Molecular Phylogenetics and Evolution 132 (2019) 117–137

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

On a new species of titi monkey (Primates: *Plecturocebus* Byrne et al., 2016), from Alta Floresta, southern Amazon, Brazil

Jean P. Boubli[a,b,*,1], Hazel Byrne[c,1], Maria N.F. da Silva[b], José Silva-Júnior[d], Rodrigo Costa Araújo[b,e], Fabrício Bertuol[e], Jonas Gonçalves[b], Fabiano R. de Melo[f,g], Anthony B. Rylands[h], Russell A. Mittermeier[h], Felipe E. Silva[a,i], Stephen D. Nash[j], Gustavo Canale[k], Raony de M. Alencar[l], Rogerio V. Rossi[k], Jeferson Carneiro[m], Iracilda Sampaio[m], Izeni P. Farias[e], Horácio Schneider[m], Tomas Hrbek[e]

[a] *University of Salford, United Kingdom*
[b] *Instituto Nacional de Pesquisas da Amazônia, Brazil*
[c] *UCLA Institute for Society and Genetics, USA*
[d] *Museu Paraense Emílio Goeldi, Brazil*
[e] *Universidade Federal do Amazonas, Brazil*
[f] *Universidade Federal de Goiás, Regional Jataí, GO, Brazil*
[g] *Universidade Federal de Viçosa, Viçosa, MG, Brazil*
[h] *Global Wildlife Conservation, USA*
[i] *Instituto de Desenvolvimento Sustentável, Mamirauá, Brazil*
[j] *Stony Brook University, USA*
[k] *Universidade Federal do Mato Grosso, Brazil*
[l] *PPG Ecologia e Conservação, Universidade do Estado do Mato Grosso, Brazil*
[m] *Universidade Federal do Pará, Brazil*

A R T I C L E I N F O

Keywords:
Callicebinae
Plecturocebus
moloch
New species
Alta Floresta
Amazon

A B S T R A C T

The taxonomy of the titi monkeys (Callicebinae) has recently received considerable attention. It is now recognised that this subfamily is composed of three genera with 33 species, seven of them described since 2002. Here, we describe a new species of titi, *Plecturocebus,* from the municipality of Alta Floresta, Mato Grosso, Brazil. We adopt an integrative taxonomic approach that includes phylogenomic analyses, pelage characters, and locality records. A reduced representation genome-wide approach was employed to assess phylogenetic relationships among species of the eastern Amazonian clade of the *Plecturocebus moloch* group. Using existing records, we calculated the Extent of Occurrence (EOO) of the new species and estimated future habitat loss for the region based on predictive models. We then evaluated the species' conservation status using the IUCN Red list categories and criteria. The new species presents a unique combination of morphological characters: (1) grey agouti colouration on the crown and dorsal parts; (2) entirely bright red-brown venter; (3) an almost entirely black tail with a pale tip; and (4) light yellow colouration of the hair on the cheeks contrasting with bright red-brown hair on the sides of the face. Our phylogenetic reconstructions based on maximum-likelihood and Bayesian methods revealed well-supported species relationships, with the Alta Floresta taxon as sister to *P. moloch* + *P. vieirai*. The species EOO is 10,166,653 ha and we predict a total habitat loss of 86% of its original forest habitat under a "business as usual" scenario in the next 24 years, making the newly discovered titi monkey a Critically Endangered species under the IUCN A3c criterion. We give the new titi monkey a specific epithet based on: (1) clear monophyly of this lineage revealed by robust genomic and mitochondrial data; (2) distinct and diagnosable pelage morphology; and (3) a well-defined geographical distribution with clear separation from other closely related taxa. Urgent conservation measures are needed to safeguard the future of this newly discovered and already critically endangered primate.

---

[*] Corresponding author at: School of Environment and Life Sciences, University of Salford, Salford M5 4WT, United Kingdom.
E-mail address: j.p.boubli@salford.ac.uk (J.P. Boubli).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ympev.2018.11.012
Received 14 July 2018; Received in revised form 7 November 2018; Accepted 16 November 2018

1. Introduction

Hershkovitz (1963) provided the first modern revision of the titi monkeys, *Callicebus* Thomas, 1903, indicating just three species: *torquatus* (three subspecies), *moloch* (seven subspecies), and *personatus* (three subspecies). His subsequent revisions (Hershkovitz, 1988, 1990) raised the number of species to 13, with 25 taxa overall: *torquatus* (six subspecies), *moloch*, *cinerascens*, *hoffmannsi* (two subspecies), *brunneus*, *cupreus* (three subspecies), *oenanthe*, *caligatus*, *dubius*, *donacophilus* (two subspecies), *olallae*, *modestus*, and *personatus* (four subspecies). Kobayashi and Langguth (1999) argued that the four subspecies of *personatus* (from the Atlantic forest and Caatinga of eastern Brazil), should be considered full species, and described a fifth closely related species (*coimbrai*). Van Roosmalen et al. (2002) described two new species (*stephennashi* and *bernhardi*) from the Madeira River basin and then recognised all listed titi monkey taxa as species, a stance accepted by Groves (2001, 2005). A further five species were described after the compilation of Van Roosmalen et al. (2002) taking the number of titi monkey taxa to 34 (all species), although it has been suggested that *dubius* is a junior synonym of *caligatus* (Byrne et al., 2016). Byrne et al. (2016) carried out the first appraisal of the phylogenetic relationships and divergence times and found that molecular, morphological, karyotypic, and biogeographic evidence support the division of titi monkeys into three genera: *Callicebus* Thomas, 1903, for the eastern Brazilian titis (type species *Callicebus personatus*); *Cheracebus* (Byrne et al., 2016), for the collared or widow titis of the Orinoco, Negro and upper Amazon basins (type species *Cheracebus lugens*); and *Plecturocebus* (Byrne et al., 2016), for the titis of the Amazon and the Chaco (type species *Plecturocebus moloch*).

Plecturocebus bernhardi, *P. vieirai*, and *P. moloch*, form what Byrne et al. (2018) refer to as the eastern Amazon clade of the *moloch* group. In this study, we describe a new species from this clade (Fig. 1). It was first recorded in the municipality of Alta Floresta, southern Amazon, Brazil (henceforth the Alta Floresta taxon). The town, founded in 1976 as a gateway to the colonization of the upper Rio Tapajós basin, straddles the Rio Teles Pires. There have been intensive surveys of the avifauna there since 1989, and Lees et al. (2013) describe the region and its biogeographical significance. It is today located in the centre of the arc of deforestation; the crescent-shaped, advancing agricultural frontier that is rapidly engulfing the southeast of the Amazon.

Byrne et al. (2016, 2018) found preliminary molecular and biogeographic evidence that suggest that this titi is a distinct and diagnosable taxon (referred to as *Plecturocebus* cf. *moloch* in these studies). Here, we formally describe this new taxon and give it a specific epithet. We adopt an integrative taxonomic approach (Dayrat, 2005) that includes evidence from phylogenomics, pelage characters, and locality records. Assessment of the pelage colour patterns of the titis follows the methodology employed in the description of *P. vieirai* (Gualda-Barros et al., 2012), and *P. miltoni* (Dalponte et al., 2014). We use a reduced representation, genome-wide approach (double digest restriction associated DNA sequencing, or ddRADseq) to assess phylogenetic relationships between the Alta Floresta taxon and the other species of the eastern Amazon clade of the *moloch* group (*sensu* Byrne et al., 2018).

Fig. 1. The Alta Floresta titi monkey. Photo by Fabiano Melo.

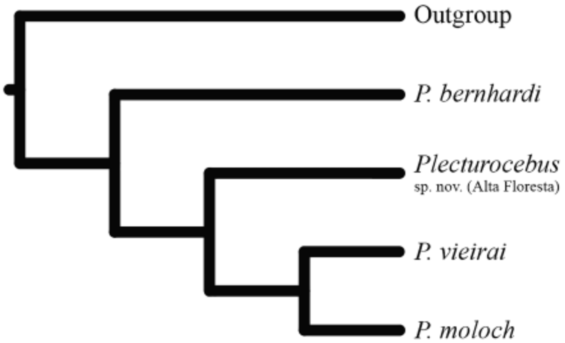


Fig. 2. Phylogenetic reconstruction showing species relationships among the eastern Amazon clade of the *moloch* group. All nodes are recovered with perfect support across all analyses, see Supp. Fig. 1 for the full phylogenies.

We propose a distribution for the new species based on available localities, and calculate its extent of occurrence (EOO; IUCN, 2012). Using predictive models of future forest loss in the Amazon, we estimate the extent of the habitat that will be lost for the Alta Floresta taxon by 2042 (or 24 years, equivalent to three titi monkey generations) under the “governance” and the more realistic “business as usual” scenarios (see Soares-Filho et al., 2006). Using the results of these predictive models, we evaluate the species’ conservation status using the IUCN Red list categories and criteria (IUCN, 2012).

2. Materials & methods

2.1. Morphological analysis

We examined skins and skulls deposited in the mammal collections of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, the Universidade Federal do Mato Grosso (UFMT), Cuiabá, and the Museu Paraense Emílio Goeldi (MPEG), Belém. The type series of the Alta Floresta lineage consists of six specimens deposited in these three collections. Comparisons were centred on species of the eastern Amazon clade of the *moloch* group, *P. moloch*, *P. vieirai*, and *P. bernhardi,* i.e., titi species phylogenetically most closely related to the Alta Floresta lineage (Byrne et al., 2016, 2018; Carneiro et al., 2016). The Alta Floresta lineage specimens were also compared with early diverging lineages of the *moloch* group: *P. hoffmannsi*, *P. miltoni*, *P. cinerascens* and with *P. baptista* (the last not analysed by Byrne et al., 2016, 2018; or by Carneiro et al., 2016). More general comparisons were also made with the other *Plecturocebus* species belonging to the western Amazon clade of the *moloch* group. Published descriptions of relevant specimens were also used for comparative purposes (e.g., Elliot, 1913; Hill, 1960; Hershkovitz, 1963, 1988, 1990; Van Roosmalen et al., 2002; Groves, 2001, 2005; Wallace et al., 2006; Defler et al., 2010; Gualda-Barros et al., 2012; Dalponte et al., 2014; and Vermeer and Tello-Alvarado, 2015). We also assessed skins of the type specimens for *emiliae* (Thomas, 1911), and *remulus*

Table 1
Summary of the final assembled ddRADseq datasets, including number of loci, parsimony informative (PI) sites, concatenated length, and dataset usage.

Dataset	No. samples	Min. coverage	No. loci	Length (bp)	Variable (bp)	Pis (bp)	Usage
divtime	13	6	466	143,563	8863	3272	BEAST
structure	16	8	504	162,962	4139	1089	Parallel-Structure
main	17	8	596	192,010	5148	1337	RAxML, MrBayes
main + RVR68	18	8	610	196,315	5358	1361	RAxML, MrBayes
main6min	17	6	1746	556,892	13,038	3112	RAxML, MrBayes

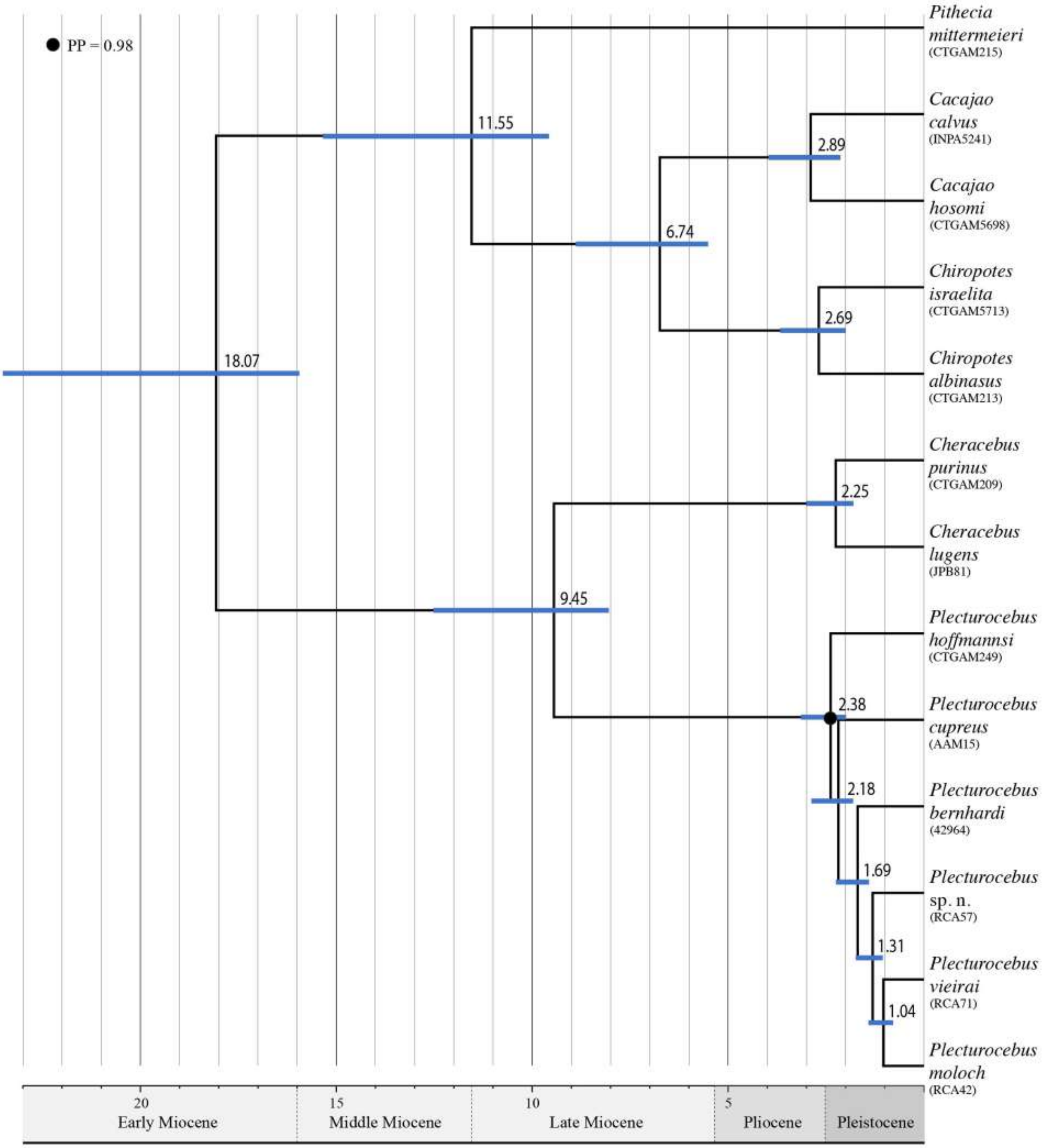


Fig. 3. A time-calibrated phylogeny for Callicebinae inferred with the ddRADseq “divtime” dataset. Unmarked nodes received full support (PP = 1.00), the black circle indicates PP = 0.98. Node bars indicate the 95% highest posterior density (HPD).

