Supplementary Materials for

Vampire bats exhibit evolutionary reduction of bitter taste receptor genes common to other bats

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Supplementary Text 1. Description of selection tests on *Calhm1* in bats.

To test whether functional relaxation of taste signaling pathway happened along with substantial reduction of taste sensation in vampire bats, we conducted three selection tests for *Calhm1* that are similar to those for *T2R*s. Briefly, we found that, 1) the average ω ratio of *Calhm1* for all branches was significantly smaller than 1 ($P = 1.9\times10^{-154}$) after comparing model A with model B (table 1); 2) the ω ratio estimated for the common ancestor of vampire bats was not significantly different from that of the rest of tree (P =0.985) (see the comparsion between model C and model D in table 1); 3) a model (model F in table 1) allowing ω variation between the ancestral branch of vampire bats and the four branches linking the three vampire bats was not significantly better fit to the data than the null model (P =0.119) (model E in table 1). For details of parameter estimates for selection tests on bat *Calhm1*, see Table S4. Together, these results suggest that *Calhm1* is under strong purifying selection in bats, and that functional relaxation on the taste signaling pathway was not observed in vampire bats.

Table S1. Species examined in this study.

Classification		Common name	Scientific name
Suborder YINPTEROCHIROPTERA	Family Pteropodidae	Geoffroy's rousette fruit bat	*Rousettus amplex*
		greenish naked-backed fruit bat	*Dobsonia inermis*
		large flying fox	*Pteropus vampyrus*
		black flying fox	*Pteropus alecto*
	Family Rhinolophidae	cyclops leaf-nosed bat	*Hipposideros cyclops*
		Pearson's horseshoe bat	*Rhinolophus pearsonii*
Suborder YANGOCHIROPTERA	Family Phyllostomidae	Gervais's fruit-eating bat	*Artibeus cinereus*
		Seba's short-tailed bat	*Carollia perspicillata*
		common vampire bat	*Desmodus rotundus*
		white-winged vampire bat	*Diaemus youngi*
		hairy-legged vampire bat	*Diphylla ecaudata*
	Family Mormoopidae	Parnell's mustached bat	*Pteronotus parnellii*
	Family Vespertilionidae	little brown bat	*Myotis lucifugus*
		David's myotis	*Myotis davidii*

Table S2. Primers used in this study.

Amplified gene	Primer name	Primer sequence (5'-3')	Primer pair[*]
T2R1	M_R41 162U19	CAGGATTGGTCTCCAGATG	forward
	M_R41_104U20	TGATCAGGCAGAGAAAGATG	forward
	M_R41_827L23	CTTAGGATTTCCTAAAATTAAGA	reverse
	M_R41 725L21	TAAAACCTAATGCAGCCATCA	reverse
T2R3	T2R20_7U18	GGACTCACAGAGTGGGTG	forward
	T2R20_48U20	GTTCTTTCTGGGAATGCTGG	forward
	T2R20_869L20	CACAAATGTCTGCTTCAGCT	reverse
	T2R20_905L19	GACTTCAGACGACCAGACT	reverse
T2R4	T2R19_56U20	CAGGACTCATTGTGAATCTG	forward
	T2R19_86U20	TGGTCAACTACAAGACTTGG	forward
	T2R19_824L22	AGAATAATGAGAACAGAATGTC	reverse
	T2R19_871L21	GAAACAGAGAATCTTCTTTGC	reverse
T2R5	T2R17_21U19	ACTGCTGATGGTGGTGGCA	forward
	T2R17_45U21	TGAATTTCTCATTGGCCTGGT	forward
	T2R17_818L20	TTTCATCCTGGGATTCCCCA	reverse
	T2R17_839L20	CAGGATTCTCTGACAAGCCT	reverse
T2R7	T2R9_16U20	AGCAACACCTTAATGATCAT	forward
	T2R9_55U20	ATGGGAATCTTAGGAAATGC	forward
	T2R9_879L20	GCACCTTTAGAAATGCTTGT	reverse
	T2R9_906L22	TCTTTTCAGGATATATGTTACT	reverse
T2R38	M_R26 87U21	GATTCTGGTCAATGTCTTCAT	forward
	T2R31_17U18	CCGTCGTCACTGTGTCCT	forward
	T2R31_76U19	TTCGTGGTGGGGATTCTGG	forward
	M_R26_712L18	GATGTGGGCCTCCAGGCT	reverse
	T2R31_919L18	CAGGATGGCGTCCACAGC	reverse
T2R39	T2R10_57U21	AACTTTCACAATTATAGGCAC	forward
	M_R28_158U19	CCACAAGTGGCAGGATCCT	forward
	T2R10_880L19	GATTGCCCAAGATCAGTAG	reverse
	T2R10_914L18	GTTGAAGCCGCTTCCAGG	reverse
	M_R28_798L20	TGGCATTGAAGATGTTGGAC	reverse
T2R40	T2R11_26U19	CGGATAAAGGCATGTCCAG	forward
	T2R11_40U22	TCCAGATTTAAAATCGTCTTCA	forward
	T2R11_893L18	TCAGGCCAGGATTGCCCA	reverse
	T2R11_917L18	ACTGCAGCCGCTTCCAGG	reverse
T2R42	T2R22_31U21	GTACTGTCAATAGCAGAATTC	forward
	T2R22_66U20	GGGAAATGTGTTCATTGGAC	forward
	T2R22_867L21	TTCAAGATTGTCTGTCTTAGC	reverse
	T2R22_927L22	CTATCTGTAAATCTGTAACAGA	reverse
T1R3	T1R3-re2-F	ACCAGGACAGCCCCTTGGT	forward
	T1R3-re2-R	GGGGCATGAAGGAGATCCAG	reverse
Calhm1(exon 1)	CALHM1e1p2-F	GCAGCGGTGAGGTGGGAGG	forward
	CALHM1e1p2-R	CCCCTCACCTGGAGATGCAG	reverse
Calhm1(exon 2)	CALHM1e2p3-F	CTCTCCCATGCAGGCACTG	forward
	CALHM1e2p3-R	GGCCACAGCTCACACTTTGC	reverse

