

Increasing the known specific richness of living mammals in Chile

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The Chilean mammal fauna is one of the best known of South America. In spite of this, in the last decade several new species have been described based on specimens collected in the country, while other species previously known elsewhere have been recorded for the first time in Chile. Here we keep on this trend by recording for the first time for Chile a species of long-tailed mouse of the genus *Oligoryzomys*. This mention is based on genetic (cytochrome b gene sequences) and morphological data gathered from several specimens collected at four localities of Quebrada de Camarones, Región de Arica y Parinacota in northern Chile. At one of these localities a specimen was live-trapped; while in the other three localities several osteological remains were recovered from owl pellets. The morphologic and genetic information robustly indicate that the revised specimens belong to the genus *Oligoryzomys*. The phylogenetic analyses show that the trapped specimens belong to *O. flavescens* s. l. However, it remains unsolved to which of two main lineages of *O. flavescens* s. l., *O. flavescens* s. s. or *O. occidentalis*, belongs the specimens from Camarones. Here we increase the known species richness of Chilean living mammals by showing that northernmost Chile is inhabited by *O. flavescens* s. l. The possibility that the specimens from Camarones represent an undescribed species cannot be ruled out. These new records indicate, once again, that much remains to be learned about basic aspects of the Chilean mammals, including which species form the local assemblages.

La fauna de mamíferos de Chile es una de las mejores conocidas de América del Sur. No obstante, en la última década se han descrito varias especies nuevas a partir de ejemplares colectados en el país, mientras que otras previamente conocidas en otros países se han registrado por primera vez para el país. En este trabajo profundizamos esta tendencia al registrar por primera vez para Chile una especie de ratón colilargo del género *Oligoryzomys*. Esta mención se basa en datos genéticos (secuencias del gen citocromo b) y morfológicos de varios especímenes colectados en cuatro localidades de Quebrada de Camarones, Región de Arica y Parinacota en el norte de Chile. En una de estas localidades se capturó un espécimen; mientras que en las otras tres localidades se recuperaron restos osteológicos de egagrópilas de lechuza. La información morfológica y genética indican de manera robusta que los ejemplares estudiados pertenecen al género *Oligoryzomys*. Los análisis filogenéticos muestran que el espécimen colectado en Camarones pertenece a *O. flavescens* s. l. Sin embargo, no es posible esclarecer a cuál de los dos linajes principales de *O. flavescens* s. l., *O. flavescens* s. s. u *O. occidentalis*, pertenece dicho ejemplar. Aumentamos la riqueza de especies conocidas de mamíferos vivientes chilenos al mostrar que *O. flavescens* s. l. habita en el extremo norte de Chile. No se puede descartar la posibilidad de que los ejemplares de Camarones representen una especie no descrita. Estos nuevos registros indican, una vez más, que queda mucho por aprender sobre aspectos básicos de los mamíferos chilenos, incluyendo qué especies forman los ensambles locales.

Keywords: Hantavirus; Rodentia; Supramyomorpha; Cricetidae; Sigmodontinae.

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Introduction

A recent list of living native mammal species indicates 163 species in Chile (D'Elía *et al.* 2020). Although this number is similar to the figures provided in the latest published lists of species (e. g., 160 in Iriarte 2008 and 157 in Yañez *et al.* 2009), the composition of the recent list differs substantially from the previous ones. Some of the differences among these lists are due to the exclusion, in the new list, of domestic varieties of wild species and species with an unconfirmed presence in the country. Additionally, most differences among lists refer to taxonomic changes, including the description

of new species and the recording in Chile of species previously known from neighboring countries. From 2014 to date, five species — two rodents and three bats — have been recorded in Chile for the first time. Zúñiga and Tancara (2014; see also Valladares *et al.* 2015) and D'Elía *et al.* (2016) reported, the presence of *Abrothrix jelskii* and *Notiomys edwardsii*, respectively (also being the first report for *Notiomys*, as currently delimited, for Chile). On the other hand, Ossa *et al.* (2015, 2018a) reported records of *Eptesicus (Histiotus) laephotis* and *Promops davisoni*. Finally, after the most recent list of Chilean mammals was published (D'Elía

