Supplementary Material

Table S1 RSCU values of different groups of genes of _L. donovani_, _L. infantum_ and _L. major_

Amino acid	Codon	_L. donovani_		_L. infantum_		_L._ _major_	
		HEG[a]	LEG[a]	HEG[a]	LEG[a]	HEG[a]	LEG[a]
Phe	UUU	0.19	1.76[c]	0.28	1.11[c]	0.26	1.13[c]
	UUC	**1.81[b]**	0.24	**1.72[b]**	0.89	**1.74[b]**	0.87
Tyr	UAU	0.05	1.59[c]	0.11	0.61[c]	0.09	0.73[c]
	UAC	**1.95[b]**	0.41	**1.89[b]**	1.39	**1.91[b]**	1.27
His	CAU	0.13	1.44[c]	0.2	0.71[c]	0.16	0.70[c]
	CAC	**1.88[b]**	0.56	**1.8[b]**	1.29	**1.84[b]**	1.3
Gln	CAA	0.04	1.47[c]	0.07	0.64[c]	0.06	0.67[c]
	CAG	**1.96[b]**	0.53	**1.93[b]**	1.36	**1.94[b]**	1.33
Asn	AAU	0.09	1.48[c]	0.15	0.73[c]	0.12	0.77[c]
	AAC	**1.91[b]**	0.52	**1.85[b]**	1.27	**1.88[b]**	1.23
Lys	AAA	0.02	1.54[c]	0.08	0.65[c]	0.05	0.68[c]
	AAG	**1.98[b]**	0.46	**1.92[b]**	1.35	**1.95[b]**	1.32
Asp	GAU	0.43	1.34[c]	0.39	0.84[c]	0.35	0.90[c]
	GAC	**1.57[b]**	0.66	**1.61[b]**	1.16	**1.65[b]**	1.1
Glu	GAA	0.07	1.36[c]	0.15	0.66[c]	0.1	0.67[c]
	GAG	**1.93[b]**	0.64	**1.85[b]**	1.34	**1.9[b]**	1.33
Cys	UGU	0.09	1.33[c]	0.11	0.68[c]	0.11	0.69[c]
	UGC	**1.91[b]**	0.67	**1.89[b]**	1.32	**1.89[b]**	1.31
Ile	AUU	0.44	1.48[c]	0.54	1.28[c]	0.44	1.28[c]
	AUC	**2.54[b]**	0.17	**2.39[b]**	1.27	**2.51[b]**	1.19
	AUA	0.02	1.35[c]	0.07	0.45[c]	0.05	0.53[c]
Gly	GGU	0.86	1.07[c]	0.67	0.86[c]	0.71	0.99[c]
	GGC	**2.91[b]**	0.97	**2.89[b]**	1.57	**2.89[b]**	1.45
	GGA	0.08	1.31[c]	0.1	0.71[c]	0.08	0.74[c]
	GGG	0.15	0.66[c]	0.35	0.87[c]	0.32	0.81[c]
Val	GUU	0.29	1.45[c]	0.23	0.85[c]	0.31	0.84[c]
	GUC	0.72[b]	0.45	1.7[b]	0.96	0.9	0.95
	GUA	0.07	1.14[c]	0.08	0.51[c]	0.08	0.54[c]
	GUG	**2.92[b]**	0.95	**1.99[b]**	1.68	2.71	1.67
Pro	CCU	0.19	1.14[c]	0.37	0.94[c]	0.28	0.94[c]
	CCC	1.2*	0.87	0.96[b]	0.79	0.98[b]	0.76
	CCA	0.08	0.99[c]	0.27	0.96[c]	0.16	0.98[c]
	CCG	**2.53[b]**	1	**2.4[b]**	1.31	**2.58[b]**	1.31

(_to be continued on the next page_)

Table S1   (_Continued_)

Amino acid	Codon	*L. donovani*		*L. infantum*		*L. major*	
		HEG[a]	LEG[a]	HEG[a]	LEG[a]	HEG[a]	LEG[a]
Thr	ACU	0.3	1.66[c]	0.25	0.78[c]	0.22	0.75[c]
	ACC	1.4[b]	0.5	1.32[b]	0.98	1.2[b]	0.9
	ACA	0.09	1.16[c]	0.25	0.99[c]	0.22	1.17[c]
	ACG	**2.21[b]**	0.69	**2.18[b]**	1.25	**2.37[b]**	1.18
Ala	GCU	0.44	1.20[c]	0.41	0.86[c]	0.45	0.88[c]
	GCC	**1.59[b]**	0.54	**1.74[b]**	0.97	**1.45[b]**	0.95
	GCA	0.08	1.34[c]	0.2	0.92[c]	0.15	0.99[c]
	GCG	**1.89[b]**	0.92	**1.65[b]**	1.26	**1.95[b]**	1.17
Ser	UCU	0.62	1.28[c]	0.49	0.99[c]	0.53	1.05[c]
	UCC	**1.87[b]**	0.63	**1.36[b]**	0.91	**1.64[b]**	0.86
	UCA	0.03	1.16[c]	0.12	0.79[c]	0.08	0.81[c]
	UCG	**1.96[b]**	0.85	**1.66[b]**	1.24	**1.79[b]**	1.18
	AGU	0.13	1.47[c]	0.18	0.64[c]	0.15	0.69[c]
	AGC	**1.38[b]**	0.6	**2.19[b]**	1.44	**1.82[b]**	1.41
Arg	CGU	0.56	1.17[c]	0.58	0.96[c]	0.59	1.02[c]
	CGC	**5.24[b]**	0.98	**4.68[b]**	1.84	**4.73[b]**	1.77
	CGA	0.02	1.40[c]	0.07	0.98[c]	0.06	0.98[c]
	CGG	0.07	1.05[c]	0.44	1.03[c]	0.44	1.03[c]
	AGA	0	0.86[c]	0.04	0.51[c]	0.03	0.53[c]
	AGG	0.12	0.54[c]	0.19	0.68[c]	0.16	0.68[c]
Leu	UUA	0	2.46[c]	0.01	0.24[c]	0.01	0.31[c]
	UUG	0.14	1.02[c]	0.24	1.03[c]	0.2	0.99[c]

