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Genetic and morphological variability of the páramo Oldfield mouse *Thomasomys paramorum* Thomas, 1898 (Rodentia: Cricetidae): evidence for a complex of species

Tesis previa a la obtención del título de Magister en Biología de la Conservación

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Certifico que la Tesis de Maestría en Biología de la Conservación del candidato Carlos Esteban Boada Terán ha sido concluida de conformidad con las normas establecidas; por tanto, puede ser presentada para la calificación correspondiente.

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Dedicado a mi hijo Joaquín

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1. RESUMEN

Revisé las pieles, cráneos, esqueletos y especímenes preservados en alcohol de *Thomasomys paramorum*, depositados en el Museo de Zoología QCAZ, Sección Mastozología, Pontificia Universidad Católica del Ecuador. Además, realicé el trabajo de campo en tres localidades adicionales. Utilicé una análisis multivariado de la variancia (MANOVA) para establecer si las diferencias morfométricas eran estadísticamente significantes. Para analizar la variabilidad morfométrica recurrir a un análisis de componentes principales incluyendo 19 medidas craneales provenientes de 88 individuos. El análisis filogenético, en base a 750 pb del gen mitocondrial cytocromo b de 26 individuos y 1202 pb del exón I del gen nuclear IRBP de siete individuos, lo obtuve bajo los citerios de análisis bayesiano y Maxima Verosimilitud, esos análisis no generaron una buena resolución filogenética para el gen IRBP. Sin embargo, los mismos criterios para el gen cytocromo b resultaron en tres topologías idénticas. Se generaron tres Clados (A, B y C) con distancias genéticas corregidas entre 3,5 y 5,7%. El análisis de componentes principales mostró que los individuos del Clado A y C se separan completamente sin que exista un sobrelapamiento entre ellos. Sin embargo, la separación entre el Clado B con respecto al A y C no es tan clara. Basados en lo resultados de esta investigación y los obtenidos en otros estudios con roedores sigmodontinos, propongo que *T. paramorum* debería considerarse como un complejo de especies que incluye tres linajes independientes.

2. ABSTRACT

I checked the skins, skulls, skeletons and specimens preserved in alcohol of *Thomasomys paramorum* deposited in the Museo de Zoología QCAZ, Sección Mastozología, Pontificia Universidad Católica del Ecuador. I also conducted field work in three additional localities. To establish if the separation in morphometric space was statistically significant, I performed a multivariate analysis of variance (MANOVA). For morphometric variability I used a principal components analysis including 19 cranial measurements of 88 individuals. The phylogenetic analysis, based on 750 pb of the mitochondrial cytochrome b gene from 26 individuals and 1202 bp of exon I of the IRBP nuclear gene from seven individuals, I obtained under the Bayesian inference and maximum likelihood criterias, these analysys did not generate a good phylogenetic resolution for IRBP gene. However, same criterias for the cytochrome b gene showed three identical topologies. Three clades were generated (A, B and C) with corrected genetic distances between 3.5 and 5.7%. The principal component analysis showed that individuals of clade A and C are completely separated without overlaping each other. However, the separation between clade B with respect to A and C is not very clear. Based on the results of this investigation and those obtained in other studies of sigmodontine rodents, I propose that *T. paramorum* should be considered as a species complex that includes three independent lineages.

3. INTRODUCTION

The genus *Thomasomys* Coues 1884 was considered part of the tribe Oryzomyini (Reig, 1986), subfamily Sigmodontinae. However, Musser and Carleton (2005) included this genus in the tribe Thomasomyini, in which they also included *Chilomys*, *Rhipidomys*, *Aepeomys*, *Phaenomys*, *Delomys* and *Wilfredomys*. Later, Smith and Patton (1999) presented analyses based on cytochrome b sequences of an extensive array of sigmodontine species. They found support for a Thomasomyini tribe that included *Thomasomys*, *Chilomys* and *Rhipidomys* and suggested that *Aepeomys* belonged to this clade. They also concluded that neither *Delomys* nor *Wilfredomy*s were closely related to the thomasomyines. A recent phylogenetic analyses based on morphological data supported the monophyly of the tribe Thomasomyini which includes *Abrawayaomys*, *Aepeomys*, *Chilomys*, *Delomys*, *Juliomys*, *Phaenomys*, *Rhagomys*, *Rhipidomys*, *Thomasomys*, *Wiedomys* and *Wilfredomys* (Pacheco, 2003).

The tribe Thomasomyini is poorly defined and one of the least known rodent groups (Pacheco, 2003). *Thomasomys* is the most variable genus in morphology and consequently its systematic retains many unsolved problems (Weksler et al., 2006). *Thomasomys* currently includes about 44 valid species endemic to Tropical Andean cloud forests from Venezuela to Bolivia (Víctor Pacheco, com. pers). Apparently, the center of diversity for the genus includes eastern Ecuador (Voss, 2003; Musser and Carleton, 2005).

Tirira (2007) listed 13 species of *Thomasomys* in Ecuador, all distributed in the highlands, from temperate forests to páramo (Albuja, 2011). Recent reports have added additional species to the country's fauna, specifically *T. praetor* (Lee et al., 2011) and *T. onkiro*

(Moreno and Albuja, 2012). Recently a specialist in Thomasomyini tribe (Victor Pacheco) reviewed the collection of the Museo de Zoología QCAZ, Sección Mastozología and determined the presence of *T. taczanowski* in Ecuador through three specimens.

Thomas (1898) described *Thomasomys paramorum* from the páramo south of Volcán Chimborazo. The species is monotypic, without described subspecies or synonyms (Voss, 2003; Musser and Carleton, 2005). This species is small in size, has small eyes, medium but evident dark brown rounded ears, which are sparsely covered with short, blackish hairs that do not contrast with the color of the head. Hands and feet are white above without darker patches over the metapodials. The vibrissae are thin, long, black, and reach slightly behind the ears. Genal vibrissae are absent, while mystacial vibrissae are long and extend posteriorly just behind the pinnae (Thomas, 1898).

The fur is soft, fine, dense, and long, usually exceeding 15 mm on the midline of the back and towards the tail. The back is uniform olive brown to reddish brown. The ventral region is pale gray to whitish cream with a distinct line between the flank and belly. Hairs on the back, belly and head are bicolored, with the base gray to dark gray. The area between the eyes and nostrils can be darker. Hind legs are long and moderately wide, clothed with silvery hair, brown or blackish on the upper side, soles are black. The claws are usually covered by whitish or silvery small ungula tufts of longer hairs. The tail may be uniform in color or bicolored; it is thick, and longer than the length of the head and body. The tail´s tip lacks a pencil or brush that characterizes other genera in the thomasomyines, appearing naked and finely scaled, but scales are clothed by short, fine and small hairs (Thomas, 1898; Voss, 2003).

