gatesy, S. M. and Pollard, N. S. (2011). Apples, oranges, and angles: comparative kinematic analysis of disparate limbs. *J. Theor. Biol.* **282**, 7-13.
- Godden, D. H. (1974). The physiological mechanism of catalepsy in the stick insect *Carausius morosus*. *Br. J. Comp. Physiol.* **89**, 251-274.
- Grabowska, M., Godlewska, E., Schmidt, J. and Daun-Gruhn, S. (2012). Quadrupedal gaits in hexapod animals - inter-leg coordination in free-walking adult stick insects. *J. Exp. Biol.* **215**, 4255-4266.
- Graham, D. (1985). Pattern and control of walking in insects. *Adv. In Insect Phys.* **18**, 31-140.
- Higham, T. E. and Jayne, B. C. (2004). Locomotion of lizards on inclines and perches: hindlimb kinematics of an arboreal specialist and a terrestrial generalist. *J. Exp. Biol.* **207**, 233-248.
- Jeck, T. and Cruse, H. (2007). Walking in *Aretaon asperimus*. *J. Insect Physiol.* **53**, 724-733.
- Karg, G., Breutel, G. and Bässler, U. (1991). Sensory influences on the coordination of two leg joints during searching movements of stick insects. *Biol. Cybern.* **64**, 329-335.
- Krause, A. F. and Dürr, V. (2012). Active tactile sampling by an insect in a step-climbing paradigm. *Front. Behav. Neurosci.* **6**, 30.
- Mujagic, S., Krause, A. F. and Dürr, V. (2007). Slanted joint axes of the stick insect antenna: an adaptation to tactile acuity. *Naturwissenschaften* **94**, 313-318.
- Pfeiffer, F. and Cruse, H. (1994). Bionik des laufs - technische umsetzung biologischen wissens. *Konstruktion* **46**, 261-266.
- Ritzmann, R. E. and Büschges, A. (2007). Adaptive motor behavior in insects. *Curr. Opin. Neurobiol.* **17**, 629-636.
- Ritzmann, R. E., Pollack, A. J., Archinal, J., Ridgel, A. L. and Quinn, R. D. (2005). Descending control of body attitude in the cockroach *Blaberus discoidalis* and its role in incline climbing. *J. Comp. Physiol. A* **191**, 253-264.
- Schmidt, A. and Fischer, M. S. (2011). The kinematic consequences of locomotion on sloped arboreal substrates in a generalized (*Rattus norvegicus*) and a specialized (*Sciurus vulgaris*) rodent. *J. Exp. Biol.* **214**, 2544-2559.
- Schütz, C. and Dürr, V. (2011). Active tactile exploration for adaptive locomotion in the stick insect. *Philos. Trans. R. Soc. B* **366**, 2996-3005.
- Stoessel, A. and Fischer, M. S. (2012). Comparative intralimb coordination in avian bipedal locomotion. *J. Exp. Biol.* **215**, 4055-4069.
- Theunissen, L. M. and Dürr, V. (2013). Insects use two distinct classes of steps during unrestrained locomotion. *PLoS ONE* **8**, e85321.
- Theunissen, L. M., Bekemeier, H. H. and Dürr, V. (2014a). *Stick Insect Locomotion Data*. Bielefeld, Germany: Bielefeld University. doi: 10.4119/unibi/cit/ec.2013.3; <http://movement.cit-ec.de>
- Theunissen, L. M., Hertrich, M., Wiljes, C., Zell, E., Behler, C., Krause, A. F., Bekemeier, H. H., Cimiano, P., Botsch, M. and Dürr, V. (2014b). A natural movement database for management, documentation, visualization, mining and modeling of locomotion experiments. In *Proceedings of the Third International Conference, Living Machines 2014, Milan* (ed. A. Duff et al.), pp. 308-319. Heidelberg: Springer.
- Theunissen, L. M., Vikram, S. and Dürr, V. (2014c). Spatial co-ordination of foot contacts in unrestrained climbing insects. *J. Exp. Biol.* **217**, 3242-3253.
- Vidal-Gadea, A. G., Rinehart, M. D. and Belanger, J. H. (2008). Skeletal adaptations for forwards and sideways walking in three species of decapod crustaceans. *Arthropod Struct. Dev.* **37**, 95-108.
- Watson, J. T., Ritzmann, R. E., Zill, S. N. and Pollack, A. J. (2002). Control of obstacle climbing in the cockroach, *Blaberus discoidalis*. I. Kinematics. *J. Comp. Physiol. A* **188**, 39-53.
- Weihmann, T. and Blickhan, R. (2009). Comparing inclined locomotion in a ground-living and a climbing ant species: sagittal plane kinematics. *J. Comp. Physiol. A* **195**, 1011-1020.
- Wendler, G. (1964). Laufen und Stehen der Stabheuschrecke: Sinnesborsten in den Beingelenken als Glieder von Regelkreisen. *Z. Vgl. Physiol.* **48**, 198-250.
- Wilson, D. M. (1966). Insect walking. *Annu. Rev. Entomol.* **11**, 103-122.



**Fig. S1. Body velocity decreases from walking to climbing.** Average velocities per trial of *Carausius* (top), *Aretaon* (middle) and *Medauroidea* (bottom) for the different conditions (Flat; Low; Middle; High) and for the pooled data (All). The box plots show the medians (red), inter-quartile range (blue) and entire ranges. Red dots are outliers. Black dotted line indicates median velocity of all *Carausius* trials. *Carausius* walked faster than the other two species. *Medauroidea* was slowest (despite being the largest) and showed hardly any change in walking velocity across conditions. The number of animals and trials differs between species: *Carausius*  $N=9$  ( $n=323$ ); *Aretaon*  $N=8$  ( $n=186$ ); *Medauroidea*  $N=6$  ( $n=167$ ).

Table S1: Statistics for the mean swing directions

Genus	length	HL					ML					FL				
		dir	r	SD	p	z	dir	r	SD	p	z	dir	r	SD	p	z
<i>Carausius</i>	long	-2	0.97	0.23	<0.001	4879	1	0.97	0.26	<0.001	4813	-2	0.91	0.45	<0.001	4048
	short	42	0.19	1.83	<0.001	23	25	0.13	2.01	<0.001	18	-60	0.11	2.10	<0.001	21
<i>Aretaon</i>	long	1	0.97	0.24	<0.001	3116	-2	0.97	0.24	<0.001	3140	2	0.93	0.39	<0.001	2833
	short	19	0.40	1.36	<0.001	44	31	0.44	1.27	<0.001	89	-14	0.29	1.56	<0.001	65
<i>Medauroidea</i>	long	0	0.98	0.20	<0.001	1147	0	0.97	0.24	<0.001	1218	3	0.90	0.47	<0.001	856
	short	32	0.18	1.85	<0.001	31	70	0.12	2.04	<0.001	26	-23	0.11	2.11	<0.001	15

Mean swing direction vectors differ between long and short steps. The direction (dir [°]), the length (r), the standard deviation (SD), the p-value and the z-value of the circular statistics are given for short and long steps of each leg type and of each species.