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Supplemental Information

Diversity in Morphology and Locomotory Behavior Is Associated with Niche Expansion in the Semi-aquatic Bugs

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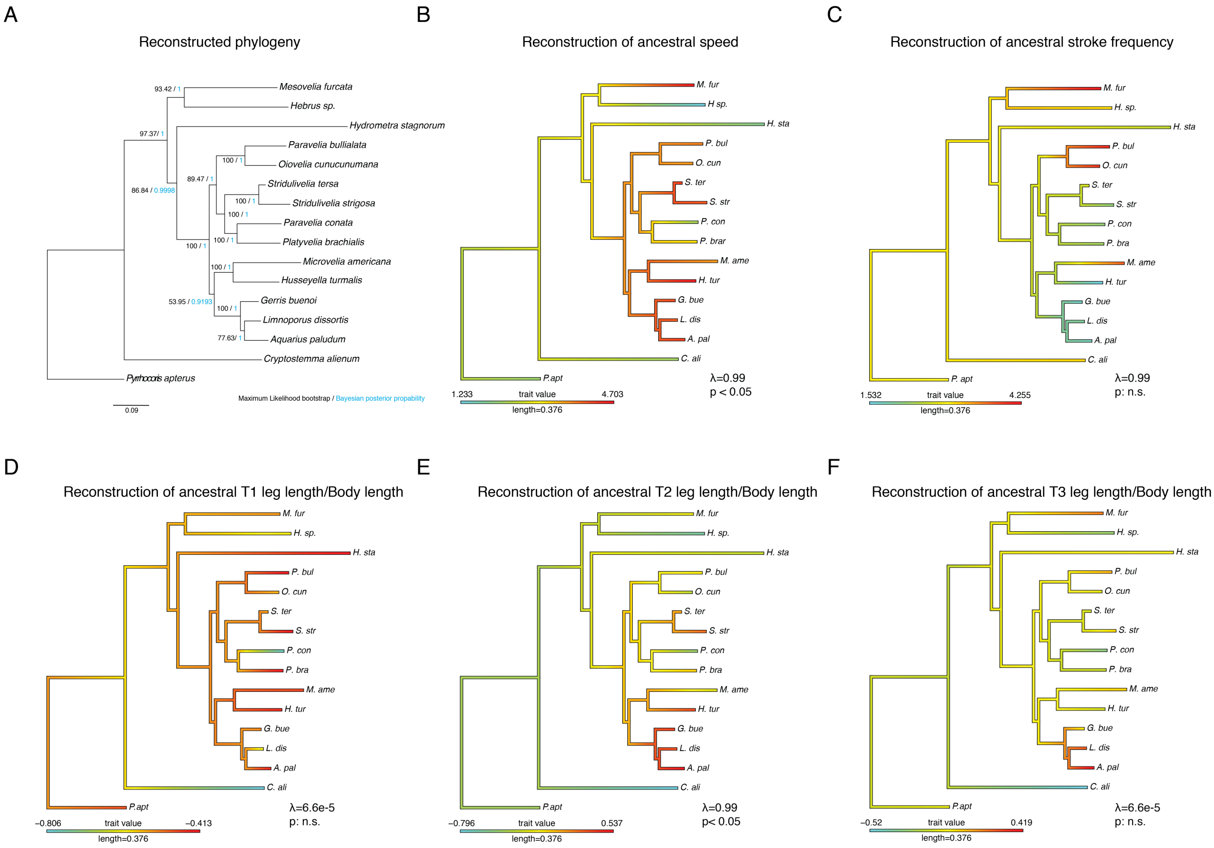

Figure S1: Phylogeny and ancestral character state reconstruction. Related to Figure 1, Figure 2 and Figure S4, Table S1, Table S2.

(A) Phylogenetic relationships between species sampled for this study. Phylogenetic construction of our sample using both Maximum Likelihood (bootstrap in black) and Bayesian methods (posterior probability in blue). (B) Phylogenetic reconstruction of ancestral state of speed of locomotion in the Gerromorpha and terrestrial outgroups. Increased speed is a derived state and correlates with preference for water surface habitat. (C) Phylogenetic reconstruction of ancestral state of stroke frequencies. Increased stroke frequency is a derived state and correlates with preference for water surface habitat for species using the tripod gait but not for species using the rowing gait. Phylogenetic reconstruction of ancestral state of the ratio leg length by body length for the foreleg (D), the midleg (E) and the hindleg (F). The elongated midleg is a derived state and correlates with preference for water surface habitat. Phylogenetic signals λ and associated significance are indicated for each ancestral reconstruction.

