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A new species of fish-eating rat, genus *Neusticomys* (Sigmodontinae), from Ecuador

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Abstract

Background: In this study, the genetic substructure and morphology of the species *Neusticomys monticolus* was evaluated. A nuclear marker and mitochondrial maker were used to examine phylogeographic structure and to estimate genetic distances. Two statistical measurement analyses were applied to morphological data.

Results: These data recovered two morphologically distinct phylogeographic groups corresponding to populations on the eastern and western slopes of the Andes. Further, these eastern and western Andean slope populations of *N. monticolus* are 8.5 % divergent using sequence data from cytochrome-*b* (0.8 % divergent in the interphotoreceptor retinoid-binding protein gene).

Conclusions: Populations currently assigned to *N. monticolus* constitute a species complex. The name *N. monticolus* is here restricted to western Andean slope populations. Populations on the eastern slope of the Andes are assigned to a new species, to which the authors assign the name *Neusticomys vossi* sp. nov.

Keywords: Andes, Cricetidae; Ecuador; Ichthyomyini; Muroidea; *Neusticomys monticolus*; *Neusticomys vossi* sp. nov

Background

Sigmodontine rodents constitute a diverse group of New World rodents; extant diversity encompasses ca. 400 species arranged in 86 genera (D'Elía and Pardiñas 2015). Traditionally, sigmodontine taxa have been arranged into tribes (Reig 1980; Musser and Carleton 2005; D'Elía et al. 2007), one of which is the tribe Ichthyomyini comprising five genera and 17 species ranging from Bolivia, central Brazil, and the Guianas to southern Mexico (Jenkins and Barnett 1997; Musser and Carleton 2005). Ichthyomyines are small to medium-sized semiaquatic animalivorous rodents (Voss 1988), constituting a remarkably distinct group within the sigmodontine radiation. Several morphological features differentiate them from other sigmodontines and as such have been interpreted as supporting the monophyly of the tribe (Voss 1988). Recent phylogenetic analyses, based on nuclear DNA sequences which include representatives of only two ichthyomyine genera (*Rheomys* and *Neusticomys*), have questioned the monophyly of the tribe (Martínez et al. 2012; Parada et al. 2013; Salazar-Bravo et al. 2013). However, a recent inspection of the sequence of *Neusticomys* (EU649036) indicates that it may be a chimeric sequence that includes a fragment retrieved from an oryzomyine. This would therefore weaken the argument against a monophyletic Ichthyomyini.

The ichthyomyine genus that deviates the least from the general sigmodontine body plan is *Neusticomys* (Voss 1988); for example, on its hind feet, it lacks the stiff hairs (presumably an adaptation for swimming) found in other members of the tribe. This genus was nominated by Anthony (1921) to contain the Andean species *Neusticomys monticolus*. Almost 7 decades later, Voss (1988) subsumed the predominantly lowland *Daptomys* Anthony (1929) under *Neusticomys*. Recently, Percequillo et al. (2005) described another lowland species of *Neusticomys* from central Brazil increasing the known diversity of the genus to six species. Of these, *N. monticolus* is the most widely distributed species, being endemic to the Andes Mountains of Colombia and Ecuador, at elevations between 1800 and 3750 m (Lee et al. 2006a, 2006b; Musser and Carleton 2005). The lifestyle of the species (living near fast-moving streams) has made it difficult to collect. Individuals either are caught in pitfall traps set next to streams, or Sherman traps set in the

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stream, and they do not seem to be attracted to typical baits, rather those that are caught seem to have been attracted due to curiosity or by insects gathered around typical rodent bait (Personal observation of TEL). These characteristics result in few museum specimens of *N. monticolus* available for study, with still fewer available tissue samples. In 1988, there were ~50 known specimens of *N. monticolus* (Voss 1988), none of which had associated frozen tissue that we could identify. An additional six specimens were collected during field work conducted in Ecuador during 2003, 2005, 2007, and 2008 (Lee et al. 2008; M. Pinto personal communication).

The only taxonomic assessment of *N. monticolus* to date is that presented by Voss (1988), and no published taxonomic oriented study based on genetic data has focused on this species or any other *Neusticomys*. Here we attempt to characterize the genetic variation of *N. monticolus* by examining the nucleotide sequence of the mitochondrial cytochrome-*b* gene (*Cyt*b) and nuclear IRBP gene (*Rbp*3) of individuals from populations on both sides of the Andes. Given the already noticed morphological differences among the phylogeographic units here uncovered, we describe a new species of *Neusticomys*.

Methods

Sampling

Four field trips were conducted to survey the mammalian fauna of Ecuador. Animals were collected following methods approved by the American Society of Mammalogists Animal Care and Use Committee (Sikes 2011). Two specimens of *Neusticomys* were collected on the western side of the Andes near the type locality of *N. monticolus* in 2003 as well as an additional two from a different locality in 2008. One specimen each was collected in 2005 and in 2007 at two different localities in the eastern Andes. These specimens are housed at various natural history collections (Appendix). Further, the tissue was obtained for another member of Ichthyomyini (*Rheomys raptor*), and sequences were obtained from GenBank for eight additional members of the subfamily Sigmodontinae (one representative of seven tribes within Oryzomalia, one member of Sigmodontini) and four non-sigmodontine members of the family Cricetidae (Appendix).

