Locomotion in the pseudoscorpion *Chelifer cancroides* – forward, backward and upside down walking in an eight-legged arthropod

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Summary statement
Chelifer cancroides pseudoscorpions perform an alternating tetrapod gait during forward and backward locomotion, with consistently higher speeds during backward motion, while no fixed leg coupling occurs during upside down walking.

Abstract
While insect locomotion has been intensively studied, there are comparably few studies investigating octopedal walking behaviour, and very little is known about pseudoscorpions in particular. Therefore, we performed an extensive locomotion analysis during forward, backward and upside down walking in the cosmopolitan pseudoscorpion *Chelifer cancroides.* During forward locomotion, we observed *C. cancroides* to freeze locomotion frequently for short time periods. These microstops were barely visible to the naked eye with a duration of 100-200 ms. Our locomotion analysis revealed that *C. cancroides* performs a statically stable and highly coordinated alternating tetrapod gait during forward and backward walking, with almost complete inversion of the tetrapod schemes, but no rigidly fixed leg coordination during upside down walks with low walking speeds up to 4 body lengths per second. Highest speeds (up to 17 body lengths per second), mainly achieved by consistent leg coordination and strong phase shifts, were observed during backward locomotion (escape behaviour), while forward walking was characterised by lower speeds and phase shifts around 10%

between two loosely coupled leg groups within one tetrapod. That is, during the movement of one tetrapod group, the last and the third leg are almost synchronous in their swing phases, as are the second and the first leg. A special role of the second leg pair was demonstrated, probably mainly for stability reasons and related to the large pedipalps.

Introduction

For most animals, including arthropods, locomotion is an indispensable factor for survival in nature (Escalante et al., 2019). While the walking behaviour of hexapods has been studied in more detail, knowledge concerning eight-legged animals that exhibit an astonishing range of locomotor strategies, remains scarce (Spagna and Peattie, 2012). Previous studies focused on spiders (Wilson, 1967; Biancardi et al., 2011; Spagna et al., 2011), harvestmen (Escalante et al., 2019; 2020), and scorpions (Bowerman, 1975; Telheiro et al., 2021), inspiring the design of walking robots to locomote through complex terrains (Klaassen et al., 2002; Spagna et al., 2007). Furthermore, crayfish locomotion was investigated (Cruse and Müller, 1986; Cruse and Saavedra, 1996) and walking of tardigrades, one of the smallest (eight-)legged animals, was analysed and compared to other arthropod datasets (Nirody et al., 2021; Nirody, 2021).

Most studied eight-legged animals walk with two alternating sets of four diagonally adjacent legs (tetrapod gait, partly comparable to tripod gait in hexapods), with varying degrees of deviation due to intermediate phase relations between adjacent ipsilateral legs (metachronal wave) (Bowerman, 1975; Spagna et al., 2011; Spagna and Peattie, 2012; Weihmann, 2013). However, there are species that use one leg pair for other purposes (e.g., sensory functions or antennal illusion behaviour (Shamble et al., 2017; Escalante et al., 2019)), and in this context, similarities with insect walking patterns have been demonstrated (e.g., Shamble et al., 2017, Nirody et al., 2021). While the above species have been examined in more detail, little is known about pseudoscorpions (Spagna and Peattie, 2012), the fourth most diverse arachnid order (>3300 valid pseudoscorpion species) (Harvey, 2007; Del-Claro and Tizo-Pedroso, 2009).

Pseudoscorpions resemble scorpions in their morphology with chelated pedipalps, eight legs, and general shape, but are clearly smaller (2-5 mm body length) and lack a metasoma and terminal stinger (Weygoldt, 1969; Harvey, 1988; Donovan and Paul, 2005). They occur in almost all terrestrial environments (Harvey, 1988) such as rock crevices, under stones and barks of trees, or in human dwellings and associated buildings (Levi, 1948; Weygoldt, 1969;

Del-Claro and Tizo-Pedroso, 2009). Pseudoscorpions are active predators (Gilbert, 1951; Weygoldt, 1969) using their large pedipalps for prey capture (Schlegel and Bauer, 1994), but also for social interactions (e.g., courtship or fighting) and orientation. Pedipalps are equipped with numerous sensory sensillae and used as main sensory organs (Weygoldt, 1969; Stemme and Pfeffer, 2022). In this study, the most widely distributed pseudoscorpion species (Harvey, 2014) *Chelifer cancroides* (Linnaeus, 1758) (Fig. 1) was investigated, which is known as beneficial organism for honeybees and beekeepers, as pseudoscorpions are suitable predators of *Varroa* mites (Donovan and Paul, 2005).

Pseudoscorpions' locomotion might be comparable primarily to those of spiders and scorpions, as they also show an alternating tetrapod gait (Weygoldt, 1969). They are capable of locomotion on many different surfaces and substrates, forward, backward and upside down, the latter being possible due to the presence of two claws and a clasper (arolium) on their pretarsi (Ax, 2000). Pedipalps are extended while running forward and angled during backward walks, which could possibly be a reason for the surprising fact that a pseudoscorpion can walk faster backwards than forwards (Weygoldt, 1969; Cowles, 2018). Pseudoscorpions can bring their pedipalps into attack position in a fast 180-degree turn when touched on the opisthosoma (Weygoldt, 1969). Furthermore, some species explore their environment phoretically: they attach themselves with their pedipalps to the bodies of larger arthropods as vehicles (Beier, 1951; Poinar et al., 1998; Del-Claro and Tizo-Pedroso, 2009).

In the present contribution, we perform a locomotion analysis in the pseudoscorpion *C. cancroides*. We analyse forward, backward and upside down walking since locomotion in a structured three-dimensional environment does not always correspond to level forward walking. Further, we discuss differences in the three locomotion scenarios in an ecological context with focus on high backward speeds and general leg coordination. We hypothesize that the forward tetrapod pattern described so far is modified to varying degrees during backward and upside down walking to enable efficient and stable locomotion. Emphasis was further placed on overall walking behaviour, as well as analysis and interpretation of small stationary episodes that occurred during forward locomotion. We assume that those stops may reduce oscillating movements of the pedipalps or may serve for orientation, and therefore occur regularly. In addition, we test the hypothesis that stops may precede for changes in walking direction.

Materials and Methods

Animals and experimental sites

Pseudoscorpions of the species *Chelifer cancroides* were obtained from an old hayloft near Rinteln (Lower Saxony, Germany). Animals were kept under constant environmental conditions and without exposure to light. To analyse the walking behaviour of *C. cancroides*, videos were recorded at Ulm University (Baden-Wuerttemberg, Germany), under laboratory conditions. All data were obtained during 2019 and 2021. The experiments were conducted in compliance with the current German laws and ethical guidelines of Ulm University.

Body measurements

Body length, prosoma length, leg and pedipalp lengths of n=10 individuals were determined (Table 1). Pseudoscorpions were dissected by cutting the pedipalps off (at the proximal end of the trochanter) and severing the legs in the coxa-trochanter joints. Photos were taken through a dissection microscope (Stemi SV 6, Zeiss Microscopy GmbH, Jena, Germany) and measurements on these photos were performed with ImageJ (National Institutes of Health, Bethesda, MD, USA). For each leg, lengths of femur, patella, tibia, and tarsus were summated, and means were calculated for the four leg pairs. Pedipalp length was determined as the sum of femur, patella and chela length and mean values of left and right pedipalps were calculated. Total body length and prosoma length were measured to characterize body size (Harvey, 2014). Prosoma length was measured to exclude the influence of the animals' nutritional status, which may affect opisthosoma size. Body length of the animals, used for high-speed recording evaluations, was directly measured in the high-speed videos (n=65) with ImageJ. Additionally, total body mass of n=10 individuals was determined (Analytic balance Kern ADB 100-4 120 g, Balingen, Germany).

Video recordings

EthoVision analysis

Top view videos for EthoVision analysis were made with an area scan camera (acA1300-30gc, Basler AG, Ahrensburg, Germany; CS-Mount Computar Objective 2.8-12 mm 1:1.3 IR 1/3", Bangladesh) at a sampling rate of 30 frames per second under laboratory conditions (room temperature 26°C). Pseudoscorpions were filmed for one hour in a rectangular arena (9.5x9.5 cm, with a plastic floor and arena walls covered with escape protection to avoid climbing) to examine their general walking behaviour in a two-dimensional setting. For illumination, a ring light source (LR-18W, GEEKOTO, Hong Kong, China) was used. The animals were filmed in groups of three (n=4 videos, n=12 individuals) as well as individually

(n=4 videos). No notable differences regarding determined walking parameters were observed between individual and group recordings. In the end, we only used group recordings to analyse overall walking behaviour.

High-speed video analysis

High-speed video recordings were made with two high-speed cameras (MotionBlitz EoSense Mini1 and Mini1-1, Mikrotron, Unterschleissheim, Germany; Nikon Lens 105 mm 1:2.8 DG MACRO and Tokina Lens 100 mm 1:2.8 D MACRO, Japan) at a sampling rate of 500 frames per second under laboratory conditions (room temperature: 24-26°C). For top view recordings, pseudoscorpions were filmed while walking forwards and backwards through a linear aluminium channel, width 3 cm, wall height 7 cm. The channel floor was coated with white print paper to provide fewer reflections as well as better contrast. Furthermore, the animals were filmed while walking upside down on a transparent plastic sheet on top of the aluminium channel (generation and evaluation of the recordings was more simple). As no noticeable differences were observed between the walking behaviour on print paper and plastic sheets (Fig. S1), upside down walking was compared with the data of the forward and backward locomotion analyses. For indoor illumination, two fibre optic cold light sources (Schott KL 1500LCD, 150W, Schott AG, Mainz, Germany) were used. The animals were filmed multiple times, using at most three videos of the same individual (in total: n=97 runs, n=65 individuals). Only straight running trajectories were analysed and walking sequences with decelerations (except microstops, see below), curves and slipping steps were disregarded. Further, each high-speed video had to consist of a minimum of three complete step cycles per leg (and more steps for gait analysis and general walking behaviour analysis). All measurements were evaluated in a manual frame-by-frame analysis with ImageJ (National Institutes of Health, Bethesda, MD, USA).

Video analysis and data evaluation

General walking behaviour (EthoVision analysis)

Individuals were placed in an arena and filmed for one hour after a short acclimation period. Different parameters of the one-hour videos were analysed with EthoVisionXT (Noldus, Wagneningen, the Netherlands): distance moved; mean, minimum and maximum walking speeds; mobility states (mobile, immobile; mean, standard deviation, frequency and cumulative duration for each state). Immobile states were defined as periods where the percentage change in object area between video frames was below a defined threshold (immobility threshold <1.5%, mobile threshold ≥1.5%). EthoVision software assessed the

mobility state by comparing the locations of the pixels belonging to the tracked individual in the current frame with the pixels in the previous frame, and the number of relocated pixels was expressed as a percentage of change in object area. During video evaluation, short pausing events were recognized that occurred repeatedly and were interspaced with walking phases. To investigate these stops, high-speed recordings (n=17 videos, 20-30 step cycles per video) were analysed with EthoVision software and heat maps of the pseudoscorpions' movements were created (Fig. 2C, Movie 1). The settings described above were used and the same parameters were examined. Detailed data are provided in Tables S1 (one-hour videos) and S2 (high-speed videos). Furthermore, we calculated an index of straightness (*i1/i2*), to test the hypothesis whether *C. cancroides* changes direction after a microstop. We calculated *i1* as the shortest distance and *i2* as the total distance walked between start and endpoint of a track (n=20 tracks, 20-30 step cycles per track). A value of 1 indicates a straight track (despite microstops, therefore no changes in direction), the lower the index value, the more tortuous is the respective path between start and end point in one video (which could hint at directional changes during microstops).

Basic walking parameters (high-speed analysis)

Different walking parameters were analysed for forward, backward and upside down walking (Fig. 3) by analysing three steps of every high-speed video. **Mean walking speed** was calculated as the distance covered from the beginning of the first to the end of the third step cycle, divided by the time needed to cover this distance. **Relative walking speed** was defined as mean walking speed divided by body length. Further, the timing of every lift-off and touch-down of the eight legs was determined to obtain swing phase and stance phase durations. These values formed the basis for a large part of further evaluations. **Swing phase** duration was calculated as the difference between the time of the tarsal lift-off and touch-down. **Stance phase** duration was defined as the time when the tarsal tip touched the ground and did not move relative to the ground (see Reinhardt and Blickhan, 2014). **Stride length** was calculated as the distance between the tarsal lift-off and touch-down positions on the ground for the respective leg pair. To determine **stride frequency**, mean walking speed was divided by stride length. All parameters described so far were averaged for all eight legs (Fig. 3). In addition, swing and stance phase durations of the individual leg pairs were presented separately (Fig. 4), as here significant differences between different leg pairs occurred. Furthermore, we calculated **duty factor**, a ratio of stance phase to step cycle duration (stance plus swing phase duration of a given stride) that describes the transition from walking to running in bipeds but is also a commonly used parameter in arthropod locomotion studies (Fig. 5). Considering a particular pair of legs, a duty factor value smaller

0.5 signifies the point where aerial phases appear in the coordination pattern to achieve higher speeds (Alexander, 2003). Note that in animals walking on more than one leg pair, phase shifts between leg pairs can still avoid aerial phases for the whole animal (Wilson, 1966; Hildebrand, 1985).