[*] Each forward primer can pair with each reverse primer.

Table S3. *T2R*s of the four bats with available genome sequences. Intact genes are characterized by complete and intact ORFs, partial genes contain incomplete and intact ORFs due to incomplete genome sequences, and pseudogenes are characterized by disrupted ORFs due to nonsense or frame-shifting mutations.

Species	Gene number				Percent of pseudogenes
	Intact	Partial	Pseudogene	Total	
Myotis davidii	25	4	10	39	25.6%
Myotis lucifugus	27	0	7	34	20.6%
Pteropus alecto	13	0	13	26	50.0%
Pteropus vampyrus	14	0	10	24	41.7%

Table S4. Likelihood values and parameter estimates for likelihood ratio tests of selective pressures on bat *T2R*s and *Calhm1*. The assumption of each model was given in table 1.

	Model A	**Model B**	**Model C**	**Model D**	**Model E**	**Model F**
T2R1	ln *L*=-3427.132844	ln *L*=-3428.833944	ln *L*=-3527.065761	ln *L*=-3526.200984	ln *L*=-3627.802949	ln *L*=-3627.174896
	ω=0.8276	ω=1	ω=0.8263	ω_1=0.8430, ω_2=0.3047	ω_1=0.8730, ω_2=0.7150	ω_1=0.8695, ω_2=1.0069, ω_3=0.3313
T2R3	ln *L*=-4263.749638	ln *L*=-4275.088529	ln *L*=-4400.220476	ln *L*=-4399.407653	ln *L*=-4053.415269	ln *L*=-4052.104084
	ω=0.6585	ω=1	ω=0.6697	ω_1=0.6594, ω_2=1.8957	ω_1=0.7100, ω_2=0.7281	ω_1=0.6941, ω_2=0.8084, ω_3=3.5745
T2R4	ln *L*=-3209.936509	ln *L*=-3225.293118	ln *L*=-2621.778461	ln *L*=-2621.229081	ln *L*=-2825.868116	ln *L*=-2825.543076
	ω=0.572	ω=1	ω=0.5854	ω_1=0.6006, ω_2=0.2878	ω_1=0.6178, ω_2=0.7636	ω_1=0.6300, ω_2=0.8823, ω_3=0.3936
T2R5	ln *L*=-3428.39021	ln *L*=-3442.627536	ln *L*=-3427.53808	ln *L*=-3427.312371	ln *L*=-3430.459055	ln *L*=-3430.459055
	ω=0.5908	ω=1	ω=0.5837	ω_1=0.2643, ω_2=0.3578	ω_1=0.2726, ω_2=0.3941	ω_1=0.6036, ω_2=1.0264, ω_3=0.2059
T2R7	ln *L*=-1905.509419	ln *L*=-1910.159618	ln *L*=-1917.232499	ln *L*=-1917.232491	ln *L*=-2069.501724	ln *L*=-2069.08614
	ω=0.64228	ω=1	ω=0.6421	ω_1=0.6421, ω_2=239.4661	ω_1=0.6435, ω_2=0.2534	ω_1=0.617, ω_2=0.1052, ω_3=1.3055
T2R38	ln *L*=-2815.328795	ln *L*=-2867.976306	ln *L*=-2705.549881	ln *L*=-2705.545709	ln *L*=-3174.197658	ln *L*=-3174.677814
	ω=0.3296	ω=1	ω=0.3566	ω_1=0.3571, ω_2=0.3311	ω_1=0.3450, ω_2=0.4829	ω_1=0.3460, ω_2=0.4928, ω_3=0.5434
T2R39	ln *L*=-3071.946349	ln *L*=-3082.233326	ln *L*=-2274.85814	ln *L*=-2274.589168	ln *L*=-2643.864733	ln *L*=-2643.851076
	ω=0.6112	ω=1	ω=0.4388	ω_1=0.4282, ω_2=0.6227	ω_1=0.5368, ω_2=0.7020	ω_1=0.5374, ω_2=0.7233, ω_3=0.6518
T2R40	ln *L*=-1933.761815	ln *L*=-1981.834222	ln *L*=-1976.861014	ln *L*=-1976.848337	ln *L*=-2279.690887	ln *L*=-2278.46542
	ω=0.2643	ω=1	ω=0.2654	ω_1=0.2643, ω_2=0.3578	ω_1=0.2726, ω_2=0.3941	ω_1=0.2639, ω_2=0.5168, ω_3=0.4964
T2R42	ln *L*=-3238.165213	ln *L*=-3238.214621	ln *L*=-3213.889067	ln *L*=-3213.834829	ln *L*=-4086.161606	ln *L*=-4086.275863
	ω=0.9668	ω=1	ω=0.8962	ω_1=0.9100, ω_2=0.8327	ω_1=0.9078, ω_2=0.7592	ω_1=0.8587, ω_2=0.5558, ω_3=1.1973
Calhm1	ln *L*=-3205.467111	ln *L*=-3555.917574	ln *L*=-3255.222199	ln *L*=-3255.222033	ln *L*=-3517.697681	ln *L*=-3516.483621
	ω=0.0565	ω=1	ω=0.0557	ω_1=0.0557, ω_2=0.0001	ω_1=0.0558, ω_2=0.0683	ω_1=0.0576, ω_2=0.0390, ω_3=223.3136