Morphological analysis

The following measurements (Table 1, defined in Voss 1988) were examined for 41 specimens of *Neusticomys*

Table 1 Mean measurements and ranges (in mm) for distinct sets of specimens of *Neusticomys*; specimen QCAZ 7830 is the holotype of *N. vossi* sp. nov

	N. monticolus ♀	*N. monticolus* ♂	*N. monticolus* Antiquia	*N. vossi* sp. nov. ♀	*N. vossi* sp. nov. ♂	QCAZ 7830
TL	216.56 (196–287)	226.44 (187–313)	206.8 (191–217)	199.5 (188–211)	205.57 (193–221)	211
HBL	115.67 (95–195)	122.88 (96–211)	106.8 (100–112)	102.82 (97–110.99)	105.17 (101–108)	110.99
LT	100.89 (90–109)	103.56 (87–114)	100 (91–105)	101.25 (96–108)	99 (92–108)	108
HFC	25.56 (25–26)	26.25 (24–36)	24.8 (24–26)	26 (25–27)	25.43 (24–27)	27
EC	10 (6–12)	10.25 (8–14)	11 (11–11)	10.25 (8–13)	9.86 (9–11)	13
CIL	24.36 (23.1–25)	24.96 (24.2–25.8)	23.75 (22.7–24.9)	23.7 (23.2–25.01)	24.13 (23.4–24.9)	25.01
LD	6.01 (5.5–6.2)	6.09 (5.4–6.6)	5.76 (5.5–6.1)	5.66 (5.34–6.1)	6.04 (5.7–6.4)	6.1
LM	4.16 (4–4.91)	4.07 (3.9–4.4)	4.06 (3.9–4.1)	4.12 (4–4.2)	4.09 (4–4.3)	4.17
LIF	4.74 (4.3–5.9)	4.69 (4.4–5.06)	4.46 (4.2–4.7)	4.39 (4.11–4.7)	4.86 (4.7–5.2)	4.45
BIT	1.44 (1.3–1.75)	1.44 (1.3–1.6)	1.42 (1.4–1.5)	1.28 (1.1–1.47)	1.27 (1.2–1.4)	1.47
BIF	2.11 (1.9–2.4)	2.09 (1.9–2.3)	2.16 (2.1–2.2)	1.99 (1.97–2)	2.15 (2–2.3)	1.99
BPB	2.93 (2.7–3.1)	2.96 (2.7–3.4)	2.88 (2.5–3.3)	2.87 (2.7–3.14)	2.85 (2.6–3.1)	3.14
LN	9.65 (8.6–11.24)	9.48 (8.5–10.2)	9.74 (9.2–10.7)	9.1 (8.81–9.5)	9.46 (8.9–10.1)	8.89
BN	2.89 (2.6–3.2)	2.99 (2.7–3.2)	2.94 (2.7–3.1)	2.37 (2.27–2.52)	2.7 (2.5–3)	2.52
LIB	4.81 (4.6–5)	4.85 (4.6–5.2)	4.84 (4.6–5.1)	4.59 (4.5–4.67)	4.61 (4.4–4.9)	4.57
ZB	12.83 (12.2–13.3)	13.16 (12.3–13.4)	12.1 (12.1–12.1)	12.03 (11.73–12.49)	12.54 (11.9–13)	12.49
BB	12.13 (11.8–12.3)	12.16 (11.7–12.5)	11.38 (11.2–11.5)	11.73 (11.52–11.91)	12.13 (11.9–12.5)	11.91
BZP	1.31 (1–2.06)	1.14 (1–1.3)	1.02 (0.9–1.1)	1.17 (1.1–1.25)	1.13 (1.1–1.3)	1.25
BM1	1.45 (1.4–1.57)	1.44 (1.3–1.5)	1.44 (1.3–1.6)	1.22 (1.01–1.4)	1.38 (1.3–1.5)	1.16
HI	4.3 (2.36–4.9)	4.68 (4.1–5.2)	4.36 (4–4.7)	3.77 (3.03–4.3)	4.49 (4.2–5)	3.03
DI	1.33 (1–1.5)	1.42 (1.3–1.5)	1.42 (1.4–1.5)	1.21 (1.2–1.23)	1.3 (1.2–1.4)	1.23
BOC	7.09 (6.7–7.39)	7.04 (6.6–7.5)	6.75 (6.6–7)	6.65 (6.47–6.8)	6.85 (6.8–7)	6.63

Measurements acronyms and definitions are found in Voss (1988)

(including four specimens not presented in Voss (1988); Appendix): TL—total length, HBL—head body length, LT—length of tail, HF.C—length of hindfoot collected, E.C—length of ear collected, CIL—condylo-incisive length, LD—length of diastema, LM—length of maxillary molars, LIF—length of the incisive foramina, BIT—-breadth of the incisor tips, BIF—breadth of the incisive foramina, BPB—breadth of the palatal bridge, LN—length of nasals, BN—breadth of nasals, LIB—least interorbital breadth, ZB—zygomatic breadth, BB—breadth of braincase, BZP—breadth of the zygomatic plate, BM1—breadth of M1, HI—height of incisor, DI—depth of incisor, and BOC—breadth of occipital condyle. The combined measurement data were first examined with principle components analysis (PCA) in the R programming package (R Development Core Team 2009) to determine if distinct groups were identified. Defined groups were then analyzed with linear discriminant analysis (LDA) using the R programming package (R Development Core Team 2009). LDA was used to graphically explain differences between phylogeographic groups using multiple variables.

Molecular methods

Genomic DNA was isolated from approximately 0.1 g of either the liver or muscle, using a phenol extraction method (Longmire et al. 1997). The polymerase chain reaction (PCR) was used to amplify the complete 1143 bp of *Cyt*b for the seven specimens and up to 1266 bp of *Rbp*3. Reaction concentrations (25 μl volume) included ≤300 ng genomic DNA, 0.07 mM dNTPs, 2.86 mM MgCl, 5 μl 10× buffer, 1.25 U Taq (Go Taq, Promega, Madison, Wisconsin), and 0.286 μM of primers L14115 and H15288 (*Cyt*b; Martin et al. 2000) or A1 and B2 then A1-F and E2-B2 (*Rbp*3; Stanhope et al. 1992; Weksler 2003). Thermal profiles for PCR included an initial denaturation step at 95 °C (2 min), 30 to 40 cycles with denaturation at 95 °C (45 s), annealing at 48 °C-*Cyt*b or 54 °C-*Rbp*3 (1 min), extension at 72 °C (1 min 30 s), and a final extension cycle of 72 °C (8 min). Amplicons were purified using the QIAquick PCR purification kit (Qiagen, Inc., Valencia, California) and then sequenced using ABI Prism Big Dye Terminator v3.1 ready reaction mix (Applied Biosystems, Foster City, California) and a 3100-*Avant* automated sequencer (Applied Biosystems, Foster City, California). *Cyt*b primers L14115 and H1528 and the internal primers O400R (Hanson and Bradley 2008), F1 (Whiting et al. 2003), O700H (Hanson and Bradley 2008), and 700 L (Peppers and Bradley 2000) and *Rbp*3 primers A1 and B2 and the internal primers F and E2, 395R (Hanson et al. 2010), and C and D2 (Jansa and Voss 2000; Weksler 2003) were used for cycle sequencing at 95 °C (30 s) denaturing, 50 °C (20 s) annealing, and 60 °C (4 min) extension. Following 30 to 40 cycles, reactions were purified and precipitated in isopropanol. Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan) was used to proof sequences. DNA sequences were deposited in GenBank, and accession numbers are listed in Appendix.

