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Kinematics of males *Eupalaestrus weijenberghi* (Araneae, Theraphosidae) locomotion on different substrates and inclines

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Background: For males of several terrestrial spiders the reproductive success depends to their locomotors performances. However, their mechanics of locomotion has been scarcely investigated. Aim of this work was to describe the gait patterns, analyse the gait parameters, the mechanics of locomotion and the energy saving mechanisms of *Eupalaestrus weijenberghi* (Araneae, Theraphosidae) on different inclinations and surfaces.

Methods: Tarantulas were collected and marked for kinematic analysis. Free displacements, both at level and on incline, were recorded using two different experimental surfaces: glass and Teflon. Body segments of the experimental animals have been measured, weighted and their centre of mass experimentally determined. Through the reconstruction of trajectories of the body segments, we estimate the mechanical internal and external works and analysed the gait patterns.

Results: Four gait patterns have been described, but spiders mainly employed a walk-trot-like gait. Significant differences between the first two pairs and the second two pairs were detected. No significant differences were detected among different planes or surfaces in duty factor, time lags, stride frequency and stride length. However, postural changes were observed on slippery surfaces. The mechanical work at level was lower than expected. In all conditions, the external work, and within it the vertical work, accounted for almost all the total mechanical work. The internal work was extremely low, and did not increase with gradient.

Discussion: Our results support the idea of the two quadrupeds in series: the anterior composed by the first two pairs of limbs, with more explorative and steering purpose, and the posterior more involved in supporting the body weight. The mechanical work to move one unit mass a unit distance is almost constant among the different species. However spiders show lower values than expected. Minimizing the mechanical work could help to limit the metabolic energy expenditure that, in small animals, is relatively very high. However, the energy recovery due to the inverted pendulum mechanics only account for a small part of energy saving. Adhesive setae present in the tarsal, scopulae and claw tufts, would participate in different ways during different moments of the step cycle, compensating part of the energetic cost on gradient, and helping to maintain constant the gait parameters.

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Introduction

Movement is one of the key traits of the life of most animal species, leading them to interact with the environment, search for shelter, food, mate, and escape from predators (Alexander, 1999). The evolutionary selective pressures drove animals to display patterns of movement that are physiologically efficient, fast, adjustable or stable. Locomotion through particular environments could condition the morphology and physiology of animals (Dickinson et al., 2000).

The order Araneae shows great ability to move on a wide range of surfaces, with a broad locomotor repertoire, which include the capacity to move backwards and to turn on spot (Spagna & Peattie, 2012; Niederegger, 2013; Zeng & Crews, 2018). Considering the characteristics of the spiders, specially their control systems, they constitute an excellent model to study general features of locomotion (Biancardi et al., 2011). Pendulum mechanics has influenced the evolution of spiders that live hanging from their webs, as well as species that undertake an errant terrestrial lifestyle (Moya-Laraño et al., 2008; Blackledge et al., 2009). However, normal terrestrial walking is compared to an inverted pendulum, which would imply higher energetic costs of locomotion, with respect to suspensory “truly pendular” locomotion (Wolff, Nentwig & Gorb, 2013). The octopedal locomotion of spiders constitutes an extreme condition in terrestrial locomotion, related to the specialization of body segment groups (tagmosis). Other taxa, with more locomotory limbs, lack of such specialized segments (De la Fuente, 1994).

Locomotion was widely studied in bipedal and quadrupedal vertebrates (e.g.: Cavagna, Heglund & Taylor, 1977; Heglund et al., 1982); in hexapedal arthropods like cockroaches (Full & Tu, 1990, 1991; Kram, Wong & Full, 1997) and in functional octopedal arthropods like crabs (Blickhan & Full, 1987). More recently, researches have been carried out on spiders: the functional hexapedal harvestmen (Sensenig & Shultz, 2006) and the truly octopedal tarantulas

(Biancardi et al., 2011). Despite the huge morphological differences in size, shape, skeleton, number and position of locomotory limbs, the mechanical energy to move a unit mass for a unit distance (external work) is almost the same. These similarities suggest common design constraints in terrestrial locomotion (Full & Tu, 1990).

The mechanical work recorded in *Grammostola anthracina* (C.L. Koch, 1842) (Araneae, Theraphosidae) was lower than in other species (Biancardi et al., 2011). On the other hand, the Cost of Transport (i.e. the metabolic energy expenditure to move a unit mass for a unit distance, CoT) recorded in Theraphosidae was comparable to that of other species of the same mass (Herreid & Full, 1980; Anderson & Prestwich, 1985; Shillington & Peterson, 2002; Grossi et al., 2016a). Therefore the mechanical efficiency (mechanical work / metabolic cost) should be lower than in other species of comparable mass.

Studying locomotion of Theraphosidae and other groups of spiders, we need to considerate their leg adhesive devices (scopulae and claw tufts). These structures could play an important role in locomotion, both at level and while climbing (Niederegger & Gorb, 2006; Foelix, 2011; Spagna & Peattie, 2012; Wolff, Nentwig & Gorb, 2013; Wohlfart et al., 2014; Lapinski, Walther & Tschapka, 2015; Pérez-Miles, Perafán & Santamaría, 2015; Wolff & Gorb, 2015; Pérez-Miles et al., 2017). Most of the species of Mygalomorphae (72%) have adhesive setae, and they usually display cursorial lifestyles (Wolff, Nentwig & Gorb, 2013; Pérez-Miles et al., 2017). However, the contribution of these features to climbing is subject to controversy (Pérez-Miles, Perafán & Santamaría, 2015; Pérez-Miles et al., 2017). There are few studies of spider locomotion on incline, mainly focused on the performance at maximum speed (Moya-Laraño et al., 2009; Prenter, Pérez-Staples & Taylor, 2010; Prenter, Fanson & Taylor, 2012; Grossi et al., 2016a; Grossi et al., 2016b).

Pérez-Miles, Perafán & Santamaría (2015) analysed the role of adhesive setae to improve locomotion, on level and at different gradients of incline, in different species, using glass and Teflon as substrate for their trials. They found higher friction on glass than on Teflon (Pérez-Miles, Perafán & Santamaría, 2015). Based on this background, we hypothesized that both, the gait parameters and the mechanical work of locomotion, should present differences due to the distinct adhesion pattern. We would expect in particular the duty factor to increase on slippery surfaces, and also on severe positive gradient of inclination.

We chose males of *Eupalaestrus weijenberghi* (Thorell, 1894) to perform our experiments, and the same conditions of substrates and inclination used in previous works (Pérez-Miles, Perafán & Santamaría, 2015).

E. weijenberghi is a medium-sized tarantula very frequent in Pampean biogeographic province. Contrary to other species, adult males are larger than females and have longer legs. Juveniles and adult females live in burrows they dig in the soil of meadows. After the maturation moult and during reproductive season, adult males leave their burrows to search for females. During the reproductive season, males walk intensively day and night, mainly when the weather is cloudy and wet. Males only live about two months as adults, and during this period the main activity is searching for females and they rarely feed (Pérez-Miles et al., 2005).

Our objective was to analyse the gait patterns and parameters, the mechanics and energetic of locomotion of this species on different inclinations and surfaces. The results would be discussed in relation to the biology of this terrestrial arachnid.

Materials & Methods

Animals

The experiments were carried out with adults males of *E. weijenberghi* (n = 12; mass = 5.25 g; (0.63 SD) from Uruguay, Canelones, Salinas north, collected in March, 2013. Individuals were maintained in glass recipients of 6.5 cm in diameter by 12 cm height, with soil and water provision. They were fed weekly with *Blaptica dubia* (Blattodea, Blaberidae). Voucher specimens were deposited in the Arachnological Collection of the Facultad de Ciencias, Universidad de la República, Montevideo Uruguay (FCE-My).

Experimental procedure

Free displacements of the spiders were recorded, during the reproductive season, on four smooth surfaces: glass and Teflon on level (g0; t0), glass and Teflon on 12º incline (g12; t12). These surfaces were used in previous studies with tarantulas showing higher adhesion in glass than Teflon (Foelix, 2011; Pérez-Miles, Perafán & Santamaría, 2015). The inclination used was selected considering an average value of the possible locomotion range on Teflon (Pérez-Miles, Perafán & Santamaría, 2015). Each individual was recorded in all the experimental conditions in randomized order, resting at least 48 hours between two successive trials. Individuals were marked dorsally, at least 5 minutes before the first trial, with water based non-toxic ink. Mark positions were on the fovea, the centre of the patella and the tip of the tarsus. These points, together with the light bands in the tibia-metatarsus joint and the points of insertion of each leg on the cephalothorax (coxae), were used as landmarks (Fig. 1). During the trials the temperature was 22.3 ºC (0.6 SD).

