On the Identity of Victoria’s Mouse Opossum, *Marmosa regina* Thomas, 1898

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**ABSTRACT**

Phylogenetic analyses of molecular sequence data obtained from the holotype of *Marmosa regina* Thomas, 1898, together with a reassessment of its morphological characters indicate that this species does not belong to the subgenus *Micoureus* as previously believed. Instead, both molecular and phenotypic data are consistent with the hypothesis that *M. regina* is a senior synonym of *M. isthmica* Goldman, 1912, in the subgenus *Exulomarmosa*. Because replacing *isthmica* with *regina* would create nomenclatural confusion, we recommend maintaining current usage of the former name and suppressing usage of the latter.

**INTRODUCTION**

The species that Thomas (1898) named *Marmosa regina* after Victoria, the British monarch who had just celebrated her Diamond Jubilee, has long been problematic for students of opossum classification. Thomas’s original material consisted of a single male specimen (BMNH 98.5.15.4) collected in western Cundinamarca department, Colombia. In the course of his long professional career (which extended for another three decades), Thomas never identified any other specimen as *M. regina*, nor was additional material of *M. regina* reported by Tate (1933) in his landmark revision of *Marmosa*. Although Colombian researchers (e.g., Ramírez-Chaves et al., 2010) have occasionally reported captures of specimens identified as *M. regina*, these identifications have not been documented by published morphological descriptions, measurements, or other supporting information. Additionally, the epithet *regina* has been applied to

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material from other South American countries (e.g., by Gardner, 1993, 2005; Gardner and Creighton, 2008), but the literature contains no indication that BMNH 98.5.15.4 has ever been examined to justify such wider use of this name.

In his original description, Thomas (1898) compared *Marmosa regina* with *M. cinerea* (Temminck, 1824) and implied that the two species were closely related. Tate (1933) endorsed this notion by assigning *regina* to his “Cinerea Group” of *Marmosa*. The species in Tate’s Cine-rea Group were subsequently referred to the genus *Micoureus* (misspelled “*Micoures*”) by Reig et al. (1987), but phylogenetic classifications now rank *Micoureus* as a subgenus of *Marmosa* (Voss and Jansa, 2009; Voss et al., 2014).

Because *regina* is the oldest available name in the subgenus *Micoureus*, its status is uniquely important for determining binomial usage. Among other problematic issues, Gardner (1993) treated several nominal taxa (*germana* Thomas, 1904; *mapiriensis* Tate, 1931; *parda* Tate, 1931; *perplexa* Anthony, 1922; *phaea* Thomas, 1899; *rapposa* Thomas, 1899; and *rutteri* Thomas, 1924) as synonyms of *regina*, and Gardner (2005) later recognized *germana* (including *parda* and *rutteri*) and *rapposa* (including *mapiriensis*) as valid subspecies. The resulting geographic concept of the species extended from Colombia to Ecuador, Peru, Bolivia, and western Brazil (Gardner and Creighton, 2008: map 35). However, the holotype of *regina* is morphologically unlike any other nominal taxon currently assigned to *Micoureus* (Voss et al., 2019, 2020), so the name has, once again, been restricted to a species known only from western Cundinamarca, Colombia.

Analyses of DNA sequences have made important contributions to the current classification of *Marmosa*, but sequence data have hitherto been unavailable from the holotype of *M. regina*. Through the generosity of colleagues in the Mammal Section of the Natural History Museum in London, we were recently allowed to sample dried tissue from BMNH 98.5.15.4, and, with some considerable effort, we were able to sequence fragments of one mitochondrial gene (cytochrome *b*) and one nuclear intron (SLC38A2) from this material. Phylogenetic analyses of the resulting data, together with morphological information that we obtained by reex-amining the holotype skin and skull, provide compelling evidence that Victoria’s mouse opossum has been misclassified for many years.

