Short Communication

Filling phylogenetic gaps and the biogeographic relationships of the Octodontidae (Mammalia: Hystricognathi)

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A B S T R A C T

Endemic to South America, octodontid rodents are remarkable by being the only mammal taxa where allotetraploidy has been documented. The taxon’s extensive morpho-physiological radiation associated to niche shifts has allowed testing phylogeographic hypotheses. Using maximum likelihood and Bayesian inference analyses, applied to all nominal species of octodontids, phylogenetic reconstructions based on sequences of 12S rRNA and growth hormone receptor gene are presented. Species boundaries were determined by coalescent analyses and divergence times among taxa were estimated based on mutation rates. Two main clades associated to the Andean orogenesis were recognized. The essentially western clade comprises genera Aconaemys, Octodon, Spalacopus, and Octodontomys whereas the eastern one included genera Octomys, Pipanacoctomys, Salinoctomys, and Tympanoctomys. Genetic relationships, coalescent analyses, and genetic distance supported the specific status given to Octodon pacificus and that given to Pipanacoctomys aureus as a species of Tympanoctomys. However, these analyses failed to recognize Salinoctomys loschalchalerosorum as a valid taxon considering its position within the diversity of Tympanoctomys barrerae. Although the origin of genome duplication remains contentious, the coincidence of the basal clade split with distinctive modes of karyotypic evolution across the Andes emphasizes the role of physiographic barriers and westerlies in shaping different edaphological conditions, selective grounds, and concomitantly distinct adaptations within the octodontids.

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1. Introduction

The Octodontidae has evolved an extensive morpho-physiological radiation (Mares and Ojeda, 1982; Contreras et al., 1993; Olivares et al., 2004), karyotypic evolution (George and Weir, 1972; Gallardo, 1992) and extreme genome size variation (Gallardo et al., 1999, 2003). These contrasting features have resulted in the recognition of 14 nominal species distributed from coastal Central Chile to the eastern slopes of the Andes including Argentina and Bolivia, between 15°S and 43°S (Gallardo et al., 2007, 2009; Verzi et al., 2015a). The Octodontidae includes five monotypic genera (Octodontomys, Octomys, Pipanacoctomys, Salinoctomys, Spalacopus) and three polytypic ones (Tympanoctomys, Aconaemys, and Octodon; Gallardo et al., 2007; Teta et al., 2014), where desert specialists, above-surface dwellers, fossorial, and strictly subterranean forms can be found (Gallardo et al., 2007). The octodontids exhibit intra- and interpopulational karyotypic stability, but extensive interspecific differences ranging from 2n = 38 (Octodontomys gliroides), to 2n = 102 (Tympanoctomys barrerae; Gallardo et al., 2007). Germinal and somatic DNA content estimates (Gallardo et al., 2003), gene copy number (Gallardo et al., 2004, 2006), and genome in situ hybridization (Suárez-Villota et al., 2012) support whole genome duplication in the red vizcacha rat T. barrerae.

The molecular phylogenetic relationships of caviomorph rodents have supported the Octodontidae’s monophyly (Honeycutt et al., 2003; Upham and Patterson, 2012, 2015). Nevertheless, the relationships within some genera of octodontids have been so far hampered by the absence of genetic data for the insular Octodon pacificus (Hutterer, 1994) and probably by the analysis of only one specimen per species. Here, we included O. pacificus, the recently described T. kirchnerorum (Teta et al., 2014), and at least two individuals for most species. Thus, the complete phylogeny of the octodontid rodents using concatenated partial sequences of the mitochondrial 12S rRNA and the nuclear growth hormone
receptor (GHR) genes is presented. We aim to resolve the phylo-
genetic relationships within the Octodontidae and to determine the
taxonomic status of all its nominal species, with special emphasis
in *Octodon pacificus*, and *Salinomys loschalchalensisorum*, a desert
dweller known only from its original description (Mares et al.
2000). By using specific mutation rates and a relaxed Bayesian
method for multilocus data, we evaluated the trends and rhythms in
the evolution of the octodontids and by using multilocus
coalescent-based methods, we estimated its species boundaries.
Finally, the adaptive radiation of the family and its correlation with
the Andean orogenesis and Pleistocene climate shifts is further
discussed.

