

Unveiling the genetic diversity of bicolored-spined porcupines (Rodentia: Erethizontidae): a novel karyotype, population structuring, and evolutionary insights

Maria Julia Cunha Cardoso^{1,4}; Ana Lazar^{2,5}; Cintia Povill^{2,6} & Cibele Rodrigues Bonvicino^{3,7}

¹ Universidade Federal do Rio de Janeiro (UFRJ), Instituto de Biologia (IB), Departamento de Zoologia, Programa de Pós-Graduação em Genética. Rio de Janeiro, RJ, Brasil.

² Universidade Federal do Rio de Janeiro (UFRJ), Museu Nacional (MN), Departamento de Vertebrados, Laboratório de Mastozoologia. Rio de Janeiro, RJ, Brasil.

³ Fundação Oswaldo Cruz (FIOCRUZ), Instituto Oswaldo Cruz (IOC), Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres. Rio de Janeiro, RJ, Brasil.

⁴ ORCID: [0009-0006-5865-5968](https://orcid.org/0009-0006-5865-5968). E-mail: mjuliacardoso@hotmail.com

⁵ ORCID: [0000-0001-6515-6792](https://orcid.org/0000-0001-6515-6792). E-mail: ana.lgs@gmail.com

⁶ ORCID: [0000-0001-8494-8939](https://orcid.org/0000-0001-8494-8939). E-mail: cpovills@gmail.com

⁷ ORCID: [0000-0002-1948-7643](https://orcid.org/0000-0002-1948-7643). E-mail: cibele.bonvicino@gmail.com

Abstract. New World porcupines are rodents belonging to the Erethizontidae family which is divided into two subfamilies: Chaetomyiinae, represented by the genus *Chaetomys*, and Erethizontinae, which includes the genera *Erethizon* and *Coendou*. Within this family, the taxonomy of *Coendou* has proven to be particularly complex and challenging. It remains one of the most neglected among New World mammals, and the taxonomic status and phylogenetic relationships of some taxa are still controversial. In this study, we assessed the diversity of *Coendou bicolor* using the mitochondrial cytochrome b marker and described the chromosome complement of this species for the first time, increasing the knowledge about this taxon. Notably, the karyotype we described for *C. bicolor* differs from the six karyotypes reported for other species within this genus. Our findings align with previous research, confirming the separation of the three *Coendou* subgenera into distinct monophyletic clades. The median joining analysis supports these phylogenetic relationships and suggested a population structure in *C. bicolor* populations, apparently related to the rivers. Samples from northern Peru are separated from the remaining population by the Ucayali River (a tributary of the Amazonas River), while samples from southern Peru and Brazil are separated from Bolivian samples by the Madre de Dios/Beni rivers. These data highlight the potential role of these rivers as geographic barriers, contributing to the genetic differentiation of *C. bicolor* populations. Our study provides valuable insights into karyotype and genetic diversity of *C. bicolor* populations, enriching our understanding of this species.

Keywords. *Coendou bicolor*; Cytochrome b; Riverine barrier; Western Amazonia.

INTRODUCTION

New World porcupines are rodents of the Erethizontidae (Bonaparte, 1845) family, divided into two subfamilies, Chaetomyiinae with the genus *Chaetomys* Gray, 1843, and Erethizontinae with the genera *Erethizon* F. Cuvier, 1822, and *Coendou* Lacépède, 1799 (Voss, 2011). The species of *Coendou* exhibit a wide range of sizes and are characterized by their defensive spines, which cover the dorsal region of their body, and by their prehensile tail (Emmons & Feer, 1997). They are

nocturnal, arboreal, and herbivorous (Voss, 2015), eating abundant and common plant species across different successional stages in forests (Battista-da-Silva *et al.*, 2019). This adaptability allows porcupines to use urban areas, exemplified by sightings in several neighborhoods of the city of Rio de Janeiro such as Tijuca, Santa Teresa (as observed by Bonvicino), as well as in the state of Pernambuco (Leal *et al.*, 2017). Furthermore, members of *Coendou* have been found to host several parasites, including endoparasitic helminths (Cruces *et al.*, 2015), *Trypanosoma cruzi* (Siqueira *et al.*,

Pap. Avulsos Zool., 2024; v.64: e202464018

<https://doi.org/10.11606/1807-0205/2024.64.018>

<https://www.revistas.usp.br/paz>

<https://www.scielo.br/paz>

Edited by: Joyce Rodrigues do Prado

Received: 08/12/2023

Accepted: 26/04/2024

Published: 03/06/2024

ISSN On-Line: 1807-0205

ISSN Printed: 0031-1049

ISNI: 0000-0004-0384-1825



1967), *Leishmania* (Herrer, 1971; Lainson, 2010), *Giardia* and *Cryptosporidium* (Soares *et al.*, 2006). The role of *Coendou* as hosts of several parasites and their tendency to inhabit areas around urban centers (Voss *et al.*, 2001) highlight the need to enhance our knowledge about them. Accurate identification of these animals is crucial to understanding their role in the life cycles of these parasites. As cryptic animals, they are rarely observed in the wild and are underrepresented in mammalian collections. Consequently, there exist significant knowledge gaps concerning most porcupine species (Feijó & Langguth, 2013; Mendes Pontes *et al.*, 2013).

Brazil is the country with the greatest diversity of *Coendou*, with a total of ten species, including *C. nycthemera* (Olfers, 1818), *C. insidiosus* (Olfers, 1818), *C. spinosus* (F. Cuvier, 1823), *C. roosmalenorum* Voss & da Silva, 2001, *C. baturitensis* Feijó & Langguth, 2013, *C. speratus* Mendes Pontes *et al.*, 2013, *C. prehensilis* (Linnaeus, 1758), *C. bicolor* (Tschudi, 1844), *C. longicaudatus* Daudin, 1802, *C. melanurus* (Wagner, 1842) of which five are endemic to the country (Menezes *et al.*, 2020). Among Brazilian species, the bicolored-spined porcupine *C. bicolor* was first recorded in Brazil only in 2013, in the state of Acre (Freitas *et al.*, 2013). Subsequently, this species was also confirmed in the Brazilian state of Amazonas (Menezes *et al.*, 2020). Previous publications reported only two species of *Coendou* in the Brazilian state of Acre, namely, *C. prehensilis* and *C. bicolor* (Voss, 2015). However, a recent study based on morphology has revealed that *C. prehensilis* is a species complex, and the correct name for the specimens from Acre is *C. longicaudatus* (Menezes *et al.*, 2021).