Genetic analyses

ClustalW, MUSCLE, and manual approaches were used in MEGA4 (Tamura et al. 2007) to align nucleotide sequences and gave identical alignments. As no additional ichthyomyine *Cyt*b or *Rbp*3 sequence were available in GenBank (the one sequence of *Rbp*3 available for *Neusticomys* has been identified as chimeric and was regenerated in this paper), newly obtained sequences of *Neusticomys* and *Rheomys* were integrated into a matrix with sequences gathered from eight Sigmodontinae tribes (one representative of seven tribes within Oryzomalia, one member of Sigmodontini) and four non-Sigmodontinae tribes which were used to form the out-group. When available, we included full-length sequences gathered from a specimen of the type species of the type genus of each tribe. The matrices were analyzed using a Bayesian approach (Rannala and Yang 1996) in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). MrModeltest (Nylander 2004) was used to estimate the most appropriate model of sequence evolution (GTR + I + G). Bayesian analysis was performed with sequences partitioned by codon using site-specific gamma distribution allowing for a proportion of invariable sites. Runs, consisting of four Markov chains, were allowed to proceed for ten million generations and were sampled every 1000 generations. The first 1000 trees were discarded as "burnin" based on stabilization of likelihood scores; the remaining trees were used to compute a 50 % majority rule consensus tree and obtain posterior probability (PP) estimates for each clade. Additionally, *Cyt*b pairwise genetic distances were calculated between the recovered phylogroups using MEGA4 (Tamura et al. 2007) and the Kimura two-parameter model (Kimura 1980).

Results

Morphological data

Results of the PCA of morphometric data (Fig. 1a, b) are congruent with the genetic data in identifying two putatively allopatric groups in Ecuador, one (V) formed by specimens collected on the eastern slopes of the Andes and the other (M) formed by specimens collected on the western slopes of the Andes. In addition, a second western group, distributed north near Antioquia, Colombia, was also recovered (there is currently no genetic data available for this group). The eastern and western groups marginally overlap. This overlap decreased when age groups and sexes were examined separately (Fig. 1a, b, respectively). The two western subgroups overlap more than the eastern and western groups but still are well separated. When the three groups were examined using

