

Fig. S1. Walking parameters - single leg pair analysis.

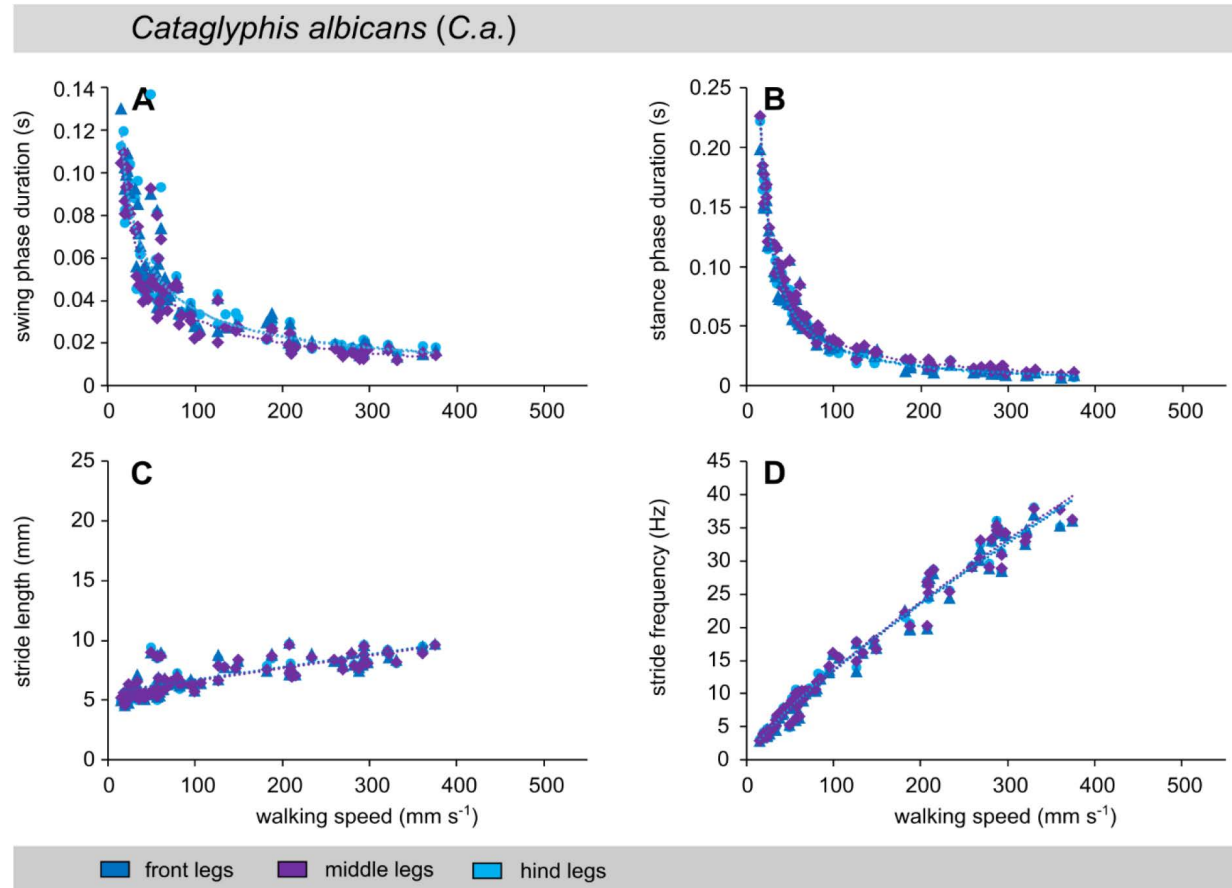


Fig. S2. Walking parameters – single leg pair analysis. (A-L) Individual values for the respective leg pairs (LP1, front legs; LP2, middle legs; LP3, hind legs). (A, E, I) Swing phase durations (power functions: **C.b.-I**: LP1: $y=0.31x^{-0.42}$, $R^2=0.86$, LP2: $y=0.20x^{-0.35}$, $R^2=0.75$, LP3: $y=0.31x^{-0.40}$, $R^2=0.83$; **C.b.-III**: LP1: $y=0.46x^{-0.48}$, $R^2=0.70$, LP2: $y=0.33x^{-0.44}$, $R^2=0.67$, LP3: $y=0.46x^{-0.48}$, $R^2=0.70$; **C.a.**: LP1: $y=0.70x^{-0.64}$, $R^2=0.92$, LP2: $y=0.65x^{-0.65}$, $R^2=0.90$, LP3: $y=0.62x^{-0.62}$, $R^2=0.87$). (B, F, J) Stance phase durations (power functions: **C.b.-I**: LP1: $y=7.63x^{-1.07}$, $R^2=0.95$, LP2: $y=9.28x^{-1.09}$, $R^2=0.96$, LP3: $y=12.25x^{-1.19}$, $R^2=0.94$; **C.b.-III**: LP1: $y=6.01x^{-0.99}$, $R^2=0.94$, LP2: $y=6.17x^{-0.97}$, $R^2=0.94$, LP3: $y=6.01x^{-0.99}$, $R^2=0.94$; **C.a.**: LP1: $y=3.58x^{-1.02}$, $R^2=0.97$, LP2: $y=3.16x^{-0.96}$, $R^2=0.98$, LP3: $y=3.84x^{-1.04}$, $R^2=0.98$). (C, G, K) Stride lengths (linear regressions: **C.b.-I**: LP1: $y=0.02x+8.21$, $R^2=0.83$, LP2: $y=0.02x+8.11$, $R^2=0.84$, LP3: $y=0.02x+8.17$, $R^2=0.83$; **C.b.-III**: LP1: $y=0.02x+9.25$, $R^2=0.76$, LP2: $y=0.02x+9.25$, $R^2=0.79$, LP3: $y=0.02x+9.25$, $R^2=0.77$; **C.a.