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Wild deer (*Pudu puda*) from Chile harbor a novel ecotype of *Anaplasma phagocytophilum*

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Abstract

Background Deer species play an important role in the enzootic cycles of several *Anaplasma* species. While in the Northern Hemisphere ticks of genus *Ixodes* are well recognized vectors of these intracellular bacteria, less is known regarding the biological cycles of *Anaplasma* spp. in South America.

Methods Using PCR protocols and Sanger sequencing, we assessed the presence of *Anaplasma* spp. in blood and ticks collected on a native deer species (*Pudu puda*) from southern Chile.

Results Based on phylogenetic analyses of the 16S rRNA, *gltA* and *groEL* genes and calculation of average sequence divergence for *groEL*, our results bring to light a novel genovariant of *Anaplasma phagocytophilum* (named strain "Patagonia"). The strain represents a novel ecotype within the *A. phagocytophilum* species complex and was detected in both *P. puda* and their ticks. Using a larger matrix, denser taxon sampling and outgroup, our maximum-likelihood- and Bayesian-inferred phylogenies for *groEL* provide an accurate picture of the topology of *A. phagocytophilum* ecotypes and their evolutionary relationships.

Conclusions This is the first report of an ecotype of *A. phagocytophilum* in South America. Our results provide novel insight into the genetic diversity and ecology of this complex of bacterial lineages. Further studies should elucidate the enzootic cycle of *A. phagocytophilum* strain "Patagonia" and assess its pathogenic potential for pudues, domestic animals and humans in the region.

Keywords Southern Pudu, *Ixodes stilesi*, Wildlife, Molecular detection, Phylogenetics, Tick-borne diseases

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Background

Alphaproteobacteria in the genus *Anaplasma* are intracellular cocobacilli of mammal blood cells transmitted by ticks of genera *Amblyomma*, *Dermacentor*, *Hyalomma*, *Ixodes* and *Rhipicephalus* [1]. *Anaplasma* spp. are infectious agents that cause diseases ranging from harmless to fatal [2, 3]. Among five species and numerous genovariants that have been identified [1], *Anaplasma phagocytophilum* is of animal and public health relevance because of tick-borne fever in ruminants and granulocytic anaplasmosis in equines, canids, felids and humans in the Northern Hemisphere [4, 5].

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The genetic diversity of *Anaplasma* spp. has been explored using the conserved 16S rRNA (*rrs*) gene [1]; however, due to its weak intraspecific discriminatory resolution [6], variable loci such as citrate synthase (*gltA*) and the heat-shock operon (*groEL*) have been selected as suitable markers for single-locus genetic analyses [1, 7, 8]. Based on these markers four ecotypes split into seven phylogenetic clusters have been proposed to compose the *A. phagocytophilum* complex in Europe, Asia and North America [1, 7, 8]. A bacterial ecotype is a monophyletic array of strains sharing a similar ecological niche [9, 10], for which the average sequence divergence among groups is significantly higher than the divergence within them for a given gene [9]. *Anaplasma phagocytophilum* ecotypes and clusters have been defined according to their genetics, geographic distribution, enzootic cycles, host preference and pathogenicity [7, 11]. For example, ticks of genus *Ixodes* and cervids constitute the ecological niche for *A. phagocytophilum* ecotypes I and II [1].

Cervids are reservoirs for *Anaplasma* spp. and are often parasitized by ticks of the genus *Ixodes* that transmit these bacteria [12]. For instance, in the Northern Hemisphere, *Ixodes scapularis* and *Ixodes pacificus* (USA), *Ixodes ricinus* (Europe), and *Ixodes persulcatus* (Eurasia) [13] are the known vectors of *A. phagocytophilum*. However, data on the epidemiology of *Anaplasma* spp. is vague in South American cervids [14–20], and restricted to few species from Brazil [14–17], Argentina [19] and Uruguay [18]. In Chile, temperate rainforests (roughly between 35° and 46° S) are the habitat for the pudu (*Pudu puda*), a deer species classified as near threatened [21], which is an important host of adults of the ticks *Ixodes stilesi* and *Ixodes taglei* [22]. Although the eco-epidemiological settings (i.e. *Ixodes* ticks and deer) for an ecotype of *A. phagocytophilum* to occur do exist in Chile, it is currently unknown whether the bacterium occupies this ecological niche in the country. In the present study, we analyzed blood and ticks collected directly from free-ranging pudues from southern Chile. Because only a few *Anaplasma* surveys performed in South American wild cervids have provided short sequences for the 16S rRNA locus (*rrs*) [14–19, 23], we performed genetic screenings with additional molecular markers to detect *Anaplasma* DNA to clarify inter- or intraspecific relationships.

