

A review of torrent frogs (*Amolops*: Ranidae) from Bhutan, the description of a new species, and reassessment of the taxonomic validity of some *A. viridimaculatus* group species aided by archival DNA sequences of century-old type specimens

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Title: 2022_Amolops_Bhutan_TableS1

Description

Analysis

Analysis ----- Distance Estimation

Scope ----- Pairs of taxa

Estimate Variance

Variance Estimation Method ----- None

Substitution Model

Substitutions Type ----- Nucleotide

Model/Method ----- p-distance

Substitutions to Include ----- d: Transitions + Transversions

Rates and Patterns

Rates among Sites ----- Uniform rates

Pattern among Lineages ----- Same (Homogeneous)

Data Subset to Use

Gaps/Missing Data Treatment ----- Complete deletion

Codons Included ----- 1st+2nd+3rd+Non-Coding

No. of Sites : 572

d : Estimate

[1] #A_formosus_BMNH1947.2.4.18

[2] #MN958740.1_Amolops_sp._4_YW-2020_voucher_KIZ012537_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[3] #MN958741.1_Amolops_sp._4_YW-2020_voucher_KIZ012534_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[4] #MN958742.1_Amolops_sp._4_YW-2020_voucher_KIZ012535_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[5] #MN958743.1_Amolops_sp._4_YW-2020_voucher_KIZ012536_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[6] #MN958739.1_Amolops_sp._4_YW-2020_voucher_KIZ012533_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[7] #MN958769.1_Amolops_medogensis_voucher_SYNU04116219_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[8] #MN958768.1_Amolops_medogensis_voucher_SYNU04116216_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[9] #MN958796.1_Amolops_sp._3_YW-2020_voucher_KIZ014067_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[10] #MN958797.1_Amolops_sp._3_YW-2020_voucher_KIZ014068_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

[11] #MN958772.1_Amolops_sp._5_YW-2020_voucher_KIZ040228_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [12] #MN958770.1_Amolops_sp._5_YW-2020_voucher_SH2789_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [13] #MN958771.1_Amolops_sp._5_YW-2020_voucher_KIZ040227_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [14] #MN958722.1_Amolops_sp._2_YW-2020_voucher_KIZ011061_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [15] #MN958721.1_Amolops_sp._2_YW-2020_voucher_KIZ016397_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [16] #MN958792.1_Amolops_viridimaculatus_voucher_KIZ047020_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [17] #MN958791.1_Amolops_viridimaculatus_voucher_KIZ047019_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [18] #MN958790.1_Amolops_viridimaculatus_voucher_SCUM050423CHX_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [19] #MN958789.1_Amolops_viridimaculatus_voucher_KIZ048488_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [20] #MN958788.1_Amolops_viridimaculatus_voucher_KIZ048487_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [21] #MN958793.1_Amolops_viridimaculatus_voucher_SCUM050402CHX_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial
 [22] #MN958794.1_Amolops_viridimaculatus_voucher_SCUM050403CHX_NADH_dehydrogenase_subunit_2_(ND2)_gene_partial_cds_mitochondrial

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
[1]																						
[2]	0.021																					
[3]	0.024	0.003																				
[4]	0.021	0.000	0.003																			
[5]	0.026	0.005	0.002	0.005																		
[6]	0.026	0.005	0.002	0.005	0.000																	
[7]	0.121	0.121	0.124	0.121	0.126	0.126																
[8]	0.121	0.121	0.124	0.121	0.126	0.126	0.000															
[9]	0.121	0.126	0.129	0.126	0.131	0.131	0.066	0.066														
[10]	0.122	0.128	0.131	0.128	0.133	0.133	0.068	0.068	0.002													
[11]	0.122	0.119	0.122	0.119	0.121	0.121	0.110	0.110	0.131	0.133												
[12]	0.126	0.119	0.122	0.119	0.121	0.121	0.114	0.114	0.135	0.136	0.003											
[13]	0.128	0.121	0.124	0.121	0.122	0.122	0.115	0.115	0.136	0.138	0.005	0.002										
[14]	0.131	0.122	0.122	0.122	0.124	0.124	0.084	0.084	0.091	0.093	0.119	0.122	0.124									
[15]	0.131	0.122	0.122	0.122	0.124	0.124	0.084	0.084	0.091	0.093	0.119	0.122	0.124	0.000								
[16]	0.124	0.119	0.122	0.119	0.124	0.124	0.082	0.082	0.089	0.091	0.126	0.129	0.128	0.066	0.066							
[17]	0.124	0.119	0.122	0.119	0.124	0.124	0.082	0.082	0.089	0.091	0.126	0.129	0.128	0.066	0.066	0.000						
[18]	0.124	0.119	0.122	0.119	0.124	0.124	0.082	0.082	0.089	0.091	0.126	0.129	0.128	0.066	0.066	0.000	0.000					
[19]	0.124	0.119	0.122	0.119	0.124	0.124	0.082	0.082	0.089	0.091	0.126	0.129	0.128	0.066	0.066	0.000	0.000	0.000				
[20]	0.124	0.119	0.122	0.119	0.124	0.124	0.082	0.082	0.089	0.091	0.126	0.129	0.128	0.066	0.066	0.000	0.000	0.000	0.000			
[21]	0.140	0.143	0.147	0.143	0.145	0.145	0.096	0.096	0.117	0.119	0.131	0.131	0.133	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093
[22]	0.140	0.143	0.147	0.143	0.145	0.145	0.096	0.096	0.117	0.119	0.131	0.131	0.133	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.000

