

## Supplementary material

### **Contrasting and complex evolutionary histories within the terapontid grunter genus *Hephaestus* revealed by nuclear and mitochondrial genes**

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**Table S1. Microsatellite diversity indices for *Hephaestus fuliginosus* and *H. tulliensis***

*n*, sample size; *A<sub>T</sub>*, total number of alleles; *H<sub>O</sub>*, observed heterozygosity; *H<sub>e</sub>*, expected heterozygosity; HWE, significant level of HWE expectation; –, insufficient sample size; values that are significant value after Bonferroni correction are indicated as: \*, *P* < 0.0001

Locus	Measure of diversity	<i>H. fuliginosus</i>					<i>H. tulliensis</i>			
		Herbert ( <i>n</i> = 9)	Burdekin ( <i>n</i> = 5)	N. Johnstone ( <i>n</i> = 11)	Murray ( <i>n</i> = 1)	Tully ( <i>n</i> = 10)	Cowley ( <i>n</i> = 1)	N. Johnstone ( <i>n</i> = 10)	Tully ( <i>n</i> = 12)	Daintree ( <i>n</i> = 2)
2Hf14	<i>A<sub>T</sub></i>	4	3	4	1	4	1	2	3	1
	<i>H<sub>O</sub></i>	0.22	0	0.11	0.00	0.25	0.00	0.00	0.00	0.00
	<i>H<sub>e</sub></i>	0.65	0.80	0.69	–	0.35	–	0.43	0.36	–
	HWE	<b>0.01</b>	0.06	<b>0.00</b>	*	0.20	*	0.14	<b>0.00</b>	*
2Hf34	<i>A<sub>T</sub></i>	2	2	4	2	2	1	2	3	1
	<i>H<sub>O</sub></i>	0.44	0.20	0.45	1.00	0.50	0.00	0.40	0.30	0.00
	<i>H<sub>e</sub></i>	0.52	0.20	0.59	1.00	0.48	–	0.44	0.28	–
	HWE	1.00	1.00	0.12	*	1.00	*	1.00	1.00	*
2Hf44	<i>A<sub>T</sub></i>	2	2	2	2	2	2	3	3	1
	<i>H<sub>O</sub></i>	0.60	0.25	0.50	1.00	1.00	1.00	0.63	0.89	0.00
	<i>H<sub>e</sub></i>	0.47	0.25	0.41	1.00	0.53	–	0.54	0.58	–
	HWE	1.00	1.00	1.00	*	<b>0.01</b>	*	0.40	0.08	*
2Hf53	<i>A<sub>T</sub></i>	7	6	7	2	5	1	7	4	4
	<i>H<sub>O</sub></i>	0.67	0.60	0.55	1.00	0.30	0.00	0.40	0.33	1.00
	<i>H<sub>e</sub></i>	0.75	0.89	0.85	1.00	0.77	–	0.88	0.74	1.00
	HWE	0.16	0.15	<b>0.02</b>	*	<b>0.00</b>	*	<b>0.01</b>	<b>0.00</b>	1.00
2Hf56	<i>A<sub>T</sub></i>	2	2	4	2	2	1	2	3	1
	<i>H<sub>O</sub></i>	0.50	0.20	0.50	1.00	0.40	0.00	0.10	0.10	0.00
	<i>H<sub>e</sub></i>	0.53	0.20	0.69	1.00	0.34	–	0.10	0.19	–
	HWE	1.00	1.00	0.50	*	1.00	*	1.00	<b>0.05</b>	*
2Hf60	<i>A<sub>T</sub></i>	3	1	5	1	2	1	5	2	1
	<i>H<sub>O</sub></i>	0.63	0.00	0.27	0.00	0.00	0.00	0.44	0.14	0.00
	<i>H<sub>e</sub></i>	0.51	–	0.67	–	0.67	–	0.71	0.14	–
	HWE	1.00	*	<b>0.00</b>	*	0.33	*	0.22	1.00	*
2Hf77	<i>A<sub>T</sub></i>	3	2	4	1	1	1	2	1	1
	<i>H<sub>O</sub></i>	0.75	0.40	0.30	0.00	0.00	0.00	0.00	0.00	0.00
	<i>H<sub>e</sub></i>	0.58	0.36	0.44	–	–	–	0.36	–	–
	HWE	0.27	1.00	0.13	*	*	*	0.11	*	*

