Molecular phylogeny of *Atractus* (Serpentes, Dipsadidae), with emphasis on Ecuadorian species and the description of three new taxa

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Abstract

We present a molecular phylogeny of snake genus *Atractus*, with an improved taxon sampling that includes 30 of the 140 species currently recognized. The phylogenetic tree supports the existence of at least three new species in the Pacific lowlands and adjacent Andean slopes of the Ecuadorian Andes, which we describe here. A unique combination of molecular, meristic and color pattern characters support the validity of the new species. With the newly acquired data, we propose and define the *A. iridescentes* species.
group, as well as redefine the A. roulei species group. The species A. iridescens is reported for the first time in Ecuador, whereas A. bocourti and A. medusa are removed from the herpetofauna of this country. We provide the first photographic vouchers of live specimens for A. multicinctus, A. paucidens and A. rouzeti, along with photographs of 19 other Ecuadorian Atractus species. The current status of A. occidentalis and A. paucidens is maintained based on the discovery of new material referable to these species. With these changes, the species number reported in Ecuador increases to 27, a number that is likely to increase as material not examined in this work becomes available and included in systematic studies.

Keywords
Pacific lowlands, biodiversity, Ecuador, groundsnakes, Atractus, phylogeny, new species

Introduction

With 140 species, Atractus is the most speciose snake genus in the world, with 33 new species described only during the last ten years (Uetz et al. 2016). Most of these new species have been described using a combination of meristic and morphometric characters (Passos et al. 2009a, 2016, Passos and Lynch 2010, Schargel et al. 2013, Salazar-Valenzuela et al. 2014). However, with the exception of the preliminary phylogeny presented in De Oliveira and Hernández-Ruz (2016), no studies have involved a phylogenetic approach to test species arrangements and boundaries.

One recent work by Passos et al. (2009a) evaluated the taxonomic status of Atractus species from the Pacific lowland of Colombia and Ecuador, using a combination of meristic, morphometric, color pattern, and hemipenial characters. These authors described three new species and provided a comprehensive review of all Atractus known to occur in the region. However, when referring to this work to compare previously unexamined material from Ecuador, it became clear to us that several Ecuadorian specimens of Pacific lowland Atractus could not be assigned to any taxa currently recognized to occur in the country. Some specimens identified as A. medusa (Passos et al. 2009a) matched the coloration of the first specimen reported in Ecuador by Cisneros-Heredia and Romero (2015), but they did not match the coloration of the holotype (Passos et al. 2009a). Other specimens were closer in coloration and lepidosis to A. iridescens (Peracca, 1860) from Colombia, and others resembled both A. microrhynchus (Cope, 1868) and A. occidentalis (Savage, 1955). To further complicate matters, the taxonomic validity of A. occidentalis and A. paucidens (Despax, 1910) was not recognized in Arteaga et al. (2013), owing to their close morphological resemblance to A. dunni (Savage, 1955) and A. modestus (Boulenger, 1894), respectively.

To resolve these pending issues and to shed light on potentially unclear species boundaries, we report on new material of Atractus from Ecuador, review current knowledge on the species occurring in the Pacific lowlands and adjacent Andean slopes, present a new molecular phylogeny, including most Ecuadorian species, and describe three new species of Atractus.
Materials and methods

Ethics statement

This study was carried out in strict accordance with the guidelines for use of live amphibians and reptiles in field research compiled by the American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists’ League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR). All procedures with animals (see below) were approved by the Centro de Investigación de la Biodiversidad y Cambio Climático (BioCamb) of the Universidad Tecnológica Indoamérica. They also were reviewed by the Ministerio de Ambiente del Ecuador (MAE) and specifically approved as part of obtaining the following field permits for research and collection: MAE-DNB-CM-2015-0017, granted to Universidad Tecnológica Indoamérica; and permit N°012-IC-FAN-DPEO-MAE, granted to the Museo Ecuatoriano de Ciencias Naturales. Specimens were euthanized with 20% benzocaine, fixed in 10% formalin or 70% ethanol, and stored in 70% ethanol. Museum vouchers were deposited at the Museo de Zoología of the Universidad Tecnológica Indoamérica (MZUTI).

Sampling

Tissue samples from 39 individuals representing 22 species (including three new species described here) were obtained throughout Ecuador. The majority of individuals were located by space-constrained visual examination of ground-level substrates (Campbell and Christman 1982). The remaining individuals were detected by turning over logs, rocks and other surface objects. All specimens included in the genetic analyses were morphologically identified according to Savage (1955, 1960), Cisneros-Heredia (2005), Passos et al. (2009a), Arteaga et al. (2013), Schargel et al. (2013) and Salazar-Valenzuela et al. (2014). We generated sequence data for samples marked with an asterisk under Appendix I, which includes museum vouchers at the Museo de Zoología de la Universidad Tecnológica Indoamérica (MZUTI), the División de Herpetología del Museo Ecuatoriano de Ciencias Naturales (DHMECN) and the Fundación Herpetológica Gustavo Orcés (FHGO).

Laboratory techniques

Genomic DNA was extracted from 96% ethanol-preserved tissue samples (liver, muscle tissue or scales) using a modified salt precipitation method based on the Puregene DNA purification kit (Gentra Systems). We amplified the 16S gene using the primers 16Sar-L and 16Sbr-H-R from Palumbi et al. (1991). Additionally, the Cytb gene was obtained with the primers L14910 and H16064 developed by Burbrink et al. (2000), whereas the gene coding for the subunit 4 of the NADH dehydrogenase was ampli-
fied with the primers ND4 and Leu developed by Arévalo et al. (1994). PCR reactions contained 2 mM (Cytb and ND4) or 3 mM (16S) MgCl₂, 200 µM dNTP mix, 0.2 µM (16S and Cytb) or 0.8 µM (ND4) of each primer and 1.25 U (16S and Cytb) or 0.625 U (ND4) Taq DNA Polymerase Recombinant (Thermo Fisher Scientific) in a 25 µL total volume. The nucleotide sequences of the primers and the PCR conditions applied to each primer pair are detailed in Appendix II. PCR products were cleaned with Exonuclease I and Alkaline Phosphatase (Illustra ExoProStar by GE Healthcare) before they were sent to Macrogen Inc (Korea) for sequencing. All PCR products were sequenced in both forward and reverse directions with the same primers that were used for amplification. The edited sequences were deposited in GenBank (Appendix I).

DNA sequence analyses
A total of 126 mtDNA sequences were used to build a mitochondrial phylogenetic tree of the genus *Atractus*. 69 were generated during this work and 57 (all available sequences for the sampled gene fragments) were downloaded from GenBank. A mitochondrial marker dataset, though less powerful to study higher-level phylogenetic relationships, was chosen because it is the most effective to successfully resolve species-level phylogenies (Patwardhan 2014). Recently published works looking to resolve intrageneric relationships within Neotropical dipsadines have done so using phylogenies that are largely based on mitochondrial data (Krysko et al. 2015, Pyron et al. 2016). Specifically, we use the gene Cytochrome-b because it is reported as the most powerful in recovering phylogenetic relationships among closely related taxa (Patwardhan 2014), which is the case for the species of *Atractus* studied here. The mitochondrial genes 16S and ND4 were used to be able to compare with *Atractus* sequences available in GenBank. Novel sequences were edited and assembled using the program Geneious ProTM 5.4.7 (Drummond et al. 2010) and aligned with those downloaded from Genbank (Appendix I) using MAFFT v.7 (Katoh and Standley 2013) under the default parameters in Geneious ProTM 5.4.7. Genes were combined into a single matrix with seven partitions, one per non-coding gene and three per protein coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 1.1.1 (Lanfear et al. 2012) and jModeltest (Darriba et al. 2012) under the Bayesian information criterion. Phylogenetic relationships were assessed under a Bayesian approach in MrBayes 3.2.0 (Ronquist and Huelsenbeck 2013). Four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 6.7 million generations and four Markov chains with default heating settings. GenBank accession numbers are listed in Appendix I. Trees were sampled every 1,000 generations, resulting in 5,000 saved trees per analysis after 25% of those were arbitrarily discarded as “burn-in.” Stationarity was confirmed by plotting the−ln L per generation in the program Tracer 1.2 (Rambaut and Drummond 2003). Genetic distances between *A. esepe* and its closest morphological relatives were calculated using the uncorrected distance matrix in PAUP 4.0 (Swofford 2002).
Molecular phylogeny of Atractus (Serpentes, Dipsadidae)

Morphological data

Our terminology for Atractus cephalic shields follows Savage (1960), diagnoses and descriptions generally follow Passos et al. (2009a), and ventral and subcaudal counts follow Dowling (1951). We examined comparative alcohol-preserved specimens from the herpetology collections at the MZUTI, DHMECN, Fundación Herpetológica Gustavo Orcés (FHGO), Museum d’Histoire Naturelle de la Ville de Genève (MHNG), Museo de Historia Natural de la Escuela Politécnica Nacional (EPN), Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ), National Museum of Natural History (USNM), Muséum National d’Histoire Naturelle (MNHN) and Museo de Zoología de la Universidad San Francisco de Quito (ZSFQ). (Table 1). Morphological measurements were taken with measuring tapes to the nearest 1 mm. When providing the standard deviation, we use the ± symbol. Sex was determined by noting the presence or absence of hemipenes through a subcaudal incision at the base of the tail.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “http://zoobank.org/”. The LSID for this publication is: urn:lsid:zoobank.org:pub:7CBF7FB1-EFEA-4DC1-8F64-5BF862694AA0. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

Results

Molecular phylogeny

The overall topology and support (Fig. 1) is similar to that of Pyron et al. (2015). We consider strong support to be posterior probability values >95%, following Felsenstein (2004). Overall, there is low support for many backbone nodes. Strong support was found for the clade colored in yellow under Fig. 1.

