

Morphological variation in *Atractustartarus* (Serpentes: Dipsadidae) from the Xingu River, east Amazon, Brazil and preliminary phylogenetic relationship in *Atractus*

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Abstract: Currently are recognized 138 species in the genus *Atractus*, distributed from the eastern region of the Isthmus of Panama to northern Argentina. In recent years, many species have been described based on external morphological differences and hemipenis. Here we described the variation in meristic character in the newly described specie *Atractus tartarus* from the eastern Brazilian Amazon from the Xingu region. Additionally we present a preliminary hypothesis of relationship with your relatives, based on marker 16SmtDNA.

Keyword: Molecular, Snakes, *Atractus tartarus*.

Abbreviations: Snout-Vent Length (SVL); Total Length (TTL); Tail Length (TL); Head Width (HW) defined as the widest part of the head; Head length (HL), definite the tip of the rostral scale to the joint angle of the square bone.

1. INTRODUCTION

The genus *Atractus* Wagler 1828, currently has 138 species [1], and is considered the richest in number of species of snakes [2,3]. Is distributed from the eastern region of the Isthmus of Panama to northern Argentina [4]. Within the main studies with genus in the Amazon we emphasize the [4] to Ecuador; [5] for Suriname; [6] for central Brazilian Amazon in Manaus region; [7] and [8] for the Colombian Amazon and [9] to Guyana and Venezuela. The taxonomy of this genus has long been based on external morphological characters as coloration pattern and cephalic plates [4, 5, 10], recently being incorporated data as hemipenis [7, 3].

In the last decade, many species have been described based on traditional morphological characteristics and/or hemipenis: Brazil [11, 12, 13, 14], Colombia [15] and Ecuador [16]. With respect to hemipenis, [4] defined two forms of this structure to *Atractus* Ecuador: 1) undifferentiated, entirely covered with small spines willing in longitudinal lines; 2) differentiated with the center of the body forming two areas, one distal and another basal.[4] also proposed the aggrupation of the genus into three groups: *A. badius* (Boie 1827), *A. elaps* (Gunther 1858) and *A. trilineatus* Wagler 1828, based on characters scalation, dentition and rows of dorsal scales.

Here we describe the variation in meristic characters within species *Atractus tartarus* Passos, Prudente and Lynch 2016, with 15 rows dorsal scales for Volta Grande do Xingu, Pará State, Brazil, and a proposal a preliminary hypothesis of phylogenetic relationships regarding the congeneric species based on 16S mtDNA fragment.

2. MATERIAL AND METHODS

Total genomic DNA was extracted by phenol-chloroform protocol [17] and resuspended with 30 µL of TE. DNA quality was verified by agarose gel electrophoresis. Polymerase Chain Reaction (PCR) was performed to amplify a fragment of 497 bp of the 16S mitochondrial gene. The PCR to fragment of the 16S mitochondrial gene (total volume of 15 µL) contained 6.7 ddH₂O µL, 1.5 µL of 25 mM MgCl₂, 25.1 µL of 10 mM dNTPs (2.5mM each dNTP), 25.1 µL of 10x buffer (75 mM Tris HCl, 50 mM KCl, 20 mM (NH₄)₂SO₄), 1.5 µL 16S A primer (5'-CGCCTGTTACCAAAACATCGCCT-3'),

1.5 µL 16S B primer (5'-CCGGTCTGAACTCAGATCACGT-3') [18], 0.3 µL of 1 U Taq polymerase, and 1 µL of DNA (30 - 50 ng/µL) under the following conditions: an initial temperature of 92°C (60 sec.), followed by 35 cycles of 92°C (60 sec.), 50°C (50 sec.) and 72°C (1.5 min.) and a final extension at 72°C for 7 min.

The sequencing reactions were performed using BigDye terminator kit as (*Applied Biosystems*).

Following the manufacturer's protocol. The primer forward 16S A (mtDNA), it was used in the sequencing reaction. The products of the sequencing reactions were precipitated with ethanol according to manufacturer's recommendations, resuspended in 10 µL of deionized formamide (ABI) and subsequently injected into the 3130xl ABI automated sequencer (*Applied Biosystems*).

For the construction of the phylogenetic tree was obtained a total of 472 bp of a specimen of *Atractus tartarus* the region of Volta Grande do Xingu, Pará State, Brazil. Additionally GenBank sequences were obtained from several species of *Atractus* and *Tretanorhinus variabilis* Duméril, Bibron and Duméril 1854, as the external group (Table 1). All sequences were aligned using the algorithm Clustal W [19] implemented in software BIOEDIT 7.2 [20]. The evolutionary molecular model TIM2 + I + G was chosen through software jModelTest [21] and a phylogenetic tree Maximum Likelihood (ML) with 10,000 replicas bootstrap was generated in software TREEFINDER [22]. The genetic distance pairwise uncorrected (p-distance) between species were calculated in MEGA 6.0 software [23].

