

Supplementary Material

The first complete mitochondrial genomes of subterranean dytiscid diving beetles (*Limbodessus* and *Paroster*) from calcrete aquifers of Western Australia

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Table S1. Coverage of the genomes sequenced for each of the subterranean species, including the final combined coverage for each of the sequences used in the study.

	no. of sequences	COVERAGE			
		Mean	Std Dev	min	max
<i>L. palmulaoides</i>	155,473	2612.5	926.7	22	4463
25542	53,960	887.9	511.8	0	2541
27821	5,767	50.9	28.4	0	167
28086	43,414	746.0	207.5	22	1157
28088	52,335	911.7	319.4	0	1555
<i>P. macrosturtensis</i>	56,958	923.7	550.0	23	2476
25544	27,253	475.9	347.4	2	1395
25545	22,303	378.3	192.0	8	951
27822	7,031	62.8	39.6	7	197
28084	96	1.6	3.2	0	25
28085	416	7.2	11.0	0	96
28089	48	0.8	1.9	0	16
<i>P. mesosturtensis</i>	7,600	66.8	291.7	4	3555
27823	7,600	66.8	291.7	4	3555
<i>P. microsturtensis</i>	8,377	118.9	181.9	2	1807
25543	5,302	91.5	103.4	0	495
27824	3,076	27.4	152.4	0	1749

Table S2. The quality scores obtained for the genomes sequenced for each of the subterranean species, including the final combined coverage for each of the sequences used in the study.

QUALITY SCORES			
	Confidence	% at least	% at least
	Mean	Q20	Q30
<i>L. palmulaoides</i>	34.4	94.5	85.5
25542	34.9	95.8	87.8
27821	524.4	96.9	96.1
28086	34.4	94.4	85.4
28088	33.8	93.2	82.7
<i>P. macrosturtensis</i>	34.3	94.2	84.8
25544	34.5	94.8	85.9
25545	33.5	93.1	81.5
27822	298.3	96.7	96.0
28084	6359.0	93.5	85.6
28085	2070.5	92.9	83.7
28089	9308.3	95.7	90.7
<i>P. mesosturtensis</i>	37.2	96.9	96.1
27823	37.2	96.9	96.1
<i>P. microsturtensis</i>	35.4	96.0	89.6
25543	34.9	95.6	87.7
27824	37.3	97.0	96.2

Table S3

AT content and AT and GC skews of the dytiscid genomes used in this study, calculated for the whole genome and each compartment of the genome (PCGs, tRNAs, rRNAs and the CR).