**MATERIALS AND METHODS**

Dried tissue from the holotype of *Marmosa regina* (BMNH 98.5.15.4) was cleaned and processed according to the procedure explained in Giarla and Voss (2020). All pre-PCR procedures (cleaning of the sample, DNA extraction, and PCR reaction preparation) took place in a laboratory space dedicated to antique DNA extraction, and where PCR products from mammals have never been present. We targeted two loci for PCR and sequencing: the mitochondrial protein-coding gene cytochrome *b* (CYTB) and an intron from the nuclear protein-coding gene solute carrier family 38 member 2 (SLC38A2). Primers were designed to amplify small (200–400 bp) portions of each locus, with each amplicon overlapping with its neighbor by at least 50 bp. Details about primer design are explained below. PCR mixtures included 13 µL of

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3 The epithet *cinerea* Temminck, 1824, is preoccupied and therefore unavailable (Gardner, 1993).
GoTaq Green Master Mix (Promega), 8 µL water, 1 µL of each primer, and 2 µL of undiluted DNA extract. A negative control, to which no DNA was added, was also included for each PCR run. Reactions were run on a thermal cycler with the following steps: initial denaturing at 95°C for 2 minutes; 42 cycles of denaturing at 95°C for 30 seconds; annealing at 50°C for 30 seconds; an extension at 72°C for 30 seconds; and a final extension at 72°C for 7 minutes. Reaction products were run on a 1% agarose gel against a DNA ladder to verify amplicon size and to ensure that the negative control reaction had failed. PCR products were purified using ExoSAP-IT (Thermo Fisher) and sent out for Sanger sequencing at GeneWiz (South Plainfield, NJ).

Several rounds of primer design for CYTB were necessary due to PCR failure and suspected amplification of a nuclear pseudogene (Numt; Bensasson et al., 2001) of CYTB. Initially, CYTB primers were designed using Primer3 (Untergasser et al., 2012) in Geneious R9 (Biomatters) against a reference consensus of sequences sampled from three Micoureus species (alstoni, constantiae, and germana; sensu Voss et al., 2020). However, while trimming sequence reads and assembling amplicons into a single contig for the entire gene, it became clear that several of the primers were amplifying a CYTB Numt. We discarded any PCR amplicon that exhibited premature stop codons, apparent heterozygous bases, or exhibited any dissimilar bases in the interval of overlap with a neighboring amplicon when assembling the larger contig. We subjected the remaining amplicons (which we assumed to be of mitochondrial origin) to nucleotide BLAST searches and discovered that they matched most closely to GenBank sequences from Marmosa isthmica. In light of this new evidence about the potential affinities of the regina holotype, we designed new primers using M. isthmica CYTB as a reference sequence (GenBank accession HM106362). As before, some of the resulting amplicons appeared to be derived from a CYTB Numt and were discarded. The remaining amplicons were combined with the assumed mitochondrial amplicons from the previous round of sequencing to produce a single contig with no heterozygous bases, no conflicting bases, and no premature stop codons. Due to the difficulty of obtaining CYTB sequence, we sought to further bolster our phylogenetic conclusions by obtaining sequences from a nuclear locus, SLC38A2. Only a single round of primer design was necessary for SLC38A2. Primers were designed using a consensus of GenBank SLC38A2 sequences from Marmosa mexicana, M. zeledoni, and M. isthmica. All successful primer pairs for CYTB and SLC38A2 are listed in tables 1 and 2, respectively.

For comparisons with the sequence we obtained from the holotype of Marmosa regina, we compiled CYTB and SLC38A2 sequences from congeneric species along with homologous sequences from two outgroups (Tlacuatzin canescens and Monodelphis brevicaudata). We only included GenBank sequences from Marmosa specimens previously identified by Gutiérrez et al. (2010) and/or Voss et al. (2014). These sequences were aligned in Geneious using MAFFT v7.309 (Katoh and Standley, 2013), and both alignments were separately subjected to maximum likelihood phylogenetic analysis in W-IQ-TREE (Trifinopoulos et al., 2016). We partitioned the CYTB alignment by codon position but did not partition SLC38A2. Nucleotide substitution models for each subset were estimated automatically in W-IQ-TREE prior to phylogenetic inference. Nodal support was estimated using 1000 ultrafast bootstrap replicates (Hoang et al., 2018).
We obtained a gap-free CYTB sequence from BMNH 98.5.15.4 (GenBank accession MT814778; 721 bp). Due to PCR failure for one internal amplicon, the SLC38A2 sequence from this specimen (GenBank accession MT814779; 570 bp) included one ca. 20 bp assembly gap relative to the reference *Marmosa isthmica* sequence. Nucleotide BLAST searches of individual amplicons from both loci matched *M. isthmica* sequences in GenBank, suggesting that we did not sequence contaminant DNA. In both the CYTB and SLC38A2 gene trees (figs. 1, 2), the holotype of *M. regina* clusters with *M. isthmica* with strong support.