2. Material and methods

Sequences of 12S rRNA and GHR genes already reported were
used to build the phylogenetic relationships within the Octodonti-
dae (Table S1). To these data sets, new GHR and 12S rRNA
sequences obtained from *O. pacificus*, *T. kirchnerorum*, *T. barrerae*,
and *A. porteri* were included (Table S1, gray cells). By using conven-
tional extraction procedures for ancient tissue (Paabo, 1989),
whole genomic DNA was extracted from *O. pacificus* paratype
(ZFMK 92.383; Hutterer, 1994). By conventional phenol-
chloroform method (Sambrook et al., 1989), DNA was extracted
from liver of nine *T. kirchnerorum*, 13 *T. barrerae*, and one *A. porteri*.
From the Mammal Tissue Collection, Universidad Austral de Chile
(see vouchers in Table S1). Fragments of 12S rRNA and GHR genes
were amplified using the primers and PCR conditions described by
Honeycutt et al. (2003). Nucleotide sequencing was conducted by
Macrogen Inc. Sequences obtained were deposited in GenBank
under accession numbers KX646495–KX646539.

Sequences were checked with Proseq v2.92 (Filatov, 2002),
and multiple alignments were done with MAFFT v5.0 (Katoh et al.,
2005) under the iterative model of global pairwise alignment (G-
INSI). The evolutionary distance between pairs of sequences was
estimated with MEGA v6.0.6 (Tamura et al., 2013), using Kimura
2-parameter model with rate variation among sites. The goodness
of fit of this model to the concatenated data set was measured also
in MEGA v6.0.6 by the Bayesian information criterion (BIC)
and corrected by Akaike information criterion. By using each single
gene sequence matrix and concatenated gene datasets, phyloge-
netic reconstructions using maximum likelihood (ML) and Baye-
sian inference (MCMC) were conducted. For best-fit partitioning
schemes and models of nucleotide substitution employed in ML
and MCMC analyses, BIC implemented in PartitionFinder v1.1.0
was used (Lanfear et al., 2012). Three ML analyses for topology
convergence were performed with GARLI v2.1 (Bazinet et al.,
2014) and their statistical nodal support was estimated by non-
parametric bootstrap with 1000 pseudoreplicates (Felsenstein,
1985). We used MrBayes v3.2.6 for two MCMC analyses with inde-
dependent runs on random trees and 106 generations sampled every
100 steps (Ronquist and Huelsenbeck, 2003). Through Tracer v1.6
(Rambaut and Drummond, 2009), the stationary phase was
checked following Nylander et al. (2004). Sample points prior to
the plateau were discarded as burn-in and the remaining trees
were combined to find the maximum a posteriori probability esti-
mated from the phylogeny. Branch support was estimated by Baye-
sian posterior probabilities.

Divergence times were estimated with BEAST v1.8.3
(Drummond and Rambaut, 2007), using the concatenated matrix
of both gene sequences and nucleotide substitution models pro-
vided by PartitionFinder v1.1.1 (Lanfear et al., 2012). Mutation
rates of 0.654% and 0.183% per million years for 12S rRNA and
GHR genes, respectively, were used. These mutation rates were
also estimated with BEAST, using (1) 12S rRNA and GHR sequences
used by Opazo (2005), (2) The GHR + I + G mutation model for 12S
rRNA and the HKY + G for GHR; and (3) the caviomorph radiation
at 41 Myr as the calibration point (Antoine et al., 2012). This cali-
boration was implemented as lognormal prior distributions with means of 0.01 and standard deviations of 0.6. We provided
a minimum bound for each distribution such that the 5% quantile
corresponds to the minimum age of the fossil while the 95% inter-
val allows both for the uncertainty of the fossil age and for the
incompleteness of the fossil record. To estimate both divergence
times and mutation rates, uncorrelated lognormal relaxed-clock
models were used to allow rate variation among branches
(Drummond et al., 2006). Bayes factor analysis (Li and
Drummond, 2012) indicated that this model received decisive sup-
port compared to an uncorrelated exponential or a strict-clock
model. Markov chains in BEAST were initialized from the tree
obtained from MCMC analyses to calculate posterior parameter
distribution, including the tree topology and divergence times.
BEAST analyses to estimate divergence times and mutation rates
were run for 107 generations, sampling every 1000th generation.
The first 10% of samples were discarded as burn-in. Convergence
to the stationary distribution and acceptable mixing were investi-
gated using Tracer v1.6 (Rambaut and Drummond, 2009).