The skull of *Thomasomys paramorum* is slender and very delicately built; bones of the braincase are exceedingly thin. The braincase is long, narrow and smoothly rounded. The front edge of the zygomatic root is nearly vertical, without projections. The muzzle is narrow and a rostral tube is absent. The interorbital region is narrow, with rounded supraorbital margins. Incisive foramina are very long, usually extending posteriorly between molar alveoli and an alisphenoid strut is present. The zygomatic plate is broad. Auditory bullae are large and conspicuously inflated (Thomas, 1898; Voss, 2003).

Tirira (2004) regarded *Thomasomys paramorum* as a species endemic to Ecuador, but Pacheco et al. (2008) mentioned its probable presence at Volcán Galeras in Nariño, southern Colombia. In Ecuador this species inhabits the upper montane forests and páramos on both sides of Andes between 2 700 and 4 300 meters, with Azuay province as the southern distributional limit.

Although the species is currently regarded as monotypic, during our review we noted differences among localities of Ecuador, particularly in ventral coloration, the color of the ventral area of the tail, as well as differences in size of adult individuals. To describe patterns of character variation, and to assess phylogenetic relationships among populations, morphometric and molecular traits were employed. The objective was to evaluate if *Thomasomys paramorum* is a single species, or represents a complex of species.

Thomasomys paramorum is considered as Least Concern species (Pacheco et al., 2208) in view of its tolerance of habitat modification, presumed large population and because it is unlikely to be declining at nearly the rate required to qualify for listing in a threatened category. Besides the distribution in Ecuador is extensive and covers several types of

habitats, including some areas that have been modified for agricultural activities. Finally, it has been recorded in some protected areas and its conservation status is considered stable (Tirira, 2007).

4. MATERIALS AND METHODS

4.1. SPECIMENS AND LOCALITIES

I examined skins, skulls, skeletons and fluid-preserved specimens of *T. paramorum* from seven localities, and deposited at the Museo de Zoología QCAZ, Sección Mastozología, Pontificia Universidad Católica del Ecuador. Additionally, three localities were visited within the known range of the species where no collections were available: Polylepis lodge, Carchi province; Casitahua, Pichincha province; and Jamanco, Napo province (Table 1). I used 88 individuals for cranial morphometric analyses (Table 2) and 26 individuals for phylogenetic molecular analyses (Table 3).

4.2. DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

The genomic DNA was extracted from liver and muscle tissue of individuals collected in the field and from tissues deposited in the QCAZ museum, where they are kept stored in 95% ethanol solution at -80 °C. Our extraction protocol was based on Bilton and Jaarola (1996), with some modifications. Both a 753 bp fragment of the mitochondrial cytochrome b gene and a 1202 bp fragment of the exon I of the nuclear IRBP gene (Interphotoreceptor Retinoid Binding Protein) were amplified (Table 4).

A polymerase chain reaction (PCR) and a standardized protocol to amplify DNA were used (Irwin et al., 1991; Jansa and Voss, 2003; Weksler, 2003; Arellano et al., 2005; Ferreira et al., 2010), although reduced annealing temperature from 52 to 48ºC. Sequencing was performed by Macrogen (Macrogen Inc., Seoul, Korea), using a 730XL 3 ("Applied Biosystems") automatic 96-well capillary sequencer.

The protocol for amplification of the cytochrome b gene was: two minutes of denaturation at 94°C, 35 cycles (one minute of denaturation at 94 °C, one minute of annealing at 48°C, followed by one minute of extension at 72°C), and five minutes of final extension at 72°C. The protocol for amplification of the IRBP gene consisted of two minutes of initial denaturation at 94°C followed for a four-stage touchdown protocol and a final five minute extension at 72°C. All stages were identical with five cycles of denaturation at 95ºC for 20 seconds and extension at 72ºC for 60 seconds. The first, second, third, and fourth stages had different lowered annealing temperatures of 58°C, 56°C, 54°C and 52°C, respectively.

In order to determine the quality of the amplification process, PCR products from the two genes were electrophoresed on 1% agarose gels stained with ethidium bromide. Any unconsumed dNTPs and primers remaining in the PCR product mixture were removed with the ExoSAP-IT method (Dugan et al., 2002).

4.3. PHYLOGENETIC ANALYSES AND SEQUENCE VARIATION

The IRBP gene has been widely used to study the phylogeny of mammals. This gene encodes a large glycoprotein which is found mainly in the matrix of interphotoreceptors of the retina (Danciger et al., 1990; Pepperberg et al., 1993). Sequences of this gene were

initially used to infer phylogenetic relationships at the order level (Stanhope et al., 1996), but more recently it also has been used for phylogenetic relationships in lower taxonomic levels (Suzuki et al., 2000; Voss and Jansa, 2000; Michaux et al., 2002; D'Elía, 2003; Jansa and Weksler, 2003; Weksler, 2003; Jansa and Voss, 2005).

The cytochrome b gene has become useful for phylogenetic and phylogeographic studies in rodents. Smith and Patton (1991, 1993 and 1999) worked in the diversification of some sigmodontine rodents. Sullivan et al. (1997, 2000) used this gene for phylogeographic studies of rodents from the Mesoamerican highlands, within species and species complexes. Smith et al. (2001) used this gene to test models of diversification in the *Abrothrix olivaceus*/*xanthorhinus* complex in Chile and Argentina. Arellano et al. (2005) used this gene in the study of the molecular systematics of Middle American Harvest mice *Reithrodontomys* (Muridae). Smith and Patton (2007) used this gene in the study of the molecular phylogenetics and diversification of South American grass mice, genus *Akodon*. Jayat et al. (2010) published about species limits and distribution of the *A. boliviensis* group in Argentina using this gene. In the case of the thomasomyines, Salazar-Bravo and Yates (2007), present molecular data based on cytochrome b sequences of some *Thomasomys* species, in the description of *T. andersoni* from Bolivia.

I analyzed 26 cytochrome b sequences of *Thomasomys paramorum*, using *T. erro* as an outgroup. *T. erro* was chosen as outgroup because it is closely related to *T. paramorum* and its sequence of cytochrome b gene was available in "Genbank". For the analysis of IRBP gene, I had only seven ingroup sequences, and used *T. baeops* as the outgroup. Both *T. erro* and *T. baeops* are closely related to the study group (ingroup), but not as closely related as any study-group members are to each other.

I used Geneious version 5.4 (Biomatters 2005 - 2013) to assemble and edit each sequence, and aligned them using the Muscle (Edgar, 2004) application in Mesquite version 2.97 (Maddison and Maddison, 2011).

A phylogenetic analysis separately for cytochrome b and IRBP sequences under the optimality criteria of Maximum Likelihood (ML) and Bayesian Inference (BI) was performed. To determine the best evolutionary model of nucleotide substitution, I used the Akaike information criterion (AIC) and Bayesian information criterion with the program JModelTest 0.1.1 (Posada, 2008). The ML analysis was conducted in GARLI version 0.951 (Zwickl, 2006). The most suitable model of nucleotide substitution for phylogenetic reconstruction through ML was chosen with the JModelTest version 0.1.1 (Posada, 2008). Nodal support was determined with 100 bootstrap replicates. Following Hillis and Bull (1993), bootstrap values >70% indicate well supported nodes.

