

A Revision of *Philander*
(Marsupialia: Didelphidae), Part 2:
Phylogenetic Relationships and Morphological
Diagnosis of *P. nigratus* Thomas, 1923

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ABSTRACT

Newly available molecular sequences and morphological data suggest that *Philander nigratus* Thomas, 1923, is a valid species. Currently known from just eight specimens collected in the Peruvian departments of Junín and Ayacucho, *P. nigratus* does not appear to be closely related to either of the congeneric taxa with which it was previously synonymized.

INTRODUCTION

The first installment of this revisionary series on *Philander* (Voss et al., 2018) summarized genetic evidence for the provisional recognition of at least eight species, two of which (*P. quica* and *P. canus*) were diagnosed morphologically, and a third (*P. pebas*) was described as new. Subsequently, Gardner and Ramírez-Pulido (2020) provided a replacement name for the species that Voss et al. (2018) called *P. pallidus*, a preoccupied combination. The species currently recognized as valid are listed in table 1 along with their junior or invalid synonyms.

Two nominal taxa of *Philander* were not included in Voss et al.'s (2018) analysis because genetic or morphological material was unavailable for analysis: *deltae* Lew et al., 2006, and *nigratus* Thomas, 1923. Material of the first remains inaccessible in Venezuelan museums, but

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TABLE 1. Species of *Philander* currently recognized as valid.

<i>P. andersoni</i> (Osgood, 1913)
<i>P. canus</i> (Osgood, 1913)
SYNONYMS: <i>crucialis</i> Thomas, 1923; <i>mondolfii</i> Lew et al., 2006; <i>olrogi</i> Flores et al., 2008
<i>P. mcilhennyi</i> Gardner & Patton, 1972
<i>P. melanurus</i> (Thomas, 1899)
SYNONYMS: <i>fuscogriseus</i> Allen, 1900; <i>griseus</i> Allen, 1901; <i>melanthero</i> Thomas, 1923
<i>P. opossum</i> (Linnaeus, 1758)
SYNONYMS: <i>frenatus</i> Olfers, 1818; <i>superciliaris</i> Olfers, 1818
<i>P. pebas</i> Voss et al., 2018
<i>P. quica</i> (Temminck, 1824)
SYNONYMS: <i>azaricus</i> Thomas, 1923
<i>P. vossi</i> Gardner and Ramírez-Pulido, 2020
SYNONYMS: <i>pallidus</i> Allen, 1901 (preoccupied)

through the generosity of colleagues at the Louisiana State University Museum of Natural Science and at the Field Museum, we were allowed to sample dried tissue from several skins of *nigratus* for DNA extraction. Analyses of the resulting sequence data together with morphological examination of Thomas's (1923) type series and other subsequently collected material suggest that *nigratus* is another valid species of *Philander*.

Materials and Methods

SOURCE OF MATERIAL: Specimens that we examined for this report are preserved in the Natural History Museum (London, BMNH); the Field Museum (Chicago, FMNH), the Louisiana State University Museum of Natural Science (Baton Rouge, LSUMZ), and the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (Lima, MUSM).

DNA EXTRACTION AND SEQUENCING: Small pieces of dried tissue were clipped from two skins of *Philander nigratus* (FMNH 65782 and LSUMZ 16399). To avoid contamination from exogenous DNA, all pre-PCR laboratory procedures were performed in a benchtop PCR enclosure with UV sterilization in a lab where mammalian DNA is never amplified and in which contaminating mammalian PCR products are unlikely to be present. For DNA extraction, PCR, and sequencing, we followed the protocol described in Giarla and Voss (2020). Nested pairs of primers were designed to span 200–300 bp pieces of the mitochondrial gene cytochrome *b* (CYTB) and two nuclear loci: breast cancer susceptibility protein (BRCA1) and interphotoreceptor retinoid binding protein (IRBP). Primers for each locus were designed using alignments of *Philander canus*, *P. mcilhennyi*, *P. opossum*, and *P. quica* sequences downloaded from GenBank. Primers were designed in Geneious R9 (Biomatters, Inc.) using the Primer3 algorithm (Untergasser et al., 2012), and only regions that exhibited sequence conservation in the reference alignments were used (appendix 1). For each PCR amplicon, chromatograms were examined, trimmed, and assembled in Geneious. To determine whether individual amplicons might be derived from contaminant DNA, each was subjected to a

standard nucleotide BLAST search (Altschul et al., 1990) against GenBank's nonredundant nucleotide database. The new sequences we obtained from *P. nigratus* have been deposited in GenBank with accession numbers MT298897–MT298900.