**MOLECULAR RESULTS**

The holotype of *Marmosa regina* consists of the skin, skull, and mandibles of an adult male specimen, all elements of which are reasonably well preserved (figs. 3, 4). The skin is over-stuffed, however, with the result that the head and feet appear disproportionately small, and the tail appears disproportionately short. The molar teeth are heavily worn (with dentine broadly exposed, even on M4), suggesting that this was a mature animal; however, the teeth are not worn below the widest part of the crowns, nor does there appear to have been sufficient interstitial wear to have affected toothrow measurements.
FIG. 1. Maximum-likelihood phylogeny for *Marmosa* based on W-IQ-TREE analysis of 72 CYTB sequences (outgroups not shown). Numbers at nodes are ultrafast bootstrap percentages (support for intraspecific relationships not shown). All species except for *M. isthmica* are cartoed with triangles whose bases are proportional to the number of individuals sampled.
FIG. 2. Maximum-likelihood phylogeny for *Marmosa* based on W-IQ-TREE analysis of 29 SLC38A2 sequences (outgroups not shown). Numbers at nodes are ultrafast bootstrap percentages (support for intraspecific relationships not shown).
According to Thomas (1898), the dorsal fur (now somewhat faded) was “buffy grey, finely speckled with brownish,” but Tate (1933: 83) described it as “near Prout’s Brown (R.),” produced by a combination of the yellowish-rusty tips overlying the gray basal portions of the hairs.” On the underside there is a broad midventral zone of self-yellowish fur bordered by lateral zones of gray-based hairs, and a well-developed gular gland is present where the throat meets the upper chest. The wrists are provided with medial and lateral carpal tubercles, of which the medial tubercle is exceptionally long (extending almost to the base of the pollex) and consists of proximal and distal segments separated by a shallow sulcus. The base of the tail is not extensively furred, the caudal skin is entirely dark (brownish, without pale markings), and the caudal scales are arranged in both annular and spiral series.

The skull is notable in dorsal view for its narrow nasal bones and well-developed postorbital processes. In ventral view, it is chiefly remarkable for the absence of palatine fenestrae and for the small, laterally compressed bullae. Although many occlusal features of the molar dentition have been obliterated by wear, the postprotocristae of M1–M3 clearly conform to the “short” morphotype, and m1–m3 lack posterior cingulids.

These qualitative features of the skin and skull support the hypothesis that *Marmosa regina* and *M. isthmica* are conspecific; in fact, no qualitative external or craniodental trait of BMNH 98.5.15.4 conflicts with the diagnosis of *M. isthmica* provided by Rossi et al. (2010). Additionally, most external and craniodental measurements of BMNH 98.5.15.4 fall within the range of morphometric variation previously documented for adult male specimens of *M. isthmica* (table 3). The few exceptions include head-and-body length—which Thomas (1898: 275) admitted was “probably stretched” by overstuffing—and a few cranial dimensions (condylobasal length, nasal length, zygomatic breadth) that suggest the holotype was an unusually large specimen.

By contrast, several traits of BMNH 98.5.15.4 are unlike those observed in members of the subgenus *Micoureus* (as diagnosed by Voss et al., 2014). Notable among such discrepancies is the presence of a gular gland (consistently absent in *Micoureus*) and caudal scales arranged in both annular and spiral series (caudal scales are spirally arranged in *Micoureus*). Additionally, the nasal bones are narrower in proportion to their length in BMNH 98.5.15.4 than in any species of *Micoureus*. For example, the ratio of nasal breadth to nasal length (NB/NL) is 0.24 in the holotype of *Marmosa regina*, but it is larger in adult male specimens of taxa formerly treated as synonyms or subspecies (e.g., 0.28–0.36 in *M. rutteri*).

No original specimen tag is now attached to BMNH 98.5.15.4, but Thomas (1898) reported that the specimen was collected by G.D. Child on 1 November 1895 in “W. Cundinamarca (Bogotá region).” The Colombian department of Cundinamarca includes the crest of the eastern Andean cordillera, as well as tropical lowlands east and west of the mountains. Bogotá is on the western side of the eastern Andes at about 2600 m above sea level, higher than most species of *Marmosa* are known to occur, so it seems probable that the specimen was collected west of the city in the valley of the Río Magdalena as inferred by Tate (1933) and Patton et al. (2000).