Figure legends:

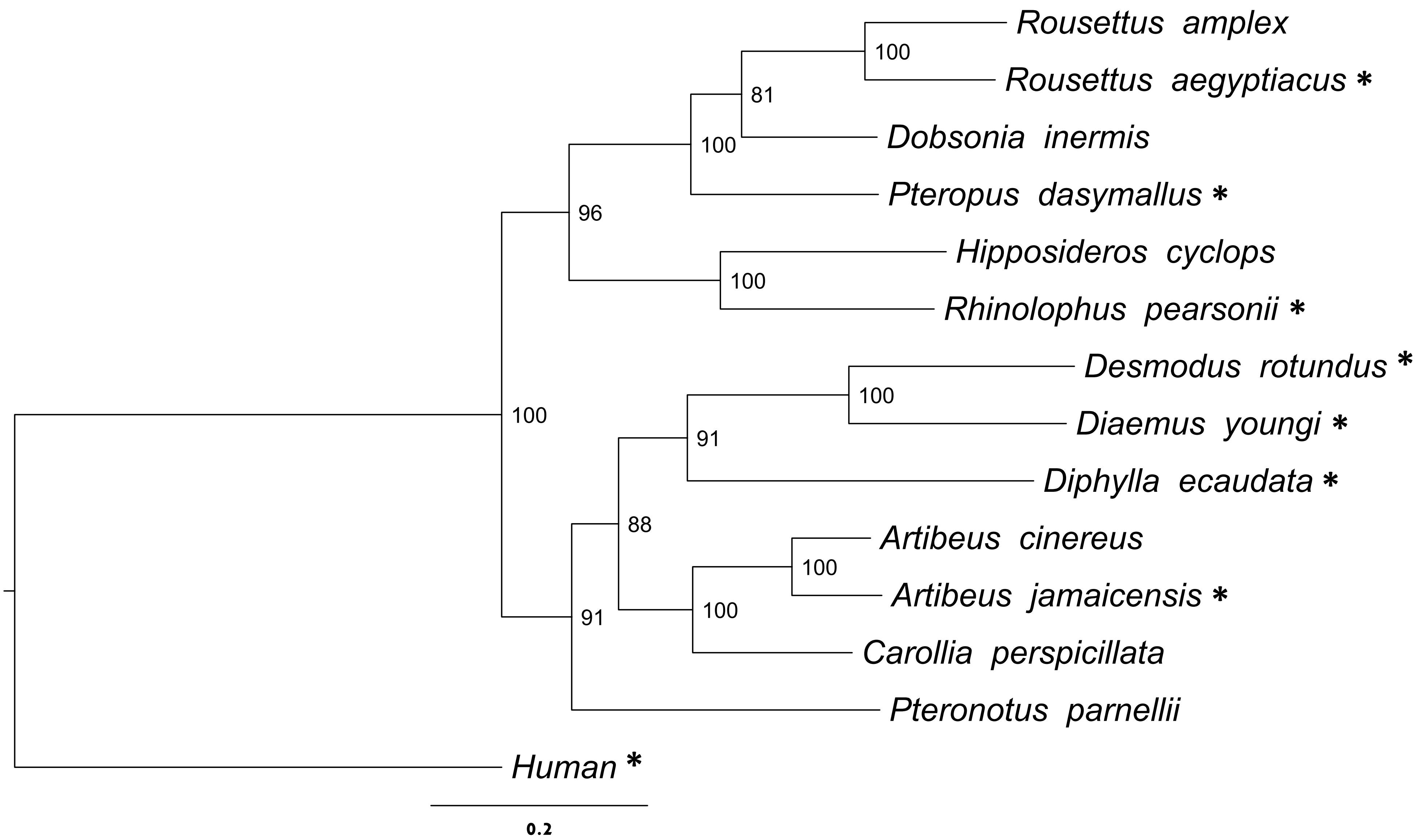
Figure S1. Phylogenetic positions of bats revealed by *Cytb* genes, which were newly sequenced or obtained from the GenBank (indicated with an asterisk). The tree was constructed with the Bayesian method, and numbers at the nodes are the Bayesian posterior probabilities as percentages.

