

Ecography

ECOG-01244

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Supplementary material

Appendix 1

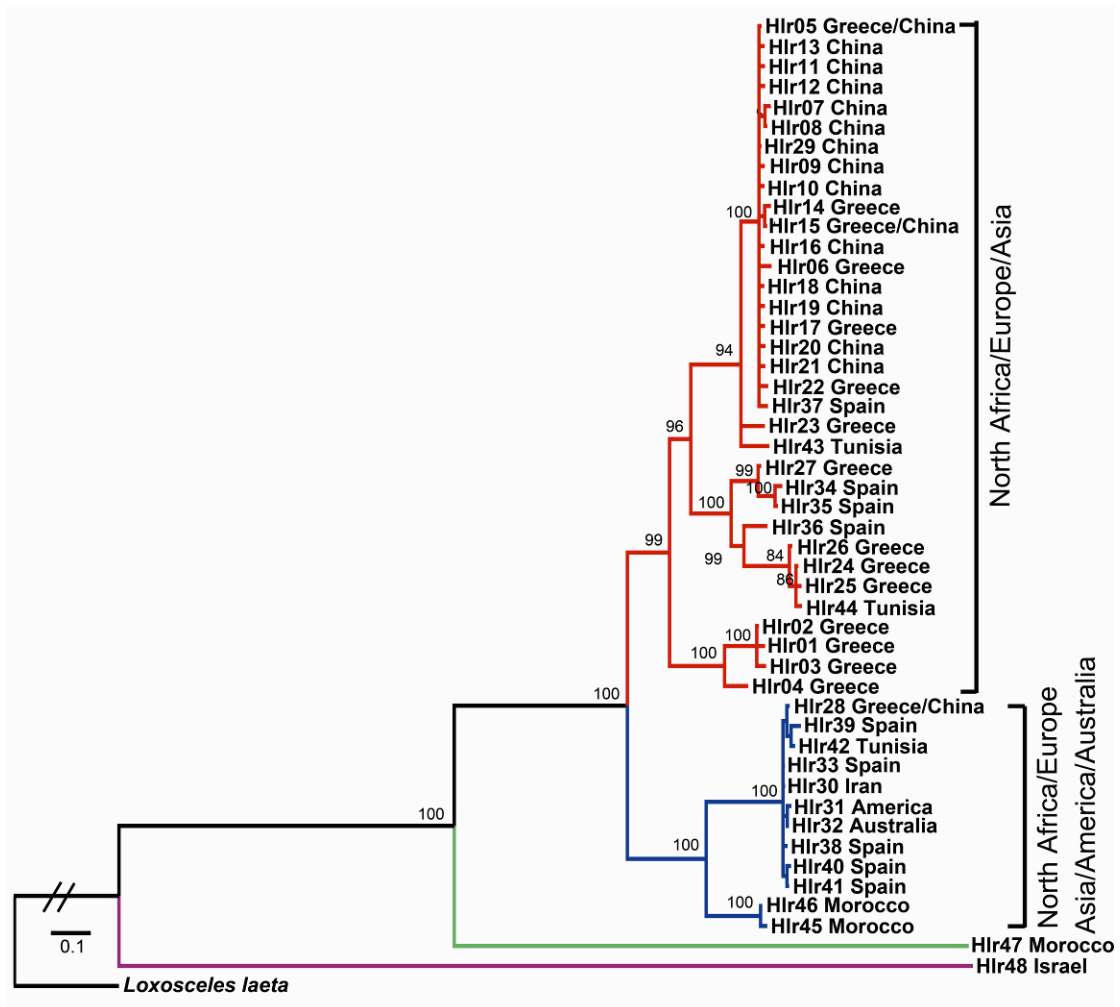


Fig. A1. Phylogenetic tree reconstructed using Bayesian inference based on the concatenated *COI* and 16S gene data. The outgroup taxon, *Loxosceles laeta*, branch is truncated as indicated by slashes. Numbers above nodes are posterior probabilities. Branch lengths indicate the expected number of substitutions per site.