Coendou taxonomy presents considerable complexity and challenges, being one of the most understudied among New World mammals. The taxonomic status and phylogenetic relationships of some taxa are still controversial (Bonvicino *et al.*, 2002; Voss, 2011; Menezes *et al.*, 2020, 2021; Voss *et al.*, 2013). The few karyological studies on *Coendou* species resulted in the description of only six karyotypes (George & Weir, 1974; Concepcion & Molinari, 1991; Bonvicino *et al.*, 2000; Bonvicino *et al.*, 2002; Silva *et al.*, 2018; Lima, 1994). This picture shows that the genetic knowledge about *Coendou* species is still incipient.

In this study, we assessed the diversity of *C. bicolor* using mitochondrial cytochrome b as a genetic marker and commented on the role of rivers in the population structure within this taxon. Notably, we provide the first description of the chromosome complement of this species, contributing to a better understanding of this taxon.

MATERIAL AND METHODS

Three specimens (LBCE22564, LBCE22568, LBCE22582) collected in the state of Acre, Brazil, have been archived in the mammal collections of the Laboratory of Biology and Parasitology of Wild Mammals Reservoirs at IOC/Fiocruz (Fig. 1). In addition, blood samples of two live specimens (LBCE21287, LBCE21289) were utilized for karyological preparations. These animals were

either rescued due to injuries ($n = 2$) and kept in quarantine or captured after they had died ($n = 3$) by the CETAS (Centro de Triagem de Animais Silvestres/Rio Branco) under the IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis). All specimens are from tributaries of the lower Purus River basin, near the municipality of Rio Branco, albeit without precise geographical coordinates. Specimens preserved in the form of skin, skull, and skeleton were deposited in the scientific mammal zoological collection of the Instituto Oswaldo Cruz/Fiocruz (LBCE). The collection and handling of these animals, along with the acquisition of biological samples, were conducted following ethical guidelines and were approved by the Council for Ethics in the Use of Animals (CEUA-UFAC) under license No. 23107.012934/2019-10, as well as having received the necessary SISBIO environmental license (ICMBIO 13373).

For karyotype preparation, we used a blood sample obtained from one *Coendou* male (LBCE21287) and one female (LBCE21289). The culture medium was prepared with 80% of RPMI 1640, 20% fetal calf serum, 20 μ L of lectin, 0.5 mL of L-Glutamine, and 60 μ L of heparin for 72 h. After 70 h we then added 0.5 mL of colchicine 10^{-5} M to stop cell division in the metaphase for 2 hours. We added KCl (0.075 M) to disrupt the cells and a fixative solution (3 methanol:1 acetic acid) to clean the material. For conventional staining, we used 2% Giemsa. The metaphases were visualized under an optical microscope with an attached camera to capture images of the best metaphases. We took at least 20 photographs to assemble the metaphases. We photographed the chromosome spreads for karyotyping, with the homologous pairs being arranged in ascending order, based on their morphology. We divided the chromosomes into two groups based on their morphology, *i.e.*, biarmed or acrocentric.

The DNA from three specimens (LBCE22564, LBCE22568, LBCE22582) was extracted from liver tissue samples preserved in absolute ethanol, using the phenol/chloroform technique, according to Sambrook *et al.* (1989). Mitochondrial cytochrome b DNA (complete sequence) was amplified with primers L14724 (Irwin *et al.*, 1991) and UMMZ04 (Jansa *et al.*, 1999) under the following conditions: one cycle of denaturation at 94°C for 2 minutes and 35 cycles of denaturation at 94°C for 30 seconds, annealing at 48°C also for 30 seconds, stretching of the DNA strand at 72°C for 1:30 minutes. The purified PCR product was subjected to sequencing reactions on automatic DNA sequencer (ABI 3730xl) using the same amplification primers and two more internal primers: Cytb-Sot in 1 and Cytb-Sot in 2 (Cassens *et al.*, 2000). The sequences generated during the current study were deposited in the GenBank repository under the numbers PP761324, PP761325 and PP761326 (Table 1).

The three newly generated sequences and 27 more from GenBank (with at least 800 bp) of all available *Coendou* species (Table 1) were manually aligned using MEGA X (Kumar *et al.*, 2018). Other two GenBank sequences from Vilela *et al.* (2009) were used as outgroups: *Erethizon dorsatum* (FJ3574280) and *Chaetomys subspinosus* (EU544660). IQ-Tree 2.1.2 (Minh *et al.*, 2020)