The resulting hypotheses of species relationships for our mitochondrial phylogenetic tree supports Savage’s (1960) assumption suggesting independent evolution of the 15 dorsal scale row lineage within Atractus, since species with this number of dorsal scale rows, like A. elaps, A. roulei and A. duboisi, belong to different lineages. However, the tree does show that A. carrioni (Parker 1930), A. lehmanni (Boettger 1898),
Table 1. Locality data for specimens examined in this study. Coordinates represent georeferencing attempts from gazetteers under standard guidelines, though some variation from the exact collecting locality will be present. Similarly, elevations are taken from Google Earth, and may not exactly match the elevations as originally reported.

| Species         | Voucher | Locality                          | Latitude   | Longitude   | Elev. |
|-----------------|---------|-----------------------------------|------------|-------------|-------|
| A. carrioni     | DHMECN 4697 | Loja, Utuana                      | -4.36642   | -79.72483   | 2517  |
| A. carrioni     | DHMECN 76 | Esmeraldas, Copa Quininde (in error) | 0.06181    | -78.72641   | 1688  |
| A. carrioni     | DHMECN 7668 | Loja, Utuana                      | -4.36642   | -79.72483   | 2517  |
| A. carrioni     | MZUTI 4194 | Loja, Utuana                      | -4.36642   | -79.72483   | 2517  |
| A. carrioni     | MZUTI 4195 | Loja, Utuana                      | -4.36642   | -79.72483   | 2517  |
| A. duboisi      | MHNG 2457.093 | Napo, Chiriboga (in error)      |            |             |       |
| A. duboisi      | MNHN 0.6147 | Ecuador                           |            |             |       |
| A. duboisi      | MZUTI 3640 | Napo, Yanayacu                    | -0.60071   | -77.88927   | 1924  |
| A. duboisi      | MZUTI 62 | Napo, Yanayacu                    | -0.59939   | -77.89050   | 2064  |
| A. dunni        | DHMECN 12769 | Carchi, Gualpi                | 0.86439    | -78.22435   | 2104  |
| A. dunni        | DHMECN 2215 | Pichincha, Río Cambugán         | 0.17697    | -78.50779   | 1828  |
| A. dunni        | DHMECN 3527 | Imbabura, Junín                  | 0.27009    | -78.64975   | 1688  |
| A. dunni        | DHMECN 3900 | Pichincha, Tambo Quinde         | 0.00967    | -78.66906   | 1870  |
| A. dunni        | DHMECN 4159 | Pichincha, Pahuma               | 0.02757    | -78.67087   | 1914  |
| A. dunni        | EPN 3127 | Santo Domingo, Chiriboga        | -0.22841   | -78.67225   | 1813  |
| A. dunni        | EPN 3128 | Santo Domingo, Chiriboga        | -0.22841   | -78.67225   | 1813  |
| A. dunni        | FHZG 357 | Santo Domingo, La Favorita      | -0.22833   | -78.75603   | 1810  |
| A. dunni        | FHZG 376 | Santo Domingo, La Favorita      | -0.22833   | -78.75603   | 1810  |
| A. dunni        | FHZG 379 | Santo Domingo, La Favorita      | -0.22833   | -78.75603   | 1810  |
| A. dunni        | FHZG 9 | Santo Domingo, La Favorita      | -0.22833   | -78.75603   | 1810  |
| A. dunni        | MHNG 2441.043 | Cotopaxi, Cutzualo           | -0.54497   | -78.91891   | 1952  |
| A. dunni        | MHNG 2457.091 | Santo Domingo, La Favorita    | -0.22841   | -78.7625    | 1813  |
| A. dunni        | MHNG 2464.03 | Cotopaxi, Otonga              | -0.41549   | -79.00480   | 2095  |
| A. dunni        | MZUTI 2189 | Pichincha, Tandayapa–Bellavista | -0.00843   | -78.67169   | 1919  |
| A. dunni        | MZUTI 3031 | Pichincha, Tandayapa Lodge     | 0.00268    | -78.68131   | 1757  |
| A. dunni        | MZUTI 4097 | Imbabura, Santa Rosa de Intag   | 0.37616    | -78.46054   | 2077  |
| A. dunni        | MZUTI 4098 | Imbabura, Santa Rosa de Intag   | 0.37616    | -78.46054   | 2077  |
| A. dunni        | MZUTI 4099 | Imbabura, Santa Rosa de Intag   | 0.37616    | -78.46054   | 2077  |
| A. dunni        | MZUTI 4100 | Imbabura, Below of Siempre Verde | 0.37782   | -78.46901   | 1974  |
| A. dunni        | MZUTI 4318 | Imbabura, Toisán               | 0.53297    | -78.52924   | 2286  |
| A. dunni        | MZUTI 4319 | Imbabura, Toisán               | 0.53297    | -78.52924   | 2286  |
| A. dunni        | ZSFQ 1513 | Santo Domingo, Guajalito       | -0.22875   | -78.82248   | 1801  |
| A. ecuadorensis | DHMECN 5101 | Tungurahua, Río Verde           | -1.40344   | -78.30099   | 1507  |
| A. elaps        | DHMECN 10179 | Morona Santiago, Tundayme    | -3.57244   | -78.46982   | 790   |
| A. gaigeae      | MHNG 2397.044 | Morona Santiago, Macas      | -2.31670   | -78.11670   | 972   |
| A. gigas        | MHNG 2250.035 | Santo Domingo, Chiriboga     | -0.22841   | -78.7625    | 1813  |
| A. gigas        | MHNG 2441.02 | Cotopaxi, Otonga              | -0.41549   | -79.00480   | 2095  |
| A. gigas        | MZUTI 3286 | Pichincha, Las Gralarias      | -0.00807   | -78.73238   | 1985  |
| A. iridescens   | DHMECN 2932 | Esmeraldas, Canande          | 0.52993    | -79.03541   | 594   |
| A. iridescens   | DHMECN 5663 | Esmeraldas, Tundaloma        | 1.18236    | -78.75250   | 74    |
| A. iridescens   | DHMECN 9633 | Esmeraldas, Canande          | 0.52993    | -79.03541   | 594   |
| A. iridescens   | EPN 13920 | Carchi, Río Blanco          | 1.18993    | -78.50413   | 223   |
| Species     | Voucher      | Locality                  | Latitude | Longitude | Elev. |
|-------------|--------------|----------------------------|----------|-----------|-------|
| A. iridescens | FHGO 10443   | Esmeraldas, Tsepi         | 0.79930  | -78.84527 | 152   |
| A. iridescens | MZUTI 3548   | Esmeraldas, Tundaloma     | 1.18166  | -78.74945 | 74    |
| A. iridescens | MZUTI 3680   | Esmeraldas, Tundaloma     | 1.18166  | -78.74945 | 74    |
| A. iridescens | MZUTI 4178   | Pichincha, Puerto Quito   | 0.11667  | -79.26661 | 143   |
| A. iridescens | MZUTI 4697   | Esmeraldas, Canande       | 0.52993  | -79.03541 | 594   |
| A. iridescens | ZSFQ 191.101109 | Esmeraldas, Tundaloma | 1.18166  | -78.74945 | 74    |
| A. lehmanni   | DHMECN 7644  | Azuay, Reserva Yunguilla  | -3.22684 | -79.27520 | 1748  |
| A. lehmanni   | DHMECN 7645  | Azuay, Reserva Yunguilla  | -3.22684 | -79.27520 | 1748  |
| A. major      | ANF 1545     | Orellana, Estación Científica Yasuní | -0.67781 | -76.39819 | 246   |
| A. major      | DHMECN 8343  | Sucumbios, Bloque 27      | 0.32273  | -76.1369  | 272   |
| A. major      | MNHN 0.6149  | Ecuador                   | -       | -         | -     |
| A. major      | MZUTI 4973   | Zamora Chinchipe, Maycu    | -4.38030 | -78.74584 | 981   |
| A. microrhynchus | DHMECN 2536 | El Oro, Buenaventura      | -3.65467 | -79.76794 | 524   |
| A. microrhynchus | DHMECN 2586 | El Oro, Buenaventura      | -3.65467 | -79.76794 | 524   |
| A. microrhynchus | FHGO 897    | El Oro, Zambo Tambo       | -3.67861 | -79.68001 | 1014  |
| A. microrhynchus | MHNG 2307.017 | El Oro, El Progreso      | -3.26998 | -79.73452 | 176   |
| A. microrhynchus | MHNG 2397.019 | El Oro, El Progreso      | -3.26998 | -79.73452 | 176   |
| A. microrhynchus | MHNG 2397.02 | El Oro, El Progreso       | -3.26998 | -79.73452 | 176   |
| A. microrhynchus | MHNG 2397.021 | El Oro, El Progreso      | -3.26998 | -79.73452 | 176   |
| A. microrhynchus | MHNG 2459.052 | El Oro, El Progreso      | -3.26998 | -79.73452 | 176   |
| A. modestus   | DHMECN 3859  | El Oro, Piñas             | -3.68041 | -79.68253 | 1019  |
| A. modestus   | EPN 13916    | Carchi, Chical            | 0.90327  | -78.16201 | 1437  |
| A. modestus   | FHGO 2936    | Pichincha, Maquipucuna    | 0.11757  | -78.67446 | 1490  |
| A. modestus   | FHGO 44      | Pichincha, Maquipucuna    | 0.11757  | -78.67446 | 1490  |
| A. modestus   | MHNG 2397.041 | Cotopaxi, Las Pampas     | -0.44036 | -78.96663 | 1590  |
| A. modestus   | MZUTI 4122   | Manabí, Jama Coaque       | -0.11556 | -80.12472 | 299   |
| A. modestus   | MZUTI 5109   | Los Ríos, Río Palenque    | -0.59273 | -79.36369 | 163   |
| A. modestus   | QCAZ 1219    | Loja, Olmedo              | -3.94994 | -79.66667 | 1545  |
| A. modestus   | USNM 285473  | Los Ríos, Río Palenque    | -0.58333 | -79.36667 | 173   |
| A. modestus   | USNM 285474  | Los Ríos, Río Palenque    | -0.58333 | -79.36667 | 173   |
| A. modestus   | MHNG 2397.028 | Cotopaxi, Las Pampas     | -0.44036 | -78.96663 | 1590  |
| A. multicinctus | MZUTI 5106 | Esmeraldas, Canandé       | 0.52581  | -79.2088  | 176   |
| A. occidentalis | EPN 13077   | Pichincha, Mindo          | -0.04872 | -78.77520 | 1277  |
| A. occidentalis | FHGO 385    | Santo Domingo, La Favorita | -0.22383 | -78.76503 | 1810  |
| A. occidentalis | MHNG 2252.079 | Cotopaxi, Las Pampas     | -0.44036 | -78.96663 | 1590  |
| A. occidentalis | MHNG 2307.068 | Pichincha, Tandapi       | -0.41522 | -78.97928 | 1455  |
| A. occidentalis | MHNG 2397.028 | Cotopaxi, Las Pampas     | -0.44036 | -78.96663 | 1590  |
| A. occidentalis | MHNG 2411.085 | Pichincha, Tandapi       | -0.41522 | -78.97928 | 1455  |
| A. occidentalis | MHNG 2411.086 | Pichincha, Tandapi       | -0.41522 | -78.97928 | 1455  |
| A. occidentalis | MHNG 2441.044 | Pichincha, Nanegalito    | 0.06181  | -78.72641 | 1688  |
| A. paucidens  | MZUTI 1385   | Pichincha, Yellow House   | -0.04492 | -78.75843 | 1504  |
| A. paucidens  | MZUTI 2649   | Pichincha, Yellow House   | -0.05199 | -78.67923 | 1325  |
| A. paucidens  | MZUTI 2650   | Pichincha, Yellow House   | -0.04371 | -78.75351 | 1520  |
| A. paucidens  | MZUTI 3323   | Pichincha, Las Gralarias  | -0.00615 | -78.73381 | 1985  |
| A. paucidens  | DHMECN 11980 | Pichincha, Pedro Vicente Maldonado | 0.05361 | -78.92109 | 938   |
| A. paucidens  | DHMECN 3975  | Santa Elena, Comuna Loma Alta | -1.83442 | -80.70291 | 72    |
| Species         | Voucher    | Locality                          | Latitude  | Longitude  | Elev. |
|-----------------|------------|-----------------------------------|-----------|------------|-------|
| *A. paucidens*   | EPN 8729   | Santo Domingo, Finca La Esperanza | -0.27160  | -79.10568  | 616   |
| *A. paucidens*   | EPN 8730   | Santo Domingo, Finca La Esperanza | -0.27160  | -79.10568  | 616   |
| *A. paucidens*   | EPN 8731   | Santo Domingo, Finca La Esperanza | -0.27160  | -79.10568  | 616   |
| *A. paucidens*   | EPN 8732   | Santo Domingo, Finca La Esperanza | -0.27160  | -79.10568  | 616   |
| *A. paucidens*   | MHNG 2309.065 | Pichincha, Puerto Quito     | 0.11667   | -79.26661  | 143   |
| *A. paucidens*   | MNHN 1906.245 | Santo Domingo, Santo Domingo | -0.25351  | -79.17297  | 554   |
| *A. paucidens*   | MZUTI 5102 | Santo Domingo, Río Cinto      | -0.09070  | -79.6794  | 524   |
| *A. roulei*      | MZUTI 4503 | Chimborazo, Vicinity of Tixán   | -2.16174  | -78.81227  | 2892  |
| *A. roulei*      | MHNG 1906.244 | Morona Santiago, Gualaquiza | -3.39914  | -78.57859  | 835   |
| *A. roulei*      | MZUTI 4916 | Carchi, Río la Plata           | 0.82381   | -80.04584  | 2256  |
| *A. roulei*      | MZUTI 4544 | Chimborazo, Vicinity of Tixán   | -2.16174  | -78.81227  | 2892  |
| *A. roulei*      | QCAZ 6256  | Azuay, Hierba Mala              | -2.76439  | -79.43816  | 3029  |
| *A. roulei*      | QCAZ 7887  | El Oro, Guanazán                | -3.44139  | -79.49147  | 2596  |
| *A. roulei*      | QCAZ 7902  | El Oro, Guanazán                | -3.44139  | -79.49147  | 2596  |
| *A. roulei*      | QCAZ 9643  | El Oro, Guanazán                | -3.44139  | -79.49147  | 2596  |
| *A. roulei*      | QCAZ 9652  | El Oro, Guanazán                | -3.44139  | -79.49147  | 2596  |
| *A. savagei*     | DHMecn 3800 | Carchi, Chilma Bajo             | 0.52993   | -78.43088  | 2058  |
| *A. roulei*      | DHMecn 3800 | Carchi, Chilma Bajo             | 0.86495   | -78.04978  | 2058  |
| *A. roulei*      | MZUTI 4916 | Carchi, Chilma Bajo             | 0.86495   | -78.04978  | 2058  |
| *A. typhon*      | MNHN 1906.244 | Morona Santiago, Gualaquiza | -3.39914  | -78.57859  | 835   |
| *A. typhon*      | MNHN 1994.1171 | Morona Santiago, Gualaquiza | -3.39914  | -78.57859  | 835   |
| *A. typhon*      | ANF 2390   | Pastaza, Tzarentza              | 0.13696   | -78.05814  | 1355  |
| *A. typhon*      | MNHN 1898.313 | Imbabura, Paramba (in error) | 0.81671   | -78.35002  | 698   |
| *A. typhon*      | MNHN 1898.314 | Imbabura, Paramba (in error) | 0.81671   | -78.35002  | 698   |
| *A. typhon*      | DHMecn 9632 | Esmeraldas, Canadé              | 0.52993   | -79.03541  | 594   |
| *A. typhon*      | FHGO 10438 | Esmeraldas, Gualpi              | 0.78173   | -79.15993  | 63    |
| *A. typhon*      | FHGO 10439 | Esmeraldas, Gualpi              | 0.78173   | -79.15993  | 63    |