et al. 2020), [Rodríguez-San Pedro *et al.* \(2022\)](#) reported the presence of the bat *Nyctinomops aurispinosus* in Chile. Similarly, the taxonomic review of the *Phyllotis xanthopygus* complex by [Ojeda *et al.* \(2021\)](#) indicates that, in addition to *P. xanthopygus s. s.* and *P. limatus*, *P. vaccarum* and a species whose name is for the moment unclear (referred to as *P. posticalis*-*P. rupestris*) are also distributed in Chile. Finally, [Novaes *et al.* \(2022\)](#) recently reviewed the species of *Myotis* present in Chile, concluding that *M. arescens* is distinct from *M. chilensis*. These recent studies bring to 167 the species of living mammals recorded in Chile.

In line with the studies mentioned above, this work increases the known richness of living native mammals to Chile, reporting for the first time a rodent species of the genus *Oligoryzomys*. Long-tailed mice, as sigmodontines belonging to the genus *Oligoryzomys* are commonly known, form a monophyletic group composed of about 32 living species ([Hurtado and D'Elía 2019](#)), distributed from northeast Mexico to the southern tip of Chile and Argentina ([Weksler and Bonvicino 2015](#)). The species recorded herein for the first time in Chile belongs to the *Oligoryzomys flavescens s. l.* species complex. This group has a wide known geographic distribution, including areas of Argentina, Bolivia, Brazil, Paraguay, Peru, and Uruguay, and is made up of two candidate species: *O. flavescens s. s.* and *O. occidentalis* ([Rivera *et al.* 2018](#); [Hurtado and D'Elía 2019](#); see below). The report of *Oligoryzomys flavescens s. l.* in Chile is based on a specimen captured and several remains recovered from owl pellets, which together came from four localities in Quebrada de Camarones, Región de Arica y Parinacota, in northernmost Chile.

Materials and methods

Study area and samples. During surveys carried out from 2017 to 2021 in Quebrada de Camarones, Arica y Parinacota Region, Chile, we collected a specimen of the genus *Oligoryzomys* and recovered skeletal remains belonging to this genus from 21 pellets of the American barn owl (*Tyto furcata*). These specimens are the basis of the mention presented in this study. Details of the collection localities are given in the Results section and in Figure 1. The collected specimen was deposited in the Colección de Mamíferos of the Universidad Austral de Chile (UACH), Valdivia, Región de Los Ríos, Chile, under the collection number UACH 8477; it was conserved as a fluid specimen after tissue aliquots were preserved in ethanol. The skeletal remains recovered from owl pellets were deposited in the Colección Zoológica de Zonas Áridas y Alto Andinas, Universidad de Tarapacá (CZZA-UTA), Arica, Región de Arica y Parinacota, Chile; the sample includes the remains recovered from 21 owl pellets deposited under the collection number CZZA-UTA 410-430.

Molecular analyses. A fragment of 801 base pairs of the mitochondrial gene encoding cytochrome b (*CytB*) of the collected specimen was sequenced using the primers MVZ 05 and MVZ 16 ([Smith and Patton 1993](#)), following the laboratory protocol described by [Cadenillas and D'Elía \(2021a\)](#). The amplified fragment was sequenced by a contract laboratory (Macrogen Inc., Korea). The sequence obtained was deposited at GenBank (OP135496) and subsequently integrated into a matrix composed of two sequences of each of the *Oligoryzomys* species delimited by [Hurtado and D'Elía \(2019\)](#). In the case of the forms in the *O. flavescens s. l.*