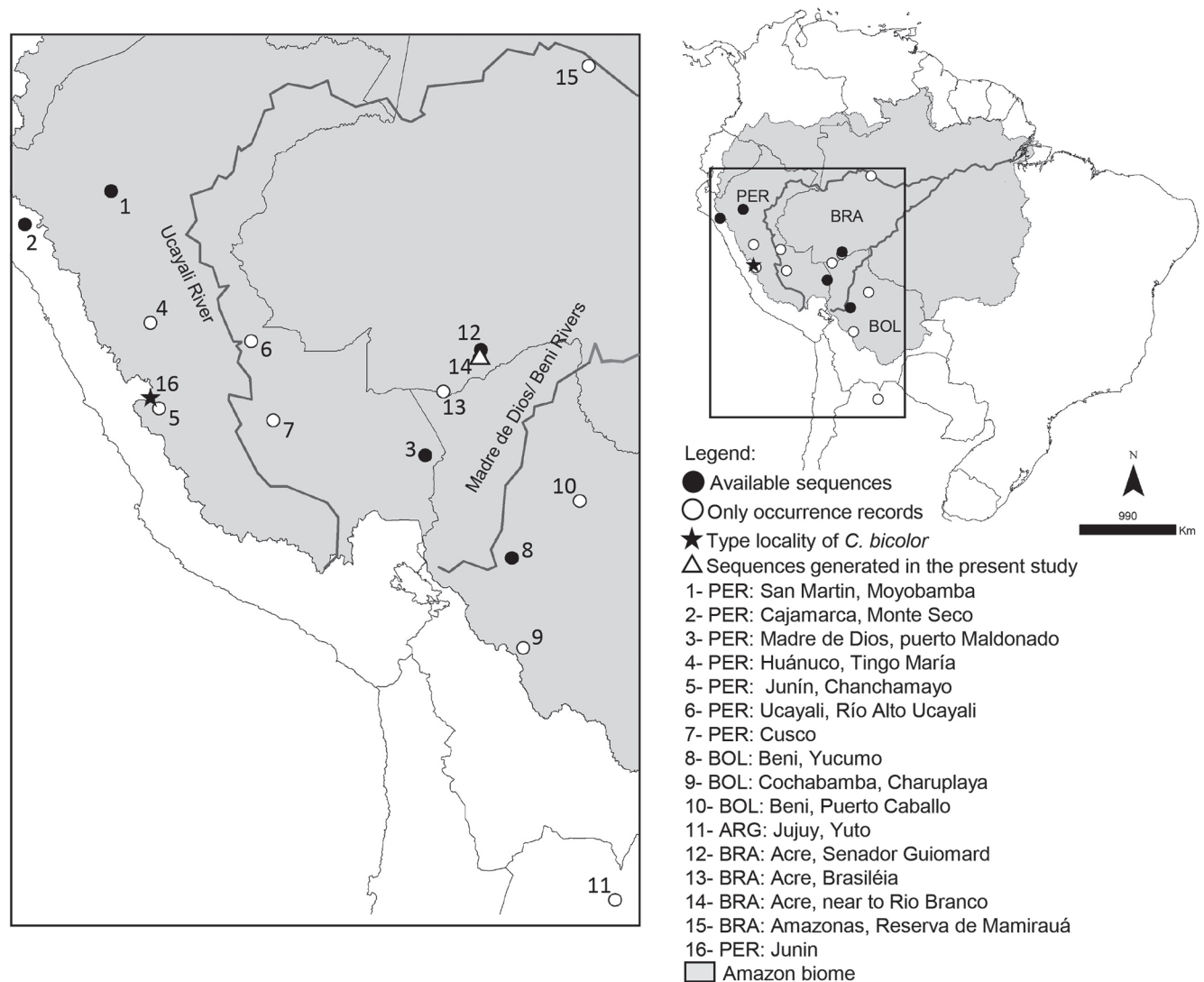


Figure 1. Map of part of South America with collecting localities of *C. bicolor* specimens with sequences on the GenBank (Black circles), generated in the present study (triangle), others register of occurrences (white circles), and type locality (star). Gray area represents the Amazon Biome. ARG = Argentina, BOL = Bolivia, BRA = Brazil, PER = Peru.

was used to generate the maximum-likelihood tree, and the best-fit evolutionary model according to the Bayesian inference criterion (BIC) was determined by ModelFinder (Kalyaanamoorthy *et al.*, 2017) included in the IQ-Tree package, where the command “-bb 50000” was applied to calculate the ultrafast bootstrap approximation (bb; Minh *et al.*, 2013) in 50,000 replicates. To confirm the clades obtained in the ML and visualize mutational steps separating each lineage, we used DNASP v. 6.12.03 (Rozas *et al.*, 2017) for identifying haplotypes (excluding sites with gaps and missing data to avoid bias in the haplotype’s distribution pattern) and Network version 10 (<https://fluxus-engineering.com>; Bandelt *et al.*, 1999) for building the haplotype network.

RESULTS

The karyotype of one male and one female *Coendou* sp. showed diploid number (2n) of 52 and fundamental number (FN) of 82 (Fig. 2). The autosome com-

plement is composed by 16 biarmed pairs varying in size from large to small, and nine acrocentric pairs varying in size from medium to small. The sexual X chromosome is large and submetacentric and the Y chromosome is small and also submetacentric.

The maximum-likelihood tree grouped all *Coendou* haplotypes with bootstrap support (bs) of 70%, divided into three main clades. One clade grouped haplotypes of the subgenus *Coendou* (*Coendou*) (bs = 95%) apart from the remaining haplotypes, divided into two clades, one including haplotypes of *Coendou* (*Caaporamys*) (bs = 66%) and the other with haplotypes of *Coendou* (*Sphiggurus*) (bs = 94%), divided into two subclades (Fig. 3). Within the clade *C. (Sphiggurus)*, haplotypes of *C. (S.) bicolor* grouped in a well-supported and structured clade (bs = 99%) with haplotypes from northern Peru grouped (bs = 97%) apart of haplotypes from southern Peru, Bolivia, and Brazil (bs = 96%, Fig. 3). The median-joining network showed the six haplotypes separated into three haplogroups connected by one median vector, the haplogroup from Acre and southern Peru, the

Table 1. *Coendou* sequences used in the molecular analysis with taxon identification, GenBank accession number, field or museum number (ID), collection locality, and reference.

Taxon	GenBank	ID	Locality	Reference
<i>C. bicolor</i>	PP761326	LBCE22564	BRA: AC, Purus river basin	This study
<i>C. bicolor</i>	PP761324	LBCE22568	BRA: AC, Purus river basin	This study
<i>C. bicolor</i>	PP761325	LBCE22582	BRA: AC, Purus river basin	This study
<i>C. villosus</i>	AF411580	MN46937	BRA: RJ, Sumidouro	Bonvicino et al., 2000
<i>C. villosus</i>	EU544661	CIT1326	BRA: ES, UHE Rosal	Vilela et al., 2009
<i>C. longicaudatus</i>	AF411581	212	BRA: MT, Chapada dos Guimarães	Bonvicino et al., 2002
<i>C. longicaudatus</i>	KC463879	USNM528360	EQU: Sucumbios, Limonocha	Voss et al., 2013
<i>C. melanurus</i>	AF411583	CRB631	BRA: RR, São João da Baliza	Bonvicino et al., 2002
<i>C. melanurus</i>	KC463862	MNHN1997.641	GF: Petit Saut	Voss et al., 2013
<i>C. insidiosus</i>	KC261591	UFES136	BRA: BA, Nova Viçosa	Mendes Pontes et al., 2013
<i>C. prehensilis</i>	HM462243	MN73383	BRA: PE, Usina Trapiche	Leite et al., 2011
<i>C. prehensilis</i>	KY784126	UFPB9412	BRA: PB, João Pessoa	Menezes et al., 2021
<i>C. speratus</i>	KC261592	UFPE1708	BRA: PE, Usina Trapiche	Mendes Pontes et al., 2013
<i>C. speratus</i>	KC261593	UFPE1709	BRA: PE, Usina Trapiche	Mendes Pontes et al., 2013
<i>C. nycthemera</i>	KC261597	UFES2079	BRA: TO, UHE Estreito	Mendes Pontes et al., 2013
<i>C. nycthemera</i>	KC463865	USNM519692	BRA: PA, Ilha de Marajó	Voss et al., 2013
<i>C. bicolor</i>	KC463858	KU144560	PER: Madre de Dios, Puerto Maldonado	Voss et al., 2013
<i>C. bicolor</i>	KC463859	MUSM9398	PER: Cajamarca, Monte Seco	Voss et al., 2013
<i>C. bicolor</i>	KC463860	FMNH203679	PER: San Martin, Mishquiyacu-Rumiyacu	Voss et al., 2013
<i>C. bicolor</i>	KC463857	AMNH 214612	BOL: Beni, Puerto Caballo	Voss et al., 2013
<i>C. ichillus</i>	KC463861	TTU115491	PER: Loreto Nuevo, San Juan	Voss et al., 2013
<i>C. mexicanus</i>	KC463863	ASNHC6407	MEX: Campeche, Jonuta	Voss et al., 2013
<i>C. baturitensis</i>	KY784124	UFPB9780	BRA: CE, Mulungu	Menezes et al., 2021
<i>C. baturitensis</i>	KY784125	UFPB9781	BRA: CE, Mulungu	Menezes et al., 2021
<i>C. quichua</i>	KC463881	KMH2218	EQU: Cotopaxi, Otonga	Voss et al., 2013
<i>C. quichua</i>	KC463882	LACM27376	COL: Cesar, San Alberto	Voss et al., 2013
<i>C. rufescens</i>	KC463884	AMNH181483	COL: Cauca, Quintana	Voss et al., 2013
<i>C. spinosus</i>	KC463885	GD252	PAR: Caazapa, Estância dos Marias	Voss et al., 2013
<i>C. spinosus</i>	KC463886	UMMZ174975	PAR: Caazapa, Estância dos Marias	Voss et al., 2013
<i>C. vestitus</i>	MG383643	IAVH7956	COL	Colmenares et al., 2017