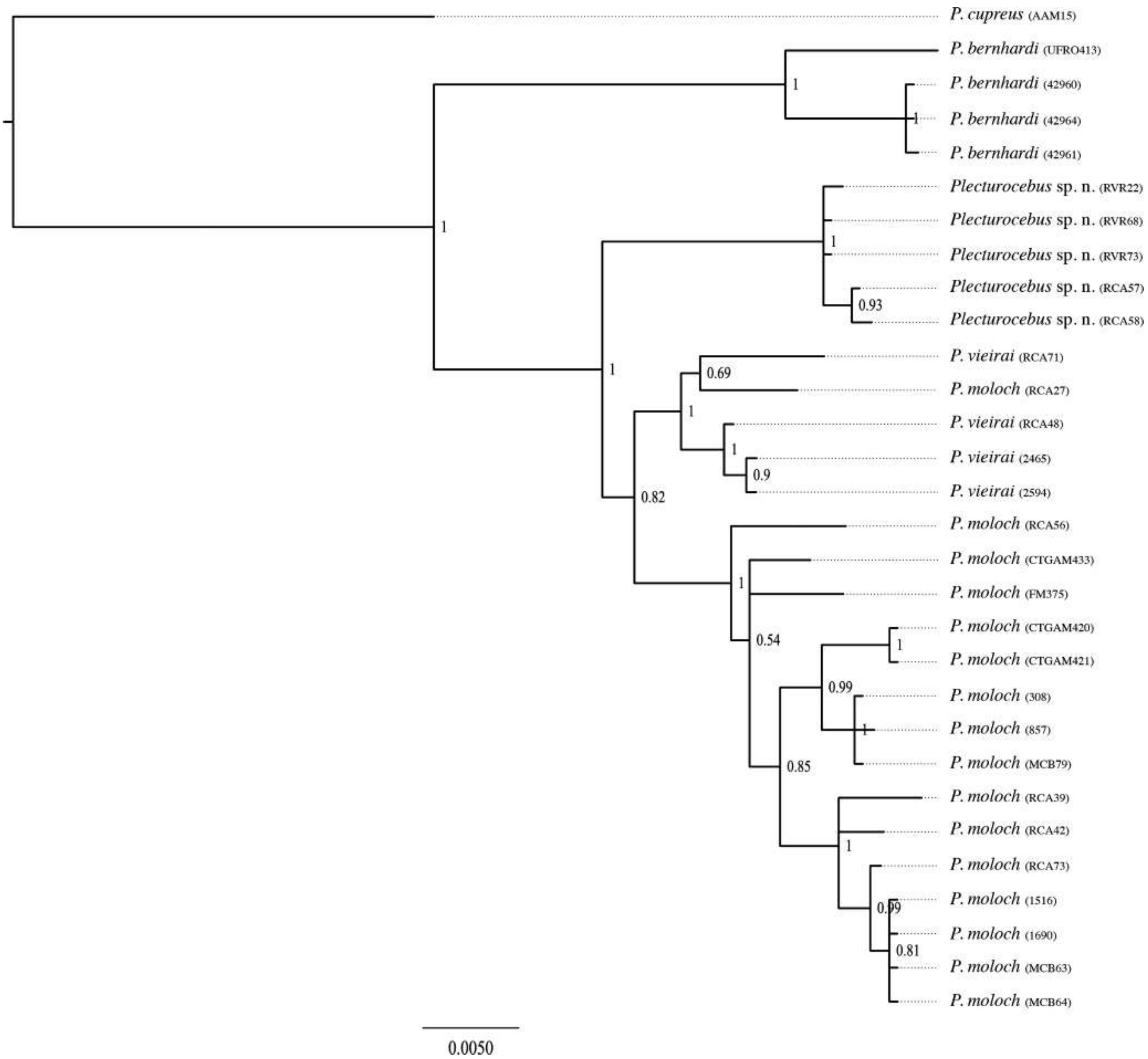


Fig. 4. Bayesian phylogeny for the eastern Amazon species of the *moloch* group inferred with the mitochondrial dataset.

(Thomas, 1908), in the Natural History Museum, London.

To describe the pelage patterns and colours, we used the same 10 chromatic regions used by Gualda-Barros et al. (2012) and Dalponte et al. (2014), as follows: (1) crown; (2) forehead; (3) sides of the face; (4) dorsum; (5) flanks; (6) throat, thorax and abdomen; (7) hands and feet; (8) limbs (external side); (9) limbs (inner side); and (10) tail. All data were collected in daylight conditions using the colour codes of Munsell Soil Colour Charts (2000).

2.2. Molecular analysis: ddRADseq

Twenty-six fresh tissue samples were collected from museum voucher specimens deposited in the following Brazilian institutions: Instituto Nacional de Pesquisas da Amazônia (INPA), Universidade Federal do Amazonas (UFAM), Universidade Federal de Rondônia (UNIR), and Museu Paraense Emílio Goeldi (MPEG). The majority of these specimens were obtained during an Amazon-wide faunal inventory project (CNPq/SISBIOTA) and recent fieldwork in Amazonia, carried out in accordance with the appropriate collection permits (IBAMA 483 license No. 005/2005 – CGFAU/LIC, ICMBio, SISBIO collecting license number 32095-1). This included 19 samples from six *Plecturocebus* taxa [*P. moloch* (N = 8), *P. vieirai* (N = 2), *P. bernhardi* (N = 3), Alta Floresta taxon (N = 4), *P. cupreus* (N = 1) and *P. hoffmannsi* (N = 1)], all of known provenance and morphologically identified (see Supp. Table 1). Two species of *Cheracebus* and five species of the subfamily Pitheciinae were included as outgroups to provide a fossil-based calibration point.

Laboratory procedures were performed at the Laboratory of Evolution and Animal Genetics (LEGAL) at the Universidade Federal do Amazonas (UFAM), Manaus, Brazil. Total genomic DNA was extracted

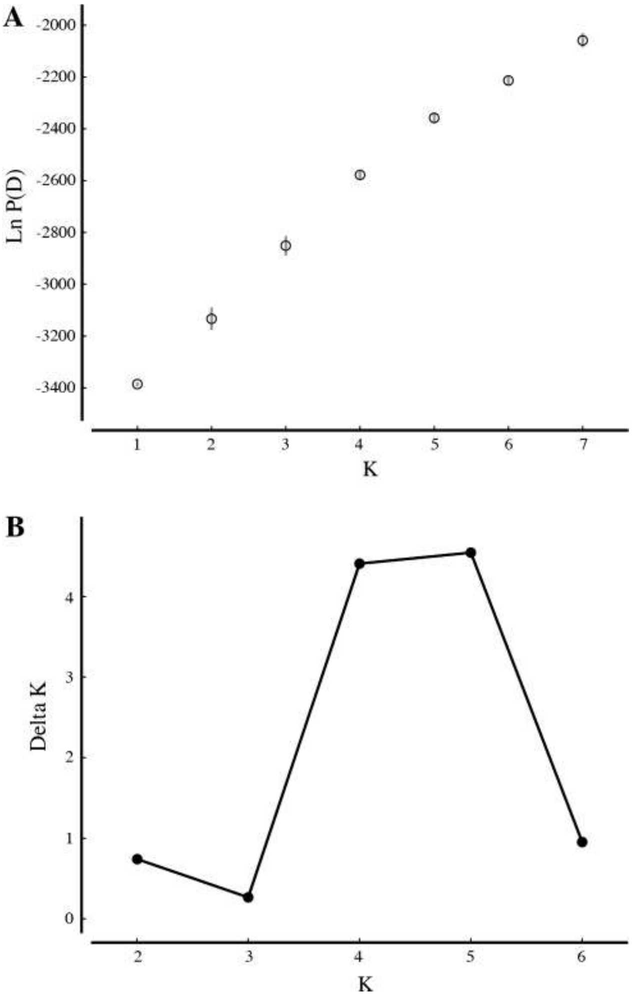


Fig. 5. (A) Mean likelihood [LnP(D) ± SD]. (B) ΔK calculated for the "structure" dataset using ParallelStructure from 6 independent runs for each value of K from 1 to 7.

from blood or muscle tissue using the standard phenol-chloroform extraction protocol of Sambrook et al. (1989). The concentration of the extracted DNA was quantified using a Nanodrop 2000 spectrophotometer (Thermo Scientific). A partial representation genomic library was constructed using the double digest restriction-site-associated DNA sequencing protocol (ddRADseq) (Peterson et al., 2012). This protocol was adapted to allow simultaneous digestion and adapter ligation, and data generation on the IonTorrent PGM (Hrbek et al., 2018; see also https://github.com/legalLab). Briefly, 200 ng of genomic DNA of each individual was digested with SdaI and Csp6I restriction enzymes (ThermoFisher) and the IonTorrent P and A adapters were ligated to the digested fragments, all in one step. The fragments were enriched via PCR; the A adapter contains a unique molecular barcode for identification of individuals and is a divergent "Y" adapter to ensure that only fragments with one P1 and one A adapter are enriched. DNA fragments in the range of 320 to 400 bp were selected using the Pippin Prep (Sage Science), owing to the ability of the IonTorrent PGM to sequence fragments up to 400 bp. The ddRADseq library was sequenced on an Ion Torrent PGM (ThermoFisher) using the 400-bp PGI 318 Ion PGM sequencing kit following manufacturers' recommendations.

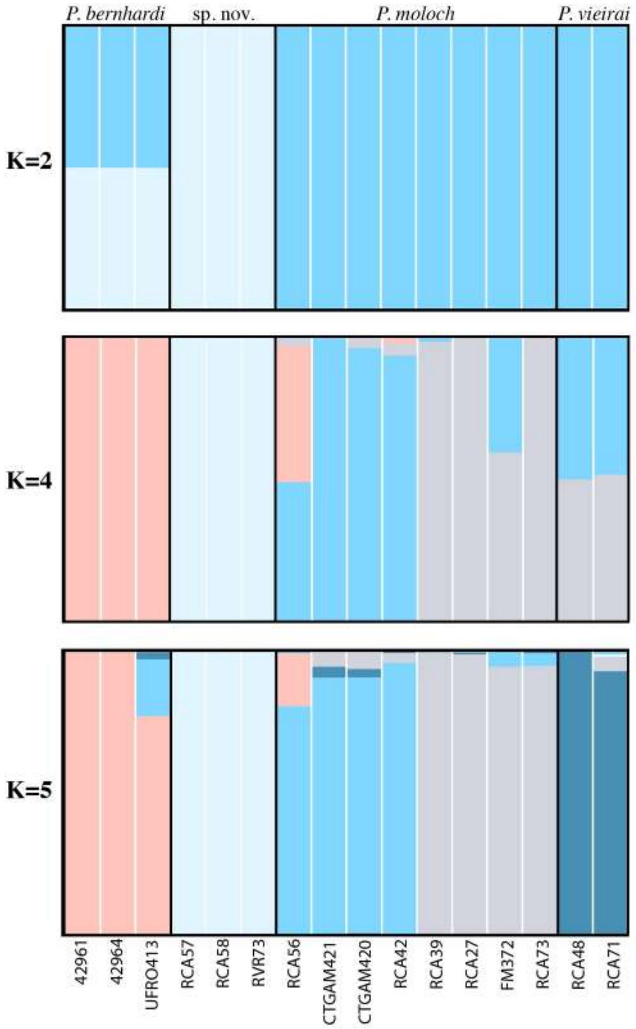


Fig. 6. Genetic structure of the eastern Amazon clade of the *moloch* group inferred from the "structure" dataset using Bayesian clustering analyses for K = 2, 4 and 5. Sample IDs are shown.

