Less effective selection leads to larger genomes
Supplemental Material

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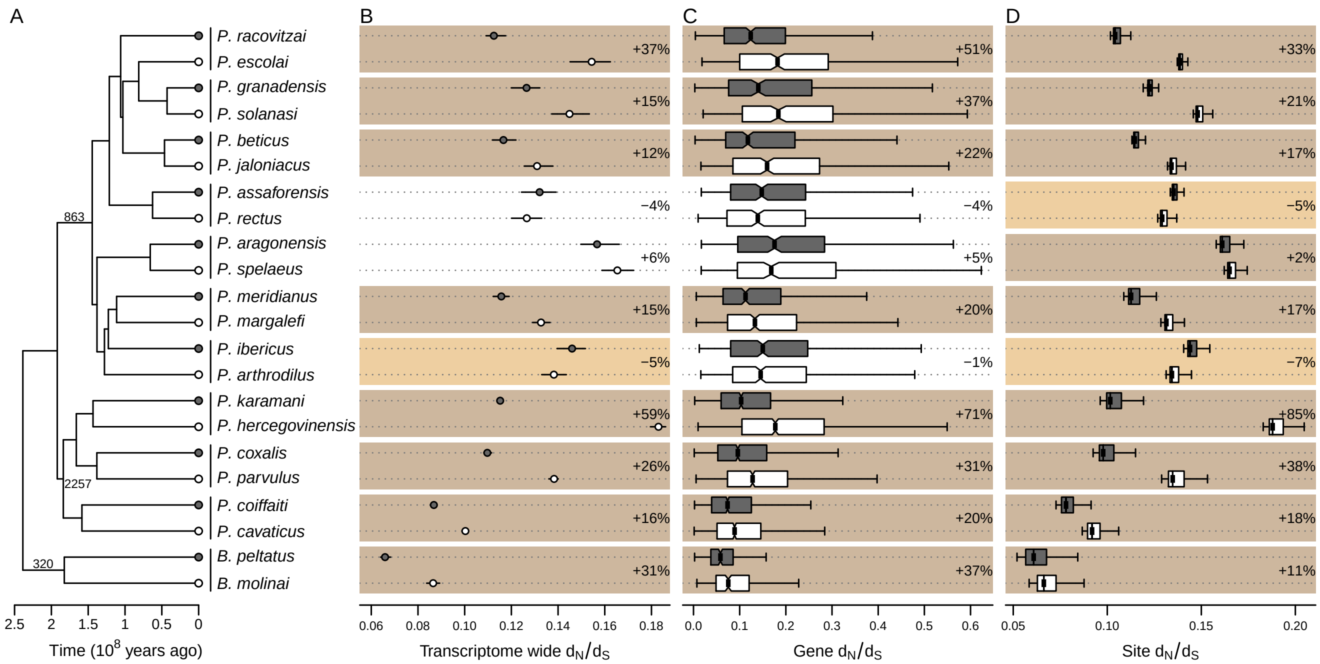

Figure S1: Evolution of the transcriptome-wide d_N/d_S (one global measure per taxa, B) and the distribution of d_N/d_S measured across genes (C) or across sites (D) for each surface and subterranean species (A). Legends are the same as in fig. 1. The percentage of variations displayed in panel C and D are between the median of the gene or site distributions, i.e. for i genes or sites, the median of $(w_{i subterranean} - w_{i surface})/w_{i surface}$ distribution.

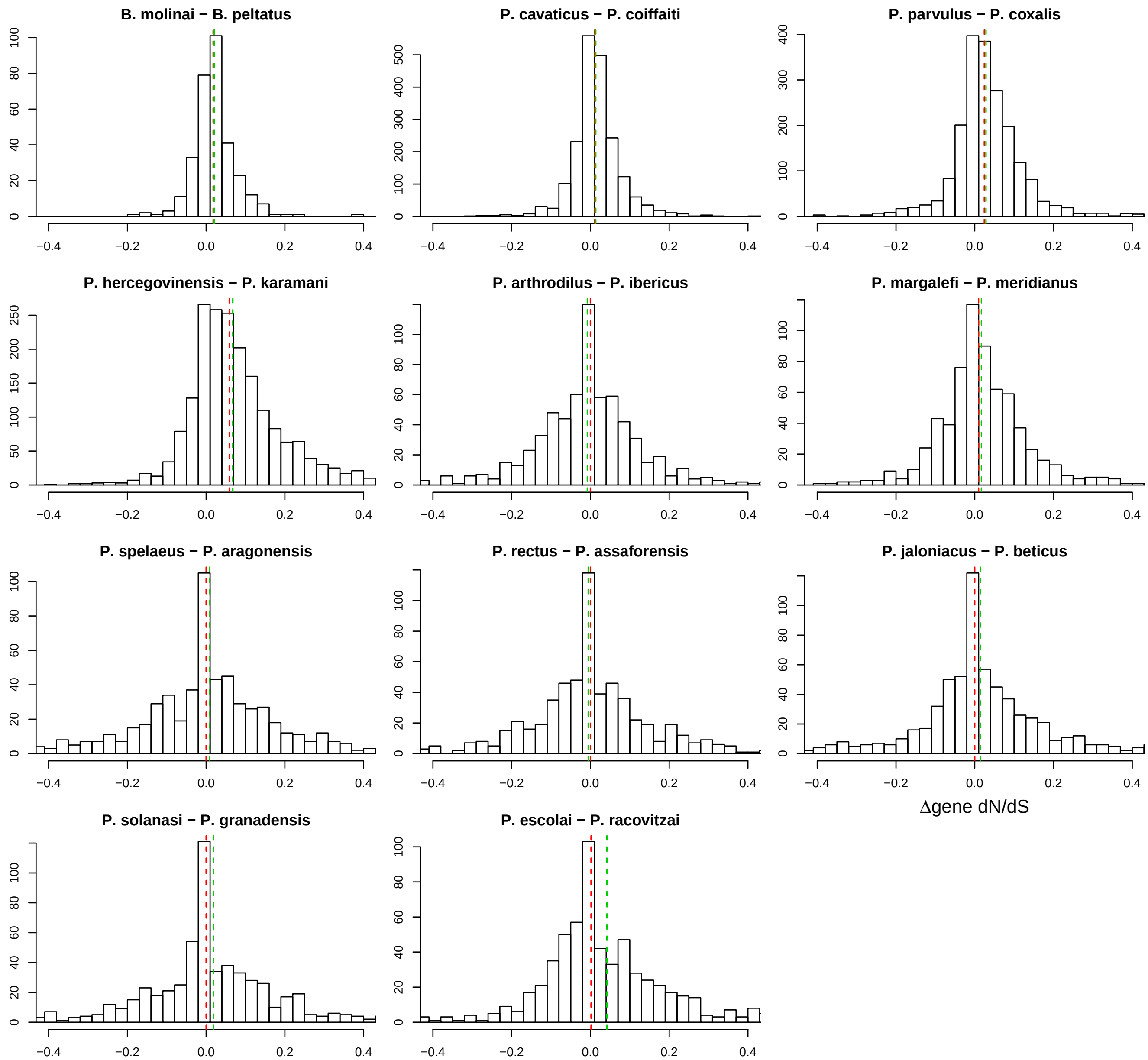

Figure S2: Distribution of gene d_N/d_S differences between subterranean and surface species ($w_{isubterranean} - w_{isurface}$). The dotted vertical lines indicate the median of the distribution (red) and the transcriptome-wide d_N/d_S (green).

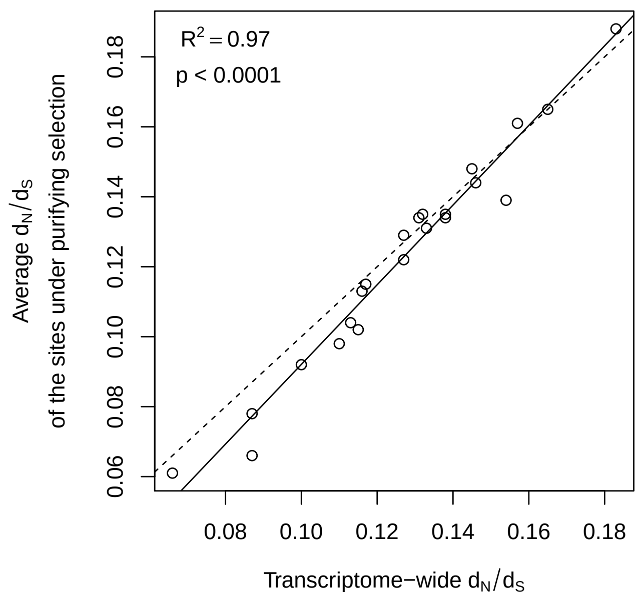

Figure S3: The transcriptome-wide d_N/d_S is a good estimate of the average d_N/d_S of the sites under purifying selection (w^-). Dash line indicates $d_N/d_S = w^-$, and the solid line indicates the linear regression. Transcriptome-wide d_N/d_S is therefore a good descriptor of the efficacy of purifying selection.

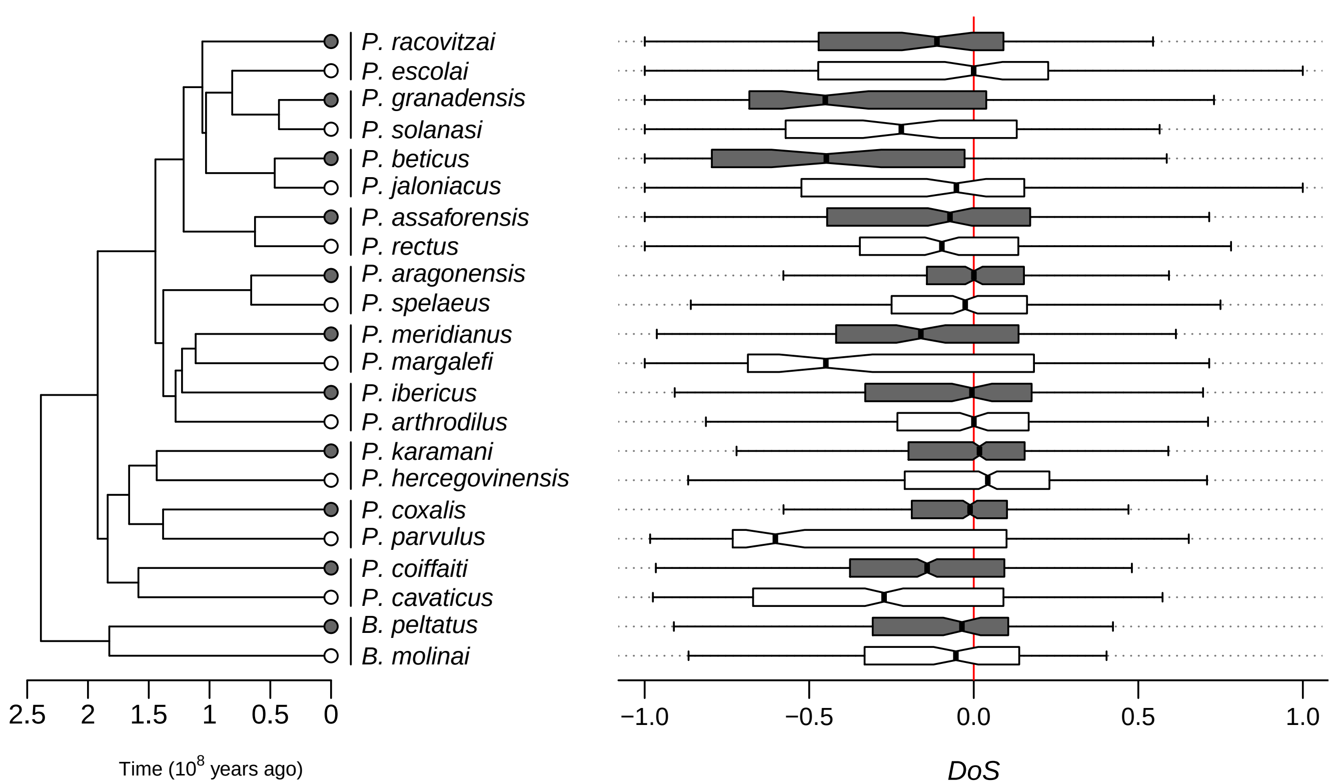

Figure S4: Distribution of the direction of selection (DoS) statistics. If positive selection was the dominant force driving d_N/d_S increase in subterranean species, DoS should increase in subterranean species (dark dots and boxes).