Legend: The countries are Ecuador (EQU), Bolivia (BOL), Colombia (COL), Venezuela (VEN), French Guiana (GF), Mexico (MEX), Peru (PER), Paraguay (PAR). Brazilian (BRA) states are: Bahia (BA), Ceará (CE), Espírito Santo (ES), Mato Grosso (MT), Paraíba (PB), Pernambuco (PE), Rio de Janeiro (RJ), Roraima (RR), São Paulo (SP), Tocantins (TO).

haplotype from Bolivia, and the haplogroup from northern Peru (Fig. 4)

DISCUSSION

The karyotype of *Coendou bicolor* with $2n = 52$ and $FN = 82$, described for the first time in this study, is different in both diploid and fundamental numbers from the other six reported for species within this genus (Bonvicino et al., 2000; Bonvicino et al., 2002; Concepcion & Molinari, 1991; George & Weir, 1974; Silva et al., 2018; Lima, 1994). Previous hypotheses had considered *C. rothschildi* as a subspecies of *C. bicolor* (Goldman, 1920: 135; Hall, 1981: 854; Corbet & Hill, 1991: 200), but posteriorly it was recognized as a valid species (Emmons & Feer, 1997: 218), with a different karyotype, $2n = 74$ and $FN = 82$ (George & Weir, 1974), despite having the same FN . Presently, *C. rothschildi* Thomas, 1902 is considered a junior synonymous of *Coendou quichua* Thomas 1899. The karyological data presented here clearly showed that *C. bicolor* karyotype ($2n = 52$ and $FN = 82$) is different from the karyotype of *C. quichua* with $2n = 74$ and $FN = 82$, as expected for different species.

Within the subgenus *C. (Coendou)*, both *C. (Co.) longicaudatus* from Pará state, Brazil, and *C. (Co.) quichua* share identical karyotypes of $2n = 74$ and $FN = 82$ (Silva et al., 2018; George & Weir, 1974). Our current knowledge indicates that species of the subgenus *C. (Co.)* share a conserved karyotype, with the same diploid and fundamental numbers. Within the subgenus *C. (Caaporamys)*, the karyotype of *C. (Ca.) melanurus* from Roraima state, Brazil, showed $2n = 72$ and $FN = 76$ (Bonvicino et al., 2002) and *C. (Ca.) vestitus* from western Venezuela showed $2n = 42$ and $FN = 76$ (Concepcion & Molinari, 1991). These karyotypes share the same $FN = 76$, but with considerably distinct diploid numbers.

Within the subgenus *Coendou (Sphiggurus)*, the karyotypes are quite distinct, specimens identified as *C. (S.) villosus* (F. Cuvier, 1823) showed $2n = 42$ and $FN = 76$ (Bonvicino et al., 2000) and the karyotype here described for *C. bicolor* showed $2n = 52$ and $FN = 82$, differing in diploid and fundamental numbers. *Coendou (S.) villosus* was considered a junior synonym of *C. (S.) spinosus* (Voss et al., 2011), a taxonomic arrangement followed by subsequent publications. This arrangement was based on the fact that the holotype of *C. villosus* was lost, the type locality was

not explicitly designated in the original description, and the posterior designation of the type locality was based on a misidentified of the type specimen (Voss *et al.*, 2011). The type of *C. villosus*, an animal characterized by its long fur, was collected by Auguste de Saint-Hilaire (Cuvier, 1823), who traveled to several places in Brazil, including Rio de Janeiro and Minas Gerais. Furthermore, a morphological study on *Coendou* populations from southeastern Brazil found three spatially coherent and morphologically distinct lineages, which can be diagnosed using a combination of discrete morphological characters (Caldara Jr. & Leite, 2012). These lineages correspond to *C. (S.) insidiosus*, *C. (S.) spinosus*, and third lineage similar to, but different from *C. (S.) spinosus*, which may represent *C. (S.) villosus*, as suggested by Caldara Jr. & Leite (2012).

The topology recovered in this study is consistent with the one reported by Menezes *et al.* (2021), who identified three morphologically distinct clades within *Coendou*: *Coendou (Coendou)* with six species (*C. baturitensis*, *C. longicaudatus*, *C. mexicanus*, *C. prehensilis*, *C. quichua*, and *C. rufescens*), *Coendou (Sphiggurus)* with five species (*C. bicolor*, *C. insidiosus*, *C. nycthemera*, *C. speratus*, and *C. spinosus*), and *Coendou (Caaporamys)*, comprising *C. melanurus*, *C. vestitus*, *C. pruinusosus*, *C. ichillus*, and *C. roosmalenorum*. In our study, we did not include samples of *C. pruinusosus*, due to the limited availability of good sequences (length more than 800 bp), and there were no sequences available for *C. roosmalenorum*. The grouping of samples from Acre with sequences from *C. bicolor* aligns with the findings of Voss (2015), who suggested