Four fixed video cameras (Sony DCR-H28E) were simultaneously used, within a space of 20x32x30 cm (Fig. 2). The frame rate was 25 Hz interlaced (50 fps), a frequency considered sufficient for this kind of analyses (Ward & Humphrey, 1981; Biancardi et al., 2011). The calibration was done with 17 markers evenly distributed in three dimensions. The synchronization of the four cameras was obtained by a sound signal, and all images were digitized and later used to reconstruct the position of each marker. The measure error in the system was 0.7±0.2 ‰, according to Barros et al. (2006). Orthogonal axes were defined as follows: the direction of x-axis agreed with the main displacement direction, z-axis agreed with height with respect to the ground and y-axis was determinate by the right-hand rule (Wu & Cavanagh, 1975).

Centre of mass determination

All the spiders used for kinematic analyses were sacrificed by means of carbon tetrachloride vapours and fixed in glass tubes with alcohol. The following right body segments were separated: cephalothorax together with abdomen, coxae and trochanters, femora; patellae jointly with tibiae; metatarsi and tarsi. The mass of each segment was determined with an analytical scale (Radwag AS 310/C2). Lengths and diameters were taken by means of an ocular micrometer (Olympus G15x) with a stereo microscope Olympus SZH. Four measures of the cephalothorax width, at the level of each coxa pair, were taken with a calliper. Further, the distances between the limb insertion and the fovea, on the medial sagittal line, was measured. Each segment, with the exception of the tarsi, was suspended from at least two points and photographed in static equilibrium (Alexander, 1985) with a Nikon D3200 camera. A fine human hair with a knot tied to a hook on one end, and the other attached to the segment with removable glue, was used to this purpose.

Digital pictures were analysed with the program ImageJ 1.49v (Rasband, 1997). A needle of known length was used to calibrate the pictures. In each picture, a straight line, extension of the hair through the mooring point and the centre of the segment, was drawn. Next, the pictures of the same segment were overlapped. We recorded the length of the segments (*ls*) and the distance from the proximal end to the point where the straight lines cross (*L*). The average relative position of the centre of mass (L_{CoM}) of each segment was calculated as: $L_{CoM} = ls/L$ (Supplementary document S1).

Data processing

All the recorded trial have been viewed and visually analysed. Only that performed in straight line, without ostensible speed changes were selected for kinematic analyses. After this first filter, the sample size was reduced to 5 spiders (n = 5, mass = 5.10 g, 0.68 SD).

Two successive step cycles were analysed in each trial. An image analysis program (Dvideow 6.3, Campinas University) (Figueroa, Leite & Barros, 2003; Barros et al., 2006) was used to synchronize the recordings and to reconstruct the 3D position of each marker. All the described landmarks were digitized in this way, with two exceptions: i) the tibia-metatarsal joints appeared blurred in the trials on Teflon, due to reflections. Therefore it has been possible to digitize them only in the trials on glass surface; ii) the *coxae*, because the insertion points of the legs were barely seen by more than one camera. In this case eight virtual markers have been calculated, according to Biancardi et al. (2011), from the fovea position.

A series of Matlab R2012 routines was built to manage and process the kinematics data. Spatial coordinates of markers were filtered with a Butterworth of order 3 and cut frequency of 6 Hz. Two multi-segment body models of the spiders have been implemented (Fig. 3). Cephalothorax segment was bounded by the eight coxae, while leg segments were delimited by a pair of the described landmarks. The first (model A) considering three segments by leg (femur, patella-tibia, tarsus-metatarsus); the second (model B) including only two leg segments (femur, and other distal segments together). In both models coxa and trochanter were considered together with cephalothorax and abdomen. Model A was used for analysing the glass trials, while model B for both, Teflon and glass. The radii of gyration of the segments were calculated by assuming a cylinder shape of segments (Biancardi et al., 2011). The 3-D position and respective masses of each L_{CoM} and fovea spatial position were used to obtain the frame-by-frame 3-D position of the total body centre of mass (b_{CoM}) (Supplementary document S1).

The trajectories of b_{CoM} were used to calculate works and energies following Willems, Cavagna & Heglund (1995). The internal work (W_{INT}) is the increases in kinetic energy of the segments arising from their speed change with respect to the b_{CoM}. The external work (W_{EXT}) is the increases in kinetic energy of the b_{CoM} with respect to the environment. W_{EXT} was obtained by summing the positive increments of total energy (E_{TOT}) with respect to time: $E_{TOT} = E_{POT} + E_{KIN.x} + E_{KIN.z}$, where E_{POT} is the potential energy of the b_{CoM}; $E_{KIN.x}$ and $E_{KIN.z}$ are the horizontal and vertical components, respectively, of the kinetic energy of the b_{CoM}. Vertical and horizontal works (W_V, W_H), components of W_{EXT}, were obtained by summing, respectively, the positive increments of vertical energy ($E_{POT} + E_{KIN.z}$), and of horizontal energy ($E_{KIN.x}$). The total mechanical work (W_{TOT}) was computed as the sum of the W_{INT} and W_{EXT} (Cavagna & Kaneko, 1977). Mechanical work was expressed as the mechanical cost of the transport, per kilogram of body mass and per unit of distance (i.e. J $Kg^{-1}m^{-1}$). The ability of the body to save mechanical energy through the interchange between E_{POT} and E_{KIN} ('energy recovery'), was calculated according to Cavagna, Thys & Zamboni (1976).

Different multi-segmented models could affect the determination of the b_{CoM} position and the estimations of the mechanical work (Pavei et al., 2017). However, the effect of different number of limb segment on the b_{CoM} trajectory (W_{EXT}) is generally negligible, especially when the limb masses / body mass ratio is small. W_{INT} could be more affected (Biancardi et al., 2011).

From the analysis of the b_{COM} trajectory were also estimated: its mean height (H_{COM}); the speed (horizontal b_{COM} displacement / time); the relative speed to body length; the stride length (L_S, horizontal b_{COM} displacement during one stride), the relative stride length ($L_{SR} = L_S/H_{COM}$); the stride frequency (F_S) and the duty factor (D_F, ratio between the duration of a foot contact interval and the stride duration).

In order to perform the gait analysis, like in other studies, the spider was considered as composed by two successive quadrupeds in series (Wilson, 1967; Root, 1985; Biancardi et al., 2011), the first begin L1-R1-L2-R2 and the second L3-R3-L4-R4 (where L and R indicate left and right and the number start from the very fore pair of legs). The gait diagram of each “quadruped” was compared with the theoretical quadrupedal gait diagrams (Hildebrand, 1966; Hildebrand, 1977). The stride coordination was evaluated by the antero-posterior sequence method (Abourachid, 2003) considering the sequence of feet footfalls (gait diagram). The parameters were: the time lag between two contralateral feet footfalls, which measures temporal coordination within each pair of fore (F) or hind (H) limbs: F_l1 (L1-R1), H_l1 (L2-R2), F_l2 (L3-R3), H_l2 (L4-R4); the time lag between fore and hind feet footfalls on the same side, for pair leg I-II (P_l1) and for pair leg III-IV (P_l2); all expressed as a percentage of the cycle duration.

All Statistical analyses were done using PAST 3.12 package (Hammer, Harper & Ryan, 2001). To compare the variables at different experimental conditions we used a two-ways ANOVA for paired values, with the angles and surfaces as independent factors. The results of the two body models have been compared by a *t*-test. Linear Pearson *r* coefficient was computed to test the correlation between speed and stride frequency. In all cases the critical p-value was set at 0.05.

To analyse locomotors footfalls patterns we performed two principal components analysis. One including the eight legs and the other considering the tarantulas as two independent quadrupeds: first involving pairs I and II and second involving pairs III and IV.

Results

A total of 40 strides were analysed, 10 strides for each experimental condition (g0; g12; t0; t12). All the raw data are given in table S2.