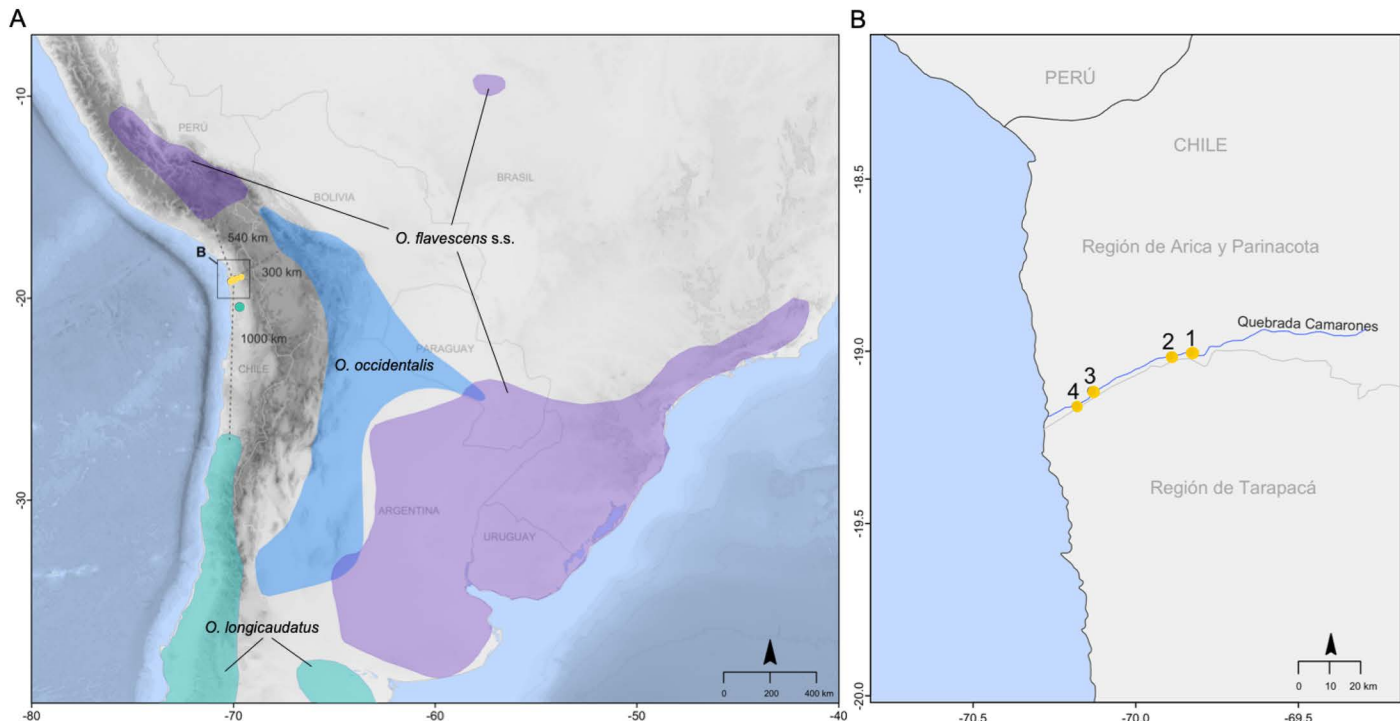


Figure 1. A) Partial map of southern South America showing the approximate distribution of the *Oligoryzomys flavescens s. l.* complex and *O. longicaudatus*, the other species of *Oligoryzomys* recognized in Chile. Approximate distances between the Chilean records of *Oligoryzomys flavescens s. l.* and the nearest of *O. flavescens s. s.* and *O. occidentalis* are indicated. The green circle indicates the locality where *O. longicaudatus* was reported by [Torres *et al.* \(2018\)](#) and that we suggest to be disregarded (details in the text). B) Partial map of northern Chile indicating the four collection localities in the Quebrada de Camarones, Región de Arica y Parinacota, Chile, where the specimens of *O. flavescens s. l.* were recorded.

