

Supplementary Materials

Supplementary Methods

Sampling: Specimens were collected using pit-trapping methods (Goodman & Ingle, 1993) with 28×30 cm (diameter×height) traps, which were inspected twice a day. The sampling areas were located in forest and forest edge shrub of the Dabie Mountains (1 064–1 273 m above sea level), Anhui Province, China.

Phylogenetic analyses: DNA was extracted from livers and muscles of the four samples using the standard proteinase K/phenol-chloroform method. PCR analysis (Sambrook et al., 1989) was performed targeting sequences of a mitochondrial molecular marker (*Cyt b*) and three nuclear markers (phospholipase C beta 4 [*PLCB4*], recombination activating protein 1 [*RAG1*], and recombination activating protein 1 [*RAG2*]). The *Cyt b* gene was amplified with primers L14724-hk3 and H15915-hk3 (Irwin et al., 1991), *RAG1* was amplified using primers F1705 and R2951, *RAG2* was amplified using primers RAG2-F220 and RAG2, and *PLCB4* was amplified using primers *PLCB4*-F and *PLCB4*-R (Wan et al., 2013). The thermal cycling conditions of the genes in the PCR program are provided in Supplementary Table S1. PCR products were sequenced by specialized biological companies.

The obtained sequences were concatenated and edited using SeqMan (DNASTAR, Lasergene v7.1) (Swindell & Plasterer, 1997). The sequences were then aligned in MUSCLE (Edgar, 2004) and further examined in MEGA6 (Tamura et al., 2013). In the R package, the *dist.dna* function in APE was used to calculate the pairwise distance matrix from the DNA sequences (Paradis et al., 2004). The DNA sequences of 95 individuals of *Uropsilus* from the mountains of Southwest China were downloaded from the National Center for Biotechnology Information (NCBI) database (Supplementary Table S2). *Sorex araneus* (Soricidae) and *Talpa altaica* (Talpinae) were used as outgroups. Bayesian inference (BI) and maximum parsimony (MP) were used for phylogenetic analysis. BI analysis was performed in MRBAYES v3.1.2 (Ronquist & Huelsenbeck, 2003). The most suitable nucleotide substitution model was specified in MrModeltest v2.4 (Nylander, 2004). A cold chain and three incremental heated chains were used for the 1 000 000 Metropolis-coupled Markov chain Monte Carlo generations, with sampling every 100 generations, which allowed the replacement parameters to be changed independently between two genes; the branch length of each gene was then proportionally estimated. MP analysis was performed in PAUP v4.0a (Swofford, 2002), using 1 000 repeats to randomly added sequences and the tree-branch reconnection (TBR) exchange algorithm. The support rate was calculated using 1 000 bootstrap repeats.

Species delimitation: BEAST v1.10.4 (Suchard et al., 2018) was used to construct Bayesian trees and estimate the time of species differentiation. Analyses were performed using four loci (*Cyt b*, *RAG1*, *RAG2*, and *PLCB4*), General Time Reversible (GTR) substitution model, gamma+invariant sites heterogeneity model, uncorrelated exponential relaxed molecular clock model, and Birth-Death process tree prior (Drummond et al., 2012; Wan et al., 2018). Based on previous research, four calibrations were used for more accurate time estimates: (1) The earliest known member of the crown clade of Talpidae and Erinaceidae+Soricidae (Roca et al., 2004)

~73 Ma was used as the split of the most recent common ancestor (MRCA). We set the prior using lognormal distribution, such that the mean age was at 73 Ma (Roca et al., 2004). (2) The ancestor of Talpidae was from the Paleocene/Eocene boundary ~47 Ma (He et al., 2017) and we set the prior using normal distribution (mean=47, standard deviation=6.08). (3) The ancestors of *Uropsilus* first appeared in the Late Miocene; the prior was set using normal distribution (mean=6.18, standard deviation=1.5); therefore, the mean age was set to 6.18 Ma and the 95% upper boundary was set to 8.65 Ma (Wan et al., 2013). (4) The earliest known *U. soricipes* is from the Early Pleistocene 2.0–2.4 Ma; thus, the prior was set with a lower boundary of 2.0 Ma (offset=2.0, mean=0.67) and higher boundary of 2.38 Ma (Agustí et al., 2001). After BEAST analysis, Trace v1.6 (Rambaut & Drummond, 2010) confirmed that all effective sample sizes (ESSs) were greater than 200.