SPECIES DELIMITATION AND PHYLOGENETIC ANALYSES: We used the cytochrome *b* sequence dataset previously compiled by Voss et al. (2018: table 2) for a species-delimitation analysis that included the sequence data newly obtained for this study as described above. The resulting matrix included 135 (127 unique) CYTB haplotypes of *Philander* together with outgroup sequences from *Chironectes*, *Didelphis*, and *Lutreolina*. We analyzed this matrix using BEAST v1.7.2 (Drummond et al., 2012) to obtain an ultrametric tree as described by Voss et al. (2018). To estimate the threshold between interspecific and intraspecific branching processes we used the R package bGMYC (Reid and Carstens 2012), a Bayesian implementation of the General Mixed Yule Coalescent model (Pons et al., 2006). To account for uncertainty in our phylogenetic analysis, we sampled 100 post-burn-in trees from the posterior distribution of CYTB trees and used them as input trees in bGMYC. We ran bGMYC for 50,000 generations, with a thinning interval of 100 and a burn-in of 40,000 generations. We allowed the threshold parameter (i.e., the number of potential species) to vary between 1 and 137, the maximum number of sequences in our CYTB dataset. A point estimate for species limits was estimated using the `bgmyc.point` function, setting the conspecificity probability threshold to 0.5.

We also analyzed a concatenated six-gene matrix that included sequences from CYTB, BRCA1, and IRBP that we obtained from FMNH 65782, as described above, plus sequences from these genes and three additional loci (Anon128, OGT, and SLC38) that had previously been obtained from exemplar specimens of other species of *Philander* and six outgroup taxa (Voss et al., 2018: table 3). The resulting matrix was concatenated in Geneious, partitioned for model-fitting with PartitionFinder2 using the Bayesian Information Criterion (Lanfear et al., 2012), and analyzed with MrBayes v3.2 (Ronquist et al., 2012) as described by Voss et al. (2018). We conducted a maximum-likelihood analysis using W-IQ-TREE (Trifinopoulos et al., 2016) using the PartitionFinder2 data subsets and allowing the program to fit its own substitution models. To determine nodal support, we conducted 1000 ultrafast bootstrap replicates. Finally, to assess the per-gene phylogenetic signal provided for the placement of *P. nigratus*, we constructed individual gene trees in W-IQ-TREE for BRCA and IRBP using the same approach as for the concatenated analysis.

MEASUREMENTS: External measurements (in millimeters, mm) are those taken in the field by collectors using either the standard American protocol (Hall, 1962) or the British method (Lankester, 1904). For specimens measured by American collectors, we transcribed total length (nose to fleshy tail tip, TL) and length of tail (basal flexure to fleshy tip, LT) from specimen labels or field notes, and we computed head-and-body length (HBL) by subtracting LT from TL. We also transcribed length of hind foot (heel to tip of longest claw), and length of ear (from notch) from specimen labels or field notes. British collectors measure head-and-body length and tail length separately (using the anus as endpoint), their measurements of hind feet do not include the claws, and the ear is measured from the crown of the head.