Speed and Gaits

The mean speed recorded during the trials was 2.53 $cm.s^{-1}$ (0.68 SD), with no significant differences between inclinations or substrates. In tables 1 and 2 are resumed the average results and the statistical comparison, respectively. Within the observed range of velocities, the stride length (L_S) was almost constant ($r=-0.419$, $p = 0.065$), while the stride frequency (F_S) significantly increased with speed ($r = 0.821$, $p = 0.000009$) (Fig. 4). The duty factor was always higher than 0.5, as in walking gaits, and did not show significant differences over the different conditions. The same occurred for stride length, stride frequency and relative stride length (Table 1 and 2). The height of the centre of mass (H_{CoM}) was the only variable affected by the interaction of experimental conditions ($F = 28.14$, $p = 0.006$), underlying a change of the limbs posture on Teflon, with respect to glass substrate, from a more upright position on level to a more sprawled one on gradient.

The gait diagrams show four different gaits, sometimes determined by the composition of different patterns in the two “quadrupeds” (Fig. 5). Walk-bound was only observed in the anterior quadruped, and was characterized by the simultaneous contact of the contralateral feet, with a phase shift of about 50% between the pairs I and II (similar to a quadrupedal bound, without an aerial phase). In walk-trot the odds limbs of the right side (I and III) move simultaneously to the even limbs of the left side (II and IV), like in a quadrupedal trot, again without an aerial phase. Diagonal and lateral walk are patterns equivalent to their quadrupedal homonym (Hildebrand, 1966; Abourachid, 2003). The time lags and D_F of each leg pair for different gaits are given in table 3.

In the principal component analysis considering the octopod (Fig. 6) the PC1 explained the 36.8% of the variance, with highest loadings of H_l1 (0.80) and P_l1 (0.53). The PC2 explained the 21.8% of the variance, with highest loadings of F_l2 (0.65) and F_l1 (0.63). No significant associations were found between the experimental conditions and the variables: duty factors and time lags (Fig. 6A). However, time lags of pairs I and II (F_l1, H_l1, P_l1) show a trend to the positive values of PC1. Duty factors associated to these time lags were placed to negative values of PC2. Conversely, posterior leg pairs did not show similar tendencies. Temporal variables explained two types of gaits (Fig. 6B). Walk-trot in four leg pairs, was observed in the negative quadrant of PC1 and positive quadrant of PC2 while walk-bound in pairs I and II was observed in the negative quadrants of PC1 and PC2.

In the principal component analysis considering quadrupeds (Fig. 7), the PC1 explained the 35.2% of the variance, with highest loadings of H_l (0.85) and P_l (0.50). The PC2 explained the 25.6% of the variance, with highest loadings of F_l (0.77) followed by the duty factors. The anterior quadruped showed lower values than the posterior quadruped in PC2, consequently a *t*-test was performed for the scores of this component. Significant differences were found between the anterior and posterior quadrupeds (*t* = -3.02, p = 0.01).

Mechanical work

The external work (W_{EXT}) overall accounted for the larger part of the total mechanical work (W_{TOT}), while the internal work component only contributed for less than 1% (Table 1 and 2). The increase of gravitational potential energy due to climbing explains the significant differences of W_V, W_{EXT} and W_{TOT} detected on gradient. The internal work (W_{INT}) was 10% to 20% higher on level than on gradient, on both surfaces (F = 9.041, p = 0.040). The horizontal work (W_H) was significantly higher on Teflon (F = 17.760, p = 0.014). The recovery was lower on gradient than on level, but the differences were not significant.

The two models produced different values of the internal work, which was generally overestimated by the simplified model B (31%). However the differences were not statistically significant (W_{INT}: t = 0.775, d.f. = 8, one-tail p = 0.230). Model B produced a slight overestimation (1.46%) of the vertical work, and therefore also of the external work, with no significant effects on the variable means (W_V and W_{EXT}: t = 0.133, d.f. = 8, one-tail p = 0.449). The effect on the total work was similar to the latter, due to the small contribution of internal work (W_{TOT}: t = 0.136, d.f. = 8, one-tail p = 0.447). Horizontal work and recovery were not affected (mean differences less than 0.5% and p values of the *t*-test near to one).

Discussion

Gait pattern

Speed changes in legged animals can be obtained adjusting the stride frequency and the stride length (Cavagna et al., 1988). Zollikofer (1994), in a research on ants, hypothesized that long legged arthropods would employ longer strides to go faster. Other authors pointed at F_S as the main determinant of speed changes in Coleoptera, Carabidae and cockroaches (Evans, 1977;

Ting, Blickhan & Full, 1994). In *E. weijenberghi*, the F_S resulted to be the main determinant factor of the speed changes, at least within the limited range we studied. This result agrees with previous findings in other tarantulas (Anderson & Prestwich, 1985; Booster et al., 2015). However in females of *G. anthracina* the L_S gradually increased within a speeds range comparable to that observed in *E. weijenberghi* (Biancardi et al., 2011). In general, animals with exoskeleton are forced to modify the F_S to change speed. The hardness of the components make difficult to modify the L_S with the participation of other body parts, as happens in animals with endoskeleton (Griffin, Main & Farley, 2004). Considering that the F_S is related with muscular work (Heglund & Taylor, 1988), and limited by the muscular physiology (Alexander, 2003), higher maximum speeds are achieved by spider species (or genders, in case of sexual dimorphism) with longer legs (Grossi & Canals, 2015; Grossi et al., 2016a).

The speed of locomotion on gradient usually tends to be slower than on level, due to the decreasing of the stride frequency (Birn-Jeffery & Higham, 2014) and increasing of the duty factor (Gabaldón, Nelson & Roberts, 2004). Both variables adapt to increase the muscular work against the gravity. In *E. weijenberghi* we did not find significant differences neither in the F_S nor in D_F, fact that could be explained by the participation of leg adhesive setae (claw tufts and scopulae) that could reduce the quantity of extra muscular work and therefore facilitate the locomotion on inclined planes (Pérez-Miles, Perafán & Santamaría, 2015).

Alternate tripodal and quadrupedal patterns, like the walk-trot, have been already described in crabs (Blickhan & Full, 1987), cockroaches running at low speed (Ting, Blickhan & Full, 1994), a caraboctonidae scorpion and an agelenidae spider (Spagna & Peattie, 2012; Fig. 8A-B) and tarantulas at different velocities (Biancardi et al., 2011). In the tarantula *G. anthracina* several gaits were observed: walk-trot combined with lateral walk at low speed; diagonal walk at medium speed and walk-trot combined with diagonal walk at high speed (Fig. 8C-E). Walk-like and trot-like gaits are included in the broad category of the symmetric gaits (Hildebrand, 1966; Abourachid, 2003). An asymmetrical gait has also been infrequently observed, a walk-bound, but only in the anterior quadruped (pairs I and II). All these gaits agree with the expected time-lags values proposed by Abourachid (2003).

Rapid and intermittent movements were reported as usual for theraphosids (Shillington & Peterson, 2002; Grossi et al., 2016b) but the sequences we select were straight trajectories and at about constant speed. Our results are in agreement with those of Biancardi et al. (2011), in the interpretation that spider locomotion patterns are complex and do not appear to show a rigid neural control. This plasticity enables rapid corrections of the locomotion pattern on irregular terrains.

According to the PCA results, the observed locomotors patterns were characterized by variations of time lags of pairs I, II and III than by variations of duty factors. Pair I has both locomotors and exploratory functions (Anderson & Prestwich, 1985; Blickhan & Barth, 1985; Foelix, 2011). Exploratory function implies less time of contact with the substrate and this could partially explain the differences in the D_F between the forelimbs and hind limbs.

In mammals, due to the weight of the head, the fore limbs supported a great proportion of the weight, involving a higher D_F than for posterior limbs (Maes et al., 2008). Our results show a more restricted pattern in posterior quadruped probably due to the important role of pairs III and IV to support the body weight of the tarantula. Furthermore, the body centre of mass in tarantulas is located between the insertion of legs III and IV, just behind the fovea (Biancardi et al., 2011). Anterior legs show more versatility considering they are involved in exploratory functions and driving displacements. Traditionally, the D_F was the usual parameter used to analyse the locomotory patterns (Farley, Glasheen & McMahon, 1993; Alexander, 2003), while the study of anterior-posterior sequence is relatively more recent (Abourachid, 2003). The principal component analysis evidenced that both approaches were important to understand the gait coordination.