We used the Bayesian Poisson tree processes (bPTP) model to infer species boundaries in the concatenated phylogenetic tree (Zhang et al., 2013). As mitochondrial genes have a higher evolution rate than nuclear genes and the effective population of the present study was insignificant (Springer et al., 2001), mitochondrial genes were not used for analysis with the BPP v3.4 program (Yang & Rannala, 2010). This is because genetic infiltration and selection may have an effect; therefore, only sequences of the three nuclear markers were used in this analysis. Based on the Splits results, the specimens were divided into 23 polyphyletic taxa and analyzed using guided tree delimitation. The tree derived from BEAST was used as the guide tree. Analysis was performed using fixed-guide tree (A10) and non-guide tree (A11) species delimitation to avoid mixing problems (Yang, 2015). Species delimitation algorithms 0 and 1 were used for A10 and A11 species delimitation, respectively. Analysis was repeated 12 times for each dataset. Each rjMCMC was run for 1 000 000 generations, with sampling every 10 generations and 10 000 generations discarded as pre-burn-in. Automatic barcode gap discovery (Puillandre et al., 2012) was used to assess species boundaries. The JC69, K80, and “simple distance” models were selected for analysis using the ABGD Web server (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>), and the minimum gap width (X) of the agent was selected as 0.25 and 0.5 for analysis (Wan et al., 2018).

Morphological analyses: Morphometric measurements were obtained from the skulls of the four collected specimens and 23 other *Uropsilus* specimens with Vernier calipers to the nearest 0.01 mm (Table 1). The specimens belonged to five recognized species. The following measurements were obtained: profile length (PL), basal length (BL), greatest neurocranium breadth (GNB), zygomatic breadth (ZB), greatest breadth of snout (GBSn), least breadth between orbits (LBO), height of braincase (HB), median palatal length (MPL), length of mandibular tooth row including first incisor (LMTR), greatest width measured at anterior labial margins of second mandibular (M²-M²), and length of mandible not including first incisor (LM). These measurements refer to standards I and V of mammalian skull measurements (Liu et al., 2013; Yang et al., 2005, 2007). IBM SPSS Statistics 22.0 was used for independent-samples T test, principal component analysis (PCA), and discriminant function analysis (DFA) (Kirkpatrick & Feeney, 2014).

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Supplementary Table S1. PCR conditions for *Cyt b*, *RAG1*, *RAG2*, and *ATP7A* genes.

Locus	Primer	Initial denaturation	Denaturation	Annealing	Elongation	Terminal elongation
<i>Cyt b</i>	L14724-hk3, H15915-hk3 (Irwin et al., 1991)	2 min at 94°C	40 s at 94°C	30 s at 50°C	45 s at 72°C	5 min at 72°C
<i>RAG1</i>	F1705, R2951 (Wan et al., 2013)	4 min at 94°C	45 s at 94°C	45 s at 48°C	2 min at 72°C	12 min at 72°C
<i>RAG2</i>	RAG2-F220, RAG2-R995 (Wan et al., 2013)	4 min at 94°C	45 s at 94°C	45 s at 53°C	2 min at 72°C	12 min at 72°C
<i>PLCB4</i>	PLCB4-F, PLCB4-R (Wan et al., 2013)	4 min at 94°	45 s at 94°C	45 s at 60°C	2 min at 72°C	12 min at 72°C

Supplementary Table S2. Samples used for molecular phylogenetic analysis in this study.

Species	Specimen voucher	Identification code	GenBank Accession Number			
			<i>Cyt b</i>	<i>RAG1</i>	<i>RAG2</i>	<i>PLCB4</i>
<i>U. investigator</i>	KIZ-028527	Uinv01	KF778154	KF778274	KF778367	KF778215
	KIZ-028530	Uinv02	KF778155	KF778275	KF778331	KF778217
	KIZ-201211136	Uinv03	MH209680	KF778271	KF778363	MH210124
	KIZ-201211149	Uinv04	MH209685	KF778269	KF778364	MH210129
	KIZ-201211160	Uinv05	MH209686	KF778272	KF778365	MH210130
	KIZ-201211169	Uinv06	MH209687	KF778273	KF778330	MH210131
	KIZ-201212135	Uinv07	MH209707	KF778270	KF778366	MH210147
<i>U. gracilis</i>	KIZ-028544	Ugra01	KF778208	KF778312	KF778368	KF778260
	KIZ-028545	Ugra02	KF778209	KF778311	KF778369	KF778259
	KIZ-028546	Ugra03	KF778206	KF778313	KF778371	KF778252
	KIZ-028547	Ugra04	KF778207	KF778310	KF778370	KF778253
	KIZ-028548	Ugra05	KF778205	KF778309	KF778372	KF778258
	KIZ-0810137	Ugra06	MH209658	MH210217	MH210339	MH210102
	KIZ-0810159	Ugra07	MH209659	MH210218	MH210340	MH210103
	KIZ-0810160	Ugra08	MH209660	MH210219	MH210341	MH210104
	KIZ-0810191	Ugra09	MH209661	MH210220	MH210342	MH210105
	KIZ-0810488	Ugra10	MH209662	MH210221	MH210343	MH210106
	KIZ-0811175	Ugra11	MH209664	MH210223	MH210345	MH210107
<i>U. soricipes</i>	KIZ-028553	Usor01	KF778191	KF778317	KF778350	KF778225
	KIZ-028554	Usor02	KF778192	KF778318	KF778351	KF778226
	KIZ-028555	Usor03	KF778193	KF778319	KF778374	KF778224
	KIZ-028556	Usor04	KF778194	KF778320	KF778373	KF778227