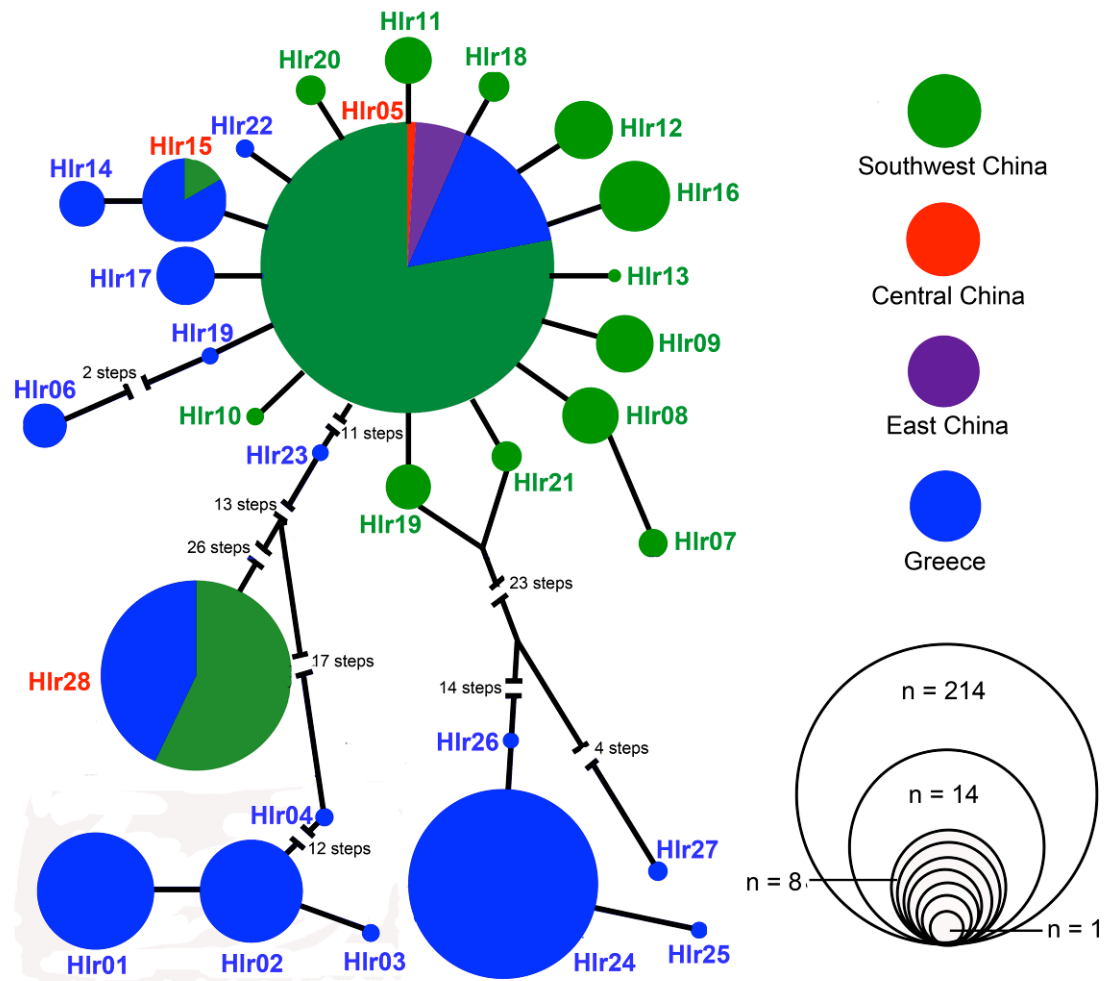


Fig. A2. Haplotype TCS network derived from concatenated mtDNA *COI* and 16S from the native Greek and invasive Chinese populations. Circle size is proportional to haplotype frequency. Shared haplotypes for Chinese and Greek individuals are marked in red.

