Evidence of a population of leaf-eared mice *Phyllotis vaccarum* above 6,000 m in the Andes and a survey of high-elevation mammals

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Biologists have long pondered the extreme limits of life on Earth, including the maximum elevation at which species can live and reproduce. Here we review evidence of a self-sustaining population of mice at an elevation that exceeds that of all previously reported for mammals. Five expeditions over 10 years to Volcán Llullaillaco on the Argentina/Chile border observed and collected mice at elevations ranging from 5,070 m at the mountain's base to the summit at 6,739 m (22,110 feet). Previously unreported evidence includes observations and photographs of live animals and mummified remains, environmental DNA, and a soil microbial community reflecting animal activity that are evaluated in combination with previously reported video recordings and capture of live mice. All of the evidence identifies the mouse as the leaf-eared mouse *Phyllotis vaccarum*, and it robustly places the population within a haplotype group containing individuals from the Chilean Atacama Desert and nearby regions of Argentina. A critical review of the literature affirms that this population is not only an elevational record for mammals but for all terrestrial vertebrates to date, and we further find that many extreme elevations previously reported for mammals are based on scant or dubious evidence.

Key words: alpine, altitude, Andes, extreme environments, leaf-eared mouse, Llullaillaco, microbial communities

Durante mucho tiempo los biólogos han reflexionado sobre los límites extremos de altura a la que las especies pueden vivir y reproducirse. Aquí presentamos nueva evidencia sobre la existencia de una población de ratones establecida a una elevación que supera todos los reports previos para mamíferos. Durante 10 años fueron realizadas 5 expediciones al Volcán Llullaillaco, ubicado en la frontera entre Argentina y Chile; observando y colectando ratones en elevaciones que van desde los 5,070 m hasta la cima de 6,739 m (22,110 feet). La nueva evidencia incluye fotografías de restos momificados, ADN ambiental y la actividad microbiana del suelo que confirman la presencia del animal, la cual fue analizada junto a videos reportados anteriormente y la captura de ejemplares vivos. Toda esta información indica que dicha población corresponde al ratón orejudo amarillento *Phyllotis vaccarum* y lo posicionan dentro de un grupo de haplotipos compuesto por individuos del Desierto de

Atacama y regiones cercanas en Argentina. La revisión crítica de la literatura demostró que esta población no solo es un récord de elevación para los mamíferos, sino para todos los vertebrados terrestres; igualmente, que los reportes de elevaciones extremas reportados para mamíferos se derivan de evidencias escasas y dudosas.

Palabras clave: altitud, ambientes extremos, Andes, comunidades microbianas, ratón orejudo, volcán Llullaillaco

A profound question in biology is what are the factors that set limits for life on earth. In montane regions, it has long been assumed that the upper elevational limits for mammals and other terrestrial vertebrates are determined by a combination of food availability and physiological tolerances to hypoxia and extreme cold. However, in the highest mountain ranges of the world, the upper range limits of montane species have seldom been precisely documented due to a lack of specimen-based survey work in remote, difficult-to-access terrain. Maximum elevation records have often been based on anecdotal reports and have rarely distinguished between resident populations and individual animals wandering high above their normal range. While many mammals and birds are able to tolerate cold and hypoxia during seasonal migrations (Storz et al. 2010; Storz and Scott 2019), there have been few reliable records of self-sustaining populations at elevations above 5,500 m. Assumptions about the environmental tolerances of animals are typically revised when their habitat and range limits are thoroughly investigated (Storz et al. 2020).

Storz et al. (2020) reported the capture and DNA sequence of a *Phyllotis xanthopygus rupestris* (now recognized as *P. vaccarum*; Jayat et al. 2021) from the summit of the Andean Llullaillaco Volcano (hereafter Llullaillaco), the second-highest active volcano in the world (6,739 m), as well as the video of a live mouse from an expedition in 2013 (6,205 m). The summit elevation was more than 1,000 m higher than previously recorded for this species (Steppan and Ramirez-Baca 2015) and a new high-elevation record for mammals. Here we provide additional information from multiple lines of evidence to demonstrate that the mouse reported in Storz et al. (2020) was not an anomaly. The new evidence from four other mountaineering/biological expeditions to Llullaillaco between 2011 and 2016 includes observations and/or photographs of live mice and mummified remains, environmental DNA of *P. vaccarum*, additional DNA sequences from mice elsewhere in northern Argentina and Chile, and environmental DNA sequences of the soil microbial community. Together, these results provide evidence for a self-sustaining population of the leaf-eared mouse *P. vaccarum* residing between 6,000 and 6,700 m, part of a continuously distributed metapopulation from sea level to the summit.

The significance of mice above 6,000 m on Llullaillaco lies in comparison to the elevation limits of other mammals. In the absence of comprehensive comparative data, we surveyed and evaluated the literature and specimen-based records of wild mammals reported at high elevations in the Andes and Himalayas. Based on these records, we conclude that *P. vaccarum* is currently the only mammalian species for which there is reliable evidence of permanent residence above 6,000 m.