	SAF-09700	Usor05	MH209649	MH210208	MH210330	MH210093
	KIZ-0905019	Usor06	MH209650	MH210209	MH210331	MH210094
	KIZ-0905021	Usor07	MH209651	MH210210	MH210332	MH210095
	KIZ-0905059	Usor08	MH209652	MH210211	MH210333	MH210096
	KIZ-0905060	Usor09	MH209653	MH210212	MH210334	MH210097
	KIZ-0905083	Usor10	MH209654	MH210213	MH210335	MH210098
	SAF-092069	Usor11	MH209719	MH210269	MH210394	MH210159
	KIZ-0905409	Usor12	MH209734	MH210284	MH210409	MH210169
<i>U. aequodonia</i>	SAF-07940	Uaeq01	MH209698	MH210253	MH210378	MH210144
	SAF-04010	Uaeq02	MH209699	MH210254	MH210379	MH210145
	KIZ-0906042	Uaeq03	MH209766	MH210315	MH210424	MH210193
	KIZ-0906074	Uaeq04	MH209767	MH210316	MH210425	MH210194
	KIZ-0906075	Uaeq05	MH209768	MH210317	MH210426	MH210195
<i>U. andersoni</i>	KIZ-Z201405738	Uand01	MH209675	MH210232	MH210354	MH210118
	KIZ-Z201405739	Uand02	MH209676	MH210233	MH210355	MH210119
	SAF-06948	Uand03	MH209738	MH210288	MH210413	MH210173
<i>U. nivatus</i>	KIZ-022473	Univ01	KF778168	KF778291	KF778356	KF778247
	KIZ-022469	Univ02	KF778166	KF778292	KF778359	KF778248
	KIZ-022974	Univ03	KF778170	KF778293	KF778360	KF778249
	KIZ-022975	Univ04	KF778167	KF778294	KF778361	KF778256
	KIZ-022976	Univ05	KF778169	KF778284	KF778362	KF778254
	KIZ-016769	Univ06	KF778171	KF778287	KF778344	KF778251
	KIZ-016783	Univ07	KF778172	KF778288	KF778345	KF778228
	KIZ-016770	Univ08	KF778173	KF778289	KF778346	KF778229
	KIZ-016768	Univ09	KF778174	KF778282	KF778347	KF778250
	KIZ-016797	Univ10	KF778175	KF778290	KF778348	KF778222

	KIZ-022467	Univ11	KF778176	KF778285	KF778352	KF778214
	KIZ-022468	Univ12	KF778178	KF778295	KF778353	KF778257
	KIZ-022470	Univ13	KF778177	KF778283	KF778357	KF778246
	KIZ-022471	Univ14	KF778179	KF778286	KF778358	KF778245
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<i>U. atronates</i>						KF778238
	KIZ-028531	Uatr01	KF778180	KF778303	KF778332	KF778237
	KIZ-028532	Uatr02	KF778184	KF778304	KF778333	KF778239
	KIZ-028533	Uatr03	KF778183	KF778308	KF778334	KF778232
	KIZ-028534	Uatr04	KF778185	KF778302	KF778337	KF778240
	KIZ-028535	Uatr05	KF778186	KF778305	KF778338	KF778230
	KIZ-028536	Uatr06	KF778181	KF778267	KF778335	KF778231
	KIZ-028537	Uatr07	KF778182	KF778268	KF778336	
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<i>U. jingdongensis</i>	KIZ-019509	Ujin01	KF778187	KF778301	KF778324	KF778233
	KIZ-019515	Ujin02	KF778189	KF778316	KF778322	KF778235
	KIZ-019517	Ujin03	KF778188	KF778307	KF778323	KF778236
	KIZ-028538	Ujin04	KF778190	KF778306	KF778354	KF778234
	KIZ-AL1303012	Ujin05	MH209638	MH210197	MH210319	MH210082
	KIZ-AL1305058	Ujin06	MH209639	MH210198	MH210320	MH210083
	KIZ-AL1305099	Ujin07	MH209640	MH210199	MH210321	MH210084
	KIZ-AL1305152	Ujin08	MH209641	MH210200	MH210322	MH210085
	KIZ-AL1305230	Ujin09	MH209642	MH210201	MH210323	MH210086
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<i>U. parva</i>	KIZ-020527	Upar01	KF778159	KF778274	KF778367	KF778219
	KIZ-020539	Upar02	KF778160	KF778275	KF778331	KF778216