**: LP1: $y=0.01x+5.81$, $R^2=0.62$, LP2: $y=0.01x+5.75$, $R^2=0.65$, LP3: $y=0.01x+5.67$, $R^2=0.63$). (D, H, L) Stride frequencies (logarithmic regressions: **C.b.-I**: LP1: $y=10.11\ln(x)-35.80$, $R^2=0.93$, LP2: $y=10.05\ln(x)-35.48$, $R^2=0.93$, LP3: $y=10.16\ln(x)-35.99$, $R^2=0.93$; **C.b.-III**: LP1: $y=8.76\ln(x)-31.11$, $R^2=0.91$, LP2: $y=8.59\ln(x)-30.37$, $R^2=0.92$, LP3: $y=8.76\ln(x)-31.11$, $R^2=0.91$; linear regressions: **C.a.**: LP1: $y=0.10x+2.95$, $R^2=0.97$, LP2: $y=0.15x+2.77$, $R^2=0.97$, LP3: $y=0.11x+2.84$, $R^2=0.97$). Statistically significant differences between the three leg pairs occurred for swing phase durations (t-test, $**P<0.05$) in *C.b.-I*, *C.b.-III* and *C.a.*, and for stance phase durations (t-test, $*P<0.05$) in *C.b.-I* and *C.a.* This characteristic of desert ant locomotion had already been described for *Cataglyphis fortis*: middle legs perform the highest swing speeds and therefore spend less time in swing phase than front and hind legs (see Wahl et al., 2015). Therefore, and due to the fact that all three ant groups exhibited this characteristic, walking parameters were averaged for all six legs in the main analysis despite small differences.

Wahl, V., Pfeffer, S. E. and Wittlinger, M. (2015). Walking and running in the desert ant *Cataglyphis fortis*. *J. Comp. Physiol. A*, **201**, 645-656. doi:10.1007/s00359-015-0999-2