For BI analyses, I used MrBayes version 3.4 (Ronquist and Huelsenbeck, 2003). Four Markov chains were run for 20 million generations and sampled every 1000 generations. The analysis was performed two times, independently. After discarding the first 1000 samples of each run as “burn-in”, the remaining trees were used to reconstruct a majority-rule consensus tree and calculate the posterior probabilities. The burn-in was determined by observing the stationary of the likelihood scores and convergence of posterior probabilities between two runs using the standard deviation of split frequencies.

Sequence variation was assessed with corrected genetic distances obtained under the Tamura Nei model (Tamura and Nei, 1993) in Geneious version 5.4 (Biomatters 2005 – 2013).

4.4. MORPHOLOGICAL MEASUREMENTS

To determine cranial morphology, 19 cranial measurements chosen based on previous taxonomic studies were selected (Voss, 1988; Musser et al., 1998; Alvarado, 2005). These included interorbital breadth (IB), occipitonasal length (ONL), greatest zygomatic breadth (ZB), crown length of maxillary toothrow (CLM1-3), breadth of zygomatic plate (BZP), length of bony palate (LBP), breadth of bony palate across first upper molars (BBP), breadth of incisive foramina (BIF), width of anterior region of the mesopterygoid fossa (WFM), length of rostrum (LR), breadth of first upper molar (BM1), breadth of rostrum (BR), height of braincase (HBC), length of diastema (LD), breadth of incisor tips (BIT), breadth of occipital condyles (BOC), occlusal length of mandibular tooth row (OLMT), postpalatal length (PL) and height of lower jaw (HLJ). I measured cranial variables from adult specimens with digital calipers to the nearest 0.01 mm and only from adult specimens. To define the age of specimens, I followed the criteria of Voss (1988).

4.5. STATISTICAL ANALYSES OF MORPHOLOGICAL DATA

I quantitatively compared 19 adult cranial measurements from 88 individuals assigned to *Thomasomys paramorum*. I estimated missing data because of broken or incomplete structures using an expectation-maximization method that estimates repeatedly missing values and adjusts to stabilize the covariance matrix (Strauss et al., 2003).

To establish if the separation in morphometric space was statistically significant, I performed a multivariate analysis of variance (MANOVA) using the 19 morphometric measurements as dependent variables and clades identified by the phylogenetic analysis of sequences as fixed factors.

The complete dataset was used to perform a principal component analysis (PCA) on the variance-covariance matrix to assess the degree of morphometric differentiation between the clades (see below), following the methodology of Anderson and Jarrín (2002). This method has been widely used in morphometric studies because it requires a small number of unrelated components to explain the increased proportion of the variance present in size (Lestrel, 2000).

For the PCA analysis, the morphometric data were log-transformed. In the analysis, a VARIMAX rotated method was used to obtain a better interpretation of the data in a two dimensional space. PC axes with eigenvalues >1 were retained for evaluated the percentage of variation of each component and the effect of the variables on each, according to the Kaiser rule (Golub and Van der Voss, 2000; Smith, 2002; Sánchez, 2009). All analyzes were conducted with SPSS Statistics 18.0.

5. RESULTS

5.1. PHYLOGENETIC ANALYSES

Bayesian and Maximum Likelihood analyses based on 1 202 characters of the exon I of the IRBP gene provided poor phylogenetic resolution; limited base variability resulted in a basal polytomy among individual sequences. Tree topologies resulting from Bayesian and Maximum Likelihood phylogenetic analyses of cytochrome b were identical. Three major clades (A, B, C) were recovered with strong nodal support (posterior probability = 100; bootstrap = ≥ 0.90; Figure 1). The corrected genetic distance, based on the Tamura Nei model (1993), ranged from 3.5 to 5.7% between clade A and B, 4.6 to 5.7% between clade

A and C and from 3.3 to 3.9 % between clade B and C (Table 5). The three clades of *Thomasomys paramorum* form a monophyletic assemblage with respect to the outgroup, *T. erro*.

Clade A contains individuals from localities in Carchi province (Lagunas del Voladero, 3600 masl and Páramo del Artesón, 3600 masl); clade B contains individuals from localities in Imbabura province (Zuleta, 2900 masl and Angochagua, 3600 masl); and clade C groups individuals from Napo province (Jamanco, 3700 masl), the boundary of Chimborazo and Morona Santiago provinces (Lagunas de Atillo, 3400 masl), Pichincha province (Casitahua, 3300 masl), and Cotopaxi province (Barrancas, 3300 masl) (Figure 2).

Clade C contains three allopatric distinct lineages. The first lineage includes specimens from Lagunas de Atillo; and the second includes specimens from Pichincha and Cotopaxi. However there is a single specimen from Jamanco which does not correspond to any of those lineages (Figure 1). Corrected genetic distances between these lineages vary from 0.02 to 2.10%, with the single specimen from Jamanco (QCAZ 12777) responsible for most of the differentiation observed (1.5 to 2.1% in relation to specimens from other subclades). The corrected genetic distances among the other specimens of the clade C (not including that from Jamanco) range from 0.02 to 0.07% (see Table 5).

5.2. MORPHOMETRIC ANALYSES

We provide means, standard errors and ranges for each measured variable in Table 6. Specimens are pooled by their molecular clade membership, with the clades (A, B, and C) exhibiting significant morphometric differences (MANOVA, $p < 0.01$). The principal component analysis showed that individuals of clades A and C are clearly separated on the bivariate PC1 and PC2 plot, with little overlap between them. However, clade B shows no clear separation with respect to the others (Figure 3).

The first three components capture most of the variation between clades, accounting for 39.56% of the total variance in the sample (19.35%, 12.42% and 7.79% respectively; Table 7). In the first component (PC1) ONL, LD and LR had the largest eigenvalues, and thus influenced the placement of individuals on that axis. In the second component (PC2) HBC, OLMT and BR were the more explanatory variables. Finally in the third component (PC3), the most explanatory variables were BIT, HBC and BM1 (see Table 7).

Members of clade A are distributed in the upper part of the first component axis, because on average, these specimens have a longer skull, a longer diastema and wider face, in relation to the individuals of clades B and C (Table 6). Members of clade C are distributed in the left part of the second component axis, again due to a higher braincase, broader rostrum, and longer occlusal length of the mandibular tooth row. Members of clade B cannot be distinguished from individuals of other clades.