Footfall geometry

In a frame-by-frame analysis, x- and y-coordinates of tarsal lift-off (**PEP**, posterior extreme position) and tarsal touch-down (**AEP**, anterior extreme position) were measured for each leg with respect to the centre of mass (COM) to compare the footfall geometry for forward, backward and upside down walking (see Seidl and Wehner, 2008; Mendes et al., 2013). COM was estimated to lie on the midline approximately near the posterior prosomal edge (base of leg pair 3, graphically determined with ImageJ, method provided in Weihmann et al., 2015 and double-checked with method by Reinhardt and Blickhan, 2014). Footfall positions relative to the COM were normalized to total body length. Standard deviations of the resulting values were used to illustrate the spread of footfall positions (Fig. 6). To analyse and illustrate footfall positions, we used ImageJ (National Institutes of Health, Bethesda, MD, USA), Excel (Microsoft Corporation, Remond, WA, USA), Sigma Plot 11.0 (Systat Software Inc., San Jose, CA, USA) and Inkscape (Inkscape 1.0).

Phase analysis

Phase plots show the coordination of the eight legs in a **circular step cycle diagram** for forward, backward and upside down walking (Wosnitza et al., 2013) and were created with the 'CircStat' Toolbox (Berens, 2009) in MATLAB (MathWorks, Inc., Natick, MA, USA). The onset of swing phase in the left hind leg (L4) was taken as reference point (Fig. 7). Note that phase plots for forward and backward locomotion at same walking speeds are illustrated together in one plot, each with L4 as reference, even though the directions of motion are opposite.

To evaluate the synchrony of tetrapod coordination, we calculated **tetrapod coordination strength (TCS[4])** (Fig. 7) (compare TCS value for insects, e.g., Wosnitza et al., 2013). The TCS[4] value is calculated as the ratio *t1/t2*, with *t1*, the time period with all four legs of one tetrapod in swing phase, and *t2*, the time period from the first swing onset in any of the legs in that tetrapod to the last swing termination in this respective tetrapod group. Higher TCS[4] values indicate a stronger synchronization of the legs in a tetrapod group (L1, R2, L3, R4 or R1, L2, R3, L4, respectively), with a value of 1 indicating perfect tetrapod coordination. The

lower the TCS[4] value, the larger is the temporal shift in swing phases within one tetrapod group during locomotion. Note that *t1* is calculated as the difference between the minimum frame of swing phase termination of the four legs of one tetrapod group and the maximum frame of swing phase onset of the tetrapod group. Therefore, negative values can occur (when the maximum frame of swing onset is larger than the minimum frame of swing termination), indicating that the four legs of one tetrapod do not share time periods with all four legs in swing phase simultaneously.

Inter-leg coordination patterns

Inter-leg coordination patterns were assessed using a frame-by-frame video analysis. Podograms were created to visualize the sequence of swing (black bars in Fig. 8) and stance (white bars) phases of the eight legs (qualitative analysis), and we quantified the inter-leg coordination by classifying each frame according to its momentary leg coordination pattern (compare Pfeffer et al., 2019). To analyse forward, backward and upside down walking behaviour, an **index number**, and a respective **index colour**, were assigned to each frame, corresponding to the momentary leg coordination. The index colour was used for illustration and the index number for statistical analysis. We defined the following categories: octopod (0, brown), septapod (1, dark purple), hexapod (2, lilac), pentapod (3, blue), tetrapod (4, light green), tetrapod* (4, dark green), tripod (5, yellow), bipod (6, light orange), monopod (7, dark orange) and aerial phase (8, red). These categories were defined by the absolute number of legs in swing or stance phase, not by concrete patterns of certain swing/ stance combinations. A distinction was made, however, between the characteristic tetrapod coordination (L1-R2-L3-R4 and R1-L2-R3-L4, light green) (Bowerman, 1975) and other tetrapod combinations (tetrapod*, dark green). For a more detailed description, see legend of Fig. 8 and Figs. S2, S3 (further gait pattern analysis with concrete inter-leg coordination patterns). To calculate gait pattern indices, we used a MATLAB environment (MathWorks, Inc., Natick, MA, USA).

Statistics

Box-and-whisker-plots, normality tests and statistical pairwise and multiple comparisons were generated in SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA). Box-and-whisker plots show the median as the box centre, the 25th and the 75th percentiles as box margins and the 10th and 90th percentiles as whiskers. To scrutinise normal data distributions, Shapiro-Wilk test was used and Levene's mean test was used to assess equal variance. The t-test was used for pairwise comparison of normally distributed data, the

Mann-Whitney Rank Sum test for not normally distributed data. For the multiple comparisons of normally distributed data, we used a One-Way ANOVA with Holm-Sidak's method as post hoc test, and for non-normally distributed data, we used an ANOVA on ranks with Dunn's method as post hoc test. Tests for correlation were performed in Excel. Significance levels are indicated by asterisks in the figures. The final editing of figures and tables was performed in Inkscape (Inkscape 1.0).

Results

In total, 65 pseudoscorpions with a body size range from 2.22 to 4.07 mm were used for high-speed recordings, twelve more animals were used for EthoVision recordings and ten individuals for body measurements (Table 1). Morphometric data provide an overview of body size (body length, prosoma length), leg and pedipalp lengths of the animals. The relationship between body and prosoma length was nearly isometric ($y=0.32x^{0.97}$, y and x representing prosoma and body length, respectively), although total body length might differ depending on the pseudoscorpions' condition (nutritional status, gravidity). Leg length increased from anterior to posterior (mean±s.d.: leg 1: 1.37±0.13 mm, leg 2: 1.47±0.20 mm, leg 3: 1.77±0.16 mm, leg 4: 2.10±0.19 mm) and pedipalps length was 3.88±0.72 mm.

General walking behaviour (EthoVision analysis)

Overall walking performance of *C. cancroides* was observed over time periods of one hour, using EthoVision software. Example trajectories of three individuals are shown in Figure 2A. Trajectories were characterized by straight but also curved shapes, with pseudoscorpions walking along the arena wall for a large part of the time. Surprisingly, *C. cancroides* was quite mobile, walking approximately 14 m h^{-1} (mean±s.d.: 13.59±0.35 m h^{-1}), which corresponds to about 4193 body lengths per hour (BL h^{-1}), with just few longer stops. Clearly shorter stationary episodes occurred much more frequently, illustrated by values for mobile and immobile states, their durations and the time courses of speed (Fig. 2B, Table S1). Immobile states lasted approximately 0.5 s (mean±s.d.: 0.48±1.92 s), with this value including both short and long stops, explaining the high standard deviation. Longer stops were spent with cleaning, resting or social interactions. Shorter stops (microstops), barely visible to the naked eye and without any noticeable body and COM movement, occurred regularly during general forward walking, although they were not obligatory. They were well documented in the high-speed recordings (Movie 1). We therefore analysed microstops in the high-speed recordings using EthoVision software in combination with a manual frame-by-frame analysis (Fig. 2C, Table S2). These data allowed accurate analysis of walking phases and microstops by analysis of 20-30 steps without turning behaviour or stops for cleaning

and social interactions. Microstops lasted between 100-200 ms (mean±s.d.: 0.16±0.04 s) and, surprisingly, all eight legs had ground contact only rarely during these stops. COM movement stopped a few milliseconds before a microstop commenced, a microstop being defined as stop without any body or leg movement. Mostly only five or six legs were in stance, and swing movements of the other legs were partly continued, but without forward COM movement. Accordingly, some legs rested in the air, and the remaining swing movement was accomplished during the next step cycle, when walking resumed at the end of the microstop. The number of microstops decreased with increasing speed (Fig. 2C). We further tested the hypothesis that pseudoscorpions tend to change their walking direction after microstops using a straightness index. We found that this is not the case. Pseudoscorpions were heading in almost the same direction after a microstop, which is demonstrated by high straightness index numbers (0.99±0.01 (n=20 tracks), min:0.95; max:1.0; 1.0 indicating straight lines).

Basic walking parameters

A characteristic walking pattern was observed during all types of locomotion, with legs placed in alternating tetrapods (Fig. 3A, Movie 2). With regard to maximum speeds, it is notable that *C. cancroides* reached considerably higher absolute and relative **walking speeds** during backward than during forward locomotion. Pseudoscorpions achieved speeds up to 30 mm s^{-1} (14 BL s^{-1}) while walking forwards, up to 40 mm s^{-1} (17 BL s^{-1}) during backward walking, and up to 10 mm s^{-1} (4 BL s^{-1}) while walking upside down (Fig. 3B).

With increasing speed, stride length and frequency increased, and swing and stance phase durations levelled off with power functions during all types of locomotion (Figs. 3, S1). **Swing phase duration** levelled off towards a value of 30 ms during forward, 28 ms during backward and 35 ms during upside down walks (Fig. 3C i). **Stance phase duration** was as short as 33 ms during forward walking and slightly shorter during backward locomotion (26 ms). Clearly higher values were observed while walking upside down with a minimum stance phase duration of 117 ms, in combination with clearly lower speeds (Fig. 3C ii). The small differences in minimum swing phase durations between upside down, forward, and backward locomotion compared to the significant differences in minimum stance phase durations were remarkable. **Relative stride length** was shortest during upside down walking and highest during backward walking, more than doubling the shortest stride lengths (Fig. 3C iii). **Stride frequency** increased up to 16 Hz during backward locomotion, compared to maximum frequencies of 14 and 7 Hz during forward and upside down walking, respectively (Fig. 3C iv).

Swing and stance phase durations differed clearly between individual leg pairs (Fig. 4). We plot only differences in swing and stance phase durations between leg pairs since differences in stride lengths and frequencies were minor. Shortest swing and longest stance phase durations were observed for leg pair 2 (LP2), followed by LP1, LP3 and LP4 during forward walking (Fig. 4A). While walking backwards, shortest swing phase durations were performed by LP2 as well, but followed by LP3, LP4 and LP1, combined with longest stance phase durations for LP2, followed by LP3, LP4 and LP1 (Fig. 4B). Same sequences in swing and stance durations were observed during upside down walking (Fig. 4C). Thus, the second leg of one tetrapod (L2 or R2) is always the first leg to touch down and the last to lift off the ground, leading to the assumption that LP2 might play an important role for stability during all types of locomotion. With increasing speed, duty factor decreased for all leg pairs. Only during backward walking, all leg pairs fell below duty factor 0.5 and therefore backward walking at highest speeds might be considered as a run (Fig. 5).

Footfall positions

Footfall patterns of the legs in their posterior extreme positions (PEP, shortly before lift-off at end of stance phase) and their anterior extreme positions (AEP; shortly after touch-down at beginning of stance) were analysed during forward, backward, and upside down walking (Fig. 6). During forward and upside down locomotion, AEP is the most anterior footfall position of the legs after swing phase and PEP is the most posterior position at the end of stance. The terms 'anterior' and 'posterior' refer to the pseudoscorpions body orientation and were also used for backward walking, where anterior and posterior positions are reversed: AEP is at the end of stance and PEP at the end of swing phase. Pseudoscorpions consistently showed AEP footfall positions in all eight legs closer to the body during backward than during forward locomotion (Fig. 6). During upside down walking both AEP and PEP footfall positions were significantly wider (ANOVA on Ranks, *P<0.05), presumably to increase static stability and ensure surface contact. A notably higher variance was observed in the PEP of LP4 during backward locomotion, while variance was quite similar for all other scenarios and footprints.

Phase analysis

Phase plots were created to analyse inter-leg coordination and its variability. Phase plots show the onset of swing phase during one step cycle in a circular illustration, with the left hind leg (L4) as reference. For all pseudoscorpions an antiphase relationship between the tetrapod groups L1, R2, L3, R4 and R1, L2, R3, L4 was observed during forward and backward locomotion (Figs. 3A, 7). Mean phase vectors of upside down plots were clearly shorter compared to forward and backward plots (Fig. 7A-C), indicating a high variance of

swing onsets (compare high variability of data points in Fig. 7A, green dots widely distributed across circumference) and more irregular leg coordination. A stronger coupling of the fourth and third legs and the second and first legs of one tetrapod group was notable during forward locomotion, with highest phase shifts at low speeds. With increasing speed, consistent leg coordination sequences L4-R3-L2-R1 and R4-L3-R2-L1 were established. Phase shifts ranged around 10% at medium speeds (Fig. 7B) and up to 14% at high speeds (Fig. 7C) between the fourth and first leg of one tetrapod (shifts at medium speeds: leg 4-3 of one tetrapod: 1%, 3-2: 8% and 2-1: 0.5%). As mentioned above, phase plots for forward and backward locomotion at similar speeds are illustrated together in one plot even though motion directions were opposite. For better comparison, mean phase vectors are mirrored on the horizontal axis (dotted blue lines, Fig. 7B, C), which compensates for the different motion directions. Variance was lowest during medium forward and fast backward locomotion. The latter indicates a comparatively strict control of leg movement and could be a reason for the observed high backward speeds. During backward walking, an inter-leg coordination sequence R1-L2-R3-L4 and L1-R2-L3-R4 was established, inverting the forward walking coordination. Phase shifts ranged up to 30% between first and fourth leg of one tetrapod (shifts at high speeds: leg 4-3 of one tetrapod: 8%, 3-2: 4% and 2-1: 15%), with a tendency towards coupling of L2 and R3 (R2 and L3) (Fig. 7C) (as higher speeds narrow spatio-temporal boundary conditions).