Raw sequence data were demultiplexed, quality filtered and assembled into *de novo* loci using the iPyRAD v.0.7.22 pipeline (https://github.com/dereneaton/ipyrad). Bases with a Phred quality score of less than 20 were turned into undetermined sites (Ns), reads were processed using the strict filter setting (= 2) with raw edges trimmed for quality, and reads with more than six undetermined sites (Ns) were discarded. These filter settings are considered to be strict in light of the 300–400 bp length of the sequenced fragments and the slight systematic underestimation of base call accuracy by the IonTorrent PGM (e.g., Bragg et al., 2013). Quality filtered reads were clustered within samples at 85% similarity. Clusters were retained if the minimum depth of coverage was at least 5×, and if the consensus sequence contained no more than ten undetermined sites (Ns), 12 heterozygous sites, and two alleles after error correction.

The generation of quality-filtered consensus sequences for within-sample clusters (steps 1–5 in iPyRAD) was performed once for all sequenced individuals. Final datasets with different combinations of taxa for downstream analyses were then constructed by clustering consensus sequences at 85% similarity across samples (steps 6 and 7 in iPyRAD) with the target individuals included. For divergence time estimation, a dataset labelled "divtime" was generated containing 13 samples with one sample from each represented species of *Plecturocebus*, and all

Fig. 7. *Plecturocebus grovesi* sp. nov. Drawing by Stephen D. Nash, used with permission.

Cheracebus and pitheciine species as outgroups. A dataset labelled "structure" was generated for a downstream Bayesian model-based clustering analysis containing all 16 specimens (except RVR68, see below) from the eastern Amazon clade of the *moloch* group (*P. bernhardi*, *P. moloch*, *P. vieirai*, and the Alta Floresta taxon). For phylogenetic inference, we generated a dataset labelled "main" containing 17 individuals, adding one *P. cupreus* sample as an outgroup to the "structure" dataset. A locus was only represented in these three datasets if it was recovered for at least ~50% of the individuals in that dataset. We also generated a larger, sparser dataset for phylogenetic inference labelled "main6min". This dataset included the same individuals as the "main" dataset, however, a locus was represented if it was recovered for ~35% of the individuals. In addition, although the specimen RVR68 from Alta Floresta was sequenced, the raw reads were of low quality and very unevenly distributed across loci, thus it was not included in these datasets. To confirm monophyly of the Alta Floresta taxon, we generated an additional dataset labelled "main + RVR68" using the exact settings from the "main" dataset but including the RVR68.

Phylogenetic inference was conducted using maximum-likelihood (ML) and Bayesian methods for the "main", "main6min", and "main + RVR68" datasets. Maximum likelihood phylogenies were inferred using RAxML v. 8.2.10 (Stamatakis, 2006, 2014) with the GTR + G (gamma) substitution model (given the quantity of data) and 1000 bootstrap replicates integrated with 200 searches for the optimal tree. Bayesian analyses were performed using MrBayes 3.2.3 (Ronquist et al., 2012) with the GTR + G substitution model. MCMC (Markov Chain Monte Carlo) convergence was checked after two independent four-chain runs of five million generations for each Bayesian inference. Convergence was assessed by examining LnL, the average standard deviation of the split frequencies between the two simultaneous runs (< 0.01), and the Potential Scale Reduction Factor (PSRF) diagnostic in MrBayes, after a burn-in of 10%.

Phylogeny and diversification times were jointly estimated for the "divtime" dataset under an uncorrelated lognormal relaxed clock model in the program BEAST v.1.8.4 (Drummond et al., 2012). A Yule speciation process and the GTR + G substitution model were used, and the ucld.mean prior was set to a diffuse gamma distribution (shape = 0.001; scale = 1000). Two replicate runs of 20 million MCMC generations, sampling every 2000 generations, were conducted. The sampling distributions of each run were visualised using Tracer v.1.6 to evaluate convergence and to verify that the effective sample size was > 200 for all parameters after a burn-in of 10%. Independent runs were combined with 10% burn-in discarded using LogCombiner v.1.8.4 and the maximum credibility tree was generated in TreeAnnotator v.1.8.4 (Drummond et al., 2012). To obtain the posterior distribution of the estimated divergence times, one calibration point on the root node (Callicebinae/Pitheciinae) was implemented with a lognormal distribution to set hard minimum and soft maximum bounds. A minimum age of 15.7 Ma was used based on the fossil *Proteropithecia* (Kay et al., 1998) (Kay et al., 1998, 1999), and a soft maximum bound was set at 26 Ma based on the fossil *Branisella boliviana* Hoffstetter, 1969, from the Deseadan fauna of La Salla (MacFadden, 1990). The standard deviation (= 0.8) and mean (= 1.016) were set such that 95% of the prior distribution falls before the maximum age to create the soft maximum bound (see Byrne et al., 2016). All phylogenetic analyses were run on the CIPRES Science Gateway v 3.3 server (Miller et al., 2010).

A Bayesian model-based clustering method was applied to investigate genetic structure among members of the eastern Amazon clade of the *moloch* group using ParallelStructure v.2.3.4 (Besnier and Glover, 2013), a multi-core implementation of STRUCTURE (Pritchard et al., 2000), on the CIPRES Science Gateway v 3.3 server. One single nucleotide polymorphism (SNP) per locus was selected and data matrices of unlinked SNPs (498 SNPs in length) in coded SNP Structure-format files were generated for the "structure" dataset by iPyRAD. All ParallelStructure analyses were conducted with the admixture model and no putative origins specified for individuals. Six runs at each value of K (ranging from one to seven) were performed with a burn-in of 100,000 steps and MCMC length of 500,000 steps. STRUCTURE HARVESTER (Earl and von Holdt, 2012) was used to examine LnP(D) and ΔK for each possible number of clusters (K). The program CLUMPP (Jakobsson and Rosenberg, 2007) was used to combine and average individual assignment probabilities across all replicates, and individual Q values were plotted and visualised using DISTRUCT 1.1 (Rosenberg, 2004).

2.3. Molecular analysis: Mitochondrial data

We also generated a mitochondrial dataset with the same individuals as in the ddRADseq dataset, as well as other individuals of the eastern Amazonian *P. moloch* clade with published sequence data (see Supp. Table 1). The complete cytochrome *b* (CYTB) locus was generated for the new specimens using the primers and amplification/sequencing strategy reported in Boubli et al. (2018). The cytochrome *c* oxidase I (COI) "barcode" was amplified for the new specimens using the C_VF1LFt1–C_VR1LRt1 primer cocktail and sequenced using the M13(−21) primer following Ivanova et al. (2007) and Ivanova et al. (2012). Consensus sequences were generated from forward and reverse reads using Geneious v7.4 (Kearse et al., 2012). Each locus was aligned independently for all specimens using the MUSCLE algorithm in Geneious v7.1 and subsequently combined in a matrix with a total alignment length of 1800 bp. The mitochondrial data matrix contained 29 individuals of the eastern clade of the *moloch* group, as well as a *P.*

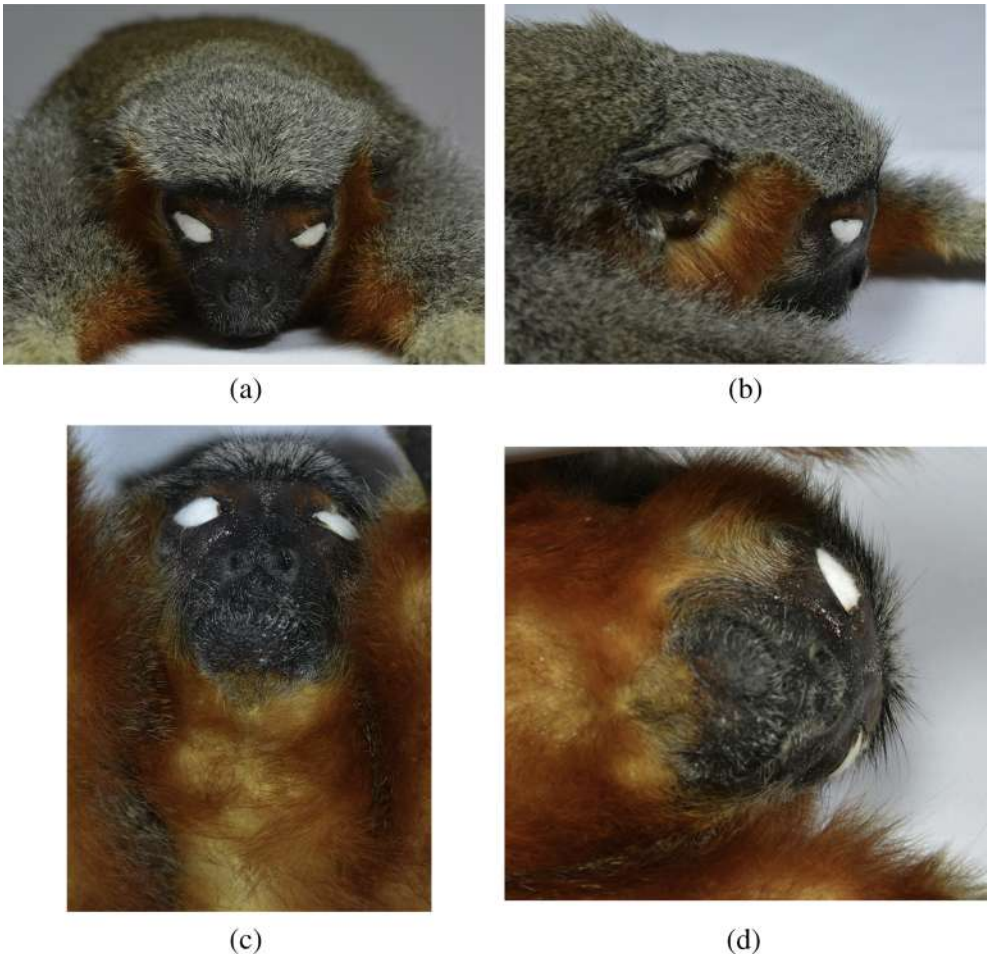


Fig. 8. Close up pictures of the head of the *Plecturocebus grovesi* sp. nov. INPA 7275 (holotype): (A) Face. (B) Side. (C) Chin and collar. (D) Cheek. Photographs by Ingrid Macedo.

cupreus specimen to root the phylogeny. GenBank accession numbers for all sequences are provided in Supp. Table 2. PartitionFinder (Lanfear et al., 2012) was used to determine the optimal partitioning scheme and the best-fit substitution models for each partition (locus and/or codon position) under the Bayesian information criterion (BIC). The PartitionFinder analyses were run using the all search algorithm and linked branch lengths.

Phylogenetic inference was conducted using maximum-likelihood and Bayesian methods. Maximum likelihood phylogenies were inferred using RAxML as outlined for the ddRADseq dataset, with a GTR + G substitution model for each codon position partition (optimal according to PartitionFinder). Bayesian analyses were performed using MrBayes 3.2.3 with substitution models selected by PartitionFinder for each codon partition (K80, HKY, HKY + G for codon position 1, 2, and 3, respectively). MCMC (Markov Chain Monte Carlo) convergence was checked after two independent four-chain runs of two million generations, as outlined above for the ddRADseq analyses.

2.4. Species distribution and threatened status evaluation

To map the distribution of the Alta Floresta taxon and calculate its extent of occurrence (EOO), we took as our base the available distribution map of *P. moloch* (*sensu lato*) from Veiga and Ferrari (2008). From this polygon we cropped what we supposed to be the distribution of *P. vieirai* and the Alta Floresta taxon based on our own field observations. We then calculated the total area of the extent of the Alta Floresta taxon by using the software QGIS 2.18.

We calculated total habitat loss to date in the newly determined range of the Alta Floresta taxon by using available historic and current satellite imagery published by PRODES (2018) for the years between 1997 and 2017. Using available forest cover projections of Soares-Filho et al. (2006), we calculated how much of the species' habitat will be lost by 2042 (in 24 years or three titi monkey generations) under two scenarios presented by the authors: (1) a "governance" scenario, i.e., assuming current deforestation trends, but with a 50% cap in forest loss due to current laws that prohibit farmers to clear more than 50% of forest in their properties, and that existing and proposed protected areas are effectively managed; (2) a "business as usual" scenario, i.e., considering current deforestation trends across the Amazon basin plus the effect of infrastructure development and low management effectiveness of protected areas. We calculated the total and relative (percentage) amount of forest loss within the Alta Floresta taxon's distribution under each scenario.

Using our data and results, we classified the taxon in terms of its threat category following the IUCN categories and criteria (IUCN, 2012). To meet the first level of a threat category, Vulnerable (VU), we should observe, estimate, infer or suspect reduction in EOO or habitat (a surrogate for population decline) of ≥30% over the longer of 10 years or three generations (IUCN, 2012). The threshold for Endangered is ≥50% and that for Critically Endangered is ≥80%.

3. Results

3.1. Molecular analyses

The ddRADseq samples had an average of 130K raw reads, which were subsequently quality filtered and clustered into an average of 10,271 stacks. Based on a minimum depth of 5×, an average of 2600 consensus sequences were retained per individual. These estimates exclude the most poorly sequenced sample, RVR68, which had 3056 stacks and 1291 consensus sequences. The larger "main6min" dataset contained 1746 ddRAD loci (with a total concatenated length of 557K bp), while the other four datasets contained between 466 and 610 loci (with a total concatenated length of 144K–196K bp). A summary of each dataset is presented in Table 1.