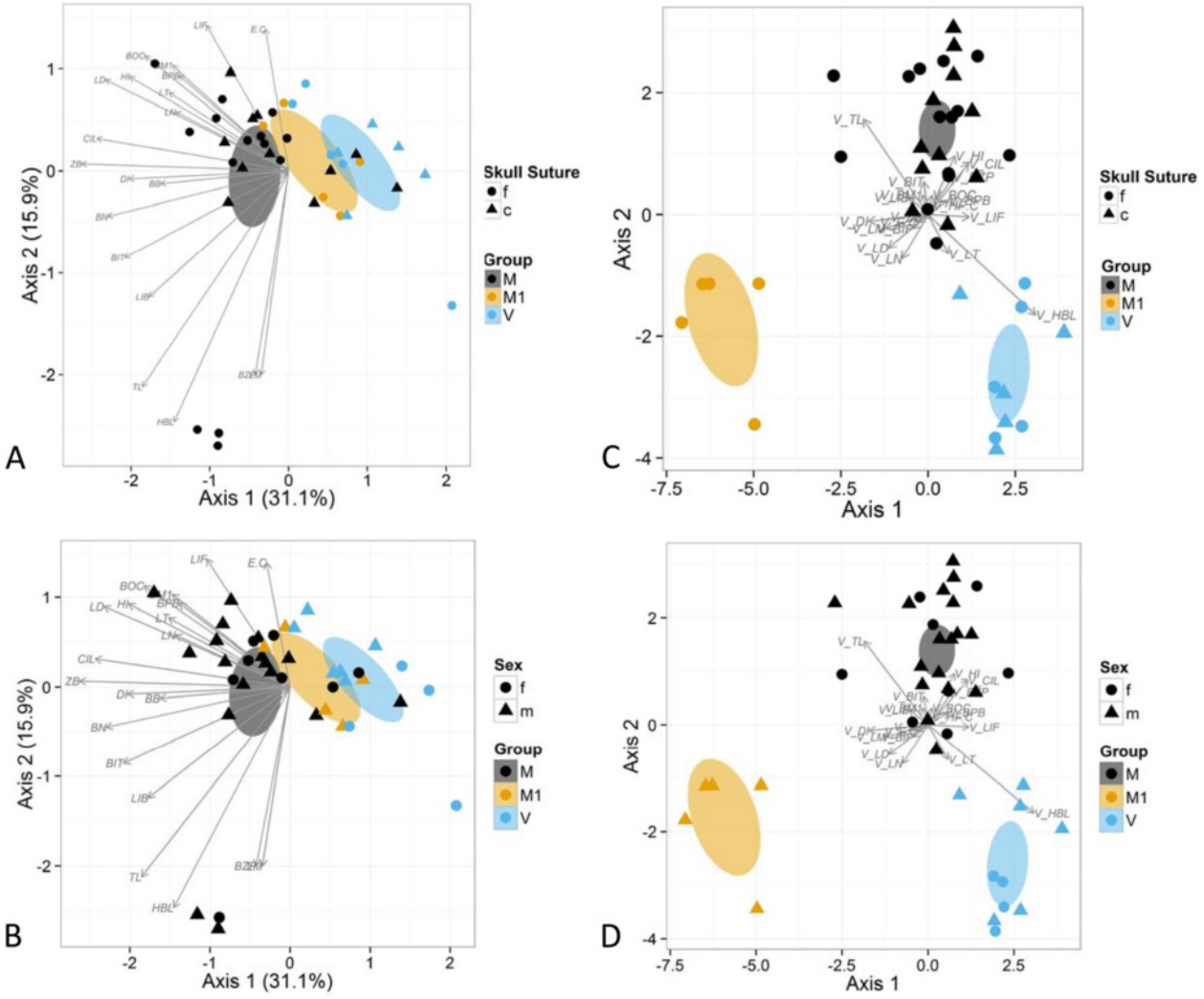

Fig. 1 Graphical representation of the PCA analyses (**a**, **b**) and LDA analysis (**c**, **d**). Vectors labeled as in Table 1. **a**, **c** Samples identified by locality and age. Eastern samples (*N. vossi* sp. nov.: *V*) are *light gray*, western samples (*N. monticolus*: *M*) are *dark gray*, specimens from Antioquia (*N. monticolus*: *M1*) are *mid-gray*. Adults (fused craniosutures) are *circles*, and subadults (closed craniosutures) are *triangles*. **b**, **d** Samples identified by locality and sex. Location symbols same as above, females—*circles*, males—*triangles*

LDA (Fig. 1c, d), the separation was complete in both age and sex analyses (Fig. 1c, d).

Genetic data

Both phylogenetic analyses of mitochondrial and nuclear DNA sequences (Fig. 2) depict a strongly monophyletic Sigmodontinae (clade I; PP = 1.00). Importantly, the tribe Ichthyomyini also is recovered as monophyletic (clade II; PP = 1.00); *Rheomys* appears sister to *Neusticomys*. Within *Neusticomys*, two distinct groups are indicated, each highly supported (PP = 1.00). The groups appear to be allopatric; clade V is distributed east of the Andes and clade M west of the Andes (Fig. 3). Average genetic distances among the eastern and western groups were 8.5 % (*Cyt*b) and 0.8 % (*Rbp*3); a much higher value than that observed within each group (eastern: 0.4 and 0.0 %; western: 0.1 and 0.1 %, respectively).

Taxonomic implications

Despite the small sample size examined, we consider that when taken together, available data provide sufficient evidence to justify recognition of an additional species of *Neusticomys*. As no name is available, we name and describe it below.

Neusticomys vossi sp. nov.

Voss' fish-eating rat urn:lsid:zoobank.org:act:FB716D66-3D57-40A0-9E48-0E8A5A9B402E

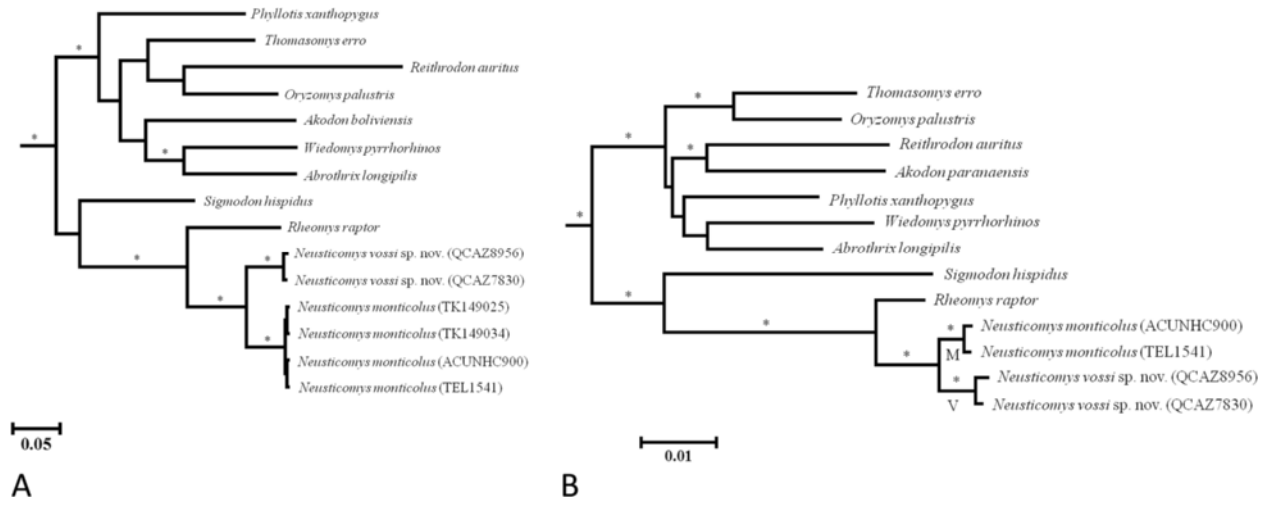

Fig. 2 Phylogenetic tree of sigmodontine rodents based on *Cytb* (**a**) and *Rbp3* (**b**) DNA sequences. Bayesian posterior probability values greater than 0.95 are represented by asterisks placed above the branch

Holotype—QCAZ 7830, an adult lactating female collected by T. E. Lee (personal catalog number TEL1846) in August 2005 with a Sherman trap placed in a forested mountain stream near a small (1 m tall) waterfall (Fig. 4). The specimen is preserved as the skin, skull and skeleton, and frozen tissue and deposited at El Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito, Ecuador.

Type locality—12 km by road northwest of Cosanga (0° 31′ 70″ S, 77° 52′ 99″ W), Napo Province, Ecuador (1900 m).

