

1 **Comparative whole-body kinematics of closely related insect species with different body**
2 **morphology**

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14 **Running title** Comparative locomotion in insects

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16 **Keywords** Stick insect, kinematics, leg movement, motion capture, whole-body
17 coordination, walking, climbing, Phasmatodea

18 **Abstract**

19 Legged locomotion through natural environments is very complex and variable. For example,
20 leg kinematics may differ strongly among species, but even within the same species it is
21 adaptive and context-dependent. Inter-species differences in locomotion are often difficult to
22 interpret, because both morphological and ecological differences among species may be
23 strong and, as a consequence, confound each other's effects. In order to understand better how
24 body morphology affects legged locomotion, we compare unrestrained whole-body
25 kinematics of three stick insect species with different body proportions, but similar feeding
26 ecology: *Carausius morosus*, *Aretaon asperrimus* and *Medauroidea extradentata* (=

27 *Cuniculina impigra*). In order to co-vary locomotory context, we introduced a gradually
28 increasing demand for climbing by varying the height of stairs on the set-up.
29 The species were similar in many aspects, for example in using distinct classes of steps, with
30 minor differences concerning the spread of corrective short steps. Major differences were
31 related to (1) antenna length, (2) segment lengths of thorax and head, and (3) the ratio of leg
32 length over body length: (1) Whereas all species continuously moved their antennae, only
33 *Medauroidea* executed high swing movements with its front legs to search for obstacles in the
34 near-range environment. (2) Whereas all species adjusted their body inclination, the range in
35 which body segments moved differed considerably, with longer thorax segments tending to be
36 moved more. (3) Finally, leg posture, time courses of leg joint angles and intra-leg
37 coordination differed most strongly in long-legged *Medauroidea*.

38

39 **1. Introduction**

40 Despite the fact that insects have become important model animals for the study of adaptive
41 locomotion (Ritzmann and Büschges, 2007), and a wide range of behavioural (Dürr *et al.*
42 2004; Cruse *et al.* 2009), biomechanical (Full *et al.* 1991) and neurophysiological (Burrows,
43 1996; Büschges and Gruhn, 2007; Büschges, 2012) studies on insect locomotion have
44 contributed to a detailed understanding of multi-legged locomotion in general, there are very
45 few studies on comparative kinematics of insect walking or climbing. Of course, there is a
46 host of information on species belonging to various taxa, most notably on cockroaches
47 (Blattodea), crickets and locusts (Orthoptera), and stick insects (Phasmatodea), and several
48 reviews have collated some of the material (e.g., Wilson, 1966; Graham, 1985; Burrows,
49 1996; Büschges and Gruhn, 2007; Cruse *et al.* 2009). Still, comparative analyses are difficult
50 to interpret if data have been collected in different settings or with different methods.

51 With regard to overall aspects of locomotion, such as certain gait characteristics (Wilson,
52 1966), the movement of the centre of mass during running (Blickhan and Full, 1993), or
53 adaptation to inclines (Birn-Jeffery and Higham, 2014), literature reviews have identified
54 commonalities among species, but they fall short of identifying causes for distinct differences
55 among species. In other words, the causes of inter-species variability in legged locomotion are
56 not understood well. In order to change this, comparative experiments on unrestrained
57 locomotion are needed. Up to now, only few such studies have linked comparative kinematics
58 to morphological differences in invertebrates. In climbing and ground-dwelling ant species,
59 postural differences occur when walking on slopes (Weihmann and Blickhan, 2009), but limb
60 kinematics were not compared. In decapod crustaceans, a combination of comparative motion
61 analysis and morphological measures indicated a link between leg segment length and
62 preferred walking direction (Vidal-Gadea *et al.* 2008). In vertebrates, where comparative
63 analyses of legged locomotion have been more common, it has become clear that both
64 morphological characters as well as ecological differences such as habitat preferences may
65 strongly affect limb kinematics. Typically, it is difficult to disentangle these two factors. For
66 example, in lizards, limb kinematics of two species may be differentially affected by slope
67 (Higham and Jayne, 2004) or may vary with habitat (Fuller *et al.* 2011). In these studies, the
68 species compared exhibit morphological differences that, at least to some extent, reflect
69 specific habitat adaptations. With regard to the interdependence of body morphology and limb
70 kinematics, Gatesy and Pollard (Gatesy and Pollard, 2011) have argued that limb segment
71 proportions necessarily pose important constraints on limb kinematics. Nevertheless,

72 constraints are not equal to determinants. For example, distantly related birds with similar
73 limb proportions but different habitat preferences were shown to have similar limb kinematics
74 in the sagittal plane, but not in the transversal plane (Stoessel and Fischer, 2012). In arboreal
75 and terrestrial rodents with similar leg segment proportions, the same kinematic adjustments
76 to climbing locomotion were found (e.g., reduced clearance), despite differences in speed and
77 gait (Schmidt and Fischer, 2011). In other words, kinematic variables associated with gait
78 differences (e.g., timing in inter-leg coordination) were species-dependent, while others were
79 not. Generally, small mammals share several important ‘overall’ kinematic features during
80 legged locomotion, e.g., scapula movement (Fischer *et al.* 2002). Nevertheless, they may
81 differ considerably with regard to limb proportions and variables of intra-leg and inter-leg
82 coordination.

83 The objective of the present study was to identify species differences in kinematics of
84 unrestrained walking and climbing insects, and to relate them to differences in morphology,
85 and body segment proportions in particular. For this, we recorded whole-body kinematics of
86 three species of unrestrained walking and climbing stick insects: *Carausius morosus*, *Aretaon*
87 *asperrimus* and *Medauroidea extradentata* (= *Cuniculina impigra*). We compared step
88 parameters, movement of the head and thorax segments, and intra-leg coordination, i.e., joint
89 angle time courses. All three species are herbivores that live in an arboreal habitat. Moreover,
90 all three species have a similar overall body structure and no obvious kinematic
91 specialisations other than differing proportions of thorax and leg segments.

92 A known morphological difference with regard to Phasmatodean locomotion concerns the
93 smoothness of tarsal attachment pads, e.g. in *Carausius* and *Medauroidea* (Bußhardt *et al.*
94 2012). Despite the structural differences, the motor control of at least two leg joints is very
95 similar in both of these species, for example the muscle structure and activity of the femur-
96 tibia joint control loop (Bässler *et al.* 1996) or the retractor unguis that moves the tibia-tarsus
97 joint (Bußhardt *et al.* 2011). The strongest difference between the three species concerns the
98 proportions of the antennae and legs, as *Medauroidea* has much shorter antennae and longer
99 legs than the other two species. Stick insects use their antennae to explore the near-range
100 environment (Dürr *et al.* 2001; Dürr and Bläsing, 2001), to sample obstacles during climbing
101 (Schütz and Dürr, 2011; Krause and Dürr, 2012) and to transfer spatial information to the
102 front legs (Schütz and Dürr, 2011). Still, kinematic properties of the antennae are conserved
103 among the Phasmatodea, irrespective of antenna length (Mujagic *et al.* 2007).

104 All three species have been studied with regard to different aspects of multi-legged
105 locomotion, including leg coordination (*Carausius*: Cruse, 1990; Dürr, 2005; Grabowska *et*
106 *al.* 2012; *Aretaon*: Jeck and Cruse, 2007), climbing and/or leg searching-movements
107 (*Carausius*: Cruse, 1976a; Dürr *et al.* 2001; *Medauroidea*: Karg *et al.* 1991; Berg *et al.*
108 2013; *Aretaon*: Bläsing and Cruse, 2004a), catalepsy (*Carausius*: Godden, 1974;
109 *Medauroidea*: Bässler and Foth, 1982), and single-joint kinematics (*Carausius*: Cruse and
110 Bartling, 1995; *Aretaon*: Frantsevich and Cruse, 1997; Jeck and Cruse, 2007).