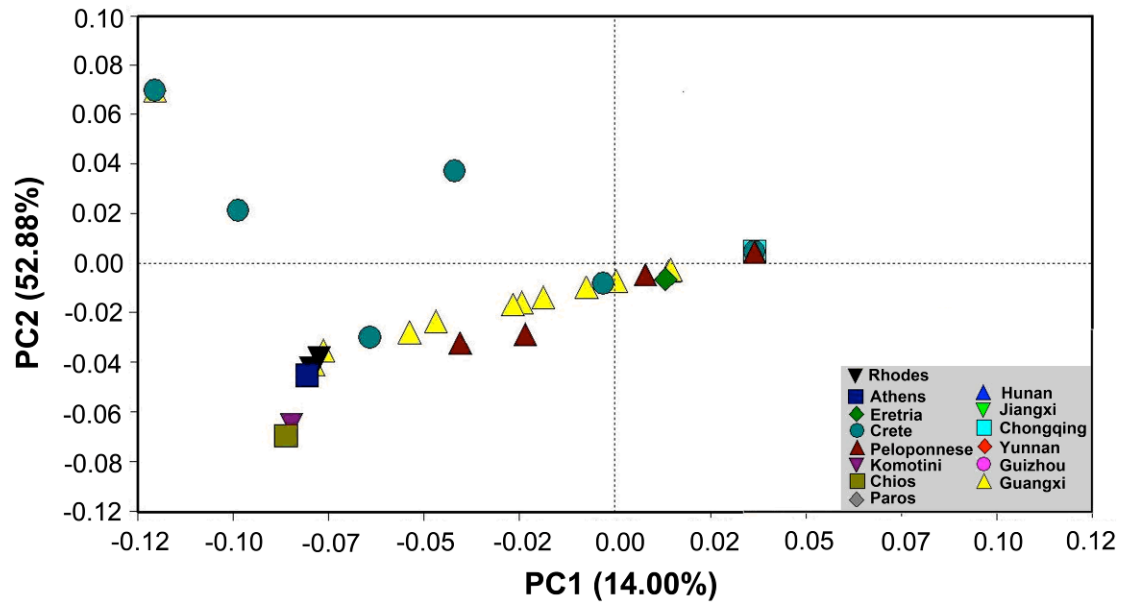


Fig. A3. Principle component analysis of the native Greek and invasive Chinese populations. The mtDNA haplogroup frequency data of eight Greek and six Chinese populations was used in this analysis. The first and second principle components (PC1 and PC2) explain 14.00 and 52.88% of the genetic variance, respectively.

Table A4. Taxon sampling for the molecular data set. The sequenced sample sizes (*N*) and sampling locations of the invasive spider *L. rufescens*.

Country	Site code	<i>N</i>	County or district, city or island, or province
China	1	2	Yuanjiang, Yuxin, Yunnan
	2	2	Qijiang, Chongqing A
	3	1	Chishui, Guizhou
	4	6	Wangmo, Guizhou
	5	6	Luodian, Guizhou A
	6	3	Ganxian, Ganzhou, Jiangxi
	7	2	Luodian, Guizhou B
	8	2	Fenghuang, Jishou, Hunan
	9	2	Ceheng, Guizhou A
	10	8	Qijiang, Chongqing B
	11	4	Ceheng, Guizhou B
	12	9	Taihe, Jian, Jiangxi
	13	5	Tiandong, Baise, Guangxi A
	14	7	Fusui, Chongzuo, Guangxi A
	15	6	Tianyang, Baise, Guangxi A
	16	6	Wuming, Nanning, Guangxi A
	17	6	Daxin, Chongzuo, Guangxi A
	18	7	Pingxiang, Chongzuo, Guangxi A
	19	2	Longzhou, Chongzuo, Guangxi A
	20	2	Debao, Baise, Guangxi A
	21	6	Tiandong, Baise, Guangxi B
	22	4	Yizhou, Hechi, Guangxi A
	23	4	Tianyang, Baise, Guangxi B
	24	4	Fusui, Chongzuo, Guangxi B
	25	5	Daxin, Chongzuo, Guangxi B
	26	9	Debao, Baise, Guangxi B
	27	2	Tiandeng, Chongzuo, Guangxi A
	28	7	Fusui, Chongzuo, Guangxi C
	29	1	Pingxiang, Chongzuo, Guangxi B
	30	3	Tianyang, Baise, Guangxi C
	31	6	Yizhou, Hechi, Guangxi B
	32	4	Tiandeng, Chongzuo, Guangxi B
	33	9	Fengshan, Hechi, Guangxi
	34	4	Tiandeng, Chongzuo, Guangxi C
	35	1	Jiangzhou, Chongzuo, Guangxi A
	36	4	Longzhou, Chongzuo, Guangxi B
	37	2	Pingguo, Baise, Guangxi
	38	3	Tiandeng, Chongzuo, Guangxi C
	39	4	Tiandeng, Chongzuo, Guangxi D
	40	4	Fusui, Chongzuo, Guangxi D