	TOTAL	T(U)	C	A	G	A+T%	AT SKEW	GC SKEW
Whole								
<i>KT876878 Acilius sp.</i>	20689.0	39.0	11.0	42.4	7.6	81.41	0.041	-0.179
<i>KT876885 Colymbetes sp.</i>	16211.0	37.2	13.4	40.2	9.2	77.34	0.039	-0.186
<i>KT876896 Hydroporus sp.</i>	23380.0	39.3	9.4	44.2	7.2	83.42	0.059	-0.136
<i>KT876897 Hydroporus sp.</i>	17693.0	38.9	11.4	42.3	7.4	81.21	0.041	-0.211
<i>KT876899 Hygrotus sp.</i>	16730.0	35.9	14.5	40.2	9.3	76.17	0.057	-0.216
<i>KT876900 Hygrotus sp.</i>	17704.0	37.6	13.0	40.2	9.2	77.81	0.034	-0.170
<i>KT876901 Hygrotus sp.</i>	17071.0	38.0	12.3	41.1	8.6	79.06	0.039	-0.175
<i>KT876902 Liopterus sp.</i>	16541.0	37.5	12.4	41.7	8.5	79.19	0.053	-0.188
<i>L. palmulaoides</i>	16862.0	36.4	14.7	40.2	8.7	76.63	0.050	-0.254
<i>P. macrosturtensis</i>	16673.0	35.8	15.5	39.3	9.4	75.09	0.046	-0.242
<i>P. mesosturtensis</i>	16663.0	37.0	14.3	40.1	8.7	77.03	0.040	-0.246
<i>P. microsturtensis</i>	16483.0	36.9	14.3	39.6	9.2	76.49	0.036	-0.221
PCGs								
<i>KT876878 Acilius sp.</i>	11212.0	43.9	11.0	33.3	11.8	77.19	-0.138	0.035
<i>KT876885 Colymbetes sp.</i>	11224.0	43.0	12.3	32.3	12.5	75.25	-0.143	0.009
<i>KT876896 Hydroporus sp.</i>	10946.0	44.2	10.4	34.3	11.1	78.51	-0.127	0.037
<i>KT876897 Hydroporus sp.</i>	11212.0	43.4	11.0	34.1	11.6	77.46	-0.121	0.026
<i>KT876899 Hygrotus sp.</i>	11218.0	41.6	13.6	31.9	13.0	73.45	-0.133	-0.021
<i>KT876900 Hygrotus sp.</i>	10979.0	42.8	12.3	32.5	12.4	75.32	-0.138	0.001
<i>KT876901 Hygrotus sp.</i>	11231.0	43.5	11.9	32.9	11.7	76.37	-0.138	-0.006
<i>KT876902 Liopterus sp.</i>	11211.0	43.7	11.0	33.4	11.9	77.10	-0.133	0.042
<i>L. palmulaoides</i>	11200.0	41.5	13.1	32.4	13.0	73.91	-0.123	-0.001
<i>P. macrosturtensis</i>	11198.0	41.5	13.9	30.8	13.8	72.30	-0.147	-0.006
<i>P. mesosturtensis</i>	11198.0	42.7	12.7	32.1	12.5	74.79	-0.143	-0.010
<i>P. microsturtensis</i>	11209.0	42.5	12.8	31.8	12.9	74.32	-0.143	0.001
tRNA								
<i>KT876878 Acilius sp.</i>	1461.0	39.2	8.5	40.7	11.6	79.95	0.019	0.154
<i>KT876885 Colymbetes sp.</i>	1467.0	38.4	9.9	39.3	12.5	77.64	0.011	0.116
<i>KT876896 Hydroporus sp.</i>	1453.0	39.8	7.7	41.2	11.3	81.00	0.016	0.188
<i>KT876897 Hydroporus sp.</i>	1452.0	39.7	7.6	41.0	11.6	80.72	0.015	0.207
<i>KT876899 Hygrotus sp.</i>	1453.0	38.3	9.2	40.1	12.5	78.39	0.022	0.153
<i>KT876900 Hygrotus sp.</i>	1445.0	38.4	9.3	40.1	12.1	78.55	0.022	0.129
<i>KT876901 Hygrotus sp.</i>	1463.0	39.1	8.9	40.6	11.4	79.70	0.019	0.125
<i>KT876902 Liopterus sp.</i>	1469.0	39.2	8.7	40.2	11.8	79.44	0.013	0.152
<i>L. palmulaoides</i>	1460.0	38.4	9.0	39.3	13.3	77.67	0.012	0.190
<i>P. macrosturtensis</i>	1454.0	38.4	8.7	41.0	11.9	79.37	0.033	0.153
<i>P. mesosturtensis</i>	1455.0	38.8	8.2	40.8	12.2	79.59	0.026	0.192
<i>P. microsturtensis</i>	1457.0	38.1	8.6	40.9	12.4	79.00	0.036	0.176