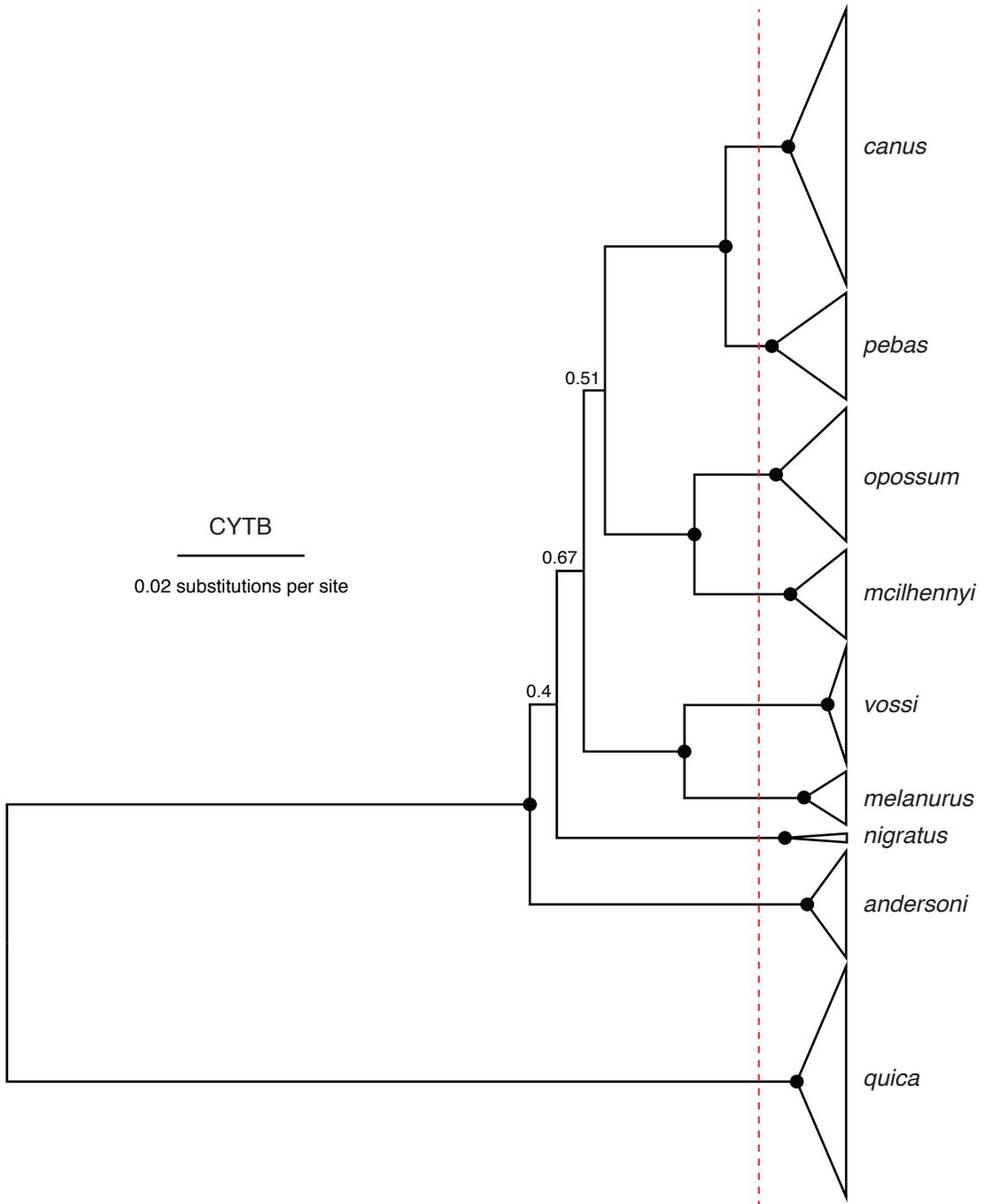


FIG. 1. Ultrametric tree from a BEAST analysis of cytochrome *b* sequences of *Philander*, with putative species cartooned as triangles. The dashed vertical red line indicates the threshold between Yule and coalescent processes as estimated by the Bayesian implementation of the general mixed Yule coalescent model (bGMYC). Bases of triangles at branch tips are proportional to the number of sequences in each clade. Filled circles at each internal node indicate strong support (PP = 1.0).

the labial margins of the M4 crowns, at or near the stylar A position), *maxillary tooththrow length* (measured from the anterior margin of C1 to the posterior margin of M4), *length of molars* (measured from the anteriormost labial margin of M1 to the posteriormost point on M4), *length of M1–M3* (measured from the anteriormost labial margin of M1 to the posteriormost point on M3), and *width of M3* (measured from the labial margin of the crown at or near the stylar A position to the lingual apex of the protocone).