*A. roulei* (Despax, 1910) and *A. pyroni* sp. n., species with 15 scale rows, form a monophyletic group that includes two more species than was suggested by Passos et al. (2013) when naming the *A. roulei* species group (Fig. 1).

*Atractus gigas* (Myers and Schargel, 2006), *A. modestus*, *A. paucidens*, *A. savagei* (Sala-zar-Valenzuela et al. 2014), *A. typhon* (Passos et al., 2009a) and *A. zidoki* (Gasc and Rodrigues, 1979) form a poorly supported clade that does not include *A. microrhynchus* and *A. iridescens*, as was suggested by Passos et al. (2009a) when naming the *A. paucidens* species group (Fig. 1). Six species, *Atractus cerberus* sp. n., *A. dunni*, *A. esepe* sp. n., *A. iridescens* *A. microrhynchus*, and *A. occidentalis*, form a strongly supported clade sister to the *A. paucidens* species group. Here, we name this lineage as the *A. iridescens* species group (Fig. 1).

*Atractus occidentalis* forms a strongly supported distinct lineage, sister to *A. microrhynchus*. Together, these two species are sister to *A. dunni*. *Atractus typhon* is shown to be the strongly supported sister lineage of *A. gigas*, as is the case for a relationship between *A. roulei* and *A. pyroni* sp. n.
**Molecular phylogeny of Atractus (Serpentes, Dipsadidae)**

Figure 1. Bayesian consensus phylogeny depicting relationships within colubrid snakes of the genus *Atractus*, summarized from 5 million post-burnin generations in MrBayes 3.2.0. The topology was derived from analysis of 2,564 bp of mitochondrial DNA (gene fragments 16S, Cytb and ND4). Numbers next to branches correspond to posterior probability values. PP values on intraspecific branches are not shown for clarity. Voucher numbers for sequences are indicated for each terminal when available.

**New taxa and systematic arrangements derived from the analyses**

We seek here to only name or redelimit *Atractus* species groups that are supported in our molecular phylogeny and share features of their coloration pattern and lepidosis. The first such groups is the clade comprising *A. cerberus* sp. n., *A. dunnii*, *A. esepe* sp. n., *A. iridescens*, *A. microrhynchus* and *A. occidentalis*. The other is the one comprising *A. carrioni*, *A. lehmanni*, *A. pyroni* sp. n. and *A. roulei*. 
**Atractus iridescens species group**

**Diagnosis.** 200–360 mm SVL *Atractus* with brown dorsal ground color bearing a pattern of dots or stripes (Fig. 2), generally 17/17/17 smooth dorsals, and 125–163 ventrals (Table 2).

**Content.** *Atractus cerberus* sp. n., *A. dunni*, *A. echidna*, *A. esepe* sp. n., *A. iridescens*, *A. microrhynchus* and *A. occidentalis*.

**Distribution.** Pacific lowlands and western Andean slopes in Ecuador and Colombia (Fig. 3).

**Comment.** Passos et al. (2009a) included *Atractus echidna*, *A. iridescens* and *A. microrhynchus* in the phenetic *A. paucidens* species group. Later, Passos et al. (2012) placed *A. microrhynchus* in the *A. multicinctus* group based on hemipenial characters. Unlike *A. paucidens* or *A. multicinctus* (Jan, 1865), however, the former three species have a brownish color pattern (Fig. 2) and also a lower number of ventral scales (Appendix III). These differences, together with the phylogenetic placement of *A. iridescens* and *A. microrhynchus* support the allocation of these species in the newly formed *A. iridescens* group.

**Atractus roulei species group**

**Diagnosis.** 300–450 mm SVL *Atractus* with olive to grayish brown dorsal ground color lacking dots and stripes, 15/15/15 smooth dorsals (occasionally 17/17/17), generally 6 supralabials (sometimes 5), and 135–161 ventrals (Table 3).

**Content.** *Atractus carrioni*, *A. lehmanni*, *A. pyroni* sp. n. and *A. roulei* (Fig. 1).

**Distribution.** Western slopes of the Andes and inter-Andean valleys in central and southern Ecuador (Fig. 4).

**Comment.** Passos et al. (2013) created the *Atractus roulei* species group to accommodate *A. roulei* and its closest morphological relative *A. carrioni*, based mainly on

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**Table 2.** Morphometric data for members of the *Atractus iridescens* species group. Codes are: V=ventrals; SC=subcaudals; D=dorsal scale rows at midbody; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth. Data is derived from Appendix III and from the literature.

| Species        | V   | SC   | D   | PO | SL | IL | MT |
|----------------|-----|------|-----|----|----|----|----|
|                | Males | Females | Males | Females |     |     |    |
| *A. cerberus*  | 152–157 | – | 25–26 | – | 17 | 2 | 7 | 7 | 7 |
| *A. dunni*     | 125–136 | 138–150 | 26–39 | 19–26 | 17 | 2 | 6–7 | 6–8 | 5–7 |
| *A. echidna*   | 127 | – | 36 | – | 15 | 2 | 7 | 7 | 6 |
| *A. esepe*     | 149 | 156 | 41 | 30 | 17 | 2 | 7 | 7 | 5 |
| *A. iridescens*| 127–150 | 135–144 | 33–42 | 25–37 | 17 | 2 | 6–7 | 6–7 | 5–6 |
| *A. microrhynchus* | 133–150 | 144–163 | 32–40 | 24–29 | 17 | 1–2 | 7 | 6–7 | 5–7 |
| *A. occidentalis* | 129–141 | 128–149 | 33–39 | 20–37 | 17 | 2 | 6–7 | 6–7 | 5–7 |
Figure 2. Photographs of some Ecuadorian species of *Atractus* in life: *A. carrioni* MZUTI 4194 (a), MZUTI 4195 (b), *A. duboisi* MZUTI 3640 (c), *A. dunni* MZUTI 4318 (d), *A. dunni* MZUTI 2189 (e), *A. elaps* AMARU SN (f), *A. gigas* MZUTI 3286 (g), *A. iridescens* MZUTI 3680 (h), *A. iridescens* QCAZ 8072 (i), *A. iridescens* MZUTI 4697 (j), *A. iridescens* MZUTI 3548 (k), *A. major* MZUTI 4973 (l), *A. microrhynchus* MZUTI 5109 (m), *A. modestus* (n), *A. multicinctus* MZUTI 5106 (o), *A. occidentalis* MZUTI 1385 (p), *A. occidentalis* MZUTI 3323 (q), *A. paucidens* MZUTI 5102 (r), *A. resplendens* MZUTI 3996 (s), *A. roulei* MZUTI 4503 (t), *A. savagei* MZUTI 4916 (u), *A. snethlageae* (v), *A. touzeti* ANF 2390 (w), and *A. typhon* MZUTI 5110.
Table 3. Morphometric data for members of the *Atractus roulei* species group. Codes are: V=ventrals; SC=subcaudals; D=dorsal scale rows at midbody; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth. Data is derived from Appendix III and from the literature.