A total of seven phylogenetic trees were reconstructed using the concatenated ddRADseq data matrices; maximum-likelihood and Bayesian (MrBayes) trees were inferred for each of the "main", "main6min" and "main + RVR68" datasets, and phylogeny and diversification times were jointly estimated for the "divtime" dataset using BEAST. The species of the eastern Amazon clade of the *moloch* group comprise four monophyletic lineages across all analyses: *P. bernhardi*, *P. moloch*, *P. vieirai*, and the new taxon from Alta Floresta. *Plecturocebus bernhardi* is the earliest taxon to diverge, followed by the Alta Floresta lineage. *Plecturocebus moloch* and *P. vieirai* are sister species. These species relationships are recovered across all trees for all datasets with full statistical support (100% bootstrap percentage, 1.0 posterior probability).

A graphical representation is presented in Fig. 2, and the full maximum-likelihood and Bayesian phylogenies with support values for the

Fig. 9. Close up pictures of upper body, hand and foot of the *Plecturocebus grovesi* sp. nov. (A) Dorsal and ventral views of the hands and upper body of: (top) type series (from left to right) INPA 7275 (holotype), UFMT 3853 and UFMT 4015; (bottom) RCA 58 and RCA 59. (B) Detail of the hands of dorsal and palmar views of INPA 7275 (holotype) (top); dorsal (middle) and palmar (bottom) views of the hands of RCA 58 and RCA 59. (C) Detail of the feet of dorsal and plantar views of INPA 7275 (holotype) (top); dorsal (bottom left) and plantar (bottom right) views of the feet of RCA 58 and RCA 59. Photographs by Ingrid Macedo.

Fig. 9. (*continued*)

"main", "main6min" and "main + RVR68" datasets are presented in Supp. Fig. 1. The same or highly similar relationships among the samples within each species are reconstructed for all phylogenetic analyses; however, many intraspecific nodes are recovered without strong support. Although the topology among *P. moloch* individuals is the most poorly resolved, three distinct lineages are consistently recovered: the specimen from the right bank of the Xingu River is the earliest diverging lineage (or forms a polytomy in some MrBayes trees); a clade containing four specimens from the north of the Tapajós-Xingu interfluve; and a clade with three specimens from the south and west of the Tapajós-Xingu interfluve (see Supp. Fig. 1).

Divergence time estimates (Fig. 3) from the "divtime" dataset are largely comparable to other published studies (Byrne et al., 2016, 2018). The subfamilies Callicebinae and Pitheciinae diverged in the early Miocene, *c.* 18.1 Ma (95% HPD = 15.9–23.5), and *Cheracebus* diverged from the ancestor of the genus *Plecturocebus* (and *Callicebus*, not represented here) in the late Miocene, *c.* 9.5 Ma (95% HPD = 8.0–12.5). Among the *Plecturocebus moloch* group taxa included in this study, *P. hoffmannsi* is the earliest to diverge at *c.* 2.4 Ma (95% HPD = 2.0–3.1), followed by *P. cupreus c.* 2.2 Ma (95% HPD = 1.8–2.9). Among the eastern Amazon clade, *P. bernhardi* is estimated to have diverged from remaining taxa at 1.7 Ma (95% HPD = 1.4–2.3), followed by the divergence between the Alta Floresta lineage and the progenitor to *P. vieirai* + *P. moloch c.* 1.3 Ma (95% HPD = 1.1–1.7). The sister species, *P. vieirai* and *P. moloch,* are estimated to have diverged 1 Ma (95% HPD = 0.8–1.4). The collared titis of the genus *Cheracebus*, *C. lugens* and *C. purinus*, diverged *c.* 2.3 Ma (95% HPD = 1.8–3.0). For the pitheciine taxa, *Pithecia* diverged *c.* 11.6 Ma (95% HPD = 9.6–15.3), and subsequently, *Chiropotes* and *Cacajao* diverged *c.* 6.7 Ma (95% HPD = 5.5–8.9).

For the mitochondrial maximum-likelihood and Bayesian phylogenies, *P. bernhardi* is the earliest taxon to diverge, followed by the Alta Floresta taxon, and both divergences are recovered with strong support (see Fig. 4 for the Bayesian phylogeny and Supp. Fig. 2 for the maximum-likelihood phylogeny). One *P. moloch* individual (RCA27) formed a clade with the four *P. vieirai* specimens, and this clade was sister to all remaining *P. moloch* individuals but with low support (78% bootstrap percentage, 0.82 posterior probability).

Bayesian clustering analyses were performed using 498 unlinked SNPs mined by iPyRAD from the ddRADseq loci in the "structure" dataset. Mean likelihood increased with each added cluster from K = 1 to K = 7 (Fig. 5a), while the Evanno ΔK method selected K = 5, closely followed by K = 4 (Fig. 5b). When K = 2 is assumed, one genetic cluster corresponded to the Alta Floresta taxon and another to *P. moloch* + *P. vieirai*, while *P. bernhardi* showed equal ancestry in both clusters (Fig. 6). At K = 4, the Alta Floresta taxon and *P. bernhardi* each

Fig. 9. (*continued*)

formed distinct clusters, and *P. moloch* and *P. vieirai* individuals showed varying levels of ancestry in the remaining two clusters, with one *P. moloch* individual (RCA56) sharing ancestry in the *P. bernhardi* cluster (Q value = 0.48). When a fifth cluster was added (K = 5), *P. bernhardi*, *P. vieirai* and the Alta Floresta lineage each formed a cluster, while *P. moloch* individuals shared ancestry across two clusters (Fig. 6). At K = 5, most individuals had very high membership coefficients in their taxon's cluster (> 0.92). The exception was a *P. bernhardi* (UFRO413) individual that shared ancestry with a *P. moloch* cluster (Q value = 0.2), and a *P. moloch* individual (RCA56) that shared ancestry

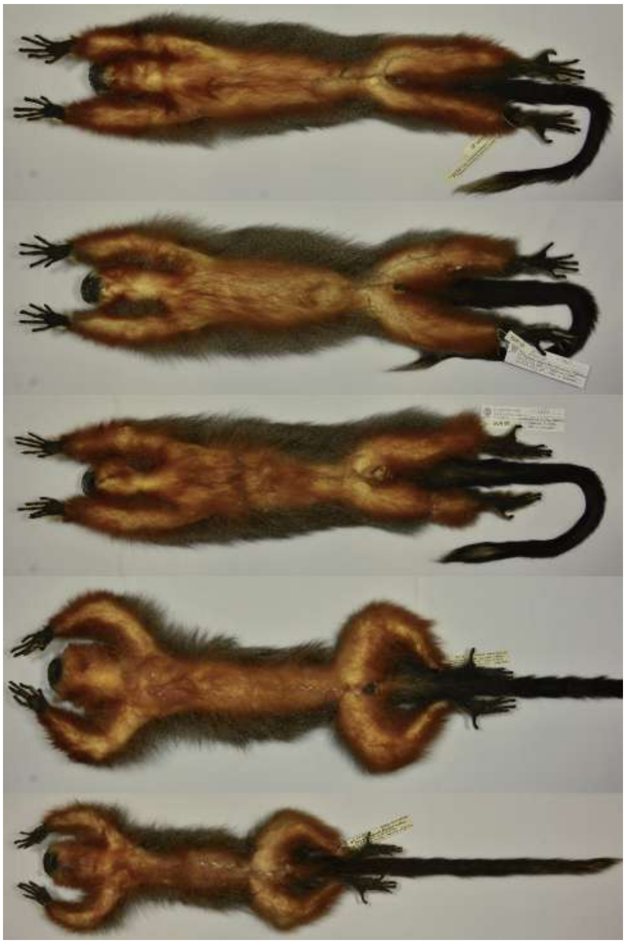


Fig. 10. Ventral view of the *Plecturocebus grovesi* sp. nov. (from top to bottom): INPA 7275 (holotype), UFMT 4015 and UFMT 3853 (paratypes), RCA 58 and RCA 59. Photos by Ingrid Macedo.

with *P. bernhardi* (Q value = 0.18). When five genetic clusters were assumed, the results of our Bayesian clustering analyses were concordant with our phylogenetic results, supporting the distinction between *P. bernhardi*, the Alta Floresta taxon, *P. vieirai* and *P. moloch* as represented in this study. Notably, the *P. moloch* specimen with the *vieirai*-like mitochondrial genome (RCA27) shared only a very small portion of ancestry in the *P. vieirai* cluster at K = 5 (Q value = 0.004). Although K = 6 is not supported by ΔK, the sixth cluster reveals genetic structuring within *P. bernhardi* that is consistent with the present and previous phylogenetic analyses (Byrne et al., 2016). At K = 6, the *P. bernhardi* individual from the Jiparaná-Roosevelt interfluve (UFRO413) and the individuals from the Guaporé Biological Reserve (south of the Rio Jiparaná; 42961, 42964) form distinct clusters with near perfect membership coefficients (results not shown).

In addition to the molecular phylogenomic evidence, morphological and geographic distribution data reject the recognition of the Alta Floresta specimens as belonging to any other species of the *moloch* group. In brief (discussed in detail below), the Alta Floresta taxon exhibits diagnostic morphological character states, including the overall greyish-agouti colour of the dorsal pelage and the contrasting colouration of hairs on face and cheeks, and is found only in the Juruena–Teles Pires interfluve, largely isolated from other *moloch* group species by these rivers (to west and east) and by the transition from Amazonian forest to Cerrado vegetation (in the south). Accordingly, we describe a new *Plecturocebus* species.

***Plecturocebus grovesi* sp. nov.** (Fig. 7).

3.1.1. Holotype

INPA 7275, field number RVR 22, skin, skull and skeleton, adult male, collected by Rogério Vieira Rossi in a forested area near Alta Floresta, left bank of the Rio Teles Pires, Mato Grosso state, Brazil (10°00′06.1″ S, 56°02′32.2″ W), 3 May 2014. Mammal collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil.

3.1.2. Type locality

The community of Novo Horizonte (10°00′06.1″ S, 56°02′32.2″ W), left bank of the Rio Teles Pires, municipality of Alta Floresta, Mato Grosso state, Brazil.

3.1.3. Paratypes

UFMT 3853, field number RVR 68, skin, skull and skeleton, adult female, collected by Rogério Vieira Rossi in a forested area near Alta Floresta (09°53′10.4″ S, 56°01′51.4″ W), left bank of the Rio Teles Pires, Mato Grosso state, Brazil, 9 May 2014. Mammal collection of the Universidade Federal do Mato Grosso, Cuiabá, Mato Grosso, Brazil.

UFMT 4015, field number RVR 73, skin, skull and partial skeleton, adult female, collected by Rogério Vieira Rossi in a forested area near Alta Floresta (09°59′02.3″ S, 56°04′22.2″ W), left bank of the Rio Teles Pires, Mato Grosso state, Brazil, 9 May 2014. Mammal collection of the Universidade Federal do Mato Grosso, Cuiabá, Mato Grosso, Brazil.

MPEG 39494, field number RA 32, skin only, sex unknown, collected by Ronaldo Alperin and Dionísio Pimentel in a forested area near Alta Floresta (09°54′S, 55°54′W – approx. coord.), left bank of the Rio Teles Pires, Mato Grosso state, Brazil, in October 1995. Mammal collection of the Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.

MPEG 24590, field number RA 34, skin and skull, adult female, collected by Ronaldo Alperin and Raimundo Rodrigues in a forested area near Alta Floresta (09°54′S, 55°54′W), left bank of the Rio Teles Pires, Mato Grosso state, Brazil, 14 October 1995. Mammal collection of the Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.

MPEG 24591, field number RA 35, skin and skull, adult male, collected by Ronaldo Alperin and Raimundo Rodrigues in a forested area near Alta Floresta (09°54′S, 55°54′W), left bank of the Rio Teles Pires, Mato Grosso state, Brazil, 14 October 1995. Mammal collection of the Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.

3.1.4. Description of the holotype

Sides of the face bright red-brown (Munsell Red 2.5YR 4/6), with yellowish cheek hairs; forehead with blackened narrow strip (approx. 0.5 cm) contrasting with agouti-grey crown (Fig. 8), slightly lighter than the grey of the legs and arms; ears with crown-like colouration; the back and flanks agouti-grey, with the central region along the back reddish brown, giving it a darker appearance than the sides; external parts of limbs agouti-grey, slightly contrasting with the back and flanks; extremities of the limbs (hands and feet) dirty white (Fig. 9), contrasting with the agouti-grey of the outer parts of the limbs; throat, chest, belly and internal parts of the limbs with bright red-brown colouration (Munsell Red 2.5YR 4/6), similar to the lateral colouring of the face (Fig. 10); black tail, distal 10 cm (approximately) is evident grey-white (Fig. 11).

3.1.5. Description of the paratypes

The variation observed in the paratypes was small, indicating that the characters are stable (Figs. 10, 12, Supp Figs. 3–5). The data

Fig. 11. Close up of the tail of the *Plecturocebus grovesi* sp. nov. (A) Detail of the dorsal (left) and ventral (right) views of the tail of INPA 7275 (holotype) (top). (B) Dorsal (left) and plantar (right) views of the feet of RCA 58. (C) Dorsal (left) and plantar (right) views of the feet of RCA 59. Photographs by Ingrid Macedo.

suggested no marked sexual dichromatism. Any observed variation appeared to be purely individual in nature.

Specimen MPEG 24590 differs from the holotype by having an agouti-grey back and flanks with the central region along the back only lightly reddish brown and the greyish-white distal part of the tail is more extensive and evident. The light grey colour of the crown is slightly darker on the specimens MPEG 24591 and 39494, slightly obscuring the sharpness of the black band of the forehead. These specimens have light yellow cheeks with a sharper contrast with the bright red-brown of the sides of the face and throat. Specimen MPEG 39494 has limbs with lighter agouti-grey outer parts and slightly lighter inner red-brown parts. Specimen MPEG 39494 has a poorly delineated margin between the proximal black parts and the light grey end of the tail. The light grey area of the tail tip of specimen MPEG 24591 is present only on its inner side.