Figure S2. Evolutionary relationships of all intact *T2R*s from the four bats with available genome sequences. A total of 211 codons were used to reconstruct a maximum-likelihood tree with the Bayesian method. The tree was rooted with the mouse *V1Rd8* and *V1Re9*, and numbers at the nodes are the Bayesian posterior probabilities as percentages. Species include *Pteropus vampyrus* (Ptva), *P. alecto* (Ptal), *Myotis lucifugus* (Mylu), and *M. davidii* (Myda).

Figure S3. Phylogenetic trees of each *T2R* gene in all bats using the Bayesian method. The Bayesian trees were rooted with the orthologous genes in humans, and numbers at the nodes are the Bayesian posterior probabilities as percentages.

Figure S4. An alignment of *T1R3* encoding the shared subunit of sweet and umami tastes in two vampire bats (*D. rotundus* and *D. ecaudata*), one megabat (*P. vampyrus*), and dog (*C. familiaris*). *D. rotundus* was sequenced previously [ref. 10 in the main text] while *D. ecaudata* was sequenced in this study (GenBank Accession no. KJ557282). Dashes indicate alignment gaps and question marks represent unamplified nucleotides, regions corresponding to transmembrane domains are boxed.

Figure S1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Dobsonia inermis
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Hipposideros cyclops
Rhinolophus pearsonii *
Desmodus rotundus *
Diaemus youngi *
Diphylla ecaudata *
Artibeus cinereus
Artibeus jamaicensis *
Carollia perspicillata
Pteronotus parnellii
Human *
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96
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100
100
91
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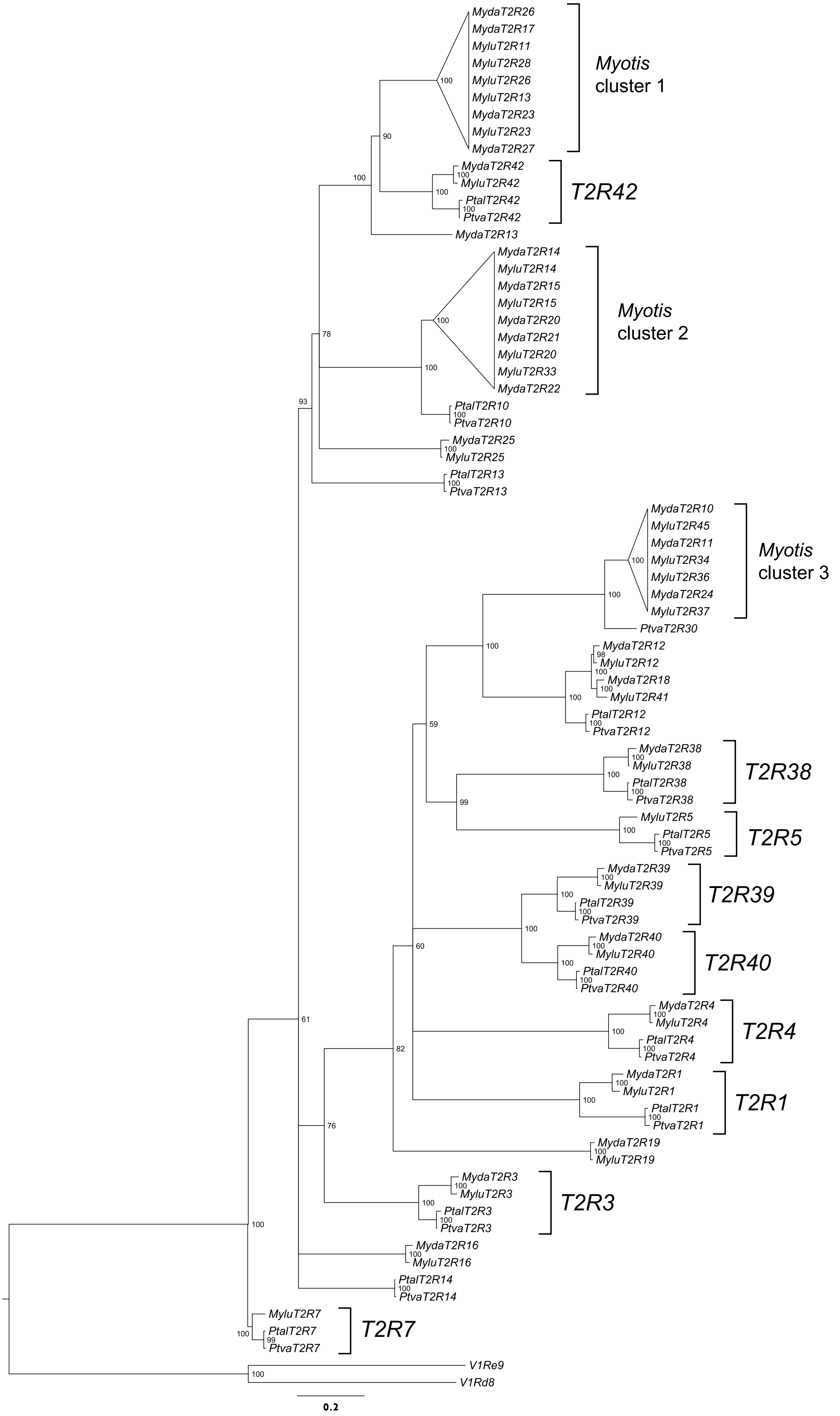
100
100
91
0.2