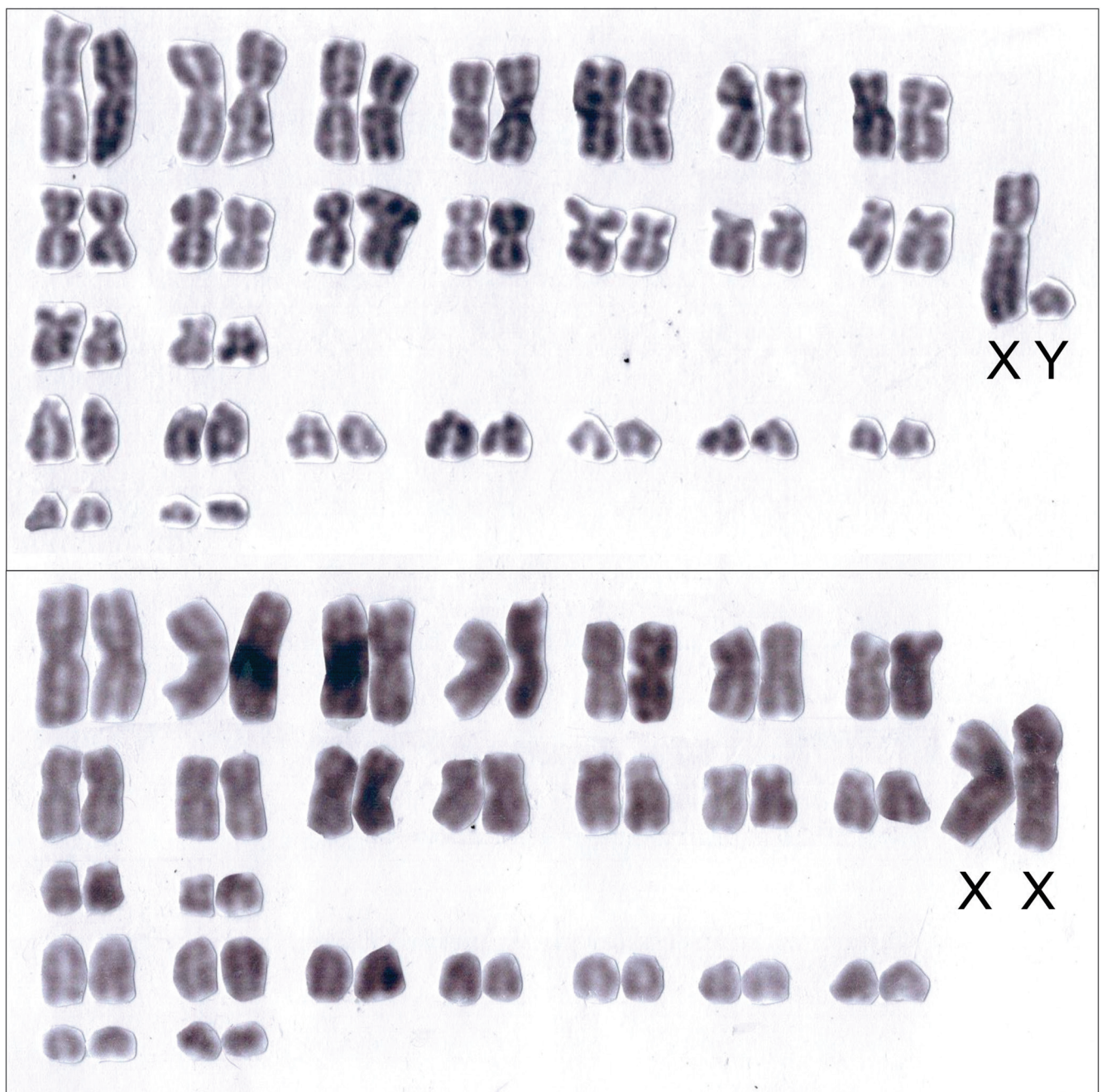


Figure 2. Conventional stained karyotypes of *Coendou (Sphiggurus) bicolor* male LBCE21287 (above) and female LBCE21289 (below) from Brazilian Acre state with $2n = 52$ and $FN = 82$. The X and Y are sexual chromosomes.

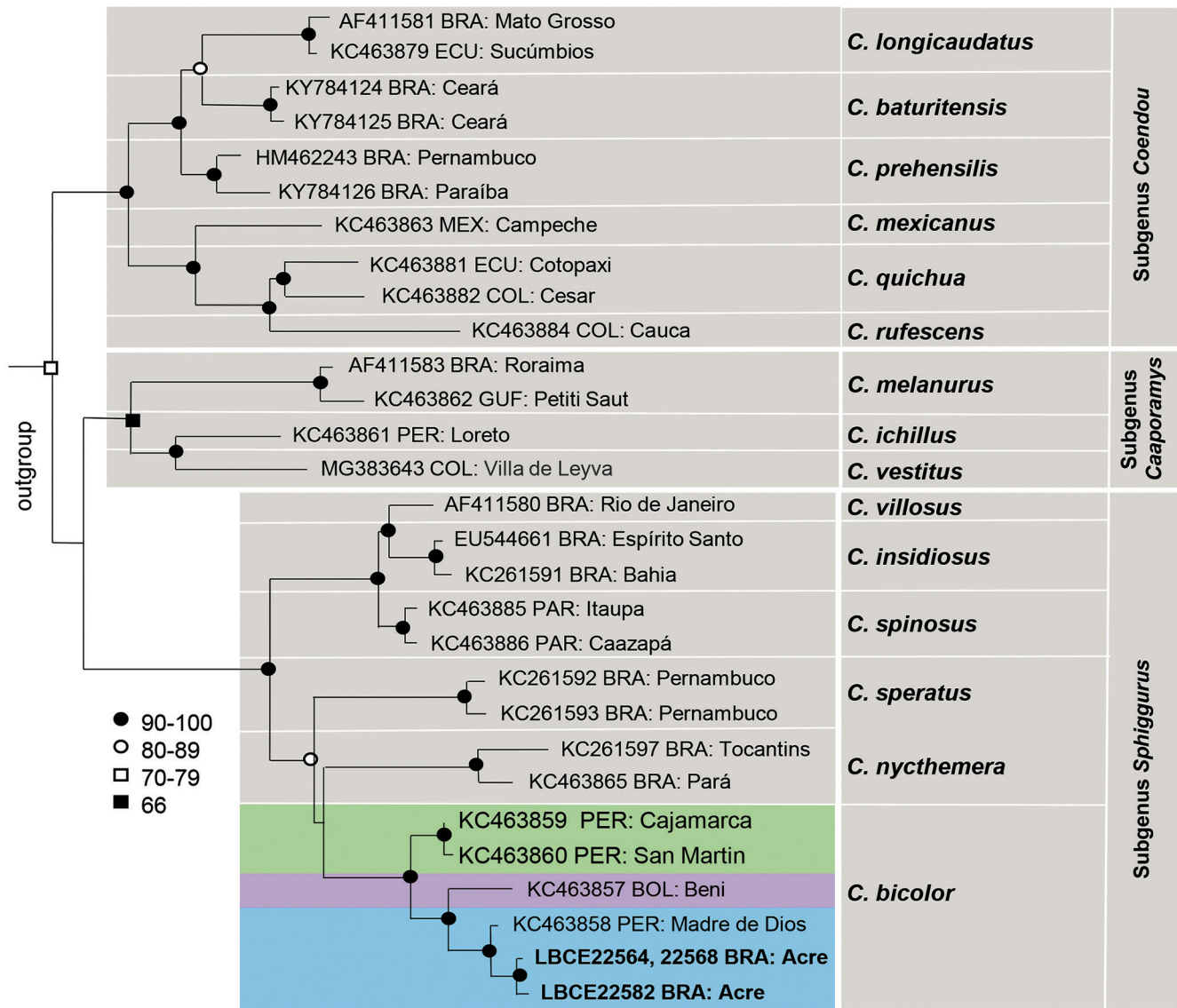


Figure 3. Maximum likelihood phylogenetic tree. Symbols near nodes represent Bootstrap values: black circles (90-100%), white circles (80-89%), white squares (70-79%), black square (66%). BOL = Bolivia, BRA = Brazil, COL = Colombia, ECU = Ecuador, GUF = French Guiana, MEX = Mexico, PAR = Paraguay, PER = Peru.

the potential occurrence of this species in the Brazilian state of Acre.