AGE CRITERIA: Unless otherwise noted below, we recorded measurements and scored qualitative morphological data from adult specimens only. Following Voss et al. (2001), a specimen was judged to be juvenile if dP3 was still in place; subadult if dP3 had been shed but P3 and/or M4 was still incompletely erupted (M4 is the last upper tooth to erupt in *Philander*); and adult if the entire permanent upper dentition (I1–5, C1, P1–3, M1–4) was fully erupted.

MOLECULAR RESULTS

We obtained complete (1149 bp) cytochrome *b* sequences for FMNH 65782 and LSUMZ 16399 and nearly complete BRCA (1865 bp out of 2046 bp) and IRBP (946 bp out of 1158 bp) sequences for FMNH 65782. The BRCA and IRBP sequences are missing data from their 5' and 3' ends; neither sequence contains internal sequencing gaps. Individual PCR amplicons assembled into contigs with no disagreements, and BLAST analyses of all amplicons closely match *Philander* sequences in GenBank.

The cytochrome *b* sequences we obtained from FMNH 65782 and LSUMZ 16399 are only 1.7% divergent from one another, and they differ from homologous sequences obtained from other taxa currently recognized as valid species of *Philander* by 3.5%–11.8% (appendix 2). In phylogenetic analyses of our CYTB dataset, FMNH 65782 and LSUMZ 16399 were recovered as a long-branched lineage that is cut by the bGMYC-inferred threshold between coalescence and cladogenesis (fig. 1). On this basis, *nigratus* merits consideration as a putative species. Nodal support values suggest that *nigratus* is not closely related to *quica* (from the Atlantic Forest), but its relationships among congeneric taxa from Amazonia (*andersoni*, *canus*, *mcilhennyi*, *opossum*, *pebas*) and the trans-Andean Neotropics (*melanurus*, *vossi*) are not effectively resolved due to weak support for several key nodes.

Concatenated analyses of the multilocus (six-gene) dataset provide strong support for a monophyletic group that includes *nigratus* along with five other Amazonian or trans-Andean species (*andersoni*, *melanurus*, *mcilhennyi*, *opossum*, *nigratus*, and *vossi*), a clade that we propose to call the Opossum Group. Sister to the Opossum Group is the strongly supported Canus Group (*canus* and *pebas*). Within the Opossum Group, *melanurus* and *vossi* form one strongly supported clade, whereas *mcilhennyi* and *opossum* form another, but the relationships of *andersoni* and *nigratus* are not convincingly resolved. The phylogenetic signal for the placement of *nigratus* in our BRCA and IRBP datasets is weak (see figs. S1 and S2 in the supplement, available online at doi.org/10.5531/sd.sp.43), indicating that its placement in the concatenated tree is largely determined by CYTB sequence variation.

As emphasized by many authors (e.g., Carstens et al., 2013; Sukumaran and Knowles, 2017), single-locus species delimitation methods are far from infallible, so the results of our GMYC analyses require testing with other kinds of data. The only data currently available for this purpose are morphological, which we obtained by comparing skins and skulls of *nigratus* with specimens of other putative species of *Philander*. We summarize those comparisons in the following account, which also formalizes our taxonomic conclusions.

TAXONOMY

Philander nigratus (Thomas, 1923)

Metachirus opossum nigratus Thomas, 1923: 603; type locality “Utcuyaco” (= Utcuyacu, ca. 11°12' S, 75°28' W; Stephens and Traylor, 1983) at 1600 m above sea level in Junín department, Peru.

Metachirops opossum nigratus: Krumbiegel, 1941: 202; name combination.

Philander opossum canus: Cabrera, 1958: 35; part (*nigratus* treated as a synonym), not *P. canus* (Osgood, 1913).

Philander andersoni: Gardner, 1993: 22; part (*nigratus* treated as a synonym), not *P. andersoni* (Osgood, 1913).