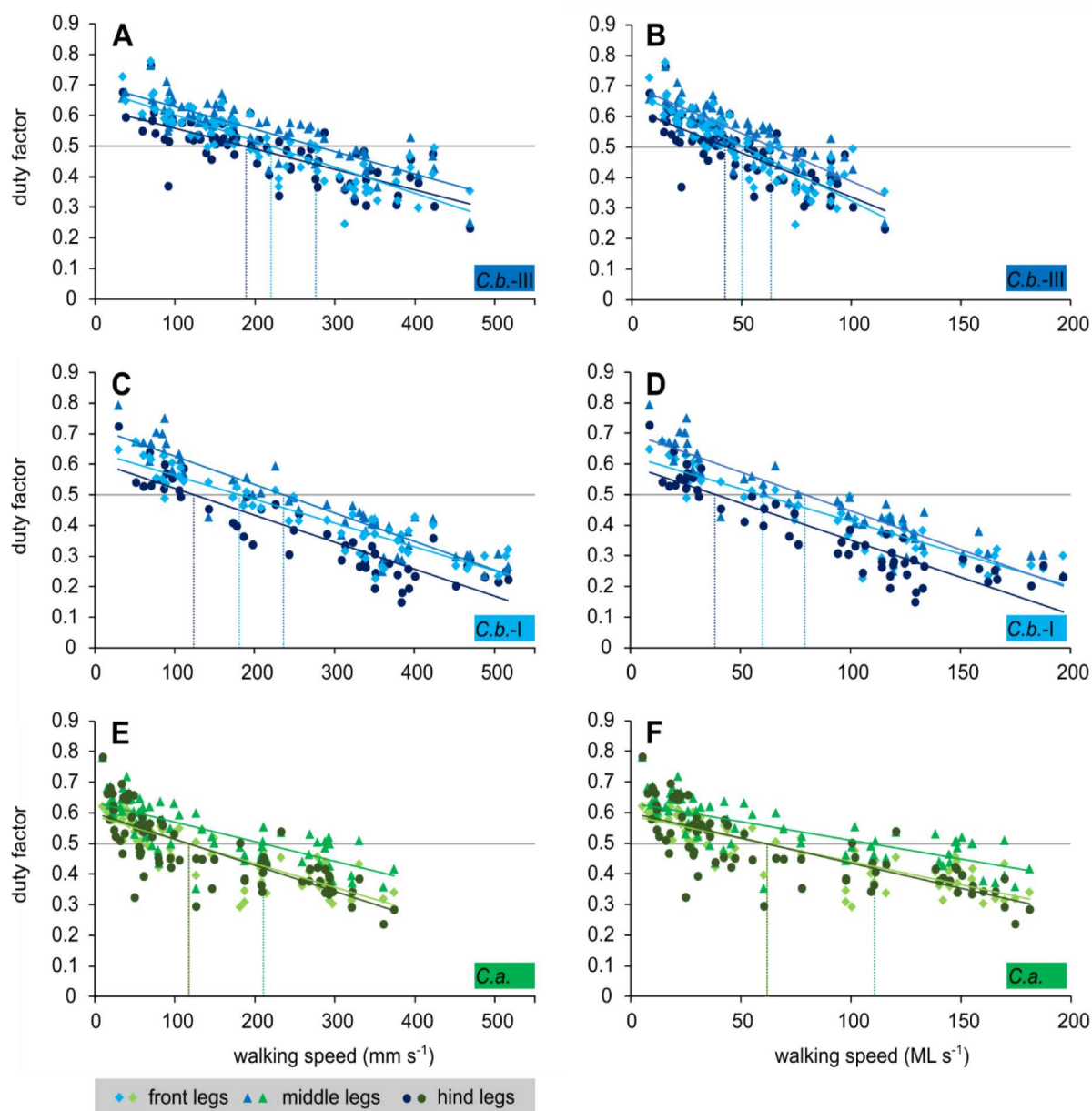


Fig. S3. Duty factor. Duty factor (ratio of stance phase to cycle period) plotted as function of **absolute** (left) and **relative** (right) walking speeds for the three leg pairs. With increasing walking speed, duty factor decreased approximately linearly for all three leg pairs in all ant groups. *C. albicans* changed from walking to running (duty factor for all leg pairs below 0.5) at the lowest absolute walking speeds, while on a relative scale this relationship was reversed, and the transition from walking to running occurred in *C. bicolor* at lower relative walking speeds than in *C. albicans*. **(A)** *C. b. -III* absolute walking speeds (duty factor dropping below 0.5: LP1: 220 mm s^{-1} , LP2: 276 mm s^{-1} , LP3: 190 mm s^{-1}); **(B)** *C. b. -III* relative walking speeds (duty factor dropping below 0.5: LP1: 50 ML s^{-1} , LP2: 63 ML s^{-1} , LP3: 43 ML s^{-1}); **(C)** *C. b. -I* absolute walking speeds (duty factor dropping below 0.5: LP1: 181 mm s^{-1} , LP2: 236 mm s^{-1} , LP3: 124 mm s^{-1}); **(D)** *C. b. -I* relative walking speeds (duty factor dropping below 0.5: LP1: 60 ML s^{-1} , LP2: 81 ML s^{-1} , LP3: 39 ML s^{-1}); **(E)** *C. a.* absolute walking speeds (duty factor dropping below 0.5: LP1: 118 mm s^{-1} , LP2: 210 mm s^{-1} , LP3: 118 mm s^{-1}); **(F)** *C. a.* relative walking speeds (duty factor dropping below 0.5: LP1: 62 ML s^{-1} , LP2: 111 ML s^{-1} , LP3: 62 ML s^{-1}). Light colors: front legs (leg pair 1, LP1), intermediate colors: middle legs (leg pair 2, LP2), dark colors: hind legs (leg pair 3, LP3).