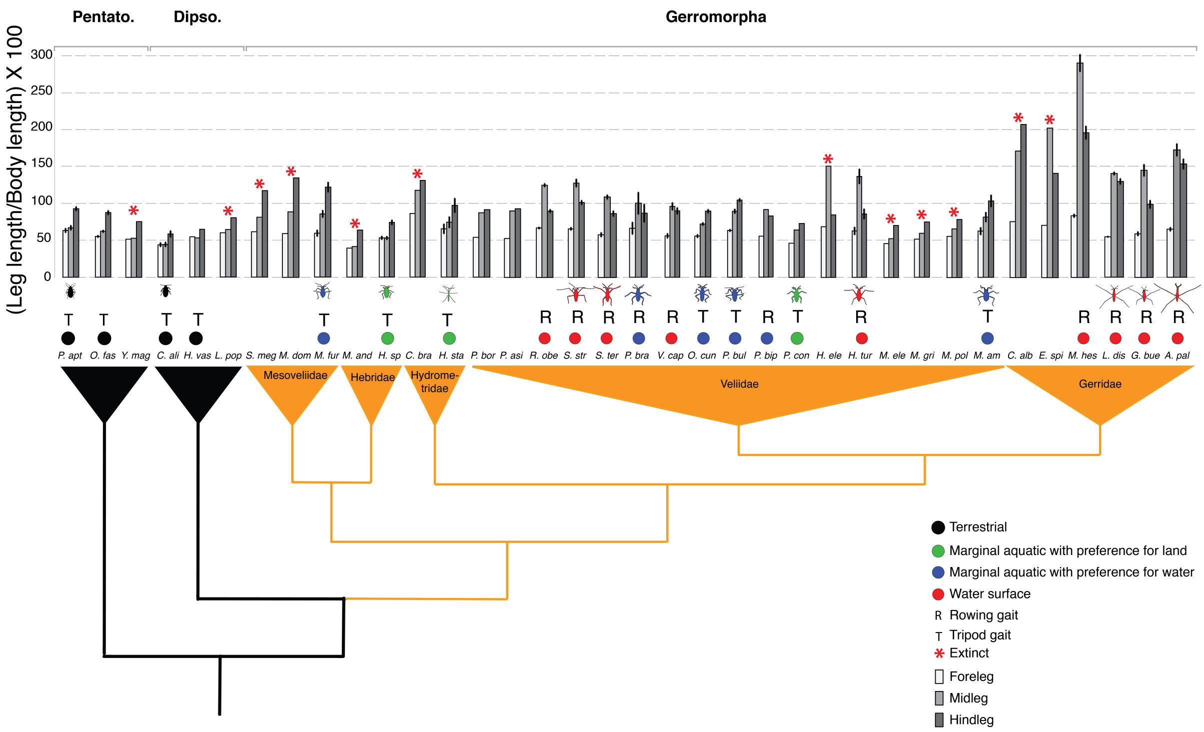

Figure S2: Ratio of leg length to body length across heteroptera including fossil record. Related to Figure 2, Table S2.

Phylogenetic relationships and ratios of leg length by body length across a sample of extant and extinct Heteropteran species. Extant Gerromorpha have generally longer legs than terrestrial relatives. *O.fas*, *Oncopeltus fasciatus*; *Y.mag*, *Yuripopovina magnifica* [S1]; *H.vas*, *Hypsipteryx vasarhelyii* [S2]; *L.pop*, *Libanohypselosoma popovi* [S3, S4]; *M.dom*, *Mesovelia dominicana* [S5]; *M.and*, *Miohebrus anderseni* [S5]; *C.bra*, *Cretaceometra brasiliensis* [S6]; *P.bor*, *Perittopus borneensis* [S7]; *P.asi*, *Perittopus asiaticus* [S7]; *R.obe, Rhagovelia obesa*; *V.cap*, *Velia caprai*; *H.ele, Halovelia electrodominica* [S8]; *M.ele, Microvelia electra* [S9]; *M.gri*, *Microvelia grimaldii* [S9]; *M.pol*, *Microvelia polhemi* [S10]; *C.alb, Cretogerris albianus* [S11]; *E.spi*, *Electrobates spinipes* [S12]; *M.hes, Metrobates hesperius*. Red asterisks indicate extinct species. Error bars represent the standard deviation.