complex, we used a sampling of sequences with a broad geographic coverage. The outgroup was formed following [Hurtado and D'Elía \(2019\)](#) with sequences representative of the main clades of the tribe Oryzomyini (*sensu* [Weksler 2006](#)): clade A, *Zygodontomys brevicauda* and *Scolomys ucayalensis*; clade B, *Handleyomys alfaroi* and *Hylaeamys megacephalus*; clade C (to which *Oligoryzomys* belongs), *Microryzomys minutus*, *Neacomys minutus*, *Neacomys paracou*, and *Oreoryzomys balneator*; clade D, *Oryzomys palustris* and *Holochilus sciureus*. Details of the sequences used, including the catalog numbers and collection localities of the sequenced specimens, are provided in the Supplementary material 1. Sequence alignment was performed with Clustal as implemented in MEGA6 ([Tamura et al. 2013](#)), with the default values for the alignment parameters. Subsequently, a visual inspection was carried out to search for potential reading frame changes. The matrix obtained was analyzed through Maximum Likelihood (ML) and Bayesian inference (BI). The ML analysis was carried out with IQ-TREE ([Nguyen et al. 2015](#)) using the W-IQ-TREE online tool (<http://iqtree.cibiv.univie.ac.at>; [Trifinoupoulus et al. 2016](#)), with the disturbance intensity set to 0.5, the term rule set to 100, and the molecular evolution model TIM2+F+R4, which was also selected using ModelFinder ([Kalyaanamoorthy et al. 2017](#)) in IQ-TREE, according to the Bayesian Information Criteria (BIC). Clade support was calculated via 1000 ultrafast bootstrap (UFB) pseudoreplicates. The BI analysis was performed with MrBayes ([Ronquist and Huelsenbeck 2003](#)) using two separate runs with four Markov chains for 1×10^6 generations, which were sampled every 1000 generations. As TIM2+F+R4 cannot be implemented in MrBayes, we used the GTR+G+I model following the recommendations of [Ronquist and Huelsenbeck \(2003\)](#). Convergence in stable log-likelihood values was verified by plotting likelihood values versus the number of generations. The first 25 % of the trees were discarded as *burn-in*; the remaining trees, sampled in the convergence zone of both runs, were used to compute a tree according to the majority rule and obtain a-posteriori probability (PP) values for each clade. Percentage of genetic difference between pairs of sequence samples were estimated based on pdistance using MEGA 6.

Morphological review. The collected specimen was identified at the genus level based on its external traits. The bone remains recovered from owl pellets were determined using the key written by [Reise \(1973\)](#) and comparative material from the Colección de Mamíferos UACH.

Results

A male specimen of *Oligoryzomys* (UACH 8477) was captured in 1) Ruta A-345 km 28, Camarones, Comuna de Camarones, Región de Arica y Parinacota (18.997300° S, 69.827450° W, 790 m; Figure 1). In addition, mandibular remains of at least 10, one, and 13 individuals assigned to the genus *Oligoryzomys* (Figure 2) were recovered from owl pellets collected, respectively, in the following three localities along the Quebrada de Camarones: 2) Approximately 600 meters south

of Ruta A-345, km 20, Camarones, Comuna de Camarones, Región de Arica y Parinacota (19.014307° S, 69.892347° W); 3) Hacienda Camarones, 7 kilometers west of Puente Cuya, Cuya, Comuna de Camarones, Región de Arica y Parinacota (19.115264° S, 70.135602° W); And 4) Puente Cuya Ruta 5, Cuya, Comuna de Camarones, Región de Arica y Parinacota (19.158704° S, 70.182612° W; Figure 1).

The mandibular remains recovered from owl pellets assigned to *Oligoryzomys* show a short and high general contour, lunar notch poorly excavated, capsular process well-developed, and upper and lower masseteric ridges converging anteriorly in V-shape under m1 (Figure 2). Similarly, the external traits of the UACH 8477 specimen (Figure 3), including its size and body metrics [(total length: 208 mm; tail length: 115 mm; foot length without/with nail: 25/27 mm; ear length: 14 mm; weight: 17 g); dorsal pelage thick, uniformly orange and brownish, ventral pelage lighter; feet without interdigital membranes; foot sole distally covered with scales, heel bare, hypothenar pad long, interdigital pads small, with pads 1 and 4 in a more proximal position than pads 2 and 4; tail sparsely haired, bicolored, covered with conspicuous epidermal scales and without a long tuft of terminal hairs] unambiguously indicate that this specimen belongs to the genus *Oligoryzomys* (see the diagnosis of the genus in [Weksler and Bonvicino 2015](#)). This identification at the genus level was confirmed by the phyloge-