| Species     | V Males | V Females | SC Males | SC Females | D | PO | SL | IL | MT |
|-------------|---------|-----------|----------|------------|---|----|----|----|----|
| *A. carrioni* | 136–151 | 143–161   | 25–34    | 18–32      | 15 | 1  | 6  | 6  | 7–10 |
| *A. lehmanni* | 141–144 | 148–153   | 25–29    | 20–21      | 15–17 | 1  | 5  | 6  | 8–11 |
| *A. pyroni*   | –       | 143       | –        | 16         | 15 | 1  | 6  | 5  | 8   |
| *A. roulei*   | 135–146 | 143–156   | 20–27    | 14–23      | 15 | 1  | 5–6| 6–7| 9–13 |
their unusual combination of 15/15/15 dorsals and 6 supralabials. Our examination of new material belonging to these two species, and material belonging to *A. pyroni* and *A. roulei* (Appendix III), shows that although the majority of specimens have indeed 6 supralabials, some specimens may have 5, compared with most Ecuadorian *Atractus* which have 7 (Appendix III). One specimen of *A. roulei* from the type locality (MZUTI 4544; Table 1) lacks a loreal scale, which was long thought (Savage 1960;
Passos et al. 2013) to be the main feature separating this species from *A. carrioni*. The syntype of *A. lehmanni* (MC 33513) revised by Savage (1960) has 17/17/17 dorsal scale rows. Specimens assignable to *A. lehmanni* have been found only in the vicinity of the type locality (hoya de Cuenca; see Table 1).
Molecular phylogeny of *Atractus* (Serpentes, Dipsadidae)...

**Figure 3.** Distribution of Ecuadorian snakes of the *Atractus iridescens* species group. Dots represent known localities.

*Atractus cerberus* sp. n.
http://zoobank.org/B93B0063-06B6-462F-8C4B-7559D9459714

**Proposed standard English name.** Cerberus Groundsnake  
**Proposed standard Spanish name.** Tierrera cancerbera

**Holotype.** MZUTI 4330 (Fig. 5a), adult male collected by José L. Vieira-Fernandes and Alejandro Arteaga on November 06, 2015 at Pacoche, province of Manabí, Ecuador (S1.06664, W80.88123; 280 m).
Figure 4. Distribution of Ecuadorian snakes of the *Atractus roulei* species group. Dots represent known localities.

**Paratopotype.** MZUTI 5108 (Fig. 5b), adult male collected by Alejandro Arteaga on September 04, 2016.

**Diagnosis.** *Atractus cerberus* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). It is included in the *A. iridescens* group due to its brown dorsal ground color (Fig. 5) and its phylogenetic position (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) two postoculaturs; (3) loreal moderate; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven infralabials, first four contacting chinshields (7) seven maxillary teeth; (8) three gular scale rows; (9)
Molecular phylogeny of Atractus (Serpentes, Dipsadidae)...

Figure 5. Adult male holotype MZUTI 4330 (a) and adult male paratopotype (b) of Atractus cerberus MZUTI 5108.

three preventrals; (10) 152–157 ventrals; (11) 25–26 subcaudals; (12) dorsal ground color brown with faint black longitudinal bands (Fig. 5); (13) venter light yellow faintly speckled with brownish pigment; (14) 212–309 mm SVL; (15) 23–36 mm TL.

Comparisons. Atractus cerberus is included in the A. iridescens species group and compared to other Pacific lowland congeners that have a brownish ground color (Fig. 2): A. boulengerii, A. dunni, A. echidna, A. esepe sp. n., A. iridescens, A. medusa, A. microrhynchus, and A. occidentalis. From A. boulengerii and A. medusa, it differs in having a striped pattern as opposed to bold black blotches (Fig. 5). From all others, it differs in having yellow ventral surfaces (as opposed to cream or dingy white) and having more than 150 ventrals in males. Finally, the dorsal pattern of A. cerberus is less clearly marked than in the majority of the known specimens of the species included in the A. iridescens group. Instead of having conspicuous spots, blotches or lines, A. cerberus has a series of feebly visible dashes made of pigment slightly darker than the surrounding ground color.

Color pattern. The dorsal ground color is brown with five feebly visible dark-brown to black longitudinal lines that are not continuous throughout the length of the body but broken into spots along some sections (Fig. 5). Between the dark longitudinal lines on each side of the body, there are fields of lighter pigment that on some sections of the body correspond to lines. The head is darker than the rest of the dorsal surfaces and is marked by a dark, irregular postocular stripe that reaches the corner of the mouth (Fig. 5). The top of the supralabials is tinged with black. The ventral surfaces are yellowish cream with scattered brownish speckling that becomes more concentrated towards the tail, which is almost completely brown. The iris is carmine and the pupil is black.

Description of holotype. Adult male, SVL 212 mm, tail length 23 mm (10.8% SVL); body diameter 6.5 mm; head length 7.9 mm (3.7% SVL); head width 4.8 mm (2.3% SVL); interocular distance 3.1 mm; head slightly distinct from body; snout–or-
bit distance 2.8 mm; rostral 1.6 mm wide, about one time broader than high; internasals 1.0 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 1.7 mm wide; frontal 2.3 mm wide, with a curvilinear triangle shape in dorsal view; parietals 2.1 mm wide, about twice as long as wide; nasal divided; loreal 1.5 mm long, about 2 times longer than high; eye diameter 1.4 mm; pupil round; supraoculars 1.4 mm wide; two postoculares; temporals 1+2, upper posterior temporal elongate, about four times longer than high, and three times as long as first temporal; seven supralabials, 3rd–4th contacting orbit; symphisial 1.0 mm wide, about twice as broad as long, separated from chin shields by first pair of infralabials; seven infralabials, 1st–4th contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 17/17/17 rows, smooth without apical pits; preventrals 3; ventrals 157; anal plate single; paired subcaudals 26.

**Natural history.** The two known specimens of *Atractus cerberus* were found in an isolated patch of deciduous lowland forest surrounded by dry lowland shrubland. MZUTI 4330 was found active on leaf litter at 19h29, in 80% closed canopy secondary forest far from streams. The night was warm and there was drizzle the night before. MZUTI 5108 was found crossing a forest trail close to an open area at 10h00 during a sunny morning after a rainy night.

**Distribution.** Known only from the type locality, Pacoche, in the Ecuadorian province of Manabí at 280–324 m (Fig. 3). This locality is 3 km airline distance from the shoreline.

**Etymology.** The specific epithet “cerberus” is derived from the name of the Greek monster Kérberos. In Greek mythology, Kérberos is a monstrous multi-headed dog that guards the gates of the underworld, preventing the dead from leaving. Here, we use this word in allusion to the type locality, at the gates of the newly formed “Refinería del Pacífico”, a massive industrial oil-processing plant that can easily be likened to the underworld.

**Conservation status.** Although *Atractus cerberus* belongs to a poorly studied genus of snakes and is known only from two specimens collected recently in a single locality, we consider this species to be Critically Endagered following B1a,b(iii) IUCN criteria because: i) its extent of occurrence is estimated to be less than 50 km² (i.e. total area of continuous semideciduous forest in the Refugio de Vida Silvestre Pacoche); ii) it has not been detected in any other locality in the province of Manabí despite numerous surveys (Almendáriz and Carr 2007, Cisneros-Heredia 2004, MECN et al. 2013); and iii) and its habitat is severely fragmented, isolated from other such habitats and declining in extent and quality due to deforestation.

*Atractus esepe* sp. n.
http://zoobank.org/F58E89A5-D398-4703-8098-7474CD6B3E6D

**Proposed standard English name.** Indistinct Groundsnake

**Proposed standard Spanish name.** Tierrera indistinta
Molecular phylogeny of Atractus (Serpentes, Dipsadidae)...

Holotype. MZUTI 3758 (Fig. 6), adult male collected by Alejandro Arteaga on September 12, 2014 at Caimito, Esmeraldas Province, Ecuador (N0.69620, W80.090472; 102 m).

Paratopotype. MZUTI 3759, adult female collected by Jaime Culebras.

Diagnosis. Atractus esepe is placed in the genus Atractus as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). It is included in the A. iridescens group due to its brown dorsal ground color and its phylogenetic position (Figs 1, 6). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) two postoculars; (3) loreal long; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven infralabials, first four contacting chinshields (7) seven maxillary teeth; (8) 2–3 gular scale rows; (9) 2–3 preventrals; (10) 149 ventrals in the male holotype, 156 in the female paratype; (11) 41 subcaudals in the male holotype, 30 in the female paratype; (12) dorsal ground color brown with a pattern of complete (MZUTI 3759) or broken (MZUTI 3758) (Fig. 6a) dark lines running parallel along each side of the body and separated from each other by a cream line, but rendering the appearance of a row of dorso-lateral blotches in the broken pattern (MZUTI 3758); (13) venter cream faintly speckled with brownish pigment (Fig. 6b); (14) 232–241 mm SVL; (15) 34–53 mm TL.