Materials and Methods

Locality.—Llullaillaco is a 6,739 m stratovolcano that straddles the Chile–Argentina border at the edge of the Atacama Desert at 24°43.2′S, 68°32.2′W. It is the second-highest active volcano on earth, with last recorded eruptions in 1854, 1868, and 1877 (de Silva and Francis 1991). Llullaillaco has one of the highest known lakes (the recently reported and informally named Lago Llullaillaco) at 6,170 m on its western flank (Arán et al. 2021) and the world's highest archaeological site just below the summit (Reinhard 1999; Reinhard and Ceruti 2010). Because the upper mountain is characterized by low atmospheric pressure, extreme temperature fluctuations, extreme aridity, intense UV radiation, and a total absence of vascular plants, it is considered one of Earth's closest analogs to Mars (Schmidt et al. 2018).

Observations and documentation.—In January 2011, M. Farson discovered and photographed the mummified carcass of a sigmodontine mouse (Fig. 1A) on the Chilean side of Llullaillaco at approximately 6,200 m, above Lago Llullaillaco (exact location not recorded). Also in January 2011, archaeologist Christian Vitry photographed a mouse carcass at 6734 m, just 5 m below the summit (Storz J., personal communication, 15 August 2019). In January 2013, during a second expedition, M. Farson encountered a live sigmodontine mouse near the location of the carcass he discovered in 2011. He tracked it for 5 min and obtained 60 s of video recording (Supplementary Data SD1; Fig. 1B). The next day Farson and T. Bowen revisited the site, briefly glimpsed the mouse—or a second individual—and recorded the GPS coordinates as 24°43.052′S, 68°33.323′W, elevation 6,205 m. They photographed multiple tracks in the snow of well-used runways and aeolian (wind-transported) plant debris lying on the snow. The video indicated that the mouse was a member of the genus *Phyllotis*.

In March 2016, G. Zimmerman briefly spotted a live mouse emerging from a probable burrow, also near Lago Llullaillaco, and recorded the GPS coordinates as 24°43.121′S, 68°33.323′W, elevation 6,154 m. He collected two bulk soil samples, one on the animal's runway just outside the burrow entrance, and the other in undisturbed soil beyond the runway. These were later split into six 0.3-g subsamples and used for DNA extraction and sequencing, as described below.

As previously reported, in February 2020 J. Storz and M. Quiroga-Carmona trapped extensively on the Chilean side of Llullaillaco. At the base of the mountain, at 5,070 m, they captured both *P. vaccarum* and *P. limatus* (Lima leaf-eared mouse). On the very summit, at 6,739 m, Storz hand-captured a single *Phyllotis* specimen (video available in Storz et al. 2020). Examination of cranial morphology and comparative analysis of mtDNA sequences confirmed the identity of the mouse,

Fig. 1.—Visual observations of mice above 6,000 m in multiple years. (A) Mummified mouse 2011, approx. 6,200 m, by Matthew Farson; (B) screen capture of video recording by Matthew Farson, 2013, 6,205 m (Storz et al. 2020); (C) screen capture of summit mouse from video by Mario Pérez Mamani, 2020, 6,739 m (Storz et al. 2020).
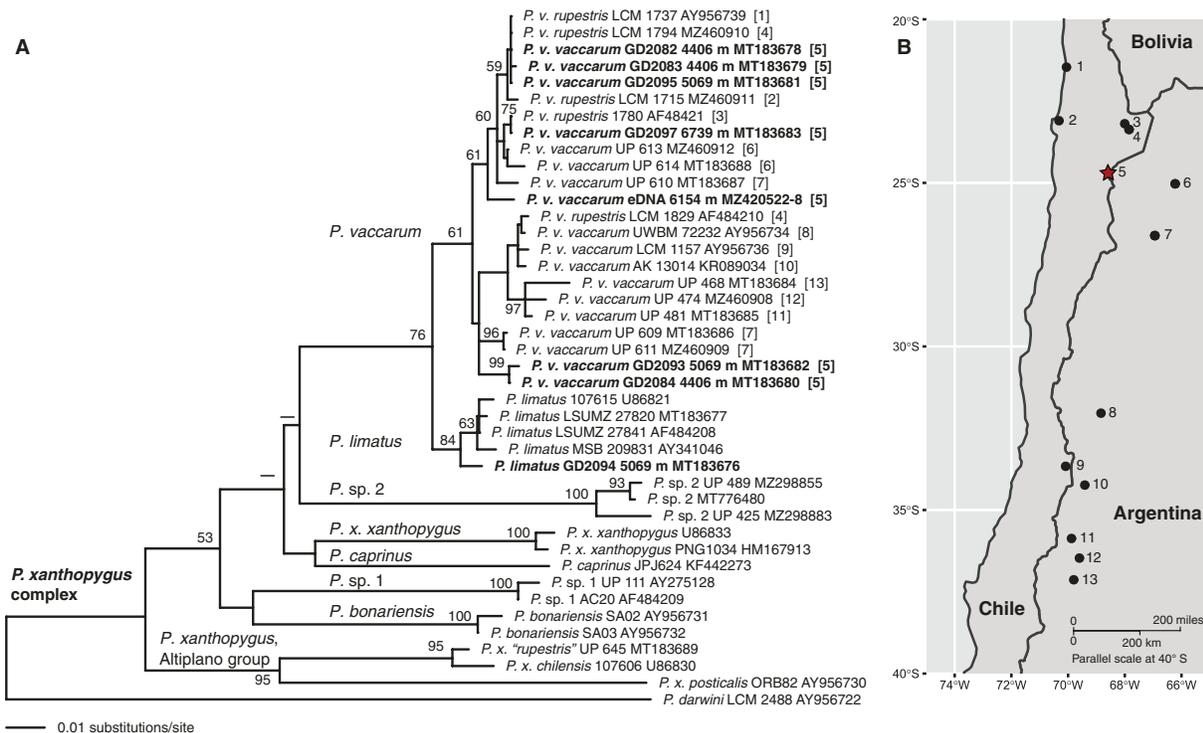