Our results suggested a geographic structure within *C. bicolor* populations, aligning with the distinct vegetational physiognomies. Individuals belonging to haplogroups from Brazil/southern Peru (Madre de Dios) primarily inhabit the lowlands of the Amazon Forest, while those in haplogroups from northern Peru (Cajamarca and San Martin) are predominantly distributed in the highlands of the Andes. On the other hand, the Bolivian lineage (from Beni) is associated with wet savanna environments. The rivers are apparently important geographical components influencing this structure. Individuals from northern Peru are separated from the remaining populations by the Ucayali River (a tributary of the south bank of the Amazonas/Solimões River). Similarly, individuals from southern Peru (Madre de Dios) and Brazil are geographically separated from Bolivian individuals by the Madre de Dios/Beni rivers. These findings suggest that these rivers could be working as effective geographic barriers shaping the distribution of different populations

within this species. The role of these rivers as geographic barriers has already been found by other authors, who observed that the Amazonian rivers are important factors in the distribution of some species of didelphid marsupials, such as the Amazon River separating *Caluromys philander philander* in the north from *C. philander affinis* and *C. philander dichurus* in the south. Furthermore, two distinct clades have been identified in *Marmosops noctivagus*, one from the southern Amazonas-Solimões and eastern Ucayali rivers, and another from northern Amazonas-Solimões, western Ucayali rivers (Bonvicino et al., 2022).

In conclusion, our study reveals a unique karyotype in *Coendou bicolor*, featuring $2n = 52$ and $FN = 82$. This distinct karyotype sets *C. bicolor* apart from its congeners, reinforcing its species status and refuting earlier hypothesis of co-specificity with *C. quichua*. Geographically, we observe a pronounced population structure, influenced by rivers, and defined based on distinct vegetational physiognomies, with the Ucayali and Madre de Dios/Beni rivers acting as potential geographic barriers. These

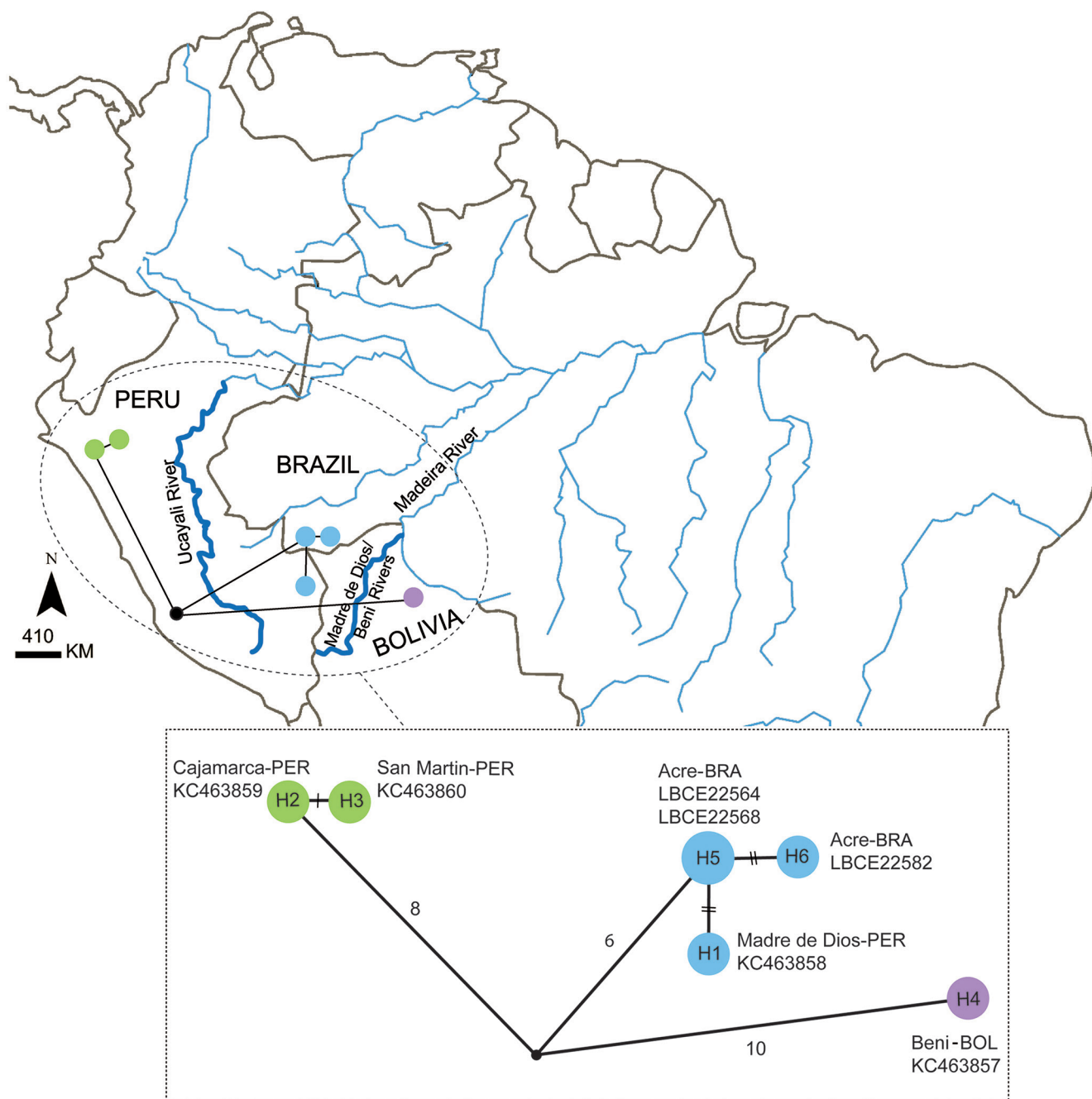


Figure 4. Topology of median joining on top of the map showing the current separation of *C. bicolor* populations by the rivers Ucayali and Beni/Madre de Dios. Circles are haplotypes and its dimension are proportional to the number of shared sequences. Numbers in the segments connecting the circles are nucleotide substitutions and black circle is the median vector. For haplotypes localities see figure 2. BOL = Bolivia, BRA = Brazil, PER = Peru.

insights enhance our understanding of biodiversity, emphasizing the role of geographic features in population distribution and diversification.

AUTHORS' CONTRIBUTIONS: CRB: Conceptualization, Funding Acquisition; MJC, CP, CRB: Data Curation; MJC, AL, CP, CRB: Formal Analysis, Writing – original draft; MJC, AL, CRB: Investigation. All authors actively participated in the discussion of the results; they reviewed and approved the final version of the paper.