	KIZ-028526	Upar03	KF778156	KF778271	KF778363	KF778220
	KIZ-028528	Upar04	KF778158	KF778272	KF778365	KF778221
	KIZ-028529	Upar05	KF778157	KF778273	KF778330	KF778218
	KIZ-TC1304166	Upar06	MH209736	MH210286	MH210411	MH210171
	KIZ-TC1304268	Upar07	MH209737	MH210287	MH210412	MH210172
<i>U. binchuanensis</i>	KIZ-BC1110001	Ubin01	MH209643	MH210202	MH210324	MH210087
	KIZ-BC1110012	Ubin02	MH209644	MH210203	MH210325	MH210088
	KIZ-BC1110129	Ubin03	MH209645	MH210204	MH210326	MH210089
	KIZ-BC1110180	Ubin04	MH209646	MH210205	MH210327	MH210090
	KIZ-BC1110202	Ubin05	MH209647	MH210206	MH210328	MH210091
	KIZ-BC1110002	Ubin06	MH209648	MH210207	MH210329	MH210092
<i>U. rubustus</i>	KIZ0905169	Urub01	KF778161	KF778280	KF778376	KF778212
	KIZ0905186	Urub02	KF778162	KF778277	KF778375	KF778213
	KIZ0905189	Urub03	KF778164	KF778278	KF778328	KF778211
	KIZ0905198	Urub04	KF778165	KF778279	KF778329	KF778210
	KIZ0905238	Urub05	KF778163	KF778281	KF778377	KF778223
<i>U. qinlingensis</i>	KIZ0509185	Uqin01	KF778195	KF778266	KF778376	KF778212
	KIZ0509335	Uqin02	KF778199	KF778276	KF778375	KF778213
	KIZ0509486	Uqin03	KF778196	KF778314	KF778328	KF778211
	KIZ0509516	Uqin04	KF778197	KF778296	KF778329	KF778210
	KIZ0509577	Uqin05	KF778198	KF778315	KF778377	KF778223
<i>U. wulingensis</i>	SAF-06040	Uwul01	MH209692	MH210247	MH210371	MH210136
	SAF-06042	Uwul02	MH209693	MH210248	MH210372	MH210137
<i>U. sp. 1</i>	KIZ-C204112	Usp1	MH209691	MH210246	MH210370	MH210135
<i>U. sp. 2</i>	SAF-12098	Usp2	MH209720	MH210270	MH210395	MH210160
<i>Uropsilus dabieshanensis</i> sp. nov.	AE1612YLP017		MT199125	MT211605	MT211608	MW321508