Philander andersoni andersoni: Hershkovitz, 1997: 61; part (*nigratus* treated as a synonym), not *P. andersoni* (Osgood, 1913).

TYPE MATERIAL: The holotype (by original designation, BMNH 0.7.7.62) consists of the skin and skull of an old adult female (figs. 3, 4) collected at Utcuyacu by P.O. Simons (original number 947) on 21 April 1900. Two other specimens mentioned by Thomas (1923) are paratypes; both were collected by Jean Kalinowski at “Chanchamayo” and were obtained by the BMNH in 1894 from the Branicki Museum in Warsaw.³ One paratype (BMNH 94.10.1.16) consists of the skin of a young (possibly subadult) individual of unknown sex without an accompanying skull, whereas the other (BMNH 94.10.1.17) is the skull of an old adult of unknown sex without an accompanying skin.

DISTRIBUTION AND SYMPATRY: *Philander nigratus* is known only from the eastern Andean foothills at recorded elevations of 1000–1600 m in the Peruvian departments of Ayacucho and Junín. It is not known to occur sympatrically with other congeneric species.

MORPHOLOGICAL CHARACTERS: Dorsal pelage short (not shaggy), about 12–17 mm long middorsally (mean = 15 mm) and more or less uniformly dark gray (sometimes indistinctly darker middorsally but never with a distinct middorsal stripe); fur of crown (between the ears) clear blackish in some specimens (e.g., BMNH 0.7.7.62, LSUMZ 16398) but grizzled gray in others (e.g., MUSM 71, FMNH 65782); pale preauricular spot absent in most specimens but indistinct in one (FMNH 65782); ventral fur frosted dark gray (the individual hairs grayish basally,

³ Although Chanchamayo is the name of a province in northern Junín department, its use by 19th-century collectors is generally understood to refer to the valley of the Río Chanchamayo, a tributary of the Río Perené. According to Berlepsch and Stolzmann (1896), Kalinowski collected at three localities in the valley of the Chanchamayo: La Merced (11°03'S, 75°19'W, ca. 800 m; Stephens and Traylor, 1983), La Gloria (coordinates unknown; ca. 975 m), and Borgoña (ca. 11°05'S, 75°20'W, ca. 795 m; Stephens and Traylor, 1983). None of these localities, however, is indicated on the labels that accompany the specimens in question.



FIG. 3. Dorsal and ventral views of the holotype skin (BMNH 0.7.7.62) of *Philander nigratus*.



FIG. 4. Dorsal and ventral views of the holotype skull (BMNH 0.7.7.62) of *Philander nigratus*.

TABLE 2. External and craniodental measurements of adult specimens of *Philander nigratus*.

	BMNH 0.7.7.62 ^a	LSUMZ 16399	FMNH 65782	LSUMZ 16398
Sex	female	female	male	male
Head-and-body length ^b	“265”	270	“280”	275
Tail length ^b	“275”	300	“275”	300
Hind foot ^b	“40”	45	“47”	48
Ear ^b	“35”	35	“30”	34
Condylobasal length	68.2	71.2	76.6	—
Nasal length	35.5	34.6	40.6	35.9
Nasal breadth	7.8	9.3	8.6	8.1
Least interorbital breadth	12.0	13.2	14.7	12.8
Least postorbital breadth	9.3	9.7	9.2	9.2
Zygomatic breadth	32.4	36.5	—	—
Palatal length	41.9	44.2	48.7	43.2
Palatal breadth	20.6	21.0	23.1	21.6
Maxillary tooththrow length	30.3	30.5	34.5	30.7
Length of molars	14.8	—	16.4	14.7
Length of M1–M3	12.1	—	13.8	12.5
Width of M3	4.2	4.3	4.6	4.4

^a Holotype.

