Parasitology

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Prevalence and molecular characterization of *Sarcoptes scabiei* from vicuñas (*Vicugna vicugna*) from Southern Peruvian Andes

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Research Article

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Abstract

Sarcoptic mange is a disease caused by an infectious parasite in the vicuñas (*Vicugna vicugna*) from South America. Although molecular studies have provided much information about the epidemiology of this disease, this information is still unknown in vicuñas. This study determined the prevalence and molecular characterization of *Sarcoptes scabiei* from vicuñas from Southern Peruvian Andes. During the 2018 shearing season, 181 vicuñas were clinically evaluated for lesions compatible with mange. *Sarcoptes scabiei* was detected in 35 (19.3%) vicuñas, and 50 mites from 25 vicuñas were selected for molecular analyses of the mitochondrial (cox1) and nuclear (ITS2) genetic markers. Molecular analyses of the cox1 and ITS2 sequences showed an identity of 94–99% and 99.8–100% with previous *S. scabiei* sequences registered in the GenBank, respectively. Sequence polymorphisms were more evident in the ITS2 than in the cox1, but only the cox1 had an association with the host. Phylogenetic analysis of *S. scabiei* cox1 sequences from vicuñas showed a cluster with *S. scabiei* cox1 sequences from canids, suggesting that the origin of *S. scabiei* from vicuña is associated with canid mites. This research is the first molecular analysis of *S. scabiei* from vicuñas. Future molecular studies will be necessary to determine the species variety, geographic segregation and host–parasite adaptation for this vicuña's mite.

Introduction

The vicuña (*Vicugna vicugna*) is a wild South American camelid distributed in the high areas of the Andes, from Ecuador to the north of Argentina, covering areas of Peru, Bolivia and Chile (Wheeler, 2012). The vicuña is one of the animals of the world that has its very fine fibre (Sahley *et al.*, 2007). Due to this, the vicuña was an animal threatened due to poaching. This illegal activity reduced the population of animals in the 1950s, reaching around 10 000 animals throughout South America. Strategies carried out to conserve the vicuña population in Peru have been successful (Wheeler, 2012). Currently, the vicuña population is about 208 000 animals throughout Peru (Acebes, 2020). However, the increase in the vicuña population was accompanied by the appearance or increase of some diseases, such as sarcoptic mange (Gomez-Puerta *et al.*, 2013; Montecino-Latorre *et al.*, 2020).

Sarcoptic mange, caused by *Sarcoptes scabiei*, is a common infectious parasite disease in domestic and wild mammals (Pence and Ueckermann, 2002; Arlian and Morgan, 2017). Infected animals manifest typical signs such as hair loss, dermal scabs and pruritus (Pence and Ueckermann, 2002). Currently, sarcoptic mange is documented in new geographic areas and new hosts, mainly wild mammals, and it is considered an emerging wildlife disease (Astorga *et al.*, 2018; Escobar *et al.*, 2021).

Sarcoptic mange is often considered an animal welfare issues, and can be a conservation issue in some circumstances [e.g. as seen in wolves, foxes, coyotes and wombats (Pence and Ueckermann, 2002; Fraser *et al.*, 2016; Astorga *et al.*, 2018)]. In South America, this disease is considered a severe problem for the vicuña population. Some studies showed up to 37% prevalence in vicuñas (Castillo *et al.*, 2019), and the disease was also associated with the mortality of vicuñas in a Peruvian National Reserve (Korswagen, 2016).

Molecular studies have provided much information about the epidemiology of this disease. Use of several gene loci such as internal transcribed spacer 2 (ITS2), ribosomal 12S and 16S RNA (rRNA) and mitochondrial cytochrome c oxidase subunit I (cox1) genes has helped to molecular typing of *S. scabiei* (Zahler *et al.*, 1999; Walton *et al.*, 2004; Amer *et al.*, 2014; Li *et al.*, 2018). These studies have shown specific genotypes of *S. scabiei* for some populations and species of mammals (Oleaga *et al.*, 2013; Amer *et al.*, 2014). Likewise, the use of microsatellites has helped to give more precise and solid results regarding the mite population in the same host species or individual compared with ribosomal and mitochondrial markers (Oleaga *et al.*, 2013; Moroni *et al.*, 2021).