rRNA

<i>KT876878 Acilius sp.</i>	2150.0	42.5	6.4	38.8	12.2	81.35	-0.045	0.312
<i>KT876885 Colymbetes sp.</i>	2121.0	42.5	6.5	38.8	12.2	81.33	-0.046	0.308
<i>KT876896 Hydroporus sp.</i>	1974.0	42.2	6.6	39.5	11.8	81.66	-0.033	0.282
<i>KT876897 Hydroporus sp.</i>	1984.0	42.0	6.4	39.9	11.7	81.91	-0.026	0.298
<i>KT876899 Hygrotus sp.</i>	2145.0	43.7	6.1	37.4	12.9	81.07	-0.078	0.360
<i>KT876900 Hygrotus sp.</i>	2143.0	42.6	6.3	39.2	12.0	81.75	-0.042	0.315
<i>KT876901 Hygrotus sp.</i>	2103.0	43.3	6.6	38.2	12.0	81.46	-0.062	0.292
<i>KT876902 Liopterus sp.</i>	2144.0	44.2	6.0	37.4	12.4	81.58	-0.083	0.347
<i>L. palmulaoides</i>	2103.0	42.1	6.3	37.6	14.0	79.65	-0.057	0.379
<i>P. macrosturtensis</i>	2111.0	41.7	6.3	37.6	14.4	79.25	-0.052	0.393
<i>P. mesosturtensis</i>	2101.0	41.8	6.0	38.3	13.9	80.06	-0.044	0.394
<i>P. microsturtensis</i>	2106.0	41.8	5.9	38.4	13.9	80.20	-0.042	0.400

CR