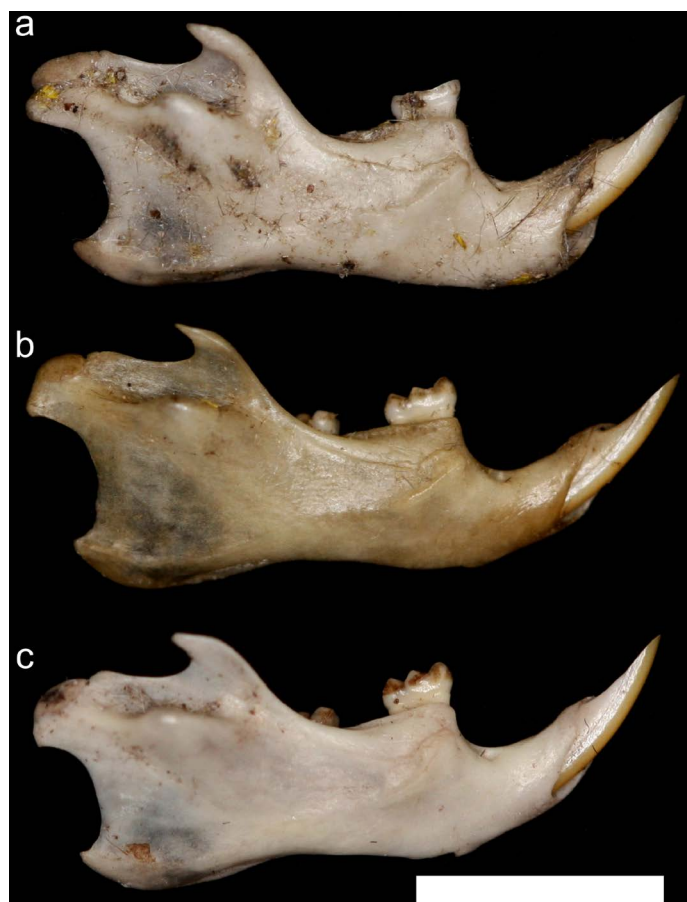


Figure 2. Right mandibles of three specimens of *Oligoryzomys flavescens* s. l. collected at three localities (a: 4, b: 3, and c: 2 in Figure. 1) in Quebrada de Camarones, Región de Arica and Parinacota, Chile. The bar indicates 5 mm.

netic analyses. In the trees obtained by BI (Figure 4) and ML, whose topologies are similar, the haplotype recovered from the Camarones specimen is part of the clade (PP = 0.99; UFB = 100) corresponding to the *O. flavescens s. l.* complex. Within this clade, the position of the haplotype from Camarones varies depending on the analysis. In the BI tree (Figure 4b) the haplotype from Camarones (*i. e.*, UACH8477) appears in a relationship lacking significant support (PP = 0.65), as sister to the clade corresponding to *O. flavescens s. s.* (recovered in both analyses: PP = 0.85; UFB = 79). In the ML tree (Figure 4c), the haplotype from Camarones is recovered as sister (UFB = 89) to the clade formed by the haplotypes of *O. occidentalis* (recovered in both analyses: PP = 0.99; UFB = 99). The mean genetic distance observed between the haplotype of the Chilean specimen and the haplotype sample of *O. flavescens s. s.* is 2.92 %, while the mean value of the comparison between the haplotype of the Chilean specimen and the sample of haplotypes of *O. occidentalis* is 3.03 %. Finally, the observed divergence between the samples of *O. flavescens s. s.* and *O. occidentalis* haplotypes is 3.27 %.



Figure 3. Specimen of *Oligoryzomys flavescens s. l.* (UACH 8477) collected in Quebrada de Camarones (locality 1 in Figure 1), Arica y Parinacota, Chile.

