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Genetic and Morphological Analyses Confirm the Presence of *Cebuella niveiventris* (Platyrrhini, Callitrichidae) in Bolivia

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Introduction

Recent advances in molecular techniques have led to the discovery that animals with similar morphology and pelage can represent genetically distinct and divergent lineages (Zinner & Roos, 2014). It is important to identify these "cryptic species," as one aim of conservation programs is to protect genetically distinct populations. Traditionally, researchers classified animals in the genus *Cebuella* as one species divided into two subspecies (*C. pygmaea pygmaea* and *C. p. niveinventris*; reviewed in Porter *et al*., 2021). These classifications have recently changed, because analyses of molecular data (Boubli *et al*., 2021) and comparisons of cranial measurements across populations (Porter *et al*., 2021) indicate that these two subspecies should be elevated to the species level. Additional analyses of museum skins and photographs of wild animals reveal that marmosets can be divided into five pelage types, two distinct to *C. pygmaea*, two distinct to *C. niveiventris*, and one found in both species (Porter *et al*., 2021). These previous studies did not include specimens from the southern end of the genus's distribution range (Fig. 1). Therefore, we examined the mitochondrial DNA and pelage patterns of individuals at one site in Bolivia to determine which species is present in that region.

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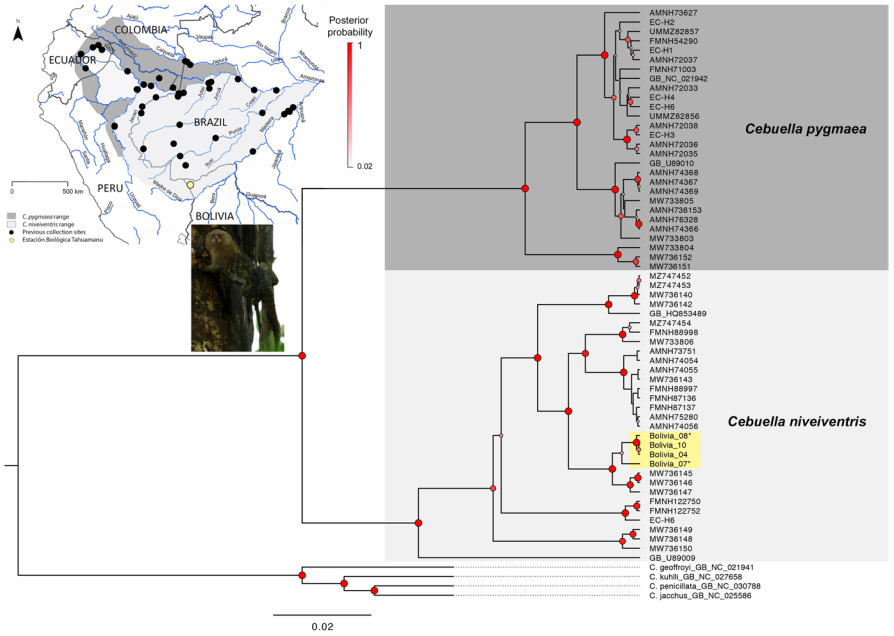

Fig. 1 Upper left, proposed geographic distribution of the two species of *Cebuella* based on mtDNA from samples collected across the region (Porter *et al*., 2021); middle, photo of *Cebuella* in this study (photograph by L. Porter); right, Bayesian maximum clade credibility tree of *Cebuella pygmaea* and *C. niveiventris* inferred from the concatenated mitochondrial cyt *b* and control region sequences in BEAST. The size and color of the circle on each node represent posterior probability support for that group (as in the scale located at the top center), with large red nodes indicating very strong support, medium-sized pink nodes indicating medium support, and small light pink nodes indicating low support. The scale bar refers to nucleotide substitutions per site. Museum specimens are referred by their accession numbers in each collection (for consistency with Porter *et al*., 2021 and Boubli *et al*., 2021). AMNH = American Museum of Natural History, FMNH = Field Museum of Natural History, UMMZ = University of Michigan Museum of Zoology. For samples from free-ranging individuals from Ecuador (EC), all distinct haplotypes in the concatenated dataset are included (i.e., EC_H1 to EC_H7). We refer to GenBank sequences as GB with their respective accession numbers. Samples shaded in yellow were collected at the study site in Bolivia. *Specimens for which we only amplified the cyt *b* fragment

Methods

We noninvasively collected 92 fecal samples from one group of *Cebuella* at the Estación Biológica Tahuamanu, Department of Pando, Bolivia (11° 25′ 30″ S, 69° 0′ 13′′ W; Fig. 1). We collected samples over the course of 6 days by placing netting beneath one of the group's exudate trees. We took photographs of individuals' pelage patterns opportunistically and later compared them to published pelage types (Porter *et al*., 2021).