	41	1	Tiandeng, Chongzuo, Guangxi E
	42	5	Daxin, Chongzuo, Guangxi C
	43	1	Tianyang, Baise, Guangxi D
	44	4	Daxin, Chongzuo, Guangxi D
	45	5	Daxin, Chongzuo, Guangxi E
	46	4	Longzhou, Chongzuo, Guangxi C
	47	2	Tiandeng, Chongzuo, Guangxi F
	48	2	Luchuan, Yulin, Guangxi
	49	5	Wuming, Nanning, Guangxi B
	50	1	Fusui, Chongzuo, Guangxi E
	51	5	Tiandeng, Chongzuo, Guangxi G
	52	5	Dahua, Hechi, Guangxi
	53	3	Jiangzhou, Chongzuo, Guangxi B
	54	1	Lingchuan, Guilin, Guangxi
	55	1	Liujiang, Liuzhou, Guangxi
	56	2	Wuming, Nanning, Guangxi C
	57	1	Jiangzhou, Chongzuo, Guangxi C
Australia	58	1	Adelaide, Hawthorn
Iran	59	1	Shriaz
	60	1	Tehran
Israel	61	2	Tel Aviv
Greece	62	2	Crete, Iraklion B
	63	2	Crete, Chania
	64	1	Rhodes B
	65	6	Peloponnese, Messini A
	66	1	Crete, Iraklion C
	67	11	Komotini
	68	9	Crete, Iraklion D
	69	10	Peloponnese, Messini B
	70	10	Crete, Iraklion E
	71	2	Peloponnese, Messini C
	72	2	Crete, Nikolaos
	73	1	Crete, Iraklion F
	74	1	Chios
	75	1	Paros
	76	10	Eretria, Diamanto Rooms
	77	3	Peloponnese, Messini D
	78	16	Rhodes A
	79	3	Athens
	80	2	Crete, Iraklion A
America	81	1	Manhattan, NY
Spain	82	1	Sierra Gorda, Cartagena
	83	1	Monte Arabì, Yecla, Murcia
	84	1	Torrejon de Adroz

	85	1	Alicante
	86	1	Denia
	87	1	Ciudad Real
	88	1	Oropesa
	89	1	Chumilla
	90	1	Collado Bermejo, Córdoba
	91	1	Purias
	92	1	Barcelona
	93	2	Mostoles
	94	1	Villamarxant
	95	1	Almería, Cabo de Gata
	96	1	Aimería, Desierto de Tabernas
	97	1	Cartagena, Bajo Piedra
	98	1	Siles, Jaen, Cueva del Castillo
	99	1	Gran Canaria, Firgas
Tunisia	100	1	Ousseltia
	101	1	Beja
	102	1	Testour
Morocco	103	1	Bouizakarne
	104	3	Asni

Table A5. Summary of genetic diversity in *L. rufescens* from China and Greece based on mtDNA and rDNA. Number of isolates (ni), polymorphic sites (S), parsimony informative sites (PIS), number of haplotypes (nh), nucleotide diversity (π), average nucleotide polymorphism (θ), haplotype diversity (h), average number of pairwise differences (k), Fu's FS and Tajima's D are shown. JX, Jiangxi; HN, Hunan; CQ, Chongqing; GZ, Guizhou; GX, Guangxi; YN, Yunnan.