The Chilean localities where *Oligoryzomys flavescens s. l.* has been recorded in Chile are in Quebrada de Camarones, in an area within the vegetation unit Desierto Absoluto, in the northern portion of the Atacama Desert. Basically, Quebrada de Camarones is a deep canyon that channels the Camarones River, which runs from the western Andean foothills across the desert and reaches the Pacific Ocean. The local vegetation, a xeric shrubland, limits to a strip a few meters wide that stretches along both sides the river and includes shrubs and herbs (*e. g.*, *Atriplex* spp, *Ephedra breana*, *Cistanthe celosioides*, *Diplostephium meyenii*, *Senecio reicheanus*) and, in some areas, also trees such as *Acacia cavens*, *Geoffroea decorticans*, or *Prosopis tamarugo*. The collection site of the specimen UACH 8477 is largely impacted by anthropic activities (*e.g.*, human constructions, livestock, crops). In addition to *Oligoryzomys flavescens s. l.*, specimens of *Abrothrix* sp., *Phyllotis* sp., *Thylamys pallidior*, and the exotic species *Mus musculus* and *Rattus norvegicus* were also collected in the same location.

Discussion

This work recorded a new species of living mammal for Chile. It corresponds to a form of long-tailed mice of the complex of *Oligoryzomys flavescens s. l.*, which is widespread in South America. The taxonomy of this lineage is still unclear. Recent studies indicate that the traditional concept of *O. flavescens* (*e. g.*, [Weksler and Bonvicino 2015](#)) comprises two main mitochondrial lineages representing candidate species ([Hurtado and D'Elía 2019](#)) that exhibit different climate preferences ([Rivera et al. 2018](#)). The validation of these candidate species, which should be carried out with analyses based on morphology and/or variation of nuclear genes, is still pending. The two mitochondrial lineages of the *O. flavescens* complex have available names. *O. flavescens s. s.* (including *O. fornesi* as a synonym [Massoia, 1973](#)) corresponds to the lineage with the broadest geographic range, with records in central and northeast Argentina, Uruguay, central, southern, and southeastern Brazil, southeastern Paraguay, and southern Peru. *O. occidentalis* corresponds to the lineage distributed in central-western and northwestern Argentina, southern, central, and western Bolivia, and southern Paraguay ([Rivera et al. 2018](#); [Hurtado and D'Elía 2019](#)). The haplotype of the specimen collected in Quebrada de Camarones appears as sister to the clade corresponding to *O. flavescens s. s.* or *O. occidentalis*, depending on the analysis considered (BI or ML, respectively). However, such relationships have either non-significant (PP = 0.65) or moderate (UFB = 83) support, so the Chilean haplotype cannot be assigned with certainty to one of these lineages. Likewise, since the morphological distinction of the two main mitochondrial lineages of *O. flavescens s. l.* has not yet been evaluated, the mandibles recovered from the owl pellets from Quebrada de Camarones, which are clearly different from those of *O. longicaudatus*, cannot be assigned with certainty to either lineage of the *O. flavescens s. l.* complex. Therefore, in this work, we opted to assign the