Fig. 2.—Phylogeny and geography of Llullaillaco mice. (A) Maximum likelihood phylogeny for concatenated cytochrome b data. Numbers in [brackets] refer to populations in (B). Bootstrap percentages above branches. Sequences obtained from Llullaillaco indicated in bold. (B) Map identifying the locations of sequenced populations of *P. vaccarum*. The red star marks the location of Llullaillaco. The Llullaillaco samples are well supported as belonging to *P. vaccarum* (3-4 nested nodes with ~60% bootstrap), with most samples from the northern part of the range (populations 1-7), including the soil sample (eDNA, 6,154 m). Notably, haplotypes from mice collected at 5,069 m on Llullaillaco were identical to those from an individual at sea level, at the mouth of the Rio Loa, on the Pacific coast of Chile (population [1]) and virtually identical to the sea level Mejillones sample (population [2]).

referred to by Storz et al. (2020) as *P. xanthopygus rupestris*, but recently reclassified as *P. vaccarum* (Jayat et al. 2021).

Specimens examined.—We sequenced mice from several additional localities in far northern Chile and Argentina in order to add detail to the geographic sampling in the vicinity of Llullaillaco. Tissues stored in ethanol were acquired from

two sources, the Laboratorio de Citogenetica Mammiferos, Universidad de Chile (LCM), and the collections of Ulyses F.J. Pardiñas (UP). The latter vouchers are housed in the Centro Nacional Patagónico, Puerto Madryn, Argentina. Numbers in [brackets] refer to the populations identified on the map in Fig. 2B. Text in parentheses are GenBank accession

numbers. *Phyllotis vaccarum rupestris*—CHILE: Antofagasta; Antofagasta, Mejillones 23.1°S, 70.32°W, <50 m elevation [1], LCM 1715 (MZ460911); El Loa, Talabre, 23.367°S, 67.850°W, 3,250 m [4], LCM 1794 (MZ460910). *Phyllotis vaccarum vaccarum*—ARGENTINA: Catamarca, Laguna Blanca, 26.613°S, 66.940°W, 3,239 m [7], UP 611 (MZ460909); Mendoza, Los Frisos, El Zampal, 36.472°S, 69.648°W, 1,101 m [12], UP 474 (MZ460908); Salta, Alto Cachi, 25.029°S, 66.224°W, 3,028 m [6], UP 613 (MZ460912).

DNA extraction and sequencing of microbial communities.—Microbial communities in the runway soil sample were sequenced to test for evidence of animal-associated bacteria distinct from the autochtonous microbial community. DNA was extracted from three 0.3-g soil subsamples from each of two soil samples (for a total of six subsamples) collected in 2016 from the mouse runway at 6,154 m with a PowerSoil DNA Extraction Kit (MOBIO, Carlsbad, California) according to the manufacturer's instructions. Extracted DNA was amplified in duplicate with standard primers for both the 16S and 18S rRNA gene (Euk1391f/EukBr, Amaral-Zettler et al. 2009; 515f/806r, Caporaso et al. 2010). The amplified DNA replicates were then pooled and normalized to equimolar concentrations using SequalPrep Normalization Plate Kit (Invitrogen Corp., Waltham, MA). Sequencing was done using an Illumina MiSeq V2 (Illumina Inc., San Diego, California) with 2 × 250 bp chemistry at the BioFrontiers Sequencing Core Facility, University of Colorado, Boulder. The raw reads were de-multiplexed and quality filtered using QIIME v1.9.1 (Caporaso et al. 2010). Paired-end sequences were joined with VSEARCH (Rognes et al. 2016) and clustered into operational taxonomic units (OTUs) at 97% similarity using UCLUST (Edgar 2010). Taxonomy was assigned using QIIME's parallel_assign taxonomy blast.py script with the Greengenes 13.5 (DeSantis et al. 2006) and SILVA 128 Ref NR99 databases (Quast et al. 2013) for the 16S and 18S rRNA gene sequences, respectively. All mitochondrial and chloroplast OTUs based on this classification were removed from the bacterial data set and all bacterial OTUs were removed from the eukaryotic data set.