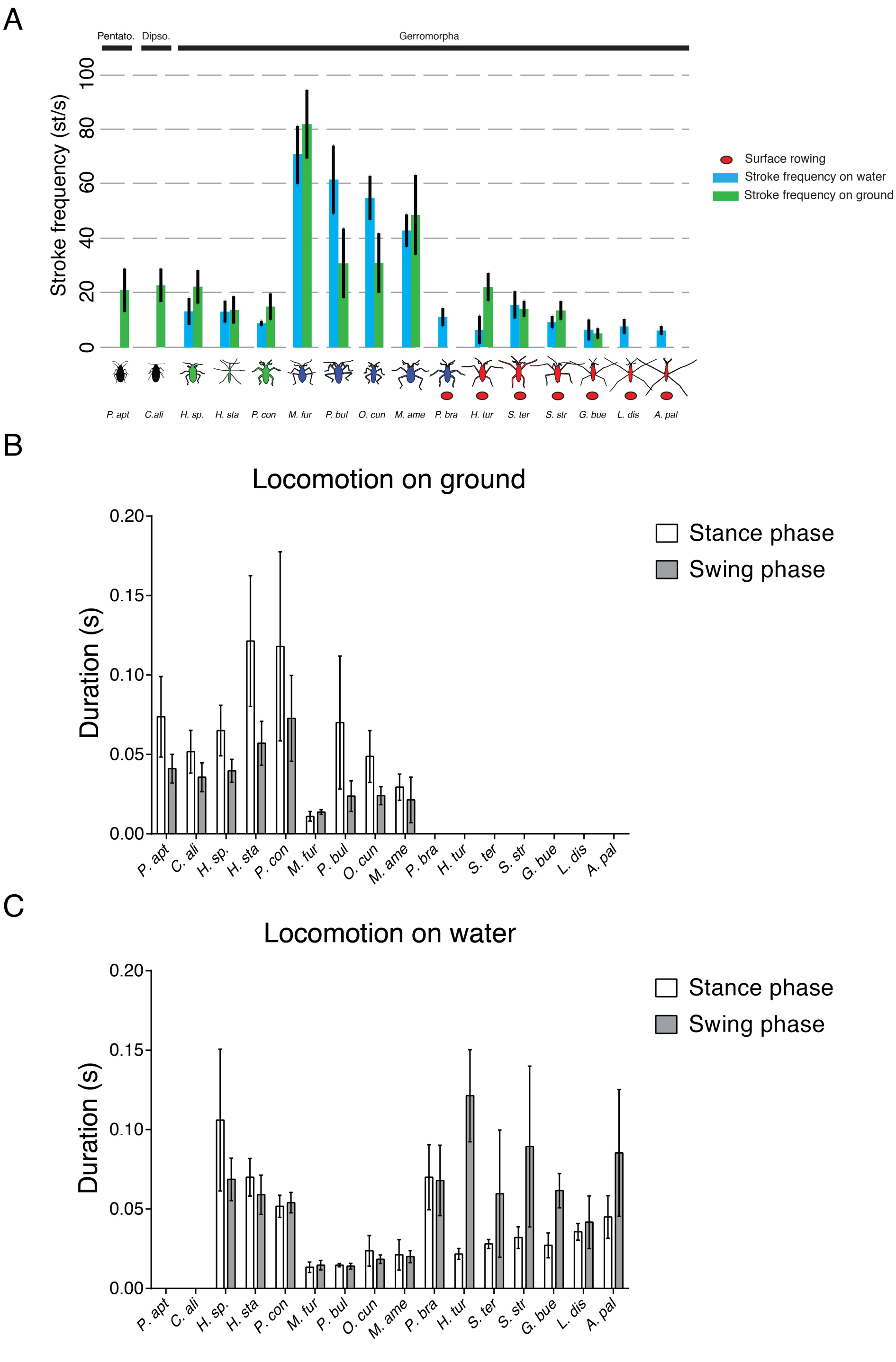

Figure S3: Stroke frequency and leg motion pattern across the Gerromorpha and terrestrial outgroups. Related to Figure 3, Figure 4 and Figure S4, Table S1, Table S3.

(A) Quantification of stroke frequencies during locomotion on ground (green bars) and on water (blue bars). Low stroke frequencies for species using the tripod gait are associated with life on ground. High stroke frequencies are associated with life on water surface for species using the tripod gait. Low stroke frequencies are associated with life on water surface only for species using the derived rowing gait. (B) Analysis of leg motion pattern on ground showing that the stance phase is longer than the swing phase for species using the tripod gait. (C) The same analysis on water showing that species with preference for water and using the tripod gait have similar stance and swing phases whereas species with preference for ground still have a longer stance phase than swing phase. Derived species using the rowing gait have a shorter stance phase compared to swing phase. This pattern is associated with life on water surface. Error bars represent standard deviation.

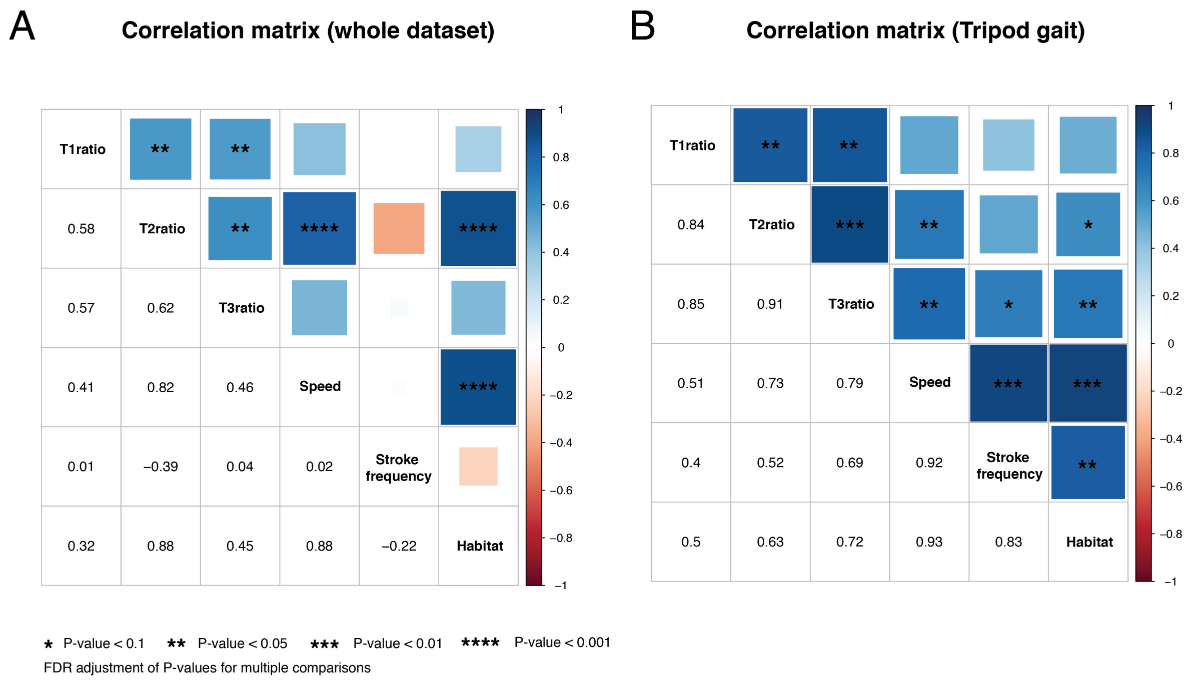

Figure S4: Matrix of correlation for the different correlation tests performed. Related to **Figure 1, Figure 2, Figure 3, Figure 4 and Figure S1, Figure S3 and Table S1.**