The molecular epidemiology of sarcoptic mange in vicuñas remains unknown. This study represents a contribution to research on the sarcoptic mange prevalence in vicuñas from

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Southern Peruvian Andes and to the molecular characterization of *Sarcoptes* mites collected from vicuñas, analysing part of the cox1 gene and the ITS2 region.

Materials and methods

Ethical statement

This study was reviewed and approved by the Forest and Wildlife Service of Peru (SERFOR) (No 246-2018-DGGSPFFS).

Animals

During May and June of 2018, a total of 181 wild vicuñas were clinically evaluated for lesions compatible with mange during the shearing season in four localities (Chilca, Palccoyo, Phacco and Sibina Sallma) located in Canchis province in Cuzco, in the Southern Peruvian Andes, at an altitude between 4800 and 5200 m above sea level (Fig. 1). During this event, commonly known as the 'chaku', all animals were clinically evaluated by veterinarians to diagnose the presence of lesions compatible with mange. For this, areas of the face, ear, chest, abdomen, axillae, groin, legs and interdigital spaces were examined since the disease occurs mainly in these areas in vicuñas (Gomez-Puerta *et al.*, 2013). In addition, skin scrapings samples from each suspected animal were collected and stored in plastic vials with 70% ethanol duly labelled, indicating the animal data and affected area.

Fig. 1. Physical map of Peru and geographic location of the study site.

Microscopic diagnosis

Some tissues from skin scraping samples were digested in tampon buffer lysis (see below DNA extraction). The mites were collected under a stereomicroscope and then mounted in glass slides using Hoyer's medium (Krantz and Walter, 2009). The mites were examined morphologically under a microscope at 100× or 400× magnification, and identified using morphological keys previously described (Fain, 1968).

DNA extraction

Some ($n = 25$) tissues (~3 mm^3) from skin scraping samples were put in a 2 mL vial and dehydrated in a vacuum centrifuge. Then, 100 μL of tampon buffer lysis (40 mM Tris-HCl, 100 mM NaCl, 20 mM EDTA, 1% SDS, 0.2 mg mL^{-1} of proteinase K, pH 7.2) was added in each tube and was incubated at 56°C for 2–3 h. After this, mites were collected from each tube under a microscope (Carl Zeiss, Germany). DNA was extracted by an individual mite using Chelex method (Gomez-Puerta *et al.*, 2016). DNA was extracted from 50 mites, two randomly selected mites from each scabietic vicuña.

PCR protocol and sequencing

A PCR was used to amplify approximately 421 base-pairs (bp) of the cox1 mitochondrial gene, using a protocol and primers (772 and 773) proposed by Navajas *et al.* (1994). Also, the complete ITS2 region was amplified using primers LGF2 (5′-CGT TTT AAA TGC AAA ATT CAA-3′) and LGR2 (5′-GCC GTT ACT AAG GGA ATC-3′) designed in this study, and the following PCR protocol reaction: 4 μL of DNA, 10 μM of each primer, 25 μL of Dream Taq Green PCR Master Mix (2×) (Thermo Fisher Scientific, USA) and ultrapure water in a final volume of 50 μL. Thermal cycler conditions were 94°C for 30 s, 45°C for 30 s and 72°C for 30 s, repeated 36 times. The PCR products were visualized by 1.5% agarose gel electrophoresis staining with ethidium bromide.

All PCR-positive samples, corresponding to 50 mites, were purified and sequenced using an ABI 3100 automated sequencer (Applied Biosystems, USA). The accuracy of the sequence was confirmed by bi-directional sequencing of two separate PCR products. The sequences were analysed using the software ChromasPro 2.1.8 (http://technelysium.com.au/wp/chromaspro). All sequences were compared with reference sequences from the GenBank using the Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The MEGA-X software (http://www.megasoftware.net/) was used to construct a phylogenetic tree according to the Maximum Likelihood method using the Tamura three-parameter model (Tamura, 1992; Kumar *et al.*, 2018). All sequences were subjected to 1000 bootstrap replications for the accuracy of the constructed phylogenetic tree. Unique nucleotide sequences of the cox1 gene and ITS2 region of *S. scabiei* from vicuñas were deposited in the GenBank under accession numbers MZ569438 to MZ569487 for cox1 and MZ574503 to MZ574550 for ITS2.