^b Scare quotes indicate measurements taken by the British method.

but with paler tips); scaly part of tail $\leq \frac{1}{4}$ white (unpigmented) distally. Nasal bones long, extending between postorbital processes in some specimens (e.g., BMNH 0.7.7.62, 28.5.1.20), but much shorter in others (MUSM 71, LSUMZ 16399); posterior margin of nasals laterally notched in some specimens (e.g., BMNH 0.7.7.62) or without posterolateral notches (LSUMZ 16398). Third upper premolar (P3) labial cingulum incomplete in most specimens but complete in one (LSUMZ 16399); crown length of upper molar series 14.7–16.4 mm ($N = 5$).

COMPARISONS: *Philander nigratus* requires close comparison with two other western-Amazonian species of the Opossum Group—*P. andersoni* and *P. mcilhennyi*—which it somewhat resembles in size and coloration (Gardner and Patton, 1972; Patton and da Silva, 1997). Additionally, *P. nigratus* merits comparison to *P. canus*, a taxon with which it was once synonymized, and with which it might reasonably be expected to occur sympatrically. Wider comparisons (e.g., with eastern Amazonian, trans-Andean, or Atlantic Forest taxa) seem unnecessary in the absence of compelling molecular support for close relationships to other species.

Philander nigratus differs externally from *P. andersoni* by lacking the distinct blackish mid-dorsal stripe that is invariably present in the latter species (illustrated by Voss et al., 2018: fig. 10). Additionally, the pale preauricular spots that are consistently present in *P. andersoni* are absent or indistinct in *P. nigratus*, and the scaly part of the tail, which is $\frac{1}{2}$ to $\frac{2}{3}$ white in *P. andersoni* is $\frac{1}{4}$ white in *P. nigratus*. The two species are craniodentally similar in qualitative

TABLE 3. Morphological comparisons among four western Amazonian species of *Philander*.

	<i>P. nigratus</i>	<i>P. andersoni</i>	<i>P. mcilhennyi</i>	<i>P. canus</i>
Dorsal pelage color	uniformly dark gray	pale gray with black middorsal stripe	usually blackish	uniformly pale gray
Middorsal fur length	12–17 mm	9–16 mm ^a	12–22 mm ^a	8–15 mm
Ventral pelage color	dark	variable	usually dark	self-white or -cream
Crown fur	variable	black	black	grizzled gray
Preauricular spot	absent or indistinct	present	variable	variable
Tail coloration	≤¼ white	≥½ white	≥½ white	½ to ½ white
Nasal bones	variable	usually long ^b	usually long ^b	short ^c
P3 labial cingulum	usually incomplete	usually complete	complete	complete
Length of molars ^d	15.1 ± 0.7 mm 14.7–16.4 mm (N = 5)	14.0 ± 0.5 mm 12.8–14.9 mm (N = 44)	14.9 ± 0.6 mm 13.8–15.9 mm (N = 23)	13.0 ± 0.4 mm 12.2–13.7 mm (N = 43)

^a Dorsal pelage length is sexually dimorphic (Voss et al., 2019: table 22).

^b Extending posteriorly to or between the postorbital processes.

^c Not extending posteriorly to or between postorbital processes.

^d Tabulated statistics include the mean plus or minus one standard deviation, the observed range, and the sample size (sexes combined).

features, but P3 usually has a complete labial cingulum in *P. andersoni*, whereas P3 has an incomplete labial cingulum in six of the seven specimens of *P. nigratus* that we were able to score for this trait. Morphometrically, however, *P. nigratus* is substantially larger than *P. andersoni*, a size difference that is most noticeable in molar measurements, some of which have almost nonoverlapping observed ranges for these species (table 3).

Philander nigratus differs in dorsal pelage color from *P. mcilhennyi*, which is either darker overall in dorsal pelage color (almost completely blackish) or has a distinct blackish middorsal stripe and pale-grayish flanks (Voss et al., 2019: fig. 19). Although the two species overlap broadly in dorsal pelage length, this is at least partly due to sexual dimorphism in *P. mcilhennyi* (females having longer, shaggier middorsal fur than males; Voss et al., 2019); the single dorsal-pelage length measurement we obtained for an adult female *P. nigratus* was just 16 mm, whereas some adult females of *P. mcilhennyi* have middorsal fur that is 19–21 mm long. The most conspicuous and consistent external difference, however, is tail coloration: whereas the scaly part of the tail is usually ½ to ⅔ whitish distally in *P. mcilhennyi*, no more than ¼ of the tail is whitish distally in *P. nigratus*. These two species are craniodentally similar, and they have broadly overlapping measurements, but P3 has a complete labial cingulum in *P. mcilhennyi*, whereas the labial cingulum of P3 is usually incomplete in *P. nigratus*.