(A) Matrix of correlation for the whole dataset (tripod gait and rowing gait) and for (B) the dataset of species using only the tripod gait. Squares and number indicates the rho of the Sperman correlation test conducted with phylogenetic correction (PIC). FDR adjustment of P-values for multiple comparisons is applied. Stars indicate the P-values.

Species	Speed on ground (cm/s)	Speed on ground (bl/s)	Speed on water (cm/s)	Speed on water (bl/s)	Stroke frequency on ground (st/s)	Stroke frequency on water (st/s)	Mean weight (mg)	Energy per stroke ((mJ/mg)/st)	P-Value	Energy per second ((mJ/mg)/s)	P-Value
Pyrrhocoris apterus	8.6±3.2 (N=14)	9.1±3.4 (N=14)	NA	NA	20.4±7.7 (N=14)	NA	NA	NA	NA	NA	NA
Cryptostemma alienum	2.3±0.7 (N=14)	9.4±2.8 (N=14)	NA	NA	23.5±6.3 (N=14)	NA	NA	NA	NA	NA	NA
***Hebrus* sp.**	1.2±0.9 (N=10)	6.0±4.4 (N=10)	1.3±0.6 (N=10)	6.4±2.7 (N=10)	22.6±6.2 (N=10)	13.4±5.0 (N=10)	NA	NA	NA	NA	NA
Hydrometra stagnorum	8.0±4.4 (N=10)	7.6±4.2 (N=10)	8.1±2.6 (N=10)	7.8±2.5 (N=10)	13.7±4.9 (N=10)	13.1±3.9 (N=10)	NA	NA	NA	NA	NA
Paravelia conata	4.7±1.5 (N=10)	8.9±2.8 (N=10)	5.0±0.04 (N=3)	9.4±0.9 (N=3)	15.1±4.8 (N=10)	8.9±0.9 (N=6)	NA	NA	NA	NA	NA
Mesovelia furcata	26.1±9.3 (N=10)	74.4±37.6 (N=10)	33.1±7.5 (N=12)	103.6±23.6 (N=12)	82.2±12.7 (N=10)	71.2±10.7 (N=12)	1.16	1.3318e-05	0.08 n.s.	2.0058782e-2	4.6e-4 ***
Oiovelia cunucunumana	8.4±4.5 (N=10)	20.6±11.0 (N=10)	16.4±3.3 (N=8)	40.2±8.1 (N=8)	31.1±10.9 (N=10)	55.0±8.0 (N=10)	2.92	8.06773e-06		1.4839771e-2	
Paravelia bullialata	8.9±5.1 (N=10)	23.0±13.3 (N=10)	18.3±3.6 (N=10)	47.4±9.2 (N=10)	30.4±12.6 (N=10)	60.7±12.3 (N=10)	2.925	1.17074e-05		2.3499226e-2	
Microvelia americana	9.5±4.7 (N=9)	33.5±16.5 (N=9)	11.2±2.3 (N=8)	39.5±8.0 (N=8)	48.2±14.4 (N=10)	42.4±5.9 (N=9)	1.53	3.6114e-06		7.093716e-3	
Stridulivelia strigosa	3.8±1.2 (N=10)	6.8±4.5 (N=10)	28.1±6.5 (N=10)	64.4±15.0 (N=10)	13.3±3.4 (N=10)	9.1±2.2 (N=10)	2.733	2.21034e-05		2.92093e-4	
Stridulivelia tersa	5.3±1.7 (N=10)	11.0±3.5 (N=10)	37.1±8.1 (N=11)	76.3±16.6 (N=11)	14.1±2.9 (N=10)	15.5±4.9 (N=22)	3.65	5.06027e-05		3.276652e-3	
Platyvelia brachialis	NA	NA	12.4±4.7 (N=18)	26.5±10.1 (N=18)	NA	10.6±3.2 (N=35)	6.233	5.98386e-06		5.43207e-4	
Husseyella turmalis	2.8±0.9 (N=9)	13.8±4.3 (N=9)	23.2±5.5 (N=10)	113.0±26.8 (N=10)	21.2±4.9 (N=9)	6.1±5.0 (N=10)	0.5	8.38042e-07		3.00e-05	
Limnopor	NA	NA	80.6±8	59.3±6	NA	7.6±2.	31.1	1.38796		9.90729	

us dissortis			.8 (N=8)	.5 (N=8)		6 (N=8)	83	e-04		e-4	
Gerris buenoi	1.3±0.7 (N=8)	1.7±0.9 (N=8)	53.7±12.7 (N=8)	69.8±16.6 (N=8)	5.0±1.8 (N=8)	6.3±3.7 (N=8)	12.2	7.87642e-05		5.82314e-4	
Aquarius paludum	NA	NA	104.1±12.2 (N=8)	74.4±8.7 (N=8)	NA	6.1±1.6 (N=8)	34.667	2.23603e-04		8.10206e-4	

Table S1: Data of locomotion characteristics across the sample of species tested. Related to Figure 1, Figure 2, Figure 4, Figure S1, Figure S3. Mean values for speed in centimeters per second (cm/s) and body length per second (bl/s) and stroke frequency in number of strokes per second (st/s), on ground and water for species analyzed with sample size (N). ± Indicates the standard deviation. NA: not available. Energy expenditure per stroke and per second for each species with the associated mean weight. Highlighted in clear grey are species using the tripod gait and in dark grey are species using the rowing gait. Student t-tests are performed to compare the mean energy expenditure per stroke and per second between the two ways of locomotion.

