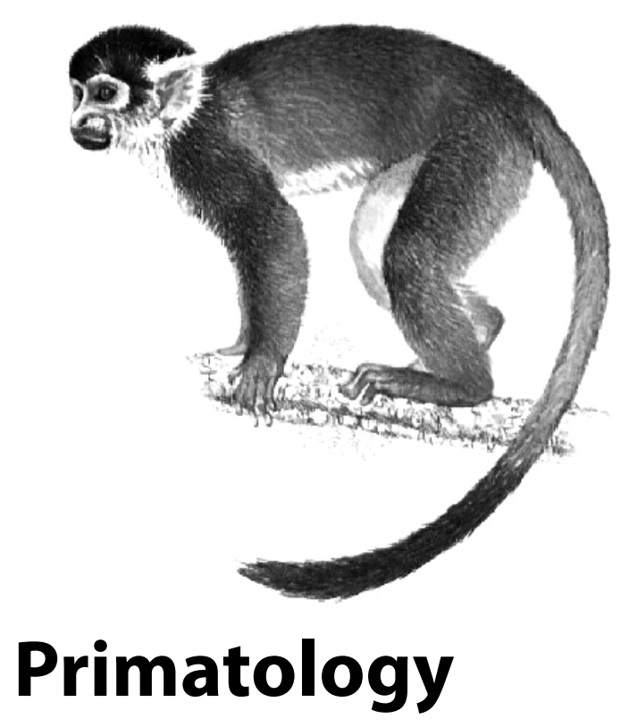
Complete mitochondrial DNA sequences lend insight into the evolutionary history and biogeography of Central American squirrel monkeys.



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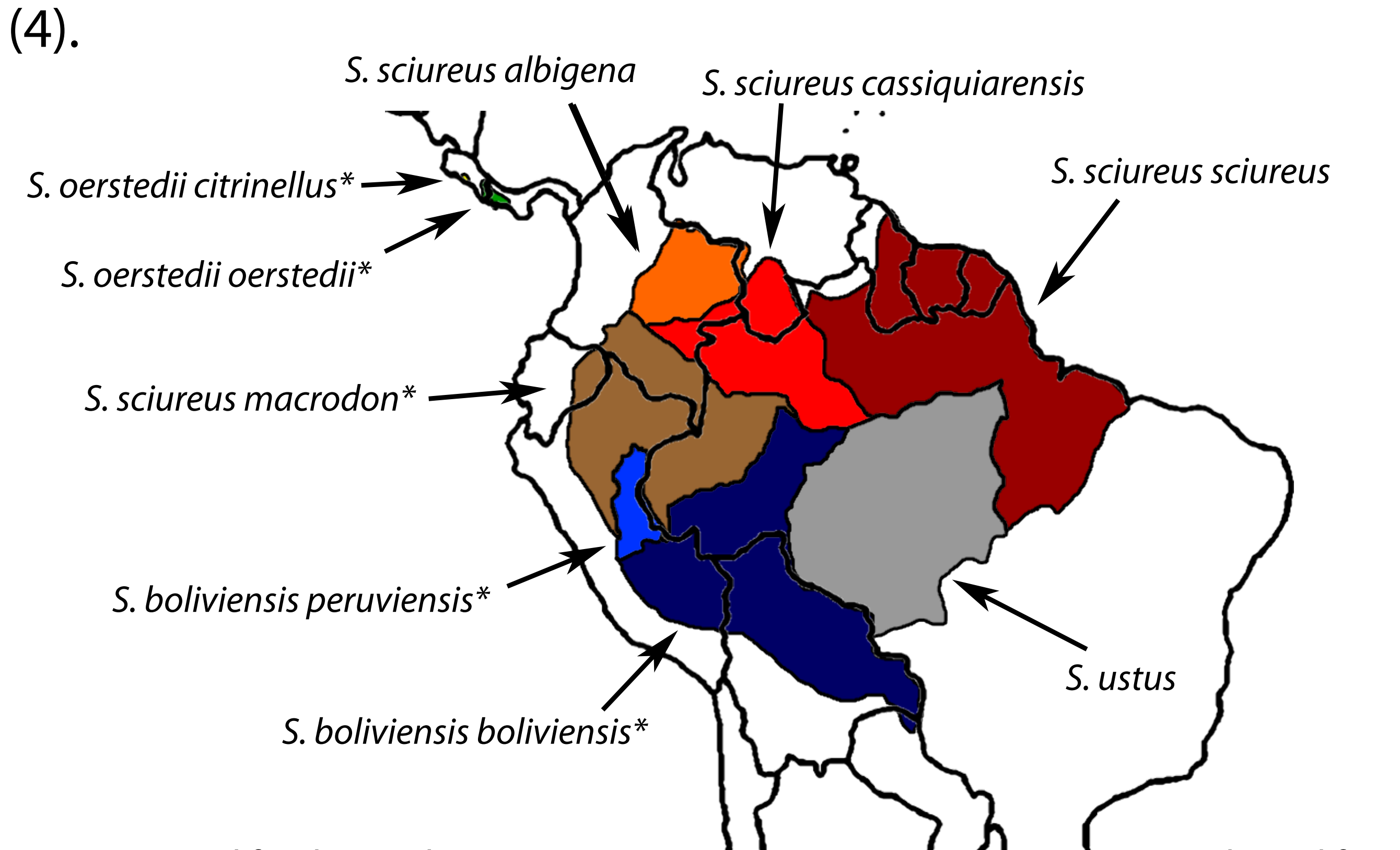
Introduction