Diagnosis—As stated by Percequillo et al. (2005), species of *Neusticomys* are difficult to diagnose using presumptive autapomorphies; rather, unique combinations of character states are operationally useful for species recognition. *Neusticomys vossi* sp. nov. is a species of Ichthyomini, that can be distinguished from other ichthyomyine species by its smaller size, reduced hallux, single cusped last molar, large interparietal bone, and an apparent less advanced aquatic specialization, and can be distinguished from other congeners (except *N. monticolus*) by having dull grayish rather than brownish pelage and by occipital condyles not projecting posteriorly beyond rest of occiput. *N. vossi* sp. nov. can be distinguished from *N. monticolus* by its smaller overall size and by its narrower incisors, braincase, occipital condyles, and rostrum as well as shorter condylo-incisive length, length of diastema, length of maxillary molars, zygomatic breadth, breadth of braincase, and breadth of occipital condyles.

Holotype measurements—Head body length (103 mm), tail length (108 mm), hindfoot (27 mm), ear (13 mm), breadth of nasals (2.52 mm), length of nasals (8.89 mm), depth of incisor (1.23 mm), length of incisors (3.03 mm), breadth of M1 (1.16 mm), and the length of the incisive foramina (4.45 mm).

Description—Dull grayish black pelage (Fig. 5). Ventral pelage color often slightly lighter than dorsal pelage. Mystacial vibrissae and oral margins usually silver. Tail uniformly colored with whitish hairs along ventral surface and occasionally a white tip. Some individuals may have white midpectoral blazes and irregular whitish dorsal spotting. Manus and pes covered with whitish silver hairs turning to dark at the wrist, ankles, and metapodials.

Rostrum slender with threadlike zygomatic arch (Fig. 6). Infraorbital foramina very large. Palatal foramina very large and medially broad, reaching the anterior lobe of large palate, well surpassing the posterior end of the toothrow. Square mesopterygoid fossa lacking a median palatine process. Braincase broad, flat, and smooth. Coronoid process long and narrow. No ridges on frontals or parietals. Small depression at base of nasals, plane of nasals continuous with frontals. Large interparietal bone. Occipital condyles not projecting posteriorly beyond rest of occiput, and not exposed dorsally. Well-developed gnathic process. Peglike process on maxillary roots of zygomata external to molars. Upper incisors ungrooved and orthodont. Parallel upper molar rows. Molar cusps nearly opposite. High crowned molars with deep flexes almost touching in the middle line of molars. M1 much larger than M2 and M3. M1 with three similar-sized lobes M2 with paracone-protocone pair much wider than the metacone-hypocone pair, M3 reduced.

Comparisons—Voss (1988) noted that specimens from Papallacta in the eastern Andes, referred here to *N. vossi* sp. nov., average slightly smaller than *N. monticolus* from Guarumal in the western Andes; the same is true for the

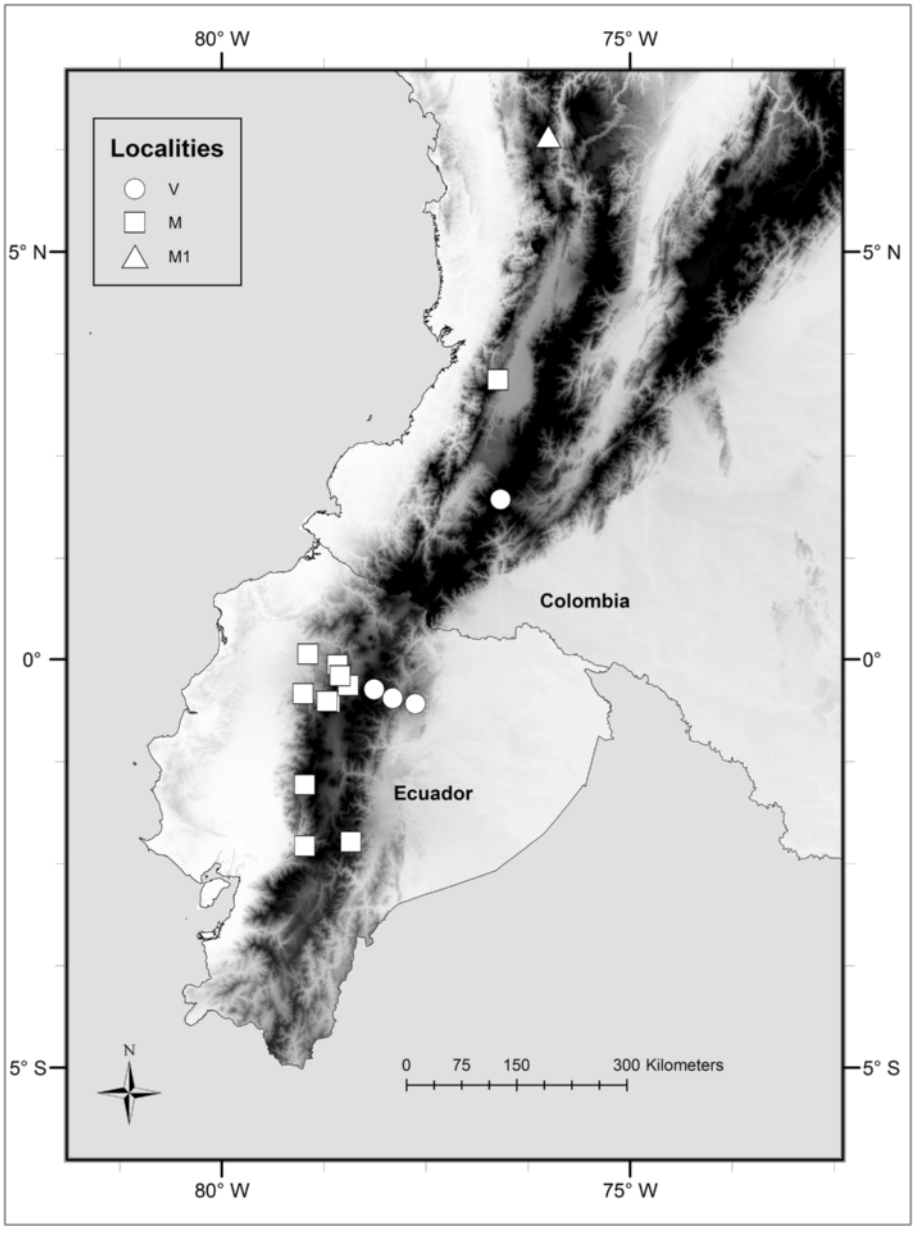

Fig. 3 Map of recording localities of specimens of *Neusticomys* analyzed in the present study. Eastern samples (*N. vossi* sp. nov.: *V*) are *circles*, western samples (*N. monticolus*: *M*) are *squares*, and specimens from Antioquia (*N. monticolus*: *M1*) are *triangles*