Philander canus (redescribed and illustrated by Voss et al., 2018) differs from *P. nigratus* by its pale-gray dorsal pelage, self-whitish ventral pelage, and longer white tail tip. *Philander canus* is also a substantially smaller animal than *P. nigratus* (e.g., with nonoverlapping molar tooththrow measurements: 12.2–13.7 mm versus 14.7–16.4 mm). Additionally, the nasal bones are consistently short in *P. canus* (never extending posteriorly to or between the postorbital

processes) and the labial cingulum of P3 is always complete, whereas the nasal bones are longer in about half the examined specimens of *P. nigratus*, which usually also has an incomplete labial cingulum on P3.

SPECIMENS EXAMINED ($N = 8$): **Peru**—*Ayacucho*, Huanhuachayo (LSUMZ 16399), San José (LSUMZ 16398); *Junín*, Chanchamayo (BMNH 94.10.1.16, 94.10.1.17; FMNH 65782), Ináñez (BMNH 28.5.1.21), Peñablanca (MUSM 71), Utcuyacu (BMNH 0.7.7.62).

DISCUSSION

Although phenotypic differences among *Philander nigratus* and the other congeneric species with which it has been synonymized or compared by authors are not as numerous nor as consistent as could be wished, they seem sufficient to support the hypothesis that this is a distinct species and not simply a divergent mtDNA haplogroup. To our knowledge, these are the first taxonomic comparisons based on firsthand examination of Thomas's (1923) original material, which perhaps explains why we have arrived at different conclusions than previous researchers. We acknowledge that pairwise cytochrome *b* distances among *P. nigratus* and other members of the Opossum Group are not large by comparison with distances among congeneric species of small opossums (e.g., members of *Monodelphis* and *Marmosa*), which are typically in the range of 9%–15%, but species of *Philander* with small pairwise distances are known to maintain their phenotypic differences in sympatry (e.g., *P. canus* and *P. pebas*, only 1.8% divergent; Voss et al., 2018), so this statistic is not an infallible indicator of lineage independence.

With so few specimens available, it is impossible to be confident about the geographic distribution of *Philander nigratus*, which could be more widely distributed along the eastern foothills of the Peruvian Andes than our material suggests. However, we note that another didelphid species (*Marmosops juninensis*; see Díaz-Nieto and Voss, 2016) is restricted to the same general region, which might eventually prove to be an area of endemism for small mammals as revisionary research proceeds with other taxa. For the moment, however, *P. nigratus* seems remarkable as having an exceptionally small geographic range for a large opossum.

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APPENDIX 1

PRIMER PAIRS USED TO AMPLIFY THREE GENES
FROM DRIED TISSUE OF *PHILANDER NIGRATUS*

Breast Cancer Activating 1

Amplicon	Forward	Reverse
1	Phil-BRCA1-118F GCTGGGAAGACCTCAGATGC	Phil-BRCA1-441R GGAGTCAGCCACCAAGTCAG
2	Phil-BRCA1-349F GCAGAGATGGCTGGTGTCTT	Phil-BRCA1-629R ACAGGCTCTGGAGGGATCAA
3	Phil-BRCA1-529F ACCTACCATAGAAAATCAGTTCACAC	Phil-BRCA1-849R CACTTCCTCTACGTGGCCAT
4	Phil-BRCA1-764F ACCAAGAACAAAATGGCCAAGT	Phil-BRCA1-1113R CCTTATCTCTTCACTGCTGGGA
5	Phil-BRCA1-1009F ACCAGGATTGTCCATGAGCT	Phil-BRCA1-1342R CAGTGACACTGCAGCTAGCT
6	Phil-BRCA1-1267F GGAAATACACCTGCCTGTGC	Phil-BRCA1-1587R GGGTTCCTTGAGGGTTTCCA
7	Phil-BRCA1-1478F GGGACACTCAGGACAGTACC	Phil-BRCA1-1825R CCTGCTGACGTAAAGCCTGA
8	Phil-BRCA1-1761F GGGTTCCTAGTCCAAGGCAGG	Phil-BRCA1-2023R CACAGTCATTGCTACCATGCA