CONFLICT OF INTEREST: The authors declare there are no conflicts of interests.

FUNDING INFORMATION: This research was supported by the following Brazilian institutions: Coordenação de Aperfeiçoamento de Pessoal

de Nível Superior (CAPES) to MJC; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to CRB (grants # 304498/2014-9 and 312446/2018-7), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) to CRB (E26/201.200/2014 and E-26/210.047/2014).

ACKNOWLEDGMENTS: We are grateful to J.T. Wagatsuma, E.C. das Neves and M.H.V. Nunes, veterinary anesthesiologists at the Veterinary Medicine Teaching Unit of the Federal University of Acre, for supervising the anesthetic protocol and sedation of the animals for blood sample collection. We also thank D.S.S. Araujo for supporting the collection of blood samples, R. Verde for supporting the collection of muscle tissue samples, and preparation of voucher specimens. Elaine Christina Oliveira do Carmo for overall coordination of sample collection activities.

REFERENCE

- Bandelt, H.J.; Forste, P. & Röhl, A. 1999. A Median-joining network for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16: 37-48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>.
- Batista-da-Silva, J.A.; Barcellos, S.J.A. & Santori, R. 2019. Use of *Elaeis guineensis* (Arecaceae: Arecaceae) as shelter and food resource by *Coendou spinosus* (Rodentia: Erethizontidae) in a mangrove swamp. *Oecologia Australis*, 23(4): 1104-1108. <https://doi.org/10.4257/oeco.2019.2304.31>.
- Bonaparte, C.L.P. 1845. *Catalogo metodico dei mammiferi Europei*. Milão, Coi Tipi di Luigi di Giacomo Pirola. <https://doi.org/10.5962/bhl.title.77311>.
- Bonvicino, C.R.; Almeida, F.C. & Cerqueira, R. 2000. The karyotype of *Sphiggurus villosus* (Rodentia: Erethizontidae). *Studies on Neotropical Fauna and Environment*, 35(2): 81-83. [https://doi.org/10.1076/0165-0521\(200008\)35:2;1-9;FT081](https://doi.org/10.1076/0165-0521(200008)35:2;1-9;FT081).
- Bonvicino, C.R.; Lazar, A.; Povill, C.; Caramaschi, F.P.; de Freitas, T.P.T.; Crisóstomo, C.F.; Botelho, A.L.M. & D'Andrea, P.S. 2022. Phylogeny of Didelphid marsupials (Didelphimorphia) from Acre, western Amazonia. *Mammalian Biology*, 102: 1997-2015. <https://doi.org/10.1007/s42991-022-00314-7>.
- Bonvicino, C.R.; Penna-Firme, V. & Braggio, E. 2002. Molecular and karyologic evidence of the taxonomic status of *Coendou* and *Sphiggurus* (Rodentia: Hystricognathi). *Journal of Mammalogy*, 83(4): 1071-1076. [https://doi.org/10.1644/1545-1542\(2002\)083<1071:MAKEOT>2.0.CO;2](https://doi.org/10.1644/1545-1542(2002)083<1071:MAKEOT>2.0.CO;2).
- Caldara Jr., V. & Leite, Y.L.R. 2012. Geographic variation in hairy dwarf porcupines of *Coendou* from eastern Brazil (Mammalia: Erethizontidae). *Zoologia*, 29(4): 318-336. <https://doi.org/10.1590/S1984-46702012000400005>.
- Cassens, I.; Vicario, S.; Waddell, V.G.; Balchowsky, H.; Van Belle, D.; Ding, W.; Fan, C.; Lal Mohan, R.S.; Simões-Lopesi, P.C.; Bastida, R.; Meyer, A.; Stanhope, M.J. & Milinkovitch, M.C. 2000. Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. *Proceedings of the National Academy of Sciences of the United States of America*, 97(21): 11343-11347. <https://doi.org/10.1073/pnas.97.21.11343>.
- Concepcion, J.L. & Molinari, J. 1991. *Sphiggurus vestitus pruinosus* (Rodentia: Erethizontidae): the karyotype and its phylogenetic implications, descriptive notes. *Studies on Neotropical Fauna and Environment*, 26(4): 237-241. <https://doi.org/10.1080/01650529109360858>.
- Corbet, G.B. & Hill, J.E. 1991. *A World List of Mammalian Species*. 3.ed. London, Natural History Museum Publications.
- Cruces, C.; Cher, J.; Mendoza-Vidaurre, C.; Iannaccone, J.; Sáez, G.; Alvarino, L. & da Silva, R.J. 2015. First record of *Heligmostrongylus sedecimradiatus* Linstow, 1899 (Rhabditida: Heligmonellidae), in bicolored-spined porcupine *Coendou bicolor* (Tschudi, 1844) (Rodentia: Erethizontidae) in Peru. *Neotropical Helminthology*, 9(2): 367-370. <https://doi.org/10.24039/rnh201592846>.
- Cuvier, F.G. 1823. Examen des espèces formation des genres ou sous-genres Acanthion, Éréthizon, Sinéthère et Sphiggure. *Mémoires du Muséum d'Histoire Naturelle* (Paris), 9: 413-484.
- Emmons, L.H. & Feer, F. 1997. *Neotropical Rainforest Mammals: A field guide*. Chicago, The University of Chicago Press.
- Feijó, A. & Langguth, A. 2013. Mamíferos de médio e grande porte do Nordeste do Brasil: Distribuição e taxonomia, com descrição de novas espécies. *Revista Nordestina de Biologia*, 22(1-2): 3-225.
- Freitas, M.A. de; de França, D.P.F. & Veríssimo, D. 2013. First record of the Bicoloured-spined Porcupine *Coendou bicolor* (Tschudi, 1844) for Brazil. *Check List*, 9(1): 94-96. <https://doi.org/10.15560/9.1.94>.
- George, W. & Weir, B.J. 1974. Hystricomorph chromosomes. *Symposia of the Zoological Society of London*, 34: 79-108.
- Goldman, E.A. 1920. Mammals of Panama. *Smithsonian Miscellaneous Collections*, 69: 1-309.
- Hall, E.R. 1981. *The mammals of North America*. 2. ed. New York, John Wiley and Sons.
- Herrer, A. 1971. *Leishmania hertigi* sp. n., from the tropical porcupine, *Coendou rothschildi* Thomas. *The Journal of Parasitology*, 57(3): 626-629. <https://doi.org/10.2307/3277928>.
- Irwin, D.M.; Kocher, T.D. & Wilson, A.C. 1991. Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution*, 32(2): 128-144. <https://doi.org/10.1007/BF02515385>.
- Jansa, S.A.; Goodman, S.M. & Tucker, P.K. 1999. Molecular phylogeny and biogeography of the native rodents of Madagascar (Muridae: Nesomyinae): a test of the single-origin hypothesis. *Cladistics*, 15(3): 253-270. <https://doi.org/10.1111/j.1096-0031.1999.tb00267.x>.
- Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.; Von Haeseler, A. & Jermini, L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14(6): 587-589. <https://doi.org/10.1038/nmeth.4285>.
- Kumar, S.; Stecher, G.; Li, M.; Knyaz, C. & Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6): 1547-1549. <https://doi.org/10.1093/molbev/msy096>.
- Lainson, R. 2010. The Neotropical *Leishmania* species: a brief historical review of their discovery, ecology and taxonomy. *Revista Pan-Amazônica de Saúde*, 1(2): 13-32. <https://doi.org/10.5123/S2176-62232010000200002>.
- Leal, E.S.B.; Gomes-Silva, F.F.; Lyra-Neves, R.M. de & Telino-Júnior, W.R. 2017. Range extension and first record of *Coendou speratus* Mendes Pontes et al. 2013 (Rodentia, Erethizontidae) from a cloud forest enclave in northeastern Brazil. *Check List*, 13(6): 951-957. <https://doi.org/10.15560/13.6.951>.
- Leite, Y.L.R.; Júnior, V.C.; Loss, A.C.; Costa, L.P.; Melo, E.R.A.; Gadelha, J.R. & Pontes, A.R.M. 2011. Designation of a neotype for the Brazilian porcupine, *Coendou prehensilis* (Linnaeus, 1758). *Zootaxa*, 2791: 30-40. <https://doi.org/10.11646/zootaxa.2791.1.2>.
- Lima, F.S. 1994. Cariótipos em espécies de Dasyproctidae e Erethizontidae, com discussão da evolução cromossômica (Rodentia, Caviomorpha). *Brazilian Journal of Genetics*, 17: 135.
- Mendes Pontes, A.R.; Gadelha, J.R.; Melo, E.R.A.; De Sá, F.B.; Loss, A.C.; Caldara Jr., V.; Costa, L.P. & Leite, Y.L.R. 2013. A new species of porcupine, genus *Coendou* (Rodentia: Erethizontidae) from the Atlantic Forest of northeastern Brazil. *Zootaxa*, 3636(3): 421-438. <https://doi.org/10.11646/zootaxa.3636.3.2>.
- Menezes, F.H.; Feijó, A.; Fernandes-Ferreira, H.; da Costa, I.R. & Cordeiro-Estrela, P. 2021. Integrative systematics of Neotropical porcupines of *Coendou prehensilis* complex (Rodentia: Erethizontidae). *Journal of Zoological Systematics and Evolutionary Research*, 59(8): 2410-2439. <https://doi.org/10.1111/jzs.12529>.
- Menezes, F.H.; Garbino, G.S.T.; Semedo, T.B.F.; Lima, M.; Feijó, A.; Cordeiro-Estrela, P. & da Costa, I.R. 2020. Major range extensions for three species of porcupines (Rodentia: Erethizontidae: *Coendou*) from Brazilian Amazon. *Biota Neotropica*, 20(2): 1-8, e20201030. <https://doi.org/10.1590/1676-0611-BN-2020-1030>.
- Minh, B.Q.; Nguyen, M.A.T. & Von Haeseler, A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30(5): 1188-1195. <https://doi.org/10.1093/molbev/mst024>.
- Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; Haeseler, A.V. & Lanfear, R. 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, 37(5): 1530-1534. <https://doi.org/10.1093/molbev/msaa015>.