Phase shifts are also reflected in **tetrapod coordination strength** (**TCS[4]**). TCS[4]-values of *C. cancroides* were mostly between 0 and 0.6 during forward and backward locomotion, which is consistent with the phase shifts between the individual legs. Highest values were reached during forward walking at medium speeds (Fig. 7D). TCS[4]-values were noticeably lower during upside down walking, with negative TCS[4]-values (compare phase plots) indicating irregular leg coordination.

Inter-leg coordination patterns
Leg coordination patterns were analysed by assigning an index number and colour to each video frame, according to the momentary leg coordination (number and colour code, see legend Fig. 8). Podograms of forward, backward and upside down walks were created, illustrating the alternation between swing and stance phases of the individual legs over time as black (swing) and white (stance) bars along with the corresponding colour code (Fig. 8A-C, qualitative analysis). The proportion of every pattern was determined by indexing and evaluation of index frequencies in adjacent speed and locomotion type bins (quantitative analysis, Fig. 8D, E). Note that coordination patterns were defined by the number of legs in swing or stance and not by particular patterns of swing-stance combinations. Tetrapod

coordinations were however divided into typical tetrapod gait coordination (L1-R2-L3-R4 or R1-L2-R3-L4, light green) (Bowerman, 1975) and other tetrapod combinations (tetrapod*, dark green). In addition to the analysis without consideration of particular leg combinations, we performed an analysis with concrete coordination patterns to obtain an overview of the proportions of specific patterns (Figs. S2, S3).

An alternating tetrapod gait was observed during forward and backward locomotion, and partly during upside down walking (Fig. 8A-C). For qualitative analysis, podograms of one walking sequence each are shown, with a higher variability of coordination patterns at the beginning of a walking bout, that is, in the initial phase of locomotion after pausing. During forward and backward walks, a tetrapod pattern was established, with clearly more tripod and bipod situations in the initial phase of backward walking. Tetrapod coordination dominated during forward walking in all speed classes. With increasing speed, a decrease of pentapod and hexapod situations and an increase of patterns with more than four legs in swing phase occurred (tripod: 6%, 17% and 29%; bipod: 0%, 1% and 5% at 0-5, 5-10 and 10-15 BL s^{-1}, respectively) (Fig. 8D, compare index in Fig. 8E). While walking backwards, tetrapod coordination was partially replaced by tripod (34%) and bipod (9%) situations, leading to index values between 4 and 5 (Fig. 8E). In comparison, upside down locomotion was characterised by a pattern with no more than four legs in swing at any time and a high fraction assigned to octopod coordination (>30%), periods with all eight legs on the ground. This was never observed to such an extent during forward or backward walking. A tetrapod pattern is only poorly recognisable (blue and red frames, Fig. 8C), with considerable deviations and a high fraction of coordinations with less than four legs in swing (>90%, compare number index, values between 1 and 2), probably preventing falls during upside down walking.

In summary, pseudoscorpions' locomotion is characterised by a stable alternating tetrapod gait, mainly in combination with patterns with one or two legs more or less in stance while walking forwards and backwards. Phase analysis revealed a coupling of two legs of each tetrapod at a time, mainly during forward and partly during backward locomotion. By comparison, small steps in combination with wider footprints, irregular leg coordination and usually more than four legs in stance enabled upside down walking.

Discussion

Eight-legged animals show a wide range of locomotor strategies, such as level but also curved forward, backward, or upside down walking, running, and crawling in a wide variety of three-dimensional structured environments (Spagna and Peattie, 2012). Basic patterns been described so far (e.g., Bowerman, 1975; Cruse, 1990; Müller and Cruse, 1991; Cruse and Saavedra, 1996; Spagna et al., 2011; Spagna and Peattie, 2012), but little is known compared to hexapod locomotion. In this context, we investigated locomotor behaviour of the cosmopolitan pseudoscorpion *Chelifer cancroides* (Fig. 1). Although pseudoscorpion biology has been studied for decades (Kästner,1927; Strebel, 1937; Levi, 1948; Weygoldt; 1969), their locomotion has not been addressed in detail. They can walk on smooth surfaces, forwards as well as backwards and also upside down (Strebel, 1937).

Walking with microstops

Throughout all observations, *C. cancroides* performed short stationary episodes (microstops) during forward walking, without any visible body movement during these stops (e.g., for cleaning or searching). This behaviour was particularly conspicuous since the animals walked a few steps at rather high speeds, in relation to their body size and associated leg length, and then stopped abruptly for 100-200 milliseconds (Fig. 2C). COM movement stopped a few milliseconds before these microstops (defined as stops without any body-movement, including leg movements), mostly with only five or six legs in stance. Swing movements of the remaining legs were partly continued, but without forward movement of the COM ('freezing of forward movement'). These microstops, barely visible to the naked eye, could play an important role in orientation and navigation performance of pseudoscorpions, as the animals might use these stops to perceive their chemosensory environment. *C. cancroides* is equipped with a pair of lateral eyes with only few photoreceptors. These eyes are suggested to serve as light detectors but not to generate sharp images (Demoll, 1917; Weygoldt, 1969; Lehmann and Melzer, 2018). Thus, *C. cancroides* most likely uses its pedipalps equipped with numerous sensory sensilla to orient, locate prey, and interact with conspecifics (e.g., Weygoldt, 1969, Harvey, 2014; Cowles, 2018). Along these lines, a distinct chemosensory and mechanosensory pathway associated with the pedipalps has been described (Stemme and Pfeffer, 2022). Accordingly, on the one hand, microstops could serve orientation and readjustment of sensory systems. Moving too fast over longer distances without microstops may compromise effective orientation, namely, the detection of chemo- or mechanosensory stimuli, and thus assessment of current habitats as well as food sources. In this regard, we rejected the hypothesis that stationary episodes occurred in combination with a change in walking direction, as *C. cancroides* maintained its direction after microstops. On the other hand,

microstops might also be related to the pseudoscorpions' pedipalps: Stationary episodes could be important for mechanical stability as they may be useful to reduce possible oscillating movements of the large pedipalps. Surprisingly, we observed a possible correlation between speed and microstops as the number of stops decreased with speed, which in turn challenges the 'stability' hypothesis. To explicitly test these hypotheses, further experiments with higher sample sizes are necessary.

Interestingly, in the ant-mimicking jumping spider *Myrmarachne formicaria*, approximately 100 ms locomotion breaks were observed during walking, in combination with antennal illusion behaviour (forelegs are elevated mimicking ant antennae, Shamble et al., 2017). Those stops have been described as brief enough to challenge the visual systems of many predatory species in determining when these animals are stationary (Shamble et al., 2017). As microstops have a similar speed regime as ant mimicry in *M. formicaria*, one could assume that mimicry is a plausible cause, and microstops are a result of selective pressure imparted by different predator types (Shamble et al., 2017). Moreover, microstops may also be compared with crayfish behaviour eventually showing prolonged swing phases, which is assumed to correct inter-leg coordination (Cruse, 1990). However, such stationary episodes are poorly studied. They probably occur only rarely in animals, or in different behavioural contexts, and could obviously serve multiple purposes.

While running backwards, microstops were not observed in *C. cancroides*, which is certainly related to the fact that backward locomotion is a general escape behaviour (Land, 1972; Cowles, 2018). Stationary episodes appear inappropriate in escape situations and would reduce survival chances. Moreover, backward walking is a behaviour performed only over shorter distances, and further, pedipalps might oscillate less in the more angled position as assumed during backward locomotion. The high backward speeds and backward escape as part of general walking behaviour appear appropriate, as *C. cancroides* can thus turn towards the danger with its large pedipalps.

Speed-dependent locomotion performance

C. cancroides achieved speeds up to 4 body lengths per second during upside down walking, 14 BL s^{-1} during forward walking, and even higher speeds during backward locomotion, up to 40 mm s^{-1} or 17 BL s^{-1}. Although these maximum speeds are not the preferred walking speeds of *C. cancroides*, these speeds in combination with precise leg coordination might be decisive for survival (Meyer-Vernet and Rospars, 2016). Indeed, individuals of *C. cancroides* appeared quite active during the one-hour observation period, walking approximately 14 m h^{-1} (mainly forwards, with short backward walking episodes, e.g.,

after contact with other individuals; upside down walking was prevented by the experimental setup). In their natural environment, pseudoscorpions occur in various three-dimensionally structured terrestrial habitats (Weygoldt, 1969; Harvey, 1988), leading a cryptic and typically crepuscular life. Therefore, our data might represent walking behaviour caused by experimental circumstances like searching for a hiding place, rather than reflect general activity patterns of *C. cancroides.*

An alternating tetrapod gait pattern, with two sets of four legs moving in anti-phase to one another (Fig. 3A), was observed during all types of locomotion (to different extents) and was already described for spiders (Wilson, 1967; Biancardi et al., 2011), scorpions (Bowerman, 1975), mites (Wu et al., 2010; Rubin et al., 2016) and several other arachnid orders (Spagna and Peattie, 2012). Stride length and frequency increased with speed, with stride frequency as main determinant of speed changes (Fig. 3C iii, iv). This result agrees with previous studies in tarantulas (Anderson and Prestwich, 1985; Booster et al., 2015; Silva-Pereyra et al., 2019). It was further notable that swing phase duration levelled off with speed towards a value of 30 ms in each locomotion type, though at considerably different velocities. Pseudoscorpions thus performed fast swing movements already at medium speeds, probably to maximise ground contact time, as described previously for wood ants (Reinhardt and Blickhan, 2014). This was most obvious during upside down walking, with swing duration levelling off with a power function towards minimum values already at lowest speeds to maximise surface contact and avoid dropping off the ceiling (Fig. 3C i). The natural habitat of *C. cancroides* is indeed highly structured (e.g., leaf litter, bark), resulting in habitual risks in mechanical stability, which may explain the fast and brief swing movements. Swing duration is limited, though, to a minimum of about 30 ms due to contraction and relaxation kinetics of leg muscles and tissue viscosity (Rubin et al., 2016). Surprisingly, leg pair 2 (LP2) showed shortest swing and longest stance phase durations during all locomotion types (compare duty factor, Fig. 5), raising the idea that LP2 may be the most important leg pair for stability and speed control, and thus for locomotion in general. Pseudoscorpions' pedipalps exceed total body length (Fig. 1), which certainly has influences on locomotion behaviour and requires a stable locomotor strategy. A special role of the middle legs during hexapod locomotion was already described for *Cataglyphis* ants (Wahl et al., 2015), and might be comparable to the role of LP2 during locomotion in pseudoscorpions. Middle legs of the fast running desert ant *Cataglyphis fortis* performed shortest swing phases, longest stance phases, and were the last legs underscoring duty factor 0.5 (start of aerial phases), leading to the assumption that middle legs exert the biggest influence on speed and locomotion in general (Wahl et al., 2015).

Tetrapod walking with leg pair coupling

Previous studies on scorpions described a stereotyped metachronal tetrapod pattern during forward locomotion, with the front leg of one tetrapod moving about 10% out of phase with the corresponding rear leg (Bowerman, 1975). By comparison, we observed a clear coupling of two leg groups (4/3 and 2/1) within one tetrapod of *C. cancroides*. The fourth and third legs swing almost in phase (1% time shift), as do the second and first legs (0.5% time shift). Between these two coupled leg groups there is a larger phase shift of about 8% between the third and second legs' swing movements (Fig. 7B). Two loosely coupled groups of legs were also described for the spider *Cupiennius salei*, with higher stride frequencies of the hind legs' (LP3 and LP4) and a dissolved symmetrical stepping pattern (Weihmann, 2013). Furthermore, a stable 'diagonal' coupling was also observed in simulations of tetrapod gait insect walking (Schilling and Cruse, 2020). However, the function of variations in leg coordination among different animal groups and the importance of metachrony are not yet fully understood (Spagna and Peattie, 2012). Phase shifts of front legs (L1, R1 and L2, R2) in *C. cancroides* could indicate their major role in stabilising the animal and balancing pedipalp movements. While walking backwards, by comparison, highest phase shifts range up to 30% between first and fourth leg of one tetrapod. This may again allow higher speeds without losing static stability (Hildebrand, 1985). No comparable leg synchronization was observed during upside down walking, related to the fact that a tetrapod coordination with phase shifts comparable to forward and backward walking may lead to deficient surface contact.

Statically stable locomotion

The use of specific gaits has been suggested to be an important feature for efficient and stable locomotion in animals (Hoyt and Taylor, 1981; Hildebrand, 1989; Aoi et al., 2013). It is not always simple though to determine with certainty whether an animal is walking, running, or using an intermediate gait (Spagna et al., 2011). To date, there are very few data available regarding gaits in eight-legged animals, and the method of (octopedal) 'gait' indexing used in this study, and established in hexapod locomotor analysis (e.g., Mendes et al., 2013; Pfeffer et al., 2019), has not yet been used in this context. Previous studies described the alternating tetrapod gait, partly with variation at higher speeds and therefore defining either one or two gaits (Spagna and Peattie, 2012). In spiders, for example, a transition between a slow and a fast gait was observed, with increasing variation in tetrapod coordination at higher speeds (Spagna et al., 2011, Weihmann, 2013). Scorpions, by contrast, are known to use one continuous statically stable tetrapod gait (Bowerman, 1975; Telheiro et al., 2021). In hexapods, by comparison, different patterns merge into each other smoothly in a velocity-dependent continuum (Hughes, 1952; Schilling et al., 2013; Dürr et al., 2018), with tripod coordination as main walking pattern (Zollikofer, 1994).