Figure S2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MydaT2R27
100
90
Myotis cluster 1
MydaT2R42
100
MyluT2R42
100
PtalT2R42
100
PtvaT2R42
T2R42
100
MydaT2R13
MydaT2R14
MyluT2R14
MydaT2R15
MyluT2R15
MydaT2R20
MydaT2R21
MyluT2R20
MyluT2R33
MydaT2R22
100
Myotis cluster 2
78
100
PtalT2R10
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PtvaT2R10
93
MydaT2R25
100
MyluT2R25
PtalT2R13
100
PtvaT2R13
MydaT2R10
MyluT2R45
MydaT2R11
MyluT2R34
MyluT2R36
MydaT2R24
MyluT2R37
100
100
Myotis cluster 3
PtvaT2R30
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MydaT2R12
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MyluT2R12
100
MydaT2R18
100
MyluT2R41
100
PtalT2R12
100
PtvaT2R12
59
MydaT2R38
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MyluT2R38
100
PtalT2R38
100
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MyluT2R5
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PtalT2R5
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T2R5
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60
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MyluT2R40
100
PtalT2R40
100
PtvaT2R40
T2R40
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MydaT2R4
100
MyluT2R4
100
PtalT2R4
100
PtvaT2R4
T2R4
82
MydaT2R1
100
MyluT2R1
100
PtalT2R1
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MydaT2R19
100
MyluT2R19
MydaT2R3
100
MyluT2R3
100
PtalT2R3
100
PtvaT2R3
T2R3
100
MydaT2R16
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MyluT2R16
PtalT2R14
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PtvaT2R14
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PtvaT2R7
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V1Re9
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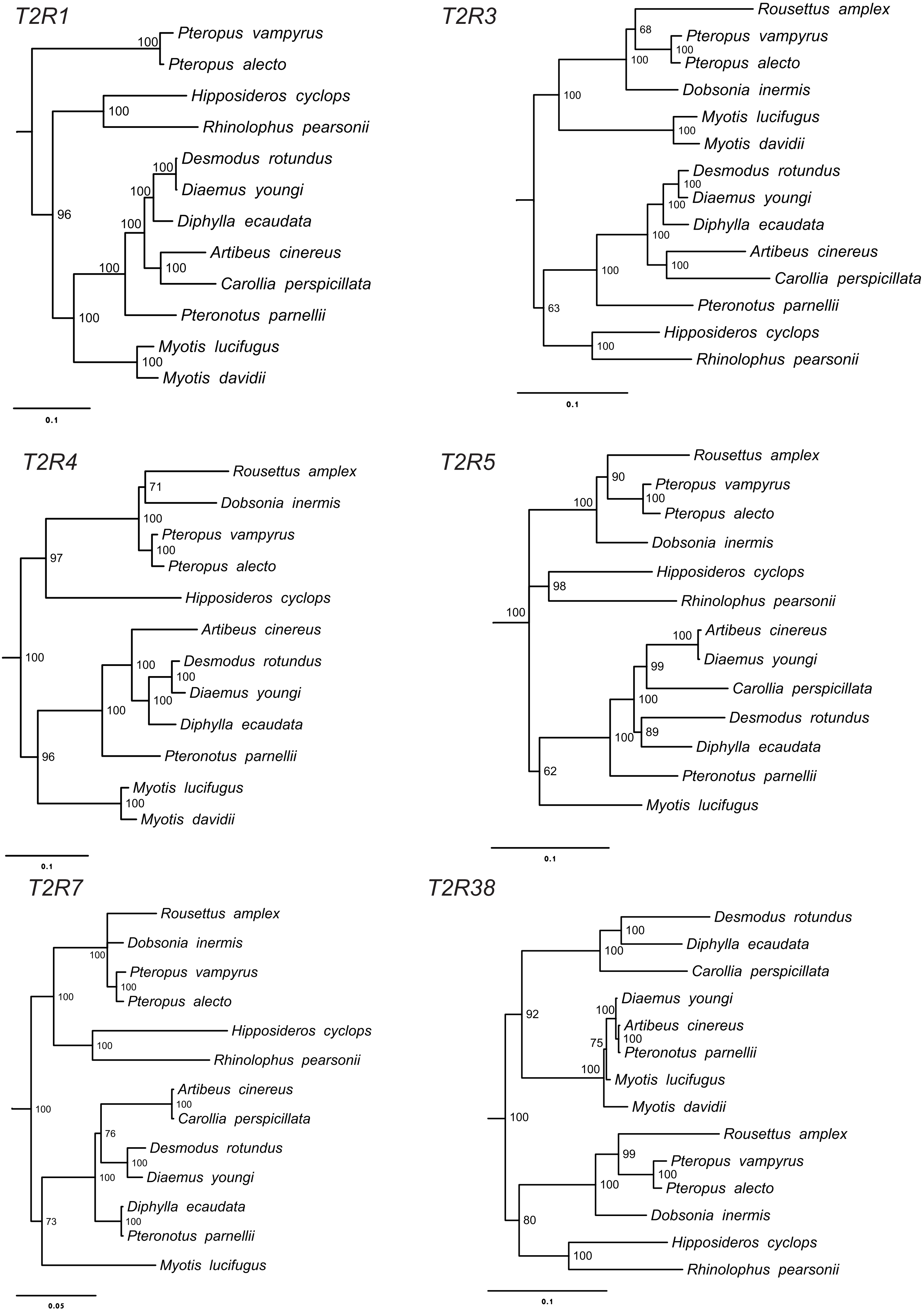
V1Rd8
0.2