Comparisons. Atractus esepe is included in the A. iridescens species group and compared to other Pacific lowland congeners who have a brownish ground color (Figs 2, 5): A. boulengerii, A. cerberus, A. durni, A. echidna, A. iridescens, A. medusa, A. microrhynchus, and A. occidentalis. From these, A. microrhynchus and A. occidentalis have striped pattern and cream ventral surfaces similar to that of A. esepe, but they occur parapatrically (Fig. 3) and can be distinguished from A. esepe by a genetic divergence of 5.3–5.7% in a 506 bp

Figure 6. Adult male holotype of Atractus esepe MZUTI 3758 in dorsal (a) and ventral (b) view. Scale = 1 cm.
long fragment of the mitochondrial Cytb gene and by having a greater number of subcaudal scales in males (Table 2). Furthermore, adult specimens of *A. microrhynchus* have light brown dorsal surfaces instead of dark brown, and their pattern can be better described as a series of blotches rather than broken longitudinal lines. Specimens of both *A. esepe* and *A. occidentalis* have a pattern of longitudinal lines, but *A. esepe* has a greater number of ventral plus caudal scales than *A. occidentalis* (more than 180 in *A. esepe*) (Table 2).

**Color pattern.** The dorsal ground color is dark brown with either six longitudinal black lines separated by lighter areas or a pattern of dark brown longitudinally arranged spots that correspond to the longitudinal lines. On each side, the line or series of dark spots along the 2nd and 3rd dorsal scale row is feebly visible, but the other lines or spots are conspicuous. The dorsal surface of the head is dark brown and there is a clearly marked dark postocular stripe running from behind the eye to the edge of the mouth (Fig. 6). The ventral surfaces are dingy white, finely speckled with brown pigment that becomes more concentrated towards the tail. The iris is carmine and the pupil is black.

**Description of holotype.** Adult male, SVL 232 mm, tail length 53 mm (22.8% SVL); body diameter 7.0 mm; head length 7.9 mm (3.4% SVL); head width 4.8 mm (2.2% SVL); interocular distance 3.4 mm; head slightly distinct from body; snout–orbit distance 3.3 mm; rostral 1.8 mm wide, about one time broader than high; internasals 0.9 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 1.9 mm wide; frontal 2.2 mm wide, with a curvilinear triangle shape in dorsal view; parietals 2.1 mm wide, about twice as long as wide; nasal divided; loreal 2.5 mm long, about 3 times longer than high; eye diameter 1.5 mm; pupil round; supraoculars 1.2 mm wide; two postoculars; temporals 1+2, upper posterior temporal elongate, about four times longer than high, and three times as long as first temporal; seven supralabials, 3rd–4th contacting orbit; symphysial 0.8 mm wide, separated from chin shields by first pair of infralabials; seven infralabials, 1st–4th contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 17/17/17 rows, smooth without apical pits; preventrals 3; ventrals 149; anal plate single; paired subcaudals 41.

**Natural history.** The two known specimens of *Atractus esepe* were found actively foraging among soil and roots in secondary evergreen lowland forest at least 400 m from the nearest natural body of water. They were found by night at 20h00 after a warm, sunny day.

**Distribution.** Known only from the type locality, Caimito, in the Ecuadorian province of Esmeraldas at 102 m (Fig. 3). This locality is 1.3 km airline distance from the shoreline.

**Etymology.** The specific epithet *esepe* is derived from the Spanish pronunciation of “sp.”, which is the abbreviation for the Latin word *species*. Here, we use this word in allusion to how the majority of Ecuadorian researchers refer to *Atractus* specimens found in the field.

**Conservation status.** We consider *Atractus esepe* to be Data Deficient following IUCN criteria because it is known only from its type locality but its occurrence in the biogeographic Choco suggests that it might as well be present in other localities. The
Molecular phylogeny of Atractus (Serpentes, Dipsadidae)...

Chocoan forests of Caimito do not appear to be isolated from other similar habitat by geographical or ecological barriers. Therefore, we consider there is inadequate information to make a direct, or indirect, assessment of its extinction risk based on its scarce distribution data.

*Atractus pyroni* sp. n.
http://zoobank.org/36145E29-02B6-4C66-A097-44EFC1BC3A92

**Proposed standard English name.** Pyron’s Groundsnake

**Proposed standard Spanish name.** Tierrera de Pyron

**Holotype.** MZUTI 5107 (Fig. 7), adult male collected by José L. Vieira-Fernandes and Carlos Durán on May 23, 2016 between Balzapamba and Bilován, province of Bolívar, Ecuador (S1.83601, W79.13322; 2026 m).

**Diagnosis.** *Atractus pyroni* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic (Fig. 1) and morphological (Table 3) evidence. It is included in the *A. roulei* group due to its 15/15/15 dorsal scale rows and its phylogenetic position (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 15/15/15 smooth dorsals; (2) one postocular; (3) loreal long; (4) temporals 1+2; (5) six supralabials, third and fourth contacting orbit; (6) five infralabials, first four contacting chinshields (7) eight maxillary teeth; (8) 3 gular scale rows; (9) 2 preventrals; (10) 143 ventrals; (11) 16 subcaudals; (12) dorsal ground color dark brown with a series of light golden brown paravertebral scales running along the entire dorsum (Fig. 7); (13) venter dark brown with scattered scales of a lighter color; (14) 443 mm SVL; (15) 34 mm TL.

**Comparisons.** *Atractus pyroni* is compared to members of the *A. roulei* species group: *A. carrioni*, *A. lehmanni*, and *A. roulei* (Fig. 2). From *A. carrioni*, it differs by having a loreal. From *A. lehmanni* and *A. roulei*, it differs in size and color pattern. *Atractus pyroni* is 443 mm in SVL; whereas *A. lehmanni* is 262–321 in SVL, and *A. roulei* is 230–396. Both *A. lehmanni* and *A. roulei* have uniform dorsal ground color, whereas *A. pyroni* has a distinct dorsal bicolored pattern (Fig. 7). Finally, in life, *A. pyroni* is darker than the remaining members of the *A. roulei* species group and has a ventral pattern that, instead of having fine speckles, has conspicuous scattered blotches of a contrasting color.

**Color pattern.** The dorsal ground color is blackish with a dark vertebral (mid-dorsal) scale row flanked by a dark yellow scale row on either side (the 7th dorsal scale row), irregularly adjoined by one to few additional yellow scales on the 6th dorsal scale row, rendering an appearance of an irregularly edged mid-dorsal striped pattern (Fig. 7). The dorsal and lateral surfaces of the head are dark grayish brown and the labials are dark mustard yellow. All ventral surfaces are glossy grayish black except for the throat and some scattered blotches, which are dark mustard yellow.

**Description of holotype.** Adult female, SVL 443 mm, tail length 34 mm (7.7% SVL); body diameter 11.6 mm; head length 14.4 mm (3.3% SVL); head width 9.8 mm
Figure 7. Adult female holotype of *Atractus pyroni*. MZUTI 5107. Scale = 1 cm.

(2.2% SVL); interocular distance 5.1 mm; head slightly distinct from body; snout–orbit distance 5.7 mm; rostral 2.8 mm wide, about two times broader than high; internasals 1.5 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 2.8 mm wide; frontal 3.5 mm wide, with a curvilinear triangle shape in dorsal view; parietals 4.0 mm wide, about twice as long as wide; nasal divided; loreal 3.7 mm long, about 3 times longer than high; eye diameter 1.8 mm; pupil round; supraoculars 2.1 mm wide; one postocular; temporals 1+2, upper posterior temporal elongate, about five times longer than high, and twice as long as first temporal; six supralabials, 3rd–4th contacting orbit; symphisial 2.4 mm wide, separated from chin shields by first pair of infralabials; five infralabials, 1st–4th contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 15/15/15 rows, smooth without apical pits; preventrals 2; ventrals 143; anal plate single; paired subcaudals 16.

**Natural history.** The only known specimen of *Atractus pyroni* was found dead on a dirt road surrounded by silvopastures and remnants of native montane cloudforest.

**Distribution.** Known only from the type locality, between Balzapamba and Bilován, in the Ecuadorian province of Bolívar at 2026 m (Fig. 7).

**Etymology.** Named after R. Alexander Pyron, one of the most prolific contemporary herpetologists, in recognition of his invaluable contribution to systematics and evolution of the world’s reptiles.

**Conservation status.** We consider *Atractus pyroni* to be Data Deficient following IUCN because there is inadequate information to make a direct, or indirect, assessment of its extinction risk based on its scarce distribution data.
Discussion

Species relationships and taxonomy in the colubrid snake genus *Atractus* are still far from being resolved, and many infrageneric groups are either non-monophyletic, or poorly supported and weakly placed, which may reflect inadequate sampling of taxa (only 30 out of 140 species are included) or characters (only 1 locus is used). No monophyly was found for the groups defined by Savage (1960), which, until further phylogenetic evidence is accumulated or unambiguous diagnostic characters are defined, should not be used.

From the five members of the *A. paucidens* species groups of Passos et al. (2009a) that were sampled in our phylogeny, only *A. paucidens*, *A. savagei*, and *A. typhon* cluster together. *Atractus microrhynchus* and *A. iridescens* belong to another lineage, which is here named the *A. iridescens* species group. This group includes the aforementioned two species plus *A. cerberus*, *A. dunni*, *A. echidna*, *A. esepe*, and *A. occidentalis*. From the species included in this group, we expand the known distribution of all their members (Fig. 3). However, we do not include the specimens ANSP 18114 nor ANSP 26316, from the vicinity of Huigra and identified as *A. occidentalis* by Savage (1960), because their description disagrees with the observed morphological variation reported for *A. occidentalis* in this work. Upon a visit to Huigra, a dry valley dominated by xeric vegetation and rocky outcrops, it became clear to us that it is unlikely for a species like *A. occidentalis*, which is found in evergreen lower-montane forests (Arteaga et al. 2013), to occur in an isolated dry habitat type ca. 250 km airline distance south of the type locality.

We also re-delimit the *A. roulei* species group of Passos et al. (2013) to include *A. carrioni*, *A. lehmanni*, *A. roulei* and *A. pyroni*. We expand the known distribution of *A. roulei* (Fig. 4), but do not include specimen AMNH 17492 from San José de Chimbo (Savage 1960) in the map because this specimen might actually be *A. pyroni* given the morphological similarities between the two species and the geographical proximity to the type locality of *A. pyroni*. Reports of *A. lehmanni* from Colombia (Passos et al. 2009b) are likely misidentifications since *A. lehmanni* has not been registered in Ecuador outside the type locality.