- Rozas, J.; Ferrer-Mata, A.; Sánchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E. & Sánchez-Gracia, A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34(12): 3299-3302. <https://doi.org/10.1093/molbev/msx248>.
- Sambrook, J.; Fritsch, E.F. & Maniatis T. 1989. *Molecular cloning: A laboratory manual*. 2. ed. New York, Cold Spring Harbor Laboratory Press.
- Silva, R.S.; Gomes A.J.B. & Oliveira, E.H.C. 2018. Análise cariotípica através de citogenética clássica e molecular na espécie *Coendou prehensilis* (Mammalia, Rodentia). *Semina: Ciências Biológicas e da Saúde*, 38: 72. <https://doi.org/10.5433/1679-0367.2017v38n1suplp72>.
- Siqueira, A.F.; Ferriolli Filho, F. & Barretto, E.M.P. 1967. Studies on wild reservoirs and vectors of *Trypanosoma cruzi*. XV – Natural infection of a porcupine, *Coendou insidiosus insidiosus* (Kuhl, 1820) by cruzi-like trypanosome. *Revista do Instituto de Medicina Tropical de São Paulo*, 9(3): 155-162.
- Soares, J.F.; da Silva, A.S.; Oliveira, C.B.; da Silva, M.K.; Mariscano, G.; Salomão, E.L. & Monteiro, S.G. 2006. Parasitism by *Giardia* sp. and *Cryptosporidium* sp. in *Coendou villosus*. *Ciência Rural*, 38(2): 548-550. <https://doi.org/10.1590/S0103-84782008000200043>.
- Vilela, R.V.; Machado, T.; Ventura, K.; Fagundes, V.; Silva, M.J.J. & Yonenga-Yassuda, Y. 2009. The taxonomic status of the endangered thin-spined porcupine, *Chaetomys subspinosus* (Olfers, 1818), based on molecular and karyologic data. *BMC Evolutionary Biology*, 29(9): 1-17. <https://doi.org/10.1186/1471-2148-9-29>.
- Voss, R.S. 2011. Revisionary Notes on Neotropical Porcupines (Rodentia: Erethizontidae) 3. An annotated Checklist on the species of *Coendou* Lacépède, 1799. *American Museum Novitates*, 3720: 1-36. <https://doi.org/10.1206/3720.2>.
- Voss, R.S. 2015. Superfamily Erethizontoidea Bonaparte, 1845. In: Patton, J.L.; Pardiñas, U.F.J. & D'Elía, G. (Eds.). *Mammals of South America, volume 2*. Chicago, The University of Chicago Press., p. 786-805.
- Voss, R.S.; Hubbard, C.S. & Jansa, A. 2013. Phylogenetic relationships of New World Porcupines (Rodentia, Erethizontidae): implications for taxonomy, morphological evolution, and biogeography. *American Museum Novitates*, 3769: 1-36. <https://doi.org/10.1206/3769.2>.
- Voss, R.S.; Lunde, D.P. & Simmons, N.B. 2001. The Mammals of Paracou, French Guiana: A Neotropical Lowland Rainforest Fauna part 2. Nonvolutant Species. *Bulletin of the American Museum of Natural History*, 263: 3-236. [https://doi.org/10.1206/0003-0090\(2001\)263<0003:TMOPF-G>2.0.CO;2](https://doi.org/10.1206/0003-0090(2001)263<0003:TMOPF-G>2.0.CO;2).