Locus	Measure of diversity	<i>H. fuliginosus</i>						<i>H. tulliensis</i>		
		Herbert ( <i>n</i> = 9)	Burdekin ( <i>n</i> = 5)	N. Johnstone ( <i>n</i> = 11)	Murray ( <i>n</i> = 1)	Tully ( <i>n</i> = 10)	Cowley ( <i>n</i> = 1)	N. Johnstone ( <i>n</i> = 10)	Tully ( <i>n</i> = 12)	Daintree ( <i>n</i> = 2)
2Hf82	$A_T$	4	1	4	1	1	1	1	1	2
	$H_O$	0.17	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.50
	$H_e$	0.80	–	0.74	–	–	–	–	–	0.50
	HWE	<b>0.00</b>	*	<b>0.00</b>	*	*	*	*	*	1.00
2Hf85	$A_T$	2	1	2	2	2	1	1	1	2
	$H_O$	0.33	0.00	0.17	1.00	0.25	0.00	0.00	0.00	0.00
	$H_e$	0.30	–	0.41	1.00	0.23	–	–	–	0.67
	HWE	1.00	*	0.27	*	1.00	*	*	*	0.33

**Table S2. Frequency and distribution of *Hephaestus* haplotypes for the ATPase, 16S and S7 genes**

Abbreviations for river names as in Fig. 2 in the main paper

Species	Haplotype number	GenBank accession number	Locality																									
			A	Bl	Buc	Bur	Ca	Co	Dai	Dal	F	Gi	Go	H	Ja	Jo	K	L	Mi	Mu	N	Ol	Or	PNG	R	S	T	W
ATP8																												
<i>H. carbo</i>	33	KT995409																	4			7			1			
	34	KT995408		1	1								3															
	35	KT995407			1								1															
	36	KT995406													4													5
<i>H. fuliginosus</i>	1	KT995412																									1	
	2	KT995413																									1	
	3	KT995414	9					5	4			2							4		1					5	1	
	4	KT995415																								1		
	5	KT995416																	1									
	6	KT995417																										
	7	KT995418																										
	8	KT995411						8						3														
	9	KT995410											1															
	15	KT995419										1																
	16	KT995420										#																
	19	KT995432																							5			
	21	KT995423						7							4		#				1						#	
	22	KT995422																									1	
23	KT995424																											
24	KT995421																											
<i>H. habbemai</i>	31	KU057350																							3			
	32	KU057349																							1			
<i>H. jenkinsi</i>	10	KT995427																								2		
	11	KT995428																								2		
	12	KT995426																								1		
	13	KT995429																								#		
	14	KT995430																								1		
<i>H. roemeri</i>	17	KT995425											2															
	18	KT995431																								1		
	19	KT995432																								1		
	20	KT995433																								2		