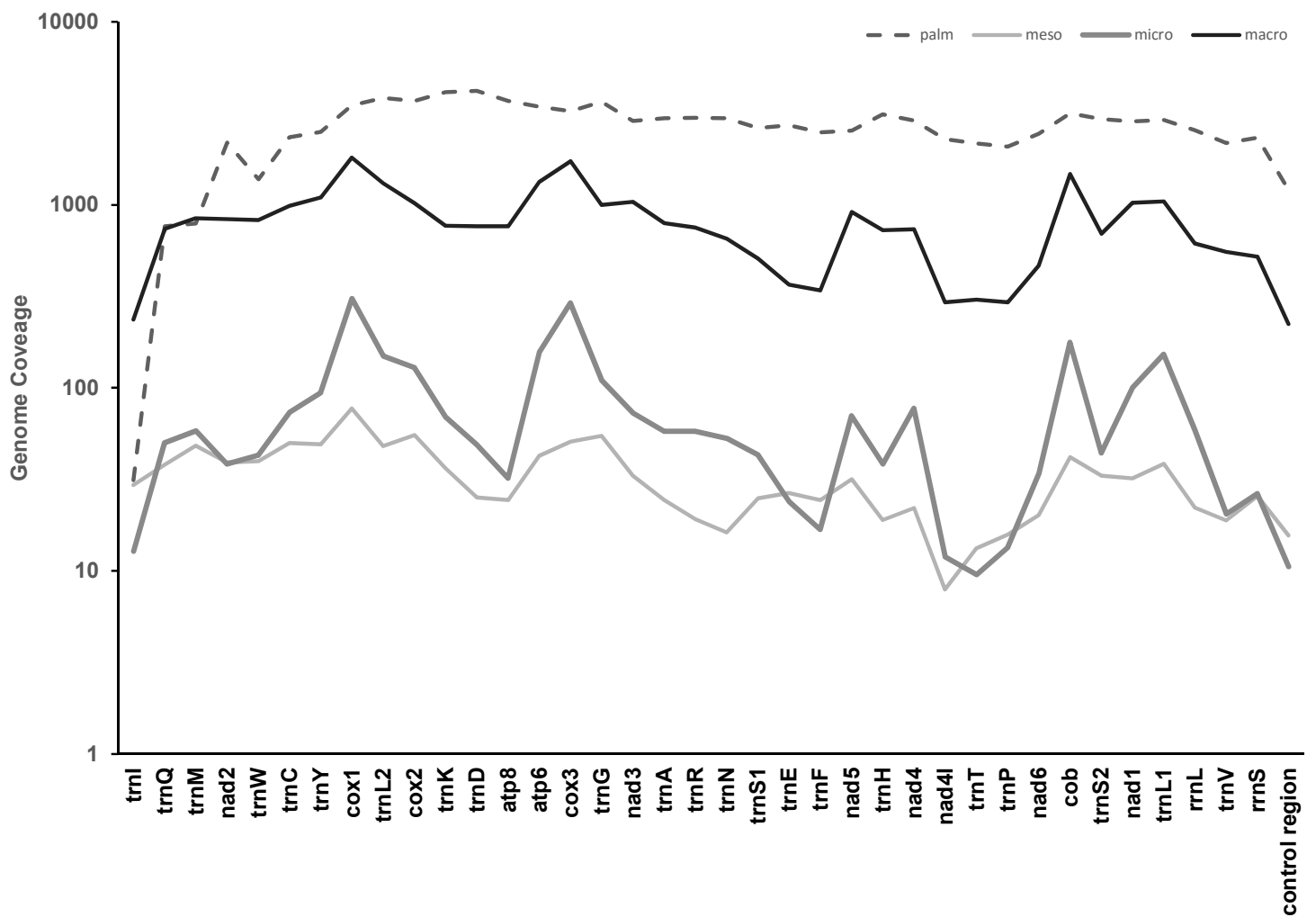
<i>KT876878 Acilius sp.</i>	4991.0	41.3	7.1	47.8	3.9	89.08	0.073	-0.292
<i>KT876885 Colymbetes sp.</i>	1263.0	41.8	6.6	46.6	5.1	88.36	0.054	-0.129
<i>KT876896 Hydroporus sp.</i>	4991.0	41.3	5.5	48.6	4.5	89.96	0.082	-0.106
<i>KT876897 Hydroporus sp.</i>	3165.0	44.8	6.0	47.4	1.8	92.20	0.028	-0.530
<i>KT876899 Hygrotus sp.</i>	4903.0	42.3	9.9	42.6	5.2	84.91	0.005	-0.305
<i>KT876900 Hygrotus sp.</i>	4717.0	44.8	6.4	44.2	4.6	88.93	-0.006	-0.161
<i>KT876901 Hygrotus sp.</i>	1521.0	43.7	5.8	46.4	4.1	90.07	0.029	-0.166
<i>KT876902 Liopterus sp.</i>	3093.0	44.0	6.1	45.8	4.1	89.85	0.020	-0.197
<i>L. palmulaoides</i>	1068.0	45.4	10.2	39.0	5.3	84.46	-0.075	-0.313
<i>P. macrosturtensis</i>	4456.0	42.5	9.9	42.4	5.2	84.92	-0.001	-0.310
<i>P. mesosturtensis</i>	2978.0	42.3	9.9	42.5	5.3	84.79	0.002	-0.302
<i>P. microsturtensis</i>	1498.0	42.4	10.2	41.7	5.7	84.05	-0.009	-0.280