In this context, it is important to realise that 'real gaits' are not simply defined by leg kinematics but also by changes in body dynamics (Nirody, 2021). The latter was not investigated here since only minimal changes in COM movement were observed and speed-dependent gait changes were not expected in *C. cancroides*. Thus, we consider inter-leg coordination patterns, not gaits as described for quadrupeds, for example.

Our results underscore the use of a tetrapod gait over the entire speed range in the pseudoscorpion during forward walking. The tetrapod is combined with coordination patterns where one or two more legs are in stance, or one or two fewer legs support the animal. These patterns merge into each other in a form of 'velocity-dependent continuum' reminiscent of hexapod locomotion (Dürr et al., 2018). In *C. cancroides*, alternating tetrapod coordination (with tetrapods R1-L2-R3-L4 and L1-R2-L3-R4) is the major coordination pattern in more than 40% of observations, supplemented by penta- and hexapod patterns at low speeds and tripod and bipod situations at higher speeds. This is mainly due to a temporal overlap or separation of the two tetrapod groups, respectively. The fraction of tetrapod* coordination - combinations with four legs in stance other than the classic tetrapod gait - was always about 15%, supporting the above interpretation since tetrapods* were mainly combinations of three legs of one tetrapod and one leg of the other tetrapod (see Figs. S2, S3). In essence, the tetrapod pattern exhibits continuous variation according to actual speed, depending on the degree of overlap of the two tetrapod groups.

When comparing *C. cancroides* with other eight-legged animals studied so far, the different ratios of body length to leg length are particularly striking. Spiders, such as *C. salei*, have extremely long legs with body-to-leg length values of around 0.65 (calculated from values in Weihmann et al., 2010; Weihmann, 2013) while this ratio is 2.1 for the second leg of *C. cancroides*, indicative of comparatively short legs. It is probably neither the most long-legged nor the most short-legged octopod walkers that can extend their leg coordination into the realm of aerial phases at high speeds. Fast running spiders may indeed exhibit aerial phases, though (Spagna et al., 2011), as do a number of hexapods such as desert ants with a body-to-leg length ratio of 1.3 (Tross et al., 2021). Other properties such as muscle endowment, body mass and body mass distribution will certainly have an impact here.

Intriguingly, our backward walking analysis revealed that tetrapod coordination is the major pattern in this situation, too. The distinctly higher backward speeds were achieved by a tetrapod pattern in combination with tripod and bipod situations. However, bipod combinations are the exception and high speeds are mainly achieved through precise leg coordination. Surprisingly, pseudoscorpions do not always have to stick to an alternating

tetrapod pattern: The tetrapod pattern is resolved during upside down walks and replaced mostly by combinations with more than four legs in stance in order to reduce the risk of falling off.

Furthermore, we analysed footfall positions (compare Seidl and Wehner, 2008; Mendes et al., 2013; Tross et al., 2021). Most intriguingly, our results indicated clear differences in footfall positions between the different locomotor types, with wider footprint positions, in both AEPs and PEPs, during upside down locomotion and with AEPs closer to the body during backward walking, probably due to angled pedipalp posture. Legs placed in sprawled positions around the body might provide the most stable posture and best surface contact (Alexander, 1971; Ting et al., 1994), while positions closer to the body might indicate less statically stable walking, but potentially faster speeds. Following the formula provided by Weihmann (2013), we specified relative body height by the quotient of body height and the distance to the anterior rim of the supporting leg polygon. Small relative body height values indicate higher static stability (Weihmann, 2013). This quotient was around 0.4 during forward walks, slightly higher when walking backwards, and slightly below 0.3 during upside down walking. Similarly small values were calculated for the stick insect *Carausius morosus* (0.32) and the spider *C. salei* (0.29) during forward locomotion while values in other insects were never below 0.49 (Graham and Cruse, 1981; Weihmann, 2013). In conclusion, upside down walking is mainly possible due to wide footprint positions and a slow modified tetrapod gait with more than four legs in stance. Claws and the arolium on the pseudoscorpions' pretarsi (Ax, 2000) allow stable surface contact during stance phases.

Backward and upside down walking
The present study demonstrated the highly controlled and intriguingly fast backward walking behaviour of *C. cancroides*, characterised by an inversion of the forward tetrapod pattern with a special role of LP2 comparable to that in forward locomotion. The general ability to walk backwards has been documented in many eight-legged animals but not characterised in detail in most cases. Backward walking was mainly described for different spiders, such as *Metaphidippus harford* (Land, 1972) or *Grammostola mollicoma (*Biancardi et al., 2011). Those spiders walk forwards and backwards at same speeds and are able to perform backward walking as easily as forward locomotion by reversing their tetrapod footfall scheme (Biancardi et al., 2011). In this context, a change from tetrapod to tripod walking by not using the first leg pair has been observed in jumping spiders (Land, 1972). By comparison, six-legged stick insects (e.g., *C. morosus*) walk backwards when trying to escape (Graham and Epstein, 1985), and in rare cases they walk backwards spontaneously (Jeck and Cruse, 2007). Their stepping pattern was described as irregular, but with ipsilateral legs still

swinging in the typical sequence from front to middle to hind leg. Other hexapods, such as *Drosophila*, use a loosely coordinated pattern with only little amount of tripod and tetrapod coordination (Bidaye et al., 2014; Feng et al., 2020). *Cataglyphis* desert ants show no rigidly fixed leg coupling during backward walking, doing so in combination with load carrying (Pfeffer et al., 2016).

We investigated upside down locomotion, which has rarely been studied to date. Several arthropods, such as locusts (Duch and Pflüger, 1995), ants (Federle and Endlein, 2004), or pseudoscorpions (this study), and other animal groups, too (e.g., geckos, Song et al., 2020) are able to walk upside down. This is possible mainly through adhesive structures on their feet, as an adaptation to complex three-dimensional environments. Besides the examination of adhesive structures, this mode of locomotion is of interest in robotics, as robots' maneuverability not only requires the capacity to control body and leg movement and respond to environmental conditions but also the ability to form stable contacts to a variety of substrates (Daltorio et al., 2006, Song et al., 2020). Our results revealed an upside down walking strategy with an inter-leg coordination clearly adapted to slow and stable locomotion allowing long adhesive surface contact. Accordingly, *C. cancroides* is able to resolve its typical tetrapod pattern and flexibly adapt the walking behavior to environmental conditions. Local sensory feedback circuits might be important to adjust the pseudoscorpions' movement to upside down walking, while the characteristic tetrapod gait might be controlled by central pattern generators to some extent (Ritzmann and Büschges, 2007; Ritzmann and Zill, 2017). In general, one should also consider that there may be no 'gaits' represented by a corresponding neural structure but rather a decentralized 'free-gait' controller whose basic task is to deal with irregular substrates (Schilling and Cruse, 2020).

Conclusion

In summary, *C. cancroides* performed a statically stable alternating tetrapod gait during both forward and backward walking. This appears reasonable in view of the highly structured three-dimensional habitat of pseudoscorpions. A dynamic locomotion strategy with high speeds might easily lead to stumbling and falling in a complex environment. In this context, characteristic inter-leg coordinations and phase relations, wide footprint positions and a special role of LP2 might play a major role in locomotion of *C. cancroides* with its large pedipalps. Intriguingly, highest speeds were observed during backward walking, which is an escape behavior, and rendered possible mainly by accurate leg coordination and strong phase shifts between leg movements. By comparison, no rigidly fixed leg coupling in combination with low speeds characterised upside down walking in order to maintain surface contact and avoid falling. Altogether, we verified the hypothesis that the forward tetrapod

pattern is adapted to different extents during backward and upside down walking. In addition, our study confirmed that forward movement of *C. cancroides* was regularly interrupted by short microstops. Further analysis may help to determine their functional role and investigate the possible relationship between speed and microstops.

Data availability

N/A

Author contributions

Conceptualisation by S.E.P. and T.S.; experiments were planned and carried out by S.E.P. and J.T.; data were analysed by J.T. and discussed and interpreted by J.T., S.E.P., T.S. and H.W.; the article was written by J.T., S.E.P., T.S. and H.W.

Symbols and abbreviations

BL (body length); COM (centre of mass); AEP (anterior extreme position); PEP (posterior extreme position); TCS[4] (tetrapod coordination strength); Description of legs: L(left), R(right), leg numbers: 1, 2, 3, 4; Description of leg pairs: LP (leg pair), leg pair numbers: 1, 2, 3, 4