Figure S3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Pteropus alecto
Hipposideros cyclops
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Rhinolophus pearsonii
100
Desmodus rotundus
100
Diaemus youngi
96
100
Diphylla ecaudata
Artibeus cinereus
100
100
Carollia perspicillata
100
Pteronotus parnellii
Myotis lucifugus
100
Myotis davidii
0.1
T2R3
Rousettus amplex
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Pteropus vampyrus
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100
Pteropus alecto
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Dobsonia inermis
Myotis lucifugus
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Myotis davidii
Desmodus rotundus
100
Diaemus youngi
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Artibeus cinereus
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100
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Hipposideros cyclops
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T2R4
Rousettus amplex
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Dobsonia inermis
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Pteropus vampyrus
97
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Pteropus alecto
Hipposideros cyclops
Artibeus cinereus
100
100
Desmodus rotundus
100
Diaemus youngi
100
100
Diphylla ecaudata
96
Pteronotus parnellii
Myotis lucifugus
100
Myotis davidii
0.1
T2R5
Rousettus amplex
90
Pteropus vampyrus
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Pteropus alecto
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Hipposideros cyclops
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100
Rhinolophus pearsonii
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Diaemus youngi
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Carollia perspicillata
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Myotis lucifugus
0.1
T2R7
Rousettus amplex
Dobsonia inermis
100
Pteropus vampyrus
100
100
Pteropus alecto
Hipposideros cyclops
100
Rhinolophus pearsonii
Artibeus cinereus
100
100
Carollia perspicillata
76
Desmodus rotundus
100
100
Diaemus youngi
Diphylla ecaudata
100
73
Pteronotus parnellii
Myotis lucifugus
0.05
T2R38
Desmodus rotundus
100
100
Diphylla ecaudata
Carollia perspicillata
Diaemus youngi
100
92
Artibeus cinereus
100
75
Pteronotus parnellii
100
Myotis lucifugus
Myotis davidii
100
Rousettus amplex
99
Pteropus vampyrus
100
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Pteropus alecto
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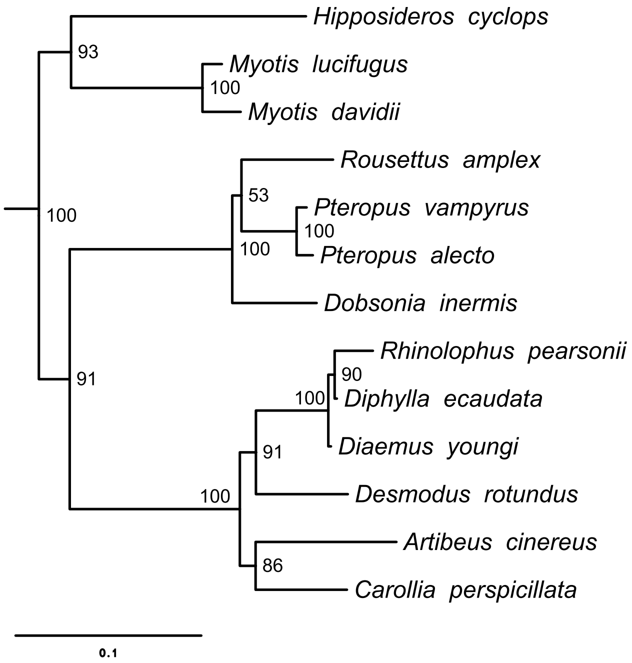
100
Rhinolophus pearsonii
0.1