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4 Ridgway’s (1912) color swatch for Prout’s Brown is a distinctly reddish hue.

5 See Voss et al. (2020) for descriptions and illustrations of these dental characters. Several nominal species in the subgenus *Micoureus* formerly treated as synonyms or subspecies of *regina* have long postprotocristae on M1–M3 and have posterior cingulids on m1–m3.
estingly, Rossi et al. (2010) reported a specimen of *M. isthmica* from a locality recorded as “Magdalena River, W. Bogotá,” a record that provides independent corroboration that the species is, in fact, present at or near the place where the holotype of *M. regina* was probably collected.

**DISCUSSION**

The molecular data analyzed in this report, together with morphological and geographical information gleaned from the holotype, provide compelling evidence that *Marmosa regina* is a senior synonym of the species currently known as *M. isthmica*. There remain two questions, one academic, the other practical, that we address in sequence.

As a matter of academic interest, how did this synonymy remain so long undetected? Thomas’s (1898) initial comparison of *Marmosa regina* with *M. cinerea* was obviously motivated by their similarity in size, all other species of *Marmosa* known at the time being substantially smaller. Tate’s (1933) subsequent classification of *regina* in the “Cinerea Group” of *Marmosa*, was perhaps less defensible, inasmuch as several large species of *Marmosa* in other groups had been described by then, including *isthmica* (ranked as a subspecies of *M. ruatanica*).
FIG. 4. Dorsal and ventral views of holotype skull of *Marmosa regina* (BMNH 98.5.15.4) and its skull-vial label.
in his “Murina Group”). However, most of us are biased by precedent, and Tate’s informally recognized groups were mostly based on Thomas’s concepts of relatedness. When Reig et al. (1987) resurrected the generic name *Micoureus* for Tate’s Cinerea Group, yet another precedent was set. Later, after several nominal taxa in *Micoureus* had been treated as synonyms or subspecies of *regina* by Gardner (1993, 2005), it seemed only natural to consult their phenotypes as representative of the species. The most that can be said for Voss et al. (2014), who should have known better, is that the holotype was not at hand when they proposed a diagnosis for the subgenus *Micoureus*. Throughout this sorry chain of events, it simply never occurred to anyone to compare the type of *Marmosa regina* with representative material of *M. isthmica*.

The second, practical question concerns usage: what should we now call the species currently known as *Marmosa isthmica*? A straightforward application of the Principle of Priority (ICZN, 1999: Article 23) would require that this taxon now be called *M. regina*, but doing so would have unfortunate consequences. Whether treated as a full species (e.g., by Enders, 1935; Rossi et al., 2010) or as a subspecies (e.g., by Tate, 1933; Hall, 1981), *isthmica* has long been recognized as a valid taxon; a Google Scholar search (https://scholar.google.com; accessed in March 2020) with keywords Marmosa + isthmica recovered >100 references on topics ranging from taxonomy to ecology, behavior, and parasitology of Central American mouse opossums. By contrast, *regina* has been consistently used for almost three decades (after Gardner, 1993) as the senior synonym of several South American taxa, including the species now recognized as *M. germana*, *M. rapposa*, and *M. rutteri*; a Google Scholar search with keywords Marmosa + regina recovered >240 references published since 1993 on a very wide range of topics, but almost all relating to Amazonian or eastern-Andean mouse opossums.

To now replace *isthmica* with *regina* would inevitably result in widespread confusion and disrupt prevailing usage. In such situations, the Code (ICZN, 1999: Article 23.2) is clear:

> In accordance with the objects of the Code … the Principle of Priority is to be used to promote stability and it is not intended to be used to upset a long-established name in its accustomed meaning by the introduction of a name that is its senior synonym.

Because the present case does not meet the criteria by which we could declare *isthmica* to be a nomen protectum and *regina* a nomen oblitum (ICZN, 1999: Article 23.9), it is necessary for us to refer it to the ICZN with a recommendation that usage of *regina* be suppressed. This we will soon do, but in the interim we recommend that current usage of *M. isthmica* be maintained.

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