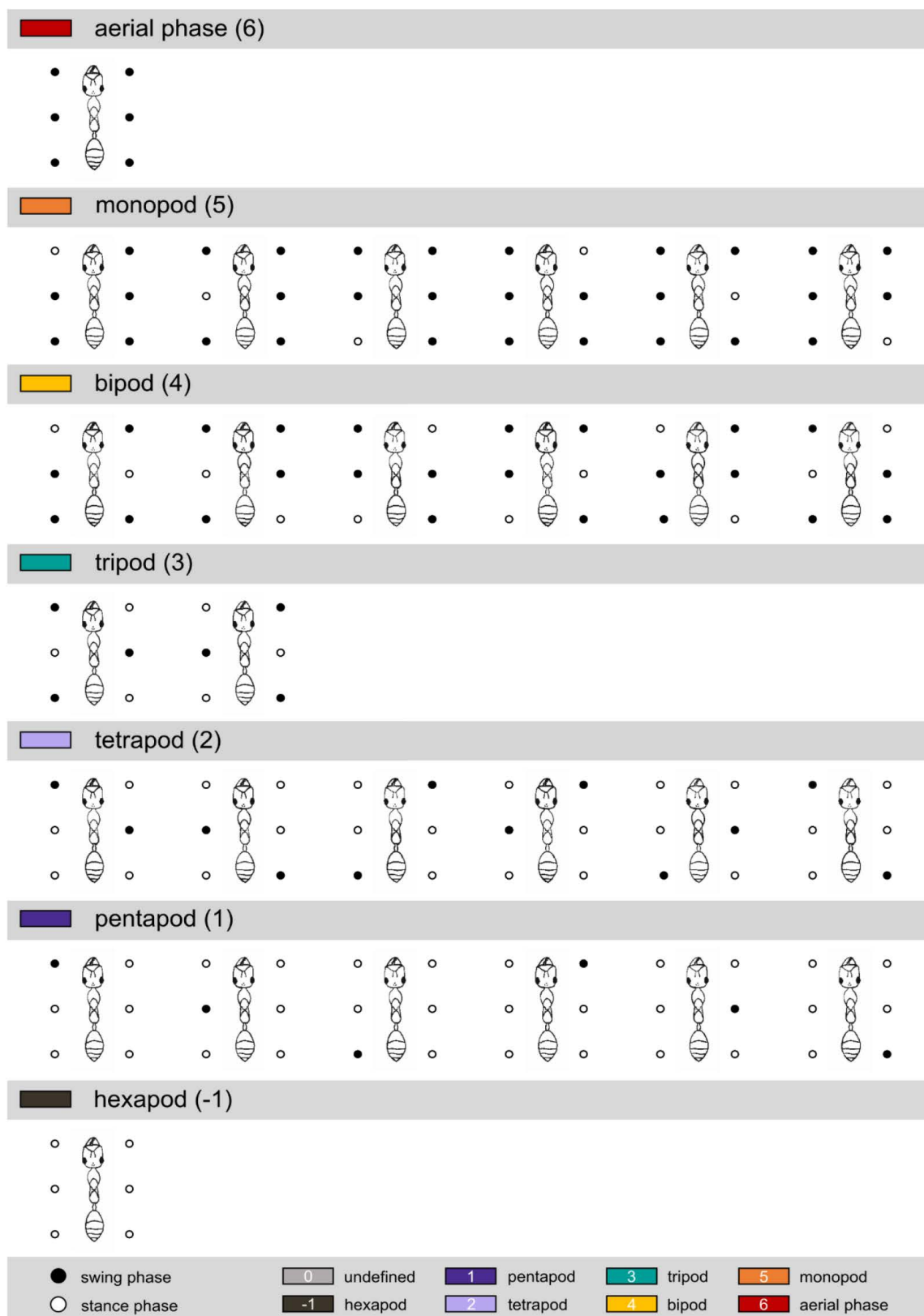


Fig. S4. Gait patterns. Every video frame was assigned a (momentary) leg coordination pattern (colour and number), according to the combination of the six legs in swing and stance phase. If none of the described combinations was applicable, the frame was assigned 'undefined' (grey, 0). Note that the walking patterns are defined by a certain combination of specific legs in air and on the ground and not only by the absolute number of legs in swing or stance phase.