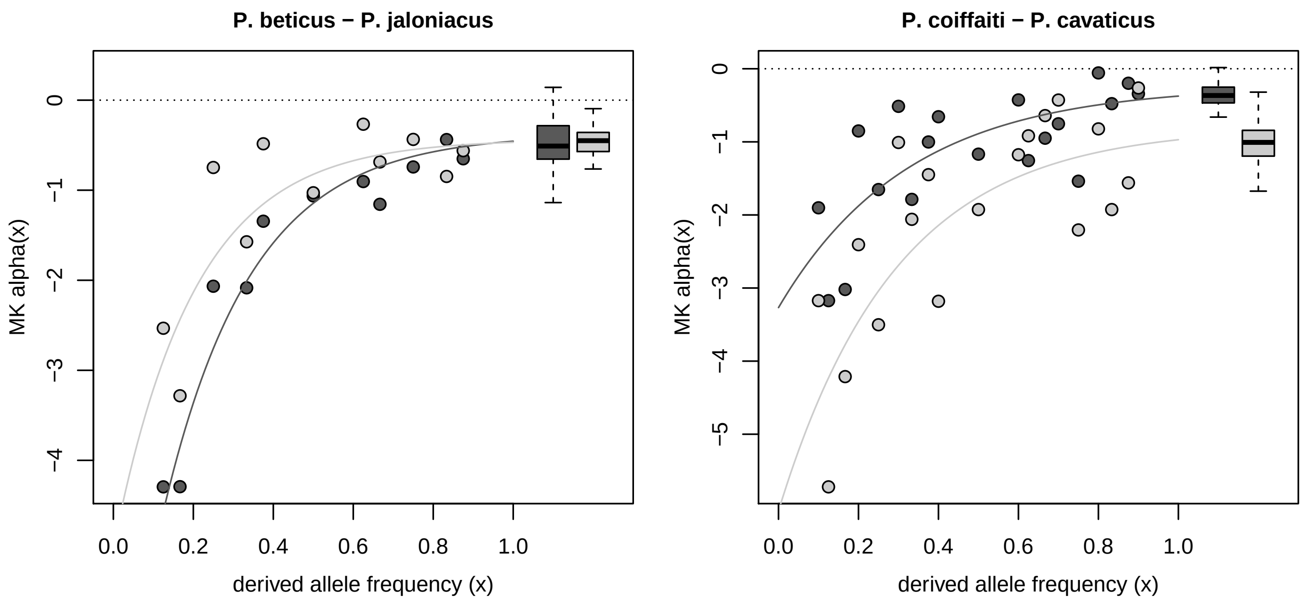

Figure S5: Estimation of the McDonald-Kreitman rate of adaptation (α) as a function of the derived allele frequency for 2 pairs of surface (dark gray) and subterranean (light gray) species. The curves represent the fitted function $\alpha(x) = a + be^{-cx}$. The boxplots represent the distribution of the asymptotic $\alpha(1)$ using 100 bootstrap pseudo-replicates of the SNPs used to reconstruct the unfolded SFS.

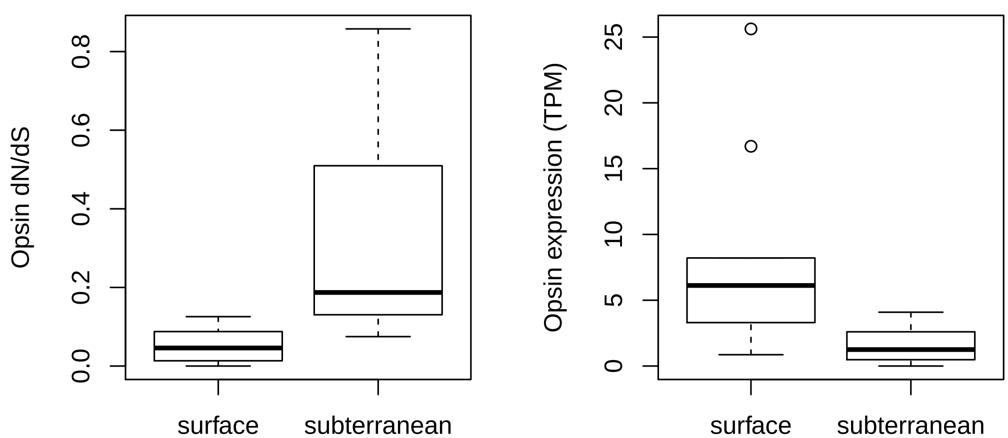

Figure S6: Opsin d_N/d_S and expression as measured in the surface and subterranean species. Expression is measured as transcript per million (TPM).

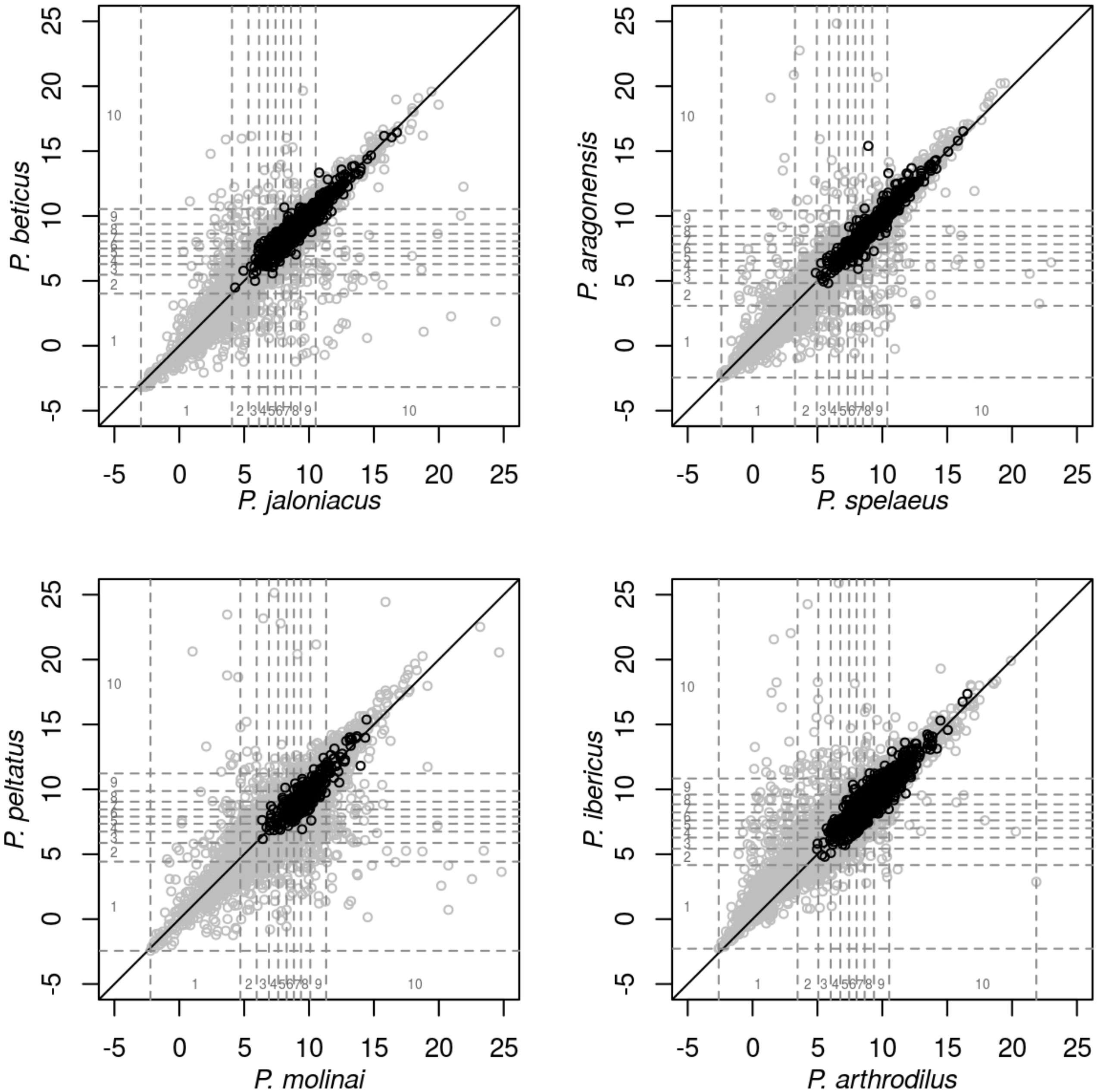

Figure S7: Comparisons of transcriptome-wide gene expression between 2 species of the same pair (Y=surface, X=subterranean). The expressions are normalized log2 read counts. All the genes present in both species are displayed in grey, while the set of conserved single copy genes used to compute d_N/d_S are in black. Ten different expression classes are delimited using dotted grey lines.