6. DISCUSSION

With more than 2277 species, the order Rodentia is the most diverse taxon of mammals (Hedges and Kumar, 2009), and also one of the groups with more uncertain taxonomy. Species assignment based on morphological data solely is often difficult, so identifying rodents at the specific level can be a substantial challenge (Galan et al., 2012). Based on molecular, cytogenetic, and morphometric studies, new species or species complexes that had been previously identified as a single lineage due to morphological similarity, are now routinely recognized on the basis of newly collected specimens and study of museum collections (Ceballos and Ehrlich, 2006; D'Elıa and Pardiñas, 2007; Reeder et al., 2007).

The rodents of Ecuador, have been poorly studied, with few studies that include lists of species at individual localities, notes on range extensions or records of species not yet known in the country. In addition to this study, a few researches using molecular assays have been conducted with rodents of Ecuador. Salazar-Bravo and Yates (2007) reported cytochrome b sequences for some species of *Thomasomys* from Ecuador, among 12 other species in the genus. Lee et al. (2011) reported cytochrome b sequences for some species of *Thomasomys* (including *T. paramorum*) from specimens collected at Sangay National Park in Ecuador. Finally, Chávez (2012), examined the taxonomic identity of populations of the *Reithrodontomys mexicanus* complex in Ecuador, using the same two genes we employ herein.

With respect to *Thomasomys*, this genus is often regarded as a group undergoing rapid speciation, with the eastern foothills of Ecuador a center of diversity and endemism (Voss, 2003; Musser and Carleton, 2005). There are few studies using molecular tools on this

genus; Smith and Patton (1999) were the first to include sequences of *Thomasomys* in their analyses of phylogenetic relationships among sigmodontine rodents using cytochrome b sequences. Their study included seven species of *Thomasomys*, all from Peru. Subsequently, D'Elia et al. (2006) clarified the affinities of *Rhagomys*, but they included only a single *Thomasomys* sequence, and again no species from Ecuador. Salazar-Bravo and Yates (2007) presented molecular data based on cytochrome b of 15 species of *Thomasomys*, including three species from Ecuador: *T. baeops*, *T. caudivarius* and *T. cinnameus*. However, only the study of Lee et al. (2011) reported sequences of *T. paramorum*, the species under investigation.

So far, the true diversity of *Thomasomys* in Ecuador is unknown. Voss (2003) described a new species (*T. ucucha*) in the area of Papallacta, Napo Province; Lee et al. (2011) reported for the first time *T. praetor* from the Atillo lagoons on the border between Chimborazo and Morona Santiago provinces, and more recently Moreno and Albuja (2012) reported for the first time the presence of *T. onkiro* in the province of Zamora Chinchipe. For this reason, all research involving species within the genus *Thomasomys* in Ecuador are important in terms of overall diversity and conservation. For example, Myers et al. (2000) indicated that it is important to know the true diversity of the worldwide hot-spots of mammalian diversity, of which the Andes of Ecuador represent one.

The molecular analyses used in this study to determine differences among populations of *Thomasomys paramorum*, reflect the separation of three clades with corrected genetic distances between 3.3 and 5.7% for cytochrome b. The genetic distance and branch lengths suggest that the three clades might each represent separate taxa. In contrast, the analyses of the IRBP gene did not produce a good phylogenetic resolution. However, it is known that

the nuclear genes evolve more slowly because these genes accumulate mutations gradually (Lewin, 2004).

Baker and Bradley (2006) assessed whether the degree of cytochrome b sequence divergence in mammals can be used for species-level differentiation. With respect to rodents (Sigmodontines and Peromyscines) they found that intrapopulation divergence values typically ranged from 0.0 to 1.4%. However, other molecular studies of Sigmodontine rodents have reported values ranging from 0 to 3.87% (Smith and Patton, 1991, 1993, 1999; Patton et al., 2000; D'Elía, 2003; D'Elía et al., 2008; Catzeflis and Tilak, 2009).

Many rodent species inhabiting the Andes exhibit small genetic divergences due to recent speciation (Smith and Patton, 2007). For example some Akodontine lineages have uncorrected cytochrome b distances of 2% yet are recognized as distinct species (Smith and Patton, 1991; Smith and Patton, 1993). Arellano (2005) acknowledge *Reithrodontomys espectabilis* and *R. gracilis* as different species, although they found an uncorrrected genetic distance from cythocrome b gene, among 1.2% and 1.3%. The authors explained that the small value of genetic distance observed was due to recent speciation

In this study, we found divergences between 3.3 and 5.7% among the three clades, thus each clade obtained may be considered a different species, rather than belonging to a single species as currently understood. Is important to note that the specimen from Jamanco (QCAZ 12777) exhibits corrected genetic distances of 1.5 to 2.1% in relation to the other specimens that form clade C, which may suggest it corresponds to a different lineage (see Arellano et al., 2005). However, since we have just one specimen from Jamanco and from

Napo province in general, we cannot affirm that it corresponds to a distinct entity that could be considered a distinct taxon. We need more specimens from that province to resolve its status.

Each of the three clades has strong internal geographical congruence, since haplotypes of the different clades are completely non-overlapping in space. Our study shows that molecular divergence of *Thomasomys paramorum* has been strictly geographical rather than ecological (as along an elevation gradient); so the diversification fits an allopatric model of speciation. In this model, an ancestral species with a broad and continuous distribution is hypothesized to have undergone differentiation triggered by a vicariant event, resulting in divergent distributions where gene flow vanishes with increases in genetic divergence until different species result (Patton, 1986; Reig, 1986; Patton and Smith, 1992).

Molecular divergence may be also explained by a dispersal model in which a taxon evolves from a center of origin by dispersing out from there. Thus a founder population is established through normal dispersal but at some point, and due to an environmental, geological, climatic or anthropic factor, the migration stops (Gillespie and Clague, 2009). So, the gene flow no longer occurs and populations diverge to produce different species.

Members of the three clades occupy similar habitats at their respective localities, especially herbaceous páramo and shrub páramo. Only at Zuleta, Imbabura (clade B), some specimens were obtained in remnant patches of high montane evergreen forest; and in the case of clade A, specimens were obtained in frailejones páramo.

In the PCA, the first three components capture 39.56% of the total variance in the sample, which is lower than the usual percentage in morphometric studies. However, there is a clear morphometric separation between the individuals of clades A and C. Nevertheless, the individuals of clade B are co-distributed among members of the other clades without clear separation. Apparently, the morphological identity of clade B has not yet become sufficiently defined, although the small sample available may limit the ability to differentiate this group in comparison to individuals of the other two clades and perhaps, this is the reason for the lack of distinction.

From the three clades obtained in this study, at least two (clades A and B) should be inside a threatened category. According to the evaluation criteria of Threatened Species (IUCN, 2000), these clades must be considered vulnerable because its area of occupancy is less than 20 000 km2 and it has less than five known occurrence localities within its range (criteria D2).

My results show that more effort needs to be conducted in order to understand the real diversity in the highlands of Ecuador, especially of those genres grouping several cryptic species and should be done not only in the field, but reviewing the available museum collections. These studies may define the presence of new species for Ecuador and also assess their conservation status. Without knowing the true limits in different lineages, some of which may be mistakenly considered as least concern species. So, is evident that this kind of investigations, can improve the conservation of species process.