111 Since none of these data have been acquired using the same combination of setup and method,
112 and many of them concern tethered, planar walking only, direct comparison of the results is
113 not justified without simplifying assumptions. Moreover, none of the above studies
114 systematically varied the setup structure to include both walking and climbing trials. Finally,
115 the movement of all thorax segments during climbing has never been measured before in stick
116 insects. However, this is important, since prothorax inclination has been shown to affect
117 climbing performance in cockroaches (Ritzmann *et al.* 2005).

118 To the best of our knowledge, we provide the first comparative study of whole-body
119 kinematics of unrestrained insect locomotion. A representative subset of the data has been
120 published (Theunissen *et al.* 2014a) as part of an initiative to establish an open-access natural
121 locomotion database (Theunissen *et al.* 2014b).

122 2. Results

123 With regard to our main objective, we selected three species of stick insects (Phasmatodea)
124 that differ in size and body proportions (Fig. 1), and readily engage in locomotion under
125 experimental conditions: *Carausius morosus*, *Medauroidea extradentata*, and *Aretaon*
126 *asperrimus* (subsequently referred to by the genus only). Although *Carausius* and
127 *Medauroidea* are more closely related to each other systematically than *Aretaon* is to either of
128 them (Bradley and Galil, 1977), the overall body proportions are more similar between
129 *Carausius* and *Aretaon*, with *Medauroidea* standing out with particularly long legs and short
130 antennae (Fig.). Among the three species, *Medauroidea* is also the largest in size and the
131 heaviest (Table 1). In all three species, the centre of mass (CoM) is located close the thorax-
132 coxa joints of the hind legs. Since the exact location of the CoM may vary with physiological
133 state, abdominal posture and, in females, number of eggs, we did not measure its location
134 quantitatively. However, qualitatively, there is a clear difference between the three species: in
135 *Carausius*, the CoM is located slightly posterior to the hind leg coxae (corroborating

136 measurements by Cruse, 1976b), in *Aretaon* it is slightly anterior, whereas in *Medauroidea* it
137 is located almost between the hind leg coxae, intermediate to the CoM locations of the other
138 two species.

139 Assuming that the absolute size differences of the body segments are of minor importance to
140 the control of locomotion, we focussed on differences in proportions. Accordingly, we
141 normalised all body segment lengths to the length of the metathorax (Table 1, T3). With
142 regard to the main body, the abdomen and mesothorax are (proportionally) longest in
143 *Carausius*, whereas the prothorax (T1) and head are longest in *Aretaon*. With regard to the
144 legs, the segments femur (fem) and tibia (tib) are longest in *Medauroidea*, whereas the
145 proximal coxa (cox) is longest in *Aretaon*. The antennae of *Carausius* and *Aretaon* reach at
146 least as far as the front leg tarsi (see also Fig. B, C). In contrast, *Medauroidea* has very short
147 antennae, particularly in females. Assuming that these differences in proportions can affect
148 the static stability, leg coordination, and the functional use of the legs in natural locomotion,
149 we will test the following hypotheses:

- 150 I. Owing to its long and, therefore, relatively heavy abdomen, the hind legs of
151 *Carausius* should stay more retracted than in the other species: this could contribute
152 static stability of the body.
- 153 II. The different proportions of thorax segments should be reflected in different
154 movement ranges of the corresponding intersegmental joints during climbing, being
155 larger for longer segments. This would indicate the use of the leverage of long
156 segments.
- 157 III. Owing to its long legs and short antennae, *Medauroidea* should be able to compensate
158 for spatial disturbances more easily by adjusting leg posture, resulting in less
159 movement of the body axis. Moreover, the antennae should be less suited for tactile
160 exploration, simply because the front leg feet should lead the antennal tips and not the
161 other way round, as described for *Carausius* (Dürr *et al.* 2001) and *Aretaon* (Bläsing
162 and Cruse, 2004a). As a consequence, the legs of *Medauroidea* should account for
163 tactile searching behaviour during climbing.

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

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169

2.1. Differences in posture and overall climbing behaviour

170 Overall walking and climbing behaviour differed with regard to forward velocity (Fig. *S 1*).
171 Clearly, body size did not correlate with speed, as *Carausius* walked significantly faster than
172 *Aretaon* and *Medauroidea* (ANOVA, $df = 2$; $F = 19.53$, $p < 0.001$, multiple comparison).
173 Generally, forward velocity was significantly reduced during climbing (ANOVA, $df = 3$,
174 $F = 6.3$, $p < 0.001$).

175 Differences in body proportions, as described above and listed in Table 1, were reflected in
176 postural differences. This is shown by the stick figures in in Fig. 2B, where the median body
177 postures are drawn to scale. For each leg, the median posture was determined for stance
178 movements only. Whereas the body postures of *Carausius* and *Aretaon* are fairly similar, that
179 of *Medauroidea* clearly stands out by its short, adducted, less levated antennae, and by the
180 knees being held much more above the body axis, i.e., the femora being more levated.

181 Since *Medauroidea* has considerably longer legs than the other species (Fig. 2B), it took less
182 steps to climb the stairs. To illustrate this, Fig. 3 shows representative stepping patterns of
183 trials with high stairs. The first stance phases with a foot standing on the next stair are
184 coloured in red and green for left and right legs, respectively. Whereas *Aretaon* usually took
185 three steps between the stairs, and *Carausius* took two, *Medauroidea* was able to step directly
186 from one stair to the next, sometimes with an intermitted *short step* (e.g., see foot trajectory of
187 R1 in Fig. 3B). The stepping patterns of all species were highly variable, with frequent
188 intermitted short steps (Theunissen and Dürr, 2013; see section 3.3 for further analysis) and
189 steady fluctuations in inter-leg coordination, i.e., gait. This corroborates earlier findings that
190 stick insects typically do not show persistent gaits (Dürr, 2005; Grabowska *et al.* 2012).
191 Accordingly, we did not analyse gait parameters and focused on kinematics of the limbs and
192 body segments instead.

193 The most obvious difference in overall leg kinematics concerned the foot trajectories of the
194 front legs (Fig. A, B). *Medauroidea* lifted its front legs high above the body axis, whereas the
195 other two species lifted their front feet about as high as the body axis (Fig. 3, side view; red
196 and green lines hardly cross the black lines). Since the height of the body axis corresponds to
197 the animal's clearance over ground, lifting the feet higher than the body axis exceeds the
198 maximum height of obstacles beneath the body (see section 3.4 for further analysis).
199 Therefore, we suggest that *Medauroidea* used the high front leg swing movements to
200 effectively search the space ahead for obstacles and footholds that would require lifting or
201 inclining the body axis. This species difference could not be explained as an adaptive

202 response to the stairs on the setup, simply because the clearance of *Medauroidea* was largest
203 (compare side views of the average posture in Fig. B). Thus, if swing height had only reached
204 body clearance in *Medauroidea*, its front legs would have easily stepped on top of all but the
205 highest stairs immediately, i.e., without requiring any adaptation of the foot trajectory (see
206 3.4).

207 **2.2. Stick insects always incline their body axis during climbing**

208 Obviously, obstacles higher than the animal's clearance can be climbed only if the body is
209 either lifted in total (as in a push-up) or inclined. Since different strategies of climbing have
210 been reported for cockroaches (Watson *et al.* 2002), we were interested in how differences in
211 body size, leg length and obstacle height affect the pitch angle of the body axis. Moreover,
212 since the insect thorax contains two intersegmental joints, we wanted to know how these
213 joints were moved during climbing.