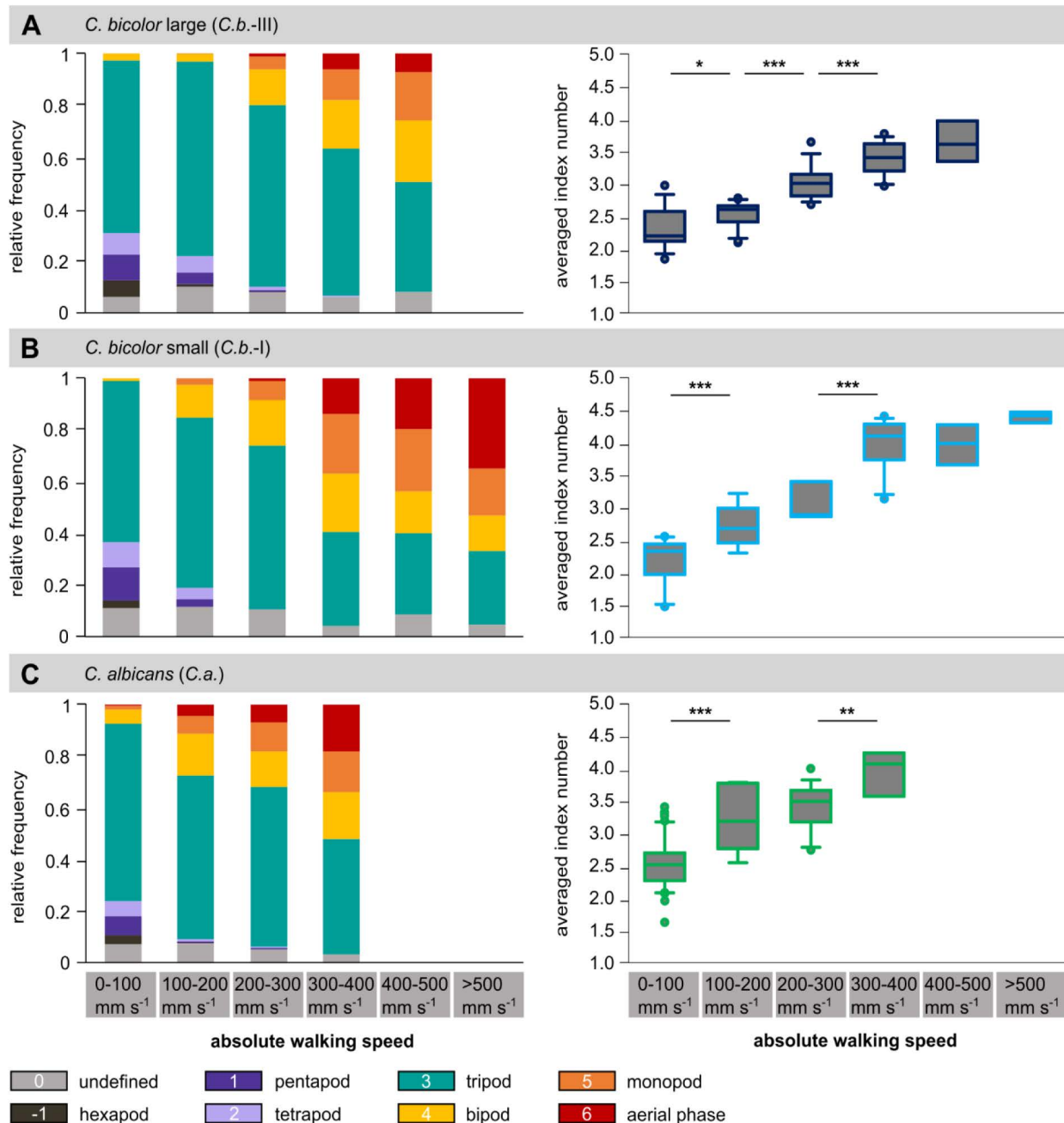


Fig. S5. Gait analysis. (A-C) Quantitative gait analysis according to the respective walking speed classes (<100 to >500 mm s⁻¹) for small ants of *C. bicolor* (A, n=57), large ants of *C. bicolor* (B, n=75) and ants of *C. albicans* (C, n=60). The data are shown as relative frequency plots (colour index, left) and averaged index number (number index, right) for the respective walking speed bins. Each video frame was assigned a colour and a number index according to the leg coordination in every particular frame (see colour and number code key at the bottom of the figure, and above Fig. S3). No data were available for the >500 mm s⁻¹ walking speed class in *C.b.-III* and for the 400-500 mm s⁻¹ and >500 mm s⁻¹ walking speed classes in *C.a.* Towards higher walking speeds (>300 mm s⁻¹), tripod coordination decreases and combinations with less than three legs in stance increase. Aerial phases start to emerge in *C. bicolor* in walking speed class 200-300 mm s⁻¹, while *C. albicans* inserts aerial phases clearly earlier (<100 mm s⁻¹). For the 300-400 mm s⁻¹ speed bin, the higher tripod fraction in *C.a.* compared to *C.b.-I* is notable (*C.b.-I*: 36.3%, *C.a.*: 47.1%), as is the steep drop in tripod fraction from the 200-300 mm s⁻¹ to the 300-400 mm s⁻¹ speed bin in *C.b.-I*. Walking speeds above 400 mm s⁻¹ are reached only by *C. bicolor*, and *C.b.-I* ants have a higher proportion of aerial phases (400-500 mm s⁻¹: *C.b.-I*: 19.8%, *C.b.-III*: 7.5%). Statistically significant differences in number index between adjacent speed bins are marked with asterisks (one-way ANOVA, Holm-Sidak method).