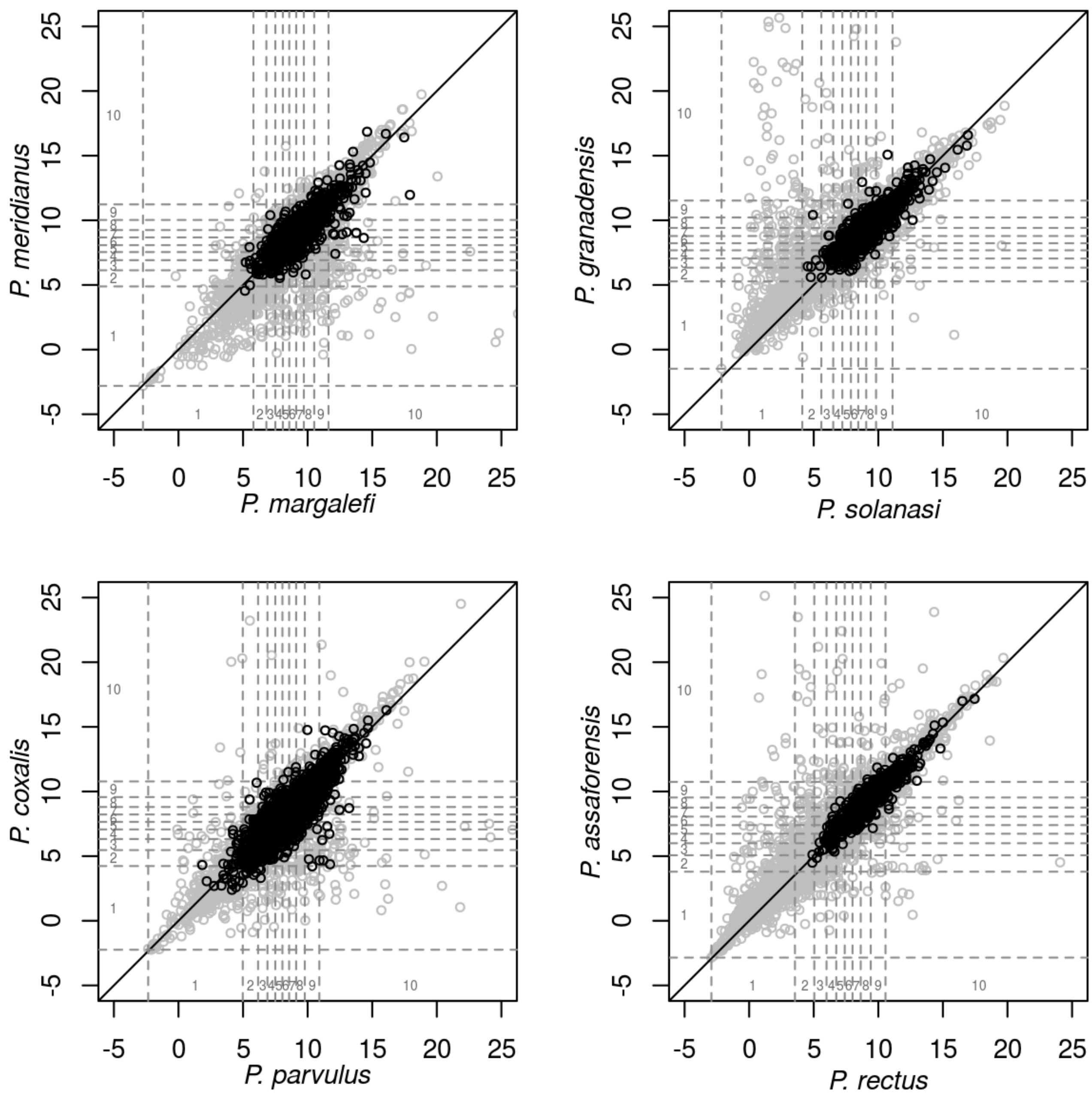

Figure S7: Continued.

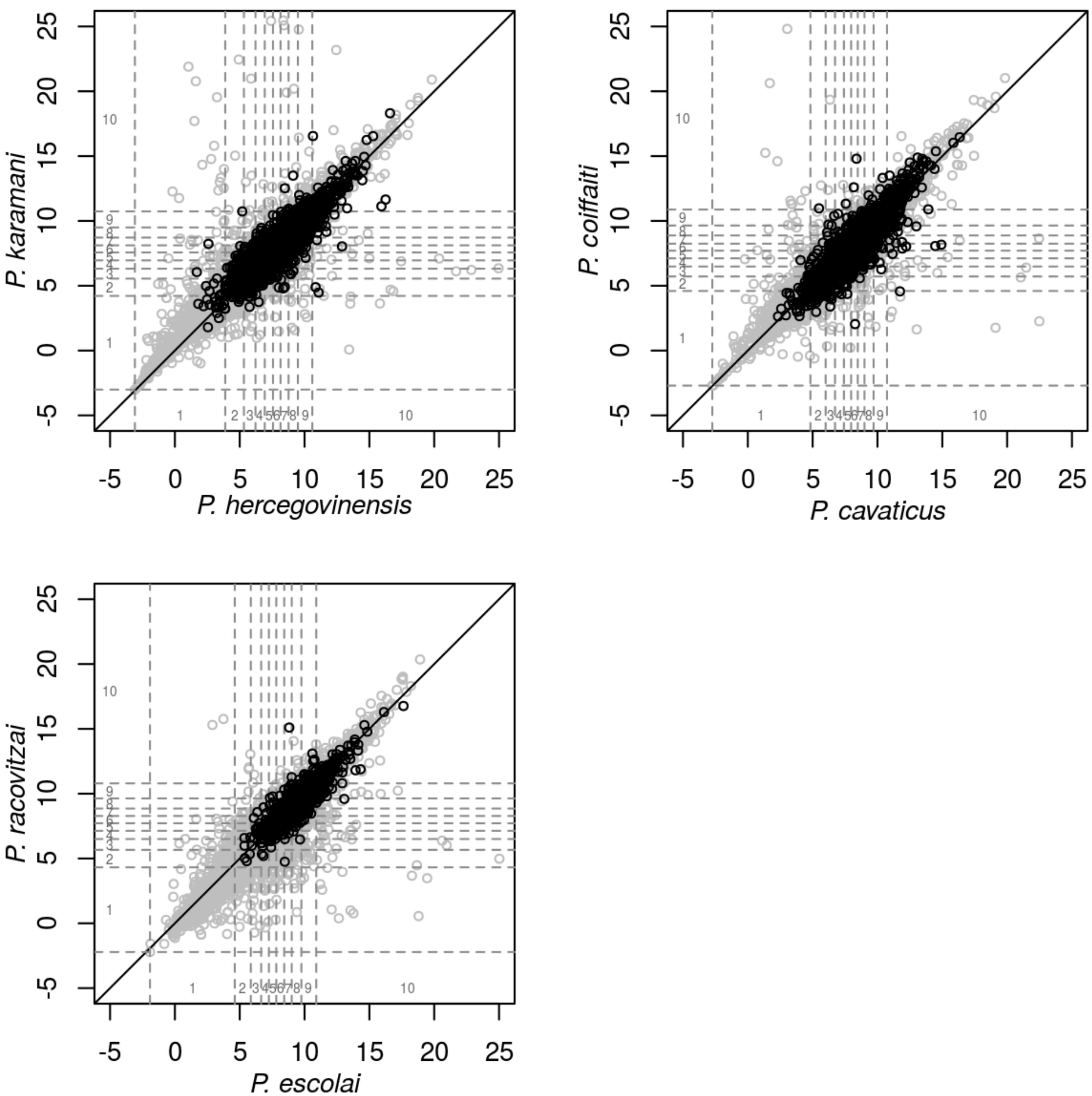

Figure S7: Continued.

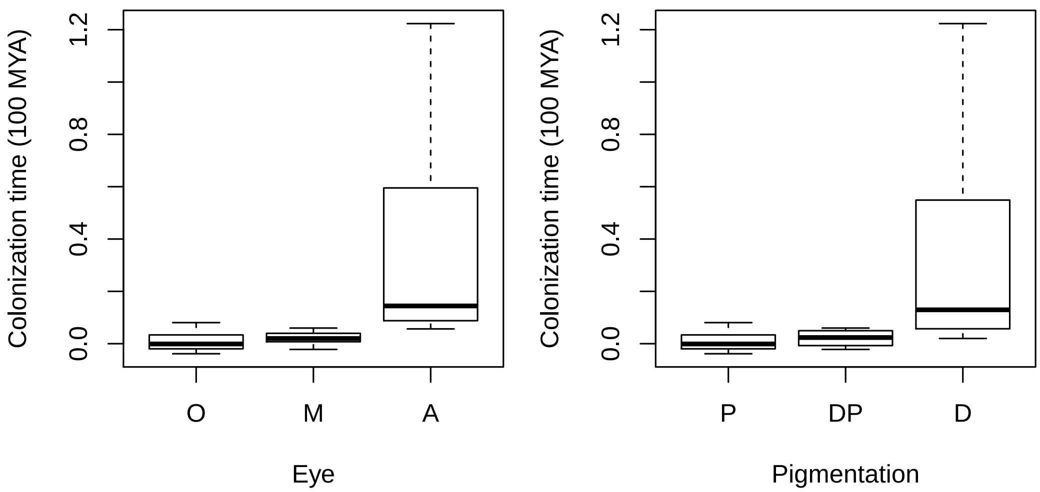

Figure S8: Covariation between eye or pigmentation phenotypes and estimated colonization times. O: oculated, M: microphtalmous, A: anophtalmous, P: pigmented, DP: partially depigmented, D: depigmented. In some pigmented or oculated species, colonization times are greater than 0, probably as a result of the incertainity in the d_N/d_S measurement.