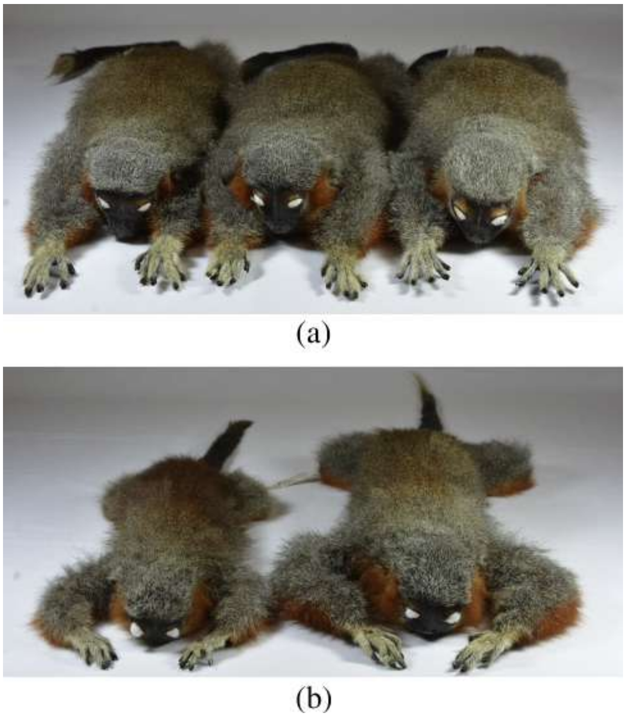
The grey colour of the crown in the specimens UFMT 3853 and UFMT 4015 was similar to that of the holotype (Fig. 13), which in all three specimens is lighter than the rest of the back and similar to the upper and lower limbs. Although it is present, the blackened band of the forehead of these two specimens is much less conspicuous than in the holotype (Fig. 8a), especially in UFMT 3853 (Fig. 12). Specimens UFMT 3853 and UFMT 4015 have light yellow cheeks (less pronounced on UFMT 3853), and more contrasting with the bright red-brown on the sides of the face and throat in UFMT 4015 (also in the holotype, INPA

Fig. 12. Dorsal view of the *Plecturocebus grovesi* sp. nov. (from top to bottom): INPA 7275 (holotype), UFMT 4015 and UFMT 3853 (paratypes), RCA 58 and RCA 59. Photographs by Ingrid Macedo.

7275) (Fig. 12). The outer part of the limbs of these specimens is agouti-grey, more similar to the colour of the head than to the back, which shows reddish tones (Fig. 11). Their tails have a weakly delineated margin between the proximal black part and the light grey extremity, which is visible in the inner and outer sides for about 12 cm or less (Figs. 14 and 15).

3.1.6. Diagnosis

Plecturocebus grovesi sp. nov. belongs to the *moloch* group (*sensu* Byrne et al., 2016). A combination of morphological characters gives the new species a unique appearance among all species of *Plecturocebus* (Fig. 16). The general colouration of the pelage is agouti-grey in the dorsal parts, with reddish tones in the median region. The agouti-grey of the back is darker than the lighter grey limbs, sides of the body, and crown. An agouti-black forehead contrasts with the agouti-grey crown, which, in turn, contrasts slightly with the back in not showing reddish tones. The sides of the face and ventral region (throat, thorax, abdomen and internal parts of the limbs) are a strong bright red-brown. The light yellow of the hairs on the cheeks is in contrast with the bright red-brown hairs on the sides of the face. The extremities (hands and feet) are lighter (dirty white), contrasting with the greyish colour of the external parts of the limbs. The tail is black but with a contrasting light grey tip (*c.* < 12 cm), the boundaries of which are diffuse.



(a)

(b)

Fig. 13. Frontal view of the *Plecturocebus grovesi* sp. nov. (A) Type series (from left to right) INPA 7275 (holotype), UFMT 3853 and UFMT 4015 (paratypes). (B) RCA 59 (left) and RCA 58 (right). Photographs by Ingrid Macedo.

3.2. Comparisons with species of the moloch group

Following Gualda-Barros et al. (2012) and Dalponte et al. (2014), we summarise in Table 2 the 10 chromatic characters used in this study for each of the compared species (see methods above). *Plecturocebus grovesi* sp. nov. resembles *P. moloch*, *P. vieirai*, *P. bernhardi*, *P. miltoni*, *P. hoffmannsi* and *P. baptista* in the general agouti-grey pattern on the dorsal surfaces (crown, dorsum, flanks and external parts of the limbs) that contrasts with the uniform reddish to brown of the ventral portions (sides of face, throat, thorax, abdomen and inner portion of limbs). This contrasting pattern varies in shades of grey and brown on the dorsum and in shades of yellow to ochre to red-brown on the ventral side of the other species.

Plecturocebus grovesi sp. nov. differs from *P. moloch* in the agouti-grey colouration of the crown and dorsal parts, with the median region of the back having more reddish tones instead of agouti-brownish. The ventral portion is entirely bright red-brown instead of light ochre (orange), a character state best observed in living specimens. The cheeks are light yellow in contrast with the throat and sides of the face, instead of uniform light ochre (orange) as in *P. moloch*. The tail is almost entirely black with only the tip being grey-white, while the tail is polymorphic in *P. moloch* with some specimens showing a blackish tail, often intermixed with pale agouti-brown to buffy at the tip and a greyish agouti base (see Fig. 17).

Plecturocebus grovesi sp. nov. differs from *P. vieirai* in the somewhat darker agouti-grey colouration of the dorsal parts instead of pale agouti-grey. The forehead has a contrasting thin black stripe in *Plecturocebus grovesi* sp. nov. and white band in *P. vieirai*. The sides of the face and throat are bright red-brown instead of white. The cheeks contrast with the throat and sides of the face instead of the uniform colouration of *P. vieirai*. The ventral parts and inner portion of the limbs are bright red-brown instead of light yellow to light orange. The tail is

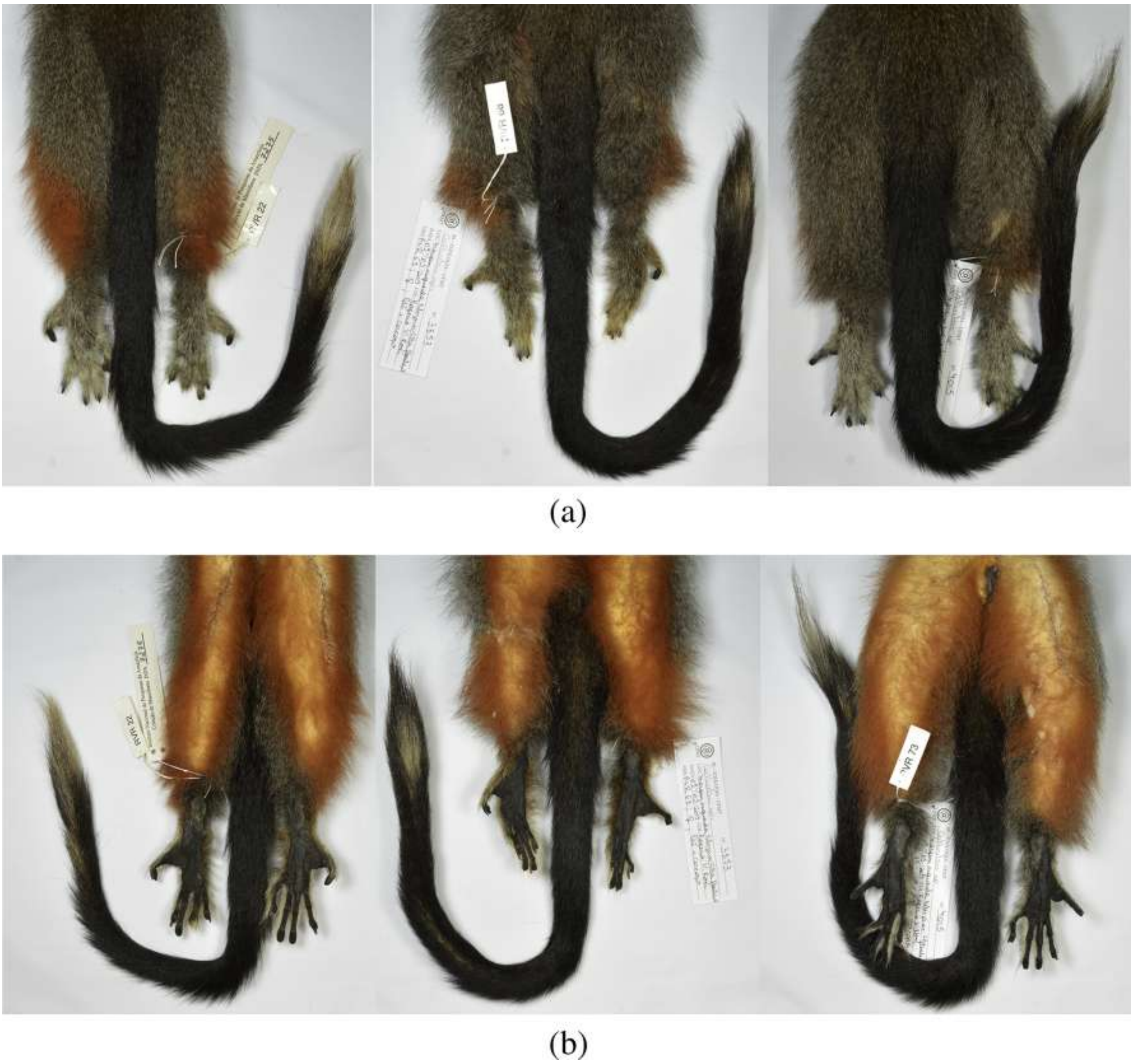


Fig. 14. Dorsal (A) and ventral (B) views of the tail of *Plecturocebus grovesi* sp. nov. Type series (from left to right): INPA 7275 (holotype), UFMT 3853 and UFMT 4015 (paratypes). Photographs by Ingrid Macedo.

black with a light white-grey end rather than the predominantly greyish tail with pale white-grey tip observed in *P. vieirai*.

Plecturocebus grovesi sp. nov. differs from *P. bernhardi* in the black stripe on the forehead, which contrasts with the crown, instead of the uniform dark grey agouti of *P. bernhardi*. The ventral parts are darker red-brown in *P. bernhardi* (Fig. 17b). The cheeks contrast with the throat and sides of the face instead of the uniform colouration of *P. bernhardi*. The general dorsal colouration is darker grey agouti in *P. bernhardi*.

Plecturocebus grovesi sp. nov. differs from *P. cinerascens* in the blackish stripe on the forehead, which contrasts with the crown instead of the uniform dark grey agouti of *P. cinerascens*. The ventral parts are bright red-brown rather than dark grey agouti, not contrasting with the dorsal parts. The cheeks contrast with the throat and sides of the face instead of the uniform agouti-grey. The hands and feet are dirty white instead of agouti-grey. The tail is black with only the light grey tip rather than evenly agouti-grey throughout.

Plecturocebus grovesi sp. nov. differs from *P. miltoni* in the uniform bright red-brown of the ventral parts instead of the dark ochre thorax and the light ochre abdomen observed in *P. miltoni.* The forehead has a contrasting thin black stripe in *P. grovesi* while *P. miltoni* shows a contrasting whitish-grey band. The cheeks contrast with the throat and sides of the face rather than the uniform colouration of *P. miltoni*. The tail is black with a light grey end instead of the uniformly orange tail of *P. miltoni*.

Plecturocebus grovesi sp. nov. is distinguished from *P. hoffmannsi* in bright red-brown instead of pale yellow ventral parts. The cheeks are light yellow contrasting with the throat and sides of the face rather than uniform light yellow. The forehead has a blackish stripe contrasting with the crown instead of the clearer and more uniform greyish agouti of *P. hoffmannsi*. The hands and feet are dirty white instead of agouti-grey. The tail is black with just the light grey end instead of blackish agouti from base to tip.

Plecturocebus grovesi sp. nov. is distinguished from *P. baptista* in the greyish agouti of the dorsum instead of dark brownish agouti. The ventral parts are bright red-brown instead of dark reddish brown. The cheeks are light yellow and contrast with the throat and sides of the face rather than the uniform reddish brown of *P. baptista*. The hands

Fig. 15. Skull of the adult male holotype of *Plecturocebus grovesi* sp. nov. (INPA 7275). Photographs by Ingrid Macedo.

and feet are off white instead of dark reddish brown speckled with grey. The tail is black with a light grey end instead of the uniform greyish agouti observed in *P. baptista*, which has a lighter grey tail than the dorsum.

Plecturocebus grovesi sp. nov. differs entirely from the remaining species of the *moloch* group in the greyish agouti of the dorsum with reddish tones in the median region, which contrasts with the bright red-brown ventral parts, instead of the general dark brown to reddish brown pattern observed in the western clade of the *moloch* group (*P. brunneus*, *P. cupreus*, *P. ornatus*, *P. discolor*, *P. caquetensis*, *P. caligatus*, *P. stephennashi*, *P. aureipalatii*, *P. toppini*).