Infraorder	Species	Habitat	T1-leg (µM)	T2-leg (µM)	T3-leg (µM)	Body (µM)	%T1-leg/Body	%T2-leg/Body	%T3-leg/Body	N	Location Or Reference
Pentatomomorpha	*Pyrrhocoris apterus*	Land	5869±353	6206±429	8596±508	9435±649	62±3	66±5	91±3	10	Lyon, France
Pentatomomorpha	*Oncopeltus fasciatus*	Land	6776±250	7628±273	10730±500	12453±426	55±1.6	61±1.8	87±3	10	Samples generously provided by Jeremy Lynch.
Pentatomomorpha	*Yuripopovina magnifica*		2530	2590	3690	4840	52	54	76	1*	[S1]
Dipsocoromorpha	*Cryptostemma alienum*	Land (can stand on water)	1078±61	1088±38	1436±104	2414±146	44±3	45±3.4	60±4.7	6	Rivière de l'Eyrieux, St Martin de Valamas, France
Dipsocoromorpha	*Hypsipteryx vasarhelyii*	Land	1088	1060	1292	1945	56	54	66	1	[S2]
Dipsocoromorpha	*Libanohypselosoma popovi*	--	560	601	749	906	62	66	83	1*	[S3]
Gerromorpha	*Sinovelia mega*	--	2780	3670	5300	4460	62	82	119	1*	[S4]
Gerromorpha	*Mesovelia dominicana*	--	1010	1510	2290	1700	59	89	135	1*	[S5]
Gerromorpha	*Mesovelia furcata*	Land/ Water (prefer water)	1859±94	2680±173	3822±299	3180±295	59±4.5	85±5	120±6.8	10	Plan d'eau de Saloniques, Vilette d'Anthon, France
Gerromorpha	*Miohebrus anderseni*		1310	1380	2130	3220	41	43	066	1*	[S5]
Gerromorpha	*Hebrus* sp.	Land/Water (prefer land)	1124±38	1124±42	1568±53	2081±47	54±2.5	54±2.5	75±3.6	9	Plan d'eau de Saloniques, Vilette d'Anthon, France
Gerromorpha	*Cretaceometra brasiliensis*		9600	13100	14600	11300	85	116	129	1*	[S6]
Gerromorpha	*Hydrometra stagnorum*	Land /water (prefer land)	6801±400	7725±398	10089±567	10425±1538	66±6.8	75±7.8	98±9.9	10	Lac de Miribel, Lyon, France
Gerromorpha	*Perittopus borneensis*	Land/ Water	1511	2440	2559	2700	56	90	95	1	[S7]
Gerromorpha	*Perittopus asiaticus*	Land/ Water	1562	2665	2753	2900	54	92	95	1	[S7]
Gerromorpha	*Rhagovelia obesa*	Water	2660±76	4959±119	3569±124	3939±68	68±1.6	126±2.5	91±2.4	10	Rivière du Nord, Montréal, Québec, Canada
Gerromorpha	*Stridulivelia strigosa*	Water	2961±110	5742±133	4564±117	4474±189	66±2.2	128±5.6	102±3.1	8	French Guiana, N 04.31572°, W -052.15396°
Gerromorpha	*Stridulivelia tersa*	Water	2767±117	5211±158	4137±231	4729±119	59±3.2	110±3.1	87±3.9	10	Brazil, N -19,07218°, W-39,79617°
Gerromorpha	*Platyvelia brachialis*	Water	3087±165	4660±351	4037±346	4678±338	66±8.3	100±15	87±12.3	3	Brazil N -18,37143°, W-40,1416
Gerromorpha	*Velia caprai*	Water	4040±142	6926±171	6484±222	7193±378	56±3.3	96±5.3	90±4.5	6	Lac de Miribel, Lyon, France
Gerromorpha	*Oiovelia cunucunumana*	Land/ Water (prefer water)	2090±48	2704±83	3363±146	3627±171	58±2.4	75±2.3	93±2.8	10	Brazil, N -20,25294°, W-44,91584°
Gerromorpha	*Paravelia bullialata*	Land/ Water (prefer water)	2579±85	3637±124	4252±89	3905±125	66±1.8	93±3.4	109±2.5	5	French Guiana, N 04.29769°, W -052.14927°