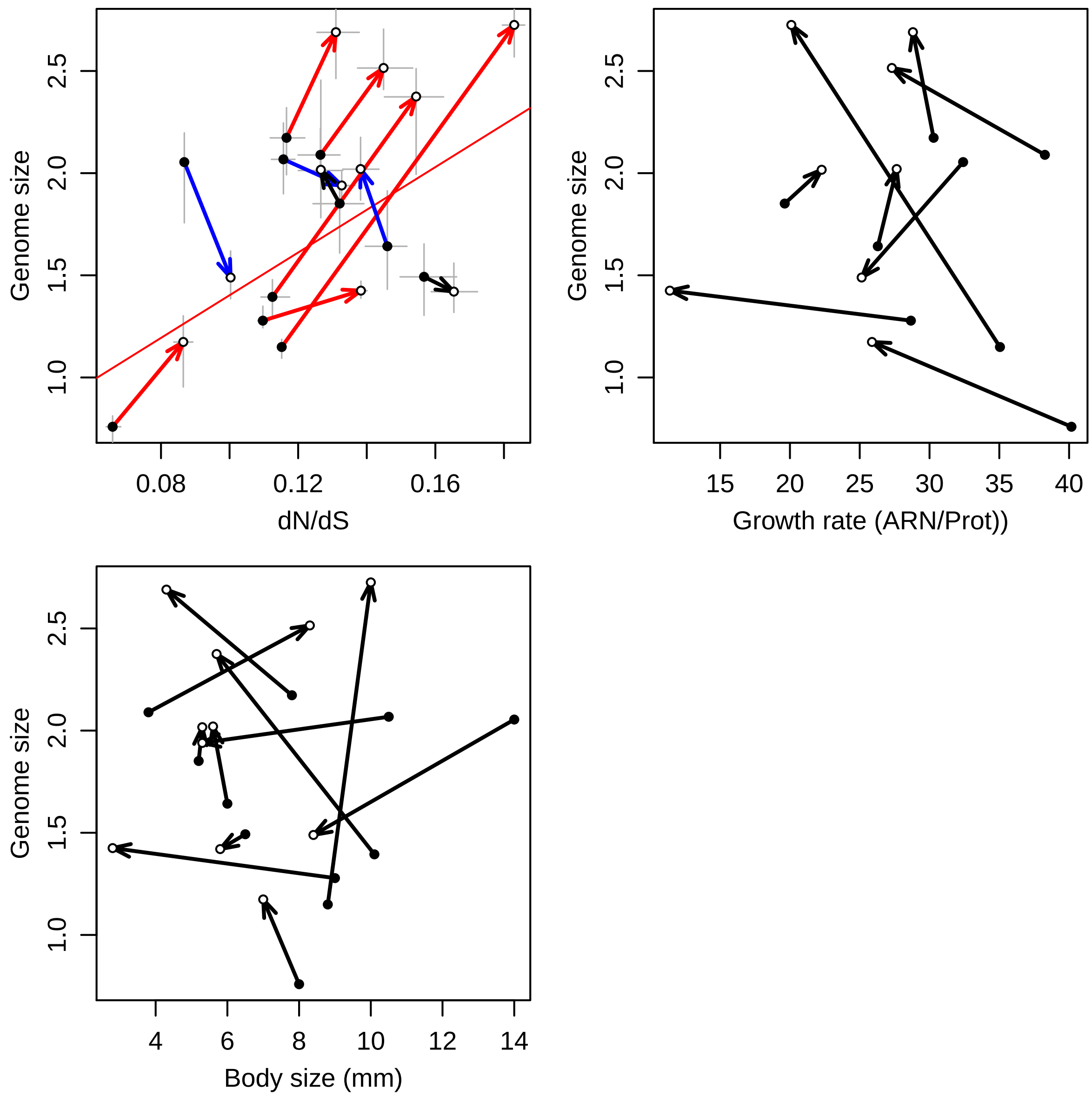

Figure S9: Relationship between genome size and selection efficacy (transcriptome-wide d_N/d_S, top left), growth rate (top right) or body size (bottom left). Variations from the surface (black circle) to the subterranean species (white circle) of a pair are displayed using arrows. For the genome size versus d_N/d_S plot, color of the arrows indicates the congruence with the MH expectation (red), a different pattern (blue) or no pattern (black). The line indicates the relationship supported by a PGLS model.

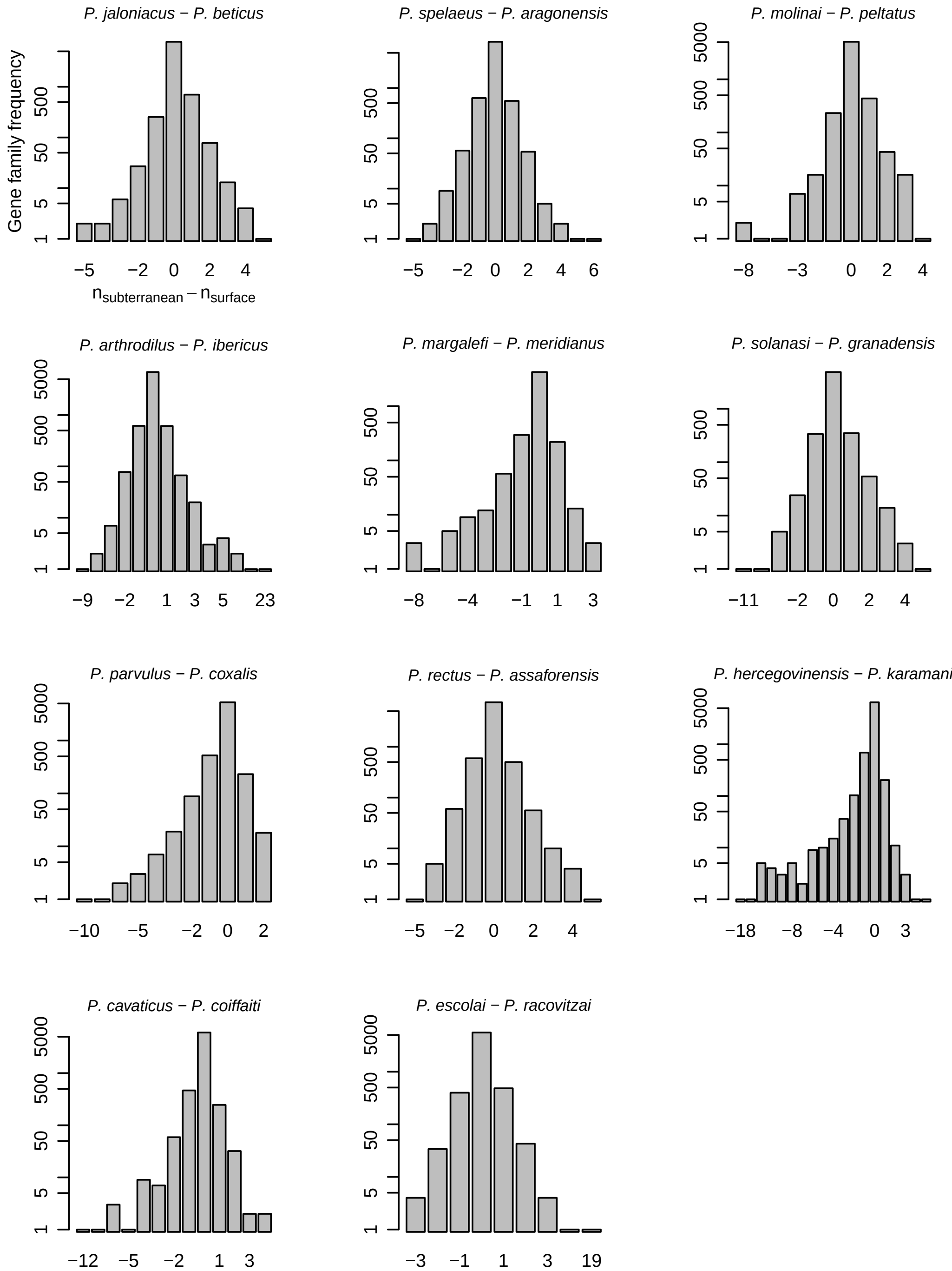

Figure S10: Testing the influence of large duplication events using the difference in the number of gene copy between the subterranean and surface species of a pair ($n_{subterranean} - n_{surface}$). In the event of a large duplication in the subterranean species, the whole distribution will be shifted toward positive values, which is not the case in any pairs.

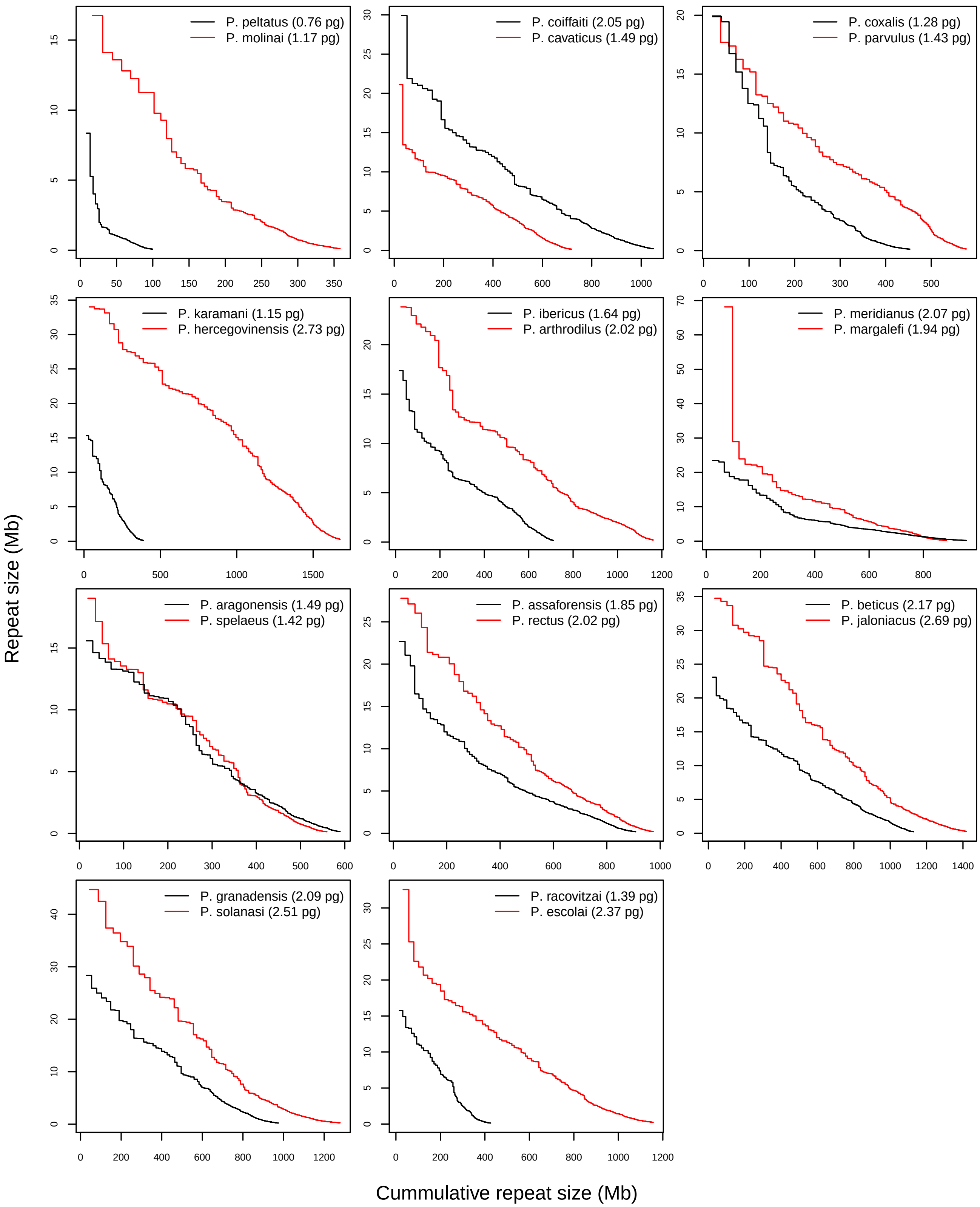

Figure S11: Repeatome size estimates using low coverage genome sequencing (0.05X) for 11 independent pairs of surface (black line) and subterranean (red line) species. Reads from highly repeated regions are sampled, clustered and assembled into elements, the size of which is estimated in megabases (Y-axis). These elements are ranked according to their decreasing contribution to genome size along the X-axis and their cumulated contributions provide an estimate of the repeatome size.