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8. FIGURES

Figure 1. *Thomasomys paramorum* phylogeny based on Bayesian inference (BI) derived from the mitochondrial gene cytochrome b. Nodal support is represented by posterior probabilities (above branches) and ML bootstrap (below branches). *T. erro* is the outgroup. The boxes to the right indicate the name of the clade. The scale bar below the phylogenetic tree represents the patristic distances. The field number, species name and locality are noted at each terminal.

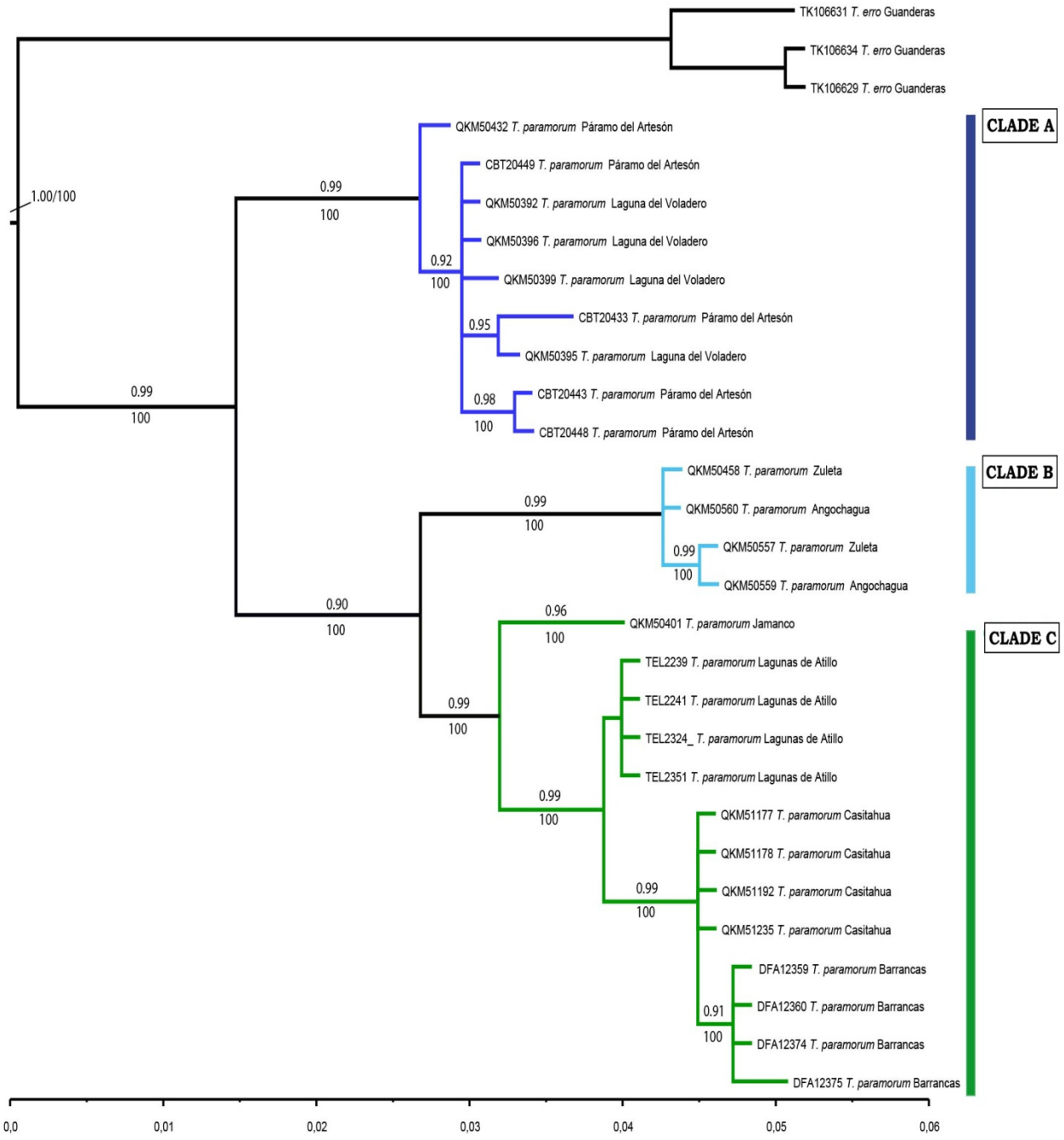


Figure 2. Geographic location of the clades obtained during this study.