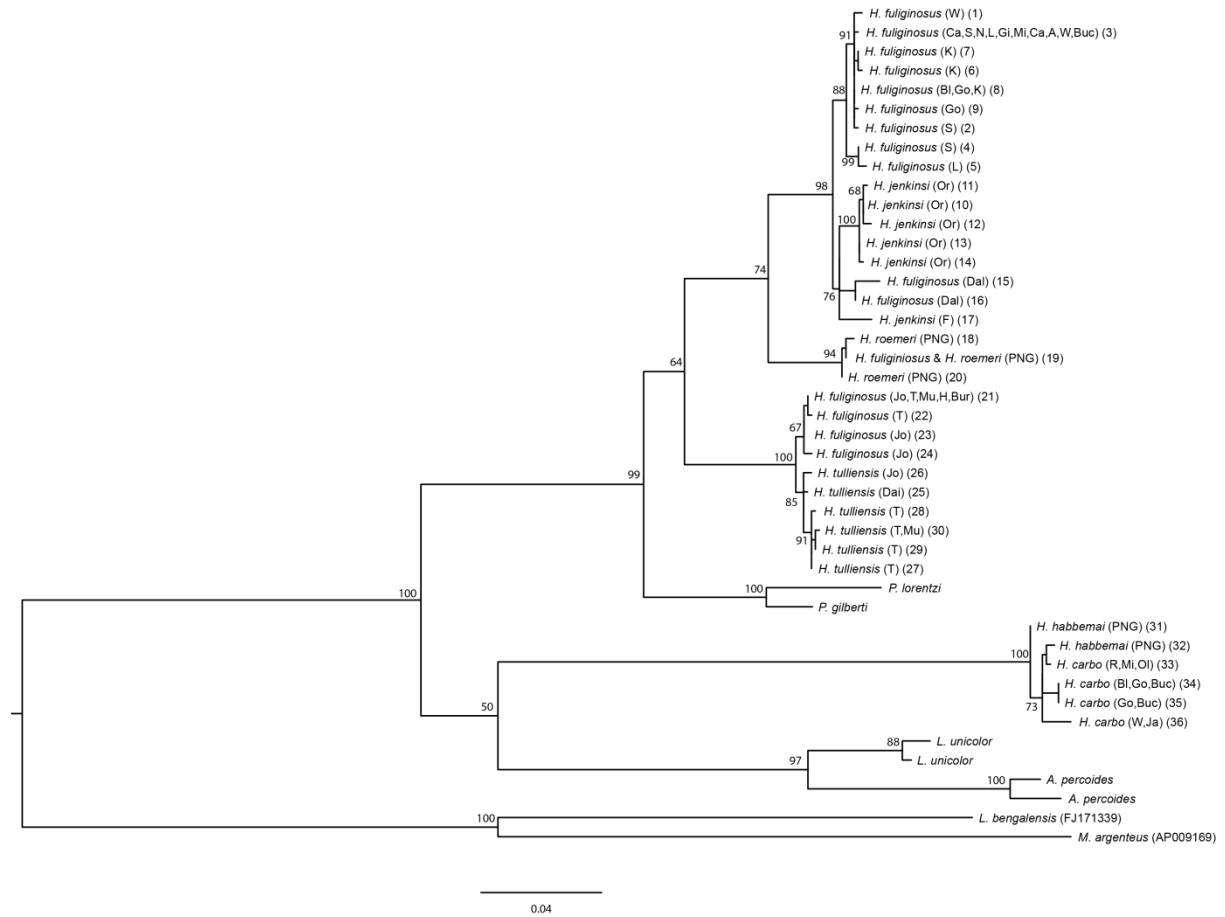
Species	Haplotype number	GenBank accession number	Locality																											
			A	Bl	Buc	Bur	Ca	Co	Dai	Dal	F	Gi	Go	H	Ja	Jo	K	L	Mi	Mu	N	Ol	Or	PNG	R	S	T	W		
<i>H. tulliensis</i>	25	KT995435							3																					
	26	KT995434																												
	27	KT995439																												
	28	KT995437																												
	29	KT995438																												
	30	KT995436																												
16S																														
<i>H. carbo</i>	5	KT995405				1																								
	6	KT934811				1																								
	7	KT934814				1																								
	16	KT934813												3															3	
	18	KT934826																					3							
<i>H. fuliginosus</i>	19	KT934812																				1								
	1	KT934818						1	2			1					1	2				1	2				2	2		
	2	KT934822															1													
	3	KT934817											1																	
	4	KT934819						1					1																	
	9	KT934816																												
	10	KT934824																						2						
	12	KT934823															1					1						1		
	13	KT934820																												
	14	KT934821																												
<i>H. habbema</i>	17	KT934825																										1		
	18	KT934826																										3		
<i>H. jenkinsi</i>	8	KT934827																										#		
<i>H. roemeri</i>	9	KT934816																										2		
	10	KT934824																										1		
<i>H. tulliensis</i>	11	KT934828																										1		
	15	KT934829																										4		
S7																														
<i>H. carbo</i>	18	KT995440																												
	1	KT995452																												
<i>H. fuliginosus</i>	2	KT995451																												
	4	KT995444																										1		
	5	KT995445																										1		
	6	KT995446																										1		
	7	KT995443																										1		