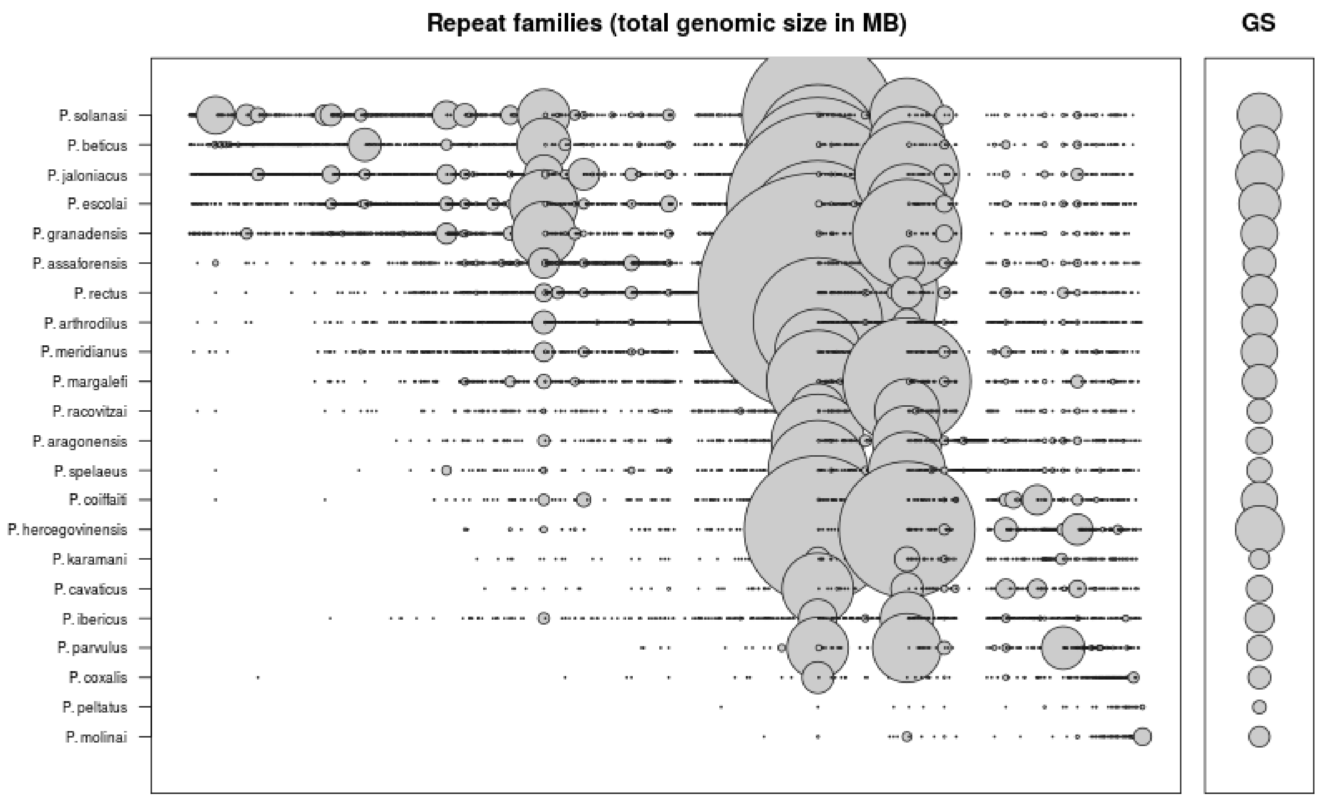

Figure S12: Repeated element frequencies in the 22 species. The table rows and columns were reordered following the first axis of a correspondance analysis. Circle areas are proportional to the total genome size of a given repeat family (min=0.01 and max=408.7 Mb). Orphan repeats were removed from the analysis. Genome sizes are represented on the right panel (min=0.76 and max=2.73 pg). On the first axis of the correspondence analysis, species are ordered following their phylogenetic proximity and not following their genome sizes.

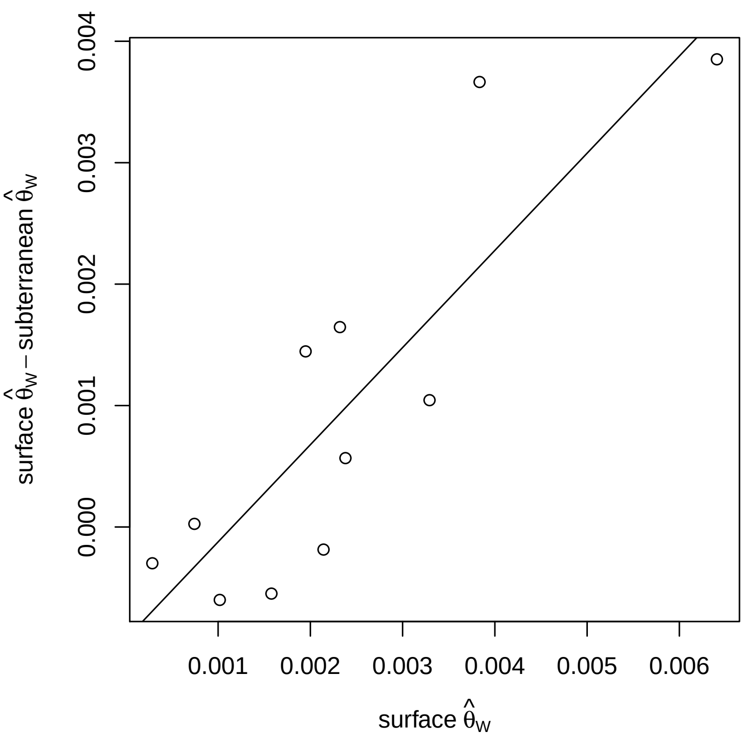

Figure S13: Differences in polymorphism between the surface and subterranean species of a pair as a function of polymorphism in the surface species. This relationship suggests that our capacity to detect changes in polymorphism subsequent to the ecological transition is strongly dependent on the polymorphism of the surface species. The line represents the linear regression with $R^2 = 0.74$, $\beta = 0.8$ and p-value $= 0.0007$.

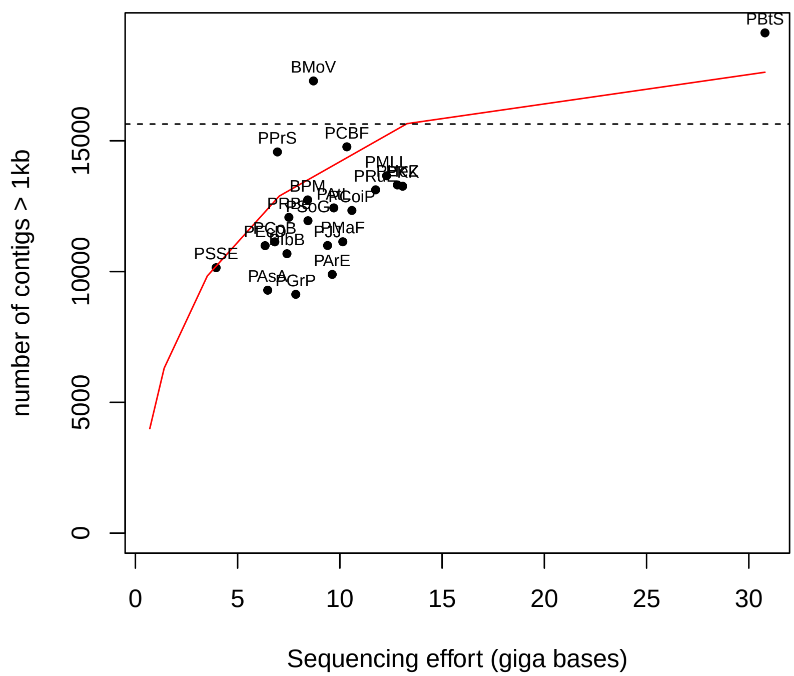

Figure S14: Transcriptome assembly quality as a function of sequencing effort. The number of component > 1kb is used as a proxy of the assembly quality. The red line represents assemblies of 2%, 5%, 10%, 25% and 50% of the original *P. beticus* reads. The dotted line represents the number of *Drosophila melanogaster* ORF > 1kb.

Table S1: Description of the 11 Aselloidea species pairs. Clade names are either Ibero-aquitanian (I), coxalis (C) or Alpine (A); ES: Ecological status (Subt: subterranean; Surf: surface); EE: evolutionary unit number as defined in Morvan *et al* 2013 *Syst. Biol.*; "pool" indicates the number of individual RNA extractions that were pooled prior to sequencing; GS: haploid genome size in picograms. Size: body size in mm. Growth rate are estimated using RNA (in ng) over protein (in μg) ratios. Taxa are listed in the same order as in figure 1 of the manuscript.

Species	clade	locality	ES	pair	EE	pool	GS	d_N/d_S	p_N/p_S	$\hat{\theta_w}$	size	growth rate
P. racovitzai Henry & Magniez, 1972	I	Arbas	Surf	1	T_110	6	1.394	0.1125	0.17299	1.02	10.10	
P. escolai Henry & Magniez, 1982	I	Salar	Subt		T_116	6	2.373	0.1544	0.18589	1.62	5.70	
P. granadensis Henry & Magniez, 2003	I	Alhama de Granada	Surf	2	T_113	6	2.089	0.1265	0.33170	0.74	3.80	38.26
P. solanasi Henry & Magniez, 1972	I	Benajoan	Subt		CD_15	6	2.514	0.1449	0.32158	0.72	8.30	27.30
P. beticus Henry & Magniez, 1992	I	Vallada	Surf	3	T_107	5	2.172	0.1166	0.33583	0.29	7.80	30.29
P. jaloniacus Henry & Magniez, 1978	I	Benichembla	Subt		T_108	7	2.689	0.1310	0.29317	0.59	4.30	28.81
P. assaforensis Afonso, 1988	I	Assofora	Surf	4	T_128	6	1.851	0.1321	0.21611	1.58	5.20	19.63
P. rectus Afonso, 1982	I	Evora	Subt		T_130	6	2.013	0.1266	0.20264	2.13	5.30	22.27
P. aragonensis Henry & Magniez, 1992	I	Burgui	Surf	5	T_147	5	1.492	0.1567	0.17180	6.41	6.50	
P. spelaeus (Racovitza, 1922)	I	Ancille	Subt		T_142	20	1.420	0.1654	0.18811	2.56	5.80	
P. meridianus (Racovitza, 1919)	I	Alfoz de Lloredo	Surf	6	T_153	6	2.067	0.1157	0.22323	1.95	10.50	
P. margalefi Henry & Magniez, 1982	I	Gestalgar	Subt		T_119	6	1.939	0.1327	0.44294	0.50	5.30	
P. ibericus (Braga, 1946)	I	Barrio	Surf	7	T_118	6	1.642	0.1460	0.18575	2.14	6.00	26.30
P. arthrodilus (Braga, 1945)	I	Legacao	Subt		T_150	6	2.019	0.1382	0.19252	2.33	5.60	27.65
P. karamani (Remy, 1934)	C	Kljuc	Surf	8	T_038	6	1.149	0.1152	0.14367	3.29	8.80	35.05
P. hercegovinensis (Karaman, 1933)	C	Zavala	Subt		T_051	6	2.725	0.1830	0.18662	2.25	10.00	20.10
P. coxalis (Dolfus, 1892)	C	La Indiana	Surf	9	T_047	6	1.278	0.1097	0.12052	3.83	9.00	28.66
P. parvulus (Sket, 1960)	C	Crnomelj	Subt		T_048	6	1.424	0.1383	0.45718	0.17	2.80	11.40
P. coiffaiti (Henry & Magniez, 1972)	A	Cauneille	Surf	10	T_044	6	2.053	0.0868	0.19055	2.32	14.00	32.41
P. cavaticus (Leydig, 1871)	A	Thoiria	Subt		T_080	6	1.489	0.1003	0.30971	0.67	8.40	25.14
B. peltatus (Braga, 1944)		Lousada	Surf	11	T_171	6	0.759	0.0659	0.12437	2.38	8.00	40.17
B. molinai Henry & Magniez, 1988		Vegacervera	Subt		T_163	8	1.173	0.0865	0.18006	1.81	7.00	25.88

Table S2: Estimates of the transcriptome-wide d_N/d_S using a branch model (w) and using a branch-site model that allows d_N/d_S variations across sites. Branch-site model allows to distinguish variation in the intensity of purifying (w^-) and positive selection (w^+) as well as their respective frequencies ($fq(w^-)$, with $fq(w^+) = 1 - fq(w^-)$). Nsites: number of codons analysed; PS: number of sites under positive selection; test: Wilcoxon test of an inscrease of w^- in subterranean species; ***: p-value < 0.0001; •: surface; ∘: subterranean.