214 Videos of representative climbing trials may be accessed through an open access data
215 publication (<http://movement.cit-ec.de/stick-insect-locomotion/data.php>; Theunissen *et al.*,
216 2014a). Concerning the dependence of body axis inclination on obstacle height, Fig. shows
217 the median pitch angle of the metathorax and its inter-quartile range with respect to the
218 position of the metathorax. During flat walking, the median pitch angles of all species were
219 positive, indicating that clearance was larger beneath the front legs than beneath the hind legs.
220 Whenever stairs were present, the pitch angle increased in front of a stair and decreased again
221 as the animals mounted the stair. The increase was proportional to the height of the stairs (Fig.
222 D). This was the case in all three species, though it differed between species, with *Aretaon*
223 showing the largest pitch angles (two-way ANOVA, species: $df = 2$, $F = 32.55$, $p < 0.001$;
224 obstacle height: $df = 3$, $F = 346.98$, $p < 0.001$). The significant interaction of the factors
225 species and obstacle height ($df = 6$, $F = 17.47$, $p < 0.001$) revealed that the slope of the
226 increase in pitch angle with obstacle height differed among species. The slope was steeper in
227 *Carausius* and *Aretaon* than in *Medauroidea* (Fig. D). Interestingly, in *Carausius* and
228 *Aretaon* the pitch angles increased significantly even for *low* stairs (8 mm; t-test: *Carausius*
229 $p < 0.001$, $df = 8$, $t = -10$; *Aretaon* $p < 0.001$, $df = 7$, $t = -9.5$), although this would not have
230 been necessary for mechanical reasons. In contrast, *Medauroidea* did not significantly
231 increase its body pitch angle for the *low* stairs (t-test: $p = 0.0853$, $df = 5$, $t = -2.14$).

232 During climbing, the pitch angles between the three thorax segments and of the neck varied in
233 a coordinated manner (Fig. ; examples for *high* stairs). In *Carausius*, the upward inclination of

234 the body segments started with an upward movement by the prothorax (Fig. 5A, T1, blue)
235 followed by the mesothorax (T2, green) and the metathorax (T3, red). The inclination of the
236 metathorax was accompanied by a sequence of downward movements (depression) of the
237 head (Hd, black) and the two anterior thorax segments (T1, T2). As soon as the pitch angle of
238 the metathorax reached the peak and reverted to its resting value, the pitch angles of head,
239 prothorax and mesothorax also reverted back to the values typical for flat walking. When
240 climbing the second stair, this sequence was repeated in the same way as on the first stair,
241 indicating that the animals did not adapt their strategy in any obvious way. Note that all pitch
242 angles were plotted against the position of the metathorax, which means that the head had
243 already passed the stair, when its angle decreased to negative values.

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

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

260 **3.3 Stick insects use two classes of steps**

261 Following the overall description of climbing behaviour and the analysis of the movements of
262 the body axis, the remaining sections will focus on the movements of the legs. At first, we
263 wondered whether the two classes of steps that were recently described for *Carausius*
264 (Theunissen and Dürri, 2013) also occur in *Aretaon* and *Medauroidea*. Indeed, the step length
265 distributions of *Aretaon* and *Medauroidea* were bimodal, too. Accordingly, we applied the

266 same method as used for *Carausius* and used the local minimum between the two peaks to
267 divide the step distributions into two distinct classes: short steps and long steps (Fig. B,C).
268 After normalization to the thorax length, i.e., accounting for size differences, there were no
269 obvious differences between the step length distributions of *Carausius* and *Aretaon*. In both
270 species long steps were used most often (Fig. A,B). Also, the two modes were located at
271 similar step lengths. This was different in *Medauroidea*, which used short steps more often.
272 Furthermore, *Medauroidea* took steps that were much longer than the thorax length and, in all
273 three leg types, the entire distributions were broader than in the other species.

274 In *Carausius*, short steps were shown to have a different function than long steps, suggesting
275 that they serve as correction steps rather than for propulsion. One of the main characteristics
276 of short steps found in *Carausius* was that their swing directions varied much more than that
277 of long steps (Theunissen and Dürr, 2013). In order to compare this property of short steps
278 among species, Fig. shows polar plots of swing directions for both short and long steps.

279 In all species, the swing movements of long steps were almost exclusively directed to the
280 front. In contrast, short steps showed much broader distributions in their swing directions.
281 Middle and hind legs swung preferably to medial-frontal direction, whereas front legs swung
282 preferably to lateral-frontal directions (Fig. , red arrows). This was consistent among species,
283 and all mean directions were statistically significant (Table S1). In *Aretaon*, short steps
284 showed less variability in their swing direction (Table S1, SD). In summary, the swing phases
285 of long steps were directed frontally in all species, whereas short steps could point into any
286 direction, even though there was a statistically significant preferred direction. The similarities
287 among species suggest that short steps share the same function in *Aretaon* and *Medauroidea*,
288 as it was postulated for *Carausius*. As yet, the lower variability of short step direction in
289 *Aretaon* indicates the presence of an additional or stronger constraint on swing direction than
290 in the other species.

291 **2.3. *Medauroidea* performs front leg searching-movements**

292 As mentioned already with regard to Fig. , long steps appeared to differ in swing height
293 among species: The front leg foot trajectories of *Medauroidea* reached high above the body
294 axis. For a more detailed analysis of this point, we defined swing height as the vertical range
295 covered by the foot during a swing movement and normalized it to the thorax length (Fig.).
296 Note that this vertical range was measured in the body coordinate system (CS) and that only
297 long steps were considered. The distributions of swing heights differed between species and

298 they were broader for the front legs than for the other legs (two-way ANOVA, species: $df = 2$,
299 $F = 55.91$, $p < 0.001$, leg: $df = 2$, $F = 50.77$, $p < 0.001$). Multiple comparison revealed that the
300 distributions were similar in *Carausius* and *Aretaon* (Fig. A,B), but swing heights were
301 significantly higher in *Medauroidea* (Fig. C). Furthermore, the front legs made significantly
302 higher swing movements than the middle and hind legs. Also, the interaction of the factors
303 *species* and *leg* was significant ($df = 4$, $F = 26.96$, $p < 0.001$), indicating that differences
304 among leg types depended on the species. In summary, *Medauroidea* made higher swing
305 movements (higher than one thorax-length) with all legs, but the front legs made particularly
306 high swing movements, reaching up to four thorax-lengths.

307 **2.4. Leg kinematics differ in the long-legged species**

308 Fig. shows the average time courses of the leg joint angles within one stride of a long step.
309 Protraction/retraction of the thorax-coxa (ThCx) joint was very similar for *Carausius* and
310 *Aretaon*, but in *Medauroidea* only the hind legs were protracted in a similar way (Fig. A). The
311 front and the middle legs were more protracted in *Medauroidea* than in the other two species.
312 At the same time, the amplitude of the protraction/retraction movement was smaller in the
313 front legs of *Medauroidea*. Also, the amplitude of pronation and supination was smallest in
314 the front legs of *Medauroidea*. Irrespective of the species, the largest amplitudes of
315 protraction/retraction and pronation/supination were observed in the middle legs (Fig. A, B).

316 The levation angles were generally similar among species and all legs were levated in the first
317 half of the swing movement and depressed in the second half (Fig. C). During stance, the
318 levation angles of the middle legs were nearly constant in *Carausius* and *Medauroidea* and
319 slightly decreased in *Aretaon* (indicating depression). In all species, the hind leg femora were
320 depressed during stance, whereas front leg femora were levated. The high swing movements
321 of the front legs of *Medauroidea* (Fig. C) were reflected by the levation of the femur at the
322 coxa-trochanter (CxTr) joint, and by the extension of the tibia at the femur-tibia (FeTi) joint,
323 both of which strongly increased at the beginning of the swing phase (Fig. C,D). For the
324 flexion/extension-angle of the FeTi-joint (Fig. D), the hind legs showed the opposite
325 behaviour of the front legs in all species. Hind legs were extended in the stance phase and
326 flexed in the swing phase, whereas the front legs were flexed in the stance phase and extended
327 in the swing phase. The former indicated pushing of the hind legs, whereas the latter indicated
328 pulling of the front legs. The middle legs were flexed in the first third of the stance phase, and
329 extended at the end of the stance phase. During swing, they were first extended and then

330 flexed. In the middle leg, the extension during swing was much stronger in *Medauroidea* than
331 in *Aretaon* and *Carausius*.