3.3. Synonyms of Plecturocebus moloch

Three previously described forms are now recognised as junior synonyms of *P. moloch*: *emiliae* (Thomas, 1911); *geoffroyi* (Miranda Ribeiro, 1914); and *remulus* (Thomas, 1908). The form described as *remulus* by Thomas (1908) appears to be a near typical *P. moloch* and a true junior synonym of this taxon. Thomas appears not to have known that it had been described previously by Hoffmannsegg (1807). The *remulus* type specimen housed at the Natural History Museum, London, was collected at Santarém, Pará, within the recognised range of *P. moloch*. Lönnberg (1939) considered it to be a junior synonym of *moloch*, and this has been followed by most authors since [e.g., Cabrera (1958), Hershkovitz (1963, 1990), de Vivo (1985) and Groves (2001)].

The type specimen for *emiliae* is also housed at the Natural History Museum, London. The individual was found in zoological gardens in Pará with no location data. It is phenotypically distinct from *Plecturocebus grovesi* sp. nov. in the strong chestnut-maroon of the dorsum, extending from the nape to the base of the tail, and the upper surface of the limbs are brownish agouti rather than grey agouti. Cabrera (1958), Hershkovitz (1963, 1990), and Groves (2001) considered it to be a junior synonym of *P. moloch.*

The form named *geoffroyi* by Miranda Ribeiro in 1914 was not formally described, but Miranda Ribeiro (1914; p.19) indicated its similarity to a plate in Geoffroy Saint Hilaire (1844), which he believed

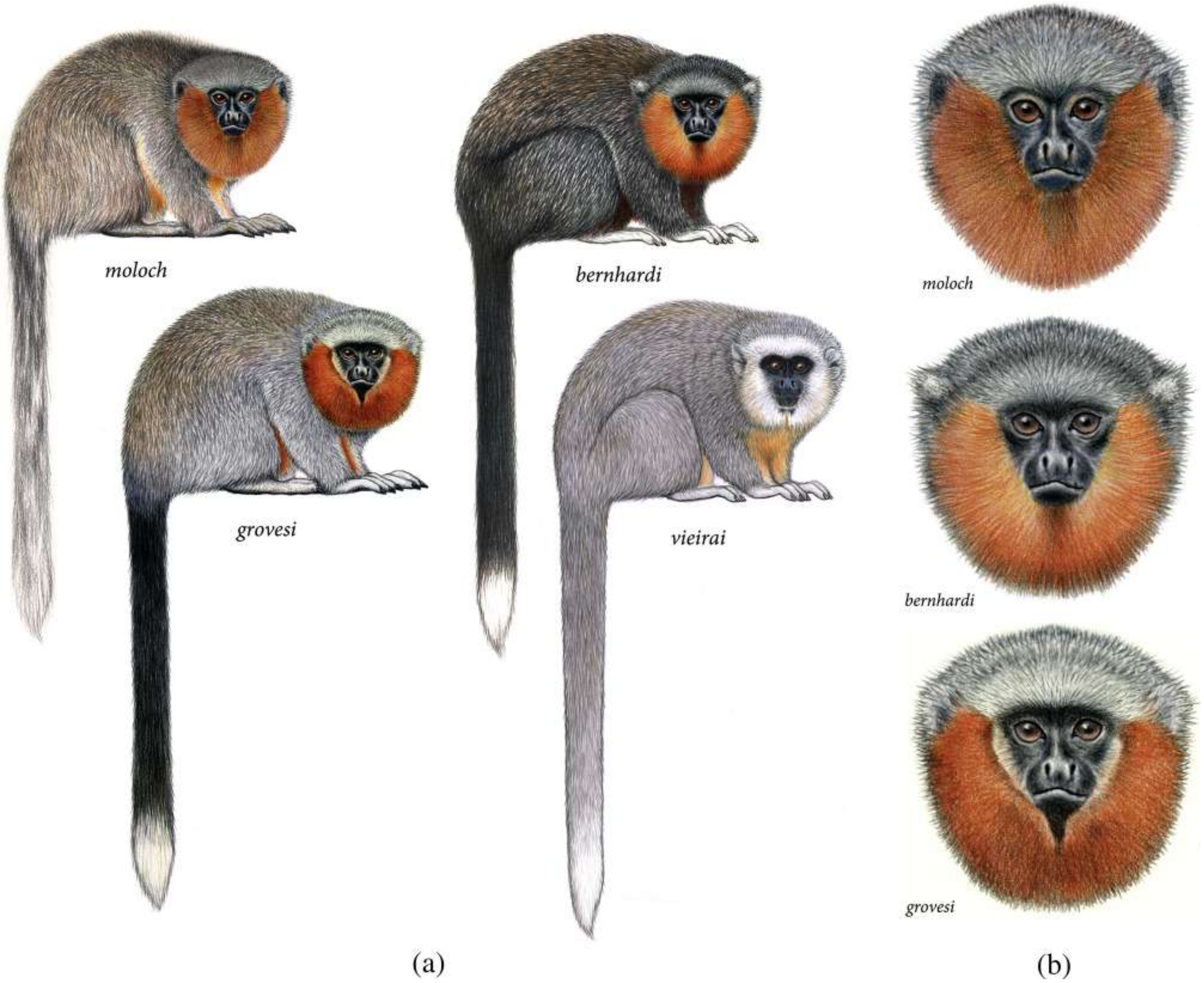


Fig. 16. The four species of the Eastern Amazon *moloch* clade (Byrne et al., 2016): (A) Full body array. (B) Faces. Drawing by Stephen D. Nash, used with permission.

was not *moloc*h Hoffmannsegg, 1807. Miranda Ribeiro first received a specimen of *geoffroyi* from an Indian at the Porto da Passagem, Pimenta Bueno, Rondônia. It lacked, however, a large part of its head and was considered useless. Miranda Ribeiro received a second specimen from the same Indian of what he believed to be the same titi, at Urupá, Rondônia, which is within what is now believed to be the distribution of *P. bernhardi* (see Byrne et al., 2018; Suppl. File 2). However, the lack of a formal description and any type specimen or specific provenance would suggest that this name should be considered a *nomen nudem* rather than a junior synonym of *P. moloch.* It might be a senior synonym of *P. bernhardi,* but like *Plecturocebus grovesi* sp. nov., *bernhardi* typically has a black tail with a whitish tip, a feature evidently lacking in *geoffroyi*. Prior to the description of *bernhardi*, *geoffroyi* was considered a junior synonym of *remulus* by Cruz Lima (1945) and Vieira (1955), of *donacophilus* by Cabrera (1958) and Hershkovitz (1963), and of *moloch* by Hershkovitz (1988), de Vivo (1985) and Groves (2001).

A combination of field studies with a phenotypic assessment of all known red-bellied museum specimens collected east of the Rio Madeira, complemented by molecular analyses, would shed light on the considerable phenotypic variation found in the taxa of the eastern clade of *Plecturocebus*.

3.4. Distribution and conservation status

Plecturocebus grovesi sp. nov. is found in the state of Mato Grosso, Brazil, in the region delimited by the rios Juruena and Arinos to the west and the Rio Teles-Pires to the east (Fig. 18). To the south, we suggest that the Amazon forest to Cerrado ecotone delimits its range (~13°S). More surveys however, are necessary to confirm the southern limits as our southernmost record is ~10°S (see Gazetteer). The area of occurrence of *Plecturocebus grovesi* sp. nov. is between that of *P. cinerascens* to the west and *P. vieirai* to the east (see updated hypothesised distributions for these taxa in Byrne et al., 2018; Suppl. File 2). We calculated the species' extent of occurrence (EOO) to be 101,667 km^2.

We estimated a loss of 42% of habitat (forest, excluding savannas) within the species range to date, or 39% of the species' total range (forest and savannas) (Table 3). In our predictive models, the species' habitat loss over the next 24 years will amount to 50% under the "governance" scenario (Table 4), and to 86% under the "business as usual" scenario (Table 5, Fig. 19).

Due to the on-going process of Protected Area downgrading, downsizing, and degazettement (PADDD) in the Brazilian Amazon and the planned hydroelectric complex of dams for this region (Bernard

Table 2
Summary of the 10 chromatic characters used in this study.

	Plecturocebus grovesi sp. nov.	*P. vieirai*	*P. moloch*	*P. bernhardi*	*P. miltoni*	*P. cinerascens*	*P. hoffmannsi*	*P. baptista*
Crown	Uniform grey agouti	Light grey agouti	Grey agouti	Dark grey agouti	Dark grey agouti	Grey agouti	Grey agouti	Blackish agouti
Forehead	Forehead with thin black narrow stripe contrasting with grey agouti crown	White or white agouti	Grey agouti	Dark grey agouti	Dark grey agouti, with whitish stripe contrasting with the crown	Grey agouti	Grey agouti	Blackish agouti
Sideburns	Bright red-brown contrasting with light yellow cheek hairs	White	Light ochre (orange)	Dark red-brown	Dark ochre (orange-brown)	Grey agouti	Yellowish	Grey agouti
Dorsum	Grey agouti slightly contrasting with external parts of the limbs	Light grey-brown agouti to light brown	Brown-grey agouti to light brown	Dark grey agouti	Uniform dark grey agouti	Reddish brown agouti with grey agouti	Grey agouti	Blackish agouti
Flanks	Grey agouti slightly contrasting with external parts of the limbs	Light grey-brown agouti	Grey agouti to light brown	Dark grey agouti	Uniform dark grey agouti	Grey agouti	Grey agouti	Blackish agouti
Neck, chest	Bright red-brown	Light yellow to light orange	Light ochre (orange)	Dark red-brown	Ochre neck, orange-brown chest, belly light ochre (orange)	Grey agouti	Yellowish	Dark red-brown
Hands and feet	Dirty white	Whitish agouti	Buffy hands, feet variable	White-grey agouti	White-grey agouti	Grey agouti	Grey agouti	Blackish agouti
Limbs (outer face)	Grey agouti (lighter than dorsum)	Light grey agouti	Pale brown to grey agouti	Dark grey agouti	Dark grey agouti	Grey agouti	Grey agouti	Blackish agouti
Limbs (inner face)	Bright red-brown	Light yellow	Light ochre (orange)	Dark red-brown	Light ochre (orange)	Grey agouti	Yellowish	Dark red-brown
Tail	Black tail, with evident grey-white at the tip	Predominantly dark grey agouti with whitish tip	Variable; typical blackish with agouti grey base and buffy to tan tip (+ intermixed)	Mostly blackish with buffy tip and agouti grey base	Light ochre (orange)	Grey agouti	Blackish agouti	Blackish agouti

Fig. 17. Comparisons among pelage of (from top to bottom): *P. moloch* (INPA/SISTAP-M 363), *Plecturocebus grovesi* sp. nov. INPA 7275 (holotype) and *P. bernhardi* (INPA 4033). (A) Dorsal view. (B) Ventral view. Photographs by Ingrid Macedo.

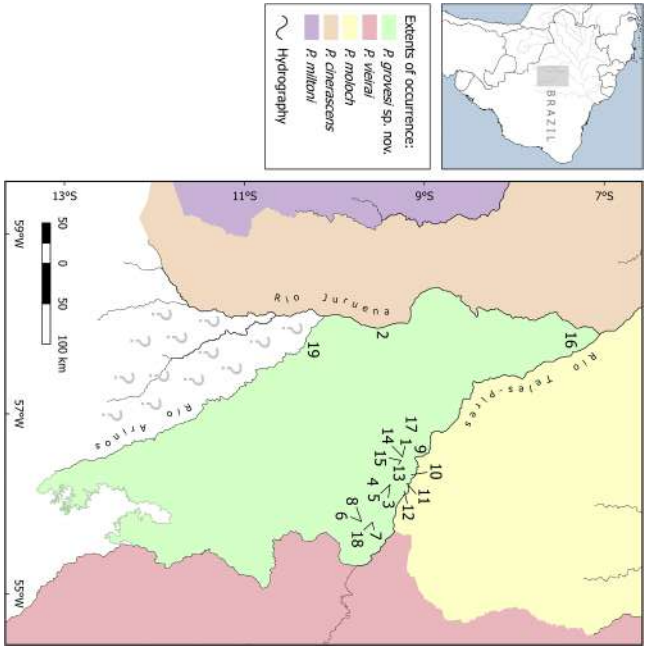


Fig. 18. Map of the geographical distributions of *Plecturocebus grovesi* sp. nov. and surrounding species of the *P. moloch* group. Numbers indicate localities in the Gazetteer.

Table 3
Results for current forest loss – PRODES (1997–2017).

CLASS	Area (km^2)	Area (ha)	%
Considering the total area of the EOO of Plecturocebus grovesi sp. nov. (including non-forested areas)			
Forest loss (1997–2017)	39,568	3,956,847	39
Remaining forest	54,358	5,435,838	53
Non-forest (rivers & savannas)	7740	773,967	8
Total area of the EOO	101,667	10,166,653	
Considering only the available habitats (forest) within the EOO of Plecturocebus grovesi sp. nov.			
Forest loss (1997–2017)	39,568	3,956,847	42
Remaining forest	54,358	5,435,838	58
Available habitat (forest) within the EOO	93,927	9,392,685	

Table 4
Results for predicted future forest loss by 2042 under a “governance” scenario. After Soares-Filho et al. (2006).