Table S4

Codon number and relative synonymous codon usage in four subterranean mt protein coding genes.

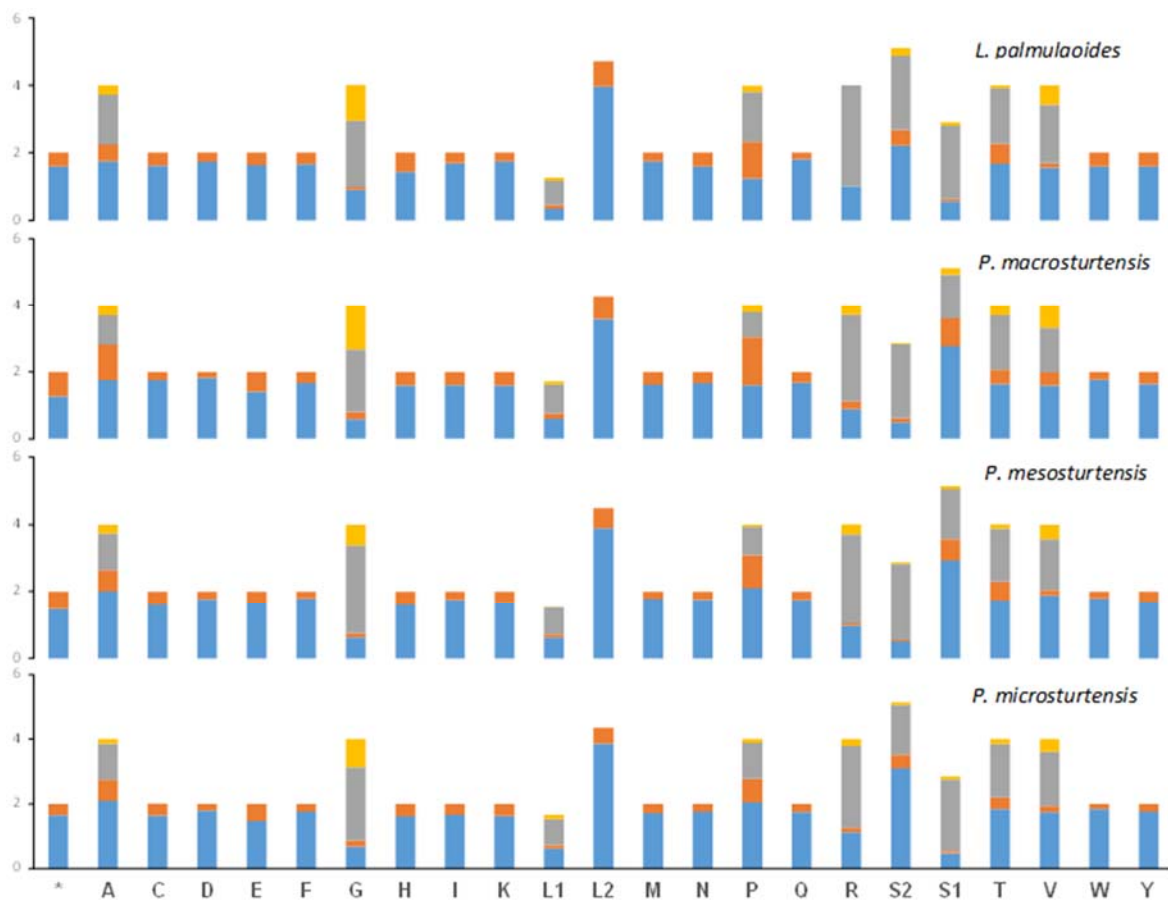
Codon	<i>P. microsturtensis</i>		<i>P. macrosturtensis</i>		<i>P. mesosturtensis</i>		<i>L. palmulaoides</i>	
	Count	RSCU	Count	RSCU	Count	RSCU	Count	RSCU
UUU(F)	330	1.75	311	1.67	342	1.78	310	1.68
UUC(F)	47	0.25	61	0.33	43	0.22	59	0.32
UUA(L)	360	3.86	337	3.59	360	3.89	363	3.96
UUG(L)	46	0.49	64	0.68	56	0.61	70	0.76
CUU(L)	57	0.61	55	0.59	56	0.61	31	0.34
CUC(L)	10	0.11	16	0.17	8	0.09	11	0.12
CUA(L)	76	0.81	82	0.87	74	0.8	66	0.72
CUG(L)	11	0.12	9	0.1	1	0.01	9	0.1
AUU(I)	322	1.66	310	1.61	340	1.74	339	1.7
AUC(I)	65	0.34	75	0.39	51	0.26	61	0.3
AUA(M)	264	1.7	242	1.62	274	1.77	263	1.75
AUG(M)	46	0.3	57	0.38	36	0.23	37	0.25
GUU(V)	69	1.74	69	1.59	75	1.88	62	1.57
GUC(V)	8	0.2	17	0.39	7	0.17	5	0.13
GUA(V)	66	1.66	58	1.33	61	1.52	68	1.72
GUG(V)	16	0.4	30	0.69	17	0.42	23	0.58
UCU(S)	140	3.11	124	2.77	128	2.93	101	2.2
UCC(S)	19	0.42	37	0.83	28	0.64	22	0.48
UCA(S)	69	1.53	59	1.32	65	1.49	101	2.2
UCG(S)	4	0.09	9	0.2	4	0.09	11	0.24
CCU(P)	64	2.05	51	1.61	67	2.09	42	1.25
CCC(P)	23	0.74	45	1.42	32	1	36	1.07
CCA(P)	34	1.09	24	0.76	27	0.84	50	1.49
CCG(P)	4	0.13	7	0.22	2	0.06	6	0.18
ACU(T)	76	1.84	69	1.64	71	1.73	74	1.69
ACC(T)	15	0.36	18	0.43	23	0.56	25	0.57
ACA(T)	68	1.65	68	1.62	65	1.59	72	1.65
ACG(T)	6	0.15	13	0.31	5	0.12	4	0.09
GCU(A)	76	2.1	62	1.75	72	2.01	57	1.75
GCC(A)	24	0.66	38	1.07	23	0.64	16	0.49
GCA(A)	40	1.1	31	0.87	39	1.09	48	1.48
GCG(A)	5	0.14	11	0.31	9	0.25	9	0.28
UAU(Y)	137	1.75	130	1.64	132	1.68	129	1.61
UAC(Y)	20	0.25	29	0.36	25	0.32	31	0.39
UAA(*)	9	1.64	7	1.27	8	1.45	8	1.6
UAG(*)	2	0.36	4	0.73	3	0.55	2	0.4
CAU(H)	58	1.61	56	1.6	57	1.58	52	1.44
CAC(H)	14	0.39	14	0.4	15	0.42	20	0.56
CAA(Q)	59	1.74	58	1.68	59	1.74	57	1.81
CAG(Q)	9	0.26	11	0.32	9	0.26	6	0.19
AAU(N)	166	1.75	158	1.67	166	1.75	159	1.61