DNA sequencing and phylogenetic analysis of mice.—For the 6,154 m runway sample, an 830 base pair (bp) region of the mitochondrial cytochrome b gene (*cytb*), ranging in position from 70 to 899 bp after the start codon, was targeted using multiple short amplicons. Twelve sets of primers were designed to target approximately 150 bp products, each with 50 bp overlap, using an alignment of all *cytb* sequences of Sigmodontinae available on GenBank. Primers were designed with up to three degenerate bases per primer and an optimal length 21 bp per primer (Table 1).

PCRs were run in individual reactions for each primer set using the Econotaq 2× Master Mix (Lucigen, Middleton, Wisconsin) in a sterile lab that had never had any rodent extractions nor rodent-sample PCRs performed there. Each reaction was 12 µl consisting of 6 µl of 2× master mix, 5 nM of each forward and reverse primer, and 1 µl of template DNA. Reaction conditions consisted of 3 min at 95°C followed by 42 cycles of 40 s at 95°C, 40 s at 58°C, and 40 s at 72°C followed by a final extension of 72°C for 7 min. Reactions were run on a 2% TBE

agarose gel stained with ethidium bromide. Successful bands were cut out and extracted with the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and sequenced in both directions on an ABI 3730 at the Florida State University DNA Sequencing Facility. Following initial results from three successful amplifications, nine additional sets of primers were designed targeting *Phyllotis*. These primers contained fewer degenerate bases per primer than in the first Sigmodontinae-target sets due to the narrower phylogenetic scope (Table 1). Individual sequencing reads from the eDNA sample have been deposited on GenBank with accession numbers MZ420522–MZ420528

Sequencing from fresh tissues targeted the entire *cytb* gene (1,144 bp) for five individuals from northern Chile and Argentina. Amplifications and sequencing followed Steppan et al. (2007) using primers P484 and P485. All sequences were individually checked for quality before further analysis. Sequences have been deposited on GenBank with accession numbers MZ460908–MZ460912.

Sequences were manually edited in Sequencer (Gene Codes Corporation, Ann Arbor, Michigan), exported, and compared to GenBank nucleotide collection using BLAST (Altschul et al. 1990). After identification, sequences were aligned to a selection of published sequences used in Storz et al. (2020). The new sequences expanded sampling in *P. vaccarum* to provide additional geographic detail, but reduced sampling in other species of *Phyllotis* because subspecific membership of the Llullaillaco samples was clearly demonstrated in Storz et al. (2020). This alignment was used to generate a maximum likelihood phylogeny using PAUP* 4.0 (build 169; Swofford 2002) under a GTR+I+Γ model of nucleotide evolution, and with 100 bootstrap replicates. Phylogenies were generated initially where each fragment was treated as an independent OTU to test that each fragment likely came from the same source sequence, and passing that test (by all clustering together among nearly identical sequences), all seven fragments were concatenated into a single sequence for analysis with a more focused phylogenetic sample. Taxonomy for haplotype groups within the *P. xanthopygus* complex follows Ojeda et al. (2021) in elevating several populations or subspecies to species level, including *P. vaccarum* (formerly a subspecies in *xanthopygus*), *P.* sp1 from the mid-elevation grasslands of the Sierra de Córdoba, and *P.* sp2 for the southern populations traditionally allocated to *P. x. vaccarum*.

Survey of high-elevation records for other species.—Comparative data on the upper elevational range limits of wild mammals provide important context for judging the significance of our observations and capture records of *Phyllotis* on Llullaillaco. Because there has never been an authoritative compilation of elevation records for wild mammals (Seimon et al. 2007), we conducted a comprehensive survey of the literature, covering historic and often anecdotal accounts as well as recent species accounts based on field studies by recognized authorities and peer-reviewed online resources (e.g., IUCN 2020). We searched the literature to determine if there have been any credible previous reports of populations of mammals at or above 6,000 m elevation. This was done by (i) searching online compendia such as *Mammalian Species* and

Table 1.—List of all primers designed for *cytb*. Primers S455–478 were designed to target Sigmodontinae and primers S483–500 were designed to target *Phyllotis*. Primers that resulted in successful sequences have an (*) at the end of the name. Degenerate sites are labeled using standard IUPAC code. Location is based on distance (bp) from the start codon of the gene.

