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SHORT COMMUNICATION

Mycoplasmal infection in a guigna (*Leopardus guigna*) from central Chile

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ABSTRACT. Routine blood analysis indicated the presence of *Mycoplasma*-like bodies in a guigna (*Leopardus guigna*). Evidence of infection with *Candidatus* Mycoplasma haemominutum was found in blood samples using PCR and DNA sequencing of the 16S rRNA gene of *Mycoplasma*. *Mycoplasma* spp. are documented in cats but their role in the transmission of *Mycoplasma* to guigna populations requires investigation.

Key words: bacteria, domestic animals, Felidae, vulnerable.

INTRODUCTION

Urbanization and natural environments is an opportunity for the transmission of infectious diseases to native wildlife (Valenzuela-Sanchez & Medina-Vogel 2014). The illegal possession of wildlife and the free-roaming of domestic species in rural areas is well known to have a substantial impact on several aspects of wild animal ecology including habitat use, activity patterns, and host-pathogen interactions (Hwang *et al* 2018). Currently, information about pathogens in populations of Chilean wildlife is limited and further research is necessary to properly understand the consequences of these infections (Llanos-Soto & González-Acuña 2019).

Haemotropic mycoplasmas (haemoplasmas) are small epierythrocytic bacteria that infect a wide variety of mammalian species, including domestic cats (Sykes 2010). Four haemoplasmas species are identified to infect domestic cats: *Mycoplasma haemofelis* (Mhm), *Candidatus* Mycoplasma the associated introduction of domestic animals in haemominutum (CMhm), *Candidatus* Mycoplasma turicensis (CMt) and *Candidatus* Mycoplasma haematoparvum-like (Sykes 2010). In Chile, mycoplasmal infection is common among domestic animals, with findings from a report by Walker *et al* (2016) indicating a prevalence of 15.1% in cats living in the southern region of the country. Nonetheless, the detection in wild species such as Darwin's Fox (*Lycalopex fulvipes*) and guigna (*Leopardus guigna*), is rather recent (Cabello *et al* 2013, Walker *et al* 2016, Di Cataldo *et al* 2020). The guigna inhabits the temperate rainforests of central and southern Chile and it is currently categorised as Vulnerable by the IUCN (International Union for Conservation of Nature) (Gálvez *et al* 2013). Here, we reported the evidence of infection with *Candidatus* Mycoplasma haemominutum (CMhm) in a guigna illegally kept as a pet.

MATERIAL AND METHODS

In August 21, 2018, a male adult guigna (*Leopardus guigna*) was confiscated by the Livestock and Agriculture Service (SAG) and brought in to the Wildlife Rehabilitation Centre, Universidad de Concepción, Chillán. The animal was being illegally kept in a warehouse by a local family from El Carmen (36°53'S, 72°01'W). On arrival, the individual did not exhibit any behavioural or physical anomalies and was unafraid of human handling. An anaesthesia protocol with 20 mg/kg of demedetomidine and 0.3 mg/kg of methadone IM was performed to collect blood from the saphenous vein for biochemical and haematological testing, as it is routinely carried out for all animals entering quarantine on their arrival to the rehabilitation centre (Tayari *et al* 2015). Blood smears were also prepared and stained with Giemsa stain for microscopic observation.

DNA extraction from blood was carried out using the DNAeasy Blood & Tissue kit (Qiagen) and according to the manufacturer's instructions. DNA templates obtained through this protocol were amplified in a thermocycler (MultiGene™ OptiMax Thermal Cycler, Labnet) and the 16S rRNA and RNAseP gene of *Mycoplasma* was targeted using primers described in table 1. The DNA sample was also analysed to detect the presence of other pathogens (table 1). The amplificated PCR products were run in 1% agarose gel, purified using the SigmaSpin ™ Post-Reaction Purification Columns (Sigma-Aldrich), and analysed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems). The sequence corresponding to the 16S rRNA was aligned to a single consensus sequence by ProSeq 3.5 software and subject to comparison with the GenBank database of National Center for Biotechnology Information (NCBI).

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Table 1. Primer pairs used for amplification of pathogens of DNA sample from guigna (*Leopardus guigna*).