AAC(N)	24	0.25	31	0.33	24	0.25	39	0.39
AAA(K)	86	1.62	88	1.6	92	1.64	89	1.75
AAG(K)	20	0.38	22	0.4	20	0.36	13	0.25
GAU(D)	59	1.79	59	1.82	59	1.76	60	1.74
GAC(D)	7	0.21	6	0.18	8	0.24	9	0.26
GAA(E)	56	1.47	52	1.41	61	1.63	63	1.64
GAG(E)	20	0.53	22	0.59	14	0.37	14	0.36
UGU(C)	26	1.63	28	1.75	26	1.58	26	1.63
UGC(C)	6	0.38	4	0.25	7	0.42	6	0.38
UGA(W)	91	1.84	86	1.76	87	1.78	79	1.61
UGG(W)	8	0.16	12	0.24	11	0.22	19	0.39
CGU(R)	15	1.11	12	0.89	13	0.96	14	1.02
CGC(R)	2	0.15	3	0.22	1	0.07	0	0
CGA(R)	34	2.52	35	2.59	36	2.67	41	2.98
CGG(R)	3	0.22	4	0.3	4	0.3	0	0
AGU(S)	21	0.47	22	0.49	21	0.48	26	0.57
AGC(S)	3	0.07	6	0.13	2	0.05	3	0.07
AGA(S)	100	2.22	99	2.21	100	2.29	99	2.15
AGG(S)	4	0.09	2	0.04	2	0.05	5	0.11
GGU(G)	36	0.67	31	0.58	32	0.61	48	0.91
GGC(G)	11	0.2	12	0.23	7	0.13	4	0.08
GGA(G)	123	2.28	99	1.86	138	2.64	104	1.96
GGG(G)	46	0.85	71	1.33	32	0.61	56	1.06



Supplementary Fig S1

Total genome coverage, for individual mt genes, by raw sequence data generated for the four subterranean beetle species (*L. palmulaoides* – palm; *P. macrosturtensis* – macro; *P. microsturtensis* – micro; *P. mesosturtensis* – meso). Codes for individual genes are as given in Fig. 1.



Supplementary Fig S2

The relative synonymous codon usage in four subterranean (*P. macrosturtensis*, *P. mesosturtensis*, *P. microsturtensis* and *L. palmulaoides*) dytiscid beetles mt genomes. Colours indicate individual codons for each amino acid. One letter amino acid code is used.