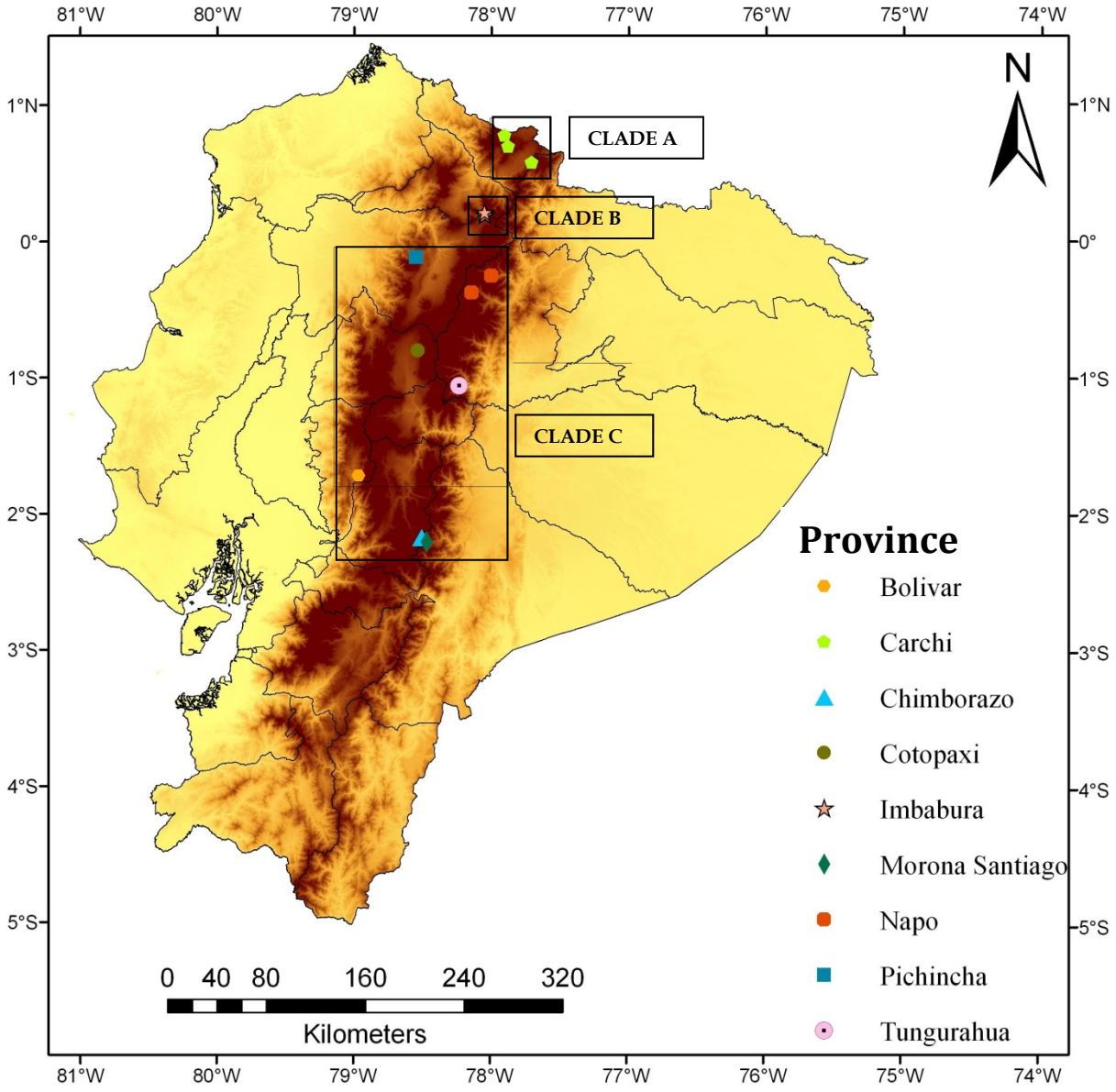
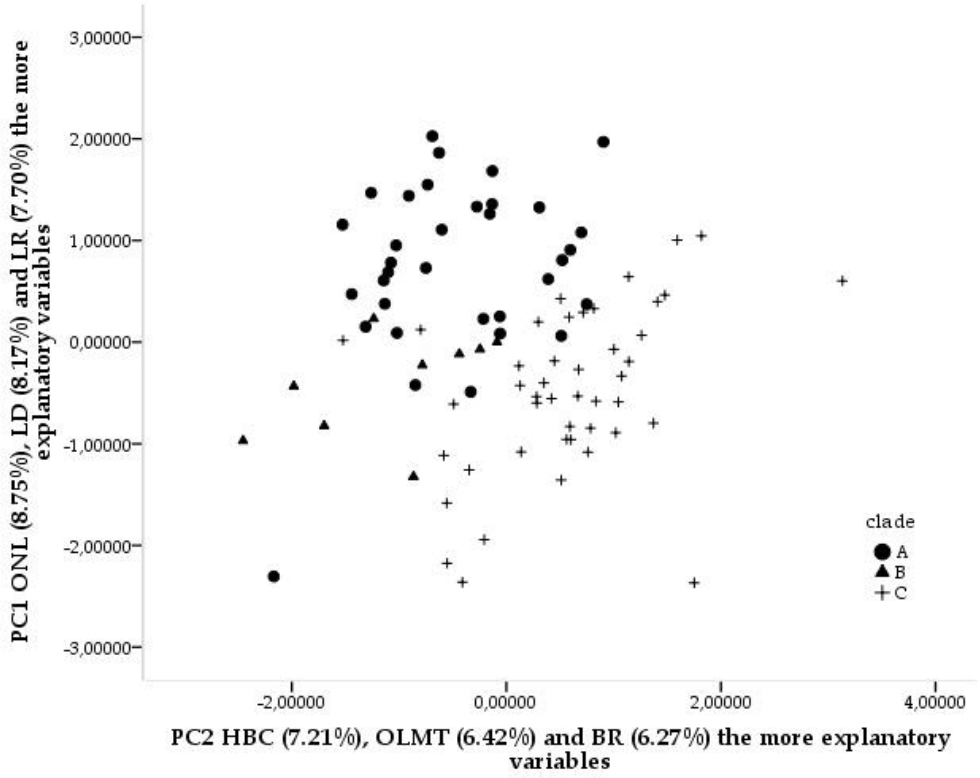


Figure 3. Principal component analysis obtained from morphological variables of adult specimens of clades A, B and C.



ONL = occipitonasal length; LD = length of diastema; LR = length of rostrum; HBC = height of braincase; OLMT = occlusal length of mandibular tooth row; BR = breadth of rostrum

9. TABLES

Table 1. Provinces and localities of specimens included in this study.

Province	Locality	Latitude	Longitude	Altitude
Carchi	Lagunas del Voladero	0.69766	–77.87739	3600
Carchi	Páramo del Artesón	0.77909	–77.90627	3600
Carchi	Polylepis Lodge	0.71878	–77.98030	3600
Chimborazo	Lagunas de Atillo	–2.17714	–78.50747	3400
Cotopaxi	Río Barrancas	–0.80011	–78.53726	3300
Imbabura	Hacienda Zuleta	0.19373	–78.05046	2900
Imbabura	Angochagua	0.21330	–78.05121	3600
Napo	Jamanco	–0.36770	–78.18804	3700
Pichincha	Cerro Casitahua	–0.02699	–78.47646	3300
Tungurahua	Lagunas de Pisayambo	–1.05839	–78.23627	3600

Table 2. List of specimens used in morphometric analyses in this study.

QCAZ	Province	Locality	Clade
9788	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9789	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9804	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9805	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9808	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9812	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9815	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9818	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9823	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
9842	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
9846	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11199	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11200	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11202	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11206	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11209	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11210	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11217	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11222	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11225	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11227	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11233	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11235	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11239	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
12572	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A

12573	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12574	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12579	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12582	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12583	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12584	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12585	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12591	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12593	Carchi	Reserva Ecológica El Ángel. Polylepis Lodge	A
12001	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12002	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12004	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12014	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12015	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12016	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12017	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12019	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12024	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
6658	Cotopaxi	Río Barrancas	C
6659	Cotopaxi	Río Barrancas	C
6660	Cotopaxi	Río Barrancas	C
6662	Cotopaxi	Río Barrancas	C
6663	Cotopaxi	Río Barrancas	C
6664	Cotopaxi	Río Barrancas	C
6665	Cotopaxi	Río Barrancas	C
6678	Cotopaxi	Río Barrancas	C