Data analysis

Sarcoptic mange diagnosis and demographic data (age, sex and geographical origin) were analysed as categorical variables. Age was categorized in younger and older than 1 year. The association of sarcoptic mange diagnosis and demographic variables was evaluated using the χ^2 test, considering 95% confidence intervals (95% CI). Data were analysed using Stata 15.0 (StataCorp, College Station, TX, USA).

Results

Lesions compatibles with mange were localized in chest, abdomen, axillae and groin zones (Fig. 2). Sarcoptic mange was

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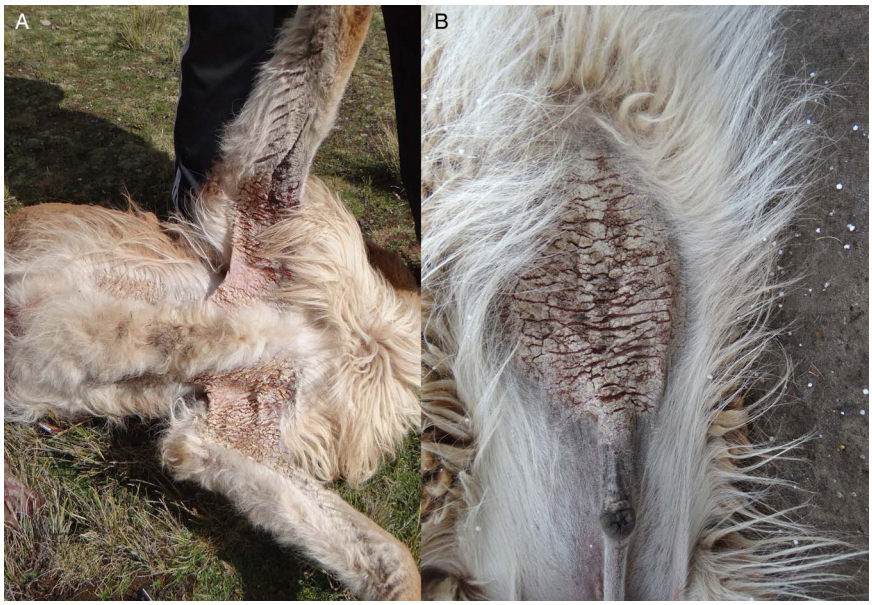

Fig. 2. Affected vicuñas showed skin lesions, mainly affecting internal legs (A), and chest and abdomen (B).

Table 1. Sarcoptic mange in vicuñas (*Vicugna vicugna*) from Cuzco in the Southern Peruvian Andes ($n = 181$) by sex, age and geographical origin as indicated by univariate analysis

		Sarcoptic mange diagnosis		
Characteristics	Total	Positive *n* (%)	Negative *n* (%)	*P*
Sex				0.548
Male	91	16 (17.6)	75 (82.4)	
Female	90	19 (21.1)	71 (78.9)	
Age				0.249
<1 year-old	56	8 (14.3)	48 (85.7)	
>1 year-old	125	27 (21.6)	98 (78.4)	
Geographical origin				0.013
Chilca	83	8 (9.6)	75 (90.4)	
Palccoyo	19	5 (26.3)	14 (73.7)	
Phacco	32	11 (34.4)	21 (65.6)	
Sibina Sallma	47	11 (23.4)	36 (76.6)	

suspected in 35 of 181 (19.3%; 95% CI 13.3–24.7%) vicuñas, leading to the collection of skin scrapings. Microscopic examination of the skin scraping samples confirmed the occurrence of *S. scabiei* in all of these samples. Infestation by *S. scabiei* did not differ between vicuñas sex and age (Table 1). Sarcoptic mange showed differences between localities, with low infestation rates from Chilca, relative to other locations (Table 1).

DNA isolates were performed on 50 *S. scabiei* individual mites from 25 infected vicuñas (two mites per vicuña). A total of 50 partial nucleotide sequences (421 bp) of the cox1 gene were obtained from these samples, and the sequences had 94–99% similarity with previous *S. scabiei* sequences recorded in the GenBank. The *S. scabiei* cox1 sequences analysis from vicuñas showed two types of sequences with a single nucleotide polymorphism, a transition cytosine (C) by thymine (T). Regarding geographical origin, vicuñas from Chilca and Sibina Sallma were parasitized with the two types of *S. scabiei* cox1 sequences. Coinfection with both sequence types was not observed in any vicuña (see Supplementary Table S1). Phylogenetic analysis by the Maximum Likelihood method showed a cluster of *S. scabiei* from vicuñas. This cluster included *S. scabiei* cox1 sequences from canids (Fig. 3A).