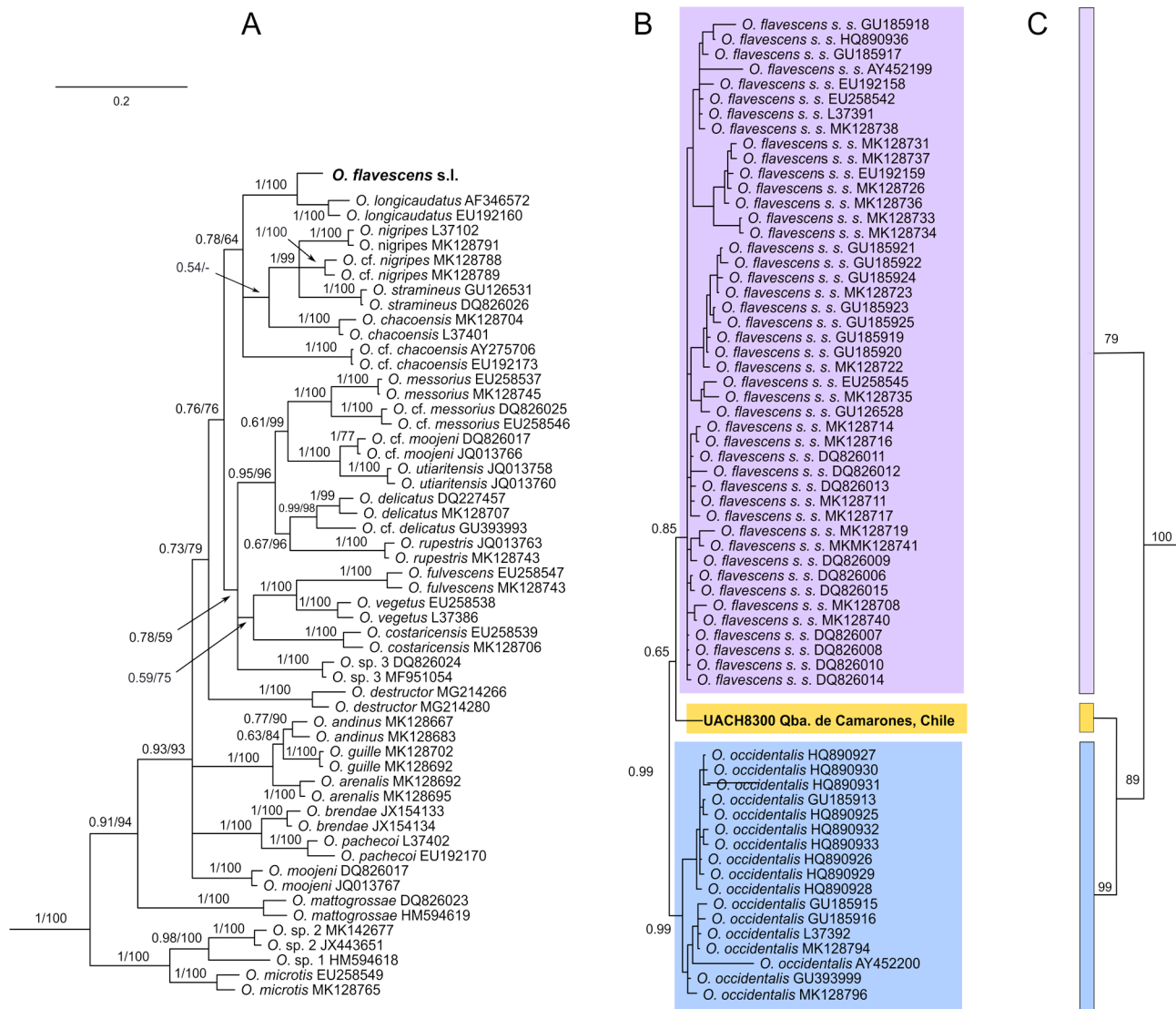


Figure 4. Phylogenetic relationships among the species of the genus *Oligoryzomys* (outgroup not shown), based on DNA sequences of the cytochrome b gene and reconstructed by Bayesian inference. A) Clade composed by species of the genus *Oligoryzomys*. Numbers indicate posterior probability (left) and ultrafast bootstrap (right) values obtained in the ML analysis ($\ln = -10641,151$). The hyphen (-) indicates that the signaled node was not recovered in the ML tree. Terminal labels indicate species and GenBank access numbers. Localities of the specimens included in the analysis are detailed in the Supplementary material. B) Details of the clade corresponding to *O. flavescens* s.l. Intraspecific support values are omitted for clarity (only posterior probability values are shown). The two major mitochondrial lineages of *O. flavescens* s.l. are indicated by colored rectangles: purple, *O. flavescens* s.l.; blue, *O. occidentalis*. The haplotype of the Chilean specimen (UACH 8477) is indicated by a yellow rectangle. C) Schematic relationships among the main lineages of *O. flavescens* s.l. found in the ML analysis (only ultrafast bootstrap support values are indicated). The colors used are as in B.

records of *Oligoryzomys* from Quebrada de Camarones to *O. flavescens* s.l. We consider that, even with the uncertainty associated with the specific identity of these specimens, it is important to report the existence of an additional species of *Oligoryzomys*, that has not been previously reported for Chile. This finding is even more relevant given that *Oligoryzomys* is a genus in which several species, including those of *O. flavescens* s.l., are reservoir of hantavirus strains (e.g., [Delfraro et al. 2003](#); [González-Iltig et al. 2014](#); [Rivera et al. 2007](#)). Finally, considering the phylogenetic uncertainty of the sample from Quebrada de Camarones and the fact that, genetically, it is almost equidistant to both lineages of the *O. flavescens* s.l. complex (2.92 % and 3.03 % relative to *O. flavescens* s.s. and *O. occidentalis*, respectively), the possibility that it represents a third species of the *O. flavescens* s.l. complex should be explored through the analysis of

additional samples. In this context, it is worth noting that in a recent analysis of species delimitation focused on this genus, [Hurtado and D'Elía \(2019\)](#) identified eight candidate species that need evaluating (for the formalization of one of these species see [Hurtado 2021](#)). Thus, the possibility that the population of *Oligoryzomys* from Camarones represents a yet undescribed species is not unexpected.

