Molecular data highlight hybridization in squirrel monkeys (Saimiri, Cebidae)

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Abstract

Hybridization has been reported increasingly frequently in recent years, fueling the debate on its role in the evolutionary history of species. Some studies have shown that hybridization is very common in captive New World primates, and hybrid offspring have phenotypes and physiological responses distinct from those of the “pure” parents, due to gene introgression. Here we used the TA15 Alu insertion to investigate hybridization in the genus Saimiri. Our results indicate the hybridization of Saimiri boliviensis peruviensis with S. sciureus macrodon, and S. b. boliviensis with S. ustus. Unexpectedly, some hybrids of both S. boliviensis peruviensis and S. b. boliviensis were homozygous for the absence of the insertion, which indicates that the hybrids were fertile.

Keywords: Saimiri, squirrel monkeys, Alu elements, hybridization.

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Osterholz et al. (2008) described the integration of an *Alu* element in *S. boliviensis*, which is absent in *S. sciureus*. In the human genome, *Alu* elements are the most abundant transposable features (Kriegs et al., 2007), and these elements are now known to comprise approximately 10% of the primate genome (Batzer and Deininger, 2002; Zhang et al., 2002). Once inserted into the genome of a species during its evolutionary history, *Alu* insertions will be present in all the descendants of that species. An *Alu* insertion is thus a single and irreversible event (Hamdi et al., 1999; Shedlock and Okada, 2000; Salem et al., 2003), and represents a marker free of homoplasies. The present study investigated the potential occurrence of hybridization in free-living populations of *S. boliviensis*, based on the presence or absence of *Alu*TA15, as described by Osterholz et al. (2008).

We examined 107 samples of *Saimiri*: two *S. sciureus macrodon*, 16 *S. collinsi*, 17 *S. ustus*, 22 *S. boliviensis peruvianus* and 50 *S. b. boliviensis* (Table 1). All the individuals sampled were born in the wild, although in some cases, the blood samples were collected in captivity. The samples of *S. collinsi* were collected from animals captured during the rescue operation of the UHE Tucurui hydroelectric reservoir in Para, Brazil (La Rovere and Mendes, 2000), and those of *S. ustus* at UHE Samuel, in Rondonia (Fearnside, 2005). The samples of *S. b. boliviensis*, *S. b. peruviensis* and *S. s. macrodon* were obtained from two captive facilities, the “Centro de Reproducción y Conservación de Primates No Humanos” (CRCP/IVITA) in Iquitos, Peru, and the “Centro Argentino de Primates” (CAPRIM) in Corrientes, Argentina. The species were identified based on the morphological characteristics described by Hershkovitz (1984). *S. b. boliviensis* has a white zone around the eyes exhibiting sparse white hairs and a flattened arch over the eyes (roman arch) while in *S. s. macrodon* the arch formed above each eye is more evident and has been named as a “gothic arch”.

While *S. b. boliviensis* and *S. b. peruviensis* have an arch that is less pronounced over the eyes (roman arch), *S. b. peruviensis* has a crown pattern on the head which is less eumelanized than that of *S. b. boliviensis*. The specimens held at CRCP/IVITA were classified as *S. boliviensis peruviensis* (roman arch) and those from the vicinity of Iquitos (Figure 1) as *S. sciureus macrodon* (gothic arch),
Table 1 - Species, specimen code, locality, geographical coordinates and origin of the specimens analyzed in the present study.