To further clarify the landscape of *Atractus* taxonomy in Ecuador, we analyze the presence of *A. medusa*, *A. melas*, *A. typhon*, *A. badius*, and *A. bocourti* in the country. Cisneros-Heredia and Romero (2015) presented the first country record of *A. medusa* in Ecuador (specimen DFCH-USFQ 191.101109 at Universidad San Francisco de Quito), based on similarities in scalation and coloration between that specimen and the holotype of *A. medusa*, form Gorgona island, Colombia. Certainly, the characters of scalation of the Ecuadorian specimen fit the diagnosis of *A. medusa*. However, they fit just as well the diagnosis of *A. iridescens* provided by Passos et al. (2009a), with the difference that the dorsal pattern of the Ecuadorian specimen resembles more the *A. iridescens* specimen, ICN 10902, pictured in Passos et al. (2009a). The dark brown ground color (as opposed to light cream), the light bordered brown blotches (as opposed to
solid black blotches), and the absence of a black nape band are all characteristics shared by DFCH-USFQ 191.101109 and the other nine specimens of *A. iridescens* presented in Appendix III, with ICN 10902 of Passos et al. (2009a). Therefore, we consider that DFCH-USFQ 191.101109 actually represents the first country record of *A. iridescens* for Ecuador. Based on this new information and re-examination of museum material, we report on 9 additional specimens (Table 1) that expand the current known distribution of this species. Cisneros-Heredia and Romero (2015) suggest that a photographic record of *Atractus cf. melas* from the Bilsa Biological Station, province of Esmeraldas, northwestern Ecuador (Ortega-Andrade et al. 2010) corresponds to *A. multicinctus*. The specimen differs from other material assigned to *A. multicinctus* in having whitish rings as opposed to red rings throughout the body (Fig. 2). Although photographic vouchers of *A. typhon* have been presented in MECN et al. (2013), we report on the first museum vouchers of the species in Ecuador (Table 1).

Finally, although Hoogmoed (1980) restricted the type locality of *A. badius* and pointed out that the upper Amazon basin specimens were misidentifications, the species has remained in Ecuadorian faunal lists (Torres-Carvajal et al. 2016), even after Schargel et al. (2013) made compelling cases to exclude this species from the upper Amazon Basin. Other snake, *A. bocourti* was included in the herpetofauna of Ecuador by Pérez-Santos and Moreno (1991) without pointing out to any museum voucher. These authors stated that although they have no information about the distribution of the species in Ecuador, its distribution in Colombia would suggest that it also occurs in Ecuador. Since there is no evidence that neither *A. badius* nor *A. bocourti* occur in Ecuador, we remove them from this country’s herpetofauna.

Our analysis of new *Atractus* material supports the evolutionary phylogenetic distinctiveness of at least 22 of the total taxa currently recognized to occur in Ecuador. To include the remaining taxa in future phylogenetic analyses will certainly help resolve species relationships and taxonomic arrangements of cis-Andean Ecuadorian *Atractus*, since the five species that were not included in the phylogeny occur in the Amazonian slopes of the Andes. However, besides including more taxa in future phylogenetic analyses, we feel that a more adequate sampling of molecular markers is needed to overcome the difficulties that mitochondrial-based phylogenies have to capture higher-lever evolutionary relationships. Certainly, future studies can benefit from a phylogeny based on both a nuclear and a mitochondrial dataset.

With these changes, the species number reported in Ecuador increases to 27: *A. carrioni* (Parker, 1930), *A. cerberus*, *A. collaris* (Peracca, 1897), *A. duboisii* (Boulenger, 1880), *A. dunni* (Savage, 1955), *A. ecuadorensis* (Savage, 1955), *A. elaps* (Günther, 1858), *A. essepe*, *A. gaigeae* (Savage, 1955), *A. gigas* (Myers and Schargel, 2006), *A. iridescens* (Peracca, 1860), *A. lehmanni* (Boettger, 1898), *A. major* (Boulenger, 1894), *A. microrhynchus* (Cope, 1868), *A. modestus* (Boulenger, 1894), *A. multicinctus* (Jan, 1865), *A. occidentalis* (Savage, 1955), *A. occipitoalbus* (Jan, 1862), *A. orcesi* (Savage, 1955), *A. paucidens* (Despax, 1910), *A. pyronii*, *A. resplendens* (Werner, 1901), *A. roulei* (Despax, 1910), *A. savage* (Salazar-Valenzuela et al., 2014), *A. snethlageae* (da Cunha & do Nascimento, 1983), *A. touzeti* (Schargel et al., 2013) and *A. typhon* (Passos et al., 2009).
We hope that the novel genetic and morphological data provided herein will promote future researchers to examine species boundaries in *Atractus*, as additional work clearly is waiting.

**Author contributions**

Conceived and designed the work: AA. Performed the analyses: AA NP. Gathered morphological data: KB JHV DFCH CRP JLVF AA. Analyzed the data: AA KM DFCH JMG. Contributed reagents/materials/analysis tools: JMG NP. Wrote the paper: AA KM JHV DFCH NP CRP JLVF JMG.

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**References**

Almendáriz A, Carr JL (2007) Lista actualizada de los anfibios y reptiles registrados en los remanentes de bosque de la Cordillera de la Costa y áreas adyacentes del suroeste de Ecuador. Escuela Politécnica Nacional, Quito, 11 pp.

Arévalo E, Davis SK, Sites JW (1994) Mitochondrial DNA-sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology 43: 387–418. https://doi.org/10.1093/sysbio/43.3.387
Arteaga A, Bustamante L, Guayasamin JM (2013) The amphibians and reptiles of Mindo. Universidad Tecnológica Indoamérica, Quito, 257 pp.

Boettger O (1898) Katalog der Reptilien-Sammlung im Museum der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt. II Teil (Schlangen). Gebrüder Knauer, Frankfurt, 160 pp.

Boulenger GA (1880) Reptiles et Batraciens recueillis par M. Emile de Ville dans les Andes de l’équateur. Bulletin de la Société Zoologique de France 5: 41–48.

Boulenger GA (1894) Catalogue of the snakes in the British Museum (Natural History), Volume II. Trustees of the British Museum, London, 382 pp.

Burbink FT, Lawson R, Slowinski JB (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (Elaphe obsoleta): A critique of the subspecies concept. Evolution 54: 2107–2188. https://doi.org/10.1111/j.0014-3820.2000.tb01253.x

Campbell HW, Christman SP (1982) Field techniques for herpetofaunal community analysis. In: Scott NJ (Ed.) Herpetological communities: a symposium of the Society for the Study of Amphibians and Reptiles and the Herpetologists League. United States Fish and Wildlife Service, Washington D.C., 193–200.

Cisneros-Heredia DF (2004) Amphibians, Machalilla National Park, province of Manabí, western Ecuador. Check List 2: 45–54. https://doi.org/10.15560/2.1.45

Cisneros-Heredia DF (2005) Rediscovery of the Ecuadorian snake Atractus dunni Savage, 1955 (Serpentes: Colubridae). Journal of the National Museum (Prague), Natural History Series 174: 87–94.

Cisneros-Heredia DF, Romero A (2015) First country record of Atractus medusa (Serpentes, Dipsadidae) in Ecuador. Herpetology Notes 8: 417–420.

Cope ED (1868) An examination of the Reptilia and Batrachia obtained by the Orton Expedition to Equador and the Upper Amazon, with notes on other species. Proceedings of the Academy of Natural Sciences of Philadelphia 20: 96–140.

da Cunha OR, do Nascimento FP (1983) Ofidios da Amazonia. As especies de Atractus Wagler, 1828, na Amazonia oriental & Maranhao (Ophidia, Colubridae). Boletim do Museu Paraense Emilio Goeldi 123: 1–38.

Darriba D, Taboada GL, Doallo R, Posada D (2012) More models, new heuristics and parallel computing. Nature Methods 9: 772–772. https://doi.org/10.1038/nmeth.2109

De Oliveira EA, Hernández-Ruz EJ (2016) Morphological variation in Atractus tartarus (Snake: Dipsadidae) from the Xingu River, east Amazon, Brazil and preliminary phylogenetic relationship in Atractus. International Journal of Research Studies in Biosciences 4: 1–7.

Despax R (1910) Mission géodésique de l’Équateur. Collections recueillies par M. le Dr Rivet. Liste des ophidiens et descriptions des espèces nouvelles. Bulletin du Muséum National d’Histoire Naturelle 16: 368–376.

Dowling HG (1951) A proposed standard system of counting ventrals in snakes. British Journal of Herpetology 1: 97–99.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thirer T, Wilson A (2010) Geneious v5.5. Biomatters. http://www.geneious.com
Dunn ER, Bailey JR (1939) Snakes from the uplands of the Canal Zone and of Darien. Bulletin of the Museum of Comparative Zoology at Harvard College 86: 1–22.

Felsenstein J (2004) Inferring Phylogenies. Sinauer Associates, Sunderland, 664 pp.

Gasc JP, Rodrigues MT (1979) Une nouvelle espèce du genre *Atractus* (Colubridae, Serpentes) de la Guyane française. Bulletin du Muséum national d’Histoire naturelle 2: 547–557.

Günther A (1858) Catalogue of Colubrine snakes of the British Museum. Taylor and Francis, London, 281 pp.

Hoogmoed MS (1980) Revision of the genus *Atractus* in Surinam, with the ressurection of two species (Colubridae, Reptilia). Notes on the Herpetofauna of Surinam VII. Zoologische Verhandelingen 175: 1–47.

Jan G (1862) Enumerazione sistematico delle specie d’ofidi del gruppo Calamaridae. Archivio per la zoologia, l’anatomia e la fisiologia 2: 1–76.

Jan G (1865) Iconographie générale des ophidiens. 10. JB Baillière et fils, Paris, 42 pp.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Krysko KL, Steadman DW, Nuñez LP, Lee DS (2015) Molecular phylogeny of Caribbean dipsadid (Xenodontinae: Alosphiiini) snakes, including identification of the first record from the Cay Sal Bank, The Bahamas. Zootaxa 4028: 441–450. https://doi.org/10.11646/zootaxa.4028.3.9

Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29(6): 1695–1701. https://doi.org/10.1093/molbev/mss020

MECN, JOCOTOCO, ECOMINGA (2013) Herpetofauna en áreas prioritarias para la conservación: El sistema de reservas Jocotoco y Ecominga. Museo Ecuatoriano de Ciencias Naturales, Quito, 408 pp.

Myers CW, Schargel WE (2006) Morphological extremes - two new snakes of the genus *Atractus* from northwestern South America (Colubridae: Dipsadinae). American Museum Novitates 3532: 1–13. https://doi.org/10.1206/0003-0082(2006)3532[1:MENSOT]2.0.CO;2

Ortega-Andrade HM, Bermingham J, Aulestia C, Paucar C (2010) Herpetofauna of the Bilsa Biological Station, province of Esmeraldas, Ecuador. Check List 6: 119–154. https://doi.org/10.15560/6.1.119

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool’s guide to PCR, version 2.0. University of Hawaii, Honolulu, 94 pp.