Primer	Sequence	Size (pb)	Organims	Reference
gltA CS78F CS323R	5'-GCAAGTATCGGTGAGGATGTAAT-3' 5'-GCTTCCTTAAAATTCAATAAATCAGGAT-3'	401	Rickettsia	Labruna *et al* 2004
EHR12SD EHR16SR	5'-GGTACCYACAGAAGAAGTCC-3' 5'-TAGACTCATCGTTTA-3'	345	Anaplasmataceae	Parola *et al* 2000
HBT-F HBT-R	5'-ATACGGCCCATATTCCTACG-3' 5'-TGCTCCACCACTTGTTCA-3'	618	*Candidatus* Mycoplasma haemominutum	Criado-Fornelio *et al* 2003
RNAsePF RNAsePR	5'-CTGCGATGGTCGTAATGTTG-3' 5'-GAGGAGTTTACCGCGTTTCA-3'	175	*Candidatus* Mycoplasma haemominutum	Tasker *et al* 2003
RNAseP-Cmh F RNAseP-Cmh R	5'-CTCTCGTCATTTCTGCAGAACGTC-3' 5´-CGCTTGCACAGTCTGAGATGA-3'	175	*Candidatus* Mycoplasma haemominutum	This study

Mycoplasma sequences reported in domestic and wild felines in the GenBank were used for phylogenetic analysis. Twenty sequences were used to perform the alignment together with the consensus sequence using the ClustalW algorithm (Thompson *et al* 1994) and phylogenetic trees were constructed based on neighbour-joining, maximum likelihood and Bayesian methods using a GTR + I + G model. Maximum-likelihood analysis was conducted using MEGA 7.0 (Kumar *et al* 2016) and Bayesian inference analysis was performed with Mr. Bayes 3.1.253 (Ronquist *et al* 2012). The data set was resampled 1,000 times to generate bootstrap values. Software FigTree 1.4.4 was used for visualisation.

RESULTS AND DISCUSSION

Results from the blood analysis showed no haematological or biochemical alterations, however, blood smears revealed the presence of *Mycoplasma*-like bodies in erythrocytes (figure 1). The sequence obtained from the guigna in this study belongs to *Candidatus* Mycoplasma haemominutum (CMhm) (Accession number: MT772012) (figure 2). Primers reported and designed for the *Mycoplasma sp* RNAseP gene did not amplify any sequences. To our knowledge, there are no reports on the amplification of the RNAseP gene for CMhm. During the analysis, sequences were clustered in different groups, namely group A (CMhm), group B (Mhf), group C (*Candidatus* Mycoplasma turicensis - CMt) and group D (*Mycoplasma sp*).

The sequence obtained in this study was positioned within group A, which is also shared with haemoplasma sequences found in *Felis silvestris catus* from Chile. The context in which the guigna was found could have resulted in increased exposure to pathogens from cats, including *Mycoplasma*. Currently, there are few studies accurately addressing *Mycoplasma* transmission in wild animals in Chile. However, the mechanism of transmission of haemoplasmas among wild and domestic cats has not been elucidated, a recent study suggests that their interaction might be not linked to exposure to haemoplasma-transmitting vectors (Sacristan *et al* 2019). *Mycoplasma* infections can cause disease in domestic cats but just in rare cases there have been reports documenting clinical signs associated with this pathogen in wild carnivores (Criado-Fornelio *et al* 2003). In this report, the guigna showed no clinical signs which is also the case for most studies in carnivores. However, the animal died two weeks

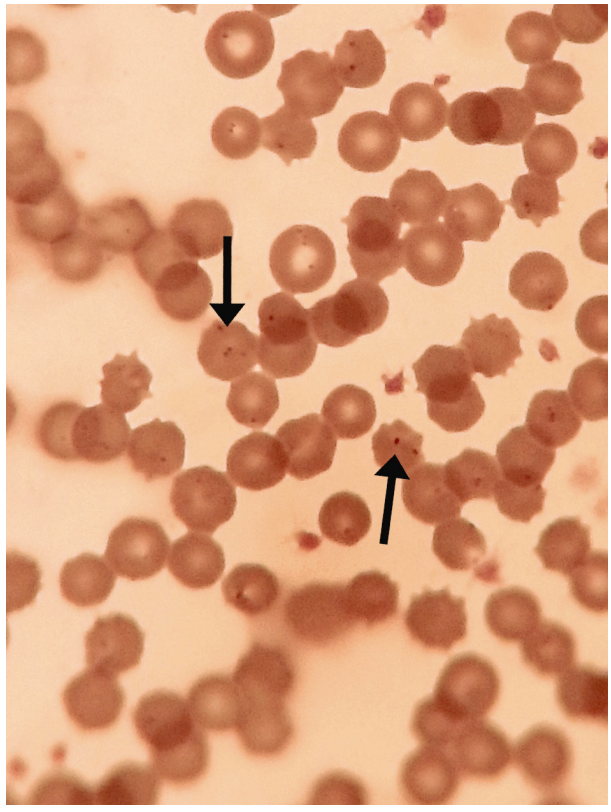

Figure 1. *Mycoplasma*-like bodies in blood of guigna (*Leopardus guigna*).