Primer Name	Location	Primer Sequence
S455	70	CCMTCCAACATYTCCTCATGATGAAA
S456	202	GYKACTGAGGAGAATGCTGT
S457*	153	CTAGCCATRCACTAYACATCCGA
S458*	301	CGTCCTACGTGRATRAATATRCAG
S459	240	CGMTAYCTACAYGCAAACGG
S460	390	CRAATGCGGTTGCYATRG
S461	329	CAGAAACATGAAAYATYGGYA
S462	481	CYAGGGTTGTKCCRATGTAGG
S463	446	TACTCTCAGCYATCCCCMTAYA
S464	593	AGRAGGTGKACTAGTACAAARGCTG
S465*	551	TCCTRCCYTTTATCATTACAGCYT
S466*	701	AGRAGCACYCCTAGKAGGTC
S467*	651	AATCCCYTTCCACCCRTAYT
S468*	800	TGTGCTGGDGTGTTGAGTGG
S469	751	GGAGACCCYGAYAATTATACM
S470	899	ATYAGAATGGATACRAKGAGGGCTA
S471	374	CAACCGCATTYGTAGGNTAYGT
S472	546	GTGRAATGCGAARAATCGWG
S473	493	TGAGGNGGATTYTCYGTAGA
S474	662	TGGAANGGRATYTTGTCTGAG
S475	592	CTNTTCCTCCACGAAACNGG
S476	750	CYCCGAGAACATCTGGGAAA
S477	685	CTNCTAGGVGTSCTCCTTTT
S478	851	ATNGATCGRAGGATRGCATA
S483	71	CCTCCARCATCTCCTCATGRTG
S484	202	GTTACTGAGGAGAATGCTGT
S485*	240	CGCTACCTACACGCAAACGG
S486*	388	CCYACGAATGCRGTTGCYAT
S487	327	ATCAGAAACRTGAAACATCGG
S488	481	CYAGGGTTGTGCCAATGTAGG
S489*	446	TACTHTCAGCCATCCCCTACA
S490*	593	GAAGGTGAACTAATACAAARGCTG
S491*	750	CGGAGACCCYGAYAATTATACC
S492	895	GAATGGATARAATGAGGGCWAGG
S493	374	CAACCGCATTCGTRGGRTATGT
S494	546	GTGAAATGCGAAGAAYCGTG
S495*	493	TGAGGGGGGTTTTCTGTAGA
S496*	662	TGGAAGGGGATTTTGTCTGAG
S497	592	CTATTYCTCCACGAAACAGGC
S498	750	CTCCGAGAACATCTGGGAAA
S499	682	GACCTYCTAGGRGTGCTCCT
S500	854	GATCGAAGGATGGCATAGGC

the *IUCN Red List of Threatened Species* (IUCN 2020) for elevation data, (ii) searching online catalogs of natural history museum collections and other portals, such as Arctos (Arctos 2011–2020), in order to locate the highest elevation voucher specimen, (iii) manually searching comprehensive print compendia such as *Mammals of South America* (Patton et al. 2015), (iv) manually searching historic and recent printed sources, including the early Himalayan mountaineering literature, and (v) where possible, tracing elevations cited in secondary sources to their original source, often finding that critical nuances were lost along the way. We searched collections of the Smithsonian National Museum of Natural History (NMNH), the Museum of Vertebrate Zoology (MVZ), and the Museum of Southwestern Biology (MSB), among many others. Unfortunately, such a search is incomplete because many museums lack online catalogs, or their voucher information is incompletely digitized. By this process, we identified 12 mammalian species, nine Himalayan and three Andean, with at least one claim of a maximum elevation of 6,000 m or greater, plus a 13th "unidentified" Himalayan rodent.

RESULTS

Molecular phylogenetics.—For the 6,154 m runway sample, three of the first 12 primer sets amplified, yielding 431 bp of sequence (149, 151, and 131 bp fragments). All three fragments most closely matched *Phyllotis* with the top hits either to individuals attributable to *P. vaccarum* (most often identified on GenBank as *P. xanthopygus rupestris*; 96–100% match) or *P. limatus* (94–98% match). The remaining nine primer sets that failed to amplify were redesigned to target *Phyllotis* and produced four additional fragments (152, 147, 147, and 171 bp). All four fragments matched most closely to *P. vaccarum* (97–100%). Across all seven fragments there was a 98.8% match to *P. vaccarum* with the next closest being *P. limatus* (96.9%). All other species of *Phyllotis* were less than 92% matches and

no species outside of *Phyllotis* were greater than 90% matches across all fragments. Four of the seven individual fragments contained polymorphic sites that were clean across both the forward and reverse sequences, indicating multiple individual haplotypes found in these soil samples. All polymorphisms were restricted to silent sites that are known to be variable within *Phyllotis*.

In the phylogenetic analysis in which each fragment from the 6,154 m runway sample was a separate OTU, six of the seven fragments fell in a polytomy that contained *P. vaccarum rupestris* and one was sister to *P. limatus*. *Phyllotis limatus* is known to be a recently diverged peripheral isolate from within a paraphyletic *P. vaccarum*, and its range extends into the Atacama Desert. Therefore, it appears that all sequences derive from multiple individuals of a single species. When concatenated into a single sequence (689 bp), the runway sample ("eDNA") from 6,154 m also nested within the clade of *P. vaccarum* (Fig. 2), that also includes haplotypes of livetrapped Lullaillaco specimens. Bootstrap support for this position was relatively high (nested series of nodes each with >60%, and the eDNA sequence was found outside the *vaccarum* clade in <1% of replicates), and the branch length was comparable to the other samples, suggesting minimal if any sequencing error. The new sequences from other localities reinforce the conclusion that the closest relatives of the Llullaillaco mice live in nearby regions of Chile (LCM 1715 from Mejiones [population 2, see Fig. 2B] near sea level and LCM 1794 from Talbre, Antofagasta at 3,250 m [4]) and Argentina (UP 611 from Laguna Blanca, Catamarca at 3,239 m [7] and UP 613 from Alto Cachi, Salta, at 3,028 m [6]).