Species	Branch			Branch-site					
	w	d_S	d_N	Nsites	w^-	w^+	fq(w^-)	PS	test
• *P. beticus*	0.117	0.0367	0.0043	266777	0.115	1.752	0.991	15	***
∘ *P. jaloniacus*	0.131	0.0300	0.0039		0.134	1.509	0.992	1	
• *P. racovitzai*	0.113	0.0689	0.0078	208775	0.104	1.164	0.996	3	***
∘ *P. escolai*	0.154	0.0208	0.0032		0.139	2.474	0.993	10	
• *P. aragonensis*	0.157	0.0214	0.0034	267427	0.161	2.104	0.992	2	***
∘ *P. spelaeus*	0.165	0.0264	0.0044		0.165	2.109	0.990	10	
• *P. ibericus*	0.146	0.0569	0.0083	268326	0.144	1.375	0.987	15	
∘ *P. arthrodilus*	0.138	0.0434	0.0060		0.134	1.264	0.991	7	
• *P. meridianus*	0.116	0.0880	0.0102	219632	0.113	1.328	0.987	15	***
∘ *P. margalefi*	0.133	0.0766	0.0102		0.131	1.500	0.983	38	
• *P. granadensis*	0.127	0.0328	0.0042	209695	0.122	3.950	0.990	35	***
∘ *P. solanasi*	0.145	0.0199	0.0029		0.148	2.601	0.993	6	
• *P. assaforensis*	0.132	0.0257	0.0034	263740	0.135	1.528	0.993	6	
∘ *P. rectus*	0.127	0.0276	0.0035		0.129	2.029	0.992	6	
• *P. coiffaiti*	0.087	0.2485	0.0216	231949	0.078	2.659	0.994	17	***
∘ *P. cavaticus*	0.100	0.1889	0.0190		0.092	1.523	0.991	15	
• *P. coxalis*	0.110	0.1493	0.0164	215844	0.098	1.316	0.988	17	***
∘ *P. parvulus*	0.138	0.1420	0.0196		0.135	1.279	0.986	17	
• *P. karamani*	0.115	0.1693	0.0195	231634	0.102	1.359	0.987	31	***
∘ *P. hercegovinensis*	0.183	0.0630	0.0115		0.188	1.401	0.981	11	
• *B. peltatus*	0.066	0.5352	0.0353	122043	0.061	1.580	0.991	4	***
∘ *B. molinai*	0.087	0.3746	0.0324		0.066	1.752	0.992	3	

Table S3: Transcriptome single nucleotide polymorphism (SNP) found in the 11 species pairs. EE: evolutionary unit, ES: Ecological status (Subt: subterranean; Surf: surface), Ngene: number of genes considered for the polymorphism scan, SNPs: synonymous SNP, SNPn: non-synonymous SNP, S: number of synonymous sites, N: number of non-synonymous sites.

Species	EE	couple	ES	ngene	SNPs	SNPn	S	N
P. racovitzai	T_110	1	Surf	230	220	133	84650	289542
P. escolai	T_116		Subt		326	204	73247	251395
P. granadensis	T_113	2	Surf	122	66	73	35132	122368
P. solanasi	CD_15		Subt		82	86	46024	158768
P. beticus	T_107	3	Surf	690	183	197	274737	936867
P. jaloniacus	T_108		Subt		404	398	271383	925563
P. assaforensis	T_128	4	Surf	676	905	657	233431	806015
P. rectus	T_130		Subt		1216	832	233519	806029
P. aragonensis	T_147	5	Surf	755	4110	2363	282681	967329
P. spelaeus	T_142		Subt		2514	1604	281863	965579
P. meridianus	T_153	6	Surf	325	608	470	124523	430339
P. margalefi	T_119		Subt		120	181	95152	330518
P. ibericus	T_118	7	Surf	351	561	360	109795	377042
P. arthrodilus	T_150		Subt		733	474	126774	434094
P. karamani	T_038	8	Surf	569	1825	906	224945	771616
P. hercegovinensis	T_051		Subt		1275	801	226679	777352
P. coxalis	T_047	9	Surf	345	1111	451	117465	403065
P. parvulus	T_048		Subt		48	74	113745	390888
P. coiffaiti	T_044	10	Surf	685	1446	952	254802	872511
P. cavaticus	T_080		Subt		413	422	254630	872530
B. peltatus	T_171	11	Surf	375	805	320	133476	451473
B. molinai	T_163		Subt		627	377	125153	425536

Table S4: Number of genes that were found in a higher, lower or identical expression class in the surface (surf) species relative to the subterranean species (subt) within the set of conserved genes used in the d_N/d_S analysis. 10 equal size expression classes were used.

surf	subt	surf > subt	surf < subt	surf == subt
B. peltatus	*B. molinai*	103	97	120
P. coiffaiti	*P. cavaticus*	693	682	882
P. coxalis	*P. parvulus*	719	710	828
P. karamani	*P. hercegovinensis*	678	738	841
P. ibericus	*P. arthrodilus*	273	280	310
P. meridianus	*P. margalefi*	317	291	255
P. aragonensis	*P. spelaeus*	183	198	482
P. assaforensis	*P. rectus*	245	242	376
P. beticus	*P. jaloniacus*	213	225	425
P. granadensis	*P. solanasi*	261	301	301
P. racovitzai	*P. escolai*	288	269	306

Table S5: Opsin d_N/d_S and estimated colonization time (Col. time) in hundreds of million years. Colonization times are negative for few surface species, presumably as a result of small error in the opsin d_N/d_S estimation.

Species	ES	d_N/d_S	Col. time
P. cavaticus	Subt	0.13	0.16
P. coiffaiti	Surf	0.04	0.01
P. coxalis	Surf	0.05	0.03
P. hercegovinensis	Subt	0.86	1.22
P. karamani	Surf	0.09	0.08
P. arthrodilus	Subt	0.52	0.64
P. ibericus	Surf	0.02	-0.02
P. margalefi	Subt	0.51	0.55
P. meridianus	Surf	0.00	-0.04
P. spelaeus	Subt	0.21	0.12
P. aragonensis	Surf	0.09	0.04
P. rectus	Subt	0.12	0.06
P. assaforensis	Surf	0.13	0.06
P. jaloniacus	Subt	0.07	0.02
P. beticus	Surf	0.00	-0.02
P. solanasi	Subt	0.16	0.06
P. granadensis	Surf	0.05	0.01
P. escolai	Subt	0.19	0.13
P. racovitzai	Surf	0.01	-0.02

Table S6: Genome sizes for 47 species of Metazoa (related to Figure 2). CO: iso country codes, LA: latitude in decimal degrees, LO: longitude in decimal degrees, EE: evolutionary unit number as defined in Morvan *et al* 2013 *Syst. Biol.*, GS: average haploid genome size in picograms, SD: GS standard deviation, ES: Ecological status (Subt: subterranean; Surf: surface). Taxa are listed in the same order than in fig. 2.

Species	Locality	CO	LA	LO	EE	GS	SD	ES	Pair
Proasellus racovitzai Henry & Magniez, 1972	Arbas	FR	42.97306	0.89889	T_110	1.394	0.066	Surf	1
Proasellus escolai Henry & Magniez, 1982	Deifontes	ES	37.32921	-3.5874	T_116	2.375	0.214	Subt	
Proasellus bellesi Henry & Magniez, 1982	El Burgo	ES	36.78994	-4.94127	T_115	3.163	0.330	Subt	
Proasellus escolai Henry & Magniez, 1982	Salar	ES	37.14075	-4.06587	T_114	2.709	0.333	Subt	
Proasellus granadensis Henry & Magniez, 2003	Alhama de Granada	ES	37.04646	-4.06266	T_113	2.090	0.108	Surf	2
Proasellus solanasi Henry & Magniez, 1972	Benajoan	ES	36.73001	-5.23871	CD_15	2.515	0.116	Subt	
Proasellus lagari Henry & Magniez, 1982	Sorbas	ES	37.0792	-2.1008	T_125	2.821	0.168	Subt	
Proasellus beticus Henry & Magniez, 1992	Vallada	ES	38.88048	-0.68871	T_107	2.173	0.118	Surf	3
Proasellus jaloniacus Henry & Magniez, 1978	Benichembla	ES	38.75669	-0.10486	T_108	2.690	0.286	Subt	
Proasellus n. sp. (Alviela)	Alcacena	PT	39.44561	-8.71215	T_127	1.985	0.196	Subt	
Proasellus assaforensis Afonso, 1988	Assofora	PT	38.90872	-9.42192	T_128	1.851	0.139	Surf	4
Proasellus rectus Afonso, 1982	Evora	PT	38.60359	-7.87318	T_130	2.017	0.268	Subt	
Proasellus ortizi Henry & Magniez, 1992	Ozana	ES	43.30385	-3.57186	T_092	1.450	0.109	Surf	5
Proasellus cantabricus Henry & Magniez, 1968	Mirones	ES	43.29376	-3.69926	T_094	1.712	0.270	Subt	
Proasellus ebrensis Henry & Magniez, 1992	Cereceda	ES	42.80023	-3.49401	T_089	1.599	0.091	Surf	6
Proasellus grafi Henry & Magniez, 2003	Rasines	ES	43.29843	-3.41971	T_091	1.149	0.068	Subt	
Proasellus aragonensis Henry & Magniez, 1992	Burgui	ES	42.70362	-1.01646	T_147	1.493	0.143	Surf	7
Proasellus spelaeus (Racovitza, 1922)	Ancille	FR	43.13806	-1.20028	T_142	1.420	0.093	Subt	
Proasellus istrianus (Stammer, 1932)	Stepani	SI	45.55225	13.85515	T_106	2.339	0.042	Surf	
Proasellus meridianus (Racovitza, 1919)	Padirac	FR	44.85809	1.75047	T_151	2.106	0.080	Surf	
Proasellus meridianus (Racovitza, 1919)	Alfoz de Lloredo	ES	43.3492	-4.18103	T_153	2.068	0.124	Surf	8
Proasellus margalefi Henry & Magniez, 1982	Gestalgar	ES	39.5969	-0.84903	T_119	1.940	0.074	Subt	
Proasellus margalefi Henry & Magniez, 1982	Albentosa	ES	40.12242	-0.76379	T_103	2.113	0.149	Subt	
Proasellus ibericus (Braga, 1946)	Barrio	PT	41.84607	-8.56764	T_118	1.642	0.177	Surf	9
Proasellus arthrodilus (Braga, 1945)	Legacao	PT	40.03177	-8.46979	T_150	2.020	0.130	Subt	
Proasellus karamani (Remy, 1934)	Kljuc	BA	43.0927	18.48516	T_038	1.149	0.040	Surf	10
Proasellus hercegovinensis (Karaman, 1933)	Zavala	BA	42.84516	17.97832	T_051	2.725	0.130	Subt	