Results for the “governance” scenario			
Class	Area (km^2)	Area (ha)	%
Considering the total area of the EOO of Plecturocebus grovesi sp. nov. (including non-forested areas)			
Predicted forest loss (up to 2042)	46,229	4,622,942	45
Remaining forest (up to 2042)	46,689	4,668,896	46
Non-forest (rivers & savannas)	7740	773,967	8
Total area of the EOO	101,667	10,166,653	
Considering only the available habitats (forest) within the EOO of Plecturocebus grovesi sp. nov.			
Predicted forest loss (up to 2042)	46,229	4,622,942	50
Remaining forest (up to 2042)	46,689	4,668,896	50
Available habitat (forest) within the EOO	92,918	9,291,839	

Table 5
Results for predicted future forest loss by 2042 under a “business as usual” Scenario. After Soares-Filho et al. (2006).

Results for the “business as usual” scenario			
Class	Area (km^2)	Area (ha)	%
Considering the total area of the EOO of Plecturocebus grovesi sp. nov. (including non-forested areas)			
Predicted forest loss (up to 2042)	80,202	8,020,219	79
Remaining forest (up to 2042)	12,720	1,272,018	13
Non-forest (rivers & savannas)	7740	773,967	8
Total area of the EOO	101,667	10,166,653	
Considering only the available habitats (forest) within the EOO of Plecturocebus grovesi sp. nov.			
Predicted forest loss (up to 2042)	80,202	8,020,219	86
Remaining forest (up to 2042)	12,720	1,272,018	14
Available habitat (forest) within the EOO	92,922	9,292,237	

et al., 2014; Fernandes et al., 2017; Ferreira et al., 2014; Pack et al., 2016; UHE Teles-Pires, 2018), the “business as usual” scenario is considered more probable. Based on this, we classify *Plecturocebus grovesi* sp. nov. as Critically Endangered (CR) under IUCN category A3c, i.e., population reduction projected, inferred or suspected to be met in the future [up to three titi monkey generations, i.e., 24 years (Veiga et al., 2011; Defler and García, 2012)], considering a decline of greater than 80% in extent of occurrence (EOO) and/or habitat quality.

3.5. Etymology

We named the Alta Floresta taxon after Professor Colin P. Groves (1942–2017) in recognition of his lifelong, preeminent contributions to mammalian taxonomy and systematics, and in particular, primate taxonomy.

4. Discussion

The results of our molecular analyses show a clear monophyly of *Plecturocebus grovesi* sp. nov., with an estimated divergence time from *P. moloch* + *P. vieirai* at 1.3 Ma in the Pleistocene. As proposed by Byrne et al. (2018), the cladogenic events that likely led to the formation of the four eastern Amazon clade species resulted from “jump-dispersal” (Matze, 2013) events from a narrow original range across already established Amazon tributaries, followed by speciation in isolation. In the case of *Plecturocebus grovesi* sp. nov., this isolation happened in the interfluvium of the rios Juruena and Teles-Pires as the ancestral population dispersed eastwards from Rondônia following the divergence of *P. bernhardi* (see Byrne et al., 2018; Fig. 6).

The *vieirai*-like mitochondrial genome of the *P. moloch* specimen RCA27 is notable and warrants further investigation into the genetic diversity of these taxa across their distributions. In this case, we suggest this pattern is likely the result of historical hybridisation or unsorted ancestral polymorphism given our ddRADseq genetic structure analyses indicate that this individual shows > 99% of its ancestry in a *P. moloch* cluster at K = 5, and only a tiny portion of ancestry in the *P. vieirai* cluster (0.4%). Future field studies and sampling across the distribution of *P. moloch* and *P. vieirai* is required to assess the relationship between these lineages. Low support in the mitochondrial phylogenies and putative evidence for more recent gene flow may suggest that the species status of *P. vieirai* requires further investigation.

The range of this newly identified species is restricted to the Juruena and Teles-Pires interfluvium in the Mato Grosso state, Brazil. This area has been severely affected by the advancing Brazilian agricultural frontier. Cattle ranching in Mato Grosso State increased by 62.4% from 2000 to 2016 (IBGE, 2016). In addition, the Brazilian government is implementing a new complex of hydroelectric plants on the rios Juruena, Arinos and Teles Pires (Agência Nacional de Energia Elétrica, Brazil, Order no. 3.208 on the 10/8/2011 and no. 3.504 on the 29/8/2011), which, along with expansion of the road network, will result in extensive habitat loss (Fearnside, 1989, 2015). We calculated that the species has already lost 42% of its original forest habitat and it is estimated that, under the “business as usual” scenario, it will lose a total of 86% of its forest habitat by 2042. The species is, thus, already Critically Endangered, and urgent measures must be put in place to safeguard its survival. Currently, there is just one protected area in the region, the Juruena National Park, located in the northernmost corner of the species range. Elsewhere, forests occupied by *Plecturocebus grovesi* sp. nov. are already largely fragmented, and the creation of new protected areas alone will not be enough to safeguard the future of this species. Areas of pasture will need to be restored to their original habitat in key areas to ensure connectivity between remaining forest patches where the species is still found. Other threatened primates are found in this region, including the black-faced black spider monkey, *Ateles chamek*, and a distinctive marmoset that has yet to be described (J. Boubli, unpubl.). The marmoset has a restricted range similar to that of *Plecturocebus grovesi* sp. nov., and is potentially similarly threatened.

5. Conclusions

Our decision to give new species status to the Alta Floresta taxon was based on: (1) clear monophyly of this lineage revealed by robust genomic evidence; (2) an exclusive combination of diagnosable pelage characters; and (3) a well defined geographic distribution with clear separation from other closely related taxa. All lines of evidence indicate that *Plecturocebus grovesi* sp. nov. is a separately evolving lineage in agreement with the unified species concept of de Queiroz (2007), and also with what Mayden (1997, p. 407) refers to as the “diagnosable and

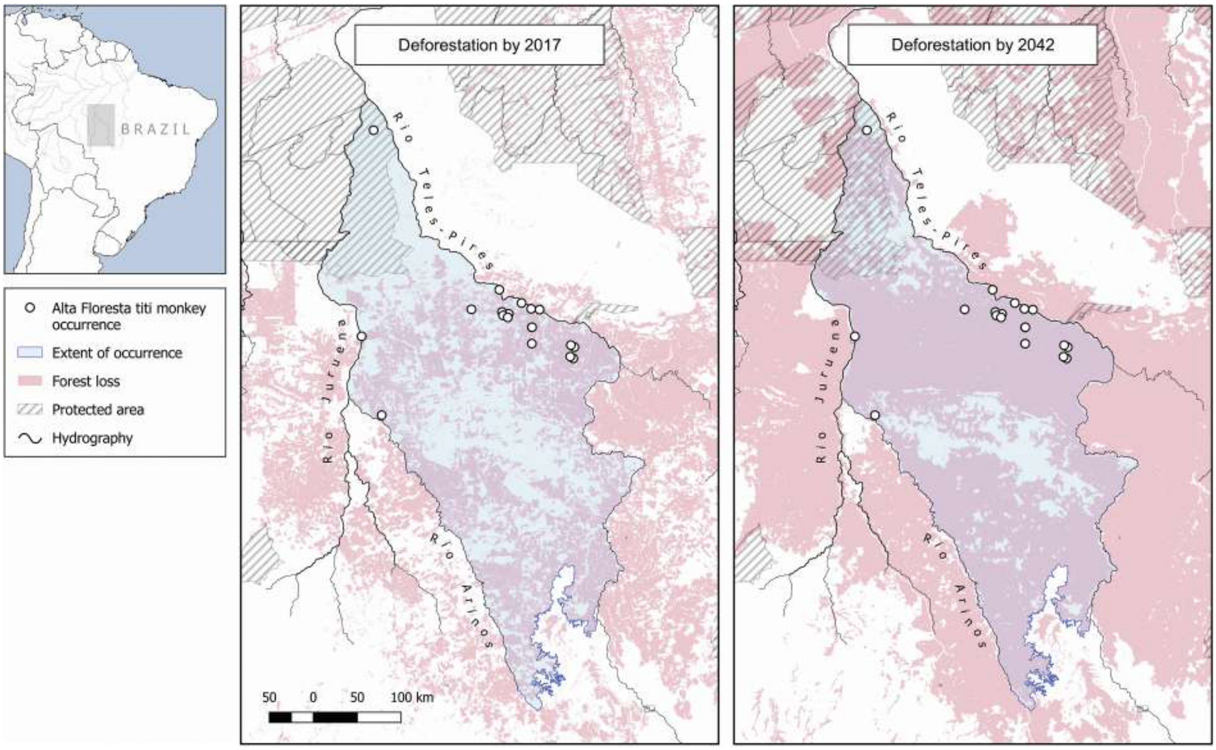


Fig. 19. Extent-of-occurrence for *Plecturocebus grovesi* sp. nov. in the Juruena/Arinos – Teles-Pires interfluvium, and the current and predicted future habitat loss for the species due to deforestation by 2042 under the "Business as Usual" scenario.

monophyly version" of the Phylogenetic Species Concept (*sensu* Cracraft, 1983). The new species is found in one of the areas of Brazil where forest is most rapidly disappearing due to the advancing agricultural frontier. Urgent conservation measures are thus needed to safeguard the future of *Plecturocebus grovesi* sp. nov.

Authors' contributions

JPB, HB conceived of the ideas; all authors contributed to the development of the ideas; JPB, RR, HB, RCA, MNF, JSJ, FB, RMA, TH collected the data; JSJ, MNFS, carried out the morphological analysis and description of the specimens; HB, FB, IF, TH performed the molecular laboratory work; HB prepared the molecular datasets and carried out the phylogenetic and data analysis; JG carried out the GIS work including the calculation of EOO and future deforestation scenarios, and produced the maps; SDN prepared Figs. 7 and 16(a and b); JPB coordinated and supervised the study; and JPB, HB and ABR led the writing. All authors read and approved the final manuscript.

Acknowledgments

Molecular analyses and field expeditions were funded by CNPq/SISBIOTA-BioPHAM (563348/2010) to IPF, CAPES/PRO-AMAZONIA/AUXPE (3261/2013) to IPF and HS), NSF/FAPESP "Dimensions of Biodiversity" (NSF 1241066 and FAPESP 12/50260-6) to Joel Cracraft and Lucia Lohman, Conservation International's Margot Marsh Primate Action Fund (6002856, granted to RCA and TH), and ARPA/ICMBio (granted to RCA). Permission to conduct fieldwork and to collect tissue samples was granted by IBAMA and ICMBio (license N° 005/2005 – CGFAU/LIC and ICMBio 32095-1). RCA receives a doctorate scholarship (CNPq 140039/2018-0) and is grateful also to managers and staff of Thaimaçu Lodge, to DEMUC/SEMA offices in Amazonas State and to the team of ICMBio bureau in Itaituba, Pará State, for supporting fieldwork in southern Amazonia. We are grateful to Consórcio Hidrelétrico Teles Pires and Biota – Projetos e Consultoria Ambiental. We are also grateful to Ingrid Macedo for producing the photos of the museum specimens (INPA), to Lucas Shepard for the edition of these photos, and Adriano Oliviera Maciel for the supplementary images of the paratype specimens (MPEG). Collection of RVR specimens was supported by the Fundação de Amparo à Pesquisa do Estado de Mato Grosso (FAPEMAT, process #477017/2011).

Appendix A. Gazetteer

ID	Locality	Municipality	State	Lat (decimal)	Long (decimal)	Type of record	Source	Specimens code
01	MT-206 highway	Paranaíta	MT	−9.526436	−56.770636	Preserved specimen	This study	RCA14
02	Juruena River, East bank	Nova Bandeirantes	MT	−9.776061	−58.205542	preserved specimen	This study	RCA15
03	Nearby Cuiabá road	Paranaíta	MT	−9.683800	−56.465519	Preserved specimen	This study	RCA2
04	MT-416 highway	Paranaíta	MT	−9.850467	−56.465519	Preserved specimen	This study	RCA57

05	Nearby Mato Grosso road	Paranaíta	MT	−9.683000	−56.463250	Preserved specimen	This study	RCA58, RCA59
06	**Novo Horizonte MT-010 highway - type locality**	Alta Floresta	MT	−10.001694	−56.042278	Preserved specimen	This study	RVR22 (holotype)
07	Nearby MT-208 highway	Alta Floresta	MT	−9.886222	−56.030944	Preserved specimen	This study	RVR68
08	MT-010 highway	Alta Floresta	MT	−9.983972	−56.072833	Preserved specimen	This study	RVR73
09	Teles Pires River, West bank	Paranaíta	MT	−9.298706	−56.797654	Sight	This study	–
10	Teles Pires River, West bank	Paranaíta	MT	−9.435402	−56.570736	Sight	This study	–
11	Teles Pires River, West bank	Paranaíta	MT	−9.494556	−56.472671	sight	This study	–
12	Teles Pires River, West bank	Paranaíta	MT	−9.502708	−56.386346	Sight	This study	–
13	Paranaíta River, East bank	Paranaíta	MT	−9.546166	−56.701805	Sight	This study	–
14	Teles Pires River, West bank	Paranaíta	MT	−9.561761	−56.759398	Sight	This study	–
15	Paranaíta River, East bank	Paranaíta	MT	−9.582353	−56.711460	Sight	This study	–
16	Almas stream, East bank of Juruena River	Apiacás	MT	−7.666667	−58.083333	Preserved specimen	Auricchio (2010)	IPBHN 207, 208, 209
17	Apiacás River, West bank	Apiacás	MT	−9.500000	−57.083333	Preserved specimen	Auricchio (2010)	IPBHN 208, 209
18	Universidade do Estado do Mato Grosso	Alta Floresta	MT	−9.866667	−56.066667	Preserved specimen	Auricchio (2010)	MPEG 24590, 24591
19	Arinos River, East bank	Juara, Paranorte	MT	−10.583333	−58.000000	Preserved specimen	Auricchio (2010)	MZUSP 11244 (SP Zoo); MNRJ 2915, 2923

Appendix B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2018.11.012.