Microbial communities.—The dominant bacteria detected by 16S rRNA gene sequencing in the 6,154 m runway sample were mostly animal-associated genera such as *Escherichia*, *Streptococcus*, and *Clostridium* (Fig. 3B). In contrast, typical soil communities found at elevations of 5,100 to 6,300 m on Llullaillaco (Lynch et al. 2012; Solon et al. 2018) contain no animal-associated bacteria (Fig. 3A). The 18S rRNA gene sequences from the mouse runway also captured a strong mammalian signal as well as several other taxa not previously

detected in soil samples from Llullaillaco, including a variety of insects and several plant groups (Fig. 4). Because the sequenced region of 18S rRNA gene is invariant across most plants and across placental mammals, finer resolution is not possible.

Survey of high-elevation records.—The 13 mammals identified by our literature search with at least one claim of a maximum elevation of 6,000 m or greater are listed in Table 2. As that table shows, most elevation figures for these species are well below 6,000 m, and in most cases the claims of 6,000 m and higher were unsubstantiated, mistakes, or equivocal for other reasons. Supplementary Data SD2 provides a detailed list and evaluation of these sources.

DISCUSSION

Evidence of a self-sustaining population above 6,000 m.—Several lines of evidence support our conclusion that leaf-eared mice *P. vaccarum* living at elevations of >6,000 m on Llullaillaco constitute the highest self-sustaining population of mammals on record. On four separate occasions in 2011, 2013, and 2016 we observed three and possibly four mice between 6,154 m and 6,205 m within a small area surrounding Lago Llullaillaco. These mice represent at least three generations because the two-to three-year time interval between observations exceeds a typical mouse generation. The carcass photographed by Vitry in 2011 at 6,734 m and the live mouse captured at 6,739 m on the summit in 2020 indicate that the population extends to the summit of Llullaillaco. Additionally, the mice trapped at multiple localities between 4,140 and 5,070 m along the western flank of the volcano in 2020 suggests a widely dispersed, continuously distributed population of *P. vaccarum*.

The bacterial community found in the soils sampled from the mouse runway at 6,154 m provides strong microbial evidence for a resident vertebrate population on Llullaillaco. Multiple previous studies have used the same environmental sequencing approach described here to thoroughly describe the soil bacterial communities between 5,100 and 6,330 m on both the Chilean and Argentinian sides of Llullaillaco (King et al. 2010;
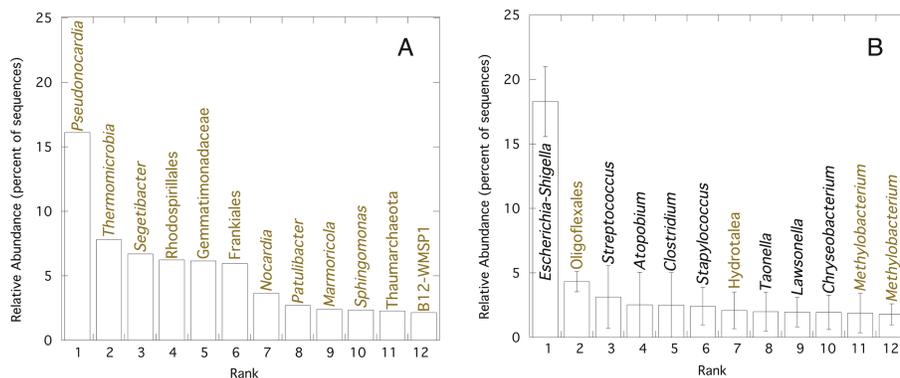

Fig. 3.—Rank abundance plots of the relative abundances of the 12 most prevalent 16S rRNA gene phylotypes in soil samples from Llullaillaco. (A) Typical Llullaillaco soil sample (data from Solon et al. 2018). (B) Mouse runway sample. Shading/colors indicate: typical soil sequence from past studies of Llullaillaco soils (gray/tan), and animal-associated phylotypes (black). Note that the mouse runway sample is dominated by animal-associated bacteria and in particular enteric bacteria that are absent from typical soils from Llullaillaco.
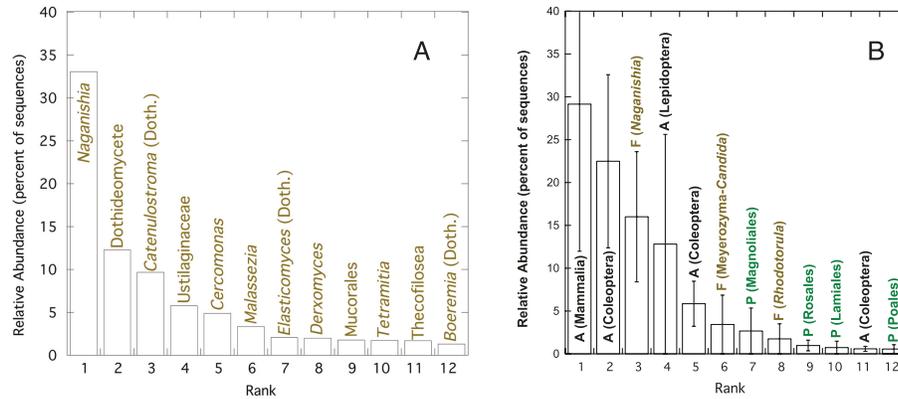
Fig. 4.—Rank abundance plots of the relative abundances of the 12 most prevalent 18S rRNA gene phylotypes in soil samples from Llullaillaco. (A) Typical Llullaillaco soil sample (data from Solon et al. 2018). (B) Mouse runway sample. Colors and letters indicate: fungal DNA ("F" tan), plant DNA ("P" green), and animal DNA ("A" black). Note that the mouse runway contains common fungi from high-elevation volcanoes (*Naganishia* and *Rhodotorula*) and many sequences from possible mouse or aeolian sources. Error bars are standard error of the mean of six subsamples from two soil samples taken from the mouse runway.