Proasellus anophtalmus (Karaman, 1934)	Dubrovnik	HR	42.68907	18.07178	CM_001	2.949	0.102	Subt	
Proasellus coxalis (Dolfus, 1892)	La Indiana	ES	36.75376	-5.20183	T_047	1.278	0.044	Surf	11
Proasellus parvulus (Sket, 1960)	Crnomelj	SI	45.60372	15.17089	T_048	1.425	0.034	Subt	
Proasellus coiffaiti (Henry & Magniez, 1972)	Cauneille	FR	43.54494	1.04227	T_044	2.054	0.174	Surf	12
Proasellus slovenicus (Sket, 1957)	Precna	SI	45.81886	15.09865	T_054	1.741	0.078	Subt	
Proasellus cavaticus (Leydig, 1871)	Thoiria	FR	46.525	5.73389	T_080	1.489	0.111	Subt	
Bragasellus peltatus (Braga, 1944)	Lousada	PT	41.28193	-8.30839	T_171	0.759	0.063	Surf	13
Bragasellus frontellum (Braga, 1964)	Aboim das Chocas	PT	41.91711	-8.44715	T_170	1.199	0.088	Subt	
Bragasellus cortesi Afonso, 1989	Vila Pouca de Aguiar	PT	41.49062	-7.64903	T_169	0.836	0.033	Surf	14
Bragasellus molinai Henry & Magniez, 1988	Vegacervera	ES	42.90665	-5.56286	T_163	1.174	0.132	Subt	
Bragasellus lagari Henry & Magniez, 1973	Gestalgar	ES	39.5969	-0.84903	T_021	1.556	0.071	Subt	
Caecidotea kenki (Bowman, 1967)	Arlington	US	38.92928	-77.11858	T_033	2.312	0.133	Surf	15
Gallasellus heilyi (Legrand, 1956)	Saint-Pierre-de-l'Ile	FR	46.02028	-0.44917	T_179	1.911	0.081	Subt	
Asellus (Asellus) aquaticus (Linnaeus, 1758)	Villeurbanne	FR	45.77986	4.86802	T_176	2.503	0.129	Surf	
Atyaephyra desmaresti (Millet, 1831)	Brissac	FR	43.84349	3.69938	CD_17	3.707	0.295	Surf	16
Typhlatia miravetensis Sanz & Platvoet, 1995	Alcala de Xivert	ES	40.25935	0.25944	CD_18	4.731	0.583	Subt	
Dugastella valentina (Ferrer Galdiano, 1924)	Gestalgar	ES	39.60081	-0.83171	CD_19	3.745	0.314	Surf	17
Gallocaris inermis (Fage, 1937)	Sauve	FR	43.94056	3.95	CD_25	7.017	0.677	Subt	
Bythinella eurystoma (Paladhile, 1870)	Le Vigan	FR	43.92985	3.59211	CD_21	0.862	0.051	Surf	18
Bythinella navacellensis Prié & Bichain, 2009	Saint Maurice de Navacelle	FR	43.8636	3.5264	CD_22	0.923	0.035	Subt	

Table S7: Correlation between the coordinates of species along the axes of a repeat community correpondance analysis (COA) and genome size and the ecological status, and phylogenetic signal associated to each axis of the COA. Repeat orphans were removed and repeats were represented using their respective total genome size (TGS). Correlations were tested by comparing phylogenetic generalized least squares models with and without the parameter of interest using a likelihood ratio test. % var: percent variation explained by this axis, coeff: PGLS coefficient, K: Blomberg et al.'s K.

COA Axis		GS		Ecological Status		Phylo. signal	
Axis	% var	p-value	coeff	p-value	coeff	K	p-value
1	12.70	0.06	-0.18	0.89	-0.04	3.76	0.00 **
2	9.10	0.75	0.06	0.43	0.12	1.59	0.00 **
3	8.10	0.13	-0.29	0.61	0.08	1.16	0.01 *
4	7.30	0.69	0.05	0.40	0.23	0.99	0.09
5	7.10	0.60	0.10	0.77	-0.05	0.82	0.10
6	6.80	0.57	0.13	0.31	0.13	0.70	0.36

Table S8: Tajima's D estimate for 2 pairs of species based on the frequencies of synonymous SNPs and associated test of mutation-drift equilibrium (H_0 : D not different than 0).

Species	status	Tajima's D	p-value
P. beticus	Surf.	2.40	0.017*
P. jalionacus	Subt.	1.67	0.095
P. coiffaiti	Surf.	1.33	0.185
P. cavaticus	Subt.	1.35	0.178

Table S9: Aselloidea sequences used to reconstruct phylogenetic relationships. EE: evolutionary unit number as defined in Morvan et al (2013 Syst. Biol.), COI: Genbank accession numbers for COI sequences, 16S: Genbank accession numbers for 16S sequences, 28S: Genbank accession numbers for 28S sequences. Bold accession numbers represent sequences obtained for this study.

SPECIES	EE	COI	16S	28S
Asellus (Asellus) aquaticus (Linnaeus, 1758)	T_172	DQ144824		DQ144738
	T_173	AY531762		DQ144740
	T_175	DQ144788		DQ144745
	T_176	DQ144816	**KC610270**	DQ144746
	T_174	DQ144885		DQ144747

Asellus (Asellus) hilgendorfii Bovallius, 1886	T_177	AY531829		DQ144750
Bragasellus comasi Henry & Magniez, 1976	T_155	JQ921528	JQ921672	JQ921863
	T_154	JQ921530	JQ921674	JQ921864
	T_157	JQ921534	**KC610271**	JQ921865
	T_158	JQ921529	JQ921673	
Bragasellus cortesi Afonso, 1989	T_169	JQ921537	JQ921676	JQ922027
Bragasellus escolai Henry & Magniez, 1978	T_160	JQ921540	**KC610272**	JQ921866
Bragasellus frontellum (Braga, 1964)	T_170	JQ921541	JQ921678	**KC610419**
Bragasellus lagari Henry & Magniez, 1973	T_025	JQ921525	JQ921671	JQ921862
	T_022	JQ921545	JQ921680	JQ921868
	T_021	JQ921547	JQ921681	JQ921869
	T_027	JQ921555	JQ921684	JQ921870
	T_026	JQ921544	JQ921679	
	T_023	JQ921549	**KC610273**	
	T_024	JQ921554	**KC610274**	
Bragasellus lagarioides Henry & Magniez, 1996	T_156	JQ921558	JQ921687	JQ921873
	T_162	JQ921562	JQ921685	**KC610420**
Bragasellus molinai Henry & Magniez, 1988	T_163	JQ921567	JQ921688	JQ921874
Bragasellus peltatus (Braga, 1944)	T_171	JQ921568	JQ921689	JQ921875
Bragasellus rouchi Henry & Magniez, 1988	T_159	JQ921571	JQ921690	JQ921876
Bragasellus stocki Henry & Magniez, 1988	T_161	JQ921524	**KC610275**	**KC610421**
Caecidotea kenki (Bowman, 1967)	T_033	JQ921575	**KC610276**	JQ921877
Chthonasellus bodoni Argano & Messana, 1991	T_074	JQ921576	**KC610278**	**KC610423**
Gallasellus heilyi (Legrand, 1956)	T_178	JQ921585	**KC610280**	JQ921881
	T_179	JQ921588	**KC610279**	**KC610424**
	T_180	JQ921582	**KC610281**	**KC610425**
Lirceolus bisetus (Steeves, 1968)	T_034	AY566533	AY570118	
Lirceolus cocytus Lewis, 2001	T_029	AY566528	AY570114	
	T_028	AY566544	AY570130	
Lirceolus hardeni Lewis & Bowman, 1996	T_036	AY566537	AY570122	
	T_035	AY566551	AY570138	
Lirceolus pilus (Steeves, 1968)	T_031	AY566539	AY570125	
	T_032	AY566554	AY570140	
Magniezia gardei Magniez, 1978	T_014	JQ921605	JQ921697	JQ921883
Mexistenasellus coahuila Cole & Minckley, 1972	T_018	AY566478	AY570149	
Proasellus aff. escolai Henry & Magniez, 1982	T_109	JQ921021	**KC610299**	JQ921884
	CD_10		**KC610298**	**KC610439**
Proasellus aff. lagari Henry & Magniez, 1982	T_117	JQ921023	JQ921700	**KC610442**
Proasellus albigensis (Magniez, 1965)	T_126	JQ921028	JQ921702	JQ921886
Proasellus anophtalmus dalmatinus (Karaman, 1955)	CM_001	**KC610503**	**KC610286**	**KC610429**
Proasellus anophtalmus (Karaman, 1934)	CD_11	**KC610502**	**KC610285**	**KC610428**
Proasellus aquaecalidae (Racovitza, 1922)	T_141	JQ921033	**KC610288**	JQ921887
	T_140	JQ921035	**KC610287**	JQ921888