Evolutionary relationships among squirrel monkeys (genus *Saimiri*) are poorly understood. Despite forming distinct geographic variants, the range of variation within *Saimiri* has been described as shallow overall (1), creating a need for greater taxon sampling in order to infer relationships. The lack of a consistent taxonomy has led to considerable confusion in both the primatological and biomedical literatures.

The phylogenetic relationship of geographically disjunct Central American squirrel monkeys (*S. oerstedii*) to South American populations remains unresolved (2). Particularly unclear is the timing of the radiation into Central America in relation to the formation of the Isthmus of Panama (ca. 3.5 Ma). While other primates are known to have been part of a migration into Mesoamerica soon after the completion of the isthmus (3), the involvement of *Saimiri* is unknown.

Here, we infer phylogenetic relationships and estimate divergence times using several newly-sequenced *Saimiri* mitochondrial DNA (mtDNA) genomes. We sample from 3 of 4 species and 6 of 8 subspecies commonly recognized (4).



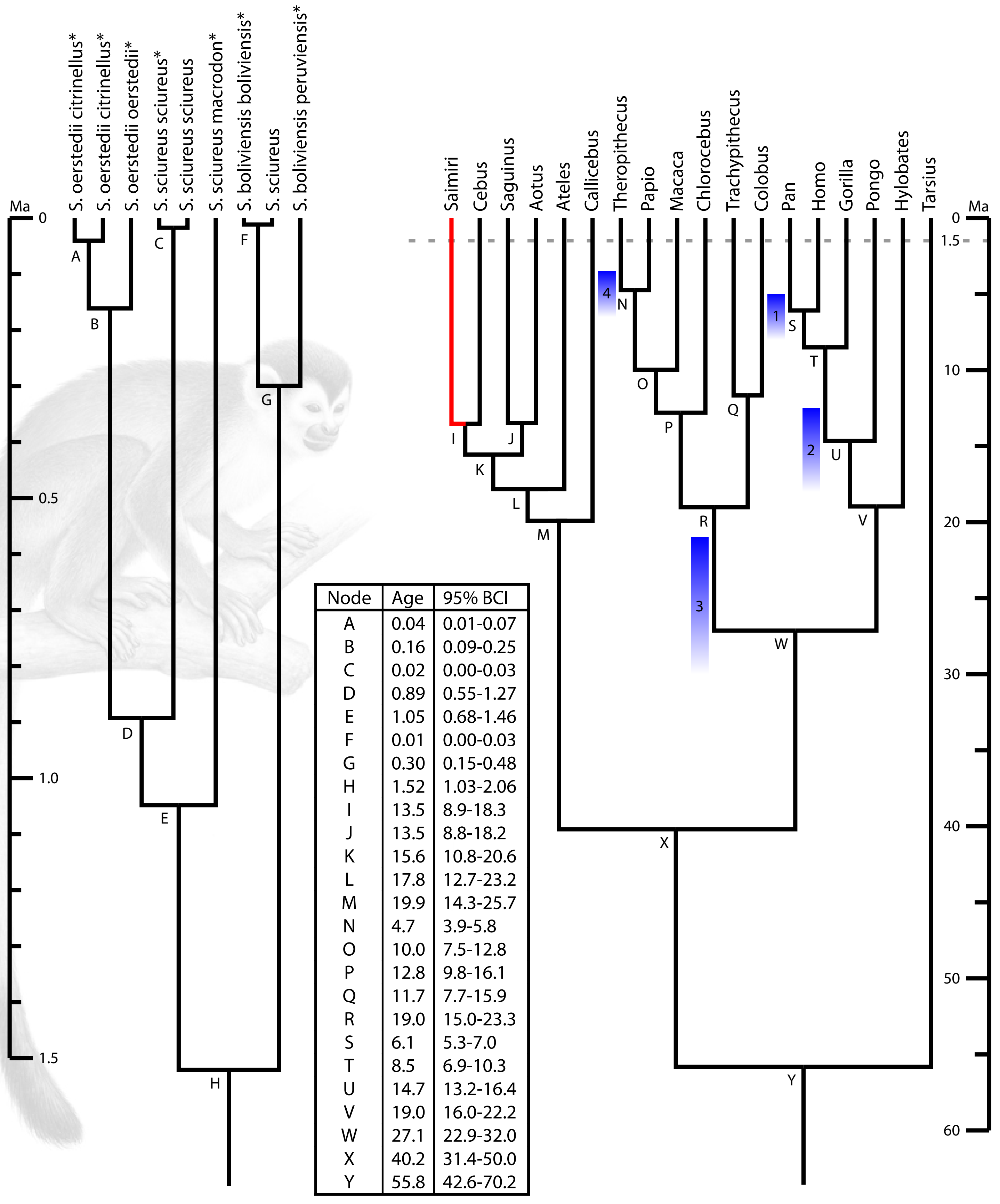
* = sequenced for this study

adapted from (4)