Figure S3 continued

T2R39



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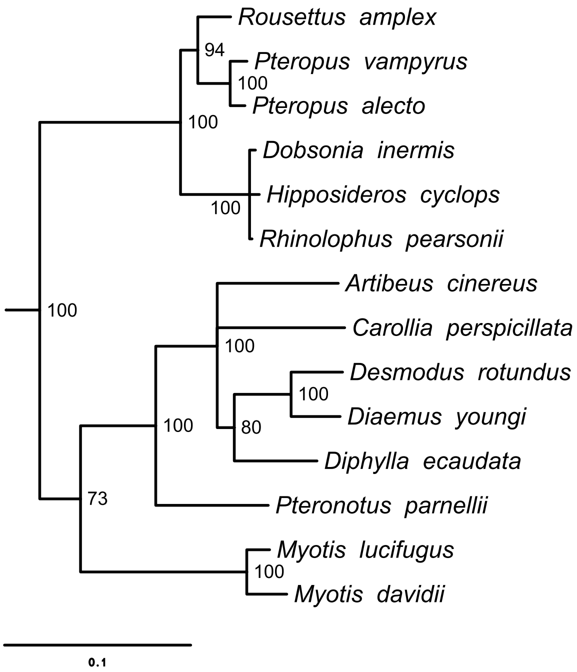

T2R42

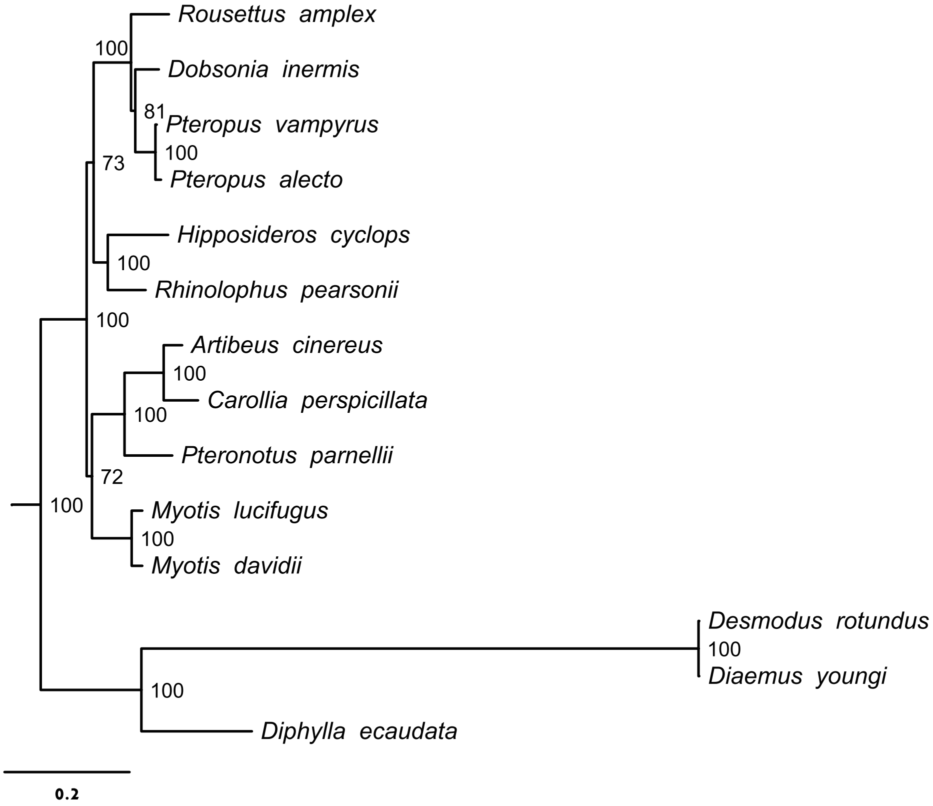

Figure S4

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                                                                                                                                                                  120
C. familiaris     GATGACCTCCTCTGCACCCAGTGTGACCAGAACCAGTGGTCCCCAGACCGGAGCACACGCTGCTTCCCCCGCAGGCTCACTTTCCTGGCATGGGGGCAGCCGGCTGTGCTGGTGCTGCTT
P. vampyrus       GATGACCTCACCTGCACCCAGTGTGACGAGGACAAGTGGTCCCCAGACCGGAGCCTGCGCTGCTTCCCCCGCAAGCTCAAGTTCCTGGCGTGGGGGGAGCCGGCCGTACTGCTGCTGCTC
D. rotundus       GATGACATTCTCTGCACCCAGTGTGAGTGGGACCACTGGTCGCCAGACAGGAGCCGGCGCTGCTTCCCCCACAGGTCCAAGTTCCTGGTGTGGGGGGAACTGGCCACACTGCTGCTGCTT
D. ecaudata       ????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????