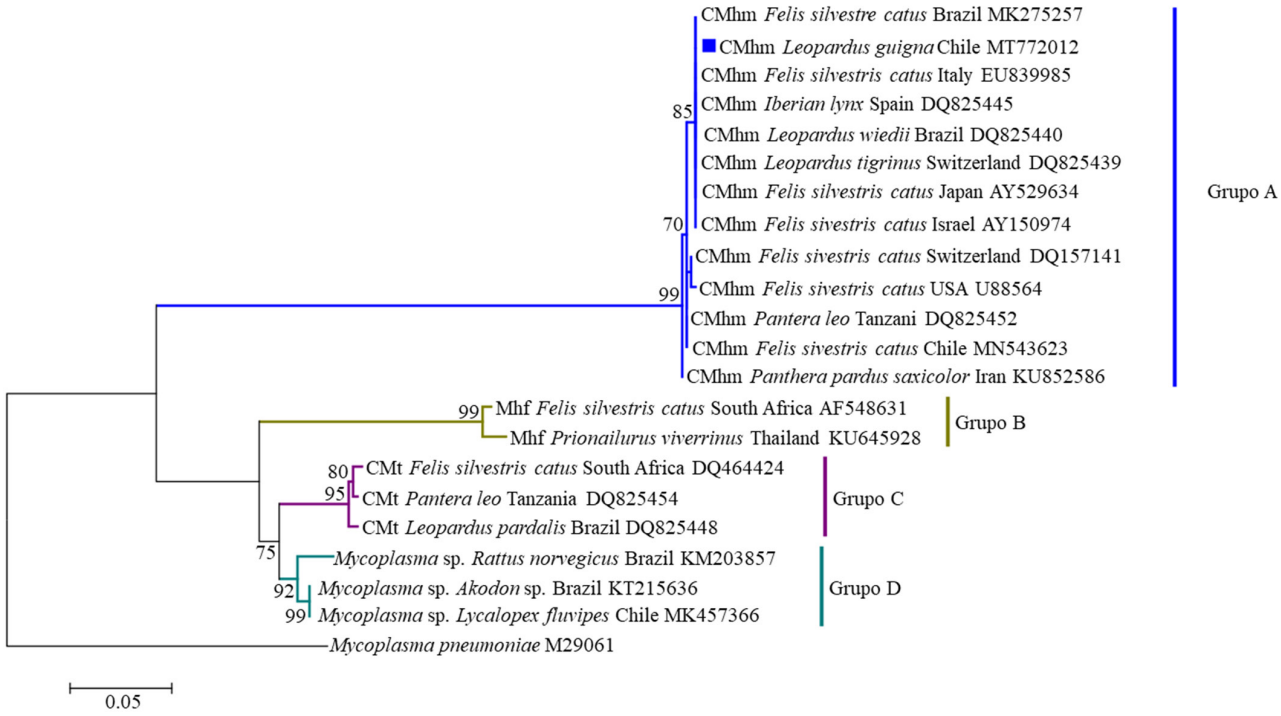

Figure 2. Maximum likelihood tree of 505 bp of the 16S rRNA *Mycoplasma* gene for guigna. *M. pneumonia* sequence was used as outgroup. The data set was resampled 1000 times to generate bootstrap percentage values and bootstrap values of ≥70 are printed at the nodes of the tree. (■) Blue square mark guigna ntST from the present study (Genbank access number MT772012). The Bayesian phylogenetic tree was congruent. The four phylogenetic (taxonomic) groups are labelled (A-D).

later of unknown causes. Mycoplasma infection was not cause of death according to necropsy. Some studies have indicated that domestic cats infected with retroviruses are more susceptible to acquire haemoplasmas and likely to develop more severe clinical signs (Luria *et al* 2004), which is concerning considering that retroviral infection is already documented in guignas in Chile (Mora *et al* 2015). Nonetheless, there is no information about guignas co-infected with haemoplasmas and retroviruses. Further investigations are needed to evaluate the health status of guigna populations along its distribution and determine whether infection with *Mycoplasma* spp. could pose a threat to guigna conservation.

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