Gerromorpha	*Paravelia bipunctata*	Land/ Water	2983	4911	4447	5348	56	92	83	1	Brazil N -20,60034°, W-45,81498
Gerromorpha	*Paravelia conata*	Land/ Water (prefer land)	2282	3167	3602	4960	46	64	73	1	Brazil N - 18.367815º , W - 40.139877º
Gerromorpha	*Halovelia electrodominica*	Water (sea)	1030	2260	1270	1500	69	151	85	1*	[S8]
Gerromorpha	*Husseyella turmalis*	Water	1296±78	2820 ±193	1769±98	2023 ±263	65±5.5	140±10.5	88±7.3	6	French Guiana, N 04.34235°, W - 052.10730°
Gerromorpha	*Microvelia electra*	--	620	710	950	1330	47	53	71	1*	[S9]
Gerromorpha	*Microvelia grimaldii*	--	910	1050	1320	1750	52	60	75	1*	[S9]
Gerromorpha	*Microvelia polhemi*	--	1060	1250	1490	1850	57	68	81	1*	[S10]
Gerromorpha	*Microvelia americana*	Land/ Water (prefer water)	1798±76	2344 ±11	2975±139	2859 ±178	68±5.2	88±6.8	112±8.2	10	Rivière du Nord, Montréal, Québec, Canada
Gerromorpha	*Cretogerris albianus*	--	1390	3150	3810	1840	76	171	207	1*	[S11]
Gerromorpha	*Electrobates spinipes*	--	1820	5210	3630	2580	71	202	141	1*	[S12]
Gerromorpha	*Metrobates hesperius*	Water	3828±80	13315±397	8990±298	4596 ±154	83±2.5	290±11.8	196±9	10	Rivière du Nord, Montréal, Québec, Canada
Gerromorpha	*Limnoporus dissortis*	Water	7371±332	18843±844	17439±1172	13580±563	54±0.9	139±2.3	128±4	10	Rivière de l'Acadie, Montréal, Québec, Canada
Gerromorpha	*Gerris buenoi*	Water	4584±140	11247±587	7679±417	7697 ±428	60±3	146±8.2	100±5.7	6	Toronto, Ontario, Canada
Gerromorpha	*Aquarius paludum*	Water	9003±721	23840±1821	21206±1793	13986±1621	65±2.6	171±8.5	152±6.9	8	Plan d'eau de Saloniques, Vilette d'Anthon, France

Table S2: Mean values for leg length and body length with corresponding ratios. Related to Figure 1, Figure 2, Figure 4, Figure S1, Figure S2. Habitat preference, number of individuals and collecting locations or references for each extant or fossil (*) species used in this study are provided. ± indicates the standard deviation. These data are plotted in Figure S2.

	Distance body to leg tip (in mm)						Amplitude of movement (in mm)					
	T1-leg		T2-leg		T3-leg		T1-leg		T2-leg		T3-leg	
	Ground	Water	Ground	Water	Ground	Water	Ground	Water	Ground	Water	Ground	Water
Hebrus sp.	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.2
	p: 0.01 *		p: 0.63 n.s.		p: 0.49 n.s.		p: 0.03 *		p: 0.40 n.s.		p: 0.08 n.s.	
Mesovelia furcata	2.1 ± 0.1	2.0 ± 0.1	2.9 ± 0.2	3.2 ± 0.2	2.7 ± 0.3	4.1 ± 0.2	1.5 ± 0.2	0.3 ± 0.3	3.0 ± 0.5	3.8 ± 0.4	2.3 ± 0.2	2.7 ± 0.5
	p: 0.03 *		p: 6.8 e-4 ***		p: 2.2 e-16 ***		p: 4.89 e-16 ***		p: 2.64 e-6 ***		p: 0.003 **	
Microvelia americana	1.5 ± 0.3	1.7 ± 0.1	2.4 ± 0.3	2.7 ± 0.2	2.2 ± 0.2	3.3 ± 0.2	0.7 ± 0.3	0.3 ± 0.2	1.1 ± 0.5	2.8 ± 0.6	1.3 ± 1.6	0.9 ± 0.5
	p: 0.22 n.s.		p: 1.8 e-4 ***		p: 2.2 e-16 ***		p: 5.09 e-5 ***		p: 8.99 e-11 ***		p: 0.41 n.s.	
Gerris buenoi	NA	2.9 ± 0.7	NA	12.3 ± 0.1	NA	8.5 ± 0.8	NA	0.5 ± 0.4	NA	15.2 ± 0.6	NA	1.0 ± 0.7

Table S3: Comparison of leg deployment and amplitude of movements during ground locomotion and locomotion on water surface. Related to Figure 3.

The locomotion of *Hebrus sp.*, *Mesovelia furcata*, *Microvelia americana* and *Gerris buenoi* are analysed. There is no important differences between ground and water locomotion for *Hebrus sp.*. The hindleg and mostly the midleg become more important during water surface locomotion in *Mesovelia* and *Microvelia*. Midleg is the most important leg for locomotion in *Gerris*. Each number is a mean of 18 measurements for *Hebrus sp.*, *Mesovelia furcata* and *Microvelia americana* and a mean of 6 values for *Gerris buenoi.* (±) indicates standard deviation. Student t- tests were performed.

Movie S1: Comparison between tripod gait and rowing gait during water surface locomotion. *Mesovelia furcata* using the tripod gait (top) and *Gerris buenoi* using the rowing gait (bottom) during water surface locomotion. Videos were taken on a grid for size reference.

Supplemental experimental procedures

Insect sampling and culture

Extant specimens were collected during fieldwork in the locations indicated in Table S2. All species were kept in water tanks at 25°C, 55% humidity, 14 hours of day light and fed on live crickets.