Table S1. Leg allometry in *Cataglyphis bicolor* and *Cataglyphis albicans*. To describe the relationship between mesosoma length and leg length we used the allometric equation $y = a \times x^b$. We estimated the scaling factor a and the exponent b for *C. bicolor* and *C. albicans* with their 95%-confidence intervals, respectively (see also Sommer and Wehner, 2012). The values are given for the allometric equation with y and x representing leg and mesosoma lengths, respectively. R^2 is the coefficient of determination. For data analysis, we used the software package (S)MATR v.2.0 (Warton et al., 2006). To convert the power function into a linear equation we used log10-transformations (i.e. $\log y = \log a + b \times \log x$). Both desert ant species have b -values near 1 (between 0.908 and 1.109), demonstrating nearly isometric scaling between leg and mesosoma lengths within the species. That means, smaller individuals have proportionally shorter legs. Generally, longer legs are illustrated by higher values of scaling factor a (see also Sommer and Wehner, 2012). Scaling factors can only be compared between species with similar b values (indicated by similar slopes in log-log plots, see Fig. 2). The allometric equation provides a good fit to the data ($R^2 > 0.93$).

		a	b	R²
<i>C. bicolor</i> (n=237)	Front leg	1.594 (1.535; 1.655)	1.091 (1.063; 1,118)	0.961
	Middle leg	1.848 (1.781; 1,918)	1.109 (1.083; 1.137)	0.964
	Hind leg	2.675 (2.581; 2.772)	1.043 (1.017; 1.069)	0.962
<i>C. albicans</i> (n=108)	Front leg	1.845 (1.787; 1.904)	0.984 (0.938; 1.038)	0.937
	Middle leg	2.065 (2.005; 2.128)	0.955 (0.911; 1.001)	0.941
	Hind leg	2.957 (2.875; 3.042)	0.908 (0.867; 0.951)	0.942