8435	Cotopaxi	Río Barrancas	C
8437	Cotopaxi	Río Barrancas	C
8438	Cotopaxi	Río Barrancas	C
8341	Cotopaxi	Río Barrancas	C
8441	Cotopaxi	Río Barrancas	C
8445	Cotopaxi	Río Barrancas	C
8454	Cotopaxi	Río Barrancas	C
11674	Imbabura	Zuleta, Comunidad de Zuleta	B
11675	Imbabura	Zuleta, Comunidad de Zuleta	B
11676	Imbabura	Zuleta, Comunidad de Zuleta	B
11677	Imbabura	Zuleta, Comunidad de Zuleta	B
11678	Imbabura	Zuleta, Comunidad de Zuleta	B
11679	Imbabura	Zuleta, Comunidad de Zuleta	B
11685	Imbabura	Zuleta, Comunidad de Zuleta	B
11687	Imbabura	Zuleta, Comunidad de Zuleta	B
11700	Imbabura	Zuleta, Comunidad de Zuleta	B
12777	Napo	Jamanco, Comunidad de Jamanco	C
12601	Pichincha	Cerro Casitahua	C
12602	Pichincha	Cerro Casitahua	C
12603	Pichincha	Cerro Casitahua	C
12606	Pichincha	Cerro Casitahua	C
12608	Pichincha	Cerro Casitahua	C
12617	Pichincha	Cerro Casitahua	C
12620	Pichincha	Cerro Casitahua	C
12621	Pichincha	Cerro Casitahua	C
12622	Pichincha	Cerro Casitahua	C

12623	Pichincha	Cerro Casitahua	C
12626	Pichincha	Cerro Casitahua	C
12627	Pichincha	Cerro Casitahua	C
12628	Pichincha	Cerro Casitahua	C
12632	Pichincha	Cerro Casitahua	C
12635	Pichincha	Cerro Casitahua	C
5783	Tungurahua	Parque Nacional Llanganates, Laguna de Pisayambo	C
5785	Tungurahua	Parque Nacional Llanganates, Laguna de Pisayambo	C
5786	Tungurahua	Parque Nacional Llanganates, Laguna de Pisayambo	C
5787	Tungurahua	Parque Nacional Llanganates, Laguna de Pisayambo	C
5788	Tungurahua	Parque Nacional Llanganates, Laguna de Pisayambo	C

Table 3. List of specimens used in the phylogenetic analyses in this study.

QCAZ	Field series	Province	Locality	Clade
9789	CBT20433	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9799	CBT20443	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9804	CBT20448	Carchi	Páramo del Artesón, Comuna La Esperanza	A
9805	CBT20449	Carchi	Páramo del Artesón, Comuna La Esperanza	A
11199	QKM50392	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11202	QKM50395	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11203	QKM50396	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11205	QKM50399	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11239	QKM50432	Carchi	Reserva Ecológica El Ángel. Lagunas del Voladero	A
11986	TEL2239	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
11987	TEL2241	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12004	TEL2324	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
12013	TEL2351	Chimborazo	Parque Nacional Sangay, Lagunas de Atillo	C
6659	DFA12360	Cotopaxi	Río Barrancas	C
6660	DFA12374	Cotopaxi	Río Barrancas	C
6664	DFA12375	Cotopaxi	Río Barrancas	C
6665	DFA12359	Cotopaxi	Río Barrancas	C
11674	QKM50458	Imbabura	Zuleta, Comunidad de Zuleta	B
11679	QKM50557	Imbabura	Zuleta, Comunidad de Zuleta	B
11680	QKM50559	Imbabura	Zuleta, Comunidad de Zuleta	B

11681	QKM50560	Imbabura	Páramo de Angochagua	B
12777	QKM50401	Napo	Jamanco, Comunidad de Jamanco	C
12565	QKM51178	Pichincha	Cerro Casitahua	C
12602	QKM51177	Pichincha	Cerro Casitahua	C
12610	QKM51192	Pichincha	Cerro Casitahua	C
12624	QKM51235	Pichincha	Cerro Casitahua	C

Table 4. Primers used for amplification of both genes used in this study.

Name of primer	Sequence
Cytochrome b	
L–14115	5'–GATATGAAAAACCATCGTTG–3'
L–14553	5'–CTACCATGAGGACAAATATC–3'
H–14541	5'–CAGAATGATATTTGTCCTCA–3'
H–14963	5'–GGCAAATAGGAARTATCATT–3'
IRBP	
A1	5'–ATGCGCGAAGGTCCTCTTGGATAAC–3'
D2	5'–TATCCCACATTGCCCGGCAGCA–3'
F	5'–CTCCACTGCCCTCCCATGTCT–3'

Table 5. Corrected genetic distances between individuals of *Thomasomys paramorum* based on the Tamura Nei model (1993) used for the phylogenetic analyses. Clades to which individuals belong are shown following Figure 1.

			Clade A									Clade B				Clade C													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Clade A	1	QCAZ 11202	0																									
	2	QCAZ 11203	0	0																								
	3	QCAZ 11205	0,001	0,001	0																							
	4	QCAZ 11199	0	0	0,001	0																						
	5	QCAZ 11239	0,004	0,004	0,005	0,004	0																					
	6	QCAZ 9789	0,001	0,001	0,003	0,001	0,005	0																				
	7	QCAZ 9799	0	0	0,001	0	0,004	0,001	0																			
	8	QCAZ 9804	0	0	0,001	0	0,004	0,001	0	0																		
	9	QCAZ 9805	0	0	0,001	0	0,004	0,001	0	0	0																	
Clade B	10	QCAZ 11674	0,055	0,055	0,057	0,055	0,052	0,057	0,055	0,055	0,055	0																
	11	QCAZ 11679	0,055	0,055	0,057	0,055	0,052	0,057	0,055	0,055	0,055	0	0															
	12	QCAZ 11680	0,055	0,055	0,057	0,055	0,052	0,057	0,055	0,055	0,055	0	0	0														
	13	QCAZ 11681	0,055	0,055	0,057	0,055	0,052	0,057	0,055	0,055	0,055	0	0	0	0													
Clade C	14	QCAZ 12777	0,054	0,054	0,055	0,054	0,05	0,055	0,054	0,054	0,054	0,037	0,037	0,037	0,037	0												
	15	QCAZ 11986	0,05	0,05	0,051	0,05	0,046	0,051	0,05	0,05	0,05	0,033	0,033	0,033	0,033	0,015	0											
	16	QCAZ 11987	0,05	0,05	0,051	0,05	0,046	0,051	0,05	0,05	0,05	0,033	0,033	0,033	0,033	0,015	0	0										
	17	QCAZ 12004	0,05	0,05	0,051	0,05	0,046	0,051	0,05	0,05	0,05	0,033	0,033	0,033	0,033	0,015	0	0	0									
	18	QCAZ 12013	0,05	0,05	0,051	0,05	0,046	0,051	0,05	0,05	0,05	0,033	0,033	0,033	0,033	0,015	0	0	0	0								
	19	QCAZ 12602	0,052	0,052	0,053	0,052	0,048	0,053	0,052	0,052	0,052	0,035	0,035	0,035	0,035	0,017	0,002	0,002	0,002	0,002	0							
	20	QCAZ 12565	0,052	0,052	0,053	0,052	0,048	0,053	0,052	0,052	0,052	0,035	0,035	0,035	0,035	0,017	0,002	0,002	0,002	0,002	0	0						
	21	QCAZ 12610	0,052	0,052	0,053	0,052	0,048	0,053	0,052	0,052	0,052	0,035	0,035	0,035	0,035	0,017	0,002	0,002	0,002	0,002	0	0	0					
	22	QCAZ 12624	0,052	0,052	0,053	0,052	0,048	0,053	0,052	0,052	0,052	0,035	0,035	0,035	0,035	0,017	0,002	0,002	0,002	0,002	0	0	0	0				
	23	QCAZ 6659	0,052	0,052	0,053	0,052	0,048	0,053	0,052	0,052	0,052	0,035	0,035	0,035	0,035	0,017	0,002	0,002	0,002	0,002	0	0	0	0	0			
	24	QCAZ 6660	0,052	0,052	0,053	0,052	0,048	0,053	0,052	0,052	0,052	0,035	0,035	0,035	0,035	0,017	0,002	0,002	0,002	0,002	0	0	0	0	0	0		
	25	QCAZ 6664	0,056	0,056	0,057	0,056	0,052	0,057	0,056	0,056	0,056	0,039	0,039	0,039	0,039	0,021	0,006	0,006	0,006	0,006	0,004	0,004	0,004	0,004	0,004	0,004	0	
	26	QCAZ 6665	0,056	0,056	0,057	0,056	0,052	0,057	0,056	0,056	0,056	0,039	0,039	0,039	0,039	0,021	0,006	0,006	0,006	0,006	0,004	0,004	0,004	0,004	0,004	0,004	0,007	0