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Figures and Table

Fig. 1: *Chelifer cancroides.* Pedipalps are angled and retracted. Measurements of total body length (magenta), prosoma length (yellow), pedipalp length (green) and leg length (blue) are shown. Measurements (Table 1) were taken in a dissected state. Scale bar: 1 mm
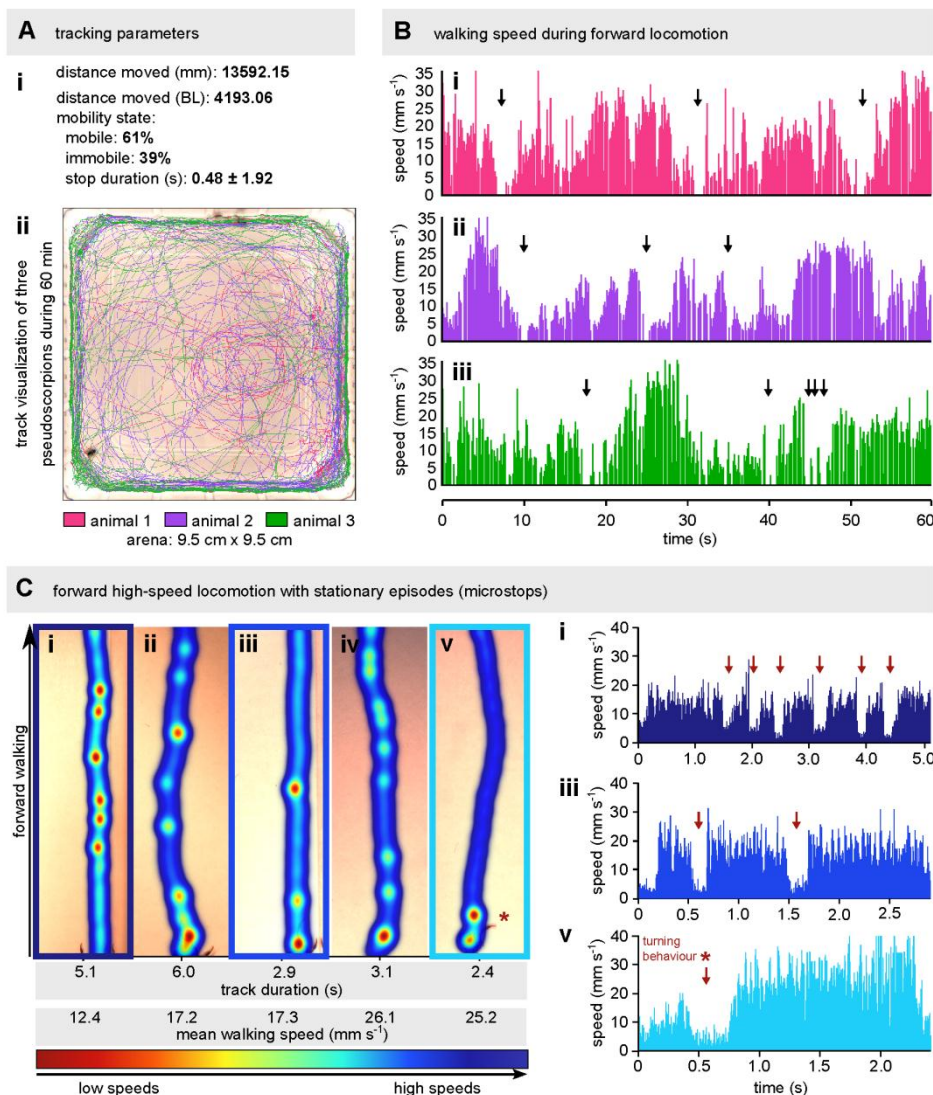
Fig. 2: General walking behaviour. (**A-B**) **One-hour video analysis.** (**A**) Tracking parameters; (**i**): distance moved in 60 minutes (mm and body lengths, BL), mobility state (mobile, immobile), and stop duration (mean ± s.d.) (n=12 individuals); (**ii**): track visualization of three *C. cancroides* individuals over a 60 minute time course. (**B**) Walking speed observed during randomly chosen time periods of 60 seconds in three individuals (**i-iii**), illustrating longer stops (e.g., for contact or turning, black arrows) and short stationary episodes (microstops) during forward locomotion. (**C**) **High-speed video analysis.** Heat maps (left) of tracks of five different individuals at different speeds (**i-v,** tendency of increasing average speeds and decreasing track durations from **i** (left) to **v** (right)). Walking phases are shown in shades of blue, microstops in shades of red and yellow. Walking speeds are plotted over track duration for **i**, **iii**, **v** (right). Microstops are marked with red arrows and turning behaviour of the pseudoscorpion with a red asterisk. For details of EthoVision analysis see Tables S1, S2.
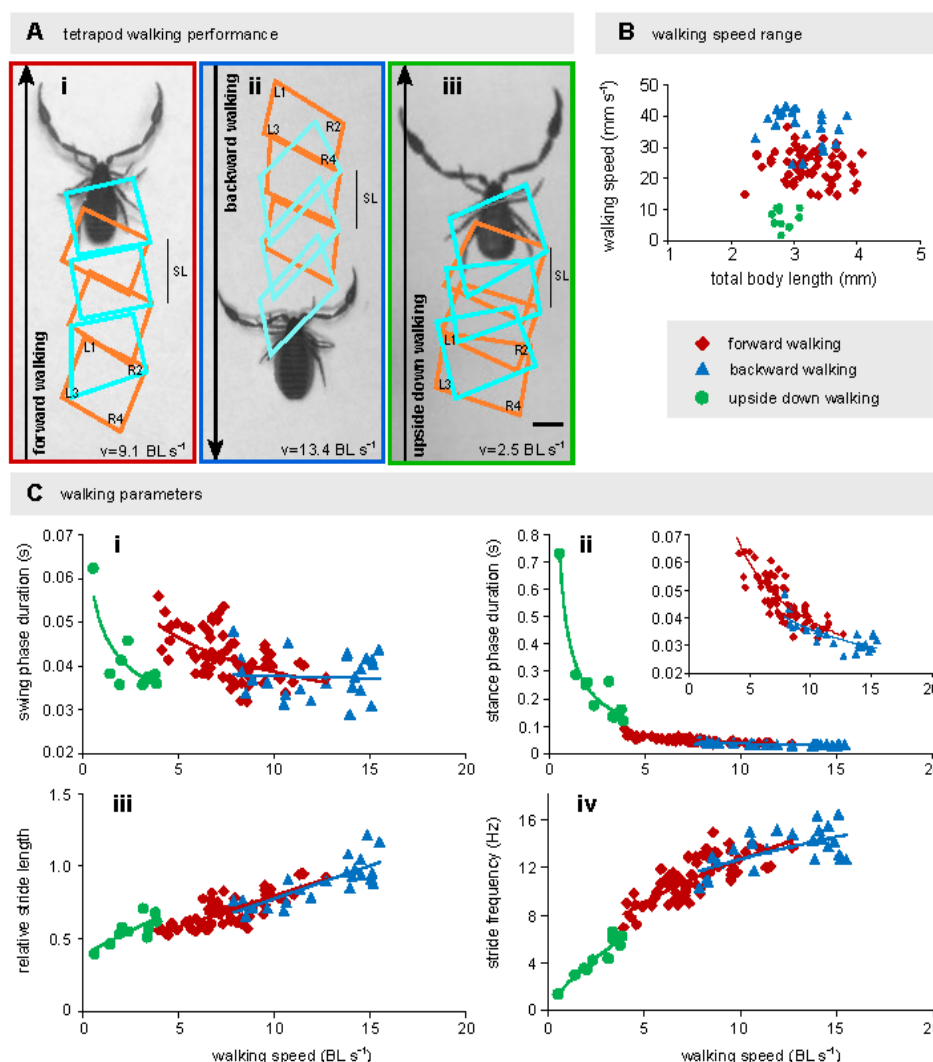
Fig. 3: Walking parameters. (**A**) Tetrapod gait. Frames from high-speed videos showing *C. cancroides* during (**i**) forward, (**ii**) backward and (**iii**) upside down locomotion. For each leg, three consecutive step cycles are illustrated. Tetrapods of L1, R2, L3 and R4 are indicated by orange squares, contralateral tetrapods (R1, L2, R3, L4) by blue squares. Stride length (SL) is indicated for R2. Scale bar: 1 mm. (**B**) Walking speed in relation to body length (forward: n=30 individuals, n=63 runs, red diamonds; backward: n=15 individuals, n=24 runs, blue triangles; upside down: n=9 individuals, n=10 runs, green circles). (**C**) Characteristic walking parameters of *C. cancroides* during forward, backward and upside down walking plotted as functions of relative speed (BL s^{-1}). Each data point represents the mean value of the four leg pairs in one video. (**i**) Swing phase duration (power functions: forward: y=0.07x$^{-0.27}$, R²=0.31, backward: y=0.04x$^{-0.04}$, R²=0.01, upside down: y=0.05x$^{-0.23}$, R²=0.67). (**ii**) Stance phase duration (power functions: forward: y=0.16x$^{-0.62}$, R²=0.71, backward: y=0.11x$^{-0.49}$, R²=0.60, upside down: y=0.42x$^{-0.83}$, R²=0.87); inset, additional detailed view. (**iii**) Relative stride length (linear regressions: forward: y=0.04x+1.91, R²=0.12, backward: y=0.05x+2.08, R²=0.23, upside down: y=0.17x+1.14, R²=0.71). (**iv**) Stride frequency (power functions: forward: y=4.14x$^{0.49}$, R²=0.61, backward: y=5.81x$^{0.34}$, R²=0.41, upside down: y=2.21x$^{0.75}$, R² = 0.96).
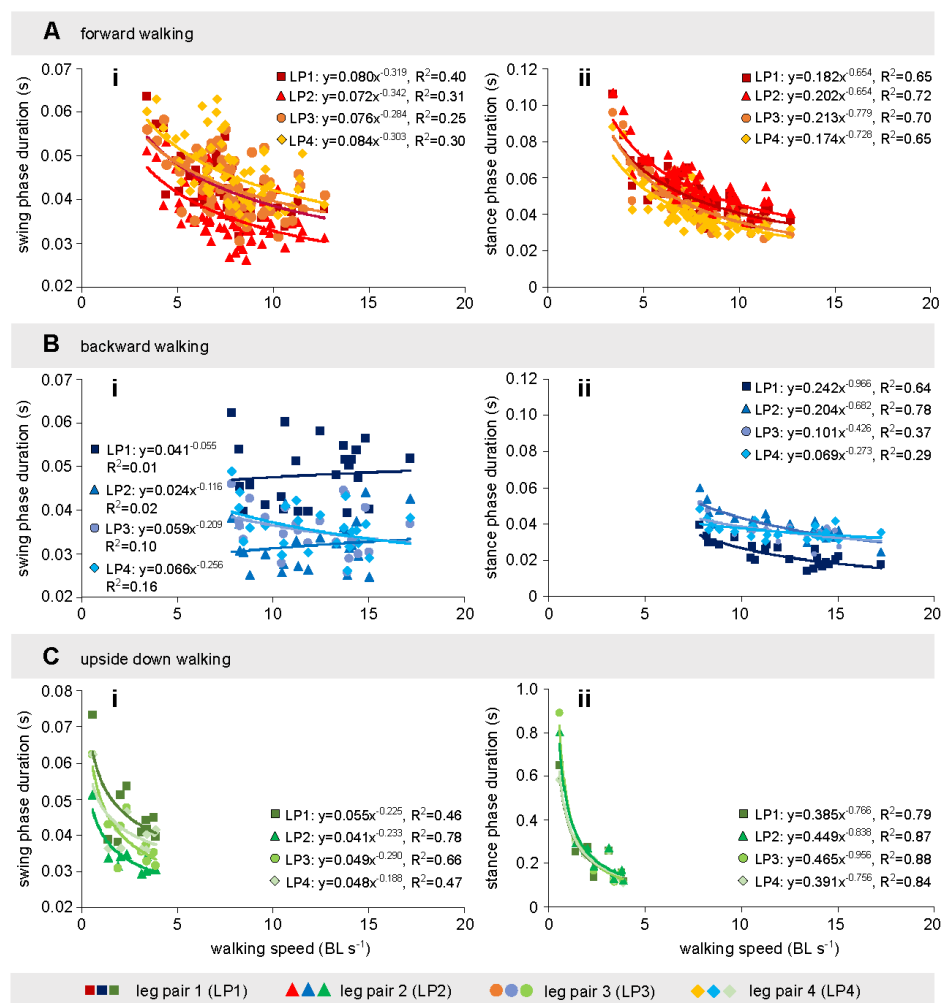
Fig. 4: Walking parameters (swing and stance phase durations) – single leg pair analysis. Single leg pair analysis of swing (**i**) and stance phase durations (**ii**) during (**A**) forward, (**B**) backward and (**C**) upside down walking (forward walking: n=30 individuals, n=63 runs, shades of red; backward walking: n=15 individuals, n=24 runs, shades of blue; upside down walking: n=9 individuals, n=10 runs, shades of green) plotted as functions of relative speed (BL s^{-1}).
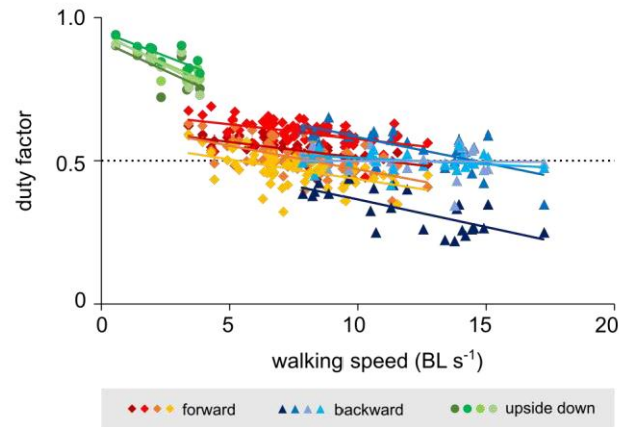
Fig. 5: Duty factor. Duty factor plotted as function of relative speed for the four leg pairs of *C. cancroides* during forward (shades of red, LP1-LP4: dark to light), backward (shades of blue) and upside down walking (shades of green). Duty factor decreased with speed for all leg pairs and during all types of locomotion. Only during backward locomotion, all leg pairs fell below duty factor 0.5.
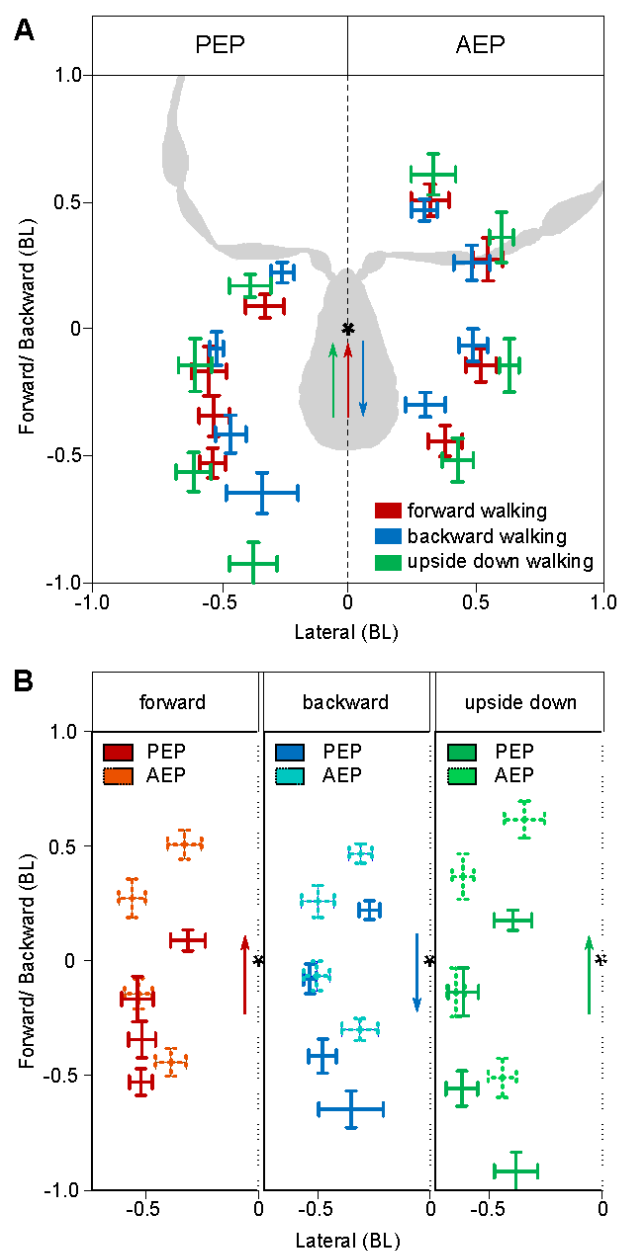
Fig. 6: Footprint geometry. Average relative footfall positions of the eight legs of *C. cancroides* with respect to the centre of mass (COM, black asterisk) during forward (red, n=6 individuals), backward (blue, n=5 individuals) and upside down (green, n=7 individuals) locomotion. For each footfall position, eight videos with three step cycles each were evaluated (n=24 steps). **(A)** Posterior extreme positions (PEP) are shown left, anterior extreme positions (AEP) are shown right. **(B)** PEP and AEP both shown for the left body side (PEP solid lines, AEP dotted lines). X- and y-axes are normalized to body length (BL).
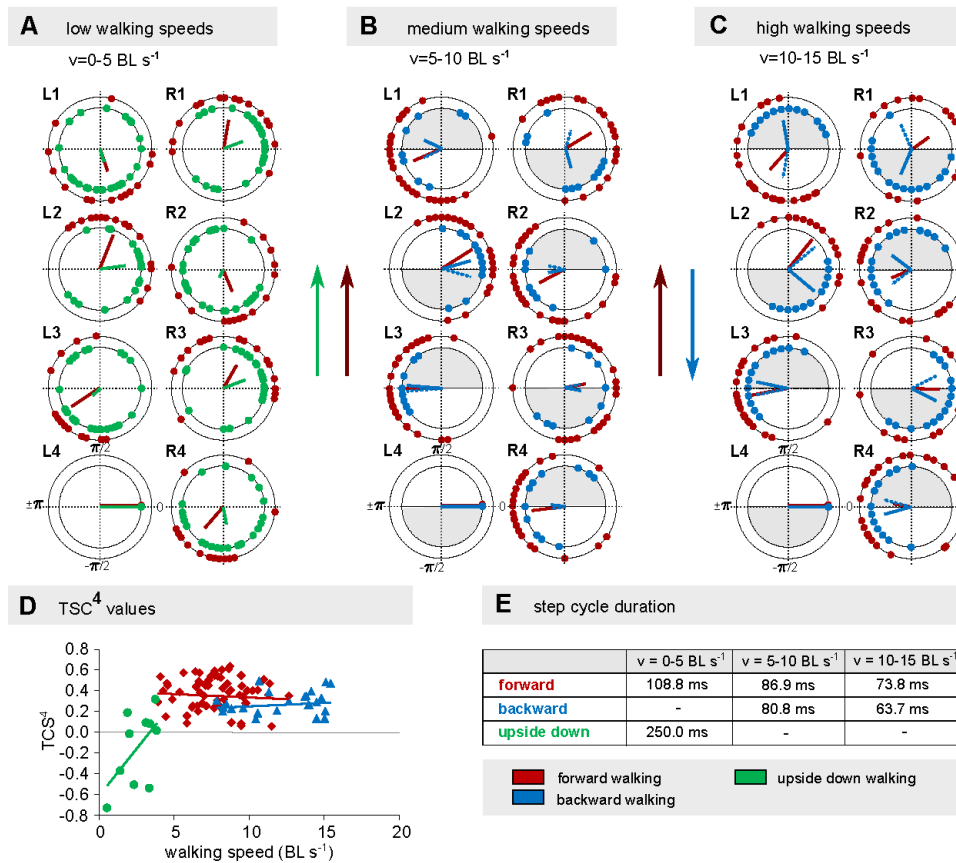
Fig. 7: Phase analysis. (**A-C**) Circular step cycle diagrams of *C. cancroides*, left hind leg (L4) serving as reference. Forward (red), backward (blue) and upside down (green) walking at different speeds: (**A**) low speeds: 0-5 BL s^{-1}, (**B**) medium speeds: 5-10 BL s^{-1}, (**C**) high speeds: 10-15 BL s^{-1}. Each data point represents the onset of swing phase with respect to L4. The mean phase vector is normalized to inner circle diameter, indicating variance of data points. For better comparison of relative phase shifts, mean phase vectors of backward walks are additionally plotted as horizontal mirror images (dotted blue lines in **B, C**). (**D**) Tetrapod coordination strength (TCS^4), plotted as function of relative speed (linear regressions: forward: y=-0.002x+0.40, R²=0.001, backward: y=0.009x+0.17, R²=0.05, upside down: y=0.18x-0.61, R²=0.32). (**E**) Legend for phase plots of forward, backward and upside down locomotion with respective circumferences (ms), representing step cycle durations.
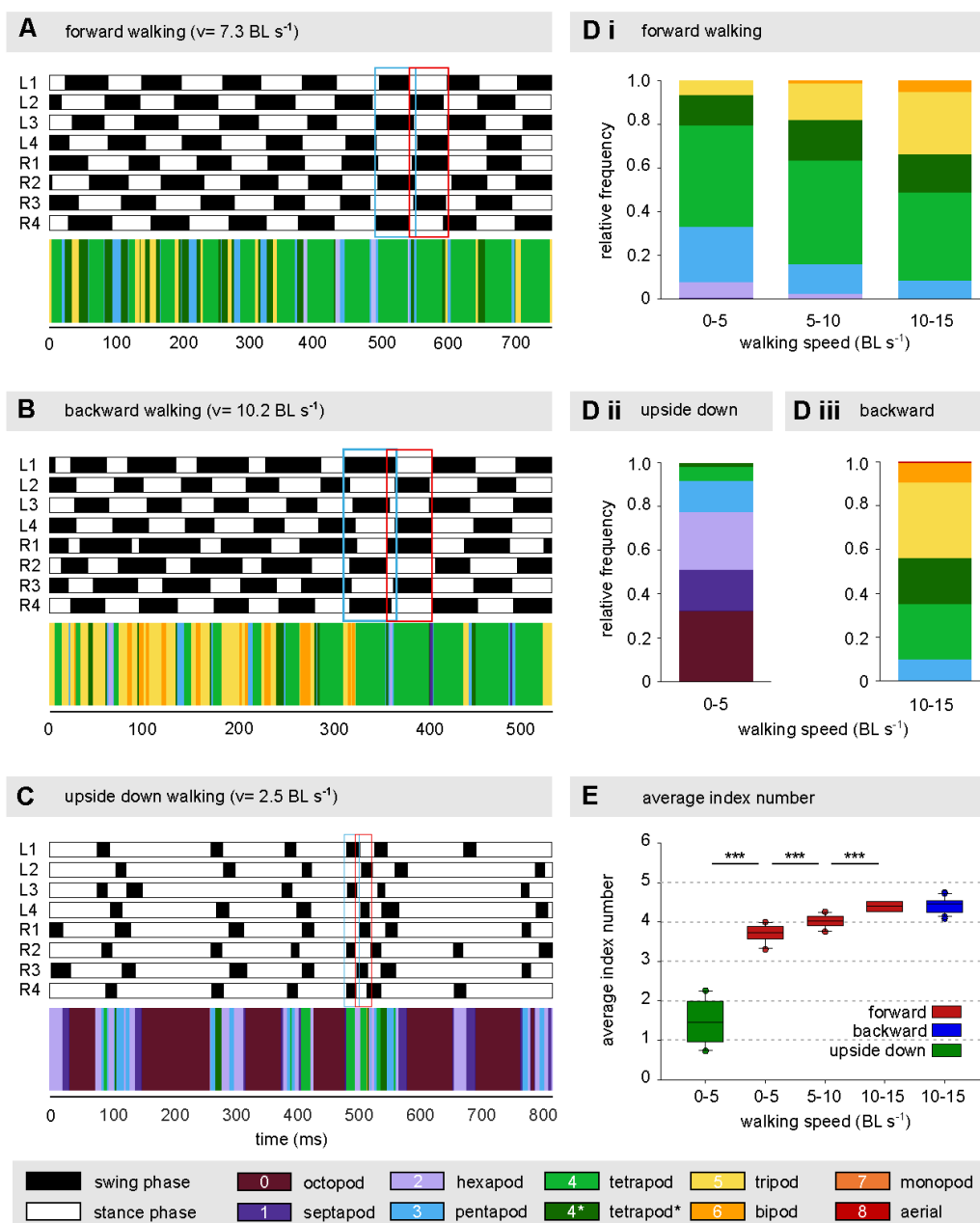
Fig. 8: Inter-leg coordination patterns. (**A-C**) Podograms recorded in *C. cancroides* during (**A**) forward, (**B**) backward and (**C**) upside down walking with corresponding colour indices plotted along the same time axes (qualitative). Podograms illustrate the patterns of the eight legs, black bars indicating swing and white bars, stance phases (L: left leg, R: right leg; 1: first, 2: second, 3: third and 4: fourth leg). Sample swing phases of two consecutive tetrapods are marked with blue and red boxes. Each video frame was assigned an index number and a corresponding colour according to the leg coordination in that frame (number and colour key at bottom of figure). (**D-E**) Quantitative analysis of leg coordination patterns according to speed classes for (**i**) forward (n=63 runs), (**ii**) upside down (n=10 runs), and (**iii**) backward locomotion (n=24 runs) (detailed analysis: Figs. S2, S3). Data are shown as relative frequency plots (**D**, colour index) and averaged index numbers (**E**, number index). Significant differences are marked with asterisks (***P<0.001).