The ITS2 region from 48 *S. scabiei* collected from 25 vicuñas was fully amplified and sequenced (total nucleotide sequence = 304 bp). The sequences were compared with previous *S. scabiei* sequences registered in the GenBank and showed an identity of 99.8–100%. A polymorphism was evidenced in the ITS2 nucleotide sequences of *S. scabiei* from vicuñas, showing a genetic variation of up to 1.4%. The polymorphic sites were represented by four transitions (C and T, alignment positions 14 and 18; A and G, alignment position 52 and 219), and five transversions (A and C, alignment positions 17 and 18; A and T, alignment positions 256 and 274; G and T, alignment position 294). This polymorphism in the ITS2 nucleotide sequences showed eight haplotypes for *S. scabiei* from vicuñas (Fig. 3B). Of these haplotypes, three were identical to those previously registered in the GenBank (Table 2, Supplementary Table S1). The remaining five corresponded to new *S. scabiei* haplotypes.

Discussion

This study indicates that sarcoptic mange is a common disease in vicuñas from Cuzco, in the Southern Peruvian Andes, showing a prevalence of 19.3%. Although epidemiological studies of sarcoptic mange in vicuñas are very limited, reports indicate prevalence ranging from 12 to 37% in Peru (Castillo *et al.*, 2019; Gomez-Puerta *et al.*, 2013). Likewise, sarcoptic mange outbreaks have been documented in the neighbouring countries, reporting outbreaks in individual vicuñas from Chile (Montecino-Latorre *et al.*, 2020) and prevalence of 46.2% (6/13 animals) in Bolivia (Ruiz, 2016) and 0.9% (4/450 animals) in Argentina (Arzamendia *et al.*, 2012). As is known, sarcoptic mange is a parasite disease with characteristic and evident clinical signs in wildlife (Escobar *et al.*, 2021). In Peru, sarcoptic mange is an important disease in vicuña populations. Recently, a die-off occurred in vicuñas from the Tanta community located in the Paisajística Nor Yauyos Cochas Reserve in the Central Andes of Peru. Of the total number of dead vicuñas in this reserve, 64% were caused by sarcoptic mange (Korswagen, 2016). This research is the first molecular study of *S. scabiei* from South American camelids in