332 To analyse the intra-leg coordination of the joints, we plotted the protraction angle as a
333 function of the other joint angles (Fig.). The resulting curves for *Carausius* and *Aretaon* were
334 of reasonably similar shape for all joint angles. In contrast, the curves for *Medauroidea* often
335 differed in shape and location, particularly in the front and middle legs. Both of these leg
336 types were protracted more strongly in *Medauroidea* than in the other species, leading to
337 displaced curves. Protraction was always correlated with supination, but the curves differed
338 for swing and stance movements, indicating slack of the rotation axis in the ThCx-joint,
339 which is commonly considered fixed in stick insects (Cruse and Bartling, 1995; Dürr et al.,
340 2004).

341 **3. Discussion**

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

350 **3.1. Use of thorax angles**

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

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

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

377 **3.2. Step parameters**

378 Stick insects do not adjust their step length to increase walking velocity (*Carausius*: Wendler,
379 1964; *Aretaon*: Jeck and Cruse, 2007). However, step length varies during turning (e.g., Dürr
380 and Ebeling, 2005) and climbing. We recently showed that *Carausius* takes two distinct
381 classes of steps: *short* steps and *long* steps (Theunissen and Dürr, 2013). Whereas *long* steps
382 support propulsion, *short* steps serve to correct for inappropriate foothold. Additional step
383 types were also identified in *Aretaon*, when describing climbing sequences across large gaps
384 (Bläsing and Cruse, 2004b). In our study, step length distributions were always bimodal,
385 indicating the occurrence of *short* and *long* steps in *Aretaon* and *Medauroidea*, too (Fig.).
386 The most characteristic feature of *short* steps is the omnidirectionality of the swing movement
387 (Theunissen and Dürr, 2013). Our results confirm this feature in *Aretaon* and *Medauroidea*
388 with significant mean swing directions to medial directions in middle and hind legs and lateral
389 directions in front legs (Fig.). The variable swing directions of *short* steps strongly suggest a
390 corrective function in all three species. However, the proportion of short to long steps differed
391 between species. In *Medauroidea*, the proportion of *short* steps was higher than in the other
392 two species. This might be explained by the function of short steps and the width of the
393 walkway (40 mm), which was adjusted to the lateral distance between contralateral legs of

394 *Carausius* and *Aretaon*. In *Medauroidea*, the distance between contralateral legs is larger,
395 resulting in more frequent foot placement on the sides of the setup. Possibly, this was one
396 reason for *Medauroidea* taking more corrective *short* steps than the other species.

397 Swing height of long steps was largest in *Medauroidea*. This was particularly pronounced in
398 the front legs, where the tarsi frequently reached high above body clearance. Such high swing
399 movements are only sensible when exploring the near range environment, allowing for
400 efficient climbing of obstacles that require the adaptation of body pitch. By doing so, the front
401 legs essentially take on the functional role of tactile feelers, which in other species is done by
402 the antennae (Dürr *et al.* 2001; Krause and Dürr, 2012). In line with this interpretation, the
403 trajectories of front leg swing movements in *Medauroidea* were similar to the trajectories of
404 the searching-movements at the end of the setup (not shown), much like it has been described
405 for *Carausius* (Dürr, 2001). All this suggests that *Medauroidea* actively uses its front legs to
406 search for obstacles in their path, thus confirming hypothesis III: *Medauroidea* appears to
407 compensate for insufficient tactile sampling of its antennae by actively involving the legs in
408 near-range searching.

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

417 **3.3. Leg kinematics**

418 The analysis of the leg joint angles was focused on *long* steps, because *short* steps occurred
419 irregularly and joint angles do not change much in *short* steps (Theunissen and Dürr, 2013).
420 Our kinematic analysis differed from several earlier studies in that we did not assume a fixed,
421 single-DoF joint axis of the ThCx-joint, but measured both the protraction/retraction and the
422 pronation/supination of the leg plane independently from each other. This was important for
423 two reasons: First, the measurement of the slanted ThCx-joint axis is complicated, as it is
424 reflected by the different published values for *Carausius* (Cruse, 1976b; Pfeiffer and Cruse,
425 1994; Cruse and Bartling, 1995). Second, it is unclear how much slack is in this joint, and

426 how much the joint axis orientation may vary during unrestrained walking and climbing.
427 Finally, the direct measurement of the two angles that specify the orientation of leg plane
428 greatly simplifies the comparison among insect, particularly when including non-
429 phasmatodean species. As yet, the different kinds of analyses somewhat complicate the
430 comparison with results of earlier studies, although the overall differences to earlier results on
431 *Carausius* (Cruse and Bartling, 1995) and *Aretaon* (Jeck and Cruse, 2007) are fairly minor.

432 The front leg searching-movements of *Medauroidea* were characterised by single high swing
433 movements and they seemed to be little influenced by the ThCx-joint. Instead, the movements
434 were dominated by high levation of the leg and large extension of the FeTi-joint. These
435 characteristics also occur when *Medauroidea* performs a sequence of searching-movements to
436 find objects (Karg *et al.* 1991; Berg *et al.* 2013). The searching-movements also affected the
437 coordination of the other legs, leading to more protracted middle legs: Whenever the front
438 legs perform high swing movements without ground contact, the other legs must account for
439 balance and stability.

440 Since several joint angle time courses were shown to vary strongly among leg types (Fig. 9,
441 see above), inter-joint coordination differed strongly between leg types, too (Fig.). With
442 regard to hypothesis I (*abdomen length is paralleled by amount of hind leg retraction*), our
443 results are not conclusive. Judged from the retraction angles, it is *Medauroidea*, the species
444 with the shortest abdomen (in proportion), that lifted-off its hind legs most posteriorly (e.g.,
445 see red stars in lower panels of Fig. 10). This contradicts hypothesis I. On the other hand,
446 *Carausius*, the species with the longest abdomen (in proportion), showed the strongest hind
447 leg extension at lift off (see black star in lower right panel of Fig. 10). This supports
448 hypothesis I, because in a retracted hind leg, extension essentially pushes the foot rearwards,
449 thus shifting the contact point away from the centre of mass (CoM) and expanding the
450 stability polygon. An assumption underlying hypothesis I is that the location of the CoM is
451 directly related to the length of the abdomen. However, the relationship between CoM
452 location (most posterior in *Carausius*, most anterior in *Aretaon*) and abdomen length (longest
453 in *Carausius*, shortest in *Medauroidea*) proved to be less straight forward than expected.
454 Moreover, the use of claws and attachment pads during locomotion might differ among
455 species, further confounding the dependence of hind leg posture at lift-off, CoM location and
456 stability. As a consequence, there may be no clear-cut relationship between abdomen length
457 and leg posture at lift-off, because the former does imply CoM location in a simple manner,
458 and the latter is not the only means by which the animal can maintain stability.

459

1.3 Conclusions

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

470

4. Material and Methods

471

4.1. Animals

472 We compared adult individuals of three species of stick and leaf insects (order: Phasmatodea,
473 Fig. 1), each one from a different taxonomic family (following the taxonomy of Bradley and
474 Galil (Bradley and Galil, 1977), including sub-order, family, sub-family and tribe): *Carausius*
475 *morosus* (DE SINÉTY, 1901), Anareolatae, Heteronemiidae, Lonchodinae, Lonchodini;
476 *Medauroidea extradentata* (BRUNNER VON WATTENWYL, 1907), Anareolatae, Phasmatidae,
477 Phasmatinae, Baculini; and *Aretaon asperrimus* (REDTENBACHER, 1906), Areolatae,
478 Bacillidae, Heteropteryginae, Obrimi. Among neurobiologists, *M. extradentata* is commonly
479 called *Cuniculina impigra*. All three species come from south-east Asia, *C. morosus* being
480 from southern India, *A. asperrimus* from Borneo and *M. extradentata* from Vietnam. In the
481 following, we will refer to the species by their genus names only.