specimens, including the holotype of *N. vossi* sp. nov., collected by us. The breadth of the occipital condyles appears nearly diagnostic between the two groups in Voss (1988) study. BOC in *N. vossi* sp. nov. samples is ≤6.9 mm with females being smaller than males. For the most part, *N. monticolus* shows a BOC of ≥6.9 mm with females being smaller than males.

Similar to *Neusticomys venezuelae* and *Neusticomys mussoi*, Voss' fish-eating rat is differentiated from *Neusticomys ferreirai*, *Neusticomys oyapocki*, and *Neusticomys peruviensis* in that the posterior edge of the inferior zygomatic root lies above the anterecone of M1. *N. vossi* sp. nov. differs from *N. oyapocki* and *N. ferreirai* in having three upper and lower molars.

Distribution—Known from four sites on the eastern slopes of the Andes in northern Ecuador and southern Colombia ranging from 1° 58′ N, 76° 35′ W in the north to 0° 33′ N, 77° 36′ W and from 1900 to 3750 m.

Etymology—*Neusticomys vossi* sp. nov. is named to honor Dr. Robert S. Voss of the American Museum of Natural History. Rob Voss is the author of a large series of key contributions towards the understanding of South

Fig. 4 View of the type locality of *Neusticomys vossi* sp. nov

American mammals; in particular, he authored a now classical monograph on ichthyomyine rodents. As such, he was the first to recognize the morphological differences between the new species described here and *N. monticolus.* As part of an ichthyomyine review, Voss (1988) wrote the following as a comment on *N. monticolus*, "The series from Guarumal together with those from nearby Las Machinas and from the Rio Pita closely resemble the Volcan Pichincha specimens, but the Papallacta series exhibits some metric differences."

Natural history—One *N. vossi* sp. nov. was recorded at Papallacta with two large embryos on the 15th of May (Voss 1988). The type collected in August is a lactating female. The type was taken by a small waterfall about 1 m tall, with traps exposed to the spray of water from the fall, which is congruent with the description by Tate (1931) of *Neusticomys* habitat. The stream was rocky and about 1 m across with fast rapids (Fig. 4). This specimen represents a low elevation record in Ecuador at 1900 m for the eastern Andes (Lee et al. 2006a). *Oreoryzomys balneator* and *Thomasomys erro* were collected in the same trap line or in nearby forests, as the type specimen (Lee et al. 2006a). Vegetation along the stream consisted of plants with large waxy leaves. Plants of the families Araceae, Arecaceae, Cecropiaceae, Chloranthaceae, Cyatheaceae, Cyclanthaceae, Flacourtiaceae, Lauraceae, Lobeliaceae, Melastomataceae, Meliaceae, Moraceae, Piperaceae, and Poaceae were found along the stream bank (Lee et al. 2006a).

Discussion

Samples from eastern and western localities were sequenced to examine phylogeographic structure of *N. monticolus*. Two highly differentiated and potentially allopatric groups were recovered; these groups are congruent with the morphological forms previously identified by Voss (1988). Externally, the two groups do not appear to present differences besides pelage color in a

Fig. 5 Skin of the holotype of *Neusticomys vossi* sp. nov. (QCAZ 7830)