Parker HW (1930) A new colubrine snake from Ecuador. The Annals and Magazine of Natural History 10: 207–209. https://doi.org/10.1080/00222933008673120

Passos P, Arredondo JC, Fernandes R, Lynch JD (2009b) Three new *Atractus* (Serpentes: Dipsadidae) from the Andes of Colombia. Copeia 2009: 425–436. https://doi.org/10.1643/CH-08-063

Passos P, Cisneros-Heredia D, Rivera DE, Aguilar C, Schargel WE (2012) Rediscovery of *Atractus microrhyynchus* and reappraisal of the taxonomic status of *A. emersoni* and *A. natans* (Serpentes: Dipsadidae). Herpetologica 68: 375–392. https://doi.org/10.1655/HERPETOLOGICA-D-11-00078.1
Passos P, Echevarría LY, Venegas PJ (2013) Morphological Variation of *Atractus carrioni* (Serpentes: Dipsadidae). South American Journal of Herpetology 2: 109–120. https://doi.org/10.2994/SAJH-D-12-00025.1

Passos P, Lynch JD (2010) Revision of *Atractus* (Serpentes: Dipsadidae) from Middle and Upper Magdalena Drainage of Colombia. Herpetological Monographs 24: 149–173. https://doi.org/10.1655/09-041.1

Passos P, Mueses-Cisneros JJ, Lynch JD, Fernandes R (2009a) Pacific lowland snakes of the genus *Atractus* (Serpentes: Dipsadidae), with description of three new species. Zootaxa 2293: 1–34.

Passos P, Prudente ALC, Lynch JD (2016) Redescription of *Atractus punctiventris* and description of two new *Atractus* (Serpentes: Dipsadidae) from Brazilian Amazonia. Herpetological Monographs 30: 1–20. https://doi.org/10.1655/HERPMONOGRAPH-D-14-00009

Patwardhan A, Ray S, Roy A (2014) Molecular markers in phylogenetic studies-A review. J Phylogen Evolution Biol 2: 1–9.

Peracca MG (1896) Sopra alcuni Ofidii nuovi o poco noti dell’America meridionale. Bollettino dei Musei di Zoologia e Anatomia Comparata della R. Università di Torino 11: 1–4.

Peracca MG (1897) Intorno ad una piccola raccolta di Rettili di Cononacco (Perù orientale). Bollettino dei Musei di Zoologia e Anatomia Comparata della R. Università di Torino 12: 1–7.

Pérez-Santos C and Moreno AG (1991) Serpientes de Ecuador. Museo Regionale di Scienze Naturali, Torino, 538 pp.

Pyron RA, Guayasamin JM, Peñafiel N, Bustamante L, Arteaga A (2015) Systematics of Nothopsini (Serpentes, Dipsadidae), with a new species of *Synophis* from the Pacific Andean slopes of southwestern Ecuador. ZooKeys 541: 109–147. https://doi.org/10.3897/zookaes.541.6058

Pyron RA, Arteaga A, Echevarría LY, Torres-Carvala O (2016) A revision and key for the tribe Diaphorolepidini (Serpentes: Dipsadidae) and checklist for the genus *Synophis*. Zootaxa 4171: 293–320. https://doi.org/10.11646/zootaxa.4171.2.4

Rambaut A, Drummond AJ (2003) Tracer version 1.4.1. http://beast.bio.ed.ac.uk

Ronquist F, Huelsenbeck JP (2013) MrBayes 3: Bayesian phylogenetic inference under mixed-models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btq180

Salazar-Valenzuela D, Torres-Carvajal O, Passos P (2014) A New Species of *Atractus* (Serpentes: Dipsadidae) from the Andes of Ecuador. Herpetologica 70: 350–363. https://doi.org/10.1655/HERPETOLOGICA-D-13-00045

Savage JM (1955) Descriptions of new colubrid snakes, genus *Atractus*, from Ecuador. Proceedings of the Biological Society of Washington 68: 11–20.

Savage JM (1960) A revision of the Ecuadorian snakes of the colubrid genus *Atractus*. Miscellaneous Publications, Museum of Zoology, University of Michigan 112: 1–86.

Schargel WE, Lamar WW, Passos P, Valencia JH, Cisneros-Heredia DF, Campbell JA (2013) A new giant *Atractus* (Serpentes: Dipsadidae) from Ecuador, with notes on some other large Amazonian congeners. Zootaxa 3721: 455–474. https://doi.org/10.11646/zootaxa.3721.5.2

Swofford DL (2002) PAUP*: Phylogenetic analysis using parsimony (*and other methods). v.4.0b10. Sinauer & Associates, Sunderland, Massachusetts. https://doi.org/10.1111/j.0014-3820.2002.tb00191.x
Appendix I

GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Novel sequence data produced in this study are marked with an asterisk (*).

| Species            | Voucher | 16S  | CYTB       | ND4       |
|--------------------|---------|------|------------|-----------|
| A. albuquerquei     | –       | GQ457726 | JQ598918 | –         |
| A. badius          | –       | AF158485 | –         | –         |
| A. carrioni        | MZUTI 4195 | KY610046* | –         | KY610094* |
| A. cerberus        | MZUTI 4330 | KY610047* | KY610073* | KY610095* |
| A. daboisi         | MZUTI 62  | KT944041 | –         | KT944059  |
| A. danna           | MZUTI 2189 | KY610048* | –         | KY610096* |
| A. dana            | MZUTI 3031 | KY610049* | –         | KY610097* |
| A. danna           | MZUTI 4318 | KY610050* | KY610074* | KY610098* |
| A. danna           | MZUTI 4319 | KY610051* | KY610075* | KY610099* |
| A. ecaudorensis    | DHMECN 5105 | –         | –         | KY610100* |
| A. elaps           | DHMECN 10179 | KY610052* | KY610076* | KY610101* |
| A. elaps           | KU 214837 | –       | EF078536  | EF078584  |
| A. esepe           | MZUTI 3758 | KY610053* | KT944052 | KY610102* |
| A. esepe           | MZUTI 3759 | KT944039 | KT944051  | KT944058  |
| A. flammigerus     | MNHN 1997.2145 | AF158471 | –         | –         |
| A. gigas           | DHMECN 3286 | KT944043 | KT944053  | KT944061  |
| A. iridescens     | DHMECN 9633 | KY610054* | KY610077* | –         |
| A. iridescens     | MZUTI 3548 | KY610055* | KY610078* | –         |
| A. iridescens     | MZUTI 3680 | KY610056* | KY610079* | –         |
| A. iridescens     | MZUTI 4178 | KT944040 | KY610080* | –         |
| A. iridescens     | MZUTI 4697 | KY610057* | KY610081* | –         |
| A. lehmanni       | DHMECN 7644 | KY610058* | KY610082* | KY610103* |
| A. major          | ANF 1545  | KT944045 | –         | KY610104* |
| A. major          | DHMECN 8343 | KY610059* | –         | KY610105* |
| A. microrhynchus   | MZUTI 5109 | KY610060* | KY610083* | KY610106* |
| A. microrhynchus   | MZUTI 4122 | KT944037 | KT944049  | KT944056  |
| A. modestus        | MZUTI 4760 | KY610061* | KY610084* | KY610107* |
| A. multicinctus    | MZUTI 5106 | KY610062* | KY610085* | KY610108* |
Appendix II

List of PCR and sequencing primers and their respective PCR conditions (denaturation, annealing, extension and number of corresponding cycles) used in this study. All PCR protocols included an initial 3-min step at 94 °C and a final extension of 10 min at 72 °C.

| Locus | Primer name | Sequence (5’-3’) | Reference | PCR profile: |
|-------|-------------|------------------|-----------|--------------|
| 16S   | 16Sar-L     | CGCCTGTTTATCAAAACAT | Palumbi et al. (1991) | 94 °C (45 sec), 53 or 56 °C (45 sec), 72 °C (1 min) [x25-30] |
|       | 16Sbr-H-R   | CCGGTCTGAACATACGCT |           |              |
| Cytb  | L14910      | GACCTGTGATMTGAAAAACCTGTTG | Burbrink et al. (2000) | 94 °C (1 min), 58 °C (1 min), 72 °C (2 min) [x30-36] |
|       | H16064      | CTTTGGTTTACACAGACATGCTTTA |           |              |
| ND4   | ND4         | CACCTATGACTACAAAAAGCTCATGTAAGAGC | Arévalo et al. (1994) | 94 °C (25 sec), 58 or 60 °C (1 min), 72 °C (2 min) [x25-30] |
|       | Leu         | CATTACCTTTACTTGGATTTCACCA |           |              |
### Appendix III

Morphometric data and sex for specimens of *Atractus* species examined. Codes are: V=ventrals; SC=subcaudals; D1–3=dorsal scale rows at neck, midbody, and vent; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth; SVL=snout-vent length (mm); TL=tail length (mm); M=Male, F=Female.