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

489

2.2. Behavioural experiments

490 The setup, recording technique and kinematic calculations were the same as described by
491 Theunissen and Dürr (Theunissen and Dürr, 2013). The essential information will be
492 reiterated here. The animals walked along a walkway (490 x 40 mm) and climbed over a
493 staircase placed at the end of it. The height of the stairs was varied pseudo-randomly trial-by-
494 trial between 0 (no stairs), 8, 24 and 48 mm, resulting in four different conditions: *flat*, *low*,
495 *middle* and *high*. The *flat* condition was used as the reference condition without spatial
496 disturbances. The *low* condition introduced two spatial disturbances, both of which were
497 lower than the animals' clearance. In the *middle* condition, the height of the stairs required a
498 change in body inclination but the top of the stairs could be reached by all species with a
499 single high swing movement. The *high* condition required climbing, and at least *Carausius*
500 and *Aretaon* had to place a foot on the vertical surface of the stairs. The four conditions were
501 tested in at least 10 trials per animal. When the animal stopped walking in front of the first
502 stair or when it climbed onto the sides of the setup, the same condition was repeated. The raw
503 data set on *Carausius* was the same as used by Theunissen and Dürr (Theunissen and Dürr,
504 2013). With the exception of Fig. 7A, which was included for reasons of comparison, all
505 aspects analysed in the present study have not been published before. The data underlying
506 Fig. A and Fig. A have been used by Theunissen and Dürr (Theunissen and Dürr, 2013) as
507 well, but without normalisation to thorax length.

508 In conjunction with the publication of a concept for an open access database comprising
509 experimental data on natural motion in animals and humans (Theunissen *et al.* 2014b), a
510 representative sample data set (comprising 36 trials, i.e., 12 per species) has been made
511 available to the public on <http://movement.cit-ec.de> (doi:10.4119/unibi/citec.2013.3;
512 Theunissen *et al.* 2014a).

513 **2.3 . Animal recording and reconstruction**

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

521 Up to 20 markers with a diameter of 1.5 mm were attached to the thorax, the head, the legs
522 and the antennae, using nail polish. For the reconstruction of the real animal, segment
523 dimensions and positions of all markers on their respective body segment were measured
524 from high-resolution photographs (0.02 mm per pixel) taken under a stereo lens (Olympus
525 SZ61T, equipped with a digital camera (Pixelink PL-B681CU). Together, the positions of the
526 markers and the body measurements were used to reconstruct the real moving animal in
527 MATLAB (Mathworks, Inc., Natick, MA, USA). Kinematic calculations were done with the
528 help of a main kinematic chain (thorax and head) and six kinematic side chains for the legs
529 (coxa, trochantero-femur and tibia). Three markers attached to the metathorax were used to
530 define the right-handed, body-fixed Cartesian CS, with its origin in the centre of the segment
531 border between the 1st and 2nd abdominal segment (in stick insects, the 1st abdominal segment
532 is fused to the metathorax). The x-axis of the resulting body CS pointed from posterior to
533 anterior, the y-axis to the left and the z-axis upward. Local, segment-fixed CS were calculated
534 for each thorax segment and the head. The roll angle described counter-clockwise rotation
535 around the x-axis, the pitch angle rotation around the y-axis and the yaw angle rotation around
536 the z-axis. Pitch angles were inverted such that positive angles described upward rotations and
537 negative angles downward rotations.

538 **2.4. Leg kinematics**

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

551 **2.5. Foot contacts and step length**

552 The position of the tibia-tarsus joint was assumed as foot position, because the tarsi could not
553 be labelled with markers and, thus, not be reconstructed. Ground contact was determined
554 based on the velocity of the tibia-tarsus-joints and the distance to the surface (see also
555 (Theunissen *et al.* 2014c) The velocity had to decrease below a certain threshold for at least 8
556 consecutive frames (40 ms). The threshold was set to 25 mm/s for *Carausius* and *Aretaon*,
557 and to 50 mm/s for *Medauroidea*. This threshold was set higher for *Medauroidea* because the
558 tibia-tarsus joint position moved considerably during stance phase. Additionally, a plausibility
559 criterion was introduced, where we calculated the distance between the foot and the surface. If
560 the velocity criterion was fulfilled and the distance between the foot and the surface was
561 below 10 mm, the leg was assumed to have ground contact. Note that this seemingly large
562 margin was only necessary to catch false positives detected by the velocity criterion, e.g.,
563 extremely slow episodes in swing movements. False negatives were a lot more unlikely
564 because they'd have required very brief stance phases and/or pronounced tibia movement
565 (marker displacement) during stance. A margin of approximately twice the tarsus length
566 proved to be useful, partly to account for the fact that our kinematics calculations estimated
567 the location of the tibia-tarsus joint and not the location of the distal tarsomeres, partly to
568 account for inaccuracies in the set-up position estimates.

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

573 **Acknowledgements**

574 We would like to thank A. F. Krause for helpful comments on the manuscript, A. Exter for
575 excellent technical assistance, and B. Otte-Eustergerling for animal care. This study was
576 supported by the EU-project grant EMICAB (FP7-ICT-270182) to VD.

577 **Author contributions**

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

582

583 **Funding**

584 This study was supported by the EU project EMICAB (Embodied Motion Intelligence for
585 Cognitive, Autonomous roBots; FP7-ICT-270182).

586

587 **References**

- 588 1. **Bässler, D., Büschges, A., Meditz, S. and Bässler, U.** (1996). Correlation between
589 muscle structure and filter characteristics of the muscle-joint system in three
590 orthopteran insect species. *J.Exp.Biol.* **199**, 2169-2183.
- 591 2. **Bässler, U. and Foth, E.** (1982). The neural basis of catalepsy in the stick insect
592 *Cuniculina impigra*. 1. Catalepsy as a characteristic of the femur-tibia control
593 system. *Biol.Cybern.* **45**, 101-105.
- 594 3. **Berg, E., Büschges, A., and Schmidt, J.** (2013). Single perturbations cause sustained
595 changes in searching behavior in stick insects. *J.Exp.Biol.* **216**, 1064-1074.
- 596 4. **Birn-Jeffery, A. V. and Higham, T. E.** (2014). The scaling of uphill and downhill
597 locomotion in legged animals. *Integr.Comp.Biol.*
- 598 5. **Bläsing, B. and Cruse, H.** (2004a). Mechanisms of stick insect locomotion in a gap
599 crossing paradigm. *J.Comp.Physiol.A* **190**, 173-183.
- 600 6. **Bläsing, B. and Cruse, H.** (2004b). Stick insect locomotion in a complex environment:
601 climbing over large gaps. *J.Exp.Biol.* **207**, 1273-1286.
- 602 7. **Blickhan, R. and Full, R. J.** (1993). Similarity in multilegged locomotion: Bouncing
603 like a monopode. *J.Comp.Physiol.A* **173**, 509-517.
- 604 8. **Bradley, J. C. and Galil, B. S.** (1977). The taxonomic arrangement of the Phasmatodea
605 with keys to the subfamilies and tribes. *Proc.Entomol.Soc.Washington* **79**, 176-
606 208.
- 607 9. **Burrows, M.** (1996). *The Neurobiology of an Insect Brain*. Oxford: Oxford University
608 Press.
- 609 10. **Büschges, A.** (2012). Lessons for circuit function from large insects: towards
610 understanding the neural basis of motor flexibility. *Curr.Opin.Neurobiol.* **22**,
611 602-608.
- 612 11. **Büschges, A. and Gruhn, M.** (2007). Mechanosensory feedback in walking: From joint
613 control to locomotor patterns. *Advances in Insect Physiology: Insect Mechanics*
614 *and Control* **34**, 193-230.
- 615 12. **Bußhardt, P., Gorb, S. N., and Wolf, H.** (2011). Activity of the claw retractor muscle
616 in stick insects in wall and ceiling situations. *J.Exp.Biol.* **214**, 1676-1684.
- 617 13. **Bußhardt, P., Wolf, H., and Gorb, S. N.** (2012). Adhesive and frictional properties of
618 tarsal attachment pads in two species of stick insects (Phasmatodea) with smooth
619 and nubby euplantulae. *Zoology* **115**, 135-141.
- 620 14. **Cruse, H.** (1976a). The control of body position in the stick insect (*Carausius morosus*),
621 when walking over uneven surfaces. *Biol.Cybern.* **24**, 25-33.
- 622 15. **Cruse, H.** (1976b). The function of the legs in the free walking stick insect *Carausius*
623 *morosus*. *J.Comp.Physiol.* **112**, 235-262.