Phylogenetic reconstruction

Sequences were retrieved from in house transcriptome and genomic sequence databases for the following markers: *12S RNA*; *16S RNA*; *18S RNA*; *28S RNA*; *Cytochrome Oxydase subunit I* (*COI*); *Cytochrome Oxydase subunit II* (*COII*); *Cytochorme Oxydase subunit III* (*COIII*); *Cytochrome b* (*cyt b*); *NADH-ubiquinone oxidoreductase chain 1* (*ND1*); *Ultrabithorax* (*Ubx*); *Sex combs reduced* (*Scr*); *Gamma interferon inducible thiol reductase* (*gilt*); *Antennapedia* (*Antp*); *Distal-less* (*dll*). All these markers were submitted to GenBank and their accession numbers can be found in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.134c4. Sequences were aligned with MAFFT version 7.017 [S13] using default parameters. The alignments were visualized, corrected and concatenated in Geneious Version 7.1.9. Phylogenetic analysis was performed with MRBAYES version 3.2.6 [S14] (1 million generations; 25% burnin) and PhyML version 3.0 [S15], using GTR model with 100 bootstraps. Concatenation of sequence alignments and phylogenetic tree in Newick format are also available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.134c4.

Habitat classification

Andersen [S16, S17] classified the various habitats of the Gerromorpha into eight classes: (1) terrestrial habitat far from water, (2) humid terrestrial habitat such as litter or humid gravel not necessary close to water, (3) marginal aquatic comprising moss, plants or rocks close to water, (4) plant-covered water surface, (5) water surface (with some plants), (6) streaming water with hood debris and foam, (7) stagnant water (such as ponds), (8) flowing water (such as streams). For simplicity, we consolidated these classes into the following four: terrestrial (Andersen's 1 and 2); marginal aquatic with preference for solid substrates (Andersen's 3); marginal aquatic with preference for water surface (Andersen's 4 and 5); and open water surface (Andersen's 6, 7 and 8). Each species was assigned to one of these four classes based on previous descriptions [S16-S20] and on the environment where we caught them.

Video acquisition, quantification of speed and stroke frequency

A set of adult individuals for each species were filmed at 2000 frames per second, both on water surface and on a solid substrate with a grid paper in the background as a calibration scale. Video acquisition was performed using the Phantom *Miro M310* Digital High Speed *Camera* and PCC Software (Vision research, Ametek). Videos were analyzed using TEMA 3.7 software (Images system) to extract speed values. To calculate speed, a mean value was extracted from a defined interval plateau phase from velocity curve along each video. This interval represents the max speed during the run of the individual. For speed, sample sizes were (on land/on water): *P. apt* (n=14/Not Applicable (NA)); *C. ali* (n=14/NA); *H.* sp. (n=10/10); *H. sta* (n=10/10); *M. fur* (n=10/12); *P. bul* (n=10/10); *M. ame* (n=9/8); *O. cun* (n=10/8); *S. str* (n=10/10); *S. ter* (n=10/11); *P. bra* (n=NA/18); *P. con* (n=10/3); *H. tur* (n=9/10); *G. bue* (n=8/8); *L. dis* (n=NA/8); *A. pal* (n=NA/8). Stroke frequency was determined as the number of strokes performed by the individuals during a given locomotion duration and converted into number of strokes per second. For stroke frequency, sample sizes were (on land/on water): *P. apt* (n=14/NA); *C. ali* (n=14/NA); *H.* sp. (n=10/10); *H. sta* (n=10/10); *M. fur* (n=10/12); *P. bul* (n=10/10); *M. ame* (n=10/9); *O. cun* (n=10/10); *S. str* (n=10/10); *S. ter* (n=10/22); *P. bra* (n=NA/35); *P. con* (n=10/6); *H. tur* (n=9/10); *G. bue* (n=8/8); *L. dis* (n=NA/8); *A. pal* (n=NA/8).

Measurements of leg length and body length

Measurements of the legs and body were performed using a SteREO Discovery V12 (Zeiss) with ZEN 2011 software (Zeiss). Body and leg length of the fossil species in (Figure S3) were extracted from the references in Table S1 and in the supplementary online information. Sample sizes used to perform these measurements were: *P. apt:* (n=10); *O. fas:* (n=10); *Y. mag:* (n=1); *C. ali:* (n=6); *H. vas:* (n=1); *L. pop:* (n=1); *S. meg:* (n=1); *M. dom:* (n=1); *M. fur:* (n=10); *M. and:* (n=1); *H. sp:* (n=9); *C. bra:* (n=1); *H. sta:* (n=10); *P. bor:* (n=1); *P. asi:* (n=1); *R. obe:* (n=10); *S. str:* (n=8); *S. ter:* (n=10); *P. bra:* (n=3); *V. cap:* (n=6); *O. cun:* (n=10); *P. bul:* (n=5); *P. bip:* (n=1); *P. con:* (n=1); *H. ele:* (n=1); *H. tur:* (n=6); *M. ele:* (n=1); *M. gri:* (n=1); *M. pol:* (n=1); *M. ame:* (n=10); *C. alb:* (n=1); *E. spi:* (n=1); *M. hes:* (n=10); *L. dis:* (n=10); *G. bue:* (n=6); *A. pal:* (n=8).