Table. Estimates of Evolutionary Divergence between Sequences
 The number of base differences per site from between sequences are shown. The analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 572 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [1].

1. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.

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Title: 2022_Amolops_Bhutan_TableS2

Description

Analysis

Analysis ----- Distance Estimation

Scope ----- Pairs of taxa

Estimate Variance

Variance Estimation Method ----- None

Substitution Model

Substitutions Type ----- Nucleotide

Model/Method ----- p-distance

Substitutions to Include ----- d: Transitions + Transversions

Rates and Patterns

Rates among Sites ----- Uniform rates

Pattern among Lineages ----- Same (Homogeneous)

Data Subset to Use

Gaps/Missing Data Treatment ----- Complete deletion

Codons Included ----- 1st+2nd+3rd+Non-Coding

No. of Sites : 448

d : Estimate

- [1] #A. formosus_BMNH1947.2.4.18
- [2] #MN961360.1_Amolops_sp._2_YW-2020_voucher_KIZ011061_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [3] #MN961359.1_Amolops_sp._2_YW-2020_voucher_KIZ016397_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [4] #MN961441.1_Amolops_sp._3_YW-2020_voucher_KIZ014068_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [5] #MN961440.1_Amolops_sp._3_YW-2020_voucher_KIZ014067_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [6] #MN961386.1_Amolops_sp._4_YW-2020_voucher_KIZ012536_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [7] #MN961385.1_Amolops_sp._4_YW-2020_voucher_KIZ012535_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [8] #MN961384.1_Amolops_sp._4_YW-2020_voucher_KIZ012534_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [9] #MN961383.1_Amolops_sp._4_YW-2020_voucher_KIZ012537_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [10] #MN961382.1_Amolops_sp._4_YW-2020_voucher_KIZ012533_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [11] #MN961416.1_Amolops_sp._5_YW-2020_voucher_KIZ040228_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [12] #MN961415.1_Amolops_sp._5_YW-2020_voucher_KIZ040227_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [13] #MN961414.1_Amolops_sp._5_YW-2020_voucher_SH2789_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [14] #MN961413.1_Amolops_medogenesis_voucher_SYNU04II6219_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [15] #MN961412.1_Amolops_medogenesis_voucher_SYNU04II6216_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
- [16] #MK568332.1_Amolops_medogenesis_voucher_SYS_a007531_cytochrome_c_oxidase_subunit_1_(CO1)_gene_partial_cds_mitochondrial
- [17] #MK568331.1_Amolops_medogenesis_voucher_SYS_a007530_cytochrome_c_oxidase_subunit_1_(CO1)_gene_partial_cds_mitochondrial
- [18] #MK568328.1_Amolops_medogenesis_voucher_SYS_a006657_cytochrome_c_oxidase_subunit_1_(CO1)_gene_partial_cds_mitochondrial
- [19] #KU243077.1_Amolops_medogenesis_isolate_KIZ06638_cytochrome_oxidase_subunit_I_(COI)_gene_partial_cds_mitochondrial
- [20] #KU243076.1_Amolops_medogenesis_isolate_KIZ06635_cytochrome_oxidase_subunit_I_(COI)_gene_partial_cds_mitochondrial

[21] #MK605597.1_Amolops_viridimaculatus_voucher_SYS:a003813_cytochrome_c_oxidase_subunit_1_(CO1)_gene_partial_cds_mitochondrial
 [22] #MK605596.1_Amolops_viridimaculatus_voucher_SYS:a003812_cytochrome_c_oxidase_subunit_1_(CO1)_gene_partial_cds_mitochondrial
 [23] #MK568311.1_Amolops_viridimaculatus_voucher_SYS_a003754_cytochrome_c_oxidase_subunit_1_(CO1)_gene_partial_cds_mitochondrial
 [24] #MK568310.1_Amolops_viridimaculatus_voucher_SYS_a003753_cytochrome_c_oxidase_subunit_1_(CO1)_gene_partial_cds_mitochondrial
 [25] #MN961438.1_Amolops_viridimaculatus_voucher_SCUM050403CHX_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
 [26] #MN961437.1_Amolops_viridimaculatus_voucher_SCUM050402CHX_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
 [27] #MN961436.1_Amolops_viridimaculatus_voucher_SCUM050423CHX_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
 [28] #MN961435.1_Amolops_viridimaculatus_voucher_KIZ048488_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial
 [29] #MN961434.1_Amolops_viridimaculatus_voucher_KIZ048487_cytochrome_c_oxidase_subunit_I_(COX1)_gene_partial_cds_mitochondrial