- 624 16. **Cruse, H.** (1990). What mechanisms coordinate leg movement in walking arthropods?
625 *TINS* **13**, 15-21.
- 626 17. **Cruse, H. and Bartling, C.** (1995). Movement of joint angles in the legs of a walking
627 insect, *Carausius morosus*. *J.Insect Physiol.* **41**, 761-771.
- 628 18. **Cruse, H., Dürr, V., Schilling, M. and Schmitz, J.** (2009). Principles of insect
629 locomotion. In: *Spatial temporal patterns for action-oriented perception in*
630 *roving robots* (eds. Arena, P. and Patanè, L.), pp. 43-96. Berlin: Springer.
- 631 19. **Dürr, V.** (2001). Stereotypic leg searching-movements in the stick insect: Kinematic
632 analysis, behavioural context and simulation. *J.Exp.Biol.* **204**, 1589-1604.
- 633 20. **Dürr, V.** (2005). Context-dependent changes in strength and efficacy of leg
634 coordination mechanisms. *J.Exp.Biol.* **208**, 2253-2267.
- 635 21. **Dürr, V. and Bläsing, B.** (2001) Antennal movements of two stick insect species:
636 Spatio-temporal coordination with leg movements. *Zoology: Analysis of*
637 *Complex Systems* **103** (Supplement III: DZG 93.1), 17.
- 638 22. **Dürr, V. and Ebeling, W.** (2005). The behavioural transition from straight to curve
639 walking: kinetics of leg movement parameters and the initiation of turning.
640 *J.Exp.Biol.* **208**, 2237-2252.
- 641 23. **Dürr, V., König, Y., and Kittmann, R.** (2001). The antennal motor system of the stick
642 insect *Carausius morosus*: anatomy and antennal movement pattern during
643 walking. *J.Comp.Physiol.A* **187**, 131-144.
- 644 24. **Dürr, V., Schmitz, J., and Cruse, H.** (2004). Behaviour-based modelling of hexapod
645 locomotion: linking biology and technical application. *Arthropod.Struct.Dev.* **33**,
646 237-250.
- 647 25. **Fischer, M. S., Schilling, N., Schmidt, M., Haarhaus, D. and Witte, H.** (2002). Basic
648 limb kinematics of small therian mammals. *J.Exp.Biol.* **205**, 1315-1338.
- 649 26. **Frantsevich, L. and Cruse, H.** (1997). The stick insect, *Obrimus asperimus*
650 (Phasmida, Bacillidae) walking on different surfaces. *J.Insect Physiol.* **43**, 447-
651 455.
- 652 27. **Full, R. J., Blickhan, R., and Ting, L. H.** (1991). Leg design in hexapedal runners.
653 *J.Exp.Biol.* **158**, 369-390.
- 654 28. **Fuller, P. O., Higham, T. E., and Clark, A. J.** (2011). Posture, speed, and habitat
655 structure: three-dimensional hindlimb kinematics of two species of padless
656 geckos. *Zoology* **114**, 104-112.
- 657 29. **Gatesy, S. M. and Pollard, N. S.** (2011). Apples, oranges, and angles: Comparative
658 kinematic analysis of disparate limbs. *J.Theor.Biol.* **282**, 7-13.
- 659 30. **Godden, D. H.** (1974). The physiological mechanism of catalepsy in the stick insect
660 *Carausius morosus* Br. *J.Comp.Physiol.* **89**, 251-274.
- 661 31. **Grabowska, M., Godlewska, E., Schmidt, J., and Daun-Gruhn, S.** (2012).
662 Quadrupedal gaits in hexapod animals - inter-leg coordination in free-walking
663 adult stick insects. *The Journal of Experimental Biology* **215**, 4255-4266.
- 664 32. **Graham, D.** (1985). Pattern and control of walking in insects. *Adv.Insect Physiol.* **18**,
665 31-140.

- 666 33. **Higham, T. E. and Jayne, B. C.** (2004). Locomotion of lizards on inclines and perches:
667 hindlimb kinematics of an arboreal specialist and a terrestrial generalist.
668 *J.Exp.Biol.* **207**, 233-248.
- 669 34. **Jeck, T. and Cruse, H.** (2007). Walking in *Aretaon asperrimus*. *J.Insect Physiol.* **53**,
670 724-733.
- 671 35. **Karg, G., Breutel, G., and Bässler, U.** (1991). Sensory influences on the coordination
672 of two leg joints during searching movements of stick insects. *Biol.Cybern.* **64**,
673 329-335.
- 674 36. **Krause, A. F. and Dürr, V.** (2012). Active tactile sampling by an insect in a step-
675 climbing paradigm. *Frontiers in Behavioural Neuroscience* **6**, 1-17.
- 676 37. **Mujagic, S., Krause, A. F., and Dürr, V.** (2007). Slanted joint axes of the stick insect
677 antenna: an adaptation to tactile acuity. *Naturwiss.* **94**, 313-318.
- 678 38. **Pfeiffer, F. and Cruse, H.** (1994). Bionik des Laufens - technische Umsetzung
679 biologischen Wissens. *Konstruktion* **46**, 261-266.
- 680 39. **Ritzmann, R. E. and Büschges, A.** (2007). Adaptive motor behavior in insects.
681 *Curr.Opin.Neurobiol.* **17**, 629-636.
- 682 40. **Ritzmann, R. E., Pollack, A.J., Archinal, J., Ridgel, A.L. and Quinn,R.D.** (2005).
683 Descending control of body attitude in the cockroach *Blaberus discoidalis* and
684 its role in incline climbing. *J.Comp.Physiol.A* **191**, 253-264.
- 685 41. **Schmidt, A. and Fischer, M. S.** (2011). The kinematic consequences of locomotion on
686 sloped arboreal substrates in a generalized (*Rattus norvegicus*) and a specialized
687 (*Sciurus vulgaris*) rodent. *The Journal of Experimental Biology* **214**, 2544-2559.
- 688 42. **Schütz, C. and Dürr, V.** (2011). Active tactile exploration for adaptive locomotion in
689 the stick insect. *Phil.Trans.R.Soc.Lond.B* **366**, 2996-3005.
- 690 43. **Stoessel, A. and Fischer, M. S.** (2012). Comparative intralimb coordination in avian
691 bipedal locomotion. *The Journal of Experimental Biology* **215**, 4055-4069.
- 692 44. **Theunissen, L. M., Bekemeier, H. H., and Dürr, V.** (2014a) Stick Insect Locomotion
693 Data. Bielefeld, Germany, Bielefeld University. Doi:10.4119/unibi/citec.2013.3
- 694 45. **Theunissen, L. M. and Dürr, V.** (2013). Insects use two distinct classes of steps during
695 unrestrained locomotion. *PLOS one* **8**, e85321.
- 696 46. **Theunissen, L. M., Hertrich, M., Wiljes, C., Zell, E., Behler, C., Krause, A. F.,**
697 **Bekemeier, H. H., Cimiano, P., Botsch, M., and Dürr, V.** (2014b) A natural
698 movement database for management, documentation, visualization, mining and
699 modeling of locomotion experiments. In: Duff, A., et al. (eds) Living Machines
700 2014 (Milan, 30.07.-01.08.2014). 308-319. Heidelberg, Springer. LNAI 8608.
- 701 47. **Theunissen, L. M., Vikram, S., and Dürr, V.** (2014c). Spatial co-ordination of foot
702 contacts in unrestrained climbing insects. *J.Exp.Biol.* **217**, 3242-3253.
- 703 48. **Vidal-Gadea, A. G., Rinehart, M. D., and Belanger, J. H.** (2008). Skeletal
704 adaptations for forwards and sideways walking in three species of decapod
705 crustaceans. *Arthropod Structure & Development* **37**, 95-108.
- 706 49. **Watson, J. T. et al.** (2002). Control of obstacle climbing in the cockroach, *Blaberus*
707 *discoidalis*. I. Kinematics. *J.Comp.Physiol.A* **188**, 39-53.
- 708 50. **Weihmann, T. and Blickhan, R.** (2009). Comparing inclined locomotion in a ground-
709 living and a climbing ant species: sagittal plane kinematics. *Journal of*