The Chilean records of *Oligoryzomys flavescens* s.l. enlarge ca. 540 km to the south and 300 km to the west the known distribution of this species complex from the Peruvian and Bolivian records, respectively (Figure 1). In addition, the Chilean records of *O. flavescens* s.l. are the first of this complex reported on the western slope of the Andes. In turn, these records are located ca. 1000 km north of the lowest-latitude record of *O. longicaudatus*, which is in the Región de Atacama of Chile ([Weksler and Bonvicino 2015](#)). In this

regard, it should be noted that [Torres et al. \(2018\)](#) reported the supposed predation of a specimen of *O. longicaudatus* by the passeriform *Agriornis micropterus* in the Reserva Nacional Pampa del Tamarugal, Región de Tarapacá, 150 km south of our records of *O. flavescens* s. l. in Quebrada de Camarones. However, the evidence presented in the work mentioned above — a gray scale photograph of a bird carrying a small, long-tailed mouse in its bill — does not allow us to determine whether it is a specimen of *Oligoryzomys*. Similarly, [Torres et al. \(2018\)](#) did not mention whether the mouse specimen was collected and deposited in a biological collection where it can be studied. Therefore we suggest that the mention of *O. longicaudatus* for such northern latitudes of Chile, about 850 km north of the northernmost records of the species confirmed with specimens, be disregarded until an unequivocal record from Pampa del Tamarugal or adjacent localities is reported.

Regarding the areas of Chile where mammal assemblages are better characterized, it is interesting to note that, except for *Notiomys edwardsii*, the mammal species previously known from neighboring countries and that have been recently reported for Chile are from the north of the country. This fact reinforces the known pattern of a higher richness of small mammal species in northern Chile ([Samaniego and Marquet 2009](#); see also [Revollo-Cadima et al. 2021](#)); therefore, further surveys and collection of specimens should be conducted in that area of the country.

Along with *Oligoryzomys longicaudatus*, *O. flavescens* s. l. is the second species of the genus *Oligoryzomys* known for Chile (refer to [D'Elía et al. 2020](#), in which the nominal forms *O. magellanicus* and *O. yatesi* are included as synonyms of *O. longicaudatus*), and the mammal species number 168 with records in the country ([D'Elía et al. 2020](#); [Ojeda et al. 2021](#); [Novaes et al. 2022](#); [Rodríguez-San Pedro et al. 2022](#)). This new record, together with those presented in recent years and the new species that have recently been proposed based on specimens collected in Chile (see a synthesis in [D'Elía et al. 2020](#); see the proposal of candidate species of *Octodon* in [Cadenillas and D'Elía 2021b](#)), indicate that the mammalian fauna of Chile is still not completely characterized. There are knowledge gaps related to basic aspects of it, such as to clarify which species constitute the mammal assemblages in several geographic areas (e. g., [Storz et al. 2020](#)). Therefore, we close this publication by reminding government officers in charge of issuing collection permits, institutional animal care and use committees, and research funding agencies that to obtain adequate characterization of mammal assemblages requires facilitating and enhancing scientific collection and taxonomic work based on collections.

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Authors' contributions. MQC, AG and GD did the field-work where the specimen was collected. PV collected the owl pellets. AG did the lab work. MQC, PV, NH and GD did the collection-based work. GD did analyses and wrote the manuscript. All authors edited and approved the text.

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Supplementary material 1

List of specimens, including their GenBank accession numbers and collection localities, analyzed in the phylogenetic analyses.

https://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/article/view/2217/Supplementary%20material_2217