Mechanical work and efficiency

In different species that has been studied, such as cockroaches (Full & Tu, 1990, 1991; Kram, Wong & Full, 1997), crabs (Blickhan & Full, 1987), quadruped mammals (Heglund et al., 1982) and bipeds (Saibene and Minetti, 2003), the mechanical work is quite constant and independent from the body mass, body shape, number of legs and skeletal type (Full & Tu, 1990). Tarantulas seem to be the exception to this rule: the external work in *E. weijenberghi* was about a half of reported for other species, in agreement with the observation of Biancardi et al. (2011) for *G. anthracina*. Possibly the hydraulic system of arachnids involved in the extension of legs is related with this difference.

Females of *E. weijenberghi* do not move more than 40 cm away from the burrow entrance (Álvarez, Perafán, & Pérez-Miles, 2006) while adult males intensively walk to search females (Pérez-Miles et al., 2005). The low magnitude of mechanical energy involved in locomotion would facilitate long displacements without feeding (Pérez-Miles et al., 2005). Indeed, there is a relationship between the mechanical work of locomotion and the metabolic energy expenditure, and low metabolic cost of transport is one of the characteristics associated to the lifestyle of wandering spider males (Grossi et al., 2016a). However, the low cost underlined these authors is an absolute value, influenced by the lower body mass of wandering males with respect to females. When compared per unit body mass, the cost of transport of smaller individuals is higher than that of larger ones, and therefore their mechanical efficiency is lower. Nevertheless, optimization of cost and efficiency is not the unique determinant of locomotion, and in some cases, when the cardiovascular or respiratory system are not performing well, inefficient yet economic (in absolute terms) locomotion patterns could be preferred (e.g. Bona et al., 2017).

The increment of W_{EXT} during locomotion on gradient was caused by the additional W_V, and resulted similar, in proportion, to that found in humans (Minetti, Ardigo & Saibene, 1993; Gottschall & Kram, 2006). This increment was slightly lower on Teflon than on glass. On Teflon the W_H was higher, probably due to lower adhesion (Pérez-Miles, Perafán & Santamaría, 2015), which could explain the difference with glass on gradient.

During horizontal displacements the variations of $E_{KIN.x}$ were much lower than the variations of $E_{KIN,y}$, as found in other arachnids (Sensenig & Shultz, 2006; Biancardi et al., 2011). This implies a low energy recovery (Gottschall & Kram, 2006), and therefore a greater energy expenditure of muscles.

The pendulum mechanics drove, even in spiders, the morphological evolution of terrestrial locomotion (Moya-Laraño et al., 2008). In bipeds (mammals and birds), the maximum recovery during horizontal displacement varies between 60-80%, while in quadruped mammals between 30-65% (Cavagna, Heglund & Taylor, 1977; Saibene and Minetti, 2003; Griffin, Main & Farley, 2004). However, in arthropods the recovery never exceeds values between 7-19% (Full & Tu, 1990; Full & Tu, 1991; Biancardi et al., 2011), and our values fall in this latter range. The number of locomotors limbs probably influences the inverted pendulum mechanics.

The internal work in *E. weijenberghi* accounted for 0.2% of the total work, lower than the 11% recorded in *G. anthracina* and then the 9-15% detected in opilions (Sensenig & Shultz, 2006). Two factors could explain that difference: i) the speed of the body segments and ii) the relative mass of the limbs in relation to body. Any increment of the segments speed would imply higher stride frequencies and consequently higher relative speeds of locomotion, and W_{INT} increases with the speed (Fedak, Heglund & Taylor, 1982; Minetti, 1998; Biancardi et al., 2011). According to the dynamic similarity hypothesis (Alexander & Jayes 1983), the Froude number is a dimensionless measure of the relative speed useful to compare the locomotion of individuals and species of different size: $Fr = v^2 g^{-1} l^{-1}$

Where v is the velocity, g the acceleration of gravity and l a characteristic measure that in spiders would be the height of the coxa joint from the ground in normal standing (Irschick & Jayne, 2000; Biancardi et al., 2011). *E. weijenberghi* displayed locomotion speeds much lower than females of *G. anthracina*. However, the equivalent speeds, expressed as number of Froude, were similar.

Moreover, relative limb mass of males *E. weijenberghi* was higher than that of females *G. anthracina* (25% vs 13% of body weight). Therefore, higher relative W_{INT} should be expected in the former species. Therefore, none of these factors seems to explain the large difference in W_{INT} between the two species. Contrary to what exposed by Minetti, Ardigo & Saibene (1993) and Minetti (1998), the W_{INT} decreased with gradient. We found a slight variation in agreement with a small decreasing of the stride frequency on glass as well as on Teflon. The W_{INT} was proportional to the F_S on both horizontal and inclined surfaces (Minetti & Saibene, 1992; Minetti, 1998).

The lower static friction found by Pérez-Miles, Perafán & Santamaría (2015), on Teflon than on glass probably explain the higher horizontal work we found on Teflon. An increment of the mean height of centre of mass was also observed on the former surface, during locomotion on level. An upright posture facilitates the contact between adhesive structures and the substrate in geckos (Higham et al., 2015). In tarantulas this posture could help the contact between claw tufts and tarsal claws and the surface, because those are the features most used during level locomotion (Pérez-Miles, Perafán & Santamaría, 2015).

Contrary, on inclines the spiders assumed a more sprawled position on Teflon than on glass, increasing the supporting area and consequently the stability (Ting, Blickhan & Full, 1994). Pérez-Miles, Perafán & Santamaría (2015) observed that, in static positive gradient, legs I and II interacted with claw tufts while legs IV also touched with distal scopulae. Birn-Jeffery and Higham (2014) suggested that animals usually extend the legs or change the pushing mode on gradient surfaces to optimize the force to overcome the gravity (Alexander, 2003). Our results suggest that *E. weijenberghi* need to increase the stability while the adhesion diminishes. Despite the adhesion implies costly mechanisms of attachment and detachment (Wolff, Nentwig & Gorb, 2013), probably these costs are less than those involved adopting a crouched posture and using extra muscular force.

The adhesion in Theraphosidae is produced by specialized setae located on the ventral face of the distal segments of the limbs. These setae are arranged on claw tufts and tarsal scopulae (Pérez-Miles, 1994). Pérez-Miles, Perafán & Santamaría (2015) and Pérez-Miles et al. (2017) proposed that different adhesive setae produce adhesion when limbs push or pull, according to the part of the limb implied and the orientation of the microtrichiae on setae. When tarantulas climb, the forelimbs pull and produce adhesion with the distal claw tufts, while hind limbs push and produce adhesion by scopulae. The contact phase of each step is composed by a braking phase, followed by a second phase of propulsion (Griffin, Main & Farley, 2004). We observed that, during the braking phase, the forelimbs were in contact with the proximal tarsi (scopulae), producing pushing adhesion, while hind limbs produced pulling adhesion contacting with distal claw tufts. Conversely, during the propulsion phase, the contacts and adhesion mechanisms work in reverse: pushing with hind limbs and pulling with forelimbs. During locomotion on positive gradient, forelimbs should maximize the pulling adhesion increasing the contact time of apical tarsi with the substrate, while hind limbs should increase the contact time with scopulae for pushing adhesion. Adhesive setae have high adaptive value for locomotion of spiders in natural environments (Lapinski, Walther & Tschapka, 2015; Pérez-Miles et al., 2017). In fact, the adhesive structures of *E. weijenberghi* show sex based differences, reflecting the different lifestyles of females and males (Pérez-Miles, Perafán & Santamaría, 2015).

Body models

The centre of mass of a multi-segment body varies by changing the relative positions of the segments, being in every instant, the weighted sum of the centres of mass of each segment (Zatsiorsky, 2002). Almost all the L_{CoM} were located close to the geometric centre of a cylinder of the same dimensions, except for the segment composed of patella and tibia, probably because the patella is wider than tibia. When considering variables affected by the movement of the distal body segments, the choice of an adequate multi-segment model is determinant. The two body models of *E. weijenberghi* built in this work have been compared in one experimental condition. The internal work was indeed the most influenced variable. Reducing the number of leg segments can affect the variation of linear and/or rotational kinetic energy. However, this simplification is necessary to analyse the movements without increasing the number of cameras and consequently the processing time (Allard, Blanchi & Aissaoui, 1995).