Table 1: Body measurements. Calculated mean values for body length, prosoma length, leg length (L1, L2, L3, L4), pedipalps length and body weight of *C. cancroides* (n=10 individuals, n=3 males, n=7 females). Minimum and maximum values in brackets.

	mean ± s.d. (min; max)
total body length (mm)	3.09 ± 0.47 (2.13; 3.84)
prosoma length (mm)	0.97 ± 0.16 (0.71; 1.21)
leg length L1 (mm)	1.37 ± 0.13 (1.24; 1.64)
leg length L2 (mm)	1.47 ± 0.20 (1.19; 1.72)
leg length L3 (mm)	1.77 ± 0.16 (1.46; 1.96)
leg length L4 (mm)	2.10 ± 0.19 (1.75; 2.41)
pedipalps length (mm)	3.88 ± 0.72 (2.98; 5.45)
body weight (mg)	2.48 ± 0.51 (1.64; 3.45)

1. General walking behaviour – EthoVision analysis

Table S1. Detailed results of EthoVision analysis (compare Fig. 2A, B). The movement of *C. cancroides* pseudoscorpions was tracked and analysed for 60 minutes using EthoVisionXT Software. For each of n=10 individuals, values were calculated for minimum, maximum and mean velocities; total distance covered; mobility state (mobile and immobile; mean ± s.d. (s), frequency (observations h^{-1}) and cumulative duration (%) for each state). Mean values are provided for all tested animals in the bottom row of the table. Immobile states were defined as periods when the percentage change in object area between video frames was below a 1.5% threshold (immobility threshold <1.5%, mobile threshold ≥1.5%).

#	velocity minimum (mm s^{-1})	velocity maximum (mm s^{-1})	velocity mean (mm s^{-1})	total distance (mm)	mobility state: mobile			mobility state: immobile		
					mean ± s.d. (s)	frequency	duration cum. (%)	mean ± s.d. (s)	frequency	duration cum. (%)
1	0	44.99	4.83	17265	0.72 ±0.91	3350	67.45	0.35 ±0.92	3350	32.53
2	0	44.68	3.62	12916	0.60 ±0.56	3694	61.80	0.37 ±0.88	3692	38.18
3	0	44.91	4.49	16052	0.78 ±0.59	3130	68.67	0.36 ±0.82	3130	31.32
4	0	44.34	2.16	7569	0.72 ±0.76	1863	37.73	1.17 ±6.71	1864	61.40
5	0	44.25	4.60	16087	0.82 ±0.63	2673	61.92	0.50 ±3.90	2645	37.37
6	0	43.75	3.80	13156	0.75 ±0.63	2993	63.03	0.42 ±2.24	2976	35.43
7	0	44.43	5.45	18284	0.76 ±0.69	3299	70.94	0.27 ±0.46	3266	25.42
8	0	42.87	4.09	14269	0.71 ±0.49	3236	64.81	0.38 ±0.84	3237	34.81
9	0	43.91	2.56	8767	0.57 ±0.48	3399	53.22	0.45 ±1.23	3394	41.80
10	0	44.79	3.21	11552	0.69 ±0.53	3001	57.13	0.51 ±1.21	3011	42.68
mean	**0**	**44.59**	**3.88**	**13592.15**	**0.71 ±0.63**	**3064**	**60.67**	**0.48 ±1.92**	**3066**	**38.09**

Table S2. Detailed results of high-speed video tracking (compare Fig. 2 C). Movements of forward walking *C. cancroides* pseudoscorpions were tracked and analysed over time periods of 2-6 seconds (corresponding to 20-30 step cycles) using EthoVisionXT Software. For each of n=17 individuals, values were calculated for minimum, maximum and mean velocities; total distance covered; mobility state (mobile and immobile; mean ± s.d. (s), frequency (observations h^{-1}) and cumulative duration (%) for each state). Mean values are provided for all tested animals in the bottom row of the table. Immobile states (microstops) were defined here as periods where no body- and COM-movement was observed. Individuals corresponding to the examples shown in Fig. 2C (i-iv) are marked in bold.