Methods

Using established methods (5), we sequenced 7 complete mtDNA genomes from 6 *Saimiri* species and subspecies sampled from across the geographic distribution and added them to a dataset of primate mtDNA genome sequences from Genbank including 2 of *Saimiri*, for one of which geographic origin was known. The 12 heavy strand protein-coding gene sequences were isolated, concatenated, then aligned. This alignment was then used for maximum likelihood analysis (*RAxML 7.2.6*), Bayesian phylogenetic inference (*MrBayes v3.1.2*), and molecular dating using the uncorrelated Bayesian relaxed clock method (*BEAST v1.5.3*). Divergence times were estimated using conservative priors and fossil calibrations shown in the table.

Results



Node	Age	95% BCI
A	0.04	0.01-0.07
B	0.16	0.09-0.25
C	0.02	0.00-0.03
D	0.89	0.55-1.27
E	1.05	0.68-1.46
F	0.01	0.00-0.03
G	0.30	0.15-0.48
H	1.52	1.03-2.06
I	13.5	8.9-18.3
J	13.5	8.8-18.2
K	15.6	10.8-20.6
L	17.8	12.7-23.2
M	19.9	14.3-25.7
N	4.7	3.9-5.8
O	10.0	7.5-12.8
P	12.8	9.8-16.1
Q	11.7	7.7-15.9
R	19.0	15.0-23.3
S	6.1	5.3-7.0
T	8.5	6.9-10.3
U	14.7	13.2-16.4
V	19.0	16.0-22.2
W	27.1	22.9-32.0
X	40.2	31.4-50.0
Y	55.8	42.6-70.2

* = sequenced for this study

Fossil Calibrations

Constraint	Divergence	Offset (Ma)	95% HPD	Fossil	Age (Ma)
1	*Homo/Pan*	5.0	5.0 - 8.0	*Ardipithecus*	5.2
				Orrorin	6.0
				Sahelanthropus	6.0-7.0
2	*Homo/Pongo*	12.5	12.5 - 18.0	*Sivapithecus*	≈12.5
3	Hominoid/cercopithecoid	21.0	21.0 - 30.0	*Morotopithecus*	>20.6
				Victoriapithecus	≈19.0
4	*Papio/Theropithecus*	3.5	3.5 - 6.5	*Theropithecus*	≈3.5

Discussion

Using the largest squirrel monkey nucleotide sequence dataset analyzed to date, we infer a strongly-supported tree topology and estimate divergence times for 9 *Saimiri* individuals sampled from across the geographic distribution. Our inferred topology places *S. boliviensis* (n=3) basal to all other squirrel monkeys. *S. oerstedii* (n=3) forms a sister relationship with *S. sciureus sciureus* (n=2), with *S. sciureus macrodon* (n=1) basal, indicating paraphyly of the *S. sciureus* species. The high similarity of the Genbank sequence of unknown geographic origin (*S. sciureus*) to Bolivian *Saimiri* indicates that it is more properly assigned to *S. boliviensis boliviensis*.

The South American sister taxon of *S. oerstedii* was found to be Guyanese *Saimiri* (*S. sciureus sciureus*), diverging 0.89 Ma (95% BCI 0.55-1.27 Ma), well after the formation of the Isthmus of Panama (ca. 3.5 Ma). The young date suggests that extant Central American squirrel monkeys are the descendents of a relatively recent radiation from South America. While more sampling is needed, the significantly more recent divergence of the two recognized subspecies of *S. oerstedii*, estimated at 160 kya (90-250 kya), is consistent with a "refugia" model of diversification.

A surprising result is the young age of the MRCA of all *Saimiri*, estimated at 1.52 Ma (1.03-2.06 Ma). Occurring well after the *Cebus*-*Saimiri* split (8.9-18.3 Ma), this leaves a substantial temporal gap in our knowledge of squirrel monkey evolution, complicated further by the description of Miocene fossils from La Venta, such as *Neosaimiri* (ca. 12.5 Ma), as exhibiting *Saimiri*-like features (6). Although some taxa were not sampled in our analysis, results from a recent molecular study (7) indicate that those taxa group with internal lineages within *Saimiri*, suggesting that our crown divergence date would be unaffected by the inclusion of additional taxa.

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