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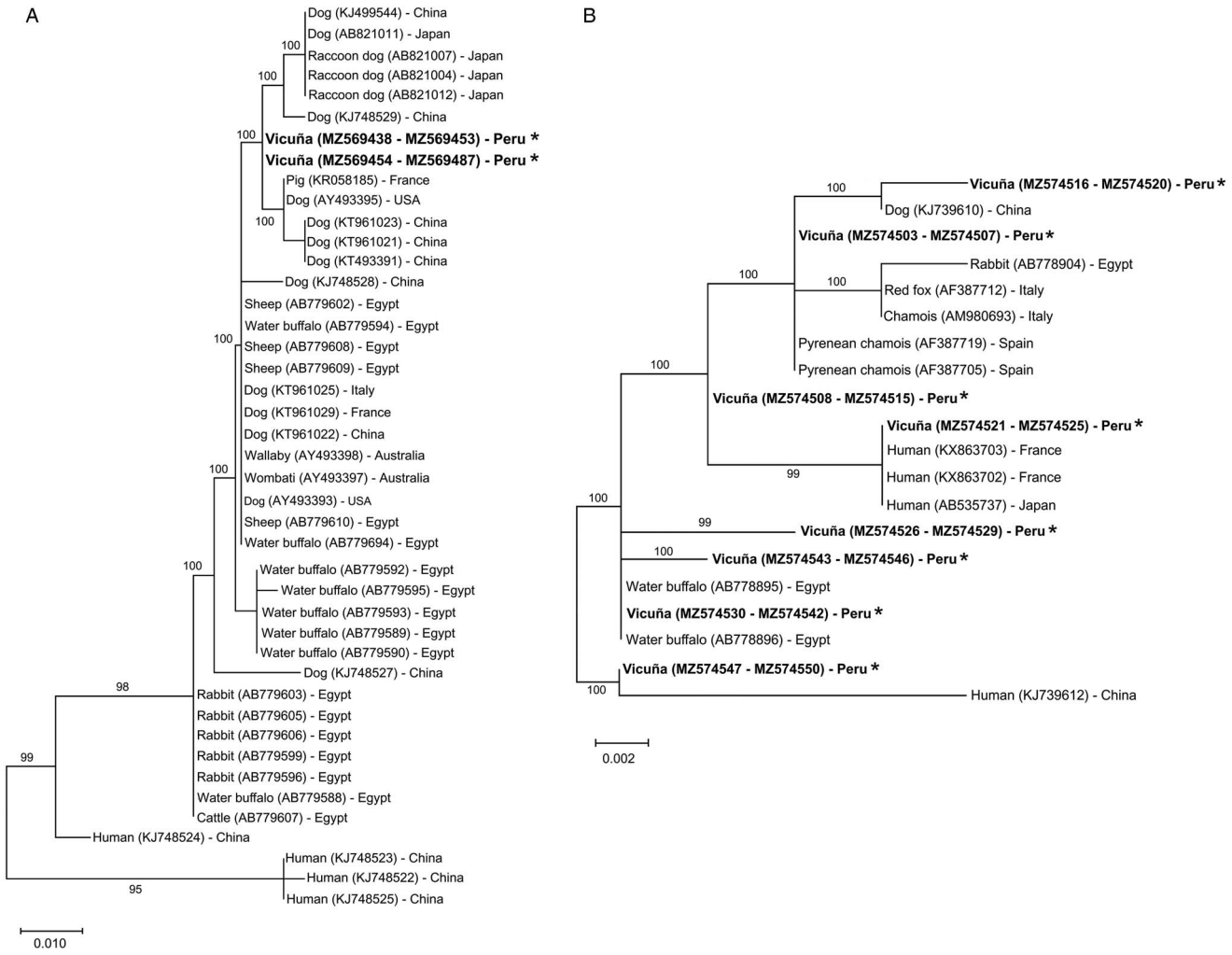

Fig. 3. Phylogenetic relationships of *Sarcoptes scabiei* collected from vicuñas (*Vicugna vicugna*) from Southern Peruvian Andes. Evolutionary analyses by the Maximum Likelihood method of the partial cox1 gene (A) and ITS-2 region (B) sequences were based on genetic distances calculated by the Tamura 3-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The trees were drawn to scale with branch lengths measured in the number of substitutions per site (scale bar for cox1 and ITS2 indicates 10 and 2 substitutions per 1000 nucleotide positions, respectively). This analysis involved 46 and 20 nucleotide sequences for cox1 and ITS2, respectively. All positions with <95% site coverage were eliminated, i.e. fewer than 5% alignment gaps, missing data and ambiguous bases were allowed at any position (partial deletion option). The sequence accession number is in parenthesis, followed by the origin country. Asterisk (*) indicates the sequences for this study.

Peru. In addition, the study contributes new *S. scabiei* reference sequences from vicuñas.

Molecular tools are widely used to understand the epidemiology, host specificity and geographic separation of *S. scabiei* (Amer *et al.*, 2014; Fraser *et al.*, 2017; Li *et al.*, 2018). As shown in other studies, the *S. scabiei* cox1 genetic marker shows nucleotide sequence polymorphisms eventually related to their host (Walton *et al.*, 2004; Amer *et al.*, 2014; Fraser *et al.*, 2017, 2019). Analysis of the mitochondrial cox1 gene of *S. scabiei* from vicuñas revealed a clade that was phylogenetically related to *S. scabiei* in canids (Fig. 3A).