                                                                                                                                                                  240
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                  GTGCTG------------CTGGGCCAGGCGCTGGCAGCCCTGGAGCTATTCATCTGGCACCAGGACAGCCCCTTGGTCCAGGCCTCG--------------------------GGCCTGC
                  ??????????????????????????????????????????????????????????ACCAGGACAGCCCCTTGGTCCAGGCCTCG--------------------------GGCCTGC

                                                                                                                                                                  360
                  CTGGGCCTTGTCTGCCTCAGTGTCCTTCTGTTCCCTGGCCAGCCGGGCCCTGCCAGCTGCCTGGCCCAGCAGCCACTGCTTCACCTTCCACTCACTGGCTGTCTGAGCACACTTTTCCTG
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                                                                                                                                                                  480
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                  CAGGCAGCTGAGGTCTTTGTGGAGGCTGAGCTGCCACCAAGCTG-GCAGACTGGCTGGGCGGCCACCTGTGCGCACCTCGGGCCTGGCTG---------------------GTGGAGGCC

                                                                                                                                                                  600
                  GCATTATGTGCCTGGTACCTGGTGGCCTTTCCACCAGAGGTGGTGACAGACTGGTGGGTGCTACCCACGCAGGTGCTGGTGCACTGCCGAGTGCGCTCCTGGATCAGCTTTGGCCTAGTG
                  GCACTGTGCACTTGGTACCTGGTAGGCTTCCCGCCAGAGGTGGTGACAGACTGGCGGGTGCTGCCCACAGAGGTGTTGGTGTCCTGCTACGTGCACTCCTGGATCAGCTTCGGCCTGGTA
                  GAGCTGTGCGCCTGGTCCCTGGGGGCCTTCCCACCCGAGGTGGTGACGAATTGGCGGGTGCTGCCCATGGAGGCTCTGGGGCACTGCTGCATGCACTCCTGGGCCAGCTTCGGCCTGCTG
                  GTGCTGTCCGCCTGGTCCCTGGGGGCCTTCCCGCCAGAGATGGTGATGAACTGGCGGGTGCTGCCCGTGGAGGCGCTGGTGCACTGCCGCGTGCGCTCCTGGGCCGGCTTTGGCCTGCTG

                                                                                                                                                                  720
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                  CACACCGCCAATGCCGTGCTGGCGTCCCTG---------GGCACCTTCCTGGTGCCCAGCTGACCTGGCCGCTGTGACAGCGTCCTCGGCCTCACCTTTGCCACACTGGCCTACTTCACC

                                                                                                                                                                  840
                  ACCTGGATCTCCTTTGTCCCTCTCTTTGCCAATGTGCATGTGGCCTACCAGCCCACTGTGCAGATGGCCGCCATCCTCCTCTGTGCCCTGGGCATCCTGGCCACCTTCCACCTGCCCAAG
                  ACCTGGATCTCTTTCGTGCCCCTCTTTGCCAACACGCACGTGGCCTACCGGCCTGCCGTGCAGATGGGCGCCAACCTCCTCTGTGTCCTGGGAATCCTGGCCACCGTCTACCTGCCTAAG
                  TCCTGGATCTCCTTCATGCCCC--TTTGCCAA--TGCACGTGGCCCACCAGACCACTGTGCAGATGGGCGCCCGCCCGCTTTGTACCCTGGGCATCCTGGTCACCTTCCACCCGCCCAAG
                  ---TGGATCTCCTTCATGCCCC??????????????????????????????????????????????????????????????????????????????????????????????????

                                                                                                                                                                  960
                  TGCTACCTGCTGCTGCAGCAGCTGGAGCTCAACAACCCGGAGTTCTTCCTAGGAGAT---------GATGCCAGAGGACAGGGCAGCAGTGGTAGTGGGGGGAAGGAGACTTAG------
                  TGCTTCCTGCTGCTGTGGCAGCCAGAGCGTAACACCCCCGAGTTCTTCCTGGGAGGGCGTCCTGGCGATACCAGGGGGCGAGGGGGCAGC---AGAGGAGGGGACGAGACTCAGGGCAAA
                  TGCTACCTGCTGCTGTGGCAGCTGGAAAGCAACGCCCCCGAGTTCTTCCTGGGAGGGCGT????????????????????????????????????????????????????????????
                  ????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????

                        969
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                  AATGAGTGA
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