| Taxa                        | Code   | Locality                            | Coordinates | Origin  |
|-----------------------------|--------|-------------------------------------|-------------|---------|
| Saimiri boliviensis boliviensis | SBB 2103 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2104 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2105 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2111 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2112 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2113 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2114 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2115 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2116 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2118 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2119 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2120 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2121 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2123 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2124 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2126 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2128 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2129 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2130 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2131 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2132 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2133 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2134 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2135 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2136 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2140 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2141 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2142 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2143 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2144 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2145 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2146 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2149 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2150 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2151 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2153 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2157 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2158 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2159 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2160 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2164 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2165 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2168 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2169 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2170 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Saimiri boliviensis boliviensis | SBB 2171 | Santa Cruz de La Sierra, Bolivia | 17°20' 64°03' | CAPRIM  |
| Taxa                     | Code     | Locality                                      | Coordinates    | Origin            |
|-------------------------|----------|-----------------------------------------------|----------------|-------------------|
| *Saimiri boliviensis boliviensis* | SBB 2174 | Santa Cruz de La Sierra, Bolivia              | 17°20' 64°03'  | CAPRIM           |
| *Saimiri boliviensis boliviensis* | SBB 2176 | Santa Cruz de La Sierra, Bolivia              | 17°20' 64°03'  | CAPRIM           |
| *Saimiri boliviensis boliviensis* | SBB 2175 | Santa Cruz de La Sierra, Bolivia              | 17°20' 64°03'  | CAPRIM           |
| *Saimiri boliviensis boliviensis* | SBB 2177 | Santa Cruz de La Sierra, Bolivia              | 17°20' 64°03'  | CAPRIM           |
| *Saimiri boliviensis peruviensis* | SBP 1893 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1906 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1908 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1915 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1916 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1917 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1918 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1919 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1920 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1922 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1923 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1925 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1926 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1929 | East bank of the Marañón river, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1931 | East bank of the Marañón river, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1932 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1933 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1934 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1936 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1937 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1939 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri boliviensis peruviensis* | SBP 1941 | East bank of the Marañón River, Peru          | 06°41' 76°08'  | CRCP/IVITA       |
| *Saimiri sciureus macrodon*     | SSM 1945 | Ucayali River (Caserio Bagazan, Quebrada Carahuayte), Peru | 07°52' 74°34' | CRCP/IVITA       |
| *Saimiri sciureus macrodon*     | SSM 1946 | Ucayali River (Caserio Bagazan, Quebrada Carahuayte), Peru | 07°52' 74°34' | CRCP/IVITA       |
| *Saimiri collinsi*              | SC 34    | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 36    | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 410   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 473   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 525   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 626   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 627   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 686   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 749   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 847   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 863   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 865   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 873   | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 1502  | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 1549  | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri collinsi*              | SC 1679  | Left bank of the Tocantins River, Pará, Brazil | 03°52' 49°42' | Free-living      |
| *Saimiri ustus*                | SU 2257  | Right bank of the Jamari River, Rondônia, Brazil | 08°56   | Free-living      |
| *Saimiri ustus*                | SU 2305  | Right bank of the Jamari River, Rondônia, Brazil | 08°56   | Free-living      |
750 base pairs (bps) is generated, but when it is absent, a TA15 insertion is present, a fragment of approximately 450 bps (Table 2). By contrast, 50 specimens presented only one band of approximately 450 bps (Table 2). By contrast, 50 specimens presented only one band of approximately 450 bps (Table 2).

| Taxa          | Code   | Locality                           | Coordinates | Origin       |
|---------------|--------|------------------------------------|-------------|--------------|
| Saimiri ustus | SU 2354| Right bank of the Jamari River, Rondônia, Brazil | 08°56 63°21' | Free-living  |
| Saimiri ustus | SU 2450| Right bank of the Jamari River, Rondônia, Brazil | 08°56 63°21' | Free-living  |
| Saimiri ustus | SU 2454| Right bank of the Jamari River, Rondônia, Brazil | 08°56 63°21' | Free-living  |
| Saimiri ustus | SU 2577| Right bank of the Jamari River, Rondônia, Brazil | 08°56 63°21' | Free-living  |
| Saimiri ustus | SU 3193| Right bank of the Jamari River, Rondônia, Brazil | 08°56 63°21' | Free-living  |
| Saimiri ustus | SU 4030| Right bank of the Jamari River, Rondônia, Brazil | 08°56 63°21' | Free-living  |
| Saimiri ustus | SU 4031| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4032| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4033| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4041| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4257| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4441| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4508| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4550| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |
| Saimiri ustus | SU 4577| Left bank of the Jamari River, Rondônia, Brazil  | 08°52 63°15' | Free-living  |

UHE Tucurui= Tocantins River; UHE Samuel= Jamari River.

while the animals at CAPRIM, captured in Santa Cruz de La Sierra, Bolivia, were all S. boliviensis boliviensis (roman arch). Some of the animals at CAPRIM were born in captivity. Further details on the specimens and the geographical distribution of each population are presented in Table 1 and Figure 1. The material analyzed in the present study was part of the sample bank maintained by the Molecular Phylogenetics Laboratory at the Bragança campus of the Federal University of Para.