#	velocity minimum (mm s^{-1})	velocity maximum (mm s^{-1})	velocity mean (mm s^{-1})	total distance (mm)	mobility state: mobile			mobility state: immobile		
					mean ± s.d. (s)	frequency	duration cum. (%)	mean ± s.d. (s)	frequency	duration cum. (%)
1	0	45.32	22.06	54.44	2.51 ±0.00	1	100.00	0.00 ±0.00	0	0.00
2	0	56.12	13.77	39.12	1.36 ±0.38	1	90.1	0.14 ±0.00	1	9.90
3	0	44.06	12.11	30.39	1.69 ±1.00	3	92.60	0.27 ±0.19	2	7.40
4	**0**	**28.10**	**12.35**	**62.74**	**0.61 ±0.39**	**7**	**86.37**	0.14 ±0.02	**6**	**13.63**
5	0	64.56	21.17	91.56	0.49 ±0.29	6	81.01	0.21 ±0.10	5	18.99
6	**0**	**34.28**	**17.21**	**92.95**	**0.88 ±0.64**	**7**	**70.46**	0.19 ±0.08	**7**	**29.54**
7	0	72.05	15.90	41.51	0.42 ±0.19	4	82.15	0.10 ±0.02	4	17.85
8	0	53.53	14.17	56.30	0.46 ±0.38	6	75.82	0.11 ±0.03	5	24.18
9	0	42.17	11.71	51.61	0.35 ±0.25	5	68.21	0.19 ±0.06	5	31.79
10	0	56.91	20.02	100.23	3.96 ±0.00	1	100.00	0.00 ±0.00	0	0.00
11	0	76.52	23.25	56.68	0.62 ±0.26	5	79.86	0.12 ±0.07	5	20.14
12	**0**	**52.06**	**17.28**	**47.70**	**0.57 ±0.41**	**3**	**80.73**	0.14 ±0.03	**3**	**19.27**
13	0	63.33	22.30	58.77	0.84 ±0.41	3	90.19	0.09 ±0.01	2	9.81
14	0	60.40	19.32	37.41	0.94 ±1.06	1	90.45	0.10 ±0.00	1	9.55
15	0	66.26	22.27	58.51	0.88 ±0.69	1	91.63	0.08 ±0.00	2	8.37
16	**0**	**56.24**	**26.05**	**101.59**	**0.26 ±0.16**	**8**	**70.96**	0.11 ±0.05	**8**	**29.04**
17	**0**	**52.82**	**25.23**	**57.42**	**1.58 ±0.00**	**1**	**86.83**	0.24 ±0.00	**1**	**13.17**
mean	**0**	**52.28**	**17.73**	**56.41**	**1.18 ±0.38**	**3.58**	**83.65**	**0.16 ±0.04**	**3.35**	**16.35**

2. Walking parameters according to absolute speed

- including walking performance on different surfaces (print paper and plastic sheet)
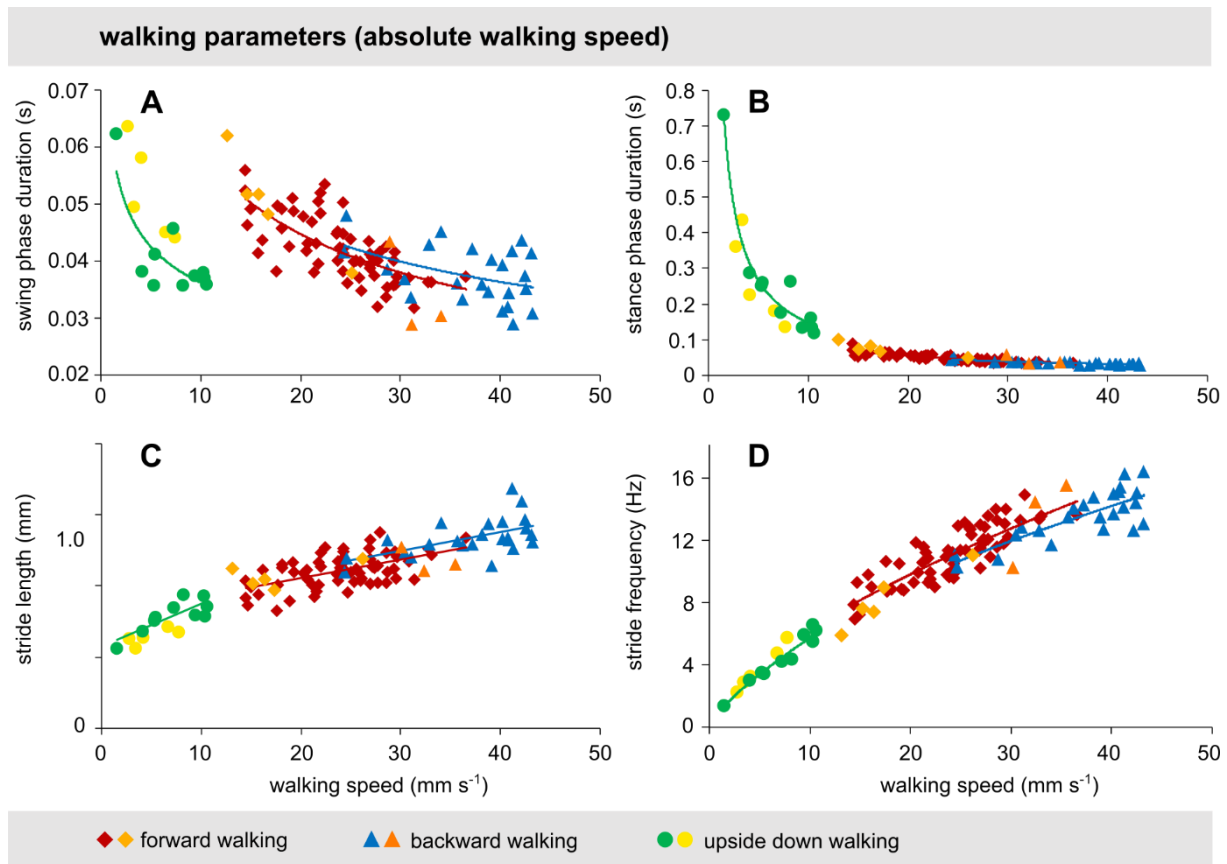

Fig. S1. Walking parameters. Characteristic walking parameters of *C. cancroides* during forward, backward and upside down walking plotted as functions of absolute speed (mm s^{-1}) (forward: n=30 individuals, n=63 runs, red diamonds; backward: n=15 individuals, n=24 runs, blue triangles; upside down: n=9 individuals, n=10 runs, green circles). Each data point represents the mean value of the four leg pairs of *C. cancroides* in one video. (**A**) swing phase duration (power functions: forward: y=0.15x$^{-0.40}$, R²=0.46, backward: y=0.12x$^{-0.33}$, R²=0.17, upside down: y=0.06x$^{-0.23}$, R²=0.65). (**B**) Stance phase duration (power functions: forward: y=0.54x$^{-0.78}$, R²=0.73, backward: y=0.44x$^{-0.72}$, R²=0.70, upside down: y=1.04x$^{-0.85}$, R²=0.90). (**C**) Stride length (linear regressions: forward: y=0.03x+1.59, R²=0.31, backward: y=0.03x+1.69, R²=0.33, upside down: y=0.06x+1.19, R²=0.65). (**D**) Stride frequency (power functions: forward: y=1.41x$^{0.65}$, R²=0.71, backward: y = 1.56x$^{0.60}$, R²=0.67, upside down: y=0.99x$^{0.76}$, R²=0.98). In order to scrutinise potential influences of the different walking substrates, (print paper and plastic sheet), high-speed recordings of forward (light orange diamonds) and backward (dark orange triangles) walks on a plastic sheet and of upside down walks (light yellow circles) on print paper were evaluated.

3. Analysis of inter-leg coordination with and without concrete patterns
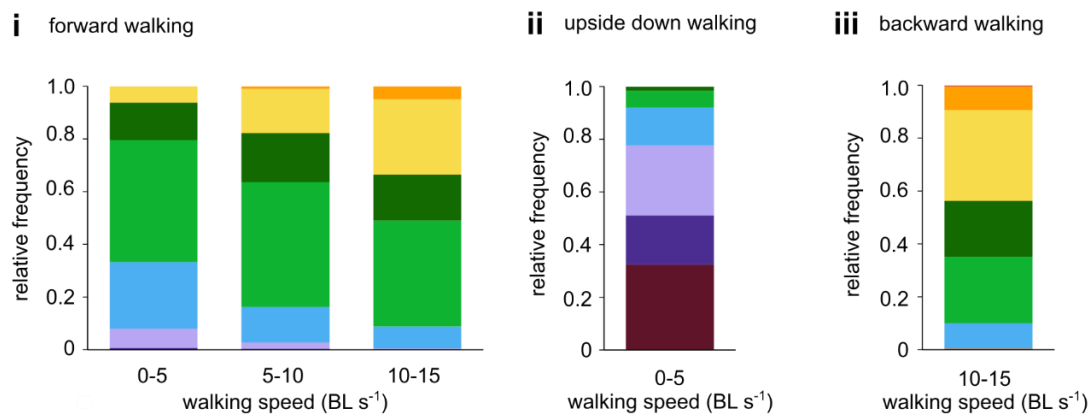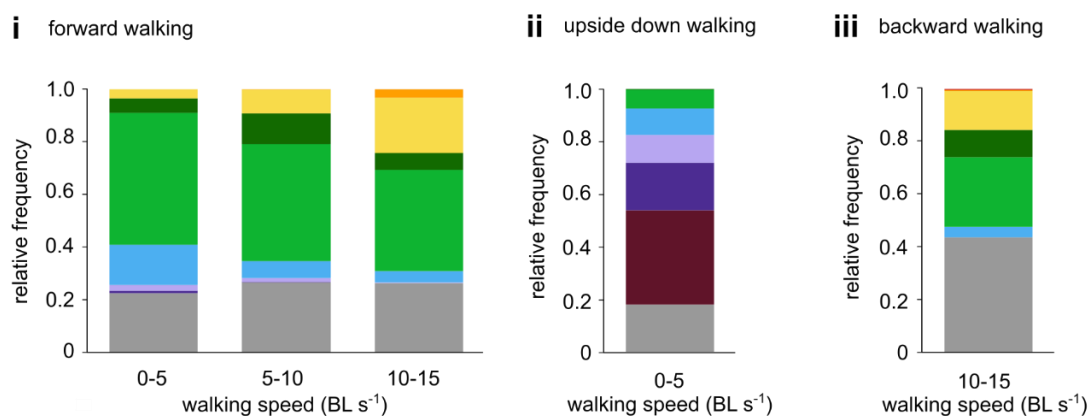

Fig. S2. Quantitative analysis of inter-leg coordination patterns with and without concrete patterns of swing and stance combinations. (**A**) Analysis without concrete patterns of forward (**i**), upside down (**ii**) and backward walking (**iii**) (detailed analysis in Fig. 8). (**B**) Analysis with concrete patterns of particular swing and stance leg combinations in forward (**i**), upside down (**ii**) and backward walking (**iii**) (see Fig. S3 for concrete inter-leg coordination patterns). Data are shown as relative frequency plots for the respective walking speed bins. Each video frame was assigned a colour index according to the leg coordination in every particular frame (see colour code key at bottom of the figure, and Fig. S3 for specific inter-leg coordination patterns). Legend in the bottom refers to both scenarios, e.g. pentapod means five legs in stance irrespective of certain leg combinations in (**A**), while in (**B**) only certain pentapod combinations of legs in stance were taken into account, see Fig. S3. Undefined patterns are mainly combinations with four (tetrapod*), three (tripod) or two (bipod) legs in stance phase, which occurred more frequently at medium and high speeds and in a multitude of combinations. Due to the large number of leg combinations in eight-legged animals, especially concerning hexapod, tetrapod*, tripod and bipod coordinations, we decided to define walking patterns by the absolute number of legs in swing or stance phase in this study (Fig. 8). Nevertheless, we also analysed leg-coordination of *C. cancroides* with concretely defined patterns (**B**). The conspicuously high proportion of undefined patterns during backward locomotion is explained by the direction of motion, as the concretely defined leg combinations are adapted to forward tetrapod walking (no anterior-posterior reversion of typical inter-leg coordination patterns, see Fig. S3).

septapod

forward slow	<0.1%	<0.1%	<0.1%	<0.1%
forward medium	<0.1%	<0.1%	<0.1%	<0.1%
forward high	0%	0%	0%	0%
backward	0%	0%	0%	0%
upside down	2.4%	1.3%	5.2%	9.2%

hexapod

forward slow	0.1%	0.6%	0.2%	<0.1%
forward medium	<0.1%	<0.1%	0.1%	<0.1%
forward high	<0.1%	<0.1%	0.2%	<0.1%
backward	0%	0%	0%	0%
upside down	2.7%	2.5%	4.0%	1.3%

pentapod

forward slow	4.0%	6.0%	3.5%	1.9%
forward medium	1.9%	3.0%	1.1%	3.7%
forward high	1.4%	0.7%	0.2%	<0.1%
backward	0.3%	1.1%	0.5%	1.9%
upside down	1.6%	3.9%	1.4%	3.3%

tetrapod

forward slow	50.2%
forward medium	44.4%
forward high	38.3%
backward	26.5%
upside down	7.1%

tetrapod*

forward slow	0.5%	<0.1%	2.6%	0.7%	0.7%
forward medium	<0.1%	0.3%	8.2%	0.5%	1.2%
forward high	<0.1%	<0.1%	4.0%	0.8%	1.0%
backward	0%	0%	2.9%	4.8%	1.4%
upside down	0%	0%	0.3%	0%	0%