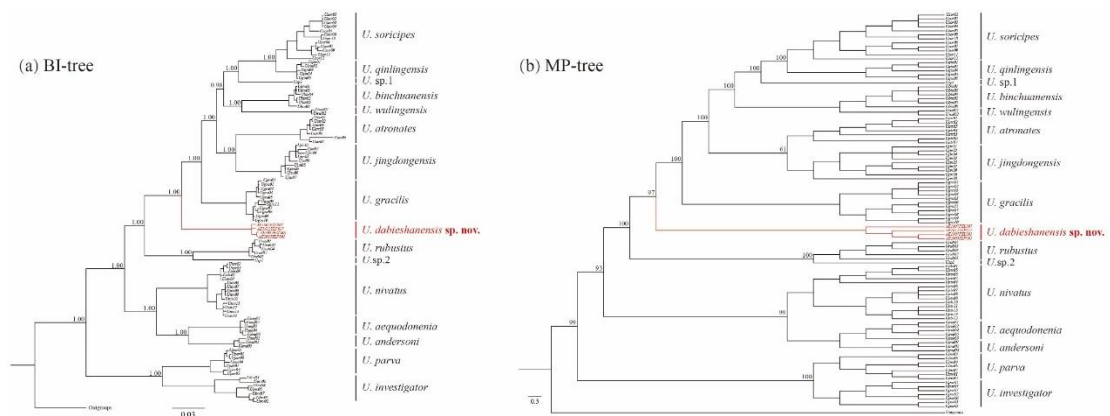
	AE1807FZL007	MT199126	MT211606	MT211609	MW321509
	AE1907FZL001	MT199127	MT211607	MT211610	MW321510
	AE2005YLP001	MT710697	MT710698	MT710699	MW321511
<i>Talpa altaica</i>		AB037602	AB176542	HG737946	AY011752
<i>Sorex araneus</i>		GU827395		JN633320	AY011754

Supplementary Table S3. Uncorrected pairwise genetic distances of Cyt *b* sequences used in this study.

Species/individual	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. AE1612YLP017																
2. AE1807FZL007	0.004															
3. AE1907FZL001	0.006	0.005														
4. AE2005YLP001	0.006	0.005	0.000													
5. <i>U. gracilis</i>	0.105	0.107	0.105	0.105												
6. <i>U. qinlingensis</i>	0.107	0.106	0.106	0.106	0.091											
7. <i>U. soricipes</i>	0.115	0.116	0.116	0.116	0.090	0.041										
8. <i>U. jingdongensis</i>	0.118	0.119	0.122	0.122	0.094	0.096	0.096									
9. <i>U. wulingensis</i>	0.124	0.125	0.129	0.129	0.119	0.114	0.115	0.109								
10. <i>U. binchuanensis</i>	0.132	0.131	0.134	0.133	0.081	0.101	0.096	0.087	0.096							
11. <i>U. atronates</i>	0.138	0.140	0.142	0.142	0.108	0.112	0.104	0.107	0.114	0.108						
12. <i>U. robustus</i>	0.146	0.143	0.144	0.144	0.145	0.153	0.152	0.145	0.152	0.150	0.169					
13. <i>U. nivatus</i>	0.155	0.153	0.160	0.160	0.126	0.141	0.139	0.148	0.142	0.130	0.149	0.150				
14. <i>U. andersoni</i>	0.163	0.157	0.160	0.160	0.149	0.154	0.149	0.161	0.159	0.152	0.164	0.145	0.105			
15. <i>U. aequodonenia</i>	0.171	0.170	0.170	0.170	0.146	0.148	0.137	0.167	0.164	0.153	0.164	0.158	0.114	0.085		
16. <i>U. parva</i>	0.177	0.176	0.176	0.176	0.177	0.172	0.175	0.204	0.183	0.191	0.185	0.184	0.179	0.188	0.182	
17. <i>U. investigator</i>	0.177	0.174	0.178	0.178	0.184	0.176	0.176	0.197	0.176	0.183	0.198	0.184	0.177	0.191	0.192	0.107

Supplementary Table S4. Results and percentage of variance explained for principal component and discriminant function analyses.

Variables	Component		Canonical axis			
	1	2	1	2	3	4
PL	0.788	0.460	-0.108	-1.228	0.248	-0.135
GNB	0.713	0.299	-0.111	1.855	0.037	0.105
BL	0.833	-0.327	1.139	-0.387	0.152	0.097
LBO	0.764	-0.330	-1.816	0.383	0.107	0.140
ZB	0.708	0.145	2.317	-1.017	0.463	0.315
M ² -M ²	0.671	-0.543	0.966	0.798	0.077	-0.297
HB	0.512	0.642	-2.012	-0.390	0.439	-0.309
GBSn	0.788	-0.390	0.752	-1.243	0.058	-0.473
MPL	0.845	0.048	-0.045	1.334	0.085	-0.227
LMTR	0.512	0.672	-1.304	0.162	0.254	-0.207
LM	0.662	-0.294	1.566	0.961	0.258	-0.279
Eigenvalues	5.655	1.937				
Total variance explained	51.41%	17.61%	77.80%	14.10%	6.3%	1.5%



Supplementary Figure S1. Molecular phylogenetic tree of *Uropsilus* based on mitochondrial-nuclear concatenated data and analyzed using Bayesian inference (a) and maximum parsimony (b) analyses.