Species	Haplotype number	GenBank accession number	Locality																										
			A	Bl	Buc	Bur	Ca	Co	Dai	Dal	F	Gi	Go	H	Ja	Jo	K	L	Mi	Mu	N	Ol	Or	PNG	R	S	T	W	
	8	KT995448																											1
	9	KT995449																											1
	11	KT995450																						1					
	12	KT995447																					1						
	13	KT995442																					2						
<i>H. habbema</i>	17	KT995441																					2						
<i>H. jenkinsi</i>	10	KT995453																					2						
<i>H. roemeri</i>	3	KT995454																					1						
<i>H. tulliensis</i>	14	KT995456																											1
	15	KT995457																											1
	16	KT995455																											4

**Table S3. Pairwise genetic differentiation index ( $F_{ST}$ ) estimates among sampling locations of eastern *Hephaestus fuliginosus* and *H. tulliensis***

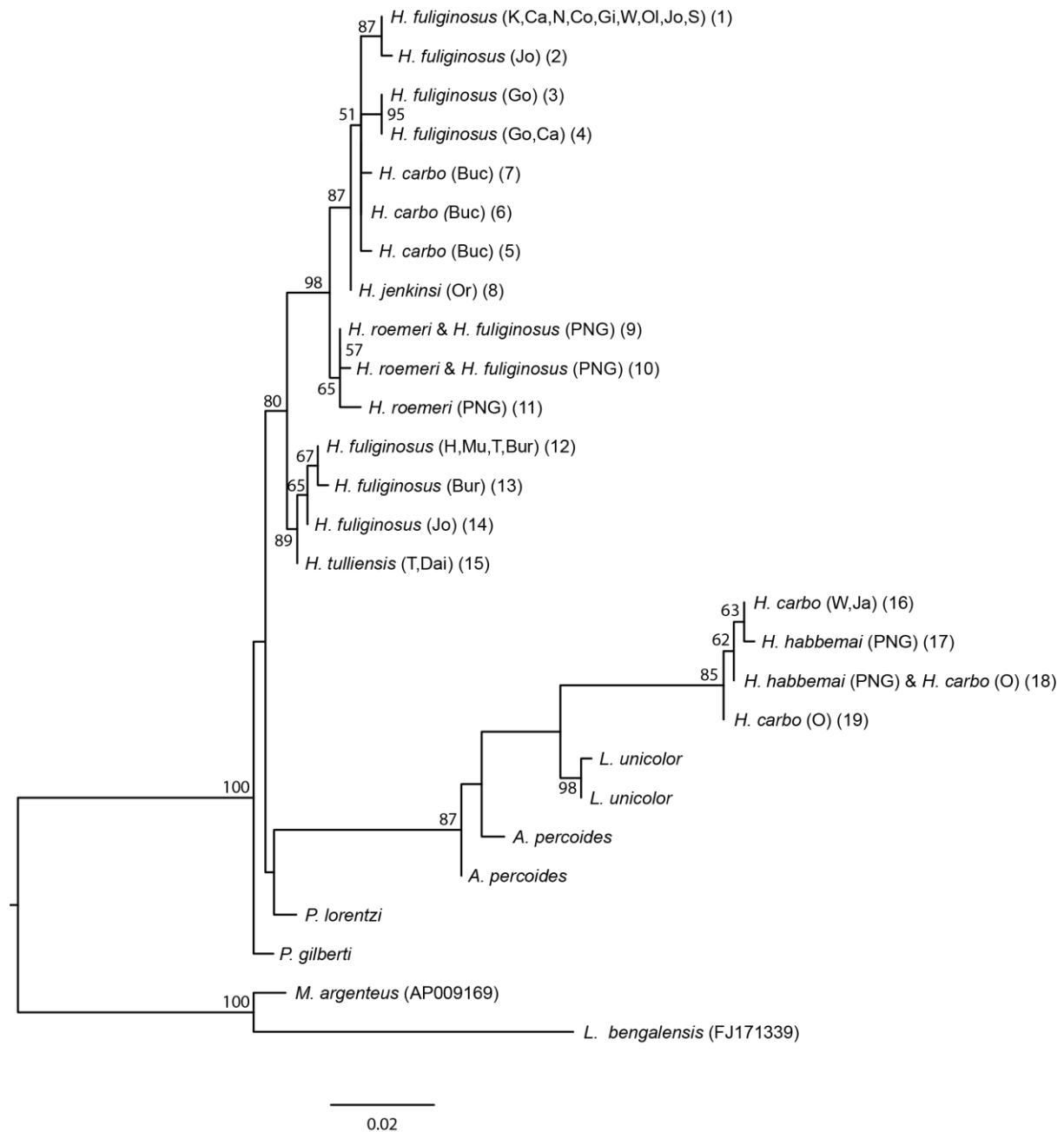
Pairwise  $F_{ST}$ -values between samples of *Hephaestus fuliginosus* from different rivers were mostly large and ranged between  $-0.0350$  to  $0.1788$ , with the Burdekin River population differing from the Herbert and Tully rivers, and the Herbert River differing from the North Johnstone and Tully rivers. All *H. tulliensis* populations were significantly different from *H. fuliginosus* populations except the Murray River, which, due to a low sample size, had very low power. Additionally, *H. tulliensis* populations from the Daintree were significantly different from *H. tulliensis* populations in both the North Johnstone and Tully rivers. Below diagonal:  $F_{ST}$  ( $\theta$ ); above diagonal:  $P$ -value: bold formatting indicates  $P < 0.001$ ; italic formatting indicates  $P < 0.05$