Proasellus aragonensis Henry & Magniez, 1992	T_147	JQ921037	JQ921705	JQ921889
	T_146	JQ921038	JQ921706	**KC610430**
Proasellus arnautovici (Remy, 1932)	T_149	JQ921205	DQ305117	**KC610358**
	T_148	DQ305138	DQ305115	**KC610451**
Proasellus arthrodilus (Braga, 1945)	T_150	JQ921046	**KC610289**	**KC610431**
Proasellus assaforensis Afonso, 1988	T_128	JQ921047	JQ921710	JQ921893
Proasellus bardaunii Alouf, Henry & Magniez, 1982	T_049	JQ921050	JQ921711	JQ921894
Proasellus bellesi Henry & Magniez, 1982	T_115	JQ921052	JQ921712	JQ921895
Proasellus beroni Henry & Magniez, 1968	T_095	JQ921055	**KC610290**	JQ921896
	T_097	JQ921057	**KC610291**	JQ921898
	T_096	JQ921059	**KC610292**	**KC610432**
Proasellus beticus Henry & Magniez, 1992	T_107	JQ921061	JQ921717	JQ921899
Proasellus boui Henry & Magniez, 1969	T_039	JQ921065	JQ921718	**KC610433**
Proasellus cantabricus Henry & Magniez, 1968	T_094	JQ921067	JQ921719	**KC610434**
Proasellus cavaticus (Leydig, 1871)	T_080	JQ921072	JQ921721	JQ921903
	T_079	JQ921076	JQ921722	JQ921904
	T_078	JQ921077	JQ921723	JQ921905
	T_081	JQ921081	**KC610168**	**KC610370**
	CD_12		**KC610293**	**KC610435**
Proasellus chappuisi Henry & Magniez, 1968	T_087	JQ921120	JQ921732	**KC610341**
Proasellus chauvini Henry & Magniez, 1978	T_131	JQ921125	**KC610294**	JQ921915
Proasellus claudei Henry & Magniez, 1996	T_098	JQ921129	JQ921735	JQ921917
Proasellus coiffaiti (Henry & Magniez, 1972)	T_044	JQ921132	JQ921736	**KC610342**
Proasellus comasi Henry & Magniez, 1982	T_112	JQ921134	JQ921737	JQ921919
Proasellus coxalis (Dolfus, 1892)	T_046	JQ921161	JQ921741	DQ144751
	T_045	JQ921141	**KC610282**	**KC610426**
	CD_13	**KC610501**	**KC610284**	**KC610427**
	T_047	JQ921158	**KC610283**	**KC610436**
Proasellus deminutus (Sket, 1959)	T_056	JQ921175	JQ921744	**KC610344**
	T_053	JQ921180	JQ921745	**KC610437**
Proasellus dianae Pesce & Argano, 1985	T_102	JQ921184	JQ921746	**KC610438**
Proasellus ebrensis Henry & Magniez, 1992	T_089	JQ921070	**KC610296**	JQ921929
	T_090	JQ921188	**KC610297**	JQ921930
Proasellus escolai Henry & Magniez, 1982	T_114	JQ921189	JQ921749	JQ921931
	T_116	JQ921192	JQ921750	JQ921932
Proasellus espanoli Henry & Magniez, 1982	T_122	JQ921194	JQ921751	JQ921933
Proasellus faesulanus Messana & Caselli, 1995	T_101	JQ921197	**KC610098**	JQ921934
Proasellus franciscoloi (Chappuis, 1955)	T_066	JQ921204	**KC610300**	JQ921936
	T_064	JQ921200	**KC610301**	**KC610440**
Proasellus grafi Henry & Magniez, 2003	T_091	JQ921207	**KC610302**	JQ921938
Proasellus granadensis Henry & Magniez, 2003	T_113	JQ921212	**KC610100**	**KC610347**
Proasellus guipuzcoensis Henry & Magniez, 2003	T_138	JQ921208	**KC610101**	JQ921940

Proasellus hercegovinensis (Karaman, 1933)	T_050	JQ921214	**KC610303**	JQ921941
	T_051	JQ921215	JQ921760	JQ921942
Proasellus hermallensis (Arcangeli, 1938)	T_104	JQ921220	**KC610304**	**KC610349**
Proasellus ibericus (Braga, 1946)	T_118	JQ921226	JQ921762	JQ921944
Proasellus intermedius (Sket, 1965)	T_055	JQ921235	JQ921763	JQ921945
Proasellus istrianus (Stammer, 1932)	T_106	JQ921239	JQ921764	**KC610441**
Proasellus jaloniacus Henry & Magniez, 1978	T_108	JQ921245	JQ921765	JQ921947
Proasellus karamani (Remy, 1934)	T_038	JQ921248	JQ921766	JQ921948
Proasellus lagari Henry & Magniez, 1982	T_125	JQ921249	**KC610306**	JQ921949
Proasellus lescherae Henry & Magniez, 1978	T_137	JQ921114	**KC610310**	JQ921911
	T_135	JQ921254	JQ921772	JQ921950
	T_133	JQ921258	**KC610307**	JQ921951
	T_136	JQ921266	**KC610309**	JQ921953
	T_134	JQ921268	JQ921771	**KC610443**
	T_132	JQ921262	**KC610308**	**KC610444**
Proasellus ligusticus Bodon & Argano, 1982	T_100	JQ921275	**KC610312**	JQ921956
	T_099	JQ921273	**KC610311**	**KC610445**
Proasellus margalefi Henry & Magniez, 1982	T_119	JQ921280	JQ921776	JQ921958
	T_103	JQ921278	JQ921775	**KC610446**
	T_120	JQ921027	JQ921701	**KC610447**
Proasellus meijersae Henry & Magniez, 2003	T_124	JQ921284	**KC610313**	**KC610448**
Proasellus meridianus (Racovitza, 1919)	T_153	JQ921309	**KC610314**	JQ921961
	T_152	JQ921316	JQ921781	JQ921962
	T_151	JQ921304	**KC610315**	**KC610353**
Proasellus micropectinatus Baratti & Messana, 1990	T_086	JQ921327	**KC610107**	**KC610354**
Proasellus n. sp. (Alviela)	T_127	JQ921337	JQ921785	JQ921967
Proasellus n. sp. (Aquamalas Pontones)	T_121	JQ921110	JQ921727	JQ921909
Proasellus n. sp. (Aquerreta)	T_139	JQ921340	JQ921786	JQ921968
Proasellus n. sp. (Boreon)	T_077	JQ921344	**KC610316**	JQ921969
	T_076	JQ921345	JQ921788	**KC610449**
Proasellus n. sp. (Cantarana)	T_072	JQ921018	**KC610295**	JQ921925
Proasellus n. sp. (Lentegi)	T_111	JQ921346	JQ921790	JQ921966
Proasellus n. sp. (Maglia)	T_065	JQ921349	JQ921791	JQ921971
Proasellus n. sp. (Martino)	T_061	JQ921350	JQ921792	JQ921972
Proasellus n. sp. (Mazandaran)	T_037	JQ921336	JQ921789	JQ921970
Proasellus n. sp. (Mescla)	T_063	JQ921353	**KC610317**	JQ921973
Proasellus n. sp. (Pesio Santa	T_075	JQ921579	**KC610277**	**KC610422**
Proasellus n. sp. (Sanguiniere)	T_062	JQ921355	**KC610318**	JQ921974
Proasellus n. sp. (Sospel)	T_073	JQ921357	JQ921795	JQ921975
Proasellus n. sp. (Verclause)	T_070	JQ921359	**KC610319**	JQ921976
Proasellus n. sp. (Verclause)	T_071	JQ921361	**KC610320**	JQ921977

Proasellus navarrensis Henry & Magniez, 2003	T_093	JQ921328	JQ921783	JQ921964
Proasellus nolli (Karaman, 1952)	T_040	JQ921332	**KC610206**	**KC610393**
Proasellus ortizi Henry & Magniez, 1992	T_092	JQ921364	JQ921798	JQ921978
Proasellus oviedensis Henry & Magniez, 2003	T_105	JQ921365	JQ921799	JQ921979
Proasellus parvulus (Sket, 1960)	T_048	JQ921366	JQ921800	**KC610355**
Proasellus pavani (Arcangeli, 1942)	T_052	JQ921370	JQ921801	JQ921981
Proasellus racovitzai Henry & Magniez, 1972	T_110	JQ921372	**KC610321**	JQ921982
Proasellus rectangulatus Afonso, 1982	T_129	JQ921373	JQ921803	JQ921983
Proasellus rectus Afonso, 1982	T_130	JQ921376	JQ921804	JQ921984
Proasellus rouchi Henry, 1980	T_082	JQ921383	JQ921805	JQ921985
Proasellus slavus (Remy, 1948)	T_043 T_041 T_042	JQ921386 JQ921389 JQ921411	JQ921806 **KC610193** **KC610212**	JQ921986 **KC610394** **KC610398**
Proasellus slovenicus (Sket, 1957)	T_054	JQ921413	JQ921810	JQ921990
Proasellus solanasi Henry & Magniez, 1972	CD_15	**KC610504**	**KC610322**	**KC610452**
Proasellus sp. (type locality of P. gourbaultae)	T_123	JQ921112	JQ921728	**KC610346**
Proasellus spelaeus (Racovitza, 1922)	CD_16 T_142	**KC610505** JQ921416	**KC610323** JQ921811	**KC610360** **KC610450**
Proasellus stocki Henry & Magniez, 2003	T_088	JQ921116	JQ921730	JQ921913
Proasellus strouhali (Karaman, 1955)	T_067 T_068	JQ921432 JQ921423	**KC610226** JQ921813	JQ921995 **KC610400**
Proasellus synaselloides (Henry, 1963)	T_083 T_084 T_085	JQ921445 JQ921449 JQ921443	**KC610240** **KC610244** JQ921817	JQ921996 JQ921998 **KC610410**
Proasellus valdensis (Chappuis, 1948)	T_069	JQ921469	**KC610147**	**KC610453**
Proasellus vandeli Magniez & Henry, 1969	T_143 T_145 T_144	JQ921488 JQ921489 JQ921486	JQ921822 JQ921823 **KC610324**	JQ922001 **KC610368** **KC610454**
Proasellus vulgaris (Sket, 1965)	T_057	JQ921493	**KC610325**	JQ922003
Proasellus walteri (Chappuis, 1948)	T_060 T_058 T_059	JQ921513 JQ921516 JQ921497	**KC610326** JQ921829 **KC610258**	JQ922004 JQ922006 **KC610415**
Remasellus parvus (Steeves, 1964)	T_030	AY566472	AY570143	
Stenasellus breuili Racovitza, 1924	T_015	JQ921607	**KC610327**	
Stenasellus buili Remy, 1949	T_012 T_011	JQ921611 JQ921613	JQ921831 JQ921832	
Stenasellus galhanoae Braga, 1962	T_019	JQ921616	JQ921833	JQ922009
Stenasellus racovitzai Razzauti, 1925	T_016 T_017	JQ921624 JQ921622	**KC610328** JQ921837	**KC610455** **KC610456**
Stenasellus virei Dollfus, 1897	T_008 T_005 T_002	JQ921627 JQ921642 JQ921635	JQ921838 **KC610332** **KC610334**	JQ922012 JQ922013 JQ922014

	T_010	JQ921645	JQ921844	JQ922015
	T_004	JQ921617	JQ921834	JQ922016
	T_182	JQ921650	JQ921849	JQ922017
	T_181	JQ921660	JQ921850	JQ922018
	T_001	JQ921669	JQ921855	JQ922019
	T_185	JQ921666	JQ921853	JQ922020
	T_184	JQ921648	**KC610333**	JQ922029
	T_006	JQ921658	**KC610329**	**KC610457**
	T_013	JQ921629	JQ921839	**KC610458**
	T_009	JQ921639	**KC610331**	**KC610459**
	T_007	JQ921664	JQ921852	**KC610460**
	T_183	JQ921667	JQ921854	**KC610461**
	T_003	JQ921654	JQ921847	
	T_186	JQ921632	**KC610330**	
Synasellus bragaianus Henry & Magniez, 1987	T_168	JQ921596	JQ921858	JQ922024
	T_165	JQ921592	**KC610335**	**KC610462**
Synasellus meijersae Henry & Magniez, 1987	T_167	JQ921598	**KC610336**	JQ922025
Synasellus n. sp. (Aboim)	T_164	JQ921602	JQ921860	**KC610463**
Synasellus n. sp. (Los Tojos)	T_166	JQ921603	**KC610337**	**KC610464**