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
[1]																													
[2]	0.096																												
[3]	0.096	0.004																											
[4]	0.080	0.080	0.080																										
[5]	0.080	0.080	0.080	0.000																									
[6]	0.022	0.107	0.107	0.087	0.087																								
[7]	0.022	0.107	0.107	0.087	0.087	0.000																							
[8]	0.022	0.107	0.107	0.087	0.087	0.000	0.000																						
[9]	0.022	0.107	0.107	0.087	0.087	0.000	0.000	0.000																					
[10]	0.022	0.107	0.107	0.087	0.087	0.000	0.000	0.000	0.000																				
[11]	0.087	0.092	0.092	0.071	0.071	0.094	0.094	0.094	0.094	0.094																			
[12]	0.087	0.092	0.092	0.071	0.071	0.094	0.094	0.094	0.094	0.094	0.000																		
[13]	0.087	0.092	0.092	0.071	0.071	0.094	0.094	0.094	0.094	0.094	0.000	0.000																	
[14]	0.089	0.089	0.089	0.058	0.058	0.092	0.092	0.092	0.092	0.092	0.096	0.096	0.096																
[15]	0.089	0.089	0.089	0.058	0.058	0.092	0.092	0.092	0.092	0.092	0.096	0.096	0.096	0.000															
[16]	0.089	0.089	0.089	0.058	0.058	0.092	0.092	0.092	0.092	0.092	0.096	0.096	0.096	0.000	0.000														
[17]	0.087	0.092	0.087	0.056	0.056	0.089	0.089	0.089	0.089	0.089	0.094	0.094	0.094	0.002	0.002	0.002													
[18]	0.089	0.089	0.089	0.058	0.058	0.092	0.092	0.092	0.092	0.092	0.096	0.096	0.096	0.000	0.000	0.000	0.002												
[19]	0.089	0.089	0.089	0.058	0.058	0.092	0.092	0.092	0.092	0.092	0.096	0.096	0.096	0.000	0.000	0.000	0.002	0.000											
[20]	0.087	0.092	0.087	0.056	0.056	0.089	0.089	0.089	0.089	0.089	0.094	0.094	0.094	0.002	0.002	0.002	0.000	0.002	0.002										
[21]	0.085	0.056	0.056	0.080	0.080	0.092	0.092	0.092	0.092	0.092	0.078	0.078	0.078	0.067	0.067	0.067	0.065	0.067	0.067	0.065									
[22]	0.085	0.056	0.056	0.080	0.080	0.092	0.092	0.092	0.092	0.092	0.078	0.078	0.078	0.067	0.067	0.067	0.065	0.067	0.067	0.065	0.000								
[23]	0.085	0.056	0.056	0.080	0.080	0.092	0.092	0.092	0.092	0.092	0.078	0.078	0.078	0.067	0.067	0.067	0.065	0.067	0.067	0.065	0.000	0.000							
[24]	0.085	0.056	0.056	0.080	0.080	0.092	0.092	0.092	0.092	0.092	0.078	0.078	0.078	0.067	0.067	0.067	0.065	0.067	0.067	0.065	0.000	0.000	0.000						
[25]	0.083	0.058	0.058	0.067	0.067	0.085	0.085	0.085	0.085	0.089	0.089	0.089	0.089	0.063	0.063	0.063	0.060	0.063	0.063	0.060	0.056	0.056	0.056	0.056					
[26]	0.083	0.058	0.058	0.067	0.067	0.085	0.085	0.085	0.085	0.089	0.089	0.089	0.089	0.063	0.063	0.063	0.060	0.063	0.063	0.060	0.056	0.056	0.056	0.056	0.000				
[27]	0.085	0.056	0.056	0.080	0.080	0.092	0.092	0.092	0.092	0.092	0.078	0.078	0.078	0.067	0.067	0.067	0.065	0.067	0.067	0.065	0.000	0.000	0.000	0.000	0.056				
[28]	0.085	0.056	0.056	0.080	0.080	0.092	0.092	0.092	0.092	0.092	0.078	0.078	0.078	0.067	0.067	0.067	0.065	0.067	0.067	0.065	0.000	0.000	0.000	0.000	0.056	0.056	0.000		
[29]	0.085	0.056	0.056	0.080	0.080	0.092	0.092	0.092	0.092	0.092	0.078	0.078	0.078	0.067	0.067	0.067	0.065	0.067	0.067	0.065	0.000	0.000	0.000	0.000	0.056	0.056	0.000	0.000	

Table. Estimates of Evolutionary Divergence between Sequences

The number of base differences per site from between sequences are shown. The analysis involved 29 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 448 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [1].

1. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.

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