| Species     | Voucher     | V | SC  | D1 | D2 | D3 | PO | SL | IL | MT | SVL | TL | Sex |
|-------------|-------------|---|-----|----|----|----|----|----|----|----|-----|----|-----|
| *A. carrioni* | DHMECN 4697 | 144 | 32 | 15 | 15 | 15 | 1 | 6 | 6 | 7 | 361 | 59 | F   |
| *A. carrioni* | DHMECN 76 | 157 | 23 | 15 | 15 | 15 | 1 | 6 | 6 | 8 | 333 | 39 | F   |
| *A. carrioni* | DHMECN 7668 | 149 | 28 | 15 | 15 | 15 | 1 | 6 | 6 | 7 | 354 | 58 | M   |
| *A. carrioni* | MZUTI 4195 | 144 | 31 | 15 | 15 | 15 | 1 | 6 | 6 | 8 | 371 | 53 | M   |
| *A. cerberus* | MZUTI 5108 | 152 | 25 | 17 | 17 | 17 | 2 | 7 | 7 | 7 | 309 | 36 | M   |
| *A. cerberus* | MZUTI 4330 | 157 | 26 | 17 | 17 | 17 | 2 | 7 | 7 | 7 | 212 | 23 | M   |
| *A. dipoosi*  | MHNG 2457.093 | 166 | 22 | 15 | 15 | 15 | 2 | 7 | 6 | 6 | 455 | 34 | F   |
| *A. dipoosi*  | MNHN 0.6147 | 164 | 17 | 15 | 15 | 15 | 2 | 8 | 7 | –  | 131 | 11 | F   |
| *A. duni*     | DHMECN 12769 | 141 | 36 | 17 | 17 | 17 | 2 | 6 | 7 | 7 | 279 | 39 | –   |
| *A. duni*     | DHMECN 2215 | 144 | 24 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 278 | 35 | F   |
| *A. duni*     | DHMECN 3527 | 141 | 24 | 17 | 17 | 17 | 2 | 6 | 6 | 6 | 352 | 48 | F   |
| *A. duni*     | DHMECN 3900 | 143 | 21 | 17 | 17 | 17 | 2 | 6 | 6 | –  | 101 | 19 | –   |
| *A. duni*     | DHMECN 4159 | 129 | 35 | 17 | 17 | 17 | 2 | 5 | 6 | 6 | 266 | 65 | –   |
| *A. duni*     | EPN 3127 | –  | –  | –  | –  | –  | –  | –  | –  | –  | 355 | 46 | F   |
| *A. duni*     | EPN 3128 | –  | –  | –  | –  | –  | –  | –  | –  | –  | 295 | 63 | M   |
| *A. duni*     | FHGO 375 | 128 | 36 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 219 | 48 | M   |
| *A. duni*     | FHGO 376 | 143 | 26 | 17 | 17 | 17 | 2 | 7 | 7 | 5 | 278 | 33 | F   |
| *A. duni*     | FHGO 379 | 132 | 35 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 297 | 61 | M   |
| *A. duni*     | FHGO 91 | 125 | 35 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 231 | 52 | M   |
| *A. duni*     | MHNG 2441.043 | 145 | 20 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 205 | 22 | F   |
| *A. duni*     | MHNG 2457.091 | 129 | 34 | 17 | 17 | 17 | 2 | 7 | 6 | 5 | 197 | 39 | M   |
| *A. duni*     | MHNG 2464.03 | 136 | 39 | 16 | 17 | 17 | 2 | 7 | 6 | 5 | 114 | 22 | M   |
| *A. duni*     | MZUTI 2189 | 134 | 29 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 189 | 28 | M   |
| *A. duni*     | MZUTI 3031 | 139 | 24 | 17 | 17 | 17 | 2 | 7 | 7 | 5 | 329 | 36 | F   |
| *A. duni*     | MZUTI 4097 | 149 | 21 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 152 | 17 | –   |
| *A. duni*     | MZUTI 4098 | 130 | 37 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 126 | 19 | –   |
| *A. duni*     | MZUTI 4099 | 140 | 25 | 17 | 17 | 17 | 2 | 7 | 7 | –  | 118 | 15 | F   |
| *A. duni*     | MZUTI 4100 | 138 | 24 | 17 | 17 | 17 | 2 | 7 | 7 | –  | 335 | 36 | F   |
| *A. duni*     | MZUTI 4318 | 136 | 34 | 17 | 18 | 17 | 2 | 7 | 7 | 6 | 242 | 53 | M   |
| *A. duni*     | MZUTI 4319 | 129 | 35 | 15 | 17 | 17 | 2 | 7 | 7 | 5 | 242 | 53 | M   |
| *A. esepe*    | MZUTI 3758 | 149 | 41 | 17 | 17 | 17 | 2 | 7 | 7 | 5 | 232 | 53 | M   |
| *A. esepe*    | MZUTI 3759 | 156 | 30 | 17 | 17 | 17 | 2 | 7 | 7 | 5 | 241 | 34 | F   |
| *A. gatgeae*  | MHNG 2397.044 | 136 | 34 | 17 | 17 | 17 | 2 | 7 | 7 | 5 | 129 | 23 | M   |
| *A. gigas*    | MHNG 2250.035 | 168 | 34 | 19 | 17 | 17 | 2 | 6 | 6 | 3 | 272 | 40 | F   |
| *A. gigas*    | MHNG 2441.02 | 177 | 31 | 17 | 17 | 17 | 2 | 6 | 6 | 5 | 1060 | 116 | F   |
| *A. iridescens* | DHMECN 2932 | 138 | 28 | 17 | 17 | 17 | 2 | 6 | 7 | 6 | 252 | 36 | F   |
| *A. iridescens* | DHMECN 5663 | 141 | 32 | 17 | 17 | 17 | 2 | 6 | 6 | 6 | 272 | 46 | F   |
| *A. iridescens* | DHMECN 9633 | 129 | 42 | 16 | 17 | 17 | 2 | 6 | 6 | 6 | 219 | 62 | M   |
| *A. iridescens* | FHGO 10443 | 139 | 32 | 17 | 17 | 17 | 2 | 7 | 7 | 5 | 204 | 32 | F   |
| *A. iridescens* | MZUTI 3548 | 131 | 34 | 17 | 17 | 17 | 2 | 7 | 7 | 6 | 200 | 44 | M   |
| Species          | Voucher   | V  | SC | D1 | D2 | D3 | PO | SL | IL | MT | SVL | TL | Sex |
|------------------|-----------|----|----|----|----|----|----|----|----|----|-----|----|-----|
| A. iridescens    | MHNG 2397 | 137| 37| 17| 17| 2  | 7  | 6  | 6  | 5  | 272 | 25 | F   |
| A. iridescens    | MHNG 2397 | 145| 20| 17| 17| 2  | 6  | 5  | 5  | 272 | 55 | M   |
| A. lehmanni      | GHGO 2936 | 165| 41| 17| 17| 2  | 7  | 5  | 11 | 20 | M   | 59 | M   |
| A. modestus      | MHNG 2397 | 146| 21| 15| 15| 2  | 6  | 5  | 5  | 200 | 23 | M   |
| A. occidentalis  | USNM 2854 | 153| 26| 17| 17| 2  | 7  | 5  | 5  | 335 | 35 | F   |
| A. occidentalis  | USNM 2854 | 163| 28| 17| 17| 2  | 7  | 5  | 5  | 212 | 21 | F   |
| A. paucidens     | MHNG 2397 | 137| 38| 17| 17| 2  | 6  | 5  | 5  | 253 | 55 | M   |
| A. paucidens     | MHNG 2397 | 129| 33| 17| 17| 2  | 6  | 5  | 5  | 122 | 23 | M   |
| A. paucidens     | MHNG 2397 | 139| 37| 17| 17| 2  | 7  | 7  | 7  | 274 | 68 | M   |
| A. paucidens     | MHNG 2397 | 134| 36| 17| 17| 2  | 7  | 6  | 6  | 223 | 35 | F   |
| A. paucidens     | MHNG 2397 | 149| 24| 17| 17| 2  | 7  | 6  | 5  | 191 | 21 | F   |
| A. paucidens     | MHNG 2397 | 134| 39| 17| 17| 2  | 7  | 7  | 7  | 332 | 67 | M   |
| A. roulei        | MHNG 2397 | 171| 43| 17| 17| 2  | 7  | 7  | 7  | 290 | 50 | M   |
| A. roulei        | MHNG 2397 | 163| 43| 17| 17| 2  | 7  | 7  | 7  | 249 | 50 | M   |
| A. roulei        | EPN 8730  | 246| 53| M   | 59 | M   | 59 | M   | 59 | M   | 59 | M   |
| A. roulei        | EPN 8731  | 237| 51| M   | 51 | M   | 51 | M   | 51 | M   | 51 | M   |
| A. roulei        | MHNG 2397 | 156| 46| 15| 15| 2  | 6  | 6  | 6  | 196 | 45 | M   |
| A. roulei        | MHNG 2397 | 186| 40| 17| 17| 2  | 7  | 6  | 6  | 262 | 42 | M   |
| A. roulesi       | MZUTI 5107| 143| 16| 15| 15| 1  | 6  | 5  | 8  | 443 | 34 | F   |
| A. roulesi       | QCAZ 6256| 135| 27| 15| 15| 1  | 6  | 6  | 9  | 337 | 48 | M   |
| A. roulesi       | QCAZ 7887| 146| 25| 15| 15| 1  | 6  | 6  | 9  | 309 | 39 | M   |
| A. roulesi       | QCAZ 7902| 156| 19| 15| 15| 1  | 6  | 7  | 11 | 392 | 37 | F   |
| A. roulesi       | QCAZ 9643| 149| 17| 15| 15| 1  | 6  | 6  | 11 | 139 | 13 | F   |
| A. roulesi       | QCAZ 9652| 143| 19| 15| 15| 1  | 6  | 6  | 13 | 230 | 21 | F   |
| A. savagei       | DHMECN 3800| 166| 25| 17| 17| 2  | 6  | 7  | 7  | 214 | 23 | F   |
| A. sibthorpeae   | MNHN 1906 | 151| 29| 17| 17| 2  | 7  | 7  | 7  | 283 | 35 | F   |
| A. sibthorpeae   | MHNG 2397 | 134| 39| 17| 17| 2  | 7  | 7  | 7  | 274 | 68 | M   |
| A. sibthorpeae   | MHNG 2397 | 134| 39| 17| 17| 2  | 7  | 7  | 7  | 274 | 68 | M   |
| A. sibthorpeae   | MHNG 2397 | 134| 39| 17| 17| 2  | 7  | 7  | 7  | 274 | 68 | M   |
### Molecular phylogeny of Atractus (Serpentes, Dipsadidae)

| Species     | Voucher         | V | SC | D1 | D2 | D3 | PO | SL | IL | MT | SVL | TL | Sex |
|-------------|-----------------|---|----|----|----|----|----|----|----|----|-----|----|-----|
| A. snethlageae | MNHN 1994.1171 | 160 | 27 | 17 | 17 | 17 | 2  | 7  | 7  | 8  | 315 | 35 | F   |
| A. touzeti   | ANF 2390        | 176 | 31 | 17 | 17 | 17 | 2  | 7  | 7  | 7  | 652 | 71 | F   |
| A. trilineatus | MNHN 1898.313  | 141 | 19 | 15 | 15 | 15 | 2  | 7  | 7  | 7  | 179 | 19 | M   |
| A. trilineatus | MNHN 1898.314  | 132 | 21 | 15 | 15 | 15 | 2  | 7  | 7  | 7  | 182 | 20 | M   |
| A. typhon    | DHMECN 9632     | 153 | 47 | 15 | 15 | 15 | 2  | 7  | 6  | 7  | 187 | 31 | M   |
| A. typhon    | FHGO 10438      | 166 | 41 | 15 | 15 | 15 | 2  | 7  | 7  | 6  | 370 | 68 | M   |
| A. typhon    | FHGO 10439      | 158 | 48 | 16 | 16 | 16 | 2  | 7  | 7  | 7  | 349 | 87 | F   |