We should finally consider that, due to methodological limitations, in this research the fovea position was used as proxy of the true centre of mass of the cephalothorax and abdomen (Biancardi et al., 2011). For *G. anthracina* no significant differences were found in the W_{EXT}, when calculated from the fixed position of a single marker on the fovea versus when it was calculated from the usual weighted position of the centres of mass of all the body segments (Biancardi et al., 2011). In humans, it has been shown that, compared to direct dynamics records, the W_{EXT} could be overestimated of the 13% using body models formed by eleven or fourteen segments, and of the 16% using a single marker close to static centre of mass (Pavei et al., 2017). In the same investigation it has been shown that the W_{INT} was not affected by the different research techniques used, involving different calculations to get the b_{CoM} (Pavei et al., 2017). The estimations of the vertical, external and total work differed less than 2%, between the two models employed. We consider that both the models proposed in this research are appropriate to perform a rigorous kinetic analysis in arachnids.

Conclusions

E. weijenberghi locomotion showed a variety of gait patterns. Nevertheless, the most frequently used gait was a walk-trot, similar to the vertebrate trot without the flight phase. Time lags and duty factors were consistent within the gaits, and provided enough information to differentiate the role of two limb groups. Indeed, the spider octopedal patterns can be interpreted as the combination of two quadrupeds in series; the anterior mainly steers the movement, while the posterior supports the body mass.

The total mechanical work to move one unit mass a unit distance was largely composed by external work, which in turn was mainly determined by a vertical component, to raise the centre of mass against gravity. Horizontal work was disproportionately smaller, determining a scarce amount of energy recovery.

On inclined locomotion the change of mechanical work was in line with others animals, but not the duty factor, speed or frequency. While on inclined and slippery surfaces, where internal and horizontal works were greater, the spiders adopted postural changes to increase the support area and stability.

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Figure 1

Male *Eupalaestrus weijenberghi*

Light blue marks were used as landmarks.

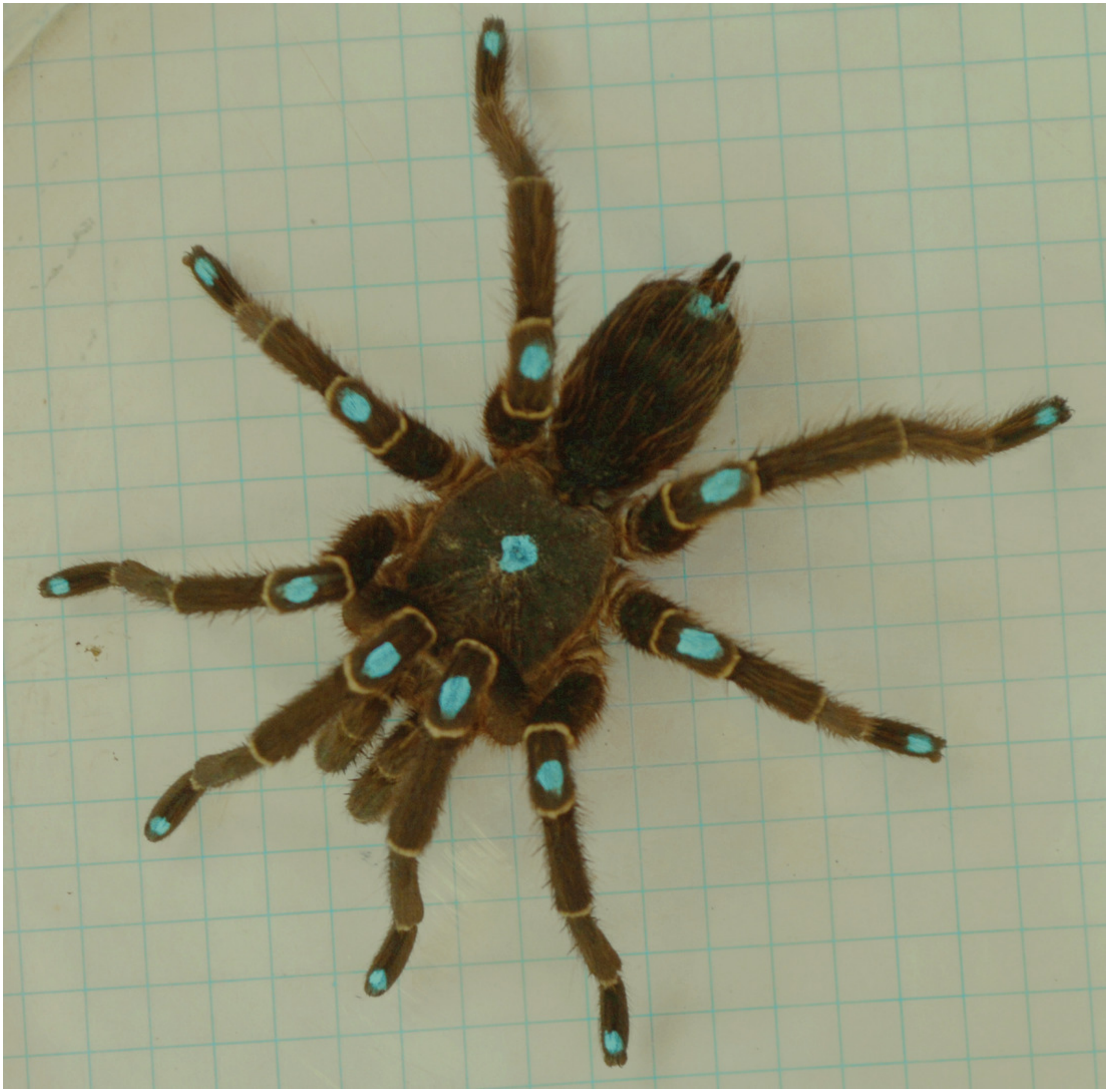

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Figure 2

Experimental set-up

Figure 3(on next page)

Models of rigid segments of the body of spiders

(A) was used for glass trials and (B) for Teflon.

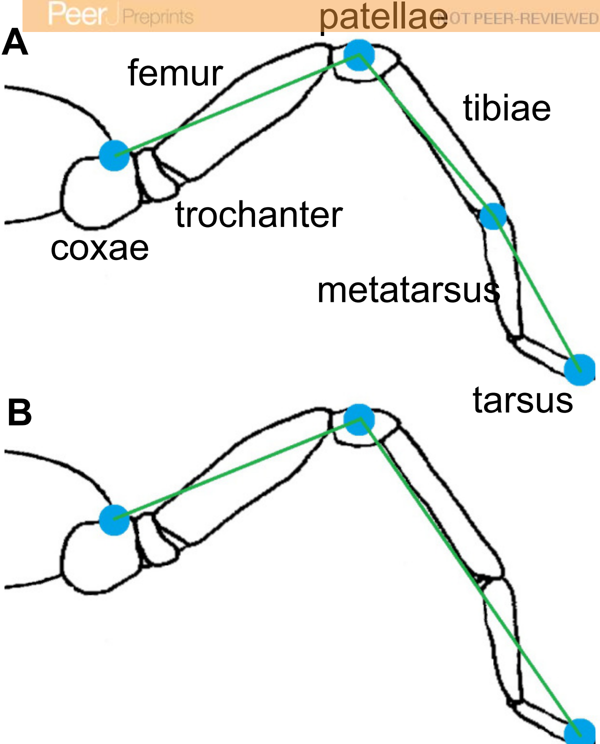
A
patellae
femur
tibiae
trochanter
coxae
metatarsus
tarsus
B

Figure 4(on next page)

Stride frequency (open circles) and stride length (full circles) on function of speed.

The dotted lines are the linear adjustments.