Table 2.—Summary of mammal species reported to exceed 6,000 m in maximum elevation, excluding least credible reports. For details, see Supplementary Data SD2.

Species	Live sightings	Tracks/sounds/hair	Not stated	Museum voucher	Confirmed or likely limits for populations
Leaf-eared mouse (*Phyllotis vaccarum*)	6,739 m	6,060 m		6,739 m	6,739 m
Large-eared pika (*Ochotona macrotis*)	6,130 m[b]			5,182 m	<6,130 m
(Unidentified rodent)		6,100 m[b]			<6,100 m
Woolly hare (*Lepus oiostolus*)	>6,035 m[b]	6,400 m[b]		5,029 m	5,400 m[a]
Snow leopard (*Panthera uncia*)			6,000 m		5,800 m[a]
Red fox/Tibetan sand fox (*Vulpes vulpes/V. ferrilata*)		6,400 m[b]			<6,000 m
Wolf (*Canis lupus*)		6,550 m[b]			<6,000 m
Yak (*Bos mutus*)	6,100 m				5,500 m
Siberian ibex (*Capra sibirica*)			6,000 m		<6,000 m
Argali or Marco Polo sheep (*Ovis ammon*)			6,100 m		<6,000 m
Bharal or blue sheep (*Pseudois nayaur*)		6,100 m[b]	6,000 m	5,182 m	~6,000 m
Bolivian pericote (*Auliscomys boliviensis*)	~6,000 m				5,200 m
Andean pericote (*Auliscomys sublimis*)			6,000 m		5,100 m

[a]IUCN estimate.
[b]Elevations from British 1921 Mt. Everest expedition, based on high-reading aneroid barometer.

Lynch et al. 2012; Solon et al. 2018; Vimercati et al. 2019). Of the hundred or so samples analyzed in those studies, none have shown a significant presence of animal-associated microbes, in marked contrast to the runway sample. Moreover, the bacteria in the runway sample that are not noted as animal-associated generally do not occur in soils elsewhere on the mountain. This may indicate that the runway bacteria are feeding off substrates in mouse waste products. Such a large shift in the composition of the runway bacterial community would likely require an accumulation of animal-deposited material over time to overwhelm DNA from the resident microbial community.

Results from the 18S rRNA gene from samples taken in the mouse runway provide even more compelling evidence for a resident population of rodents. Previous analyses of 18S rRNA gene microbial communities in Llullaillaco soils have revealed a community dominated by fungi; in fact, Solon et al. (2018) found that all of the top 12 phylotypes were stress-tolerant fungi that are likely to be able to survive in arid soils. In contrast, the top 12 phylotypes in the 6,154 m runway sample are mostly sequences from plants and animals (primarily beetles

and moths or butterflies) which may have been transported by wind, because no vascular plants are known to occur above ~5,100 m on Llullaillaco. Therefore, the DNA in the runway soils is most likely from mouse feces derived from eating plant and insect material. Since four different plant phylotypes and four different animal phylotypes were represented, mice on Llullaillaco may have access to a variety of food sources. Unfortunately, the 18S rRNA gene evolves too slowly to identify specific plants and animals, so stomach content analysis of captured mice is needed to identify specific food sources. Two of the three fungi in the mouse runway are commonly found at high elevations on Llullaillaco and other nearby volcanoes (Pulschen et al. 2015; Solon et al. 2018), whereas the third fungus ("*Meyerozyma–Candida* clade") is a close match to a copper-tolerant fungus isolated from a nearby copper mine (Villegas et al. 2009).

Phylogenetics.—All the samples from Llullaillaco, including the mouse runway, group with nearby populations from northern Chile and Argentina, suggesting that the mountain population is simply part of a general high-elevation species.

But remarkably, the closest relatives to most Llullaillaco samples include animals collected at sea level from two different sites, the mouth of the Río Loa, and the newly sequenced mouse from Mejillones. This corroborates and further refines the conclusion of Storz et al. (2020) that the Llullaillaco population may belong to a metapopulation that extends over the full 6,700 m elevation range.