Interphotoreceptor Retinoid Binding Protein

Amplicon	Forward	Reverse
1	Phil-IRBP-69-F AAATGCTGGCCAGTGCCTG	Phil-IRBP-352-R GGTCCCCATGAGCTTCTTCC
2	Phil-IRBP-269-F GTTGACTACATCCCCTGGCCA	Phil-IRBP-552-R ACCACCATGTCTTTCTCCCC
3	Phil-IRBP-381-F GCTCAGGGGGTGAAGTTTCA	Phil-IRBP-675-R CGCAGCTCCGGAGATCTAG
4	Phil-IRBP-552-F TCCTCACCAGTCACGGTACT	Phil-IRBP-838-R CCTACGCAGGGTGAGAATGG
5	Phil-IRBP-698-F ATCACTGTGCCGGTATCACG	Phil-IRBP-975-R GCGGCCAAATCTTCTCTGT
6	Phil-IRBP-819-F CCATTCTCACCCTGCGTAGG	Phil-IRBP-1044-R TCAGGCTTCAGAACCCGAAC

Cytochrome *b*

Amplicon	Forward	Reverse
1	Phil-CYTB-31F TGGCATGAAAAACCATCGTTGT	Phil-CYTB-256R TGCGGTTAATGTGTCTGATGT
2	Phil-CYTB-152F TCAGCCTGATGAAATTCGGT	Phil-CYTB-426R AGGATAACTCCAATGTTTCATGTTTCT

APPENDIX 1 *continued*

3	Phil-CYTB-347F TGCCTCTTTCTTCACGTAGG	Phil-CYTB-542R TTCCAATGTAGGGGATGGCG
Amplicon	Forward	Reverse
4	Phil-CYTB-400F AGAAACATGAAACATTGGAGTTATCCT	Phil-CYTB-689R TGCTTGATCCTGTTTCGTGGA
5	Phil-CYTB-523F CGCCATCCCCTACATTGGAA	Phil-CYTB-822R CCTAGGAGGTCTGGTGAGAAT
6	Phil-CYTB-683F TCAAGCAATCCAACAGGCCT	Phil-CYTB-939R CCTCCTAATTTATTTGGGATTGATCGT
7	Phil-CYTB-805F CTCACCAGACCTCCTAGGAGA	Phil-CYTB-1081R TCAAGTAAGGATAATTAGGTTTGCTGT
8	Phil-CYTB-1015F AGCATTCCGACCAATCTACA	Phil-CYTB-1284R GGGTGTTTCCTTCGTTGTTGG

APPENDIX 2

AVERAGE PERCENT SEQUENCE DIVERGENCE (UNCORRECTED)
AT THE CYTOCHROME B LOCUS WITHIN AND AMONG
PUTATIVE SPECIES OF *PHILANDER*

	1	2	3	4	5	6	7	8	9
1. <i>andersoni</i>	0.4								
2. <i>canus</i>	5.9	0.8							
3. <i>mcilhennyi</i>	6.1	4.1	1.2						
4. <i>melanurus</i>	6.0	4.5	5.2	0.8					
5. <i>nigratus</i>	5.3	4.4	4.4	4.2	1.7				
6. <i>opossum</i>	5.6	3.5	3.0	4.5	3.5	0.8			
7. <i>pebas</i>	5.2	1.8	3.2	4.6	4.1	3.0	1.3		
8. <i>quica</i>	11.8	11.2	10.8	10.5	11.8	11.2	9.9	0.9	
9. <i>vossi</i>	6.9	5.4	5.4	3.9	5.6	5.1	5.0	11.9	0.3

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