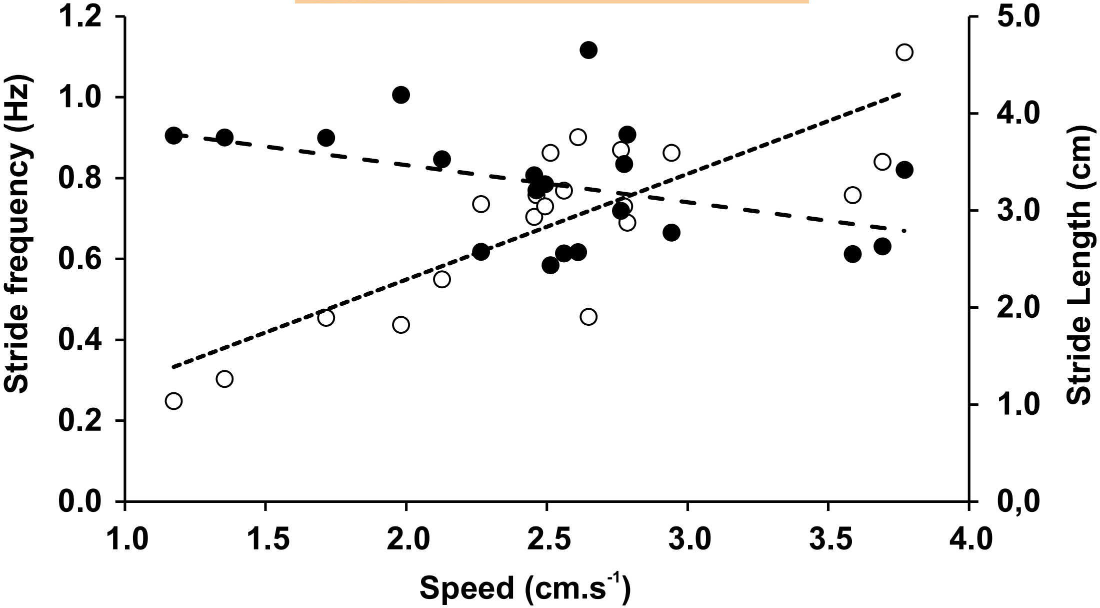

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Figure 5(on next page)

Gait diagram of footfall pattern

(A) Walk-bound in legs I and II (R1 to L2) and diagonal walk in legs III and IV (R3 to L4), recorded on g0. (B) Lateral walk in all legs, recorded on t0. (C) Walk-trot in all legs, recorded on g12.

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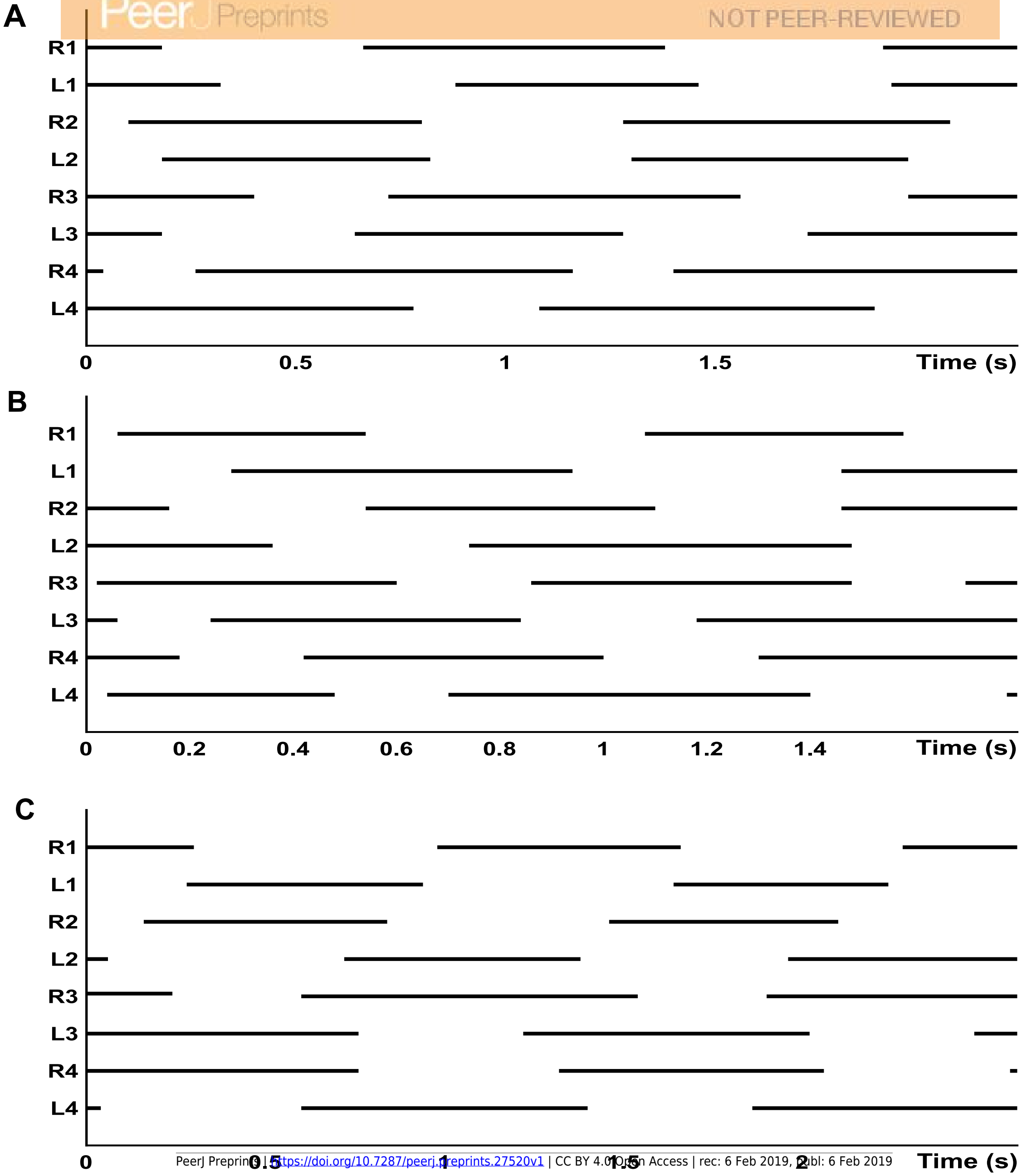
A
R1
L1
R2
L2
R3
L3
R4
L4
0
0.5
1
1.5
Time (s)
B
R1
L1
R2
L2
R3
L3
R4
L4
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Time (s)
C
R1
L1
R2
L2
R3
L3
R4
L4
0
0.5
1
1.5
2
Time (s)

Figure 6 (on next page)

Principal component analysis of temporal variables

Time lag of the pair legs I (F_l1), of pair legs II (H_l1), pair III (F_l2) and IV (H_l2); time lag of ipsilateral legs, pair I-II (P_l1), and between pair III-IV (P_l2); the duty factor of pair of legs I (D_{FFl}1), pair II (D_{FHl}1), III (D_{FFl}2) and IV (D_{FHl}2), ipsilateral of the pairs I-II (D_{FPl}1), and the III-IV (D_{FPl}2). (A) Experimental condition (full squares - g0, open circles - g12, full circles – t0, open squares – t12). (B) Gait patterns quadrupeds (full triangle – bound diagonal, open triangle – bound trot, full circles – trot trot, full squares – diagonal diagonal, full diamonds – lateral lateral, open squares - trot diagonal, open diamonds – trot lateral, open circle – diagonal trot).