Previously reported elevation records for mammals.—Here we define the record elevation of a species as the highest elevation that has sustained a population in a particular location over many generations. Previously, the species most often considered the high-elevation record holder was the large-eared pika *Ochotona macrotis* (e.g., Guinness_World_Records 2018), although it was not based on vouchered specimens. The record was established during the 1921 British Mt. Everest expedition, where multiple individuals were observed in camp at 20,100 feet (6,130 m). However, because the expedition barometers were reading high by several hundred feet (Leigh-Mallory 1922), the actual elevation of the camp and the pikas may have been less than 6,000 m. Subsequent citations of these Mt. Everest pikas at 6,400 m (*Science* 2016; Smith 2008; Smith and Johnston 2008) probably resulted from an unfortunate transposition error of the numerals from 20,100 to 21,000 feet which was then converted to metric. Although later replaced by the correct figure of 6,130 m (Smith 2016, 2018), the erroneous 6,400 m figure persists in the current IUCN Red List (Smith and Lissovsky 2016, Version 2021.2). A critical appraisal of published accounts clearly demonstrates the frequent overstatements and uncertainty of maximal records (Table 2 and Supplementary Data SD2). Notably, we find no robust evidence for any mammal species persisting above 6,000 m except for *P. vaccarum*.

We contend that *P. vaccarum* currently holds the record for the highest sustained population not only for mammals (Table 2), but for all vertebrates. It far exceeds the record for reptiles, recently established at 5,400 m on Volcán Chachani in southern Peru for the iguanine lizard *Liolaemus* aff. *tacnae* (Cerdeña et al. 2021). It also exceeds the previous record for vertebrates, a single observation of a pair of birds, alpine choughs (*Pyrrhocorax graculus*), nesting at 6,550 m below Mt. Everest in 1924 (Noel 1927).

Ecological limits.—The most critical factor that limits the maximum elevation at which populations can be self-sustaining must surely be the food supply. In the region surrounding Llullaillaco, the limit for grasses is about 4,600 m (Halloy 1991; Costello et al. 2009) and the highest known vascular plants disappear around 5,100 m. Lichens persist above 6,000 m but are scarce. Therefore, the mouse population in the Llullaillaco lake basin resides more than 1,000 m above any living food source normally associated with rodents. On Volcán Socompa, 45 km northeast of Llullaillaco, fumaroles power miniature ecosystems which in turn are thought to provide food for rodents. One such system, just below the 6,060 m summit, is based on mosses and liverworts and supports a variety of insects. In 1984, S. Halloy found rodent hair at the site which he attributed to *Phyllotis*, and a live mouse entered but escaped his trap (Halloy 1991). A thermal source may be responsible for the elevated bottom temperature of Lago Llullaillaco (Arán

et al. 2021), but no terrestrial fumarole-driven ecosystems have been discovered on the upper mountain.

A possible food source for mice above 6,000 m on Llullaillaco is wind-transported plant parts and insects. This material appears varied and substantial. As noted earlier, DNA collected at 6,154 m from the mouse runway was from four plant groups and at least four different insect species (Fig. 4). In 2013, Farson and Bowen found plant parts scattered over the snow above 6,000 m in the wake of a blizzard with gale-force winds. Mice on Llullaillaco may be among the few mammals that live entirely within the aeolian zone (Swan 1963, 1967). More thorough surveys of plants and arthropods on the mountain are needed to test this possibility.

A related question is how a species that, so far as is known, is a strictly nocturnal forager elsewhere within its range (Steppan and Ramirez-Baca 2015), is able to tolerate cold nighttime temperatures at high elevations. In fact, all sightings above 6,000 m on Llullaillaco have been during midday, suggesting that there has been a behavioral shift in this portion of its range. A shift to daylight foraging may have been facilitated by the absence of predators at this elevation and hence accomplished without increased predation mortality.

The lingering question is whether the highest elevation recorded for mice on Llullaillaco is also the maximum elevation at which mice, or any mammal, can maintain a self-sustaining population. Although *P. vaccarum* can ascend no higher on Llullaillaco than the summit, they may be physiologically capable of living higher as long as they have access to food. This was well understood by R.W.G. Hingston, medical officer and naturalist with the 1924 British Mt. Everest expedition, who observed nearly a century ago that:

> Animals will ascend the flanks of mountains so far as their accustomed food supply is obtainable....Animals are not deterred by physical inclemencies. They will brave the cold wind and the rarified atmosphere provided a suitable supply of food can be secured. This is the reason why mammals reach the farthest limits of the vegetation (Hingston 1925: 283).

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Author Contributions

Conceptualization: TB, SJS; Investigation: TB, MF, SKS, MRB, JFS, MQ-C, GD, LV, CDO, GZ; Writing—original draft: SJS, TB, SKS, MRB, JFS; Writing—review and editing: SJS, TB, LV, GD, JFS, MQ-C; Funding acquisition: SJS, SKS, GD.

Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Complete video of 2013 encounter with live mouse (49 s).

Supplementary Data SD2.—Survey and evaluation of previous accounts of mammal species above 6,000 m.

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