References

Auricchio, P., 2010. A morphological analysis of some species of *Callicebus* Thomas, 1903 (Pitheciidae – Callicebinae). Neotrop. Primates 17 (2), 47–58.

Bernard, E., Penna, L.A.O., Araújo, E., 2014. Downgrading, downsizing, degazettement, and reclassification of protected areas in Brazil. Conserv. Biol. 28, 939–950.

Besnier, F., Glover, K.A., 2013. ParallelStructure: An R package to distribute parallel runs of the population genetics program STRUCTURE on multi-core computers. 29;8(7), e70651.

Boubli, J.P., da Silva, M.N., Rylands, A.B., Nash, S.D., Bertuol, F., Nunes, M., Mittermeier, R.A., Byrne, H., Silva, F.E., Röhe, F., Sampaio, I., Schneider, H., Farias, I.P., Hrbek, T., 2018. How many pygmy marmoset (*Cebuella* Grey, 1870) species are there? A taxonomic re-appraisal based on new molecular evidence. Mol Phylogenet. Evol. 120, 170–182.

Bragg, L.M., Stone, G., Butler, M.K., Hugenholtz, P., Tyson, G.W., 2013. Shining a light on dark sequencing: characterising errors in Ion Torrent PGM data. PLoS Comput Biol. 9 (4), e1003031.

Byrne, H., Rylands, A.B., Carneiro, J.C., Lynch Alfaro, J.W., Bertuol, F., da Silva, M.N.F., Messias, M., Groves, C.P., Mittermeier, R.A., Farias, I., Hrbek, T., Schneider, H., Sampaio, I., Boubli, J.P., 2016. Phylogenetic relationships of the New World titi monkeys (*Callicebus*): first appraisal of taxonomy based on molecular evidence. Front. Zool. 13 (10), 25p.

Byrne, H., Lynch Alfaro, J.W., Sampaio, I., Farias, I., Schneider, S., Hrbek, T., Boubli, J.P., 2018. Titi monkey biogeography: parallel Pleistocene spread by *Plecturocebus* and *Cheracebus* into a post-Pebas Western Amazon. Zool. Scripta. 47, 499–517.

Cabrera, A., 1958. Catalogo de los mamíferos de América del. Sur. Rev Mus Argentino de Cienc Nat “Bernardino Rivadavia”. 4, 1–307.

Carneiro, J.C., Silva Júnior, J.S., Sampaio, I., Pissinatti, A., Hrbek, T., Messias, M., Röhe, F., Farias, I., Boubli, J.P., Schneider, H., 2016. Phylogeny of the titi monkeys of the *Callicebus moloch* group (Pitheciidae, Primates). Am. J. Primatol. 78 (9), 904–913.

Cracraft, J., 1983. Species concepts and speciation analysis. In: Johnston, R.F. (Ed.), Curr Ornithol. Springer, Boston, MA, pp. 159–187.

Cruz Lima E., 1945. Mamiferos da Amazonia – Introdução geral e primatas. Belém, Pará, Brazil: Museu Paraense Emilio Goeldi de História Natural e Etnografia.

Dalponte, J.C., Silva, F.E., Silva-Júnior, J.S., 2014. New species of tit monkeys, genus *Callicebus* Thomas, 1903 Primates, Pitheciidae), from southern Amazonia, Brazil. Pap Avulsos Zool, S Paulo. 54 (32), 457–472.

Dayrat, B., 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. 85 (3), 407–415.

Defler, T.R., Bueno, M.L., García, J., 2010. *Callicebus caquetensis*: a new and critically endangered titi monkey from southern Caquetá. Colombia. Primate Conserv. 25, 1–9.

Defler, T.R., García, J., 2012. *Plecturocebus caquetensis*. The IUCN Red List of Threatened Species 2012: e.T14699281A14699284. https://10.2305/IUCN.UK.2012-1.RLTS.T14699281A14699284.en (Downloaded 06 November 2018).

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29 (8), 1969–1973.

Earl, D.A., von Holdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4 (2), 359–361.

Elliot, D.G., 1913. A review of primates. Monograph Series. New York: American Museum of Natural History.

Fearnside, P.M., 2015. Hidreletricas na Amazônia: impactos ambientais e sociais na tomada de decisões sobre grandes obras. Editora do INPA, Manaus.

Fearnside, P.M., 1989. Brazil’s Balbina Dam: Environment versus the legacy of the pharoahs in Amazonia. Env. Manage. 13 (4), 401–423.

Fernandes, G.W., Vale, M.M., Overbeck, G.E., Bustamante, M.M.C., Grelle, C.E.V., Bergallo, H.G., Magnusson, W.E., Akama, A., Alves, S.S., Amorim, A., Araújo, J., Pillar, V.D., 2017. Dismantling Brazil’s science threatens global biodiversity heritage. Perspectives Ecol. Conserv. 15 (3), 239–243.

Ferreira, J., Aragao, L.E.O.C., Barlow, J., Barreto, P., Berenguer, E., Bustamante, M., Gardner, T.A., Lees, A.C., Lima, A., Louzada, J., Pardini, R., Parry, L., Peres, C.A., Pompeu, P.S., Tabarelli, M., Zuanon, J., 2014. Brazil’s environmental leadership at risk. Science 346, 706–707.

Geoffroy, Saint-Hilaire I., 1844. Description des mammifères nouveaux ou imparfaitement connus 2: Singes Américains. Archives du Muséum d’histoire naturelle. 4, 31–36.

Groves, C.P., 2001. Primate Taxonomy. Smithsonian Institution Press, Washington, DC.

Groves, C.P., 2005. Order Primates. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal species of the world: a taxonomic and geographic reference. Johns Hopkins University Press, Baltimore, MD, pp. 111–184.

Gualda-Barros, J., Nascimento, F.O.do., Amaral, M.K., 2012. A new species of *Callicebus* Thomas, 1903 (Primates, Pitheciidae) from the states of Mato Grosso and Pará, Brazil. Pap Avulsos Zool, S Paulo 52 (23), 261–279.

Hershkovitz, P., 1963. A systematic and zoogeographic account of the monkeys of the genus *Callicebus* (Cebidae) of the Amazonas and Orinoco River basins. Mammalia 27 (1), 1–80.

Hershkovitz, P., 1988. Origin, speciation, and distribution of South American titi monkeys, genus *Callicebus* (Family Cebidae, Platyrrhini). Proc. Acad. Nat. Sci. Philadelphia 140 (1), 240–272.

Hershkovitz, P., 1990. Titis, New World monkeys of the genus *Callicebus* (Cebidae, Platyrrhini): a preliminary taxonomic review. Fieldiana Zool. ns. 55, 1–109.

Hill, W.C.O., 1960. Primates, Comparative Anatomy and Taxonomy IV. Cebidae Part A. Edinburgh University Press, Edinburgh.

von Hoffmannsegg, G., 1807. Beschreibung vier affenartiger Thiere aus Brasilien. Magazin Gesellschaft Naturfosrschungen Freunde Berlin, pp. 83–104.

Hrbek, T., Martínez, J.G., Hernández-Rangel, S.M., Bertuol, F., de Assunção, E.N., Canton, R., de, C., Astolfi-Filho, S., Farias, I.P., 2018. Optimizing Next Generation Sequencing for biodiversity studies on the Ion Torrent PGM: an in silico and in vitro analysis. PeerJ (in revision).

IBGE – Instituto Brasileiro de geográfia e estatística. Produção pecuária municipal, volume 44. 2016. < https://biblioteca.ibge.gov.br/visualizacao/periodicos/84/ppm_2016_v44_br.pdf > (accessed 01/07/2018).

IUCN, 2012. IUCN Red List Categories and Criteria. Version 3.1, second ed. Gland, IUCN, Switzerland and Cambridge, UK.

Ivanova, N.V., Clare, E.L., Borisenko, A.V., 2012. DNA Barcoding in Mammals. In: Erickson, W.J., Kress, D.L. (Eds.), DNA Barcodes. New York, NY, USA, Springer, New York, pp. 153–182.

Ivanova, N.V., Zemlak, T.S., Hanner, R.H., Hebert, P.D.N., 2007. Universal primer cocktails for fish DNA barcoding. Mol. Ecol. Notes 7, 544–548.

Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. BMC Bioinform. 23, 1801–1806.

Kay, R.F., Johnson, D.J., Meldrum, D.J., 1998. A new pitheciine primate from the middle Miocene of Argentina. Am. J. Primatol. 45 (4), 317–336.

Kay, R.F., Johnson, D.J., Meldrum, D.J., 1999. *Proteropithecia*, new name for *Propithecia*

Kay, Johnson and Meldrum, 1998 non Vojnits 1985. Am. J. Primatol. 47, 347.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., et al., 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinforma. Appl. Note 28, 1647–1649.

Kobayashi, S., Langguth, A.L., 1999. A new species of titi monkey, *Callicebus* Thomas, from north-eastern Brazil (Primates, Cebidae). Rev. Bras Zool. 16 (2), 531–551.

Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29 (6), 1695–1701.

Lees, A.C., Zimmer, K.J., Marantz, C.A., Whittaker, A., Davis, B.J.W., Whitney, B.M., 2013. Alta Floresta revisited: an updated review of the avifauna of the most intensively surveyed locality in south-central Amazonia. Bull. B O C 133 (3), 178–239.

Lönnberg, E., 1939. Notes on some members of the genus *Callicebus*. Arkiv för Zoologi 31 (13), 1–18.

MacFadden, B.J., 1990. Chronology of Cenozoic primate localities in South America. J. Hum. Evol. 19 (1), 7–21.

Matzke, N.J., 2013. Probabilistic historical biogeography: New models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. Front. Biogeogr. 5, 242–248.

Mayden, R.L., 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge, M.F., Dawah, H.A., Wilson, M.R. (Eds.), Species: The Units of Biodiversity. Chapman & Hall, London, pp. 381–423.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, LA, p. 1–7.

Miranda Ribeiro, A., 1914. História Natural, Zoologia, Mamíferos. Comissão de linhas telegráficas estratégicas de Mato Grosso ao Amazonas 17, pp. 1–49.

Munsell Soil Colour Charts, 2000. Gretag Macbeth, New Windsor, NY.

Pack, S.M., Ferreira, M.N., Krithivasan, R., Murrow, J., Bernard, E., Mascia, M.B., 2016. Protected Area Downgrading, Downsizing, and Degazettement (PADDD) in the Amazon. Biol. Conserv. 197, 32–39.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE 7 (5), e37135.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

PRODES, 2018. PROJETO PRODES. http://www.dpi.inpe.br/prodesdigital/prodes.php (accessed 24 June 2018).

de Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56 (6), 879–886.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61 (3), 539–542.

van Roosmalen, M.G.M., van Roosmalen, T., Mittermeier, R.A., 2002. A taxonomic review of the titi monkeys, genus *Callicebus* Thomas, 1903, with the description of two new species, *Callicebus bernhardi* and *Callicebus stephennashi*, from Brazilian Amazonia. Neotrop. Primates 10 (Suppl), 1–52.

Rosenberg, N.A., 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4, 137–138.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Soares-Filho, B.S., Nepstad, D.C., Curran, L.M., Cerqueira, G.C., Garcia, R.A., Ramos, C.A., Voll, E., McDonald, A., Lefebvre, P., Schlesinger, P., 2006. Modelling conservation in the Amazon basin. Nature 440 (7083), 520–523.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. BMC Bioinform. 22, 2688–2690.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. BMC Bioinform. 30 (9), 1312–1313.

Thomas, O., 1908. Four new Amazonian monkeys. Annals and Magazine of Natural History 8th series, 2, pp. 88–91.

UHE teles-pires, http://www.uhetelespires.com.br/site/?page_id=27#body (accessed 01/07/2018).

Veiga, L.M., Ferrari, S.F., 2018. Plecturocebus moloch. The IUCN Red List of Threatened Species 2008:e.T41556A10477784. < https://doi.org/10.2305/IUCN.UK.2008.RLTS.T41556A10477784.en > . Downloaded 21 June 2018.

Veiga, L.M., Bóveda-Penalba, A., Vermeer, J., Tello-Alvarado, J.C., Cornejo, F., 2011. Plecturocebus oenanthe. The IUCN Red List of Threatened Species 2011: e. T3553A9939083. < https://doi.org/10.2305/IUCN.UK.2011-1.RLTS.T3553A9939083.en > Downloaded 06 November 2018.

Vermeer, J., Tello-Alvarado, J.C., 2015. The distribution and taxonomy of titi monkeys (*Callicebus*) in central and southern Peru, with the description of a new species. Primate Conserv. 29, 9–29.

Vieira, C. da C., 1995. Lista remissiva dos mamiferos do Brasil. Arq. Zool., S Paulo 8(10), 341–474.

de Vivo, M., 1985. On some monkeys from Rondônia, Brasil (Primates: Callitrichidae, Cebidae). Páp. Avuls. Zool, S Paulo 36(11), 103–110.

Wallace, R.B., Gómez, H., Felton, A., Felton, A.M., 2006. On a new species of titi monkey, genus *Callicebus* Thomas (Primates, Pitheciidae), from western Bolivia with preliminary notes on distribution and abundance. Primate Conserv. 20, 29–39.