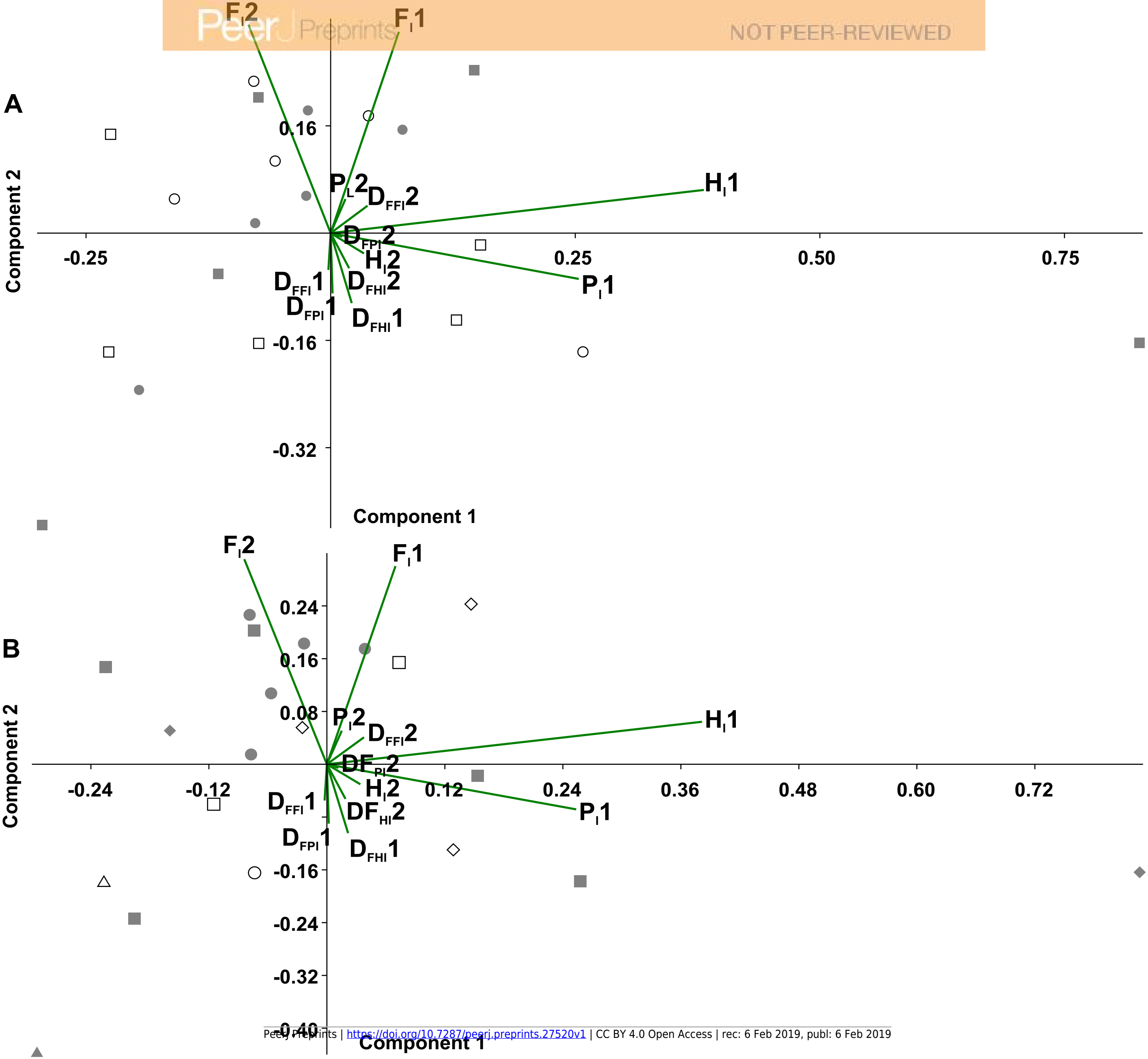

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Figure 7(on next page)

Principal component analysis of temporal variables considering the tarantulas as two quadrupeds

Time lag of fore legs (F_l), of hind legs (H_l); ipsilateral (P_l); and duty factor of the fore legs (D_{FFl}), hind legs (D_{FHl}) and ipsilateral (D_{FPl}). Gait of pair legs I and II (open squares) and pair legs III and IV (full circles).

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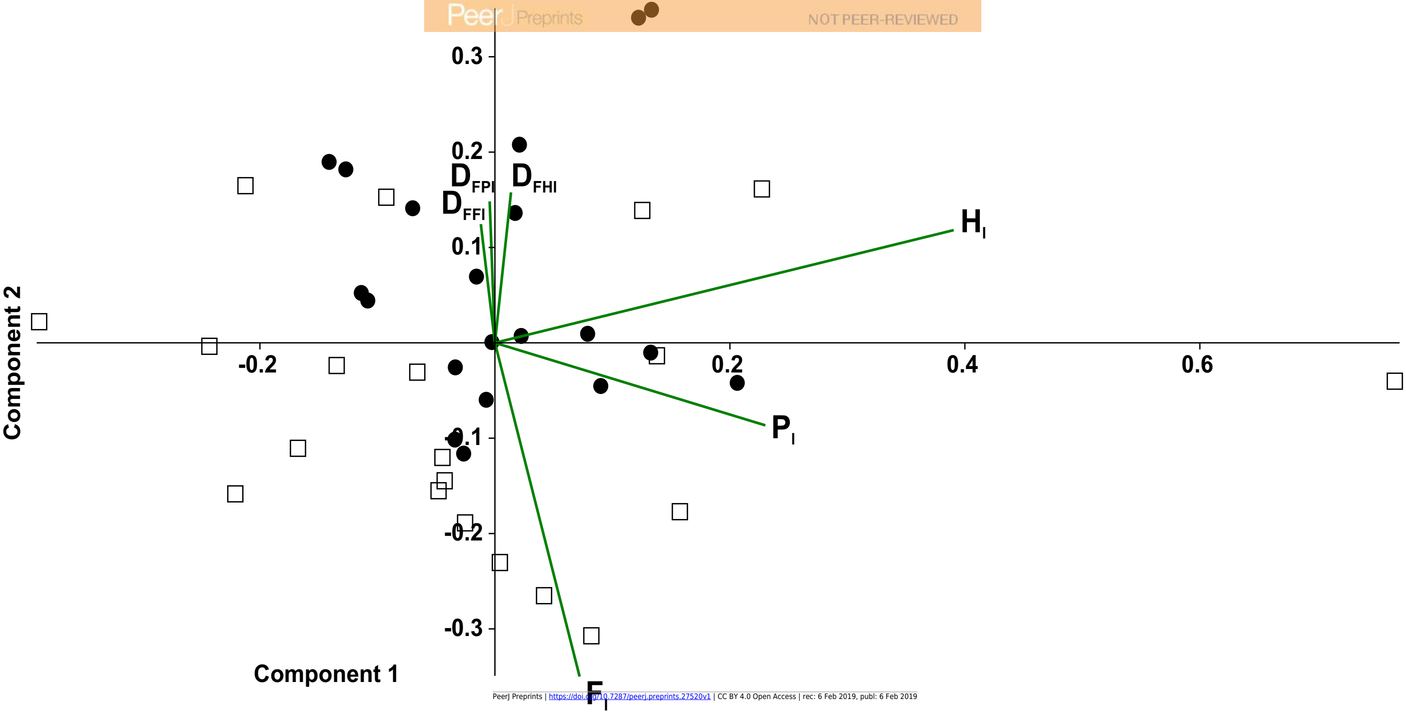
0.3
0.2
0.1
-0.1
-0.2
-0.3
-0.2
0.2
0.4
0.6
Component 1
Component 2
D_{FPI}
D_{FHI}
D_{FFI}
H_I
P_I
F_I

Figure 8(on next page)

Gait diagram of different species

(A) *Hololena adnexa* (Araneae, Agelenidae) modified from Spagna & Peattie (2012); (B) *Hadrurus arizonensis* (Scorpiones, Iuridae) modified from Root (1985), and (C-E) *G. anthracina* at three speeds modified from Biancardi et al. (2011).