Population code	ni	S	PIS	nh	π (%)	θ (%)	h	k	Fu's FS	Tajima's D
China	222	66	59	26	0.13 $\pm$ 0.03	0.31 $\pm$ 0.08	0.72 $\pm$ 0.03	4.726	-2.0840	-1.6038
East China	12	2	1	3	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.44 $\pm$ 0.16	0.576	1.2235	-0.0974
JX	12	2	1	3	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.44 $\pm$ 0.16	0.576	1.2235	-0.0974
Central China	2	0	0	1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.000	0.0000	0.0000
HN	2	0	0	1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.000	0.0000	0.0000
Southwest China	208	66	59	26	0.14 $\pm$ 0.04	0.32 $\pm$ 0.08	0.72 $\pm$ 0.03	4.935	-2.0222	-1.5822
GX	174	65	59	25	0.15 $\pm$ 0.04	0.32 $\pm$ 0.08	0.73 $\pm$ 0.03	5.151	-1.8772	-1.6092
GZ	23	2	2	3	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.63 $\pm$ 0.07	0.964	2.8970	1.5943
CQ	10	2	1	3	0.02 $\pm$ 0.01	0.02 $\pm$ 0.02	0.64 $\pm$ 0.10	0.756	2.0920	-0.4526
YN	2	0	0	1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.000	0.0000	0.0000
Greece	92	96	96	34	0.77 $\pm$ 0.06	0.53 $\pm$ 0.14	0.95 $\pm$ 0.01	27.226	2.8515	1.5095

Table A6. GenBank accession numbers for *L. rufescens* mtDNA haplotypes analyzed in this study.

<i>COI</i> haplotype			16S rDNA haplotype		
Haplotype code	GenBank accession number	Reference	Haplotype code	GenBank accession number	Reference
H01 <i>COI</i> _1	KR706261	<i>Present study</i>	H01 16S_1	KR706287	<i>Present study</i>
H02 <i>COI</i> _1	KR706262	<i>Present study</i>	H02 16S_1	KR706288	<i>Present study</i>
H03 <i>COI</i> _1	KR706263	<i>Present study</i>	H03 16S_1	KR706289	<i>Present study</i>
H04 <i>COI</i> _1	KR706264	<i>Present study</i>	H04 16S_1	KR706290	<i>Present study</i>
H05 <i>COI</i> _1	KR706265	<i>Present study</i>	H05 16S_1	KR706291	<i>Present study</i>
H06 <i>COI</i> _1	KR706266	<i>Present study</i>	H06 16S_1	KR706292	<i>Present study</i>
H07 <i>COI</i> _1	KR706267	<i>Present study</i>	H07 16S_1	KR706293	<i>Present study</i>
H08 <i>COI</i> _1	KR706268	<i>Present study</i>	H08 16S_1	KR706294	<i>Present study</i>
H09 <i>COI</i> _1	KR706269	<i>Present study</i>	H09 16S_1	KR706295	<i>Present study</i>
H10 <i>COI</i> _1	KR706270	<i>Present study</i>	H10 16S_1	KR706296	<i>Present study</i>
H11 <i>COI</i> _1	KR706271	<i>Present study</i>	H11 16S_1	GQ279113	Duncan et al. 2010
H12 <i>COI</i> _1	KR706272	<i>Present study</i>	H12 16S_1	GQ279114	Duncan et al. 2010
H13 <i>COI</i> _1	KR706273	<i>Present study</i>	H13 16S_1	GQ279157	Duncan et al. 2010
H14 <i>COI</i> _1	KR706274	<i>Present study</i>	H14 16S_1	GQ279171	Duncan et al. 2010
H15 <i>COI</i> _1	KR706275	<i>Present study</i>	H15 16S_1	GQ279170	Duncan et al. 2010
H16 <i>COI</i> _1	KR706276	<i>Present study</i>	H16 16S_1	GQ279168	Duncan et al. 2010
H17 <i>COI</i> _1	KR706277	<i>Present study</i>	H17 16S_1	GQ279156	Duncan et al. 2010
H18 <i>COI</i> _1	KR706278	<i>Present study</i>	H18 16S_1	GQ279153	Duncan et al. 2010
H19 <i>COI</i> _1	KR706279	<i>Present study</i>	H19 16S_1	GQ279151	Duncan et al. 2010
H20 <i>COI</i> _1	KR706280	<i>Present study</i>	H20 16S_1	GQ279150	Duncan et al. 2010
H21 <i>COI</i> _1	KR706281	<i>Present study</i>	H21 16S_1	GQ279118	Duncan et al. 2010
H22 <i>COI</i> _1	KR706282	<i>Present study</i>	H22 16S_1	GQ279115	Duncan et al. 2010
H23 <i>COI</i> _1	KR706283	<i>Present study</i>	H23 16S_1	GQ279116	Duncan et al. 2010
H24 <i>COI</i> _1	KR706284	<i>Present study</i>	H24 16S_1	GQ279117	Duncan et al. 2010
H25 <i>COI</i> _1	KR706285	<i>Present study</i>	H25 16S_1	GQ279145	Duncan et al. 2010
H26 <i>COI</i> _1	KR706286	<i>Present study</i>	H26 16S_1	GQ279147	Duncan et al. 2010
H27 <i>COI</i> _1	GQ279236	Duncan et al. 2010	H27 16S_1	GQ279227	Duncan et al. 2010
H28 <i>COI</i> _1	GQ279221	Duncan et al. 2010			
H29 <i>COI</i> _1	FJ986186	Duncan et al. 2010			
H30 <i>COI</i> _1	GQ279220	Duncan et al. 2010			
H31 <i>COI</i> _1	GQ279226	Duncan et al. 2010			
H32 <i>COI</i> _1	GQ279224	Duncan et al. 2010			
H33 <i>COI</i> _1	QG279232	Duncan et al. 2010			
H34 <i>COI</i> _1	GQ279215	Duncan et al. 2010			
H35 <i>COI</i> _1	FJ986187	Duncan et al. 2010			
H36 <i>COI</i> _1	GQ279212	Duncan et al. 2010			
H37 <i>COI</i> _1	GQ279233	Duncan et al. 2010			