Table 6. List of morphometric values used in this study for each clade obtained. The values are the mean ± standard deviation. Values in parentheses correspond to the minimum and maximum values.

Variable morphometric	**Abbreviation**	**Clade A n= 34**	**Clade B n= 9**	**Clade C n= 45**
Interorbital breadth	IB	4.17 ± 0.18 (4.43 – 3.53)	4.18 ± 0.18 (4.54 – 4.00)	4.44 ± 0.22 (4.82 – 3.55)
Occipitonasal length	ONL	27.63 ± 0.72 (28.99 – 25.75)	26.95 ± 0.50 (27.62 – 26.27)	27.03 ± 0.52 (28.35 – 25.56)
Greatest zygomatic breadth	ZB	14.36 ± 0.43 (15.34 – 13.57)	13.98 ± 0.31 (14.42 – 13.51)	14.02 ± 0.39 (14.96 – 12.91)
Crown length of maxillary toothrow	CLM1–3	4.12 ± 0.21 (4.36 – 3.15)	4.11 ± 0.11 (4.31 – 3.92)	4.20 ± 0.13 (4.51 – 3.87)
Breadth of the zygomatic plate	BZP	1.91 ± 0.17 (2.18 – 1.60)	1.84 ± 0.09 (1.99 – 1.67)	2.01 ± 0.13 (2.29 – 1.81)
Length of bony palate	LBP	4.28 ± 0.19 (4.80 – 3.88)	4.44 ± 0.38 (5.31 – 4.06)	4.18 ± 0.23 (4.51 – 3.35)
Breadth of bony palate across first upper molars	BBP	5.72 ± 0.36 (6.23 – 4.15)	5.74 ± 0.18 (5.95 – 5.36)	5.81 ± 0.22 (6.33 – 5.16)
Breadth of the incisive foramina	BIF	1.97 ± 0.13 (2.23 – 1.69)	1.91 ± 0.07 (1.96 – 1.77)	2.04 ± 0.19 (1.73 – 2.97)
Width of the anterior region of the fossa mesopterygoidea	WFM	1.80 ± 0.14 (2.07 – 1.45)	1.81 ± 0.08 (1.95 – 1.66)	1.78 ± 0.14 (2.01 – 1.30)
Length of rostrum	LR	10.21 ± 0.33 (9.87 – 11.01)	9.77 ± 0.51 (10.35 – 9.10)	9.91 ± 0.28 (10.67 – 9.90)
Breadth of first upper molar	BM1	1.27 ± 0.05	1.27 ± 0.06	1.31 ± 0.05

		(1.39 – 1.17)	(1.36 – 1.18)	(1.44 – 1.14)
Breadth of rostrum	BR	4.74 ± 0.20 (5.36 – 4.37)	4.48 ± 0.28 (4.95 – 4.16)	4.87 ± 0.20 (5.27 – 4.34)
Height of braincase	HBC	10.52 ± 0.36 (11.12 – 9.57)	10.37 ± 0.26 (10.65 – 9.81)	10.64 ± 0.32 (11.12 – 9.49)
Length of diastema	LD	7.21 ± 0.28 (7.88 – 6.38)	6.89 ± 0.19 (7.21 – 6.64)	6.91 ± 0.25 (7.46 – 6.39)
Breadth of the incisor tips	BIT	1.89 ± 0.18 (2.31 – 1.27)	1.95 ± 0.08 (2.09 – 1.85)	1.92 ± 0.13 (2.16 – 1.66)
Breadth of the occipital condyles	BOC	6.52 ± 0.45 (8.87 – 6.28)	6.24 ± 0.15 (6.42 – 5.97)	6.28 ± 0.17 (6.68 – 5.86)
Occlusal length of mandibular tooth row	OLMT	4.35 ± 0.15 (4.69 – 4.11)	4.25 ± 0.13 (4.04 – 4.42)	4.43 ± 0.19 (4.79 – 3.84)
Postpalatal length	PL	10.16 ± 0.36 (11.02 – 9.34)	9.67 ± 0.28 (10.64 – 9.05)	9.77 ± 0.31 (4.79 – 3.84)
Height of lower jaw	HLJ	5.93 ± 0.24 (6.92 – 5.36)	5.81 ± 0.38 (6.56 – 5.23)	5.79 ± 0.35 (7.27 – 5.07)

Table 7. Percentage of variance explained by each variable in the three components obtained.

	PRINCIPAL COMPONENTES		
Variable	**PC1**	**PC2**	**PC3**
Interorbital breadht	–2.52	6.09	0.16
Occipitonasal length	8.75	1.91	0.64
Greatest zygomatic breadth	5.25	–0.92	1.68
Crown length of maxillary toothrow	0.29	1.93	–1.74
Breadth of the zygomatic plate	–0.99	5.11	–1.51
Length of bony palate	3.51	–1.51	3.68
Breadth of bony palate across first upper molars	1.75	4.34	3.72
Breadth of the incisive foramina	1.44	5.21	–0.30
Width of the anterior region of the fossa mesopterygoidea	3.40	0.49	2.05
Length of rostrum	7.70	2.78	–2.59
Breadth of first upper molar	–0.39	5.69	4.75
Breadth of rostrum	1.69	6.27	–0.45
Height of braincase	–0.41	7.21	6.18
Lenght of diastema	8.17	–0.20	–0.54
Breadth of the incisor tips	1.04	0.06	7.08
Breadth of the occipital condyles	5.30	–0.24	0.05
Occlusal length of mandibular tooth row	1.35	6.42	1.63
Postpalatal length	6.16	0.88	–3.64
Height of lower jaw	3.20	–0.23	0.83

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