- 710 *Comparative Physiology A-Neuroethology Sensory Neural and Behavioral*
711 *Physiology* **195**, 1011-1020.
- 712 51. **Wendler, G.** (1964). Laufen und Stehen der Stabheuschrecke: Sinnesborsten in den
713 Beingelenken als Glieder von Regelkreisen. *Z.vergl.Physiol.* **48**, 198-250.
- 714 52. **Wilson, D. M.** (1966). Insect walking. *Ann.Rev.Entomol.* **11**, 103-122.
715

716 **Figure Legends and Tables**

717

718 Figure 1: The three stick insect species under study. From top to bottom: Adult female
719 *Carausius morosus* (DE SINÉTY, 1901), adult female *Medauroidea extradentata* (BRUNNER
720 VON WATTENWYL, 1907), and adult male *Aretaon asperrimus* (REDTENBACHER, 1906).
721 Photomontage. The diameter of a 1 Euro coin is 23,25 mm.

722

723 Fig. 2: ***Carausius*, *Aretaon* and *Medauroidea* have different body proportions and**
724 **postures.** A: Stick figures of each species walking on the flat setup with markers (blue)
725 attached to the body (black) and the right (green) and left (red) legs. B: Side and top views of
726 median posture during stance. The sex of the illustrated bodies are female in case of
727 *Carausius* and *Medauroidea*, and male in *Aretaon*. C: Schematics of body proportions with
728 out-stretched limbs. Relative to the thorax, *Medauroidea* has the longest legs and the shortest
729 antennae. Its leg posture differs from that of the other species. Segment lengths, but not
730 widths were scaled 1:2 in B and C.

731

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

743

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

754

755 **Fig. 5: Pitch angles of the thorax segments and head are coordinated during climbing.**
 756 The median and the quartiles of the pitch angles of the three thorax segments (T3: metathorax,
 757 T2: mesothorax, T1: prothorax) and the head (Hd) are plotted against the rear end position of
 758 the metathorax. Positive/negative angles indicate levation/depression. Only trials using the
 759 climbing condition (48 mm stairs) were considered. The vertical blue lines indicate the
 760 position of the stairs. A-C: The numbers to the top left give number of animals (N) and trials
 761 (n). Note, that the binning (5 mm) is different than in Fig. 4 (1 mm). D: Pitch angle of the
 762 head plotted against the position of the neck. Black: *Carausius*; blue: *Aretaon*; Red:
 763 *Medauroidea*. Head pitch changes nearly as much as body axis pitch (T3), but with a time
 764 shift.

765

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

772

773 Fig. 7: **Swing direction differs between long and short steps.** Circular histograms of long
 774 steps and short steps are shown for *Carausius* (top row), *Aretaon* (middle row) and
 775 *Medauroidea* (bottom row). Step direction was calculated from lift-off to touch-down in the
 776 horizontal plane of body-fixed coordinates. Due to the bin width of 30°, step directions of 0°
 777 ± 15° are considered as anteriorly directed steps. Medial steps are directed towards 90°,
 778 backward steps to 180° and lateral steps to 270°. The number of steps (n) and the radius (r) of
 779 the outer circle are given to the lower right of each plot. The statistics for the mean direction
 780 vectors (red) are given in Table S2. The length of the lower left red arrow indicates r = 1.

781

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

788

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

800

801 Fig. 10: **Joint angle movements are similar for *Carausius* and *Aretaon*, but different for**
 802 ***Medauroidea*.** Protraction angles of *Carausius* (black), *Aretaon* (blue) and *Medauroidea* (red)
 803 are plotted against supination (A), levation (B) and flexion (C) for front (FL), middle (ML)
 804 and hind (HL) legs. Crosses/stars show the beginning/end of the stance phase. The direction

805 of the stance movement is indicated by the arrow next to it. Note that protraction of the front
806 legs of *Medauroidea* (red) range less than 20°, whereas flexion ranges over around 80°.
807 Sample sizes are the same as in Fig. 9.

808

809 **Fig. S 1: Body velocity decreases from walking to climbing.** Average velocities per trial of
810 *Carausius* (top), *Aretaon* (middle) and *Medauroidea* (bottom) for the different conditions
811 (Flat; Low; Middle; High) and for the pooled data (All). The box plots show the medians
812 (red), inter-quartile range (blue) and entire ranges. Red dots are outliers. Black dotted line
813 indicates median velocity of all *Carausius* trials. *Carausius* walked faster than the other two
814 species. *Medauroidea* was slowest (despite being the largest) and showed hardly any change
815 in walking velocity across conditions. The number of animals and trials differs between
816 species: *Carausius* N = 9 (n = 323); *Aretaon* N = 8 (n = 186); *Medauroidea* N = 6 (n = 167).

817

818 Table 1: Comparison of segment sizes and proportions.

Genus	Sex		Weight	Body					HL			ML			FL			Antennae		
				Abdomen	T3	T2	T1	Head	cox	fem	tib	cox	fem	tib	cox	fem	tib	scp	ped	fla
<i>Carausius</i>	female N = 10	mean	0.955	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.
		SD	0.124	1.21	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	0.6
		x/T3		3.27	1.00	1.43	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	2.5
<i>Aretaon</i>	male 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.
		SD	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	1.4
		x/T3		3.06	1.00	1.15	0.50	0.57	0.24	1.46	1.53	0.22	1.15	1.14	0.22	1.24	1.25	0.14	0.11	3.5
<i>Medauroidea</i>	female 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.
		SD	0.243	1.64	0.42	0.30	0.16	0.42	0.26	1.81	1.65	0.26	1.14	1.19	0.15	1.50	2.41	0.25	0.11	2.2
		x/T3		2.85	1.00	1.26	0.32	0.37	0.14	1.85	2.28	0.14	1.44	1.61	0.14	2.19	2.63	0.15	0.05	0.8
	male 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.
		SD		1.92	1.31	0.90	0.17	0.66	0.17	1.83	7.44	0.17	1.43	16.32	0.16	3.40	10.71	0.28	0.18	1.4
		x/T3		2.66	1.00	1.15	0.22	0.30	0.12	2.09	2.51	0.12	1.60	1.90	0.11	2.53	3.05	0.09	0.06	1.5

819

820 Body weight, absolute and normalised segment lengths of main body axis, the legs and the antennae. HL: hind leg; ML: Middle leg; FL: front leg;
 821 T1: Prothorax; T2, Mesothorax; T3: Metathorax; cox: Coxa; fem: Femur; tib: Tibia; scp: Scapus; ped: Pedicellus; fla: Flagellum. All lengths and
 822 standard deviations (SD) are given in mm, proportions (x/T3), are given as the ratio of the respective mean of parameter x and the length of the
 823 metathorax (T3). Bold font highlights the species with the largest proportions. The mean weights and its standard deviations are given in g and were
 824 measured from ten animals per species.