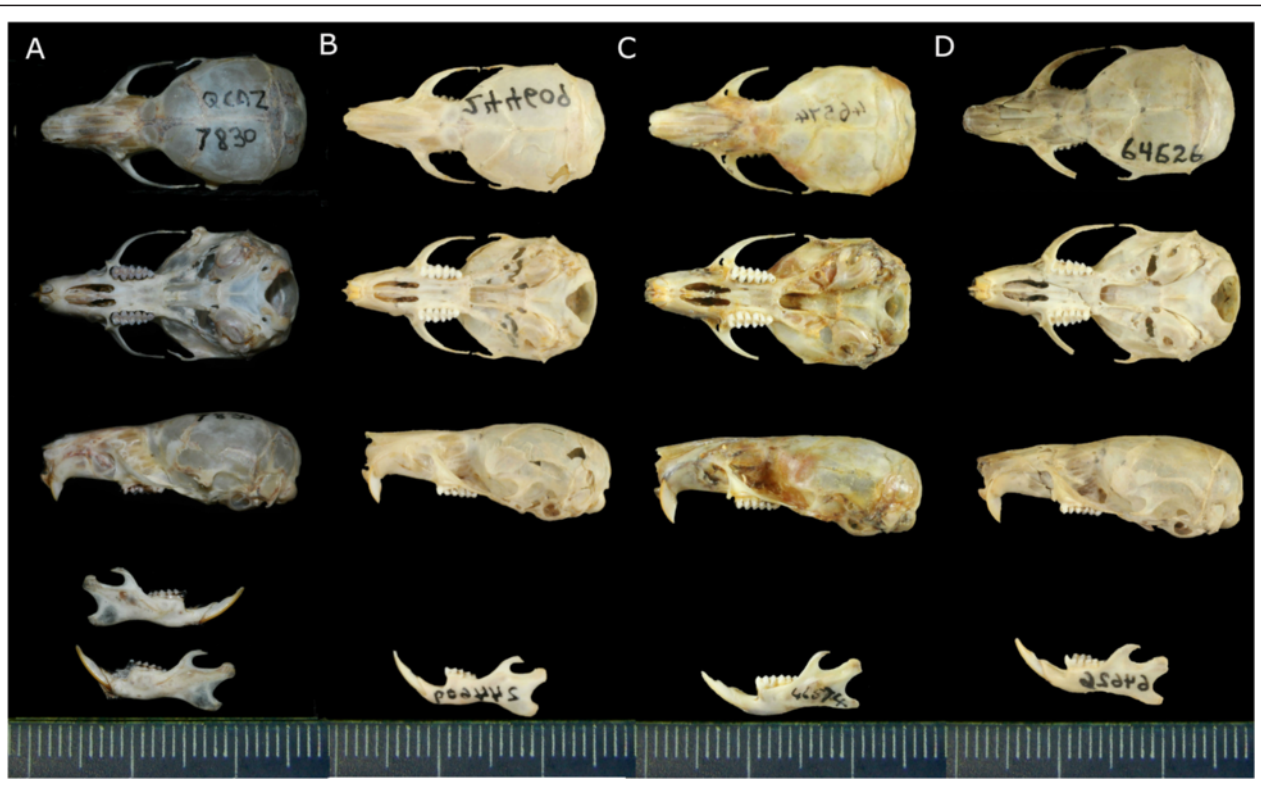

Fig. 6 Skulls and jaw for *Neusticomys vossi* sp. nov. (**a** QCAZ 7830 and **b** AMNH 244609) and *N. monticolus* (**c** AMNH 46574 and **d** AMNH 64626). **a**, **c** are the respective type specimens. All skulls are from adult females with closed cranial sutures except for **c** which is a juvenile male with open sutures

few individuals but show differences in certain cranial characteristics as well as an overall smaller size for the eastern animals (Voss 1988). However, Voss (1988) considered these differences insufficient to consider both forms as distinct species.

Genetic and genealogical results combined with morphological differences presented herein suggest that populations currently allocated to *N. monticolus* east and west of the Andes represent two distinct biological entities. Mean *Cyt*b genetic distance between the two *Neusticomys* haplogroups (8.5 %) is in the order of mean genetic distances shown between species in other groups of sigmodontine rodents (e.g., *Abrothrix* ca. 5 to 10 % [Feijoo et al. 2010; D'Elía et al. in press]; species of the *Akodon boliviensis* species group: 2.8–7.7 % [Jayat et al. 2010]; *Eligmodontia*: 4.6–11.4 % [Mares et al. 2008]; *Juliomys*: ca. 12 % [Pardiñas et al. 2008]; *Melanomys*: 4.5–7.6 % [Hanson and Bradley 2008]; *Nectomys*: 7.36 % [Hanson and Bradley 2008]; *Oligoryzomys*: 4.45–15 % [Hanson et al. 2011; Palma et al. 2010; Richter et al. 2010; Rogers et al. 2009]; *Oryzomys*: 4.5–12.1 % [Hanson et al. 2010]; *Oxymycterus*: 2.5–9.6 % [Jayat et al. 2008]); *Rhipidomys*: 4.1–12.4 % [Costa et al. 2011]; *Scapteromys*: ca. 4.5 % [D'Elía and Pardiñas 2004]; *Sigmodon*: 8.5–20.8 % [Henson and Bradley 2009]). Although the species used for comparison are not the closest possible relatives to *Neusticomys*, all belong to other sigmodontine tribes and provide a diverse array of reference points. Further, the *Rbp*3 genetic distance between the two *Neusticomys* groups (0.8 %) is significant when compared to the 1.5 % difference between them and *Rheomys* (the only other Ichthyomini examined genetically). For a frame of reference, the *Rbp*3 genetic distance between *Neusticomys* and other members of the Sigmodontinae is only 6–8 % (compared to 15–20 % in *Cyt*b). Eastern and western Andean clades of *Neusticomys* previously assigned to *N. monticolus* showed enough genetic differentiation—despite the low sample size—to warrant further examination using other source of evidence (e.g., karyological, morphological, ecological studies; see Baker and Bradley 2006; Bradley and Baker 2001). In this particular situation, the additional investigation was mostly conducted some 21 years (Voss 1988) prior to the discovery of the highly differentiated haplogroups, as well as complemented here.

In addition to the new species described above, the morphometric data further support a unique group near Antiquia, Colombia, which Voss (2015) identified in his account of *N. monticolus* saying, "The few available specimens from the western Andes (Cordillera Occidental) of

Colombia exhibit morphological differences from Ecuadorean material and may represent a distinct species." We identify this subgroup as one requiring further molecular examination.

Conclusions

Ichthyomyini is the least studied tribe of sigmodontine rodents; studies on these mice are rare in the literature, limiting in the current century to the reporting of new geographic records (e.g., Leite et al. 2007; Miranda et al. 2012; Nunes 2002; Pacheco and Ugarte-Núñez 2011), the presentation of new data in reports of mammalian surveys (e.g., Lee et al. 2006a, 2006b, 2008; Voss et al. 2001), synthesis of available knowledge for one species (Packer and Lee 2007), and the description of a new species (e.g., Percequillo et al. 2005). As such, aside from Voss (1988) study, no comprehensive phylogenetic hypothesis is available for the tribe. Therefore, currently, it is not feasible to advance either a robust biogeographic hypothesis accounting for ichthyomyine diversification or one centered on *Neusticomys*. Similarly, given the scarcity of ichthyomyines in natural history collections and the difficulty of collecting them in the field (but see Pacheco and Ugarte-Núñez 2011), the task of performing an exhaustive phylogeographic study of any ichthyomyine species may be difficult to accomplish.

The present study is the first study of ichthyomyine alpha taxonomy since Percequillo et al. (2005) described *N. ferrerai* and the first taxonomic study focused on members of the tribe Ichthyomyini using sequence data. Given the results of our morphological assessment (and that of Voss 1988), it would be informative to perform an analysis of DNA sequences from specimens collected near Antioquia, Colombia.