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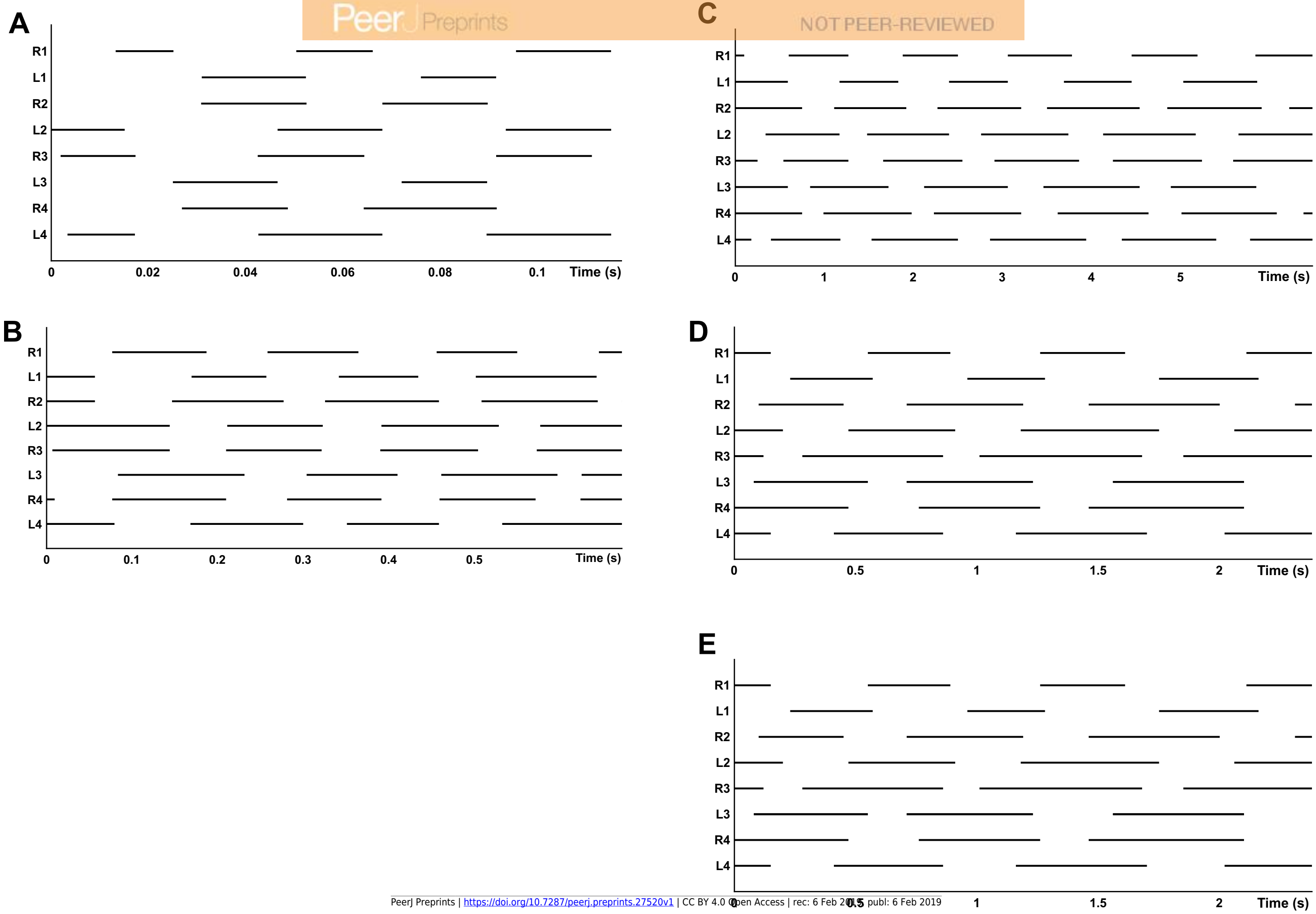
A
R1
L1
R2
L2
R3
L3
R4
L4
0
0.02
0.04
0.06
0.08
0.1
Time (s)
B
R1
L1
R2
L2
R3
L3
R4
L4
0
0.1
0.2
0.3
0.4
0.5
Time (s)
C
R1
L1
R2
L2
R3
L3
R4
L4
0
1
2
3
4
5
Time (s)
D
R1
L1
R2
L2
R3
L3
R4
L4
0
0.5
1
1.5
2
Time (s)
E
R1
L1
R2
L2
R3
L3
R4
L4
0
0.5
1
1.5
2
Time (s)

Table 1(on next page)

Results obtained in all experimental condition (mean ± standard deviation, n = 5)

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Variable	**Glass 0°**	**Teflon 0°**	**Glass 12°**	**Teflon 12°**
Speed (cm.s^{-1})	2.55±0.90	2.80±0.62	2.61±0.59	2.17±0.63
Relative speed (B_l.s^{-1})	1.11±0.38	1.23±0.29	1.03±0.37	0.94±0.25
Duty Factor (D_F)	0.61±0.01	0.58±0.04	0.60±0.05	0.59±0.04
L_S (cm)	3.04±0.68	3.53±0.69	3.17±0.68	3.31±0.51
L_{SR}	2.28±0.56	2.09±0.44	2.25±0.23	2.43±0.28
F_S (s^{-1})	0.71±0.27	0.74±0.26	0.69±0.14	0.62±0.23
H_{CoM} (cm)	1.34±0.06	1.70±0.19	1.41±0.13	1.34±0.11
W_{EXT} (mJ.kg^{-1}.m^{-1})	568±98	1202±1038	2135±128	2578±721
W_{INT} (mJ.kg^{-1}.m^{-1})	1.1±0.6	2.6±0.8	0.9±0.3	2.4±1.9
W_{TOT} (mJ.kg^{-1}.m^{-1})	569±98	1205±1038	2136±129	2578±721
Recovery (%)	6.9±3.8	10.5±9.3	2.1±0.7	1.9±0.7
W_V (mJ.kg^{-1}.m^{-1})	563±97	1205±1053	2137±129	2574±719
W_H (mJ.kg^{-1}.m^{-1})	45±16	95±40	43±9	53±25

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Table 2(on next page)

Results of the two-way ANOVA

Variable	**Gradient effect**	**Substrate effect**	**Gradient x Substrate**
Speed	F = 0.984; df = 1; p = 0.377	F = 0.146; df = 1; p = 0.722	F = 1.504; df = 1; p = 0.287
Duty Factor (D_F)	F = 0.080; df = 1; p = 0.791	F = 1.720; df = 1; p = 0.260	F = 0.460; df = 1; p = 0.535
Stride length (L_S)	F = 0.046; df = 1; p = 0.841	F = 3.166; df = 1; p = 0.150	F = 0.980; df = 1; p = 0.378
Relative stride length (L_{SR})	F = 1.000; df = 1; p = 0.374	F = 0.001; df = 1; p = 0.977	F = 3.380; df = 1; p = 0.140
Stride frequency (F_S)	F = 1.463; df = 1; p = 0.293	F = 0.138; df = 1; p = 0.729	F = 0.281; df = 1; p = 0.624
b_{COM} height (H_{COM})	F = 4.108; df = 1; p = 0.113	**F = 24.874; df = 1; p = 0.008**	**F = 28.139; df = 1; p = 0.006**
External work (W_{EXT})	**F = 19.260; df = 1; p = 0.012**	F = 4.645; df = 1; p = 0.097	F = 0.096; df = 1; p = 0.772
Internal work (W_{INT})	**F = 9.041; df = 1; p = 0.040**	**F = 33.972; df = 1; p = 0.004**	F = 1.677; df = 1; p = 0.265
Total work (W_{TOT})	**F = 19.204; df = 1; p = 0.012**	F = 4.672; df = 1; p = 0.097	F = 0.096; df = 1; p = 0.773
Recovery	F = 5.622; df = 1; p = 0.077	F = 2.038; df = 1; p = 0.227	F = 1.656; df = 1; p = 0.268
Vertical work (W_V)	**F = 19.191; df = 1; p = 0.012**	F = 4.506; df = 1; p = 0.101	F = 0.108; df = 1; p = 0.759
Horizontal work (W_H)	F = 5.642; df = 1; p = 0.076	**F = 17.760; df = 1; p = 0.014**	F = 4.090; df = 1; p = 0.113

Table 3(on next page)

Time lags and duty factors of each leg pair for different gaits (mean ± standard deviation).

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	F_l1	**H_l1**	**P_l1**	**F_l2**	**H_l2**	**P_l2**
Diagonal	0.38±0.13	0.51±0.19	0.45±0.16	0.31±0.15	0.54±0.14	0.45±0.07
Lateral	0.40±0.11	0.72±0.54	0.72±0.37	0.38±0.18	0.55±0.15	0.57±0.12
Trot	0.42±0.15	0.51±0.09	0.49±0.14	0.44±0.09	0.51±0.04	0.43±0.08
Bound	0.14±0.08	0.23±0.21	0.44±0.08			

	$D_{FFl}1$	**$D_{FHl}1$**	**$D_{FPl}1$**	**$D_{FFl}2$**	**$D_{FHl}2$**	**$D_{FPl}2$**
Diagonal	0.53±0.06	0.61±0.09	0.56±0.08	0.68±0.08	0.65±0.07	0.67±0.06
Lateral	0.49±0.04	0.58±0.10	0.52±0.08	0.66±0.06	0.71±0.04	0.69±0.04
Trot	0.50±0.02	0.51±0.04	0.52±0.04	0.63±0.07	0.65±0.04	0.64±0.04
Bound	0.53±0.04	0.60± 0.04	0.59±0.04			