825

826 Table 2: Comparative locomotion summary

		<i>Carausius morosus</i>	<i>Medauroidea extradentata</i>	<i>Aretaon asperrimus</i>
Systematics (Bradley and Galil, 1977)		Anareolatae, Heteronemiidae, Lonchodinae, Lonchodini	Anareolatae, Phasmatidae, Phasmatinae, Baculini	Areolatae, Bacillidae, Heteropteryginae, Obrimi
Size & 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 much above body height	Knees at body height
	Swing height	≈ clearance	> clearance	≈ clearance
Steps	Predominant step type	Long	Short	Long
	Long-step length	< 1.2 x T3	> 2 x T3	< 1.2 x T3
	Short step SD (HL/ML/FL)	105/115/120 deg	106/117/121 deg	78/73/89 deg
Leg joints	FL protraction range	45 deg	<i>20 deg</i>	40 deg
	ML protraction mean	0 deg	25 deg	0 deg
	HL flexion mean	100 deg	<i>80 deg</i>	85 deg
Thorax joints	Thorax inclination	1.2 deg/mm	<i>0.6 deg/mm</i>	1.1 deg/mm
	T2 levation range	25 deg	10 deg	<i>0 deg</i>
	T1 levation range	35 deg	<i>5 deg</i>	20 deg
	Head levation range	30 deg	<i>15 deg</i>	45 deg

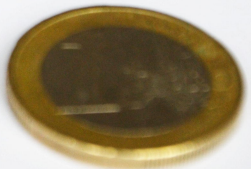
827 Data from *Carausius* only concerns females, those of *Aretaon* only concern males. Where the sex
828 of *Medauroidea* is not specified, data have been pooled for both sexes. **Largest values** are
829 typeset in bold font, *lowest values* are typeset in italics. Ant: Antenna; FL: Front leg; HL: Hind
830 leg; ML: Middle leg; SD: standard deviation; T1: Prothorax; T2: Mesothorax; T3: Metathorax.
831 Steps: Long step length is given as multiples of T3 length; Short step SD gives the angular
832 dispersion of short step direction. Leg joints: range gives the approximate joint angle range
833 shown in Fig. 10; mean gives the approximate central angle, midway between minimum and
834 maximum angle in Fig. 10. Thorax joints: Thorax inclination gives the slope of Fig. 5D; range
835 gives the approximate joint angle ranges shown in Fig. 5A-C.

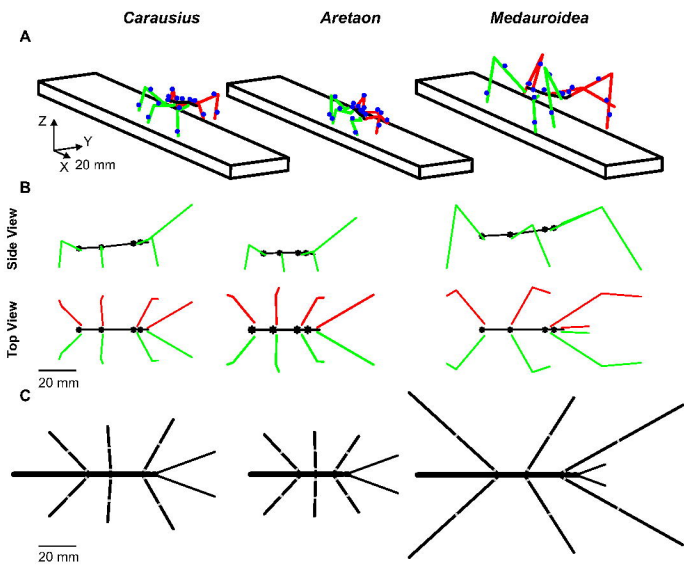
836 Table S1: Statistics for the mean swing directions

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

837

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



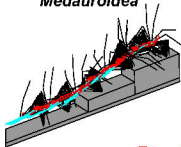
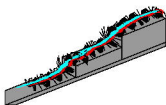
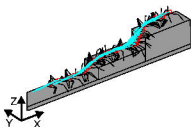


Carausius

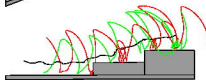
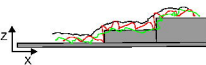
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Medauroidea

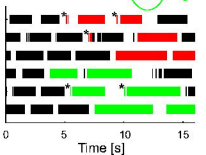
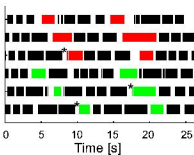
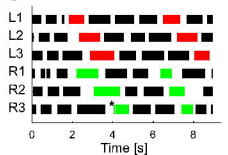
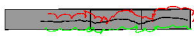
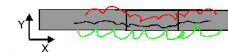
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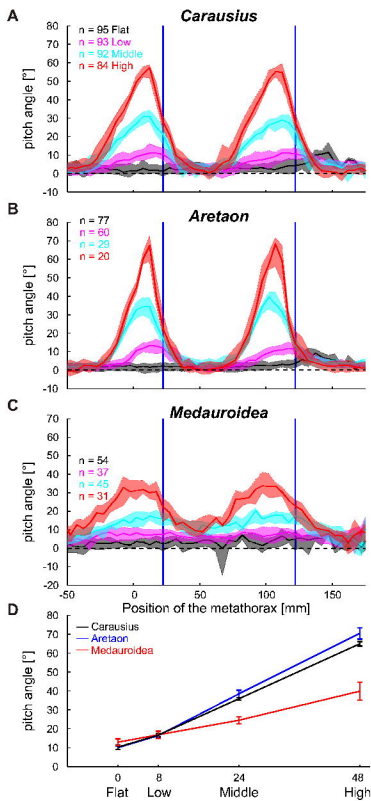


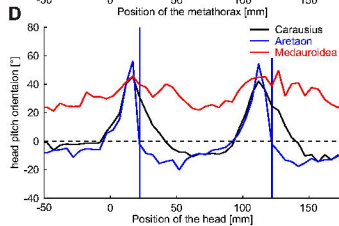
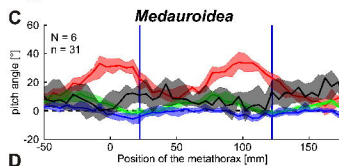
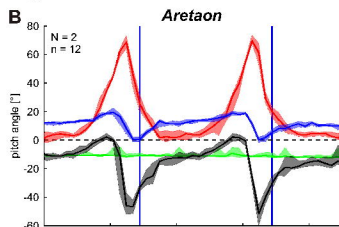
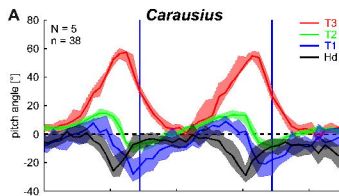
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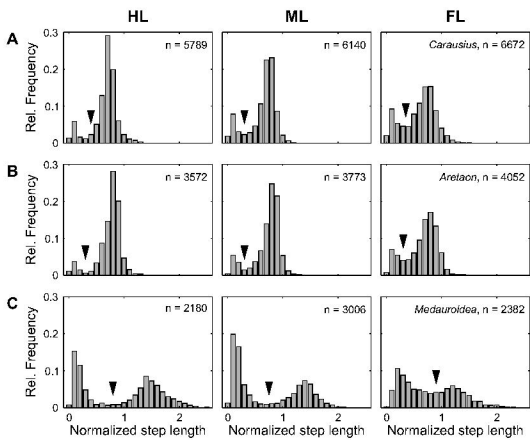


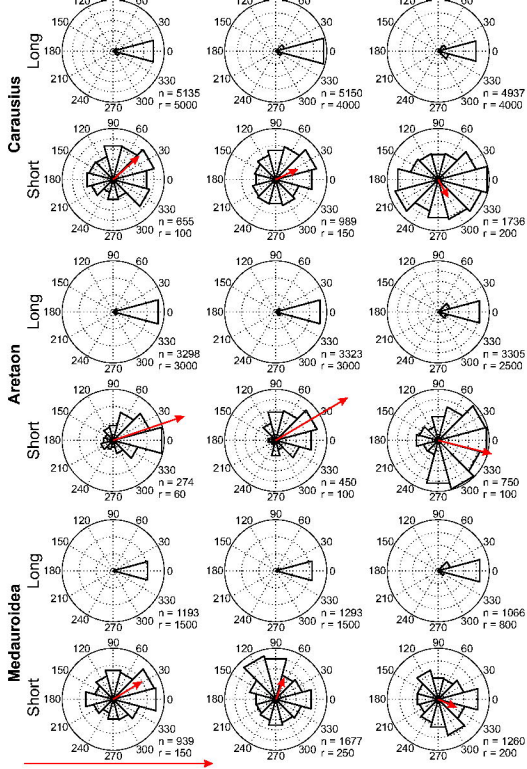
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HL

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FL