Table 1: GenBank species accession used for molecular comparison with species *Atractus tartarus* in this study

| Species | Nº in GenBank |
|---------------------------------|---------------|
| <i>Atractus albuquerquei</i> | GQ457726.1 |
| <i>A. duboisi</i> | KT944041.1 |
| <i>A. dunni</i> | KT944038.1 |
| <i>A. gigas</i> | KT944043.1 |
| <i>A. iridescens</i> | KT944039.1 |
| <i>A. major</i> | KT944045.1 |
| <i>A. resplendens</i> | KT944042.1 |
| <i>A. schach</i> | AF158486.1 |
| <i>Atractus sp.</i> | KT944040.1 |
| <i>A. trihedrurus</i> | GQ457727.1 |
| <i>A. typhon</i> | KT944044.1 |
| <i>A. zidocki</i> | AF158487.1 |
| <i>Tretanorhinus variabilis</i> | AF158529.1 |

The specimens are derived from environmental monitoring studies and were donated to the Universidade Federal do Pará (UFPA), campus de Altamira. The abbreviations used are: Snout-Vent Length (SVL); Total Length (TTL); Tail Length (TL); Head Width (HW) defined as the widest part of the head; and Head length (HL), definite the tip of the rostral scale to the joint angle of the square bone. The SVL, TTL and TL were measured with a flexible tape measure 100 cm length. All other measures were taken with the help of a digital caliper 0.01mm of the brand Mitutoyo®. Morphological descriptions and type of cephalic scales follow the literature [4, 14, 15]. The measurements of the structures pairs, were performed on the left side of the body [15]. The sex was identified by observing the hemipenis. The description of hemipenis was made using the terminology of [4] and [24].

Due we do not have access to specimen type da specie *A. tartarus*, we used the original descriptions of [25] to be compared with the specimens studied here.

3. RESULTS AND DISCUSSION

Atractus tartarus



Figure 1. Holotype of *Atractus tartarus* collected in vila Palestina, state of Pará – Brazil (Passos et al. 2016).

Morphological variation in *Atractus tartarus* of (Snake: Dipsadidae) from the Xingu River, East Amazon, Brazil and Preliminary Phylogenetic Relationship in *Atractus*

Holotype: Adult male (MNRJ 16511) collected by R.S. Bérnuls, H. Wogel, and P.S. Abe on 07 February 2008 at Vila Palestina (04°40'S, 47°56'W, ca. 200 m asl), municipality of Rondon do Pará, state of Pará, Brazil.

Specimens in this Study: Male, LZATM 935 and female, LZATM 946, collected in the region known as Volta Grande do Xingu, Pará State, Brazil (3°15'57,17'' S and 51° 57'25,03''W) in 02/10/2013 by environmental consulting firm.

Variation in Meristic and Morphometric Characters (in parenthesis): Seven infralabial (six infralabial); ventral scales 146 – 157 in male (158 ventral scales); subcaudal 29 – 38 in male (39 scales subcaudal); scale subcaudal 23 – 31 in female (32 scales subcaudal in female); long tail in males varying from 15.5 – 18.1% of SVL (18.31% of SVL). The measurement values of all variables are in the supplementary material Table 2.

Molecular data: Based on mitochondrial 16S we find a phylogenetic relationship with low values support (Figure 2), probably due to small number of available sequences in GenBank to the species of *Atractus*. However, we observe that the species with 15 rows of dorsal scales does not represent a monophyletic group, and the specie *A. tartarus* is more phylogenetically related to species *Atractus trihedrurus* (Amaral 1926) what has 17 rows of dorsal scales. With respect to genetic distance, the specie having from 4 - 6% (Table 3) differences for other species, values that are much larger than those found for other species, e.g. *Atractus resplendens* Werner 1901 and *A. duboisi* (Boulenger 1880) they have 2% of interspecific genetic distance.