STEM multilocus coalescent-based method (Kubatko et al.,
2009; Carstens and Dewey, 2010) and Bayesian approach (BPP;
Yang and Rannala, 2010) were applied to species delimitation, fol-
lowing the recommendations of Harrington and Near (2012)
and Yang (2015), respectively.

3. Results and discussion

After the amplification of 953 bp of the 12S rRNA and 888 bp of
GHR totaling 223 variable positions and 184 informative sites,
the models selected for the phylogenetic analyses were GHR + I
+ G for 12S rRNA, and HKY + G, HKY + G, and K80 + G for each
codon position, for GHR. The likelihood and Bayesian tree topo-
logies obtained from independent and concatenated dataset were
congruent among them and with previous phylogenetic studies
(Figs. 1A and S1; Gallardo and Kirsch, 2001; Honeycutt et al.,
2003). The monophyly of the Octodontidae was strongly supported
(bootstrap: 97.6%, Bayesian posterior probabilities: 0.99). Two main lineages (with high bootstrap and posterior probability sup-
ports) associated to the Andean orogenesis were recognized
(Fig. 1A). The essentially western lineage was composed by genera
Octodon, Spalacopus, Aconaemys, and *Octodontomys* whereas the
eastern one included desert specialist genera *Octomys*, *Tympan-
omys*, *Pipanacotoxys*, and *Salinomys* (Fig. 1A and B). This result is
consistent with vicariant effects proposed in the eco-
evolutionary hypothesis of Contreras et al. (1987) and with the model of karyotypic evolution that assumes a bidirectional trend
from an ancestral 58-chromosome lineage (Gallardo, 1992;
Gallardo et al., 2007). Indeed, the clade distributed essentially
on the western slope of the Andes retained diploid numbers similar
to, or lower than, the ancestral chromosomal form (Fig. 2). In
the opposite direction of karyotypic evolution, genome duplication
and the doubling of both diploid and fundamental numbers of
arms characterizes the desert-adapted genera *Tympanoctomys*
and *Pipanacotoxys* (Fig. 2; Gallardo et al., 2003, 2004). On the
other hand, the well-supported inclusion of *Octodontomys gliroides*
in the essentially western clade corroborates previous analyses
(Gallardo and Kirsch, 2001; Honeycutt et al., 2003; Upham and
Patterson, 2015; Verzi et al., 2016) and contradicts previous allega-
tions based only on cranio- dental morphology (Verzi, 2001; Verzi
et al., 2014; Candelá, 2016). Having in mind that the phylogenetic
position of *O. gliroides* remained uncertain in the two-gene analy-
uses using one individual of this species (Upham and Patterson,
our results highlight the importance of increasing sample size to prevent long branch attraction and conflicting phylogenetic trees (Hedtke et al., 2006; Gatesy et al., 2007). In fact, phylogenetic uncertainties associated to O. gliroides were solved when molecular or morphological characters were added (Upham and Patterson, 2015; Verzi et al., 2016).