Analysis of leg pattern during locomotion

The deployment of the leg (distance from the body to the tip of the leg) and the amplitude of leg movement (distance between the point where the leg starts to push on substrate and the point where it loses contact with substrate) were extracted from the high-speed videos. To measure these parameters we took 3 videos on ground and 3 videos on water for *Hebrus sp., Mesovelia furcata* and *Microvelia americana*. In each video we extracted 6 strokes (n=18) to obtain an average of leg deployment and the amplitude of leg movements for the three legs. For *Gerris buenoi* we used 6 videos with 1 stroke per video (n=6). Measurements were performed using Image J software [S21]. To measure the duration of stance phases and swing phases we extracted 6 gait cycles for each species from our high-speed videos during both locomotion on ground and locomotion on water. Then we recorded the duration of each phases using the PCC Software (Vision research, Ametek).

Reconstruction of ancestral trait

Ancestral reconstruction for habitat was performed in Rstudio version 0.99.486 using a maximum likelihood method adapted to discrete characters (ace, package ape, [S22]) and represented using phytools [S23]. The simplest model "ER", with equal transition rates across all 4 habitat categories, was the best both with AIC and likelihood comparisons (p value = 0.28 for comparison ER and SYM; failure of convergence of ARD model). The pies for ancestral nodes represent marginal ancestral states. We reconstructed the ancestral value of the quantitative characters on the internal nodes of the phylogenetic tree using contMap (ML reconstructions, package Phytools, [S23]). The resulting figures are maps of the observed and ancestral reconstructed phenotypic trait values onto the tree using a color gradient. This was done for the different variables (T1-leg/Body, T2-leg/Body, T3-leg/Body, speed, stroke frequency). The R script used and the data are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.134c4.

Inference of energy consumption

We inferred the amount of energy spent per stroke based on the procedure from [S24]. Kinetic energy (E_k in Joules) used during a stroke is determined using the following expression:

$$E_k=0.5mv^2$$

where m is the mass of the insect in grams and v the velocity generated during one stroke in meters per second. The analyses were performed using speed data extracted from the high-speed movies. We first extracted, using PCC software (Vision research, Ametek), the time of takeoff that corresponds to the interval of time when the leg starts to apply pressure on the substrate until the leg loses contact with the substrate. The distance travelled during this interval of time is recorded using ImageJ [S21] and a takeoff velocity is calculated. Pools of live insects were weighted to determine a mean weight for one individual for each species. Then the energy per stroke is calculated using the mass and the takeoff velocity. Because individuals from *Paravelia bullialata, Stridulivelia strigosa, Platyvelia brachialis,* and *Husseyella turmalis* died during the interval of time between video acquisition and weight recording, the samples were conserved in absolute ethanol and rehydrated using the procedure from [S25] to obtain body mass. Control of rehydration was performed on dead insects compare to live specimens from a control species to evaluate the accuracy of the protocol (data not shown). The numbers of individuals per species weighted are the following: *M. fur:* (n=10)*; M. ame:* (n=10)*; O. cun:* (n=10); *P. bul:* (n=4)*; S. str:* (n=6)*; S. ter:* (n=10); *P. bra:* (n=3); *H. tur:* (n=5)*; L. dis:* (n=6)*; G. bue:* (n=5); *A. pal:* (n=6). The numbers of videos used to measure the take-off velocity are the following: *M. fur:* (n=12)*; M. ame:* (n=10)*; O. cun:* (n=10); *P. bul:* (n=10)*; S. str:* (n=10)*; S. ter:* (n=22); *P. bra:* (n=12); *H. tur:* (n=10)*; L. dis:* (n=8)*; G. bue:* (n=8); *A. pal:* (n=8).

Statistical analyses

The quantitative variables (T1-leg/Body, T2-leg/Body, T3-leg/Body, speed, stroke frequency) did not follow a normal distribution (Shapiro tests), and hence were log-transformed and a mean value is calculated for each species and for each variable. The "habitat" variable is semi-quantitative and was not log transformed. Because habitat and speed did not follow a normal distribution we performed a classical non-parametric Spearman correlation test and non-parametric Spearman correlation test with Phylogenetic Independent Contrast (PIC) in order to take into account for the non independence of data points resulting from a phylogeny ([S26]; implemented in ape version 3.5 and Picante version 1.6-2 packages [S22, S27]). FDR P-value correction for multiple tests was applied (rho and P-value are indicated on figures). Parametric Pearson correlation test with and without PIC correction were also performed. These results are available in supplementary online information available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.134c4. For the quantification of leg deployment and leg movement, statistical significance between ground and water locomotion was determined by performing a Student t-tests. P-values are indicated in Table S3. Comparison of energy expenditure between species using the tripod gait and species using the rowing gait is performed using a non-parametric Wilcoxon test and graphically represented using the scatterplot3d version 0.3-37 package. Results are indicated in Table

S1. Statistical analyses were performed using RStudio Version 0.99.486 [S28]. Graphs were made using both RStudio and GraphPad Prism (version 6.01). Correlation matrixes were made using Picante version 1.6-2 package for the calculation and Corrplot version 0.77 package for the graphs [S29]. The R script used and the data are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.134c4.

Online ressources

Genbank accession numbers as well as sequence alignments for phylogenetic and tree reconstruction, the R script and dataset table used for phylogenetic and statistical analyses can be found in Dryad. Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.134c4.

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