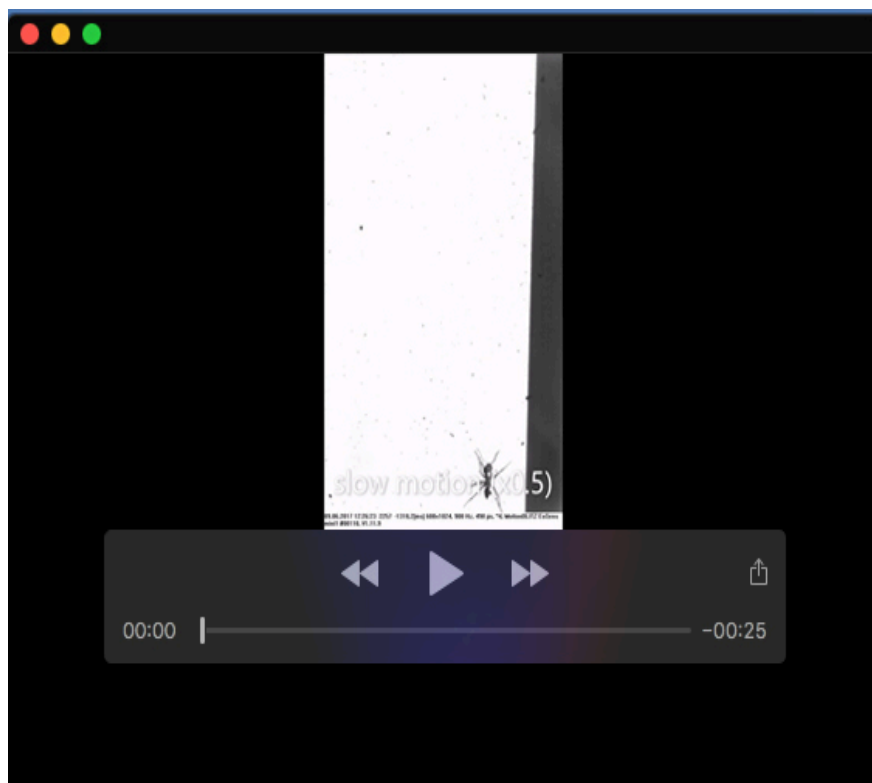
Warton, D. I., Wright, I. J., Falster, D. S. and Westoby, M. (2006). Bivariant line-fitting methods for allometry. *Biol. Rev.* **81**, 259-291. doi: 10.1017/S1464793106007007

Table S2. Leg allometry in *Cataglyphis bicolor*. To describe the relationship between mesosoma length and leg length we used the allometric equation $y = a \times x^b$ with scaling factor a and exponent b with their respective 95%-confidence intervals (see also Sommer and Wehner, 2012) (Table S1). The values are given for the allometric equation with y and x representing leg length and mesosoma length, respectively. R^2 is the coefficient of determination. Values were calculated for *C.b.-I*, *C.b.-II*, *C.b.-III* (see also Fig. 2). *C.b.-I* and *C.b.-III* have b -values slightly above 1 (1.060 to 1.261), demonstrating nearly isometric (slightly positive) scaling between leg and mesosoma lengths within the two *C. bicolor* groups. Ants of *C.b.-II* (grey) cannot be compared with *C.b.-I* and *C.b.-III* because the range of mesosoma lengths (0.5 mm) is too small and was only chosen to clearly delineate the small and large individuals and avoid overlaps impeding comparison. We also ran a test for common slope across the groups (critical P : 0.05, CI 95%): Regarding the slopes, there were no significant differences between *C.b.-I* and *C.b.-III* for the middle and hind legs (*C.b.-I* vs. *C.b.-III*: front leg: $P=0.014$, middle leg: $P=0.096$, hind leg: $P=0.116$).

		a	b	R ²
<i>C.b.-I</i> (n=51)	Front leg	1.365 (1.213; 1.535)	1.236 (1.132; 1.349)	0.906
	Middle leg	1.573 (1.389; 1.782)	1.261 (1.151; 1.381)	0.899
	Hind leg	2.325 (2.076; 2.603)	1.175 (1.075; 1.284)	0.904
<i>C.b.-II</i> (n=55)	Front leg	0.689 (0.460; 1.033)	1.731 (1.454; 2.061)	0.595
	Middle leg	0.918 (0.617; 1.366)	1.646 (1.375; 1.972)	0.567
	Hind leg	1.416 (0.986; 2.035)	1.531 (1.283; 1.827)	0.584
<i>C.b.-III</i> (n=131)	Front leg	1.685 (1.462; 1.941)	1.048 (0.957; 1.149)	0.722
	Middle leg	1.772 (1.546; 2.031)	1.133 (1.044; 1.229)	0.780
	Hind leg	2.590 (2.240; 2.995)	1.060 (0.966; 1.163)	0.716



Movie 1. Locomotion in *Cataglyphis bicolor* (original speed and slow motion). Example high-speed video of a small *C. bicolor* ant moving at an intermediate walking speed (118 ML s⁻¹ or 348 mm s⁻¹).



Movie 2. Locomotion in *Cataglyphis albicans* (original speed and slow motion). Example high-speed video of *C. albicans* ant moving at an intermediate walking speed (148 ML s⁻¹ or 288 mm s⁻¹).