Note: Codons with bold RSCU values: the codon optimally used by a particular amino acid residue.
[a]HEG and LEG: groups of potential highly expressed genes (HEGs) and lowly expressed genes (LEGs) taken from two extreme ends of Axis 1 of COA of RSCU of genes in the respective species.
[b]Codons with significantly high frequencies in HEG, compared to LEG ($P<0.01$)
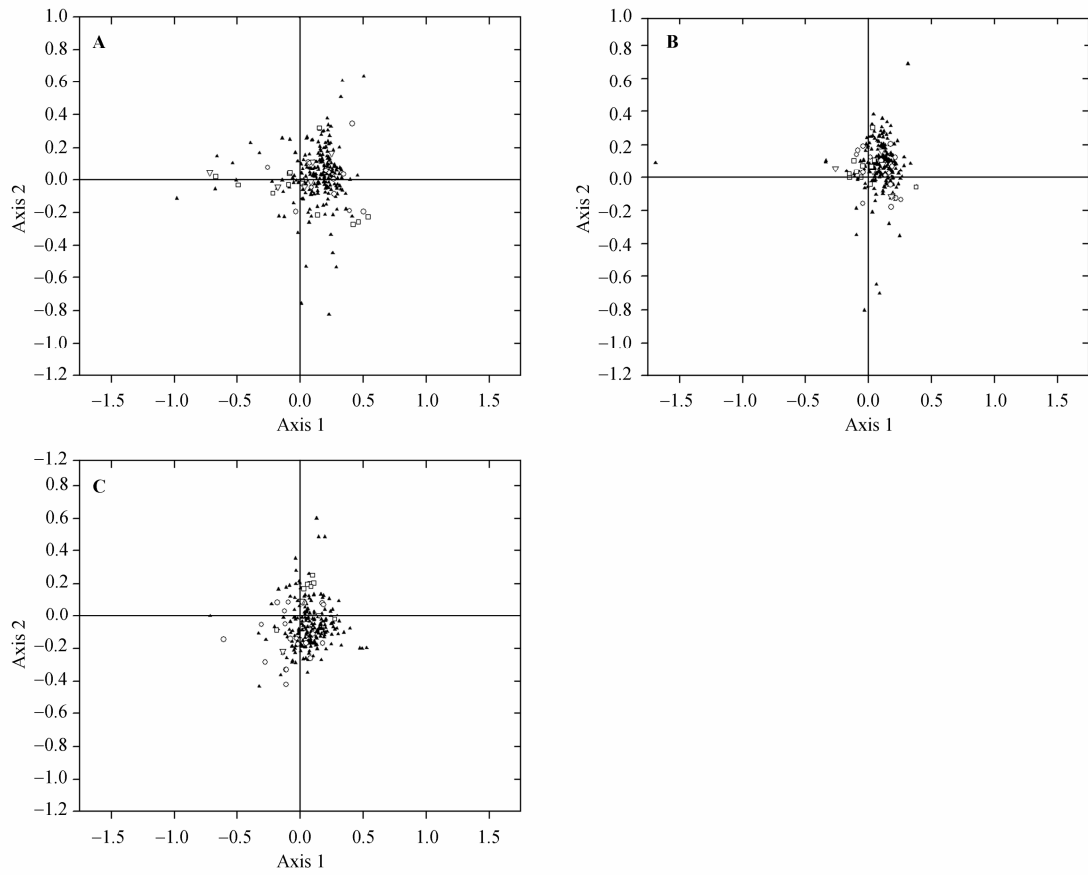[c]Codons with significantly high frequencies in LEG, compared to HEG ($P<0.01$)
*Highly expressed dataset ($0.01<P<0.05$).

Table S2   GC-content at three codon positions of HEGs and LEGs in three species of *Leishmania*

	HEGs			LEGs		
	L. donovani	*L. infantum*	*L. major*	*L. donovani*	*L. infantum*	*L. major*
GC	0.64	0.65	0.65	0.56	0.55	0.55
GC_1	0.58	0.61	0.60	0.58	0.59	0.59
GC_2	0.43	0.43	0.43	0.56	0.46	0.45
GC_3	0.92	0.91	0.92	0.55	0.60	0.60

Note: GC: overall GC composition; GC_1: GC composition at the first site of a codon; GC_2: GC composition at the second site of a codon, and GC_3: GC composition at the third site of a codon.

Figure S1 Position of homologous genes along the two major axes of variation in COA on RAAU. Position of homologous genes along Axis 1 were plotted against Axis 2 by COA on RAAU in *L. donovani* (**A**), *L. infantum* (**B**) and *L. major* (**C**). HEG, VSG and topoisomerase is represented by open circle, square and triangle, respectively. The rest of the genes (LEGs) are indicated by solid triangles.