H38 <i>COI</i> _1	GQ279219	Duncan et al. 2010
H39 <i>COI</i> _1	GQ279230	Duncan et al. 2010
H40 <i>COI</i> _1	GQ279231	Duncan et al. 2010
H41 <i>COI</i> _1	GQ279228	Duncan et al. 2010
H42 <i>COI</i> _1	GQ279229	Duncan et al. 2010
H43 <i>COI</i> _1	GQ279210	Duncan et al. 2010

Supplemental references:

Duncan, R. P. et al. 2010. Diversity of *Loxosceles* spiders in Northwestern Africa and molecular support for cryptic species in the *Loxosceles rufescens* lineage. – Mol. Phylogenet. Evol. 55: 234–248.

Table A7. GenBank accession numbers for *L. rufescens* rDNA haplotypes analyzed in this study.

28S rRNA haplotype			ITS2 haplotype		
Haplotype code	GenBank accession number	Reference	Haplotype code	GenBank accession number	Reference
H01 28S_1	KR706297	<i>Present study</i>	H01 ITS2_1	KR706299	<i>Present study</i>
H02 28S_1	KR706298	<i>Present study</i>	H02 ITS2_1	KR706300	<i>Present study</i>
			H03 ITS2_1	KR706301	<i>Present study</i>
			H04 ITS2_1	KR706302	<i>Present study</i>

Table A8. Primer sequences used in this study.