Resumen

En este estudio se evaluó la estructura genética y morfológica de la variación de la especie *Neusticomys monticolus*. Distancias genéticas y estructura filogeográfica fueron estimadas en base a secuencias de un gen nuclear y otro mitocondrial. Los datos morfológicos fueron analizados estadísticamente. Los datos indican la existencia de dos grupos filogeográficos que difieren morfológicamente y corresponden a poblaciones de las vertientes este y oeste de los Andes. Estos grupos difieren en promedio en 8.5 % en el gen del citocromo b (0.8 % en el gen de la proteína de unión interfotorreceptor retinoide). Las poblaciones actualmente asignadas a *N. monticolus* constituyen un complejo de especies. El nombre *N. monticolus* es aquí restringido a las poblaciones de la vertiente occidental de los Andes. Las poblaciones de las estribaciones orientales de los Andes son asignadas a una nueva especies, a la cual es dado el nombre *Neusticomys vossi* sp. nov.

Appendix

Specimens examined—Specimens are arranged by species and locality. Specific identification numbers (museum or collector numbers) and GenBank accession numbers (*Cyt*b/*Rbp*3; for specimens included in molecular analysis) are listed in parentheses, respectively. Museum acronyms are as follows: Abilene Christian University Natural History Collections (ACUNHC); El Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ); Departamento de Microbiología, Universidad del Valle (HTC); Field Museum of Natural History (FMNH); Museum of Southwestern Biology (MSB); Museum of Vertebrate Zoology (MVZ); The Museum of Texas Tech University (TTU, TK—tissue collection); Thomas E. Lee (TEL; vouchers available at ACUNHC or QCAZ); University of Kansas Museum of Natural History (KU); and University of Michigan Museum of Zoology (UMMZ)

Akodon boliviensis—BOLIVIA: Tarija; 4.5 km E of Iscayachi (MSB68571, *Cyt*b [GenBank:KC841367]).

Akodon paranaensis—PARAGUAY (TTU108159, *Rbp*3 [EU649035]).

Abrothrix longipilis—ARGENTINA: Río Negro, La Veranda (MVZ1554494, *Rbp*3 [AY163557]). CHILE: Araucania; Fundo Hermanos Garcia (MSB205660, *Cyt*b [GenBank:GU564083]).

Arvicola terrestri—SWITZERLAND (MVZ155884, [GenBank:AY275106/AY277407]).

Cricetus cricetus—AUSTRIA: Niederosterreich; 1 km NE Gutramsdorf (MVZ155880, [GenBank:AY275109/AY277410]).

Neusticomys monticolus—COLOMBIA: Antioquia, Santa Bárbara (FMNH71218; FMNH71220; FMNH71221; FMNH71222; FMNH71223). COLOMBIA: Valle, Pichindé (HTC1372; HTC2180; HTC2237; HTC3365). ECUADOR: Chimborazo, Pauchi (AMNH66848). ECUADOR: Cotopaxi, Reserva Otonga (TK149025, [GenBank:KF359517]; TK149034, [GenBank:KF359518]). ECUADOR: Pichincha, Guarumal (UMMZ126298; UMMZ126299; UMMZ126298; UMMZ126299; UMMZ155789; UMMZ155790; UMMZ 155791; UMMZ155793; UMMZ155794). ECUADOR: Pichincha, Las Machinas (AMNH64634; AMNH64636; AMNH64637; AMNH64638; AMNH64639). ECUADOR: Pinchincha, Rio Pita (AMNH64627; AMNH64628; AMNH64629; AMNH64631; AMNH64632). ECUADOR: Pinchincha, San Ignacio (AMNH64625; AMNH64626). ECUADOR: Pichincha, Tandayapa Valley (QCAZ6446, [GenBank:KF359515/KR105606]; ACUNHC900/QCAZ 6531, [GenBank:KF359516/KR105605]).

Neusticomys vossi sp. nov.—COLOMBIA: Huila, San Antonio (FMNH71224; FMNH71225). ECUADOR: Bolivar, Sinche (AMNH62920). ECUADOR: Napo; 11 km SE of Baeza (QCAZ7830, [GenBank:KF359513/KR105608]). ECUADOR:Napo, Papallacta (AMNH244608;

AMNH244609; UMMZ126297; UMMZ155604; UMMZ 155605; UMMZ155606). ECUADOR: Napo; Volcan Sumaco (QCAZ8956, [GenBank:KF359514/KR105607]).

Oryzomys palustris—USA: Texas; Galveston, Virginia Point (TTU82920, [GenBank:DQ185382/EU273431]).

Phyllotis xanthopygus—ARGENTINA: Río Negro, Pilcanieyeu (MVZ182703, *Rbp*3 [GenBank:AY163632]). CHILE: Tarapaca; Parinacota; Arica *Cyt*b (FMNH133830, [GenBank:U86831]).

Reithrodon auritus—ARGENTINA: Rio Negro, Las Victorias (MVZ182704, [GenBank:EU579474/AY163634]).

Rheomys raptor—COSTA RICA: Puntarenas; Monteverde Cloud Forest Reserve, Quebrada Cuecha (KU159017, [GenBank:KF359512/AY163635]).

Sigmodon hispidus—USA: Texas; Cameron, Brownsville (TK32481, *Cyt*b [GenBank:AF425199]). USA: Kansas; Ellis, Hays (OK5840, *Rbp*3 [EU635707]).

Thomasomys erro—ECUADOR: Napo; 12 km N Cosanga (ACUNHC1137/QCAZ7649, [GenBank:EU57 9476/EU649057]).

Wiedomys pyrrhorhinos—BRAZIL: Minas Gerais; Ponte do Colatino (MVZ197566, *Cyt*b [GenBank:EU579477). BRAZIL: Bahia, Jaborandi (CRB1839, *Rbp*3 [AY163644]).

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
JDH supervised the molecular work, drafted the manuscript, and processed the measurements. GD helped draft the manuscript, and proofed and modified the species description. SBA performed the molecular work and helped drafting the manuscript. SBC performed the statistical analyses. TEL and SB collected the specimens and measurements and provided guidance on the natural history of the genus. All authors read and approved the final version of the manuscript.

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