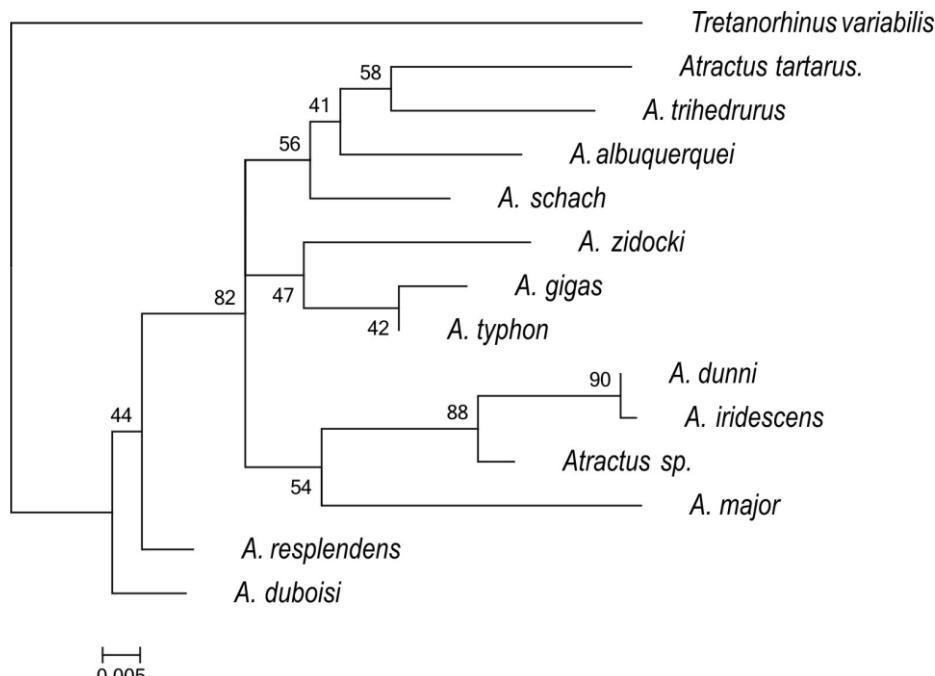


Figure2. Maximum likelihood tree showing a hypothesis phylogenetic relationship between species 17/17/17 and 15/15/15 rows of dorsal scales of genus *Atractus*, based on 16S mitochondrial marker.

Table2. Values of morphometric variables in mm of the two species in study

| Meristic characters/morphometry | Male | Female |
|---------------------------------|--------|--------|
| Snout-Vent Length | 350 | 450 |
| Length Tail | 64.09 | 52.81 |
| Total Length | 414.09 | 502.81 |
| Length Head | 10.88 | 12.94 |
| Width Head | 7 | 9.75 |
| Nose To Eye | 3.02 | 3.38 |
| Rostro To Eye | 4.36 | 4.84 |
| Intraorbital | 4.61 | 5.64 |
| Width Rostral | 2.49 | 2.46 |
| Height Rostral | 1.29 | 1.72 |
| Width Internasal | 1.36 | 1.47 |

| | | |
|------------------------------------|----------|----------|
| Height Internasal | 1.06 | 1.21 |
| Suture Internasal | 0.62 | 0.95 |
| Width Pre Front | 2.20 | 2.73 |
| Length Pre Front | 2.40 | 3.25 |
| Length of Scale Front | 3.72 | 4.09 |
| Width Front Of Scale | 3.49 | 3.95 |
| Length Supraocular | 1.83 | 2.33 |
| Width Supraocular | 1.31 | 1.88 |
| Length Parietal | 5.47 | 5.80 |
| Width Parietal | 3.86 | 3.60 |
| Length Pre Nasal | 0.87 | 1.09 |
| Width Pre Nasal | 1.39 | 1.42 |
| Length Post Nasal | 0.94 | 0.71 |
| Width Post Nasal | 1.30 | 1.29 |
| Length Loreal | 2.25 | 2.83 |
| Height Loreal | 1.08 | 1.16 |
| Diameter Eye | 2.10 | 2.31 |
| Height Post Ocular Upper | 0.79 | 1.20 |
| Length Post Ocular Upper | 0.80 | 0.99 |
| Height Post Ocular Lower | 1.16 | 1.33 |
| Length Post Ocular Lower | 0.67 | 0.80 |
| Length Temporal Previous | 2.29 | 2.75 |
| Width Temporal Previous | 1.52 | 2.14 |
| Posterior Superior Temporal Length | 2.57 | 4.82 |
| Posterior Superior Temporal Width | 1.80 | 2.95 |
| Length Seventh Supralabial | 2.46 | 2.25 |
| Width Seventh Supra Labial | 1.84 | 2.52 |
| Width Mental | 1.79 | 2.09 |
| Height Mental | 0.75 | 0.95 |
| Length Mentonianas | 4.47 | 4.44 |
| Width Mentonianas | 1.80 | 1.96 |
| Scales Ventrals | 158 | 169 |
| Scales Subcaudal Split | 39 | 32 |
| Dorsal Scales | 15/15/15 | 15/15/15 |

Table3. Genetic distance (%) pairwise uncorrected between species *Atractus* based on molecular marker 16s