The phylogenetic relationships of desert specialists using independent and concatenated data sets depict P. aureus external to T. kirchnerorum, T. barrerae, and S. loschalchalerosorum (Figs. S1 and 1A), concordant with previous analyses (Gallardo et al., 2013; Upham and Patterson, 2015). Taken together, the position of S. loschalchalerosorum within the T. barrerae’s clade (Fig. 1A) and the negligible genetic distance between them (0.3%; range: 0–0.64%) do not support S. loschalchalerosorum as a valid taxon. Species delimitation analysis further supports this conclusion (Fig. 1A). The phylogenetic relationships and taxonomic validity of S. loschalchalerosorum have been questioned since its original morphological description based on two specimens, considered as the sister taxon to P. aureus (Mares et al., 2000). Subsequent morphological and molecular analysis placed Tympanoctomys-Salinoctomys as sister taxa, with Pipanacoctomys external to them (Barquez et al., 2002; Upham and Patterson, 2015). Our molecular analyses of T. barrerae’s specimens encompassing five populations also support the close relationship proposed by Barquez et al. (2002) and Upham and Patterson (2015), but refuses the full recognition of Salinoctomys as a valid taxon (Fig. 1A). On the other hand, genetic distance among desert specialist genera average only 1.79% (range: 2.30–0.54%), contrasting with the much higher value of the remaining genera (5.26%; range: 2.53–8.64%) and falling within the range of interspecific comparisons (mean: 2.38%, range: 0.65–4.13%). Following this criterium, genetic distance data suggests Pipanacoctomys and Salinoctomys to be included within Tympanoctomys, as suggests Verzi et al. (2015a). In short, our data analyses support the notion that Salinoctomys and Pipanacotomys are not distinct from Tympanoctomys although for Verzi et al. (2015a) S. loschalchalerosorum is considered a valid species.

Consistent with previous phylogenetic reconstructions (Gallardo and Kirsch, 2001; Honeycutt et al., 2003), Octodon was recovered as sister to the unresolved Spalacopus/Aconaemys clade (Fig. 1A). In fact, our data supports the monophyly of Spalacopus and A. porteri (bootstrap: >95, Bayesian posterior probabilities: 0.99) although the monophyly of other Aconaemys species remain unsupported (Fig. 1A) and A. fuscus appears paraphyletic (Fig. 1A). Nevertheless, interspecific recognition between A. fuscus and A. sagei is supported by differences in diploid number (Fig. 2; Gallardo, 1992) and penial morphology (Contreras et al., 1993). Thus, additional sequences will be required to elucidate the phylogenetic relationships among the Aconaemys species.

The early divergence of O. degus, followed by O. pacificus characterizes the Octodon clade in which O. lunatus, and O. bridgesi form a derived sister group (Fig. 1A). These species, recognized as valid by the delimitation analysis (Fig. 1A), share pairwise distances (K2P)
ranging from 0.6% to 2.7%. *O. pacificus* was captured in 1959 and described as a new species based on morphological data (Hutterer, 1994) but no further records have been documented ever since (Pefaur and Yañez, 1980; Saavedra et al., 2003). The high genetic distance of *O. pacificus* relative to the remaining congeners (2.3%) indicate its distinctiveness. Hence, the phylogenetic relationships, species delimitation analyses, and pairwise distances support the distinctive taxonomic status of *O. pacificus*, in spite of its morphological similarity with *O. bridgesi* (Saavedra et al., 2003).