The total DNA was extracted using the Wizard Genomic kit (Promega, Madison, WI, USA) following the manufacturer’s recommendations. The region of interest (AluTA15) was amplified using the primers and the protocol described by Osterholz et al. (2008). The initial denaturation step was 2 min at 94 °C, followed by 40 cycles of denaturation (1 min at 94 °C), annealing (1 min at 58 °C), and extension (1 min at 72 °C), with a final extension step of 5 min at 72 °C. After amplification, the PCR products were separated electrophoretically in a 2% agarose gel at 60 V, 150 mA for 60 min together with a 1 kb plus DNA ladder (Invitrogen, Carsbad, CA, USA). All the fragments were stained with GelRed, as recommended by the manufacturer (Biotium, Hayward, CA, USA). Sequence reactions were conducted with a Big Dye v.3.1 kit (ABI BigDye® Terminator Mix; Applied Biosystems, Carsbad, CA, USA), conducted in an ABI 3500XL sequencer (Applied Biosystems), to confirm that the region amplified by PCR was the fragment of interest (AluTA15). The sequences were aligned and edited manually in the BioEdit program (Hall, 1999).

The primers designed by Osterholz et al. (2008) amplify fragments of distinct sizes depending on the presence or absence of the Alu insertion (AluTA15). When the AluTA15 insertion is present, a fragment of approximately 750 base pairs (bps) is generated, but when it is absent, a fragment of only 450 bps is generated. As the insertion is only present in S. boliviensis (Figure 2), in hybrids between this species and other Saimiri species, two fragments will be amplified, one with 750 bps and another with 450 bps. The AluTA15 insertion was not detected in any of the individuals identified as S. ustus (n=17) from Rondonia, S. collinsi (n=16) from Para or S. seicures macrosdon (n=2) from Peru. All 35 individuals presented only one band of approximately 450 bps (Table 2). By contrast, 50 specimens from Santa Cruz de La Sierra, Bolivia, identified as S. b. peruviensis, the ancestral lineage of S. boliviensis, which implies that all S. boliviensis should be homozygous for AluTA15 (+/+). Interestingly, all three possible combinations were found in the population previously identified as S. b. peruviensis from Peru (CRCP), with six individuals (28%) being homozygous for the insertion (+/+-), eight (36%) being homozygous for its absence (-/-), and the other eight being heterozygous (+/-), showing both bands (750/450 bps) in the gel (Figure 2). Osterholz et al. (2008) also found three possible patterns of bands (+/+; +/-; -/--) for specimens that were previously identified as S. b. peruviensis. So again, if the AluTA15 was inserted into the ancestral lineage of S. boliviensis, as proposed by Osterholz et al. (2008), it is unclear how specimens of this species could lack the insertion (-/-).

It is well known that Alu elements are replicated in a copy-and-paste way in the primate genome, and once inserted into a genome, they cannot be excised. Given this, individuals phenotypically typical of Saimiri b. peruviensis,
but heterozygous for the insertion (+/-), must be the result of natural hybridization, which would presumably have involved the geographically closest taxon, *S. sciureus macrodon*. Furthermore, the absence of the insertion (-/-) in morphologically typical *S. b. peruviensis* can only be accounted for by the crossing of hybrid (+/-) *Saimiri b. peruviensis* or crosses between a hybrid and *S. sciureus macrodon* (-/-). These crosses would generate 25% or 50% of descendants without the insertion (-/-) and with dubious or intermediate morphological characteristics, which would represent conclusive evidence that hybridization between *S. boliviensis* and *Saimiri sciureus macrodon* produces fertile offspring. However, only 10% of the 50 *Saimiri b. boliviensis* specimens were heterozygous (+/-), and probably originated from crosses with *Saimiri ustus*, due to the proximity of the geographical distribution of these species (Figure 2).