Species and location	<i>H. fuliginosus</i>					<i>H. tulliensis</i>			
	Burdekin	Herbert	N Johnstone	Tully	Murray	N Johnstone	Tully	Daintree	Cowley
<i>H. fuliginosus</i>									
Burdekin		<b>0.0000</b>	0.1261	<b>0.0000</b>	0.9910	<b>0.0000</b>	<b>0.0000</b>	<i>0.0451</i>	0.9910
Herbert	<b>0.1443</b>		<i>0.0090</i>	<i>0.0090</i>	0.9910	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.9910
N. Johnstone	0.0666	<i>0.0799</i>		0.9820	0.9460	<b>0.0000</b>	<b>0.0000</b>	<i>0.0270</i>	0.9910
Tully	<b>0.1614</b>	<i>0.1334</i>	$-0.0350$		0.1081	<b>0.0000</b>	<b>0.0000</b>	<i>0.0090</i>	0.9910
Murray	0.1788	0.0497	$-0.1072$	0.1633		0.1892	0.0901	0.9910	0.9910
<i>H. tulliensis</i>									
N. Johnstone	<b>0.4863</b>	<b>0.2458</b>	<b>0.2375</b>	<b>0.3338</b>	0.2594		<b>0.0000</b>	<i>0.0451</i>	0.5315
Tully	<b>0.4766</b>	<b>0.3668</b>	<b>0.2304</b>	<b>0.3114</b>	0.3863	<b>0.2200</b>		<i>0.0360</i>	0.4505
Daintree	<i>0.6112</i>	<b>0.3977</b>	<i>0.3305</i>	<i>0.5205</i>	0.6191	<i>0.3064</i>	<i>0.3214</i>		0.9910
Cowley	0.4135	0.1852	0.0205	0.3436	0.3333	0.0346	0.0571	0.3333	