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|----------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| <i>A. tartarus</i> -1 | | | | | | | | | | | | | |
| <i>A. albuquerquei</i> -2 | 0,04 | | | | | | | | | | | | |
| <i>A. duboisi</i> - 3 | 0,05 | 0,06 | | | | | | | | | | | |
| <i>A. dunni</i> - 4 | 0,06 | 0,05 | 0,05 | | | | | | | | | | |
| <i>A. gigas</i> - 5 | 0,05 | 0,04 | 0,05 | 0,06 | | | | | | | | | |
| <i>A. iridescens</i> - 6 | 0,06 | 0,05 | 0,06 | 0,00 | 0,07 | | | | | | | | |
| <i>A. major</i> - 7 | 0,06 | 0,06 | 0,07 | 0,06 | 0,06 | 0,06 | | | | | | | |
| <i>A. resplendens</i> - 8 | 0,04 | 0,05 | 0,02 | 0,05 | 0,04 | 0,05 | 0,06 | | | | | | |
| <i>A. schach</i> - 9 | 0,05 | 0,04 | 0,05 | 0,06 | 0,05 | 0,06 | 0,07 | 0,04 | | | | | |
| <i>Atractus</i> sp. - 10 | 0,06 | 0,04 | 0,05 | 0,03 | 0,05 | 0,03 | 0,05 | 0,05 | 0,05 | | | | |
| <i>A. trihedrurus</i> - 11 | 0,05 | 0,04 | 0,06 | 0,06 | 0,05 | 0,06 | 0,08 | 0,05 | 0,04 | 0,05 | | | |
| <i>A. typhon</i> - 12 | 0,04 | 0,04 | 0,04 | 0,06 | 0,01 | 0,06 | 0,06 | 0,04 | 0,04 | 0,05 | 0,04 | | |
| <i>A. zidocki</i> - 13 | 0,05 | 0,06 | 0,05 | 0,07 | 0,04 | 0,07 | 0,06 | 0,05 | 0,05 | 0,06 | 0,07 | 0,04 | |
| <i>T. variabilis</i> - 14 | 0,08 | 0,07 | 0,07 | 0,09 | 0,07 | 0,09 | 0,09 | 0,07 | 0,08 | 0,07 | 0,08 | 0,07 | 0,09 |

Atractus tartarus is part of the group of species *Atractus* with 15 dorsal scales rows. The species that share this condition in the dorsal scales are derived regarding those with 17 rows of dorsal scales [4]. Due to fossorial habit, becomes difficult to found with several specimens of the genus. [25] founding 16 specimens, but none had the variations recorded here. Works that link variations in morphological characters used to identify species of snakes, are important to better understand the phenotypic plasticity within a species.

[25] proposes the creation of a new group of species, group *Atractus flammigerus*, including the species: *Atractusatratus* (Passos and Lynch 2010), *A. flammigerus* (Boie, 1827), *A.fuliginosus* (Hallowell 1845), *A. major* (Boulenger 1894), *A. punctiventris* Amaral 1933, *A. schach* (Boie 1827), *A. snethlageae* Cunha and Nascimento 1983, *A. tartarus* and *A. univittatus* (Jan 1862). This group, with the exception of *A. tartarus* and *A. punctiventris*, share 17/17/17 rows of dorsal scales. Our molecular data showed that *A. tartarus* it is more related to the species *Atractus trihedrurus*, that presents 17/17/17 rows of dorsal scales, revealing a paraphyletic group.

Atractus tartarus represents a species of a mega-diverse genus with about 138 species [1], we believe that with the use of molecular markers (mtDNA), this number may increase due to the probable existence of cryptic species. Despite the recognized effectiveness of the use of genetic markers to identify cryptic species of frogs [26, 27], lizards [28, 29] and snakes [30, 31] the use of this tool is still a taboo in studies with *Atractus*, which has pronounced morphological conservatism, except in color pattern [32].

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ADDITIONAL INFORMATION (DATA OBTAINED FROM THE LITERATURE)

Atractus tartarus – Brazil: Pará: Vila Palestina. MNRJ 16511 (holotype);

Atractus albuquerquei - Brazil: Pará: Vila Nova. MPEG 12946 (holotype);

Atractus spinalis – Brazil: Minas Gerais: Morro do Pilar. MZUSP 2760 (holotype);

Atractus edioi – Brazil: Goiás: Minaçu. MZUSP 13371 (holotype);

Atractus elaps – Brazil: Rondônia: Porto Velho. MZUSP 3156;

Atractus insipidus – Rondônia: CEPB 2951

Atractus poeppigi – Brazil: Amazonas: Manaus. IMTM 1039;

Atractus potschi – Sergipe: Salgado. MZUSP 7275

Atractus reticulatus – Rio Grande do Sul: Colônia São Lorenzo. BMNH 1946.1.2.7 (holotype);

Atractus taeniatus – Rondônia. CEPB 3121;

Atractus trilineatus – Amazonas – Novo Airão. IMTM 1328;

Atractus occipitoalbus – Andes do Ecuador. ZSM 1395

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