We extracted DNA from ten randomly selected fecal samples (Bol-02, Bol-03, Bol-04, Bol-05, Bol-07, Bol-08, Bol-10, Bol-12, Bol-15, Bol-37) at the University of Michigan, using the QIAamp DNA Stool Mini Kit (Qiagen). We used two mitochondrial markers, the cytochrome *b* gene (cyt *b*) and the control region (for details

concerning which primer pairs we used to amplify and sequence mitochondrial DNA markers see Porter *et al*., 2021) to determine the phylogenetic relationships of these specimens in relationship to individuals from other geographical locations. We were able to obtain PCR products for four samples (Bol-04, Bol-07, Bol-08, and Bol-10). We purified each PCR product with ExoSAP-IT™ (Thermofisher Scientific, Waltham, MA) and submitted them for Sanger sequencing in both directions to a commercial sequencing service (Genwiz, Azenta). We used Sequencher v. 5.1 (Gene Codes, Ann Arbor, MI) to assemble DNA sequences and aligned our new sequences to previously published sequences available on GenBank. We used maximum likelihood and Bayesian inference approaches to determine the relationships among our samples and between our samples and *Cebuella* sequences publicly available (using MEGA X and BEAST 2.6.2, under the HKY + I model of evolution, and using bootstrap and posterior probabilities to determine clade and nodal support: see Porter *et al*., 2021 for details). We then used the program TreeAnnotator (version 1.10.4) to produce a maximum clade credibility tree.

Ethical Note and Data Availability

This research complied with protocols approved by Northern Illinois University's Institutional Animal Care Committee and adhered to the legal requirements for research in Bolivia. All mtDNA sequences generated in this study are publicly available in GenBank (accession numbers OQ993331- OQ993336).

Results

We obtained two *Cebuella* mitochondrial haplotypes: we were able to amplify and sequence fragments of both mitochondrial genes (889 bp of cyt *b* and 395 bp of the control region) for two samples (Bol-04 and Bol-10), and only cyt *b* (889 bp) for the remaining two samples (Bol-07 and Bol-08). Both maximum likelihood and Bayesian approaches produced concordant tree topologies and showed that the Bolivian samples nest within the *Cebuella niveiventris* clade with strong support (Fig. 1). Photographs of the pelage of individuals in the study group indicate that their abdominal regions are white, and their throats are red ocher (Fig. 1).

Discussion

Our phylogenetic analyses indicate that the Bolivian marmosets belong to the *Cebuella niveiventris* clade, thereby establishing the southern geographic range limit for this species (Fig. 1). The pelage pattern of our study animals (white abdomens and red ocher throats) is also consistent with this designation, as this pattern is observed to the north among *C. niveiventris* populations but not in *C. pygmaea* (Porter *et al*., 2021). The presence of only two mitochondrial haplotypes in the samples is consistent with a group comprised of a reproductive pair and their offspring—a composition that is

common among *Cebuella* (Soini, 1988). Although *Cebuella niveiventris* has a wide geographic range, deforestation and fires are increasingly affecting its habitat in this region (Silveira *et al*., 2022). Therefore, it is important to develop protection plans for this species, particularly in riverine habitats where it is most abundant (de la Torre *et al*., 2009). These results demonstrate how molecular and morphological data collected from free-ranging animals and museum specimens can be used to investigate the geographic distribution of cryptic primate taxa to better inform conservation strategies for their protection.

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Author Contributions LMP originally formulated the idea; LMP, SDT, PPP, LCO developed methodology; LP conducted fieldwork; LCO generated sequencing data and molecular analyses; LMP and LCO wrote the manuscript.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Inclusion and Diversity One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science.

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