It is interesting to note that *S. b. peruviensis* and *S. s. macrodon* occur sympatrically in the region between the Marañón and Tapiche rivers in the Peruvian Amazonia, whereas *S. b. boliviensis* is parapatric with *S. s. macrodon* and *S. ustus*, which are separated by the Juruá and Purus-Guaporé Rivers, respectively (Hershkovitz, 1984). However, these rivers do not constitute an effective geographic barrier to gene flow in lizards (Souza et al., 2013), primates, and other organisms (Gascon et al., 2000), which implies that there may be gene flow between the present-day ranges of the three *Saimiri* species, resulting in hybridization between *Saimiri boliviensis* and *Saimiri sciureus* or *S. ustus*, as suggested by previous authors (Hershkovitz, 1984; Thorington Jr, 1985; Silva et al., 1992, 1993; Osterholz et al., 2008) based on morphological data.

Using chromosomal data, Jones and Ma (1975) were able to distinguish between *S. b. peruviensis* and *S. s. macrodon* from the vicinity of Iquitos (Peru) and Leticia (Colombia), respectively. Both species revealed a diploid number of 2n=42, with 10 meta/submetacentric, 22 acrocentric and 10 telocentric chromosomes in *S. b.

### Table 2 - Presence (+) or absence (-) of the *Alu* TA15 insertion in the *Saimiri* specimens analyzed in the present study.

|                    | Homozygous | Heterozygous | Homozygous | Total |
|--------------------|------------|--------------|------------|-------|
| *Saimiri boliviensis peruviensis* | 8 (36%) | 8 (36%) | 6 (28%) | 22    |
| *Saimiri boliviensis boliviensis* | 0 | 5 (10%) | 45 (90%) | 50    |
| *Saimiri collinsi* | 16 (100%) | 0 | 0 | 16    |
| *Saimiri sciureus macrodon* | 2 (100%) | 0 | 0 | 2     |
| *Saimiri ustus* | 17 (100%) | 0 | 0 | 17    |
| **Total:** | 32 | 13 | 51 | 107   |

Figure 2 - Electrophoresis gel showing the distribution of the three *Alu* genotypes (+/+; +/-; and -/-) in the five subspecies sampled in the present study. A 1 kb ladder placed at both sides of the gel indicates the size in base pairs (bp) of the two amplified fragments.
peruviensis, and 10 meta/submetacentric, 20 acrocentric, and 12 telocentric chromosomes in S. s. macrodon. A hybrid produced in the laboratory between a male from Iquitos and a female from Leticia showed 10 meta/submetacentric, 11 acrocentric and 11 telocentric chromosomes. Lau and Arrighi (1976) using chromosomal banding analyses detected two nonhomologous pericentric inversions in the telocentric group of chromosomes of a squirrel monkey, Saimiri sciureus, suggesting that this individual was an intersubspecific hybrid whose parents originated from different geographical locations. Recently, Ruiz-Garcia et al. (2015) also found evidence of hybridization between Saimiri species based on mitochondrial markers (Cox1 and Cytb), emphasizing the importance of this process in the species-level diversification of this genus. In fact, these authors concluded that this genus comprises only three species, S. oerstedi, S. sciureus, and Saimiri vanzolinii, which diversified during the Pleistocene. This is consistent with the estimate of Alfaro et al. (2015), who concluded that S. boliviensis diverged from the other Saimiri species less than 1.5 Ma.

Hybridization may be a catalyst not only for speciation but also for major evolutionary innovations (Mallet 2007). Hybridization between Saimiri species appears to be common, and as Mallet (2007) concludes at page 182: “most speciation involves natural selection; natural selection requires genetic variation; genetic variation is enhanced by hybridization; and hybridization and introgression between species is a regular occurrence, especially in rapidly radiating groups”. On the basis of the evidence presented here, this appears to have been the case in Saimiri.

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