Gene	Primers code	Primer sequence	Reference
COI	LCO1490	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	Folmer et al. 1994
	HCO2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Folmer et al. 1994
	F42	5'-GCT TTC CCG CGG ATG AAT AA-3'	<i>Present study</i>
	R675	5'-ACG CTC GAG TAT CAA CGT CT-3'	<i>Present study</i>
	R847	5'-GAA TTA GCC AAG ATC ACA CCT GT-3'	<i>Present study</i>
16S	16S1471-mod	5'-GCC TGT TTA WCA AAA ACA T-3'	Crandall and Fitzpatrick 1996
	16Sbr-H-mod	5'-CCG GTY TGA ACT CAR ATC AYG T-3'	Palumbi et al. 2002
	16SA	5'-CGC CTG TTT ATC AAA AAC AT-3'	Tan et al. 1999
	16SB2	5'-CTC CGG TTT GAA CTC AGA TCA-3'	Tan et al. 1999
28S	ZX1	5'-ACC CGC TGA ATT TAA GCA TAT-3'	Bond and Hedin 2006
	A58OP1	5'-AGA GCC AAT CCT TGT CCC GA-3'	Bond and Hedin 2006
<i>Internal primers for sequencing</i>			
	65-w1r	5'-GGC AGG TGA GTT GTT ACA CA-3'	<i>Present study</i>
ITS2	CAS5p8SFC	5'-TGA ACA TCG ACA TTT YGA ACG CAC AT-3'	Ji et al. 2003
	CAS28SB1d	5'-TTC TTT TCC SCT TAY TRA TAT GCT TAA-3'	Ji et al. 2003

Supplemental references:

Folmer, O. et al. 1994. DNA primers for amplification of mitochondrial cytochrome oxidase subunit I from diverse metazoan invertebrates. – *Mol. Mar. Biol. Biotechnol.* 3: 294–299.

Crandall, K. A. and Fitzpatrick, J. E. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. – *Syst. Biol.* 45: 1–26.

Palumbi, S. R. et al. 2002. The simple fool's guide to PCR, Version 2.0. – Honolulu: privately published.

Tan, A. M. et al. 1999. Paraphyly of the *Enoplognatha* group (Araneae, Theridiidae) based on DNA sequences. – *J. Arachnol.* 27: 481–488.

Bond, J. E. and Hedin, M. C. 2006. A total evidence assessment of the phylogeny of North American euctenizine trapdoor spiders (Araneae, Mygalomorphae, Cyrtaucheniidae) using Bayesian inference. – *Mol. Phylogenet. Evol.* 41: 70–85.

Ji, Y. J. et al. 2003. Evolutionary conservation and versatility of a new set of primers for amplifying the ribosomal internal transcribed spacer regions in insects and other invertebrates. – *Mol. Ecol. Notes* 3: 581–585.

Table A9. GenBank accession numbers of *COI* and 16S rDNA genes of the haplogyne spiders used to estimate the divergence time and mtDNA substitution rate of *L. rufescens* from Greek and Chinese populations in this study.

Species	Family	<i>COI</i>	16S rDNA
<i>Dysdera erythrina fervida</i>	Dysderidae	GQ285630	GQ285606
<i>Dysderocrates silvestris</i>	Dysderidae	AF244236	AF244151
<i>Plectreurys tristis</i>	Plectreuridae	FJ607585	FJ607472
<i>Xestaspis loricata</i>	Oonopidae	KR864742	KR864748
<i>Ischnothyreus auritus</i>	Oonopidae	KR864743	KR864749
<i>Ischnothyreus peltifer</i>	Oonopidae	KR864744	KR864750
<i>Dictis striatipes</i>	Scytodidae	KR864741	KR864747
<i>Dictis denticulate</i>	Scytodidae	KR864740	KR864746
<i>Scytodes tigrina</i>	Scytodidae	KR864739	KR864745
<i>Loxosceles laeta</i>	Sicariidae	GQ279223	EU817794
<i>Sicarius terrosus</i>	Sicariidae	EU817704	EU817810
<i>Leptopholcus tipula</i>	Pholcidae	JX023545	JX023853
<i>Leptopholcus guineensis</i>	Pholcidae	JX023548	JX023856
<i>Quamtana</i> sp.	Pholcidae	JX023567	JX023883
<i>Quamtana filmeri</i>	Pholcidae	JX023607	JX023948
<i>Ariadna fidicina</i>	Segestriidae	FJ607555	FJ607447
<i>Segestria senoculata</i>	Segestriidae	JQ412457	JQ406615
<i>Segestria</i> sp. LBB-2011	Segestriidae	JN689168	JN705789