tripod

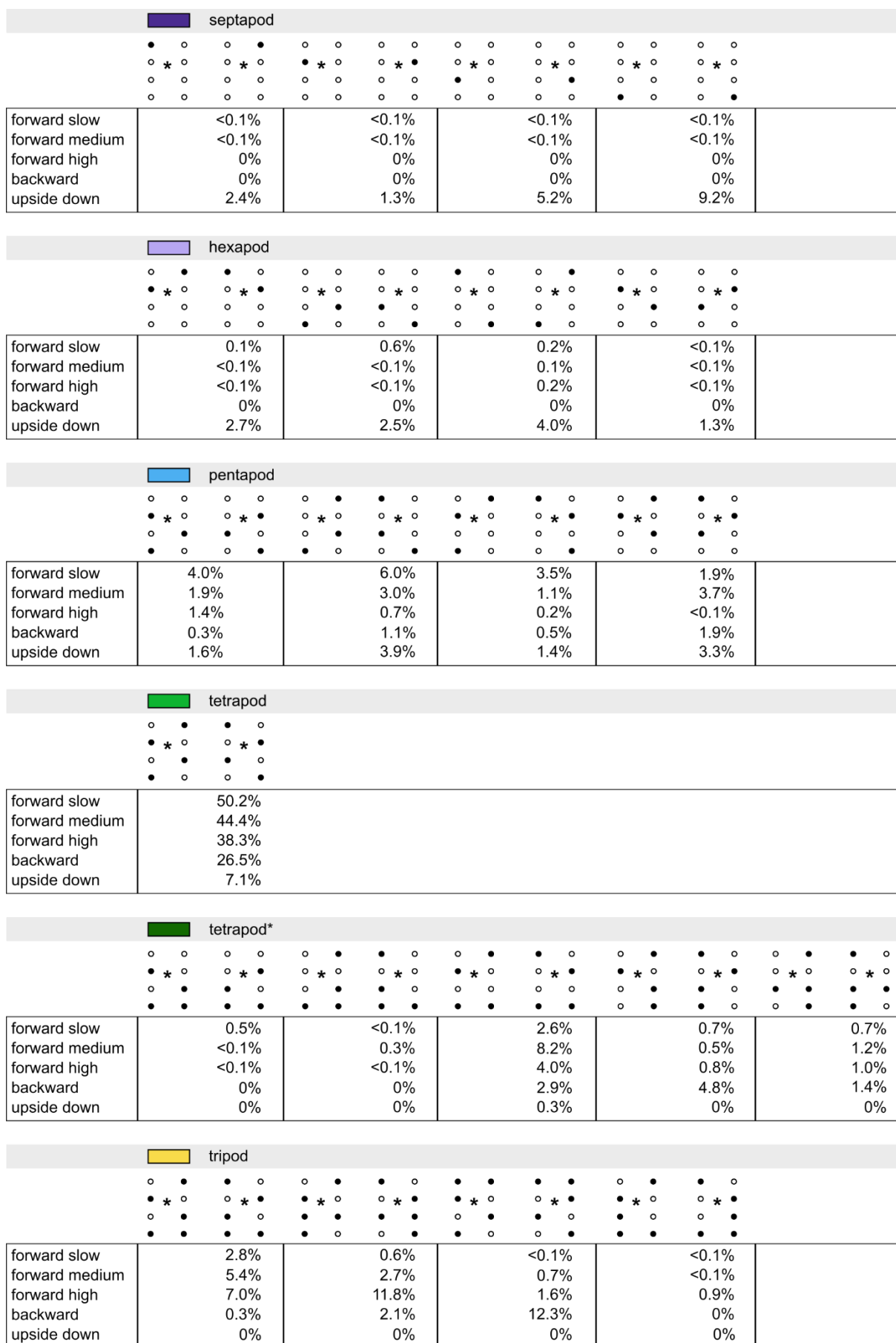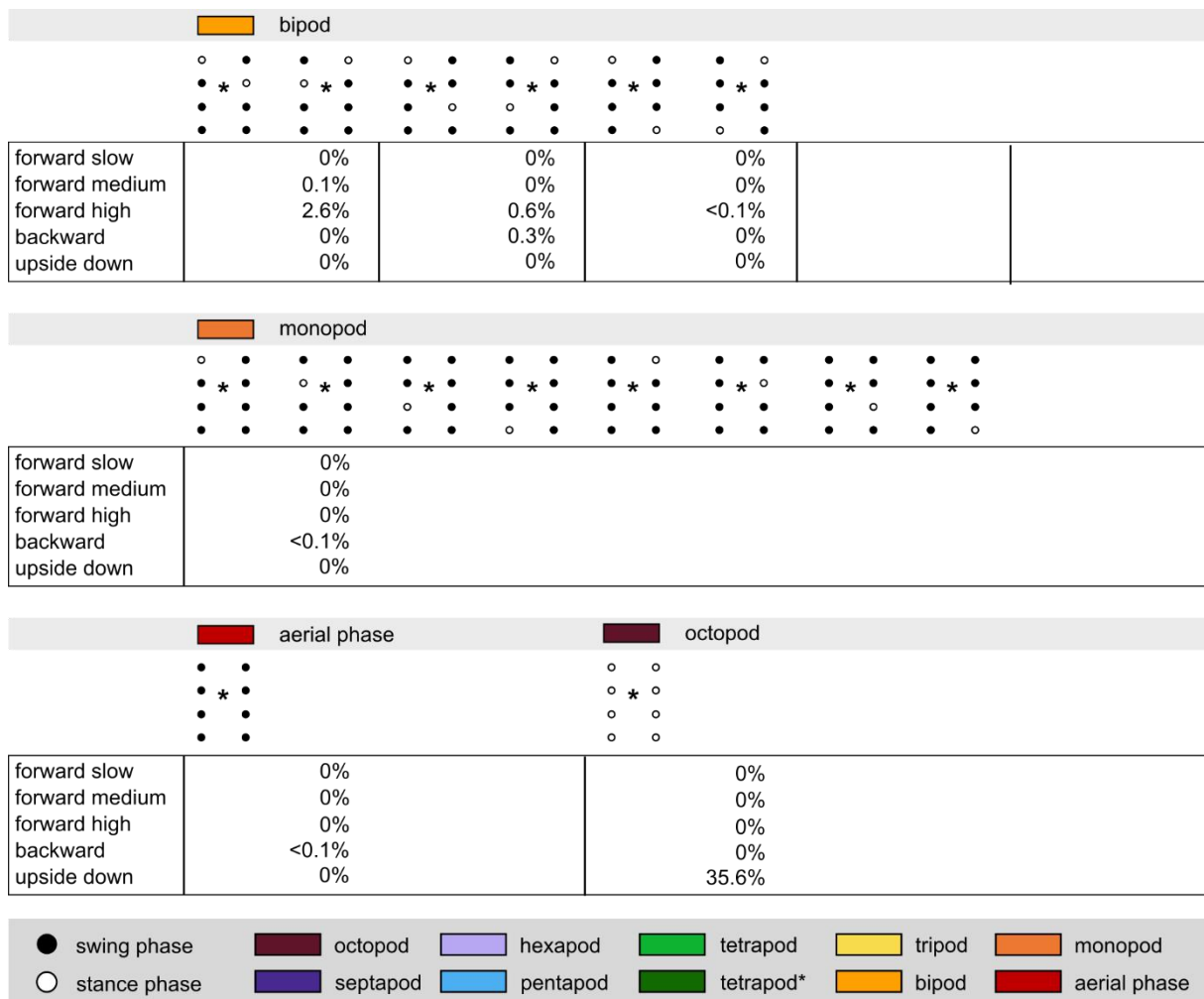|			
forward slow	2.8%	0.6%	<0.1%	<0.1%
forward medium	5.4%	2.7%	0.7%	<0.1%
forward high	7.0%	11.8%	1.6%	0.9%
backward	0.3%	2.1%	12.3%	0%
upside down	0%	0%	0%	0%

Fig. S3. Selected inter-leg coordination patterns with percentages of these combinations during forward, backward and upside down walking of *C. cancroides*. See next page for legend.

bipod				
forward slow	0%	0%	0%	
forward medium	0.1%	0%	0%	
forward high	2.6%	0.6%	<0.1%	
backward	0%	0.3%	0%	
upside down	0%	0%	0%	

monopod	
forward slow	0%
forward medium	0%
forward high	0%
backward	<0.1%
upside down	0%

aerial phase		octopod	
forward slow	0%	0%	
forward medium	0%	0%	
forward high	0%	0%	
backward	<0.1%	0%	
upside down	0%	35.6%	

| ● swing phase | | ■ octopod | ■ hexapod | ■ tetrapod | ■ tripod | ■ monopod |
| ○ stance phase | | ■ septapod | ■ pentapod | ■ tetrapod* | ■ bipod | ■ aerial phase |

Fig. S4. Selected inter-leg coordination patterns with percentages of these combinations during forward, backward and upside down walking of *C. cancroides*. Many more combinations of concrete leg coordinations exist in eight-legged animals. We thus selected the more commonly observed combinations for the current scrutiny that would also appear biomechanically useful. Every video frame was assigned a (momentary) inter-leg coordination pattern, according to a particular combination of the eight legs in swing and stance phase (swing phases: black circles, stance phases: white circles; body marked with an asterisk). If none of the selected combinations was applicable, the frame was assigned 'undefined' (grey, see Fig. S2). Note that walking patterns are defined by a certain combination of leg states and not just by the absolute number of legs in swing or stance. Percentages of the respective inter-leg combinations are given for the different speed bins during forward, backward and upside down walks (compare Figs. 8, S2). Mirrored combinations are pooled. The comparably high percentages of the third tetrapod* pattern (2.6, 8.2, 4.0%) and the first (2.8, 5.4, 7.0%) and second tripod patterns (0.6, 2.7, 11.8%) were notable during forward locomotion, as well as the comparably low tetrapod fraction during backward (26.5%) and upside down (7.1%) walking. The high percentage of the third tripod pattern during backward locomotion (12.3%) coincides with the general inversion of the tetrapod scheme, as this pattern is the horizontal mirror image of the first tripod pattern. Note that inter-leg coordination patterns are mainly defined for forward locomotion, explaining the high undefined fraction of around 40% during backward locomotion. Intriguingly, octopod coordination (stance) could mainly be observed during upside down locomotion.
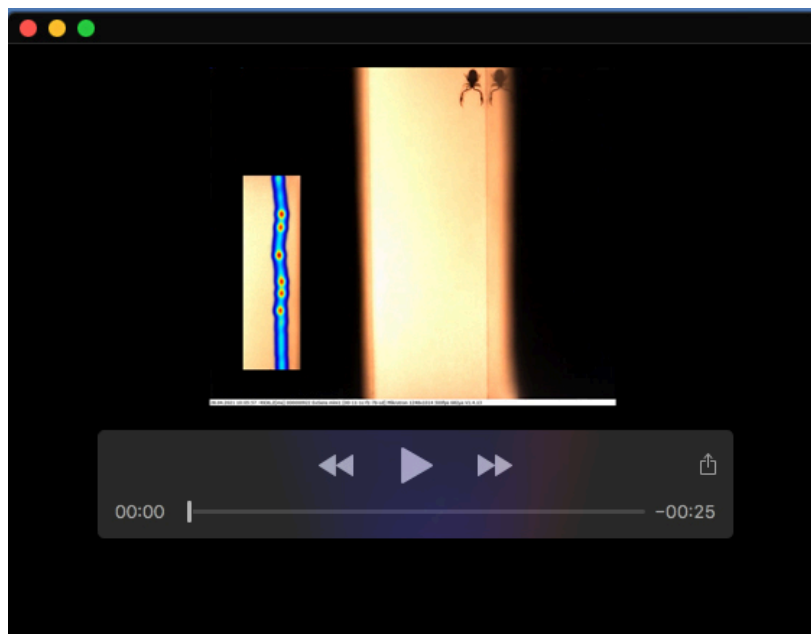
Movie 1. *C. cancroides* **forward locomotion with microstops.** Sample high-speed recording illustrating brief stationary episodes (microstops) during forward locomotion at an intermediate walking speed of 12.4 mm s^{-1} (original speed, slow motion (x0.5); with associated heat map). High-speed videos were recorded at a sampling rate of 500 frames per second (for detailed analysis see Table S2: #4, and Fig. 2C i).
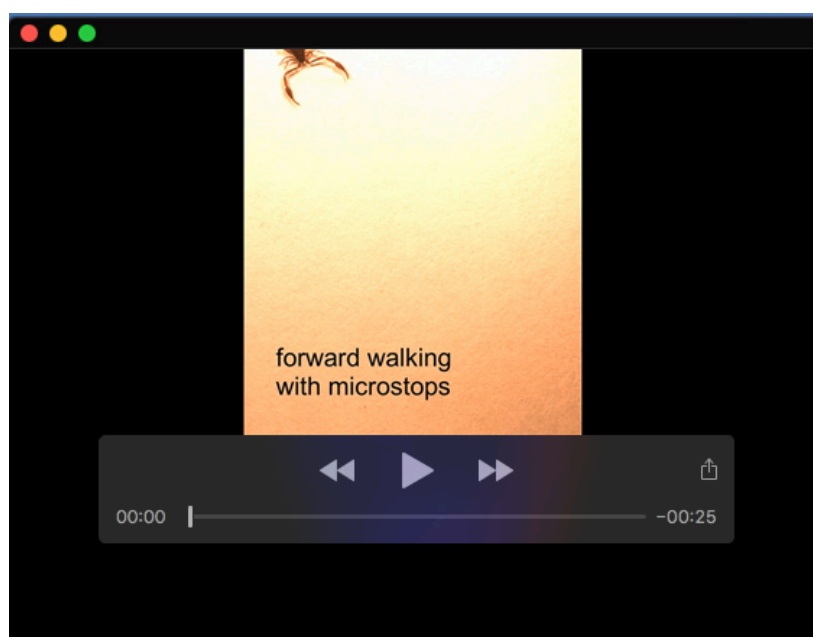
Movie 2. Walking in *C. cancroides***.** Example high-speed recordings of *C. cancroides* during forward, backward and upside down locomotion (slow motion (x0.0625)). High-speed videos were recorded at a sampling rate of 500 frames per second.