Divergence time calibrations using mutation rates are slightly older than previous ones (Opazo, 2005; Gallardo et al., 2013) and congruent in general terms with Gallardo and Kirsch (2001), Honeycutt et al. (2003), Upham and Patterson (2012, 2015), and Verzi et al. (2016) (Fig. 2, Table S2). Concordance between our calibration and those of Upham and Patterson (2015) and Verzi et al. (2016) rests on the use of a more ancient caviomorph fossil, as the one recently found in Yahuarango Formation (Antoine et al., 2012). The octodontid split into an eastern and western clade is esti-
mated at 11.08 (7.25–16.41) Myr, coinciding with the vicariant effect that triggered new ecological settings for the evolution of caviomorph rodents, as derived by the uplift of the Andes (Pascual et al., 1996; Pascual, 2006; Upham and Patterson, 2012, 2015). By affecting water vapor transportation along western South America, the dramatic effects of plate tectonics resulted in different climatic regimes across and along the mountain chain (Hartley, 2003; Hoorn et al., 2010). This basal split could be the result of the contrasting environmental conditions and barriers across the eastern and western slopes of the Andes, as suggested previously (Contreras et al., 1987; Ojeda et al., 2013) The divergence between the Octodontomys lineage and the essentially western species is estimated at 7.42 (4.62–10.9) Myr, whereas the differentiation process within this western clade started at 5.26 (3.52–7.47) Myr (Fig. 2). These estimations are consistent with the intensification of the tectonic processes initiated during the upper Miocene and Pliocene (Hinajosa and Villagrañ, 1997). The uplift of the Andes changed the physiographic context that generated new, more open biomes (Pascual and Jaureguizar, 1990; Le Roux, 2012). It has been suggested that diverse morphological adaptations of octodontids to the new emerging environments, reflect responses to these Cenozoic environmental changes (Verzi et al., 2015b, 2016).

The origin of the polytypic genera Aconaemys and Octodon is estimated at 2.91 (1.7–4.4) and 3.72 (2.2–5.7) Myr, respectively whereas the split between O. pacificus and O. bridgesi/O. lunatus is around 1.77 (0.7–3.2) Myr (Fig. 2). The oldest deposit of Mocha island are dated from the Eocene and Miocene (Tavera and Veyl, 1958) whereas extensive terraces from Pliocene and Pleistocene characterize more recent settings (Melnick et al., 2003). Although the arrival of O. pacificus to the island remains unknown, these large terraces might have been a suitable habitat for its settlement and for its differentiation from the three continental Octodon species (Fig. 1). The ancestral stock of the desert dwellers dates back to 6.10 (3.4–9.4) Myr, coinciding with the origination and subsequent division of salt flats in northwestern of Argentina (Mares et al., 2000). The divergence between Tymanotomys and Pipanacoto- mys is estimated at 2.88 (1.6–4.5) Myr. Currently, these taxa are distributed in extreme saline habitats on piedmonts, salt flats and basins, from the Pilancano salt flat (27°S) to Patagonia (43°S) (Gallardo et al., 2007, 2009; Ojeda et al., 2013). Considering this estimate and the present distribution of the desert dwellers, its ancestor could have been affected by periodic latitudinal and altitudinal shifts of the glacial ice sheets during the Pleistocene (Dynesius and Jansson, 2000; Patterson, 2010). Thus, suitable Pleistocene habitats probably contracted from south to north during periods of glacial advance, whereas distributional ranges shifted westward and southward again, during glacial retreats and warming (Verzi et al., 2002; Gallardo et al., 2013). Accordingly, glacial advances during the ice ages probably fragmented and isolated populations. With the retreat of ice during interglacial periods, divergent lineages may have re-established contact in suture zones (Schönswetter et al., 2005). Such an scenario probably accounts for the hybrid origin of T. barrerae (Suárez-Villota et al., 2012; Gallardo et al., 2013), the structured genetic pattern of several mammals including octodontids (Himes et al., 2008; González-Ittig et al., 2010; Mapelli et al., 2012), and for the origin of the isolated species, T. kirchnerorum (Gallardo et al., 2013; Teta et al., 2014).

This study stress the importance of having a complete phylogenetic of this endemic, peculiar group to fill in this fuzzy picture of octodontid relationships and to get a better understanding of genome duplication for future genomic studies (Gallardo et al., 1999; Suárez-Villota et al., 2012). It will also provides new insights into the evolution of octodontids and its temporal speciation processes dynamics associated to environmental changes derived from the Andes uplift and patterns of Pleistocene glaciations.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.08.015.

References