On the other hand, several studies worldwide have analysed the ITS2 region of *S. scabiei* from different hosts (Zahler *et al.*, 1999; Verdugo *et al.*, 2016; Peltier *et al.*, 2017; Li *et al.*, 2018). In all of them, the results indicated a greater genetic variability, decreasing over the years, compared to the cox1 gene (Amer *et al.*, 2014; Fraser *et al.*, 2016, 2017; Peltier *et al.*, 2017). In our study, the ITS2 region analysis of *S. scabiei* from vicuñas showed three known haplotypes, which have been previously identified from *S. scabiei* from Pyrenean chamois (*Rupicapra pyrenaica*) from Spain (Berrilli *et al.*, 2002), water buffalos (*Bubalus bubalis*) from Egypt (Amer *et al.*, 2014), and humans from France and Japan (Fukuyama *et al.*, 2010; Delaunay *et al.*, 2020), respectively. Likewise, five new haplotypes were identified in *S. scabiei* from vicuñas (Table 2). In addition, as other studies have demonstrated (Zahler *et al.*, 1999; Gu and Yang, 2008; Amer *et al.*, 2014), the ITS2 nucleotide sequence polymorphism of *S. scabiei* from vicuñas was not associated with the host species and the geographic location (Fig. 3B).

As with other parasitic agents, host–parasite adaptation and geographic segregation play an essential role in understanding the epidemiology of this mite. For many years, it has been believed that the *S. scabiei* transmission could occur between host species and that their descent with new genetic variability could infect new animal species (Fain, 1994; Fraser *et al.*, 2017). In recent decades, molecular studies have attempted to better explain the epidemiology of *S. scabiei* in different host species (Amer *et al.*, 2014; Fraser *et al.*, 2017, 2019; Peltier *et al.*, 2017; Bae *et al.*, 2020). These studies demonstrated the existence of various genotypes analysing different molecular markers, as the cox1 gene, suggesting some host–parasite adaptation for *S. scabiei*, as supported by this study. Furthermore, the phylogenetic analysis of the *S. scabiei* cox1 gene indicates that canids are possibly involved in transmitting sarcoptic mange to vicuñas.

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Table 2. Data information of the *Sarcoptes scabiei* isolated from vicuñas included in the molecular study

Haplotype	Geographical origin1	GenBank accession numbers	Identity with sequences from the GenBank	Previous hosts	Country of previous record	Reference
cox1						
1	Chilca Sibina Sallma	MZ569438–MZ569453	Not identical	–	–	–
2	Chilca Palccoyo Phacco Sibina Sallma	MZ569454–MZ569487	Not identical	–	–	–
ITS2						
1	Chilca Sibina Sallma	MZ574503–MZ574507	AF387719 AF387705	*Rupicapra pyrenaica*	Spain	Berrilli *et al.* (2002)
2	Palccoyo Phacco Sibina Sallma	MZ574508–MZ574515	Not identical	–	–	–
3	Phacco Sibina Sallma	MZ574516–MZ574520	Not identical	–	–	–
4	Chilca Phacco Sibina Sallma	MZ574521–MZ574525	KX863702 KX863703 AB535737	*Homo sapiens*	 France Japan	Delaunay *et al.* (2020), Fukuyama *et al.* (2010)
5	Chilca Palccoyo	MZ574526–MZ574529	Not identical	–	–	–
6	Chilca Palccoyo Phacco Sibina Sallma	MZ574530–MZ574542	 AB778895 AB778896	*Bubalus bubalis*	Egypt	Amer *et al.* (2014)
7	Chilca Phacco	MZ574543–MZ574546	Not identical	–	–	–
8	Chilca Phacco	MZ574547–MZ574550	Not identical	–	–	–

1Localities from Canchis Province, Cuzco, in the Southern Peruvian Andes.

However, to demonstrate this, it will be necessary to molecularly analyse *S. scabiei* from canids and other mammals from the Cuzco areas, adding the use of other genetic markers such as microsatellites.

The main limitations of our study are the small number of specimens analysed by each vicuña, the use of mites from the same geographic area, and not used *S. scabiei* from other host species, both domestic and wild. Although the analysis of cox1 showed an exclusive clade for vicuñas, with these limitations, we cannot conclude that *S. scabiei* mites from vicuñas or other South American camelids are exclusively adapted to these particular hosts. Therefore, it will be necessary to carry out future molecular studies such as population genetics and comparative genomics to help us determine the variety of strain, geographical segregation and the host–parasite adaptation for the *S. scabiei* from vicuñas from Peru. Likewise, this will help understand valuable and applicable concepts for the conservation of wildlife and its health, and their relationship with domestic animals and humans.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182021001931

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Conflict of interest. None.

Ethical standards. This study was reviewed and approved by the Forest and Wildlife Service of Peru (Servicio Nacional Forestal y de Fauna Silvestre – SERFOR) from Lima, Peru.

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