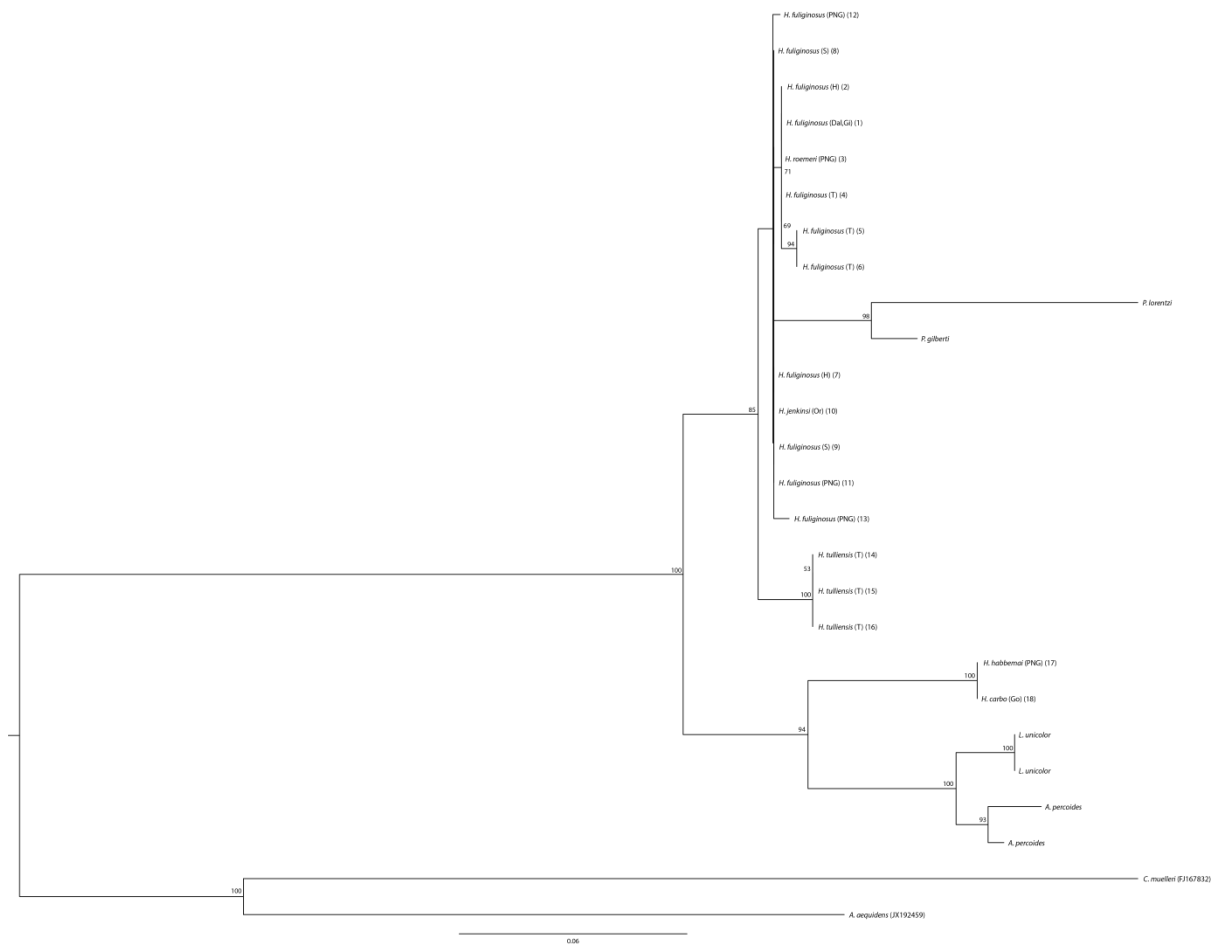


**Fig. S1.** Maximum likelihood phylogram of ATP sequence data for species within the genus *Hephaestus* and other terapontid genera. Each haplotype is labelled with its species and river of origin, with each river denoted by first initial or first and following initial(s) when first initials are shared. River of origin is not listed for species used as outgroups or for species from Papua New Guinea, which are labelled as PNG only. GenBank acquisition numbers are also shown for outgroup species (i.e. not within Terapontidae).





**Fig. S2.** Maximum likelihood phylogram of 16S sequence data for species within the genus *Hephaestus* and other terapotid genera. Location abbreviations as per Fig. S1.



**Fig. S3.** Maximum likelihood phylogram of *S7* sequence data for species within the genus *Hephaestus* and other terapotid genera. Location abbreviations as per Fig. S1.