Methods

Sample collection

During a 5-year period (2017–2022), the blood (2–4 ml) of pudues admitted to any one of two wildlife rescue centers, Centro de Conservación Chiloé Silvestre (Nal Bajo, in Chiloé Island; − 41.839786, − 73.936015° W) and Cerefas Universidad San Sebastián (Puerto Montt; − 41.469628, − 72.907159), was collected from the cephalic or saphenous vein using an evacuated tube system (Vacutainer; Beckon, Dickson, and Company, Franklin Lakes, NJ, USA) on the day of admission (Fig. 1).

In addition to blood sampling, ticks were also removed with steel tweezers from various pudues. Blood samples and ectoparasites were kept in sterile tubes containing absolute ethanol and stored at − 80 °C until processing. The morphology of ticks was examined with a Nexius-Zoom (EVO) Stereo Microscope (Euromex Microscopen B.V., Arnhem, The Netherlands) and identified according to Nava et al. [22]. The identity of *Anaplasma*-positive ticks was further validated by sequencing a fragment of

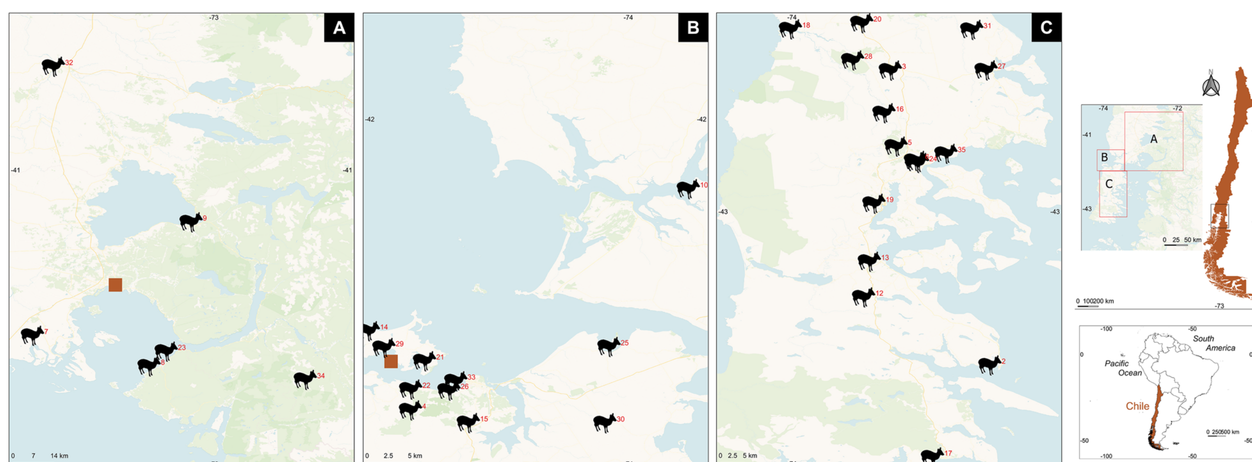

Fig. 1 Map of Chile showing the origin of rescued pudues (black icons) within the Región de Los Lagos, Chile. Brown squares indicate the rehabilitation centers. Maps were constructed with QGIS 3.18.1-Zürich (https://www.gnu.org/licenses). QGIS, Quantum Geographic Information System

the tick mitochondrial (mt) 16S ribosomal RNA (rRNA) gene [22].

DNA isolation

Genomic DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and eluted in 40 µl of buffer AE (10 mM Tris–Cl; 0.5 mM ethylenediaminetetraacetic acid [EDTA], pH 9.0). DNA was quantified with an Epoch™ Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) and assessed for quality at A260/A280 according to Khare et al. [24].

Gene amplification and sequencing

The suitability of the extracted DNA was checked by a conventional PCR (cPCR) assay targeting the mammalian glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and the tick mt 16S rRNA genes, respectively. The primers and thermal conditions used in this study together with their references are shown in Table 1. *Anaplasma* detection was achieved by implementing different nested and hemi-nested PCR protocols targeting the *rrs*, *gltA* and *groEL* genes. DNA of *Anaplasma platys* (OQ155255) was used as the positive control and nuclease-free water was used as the negative control. All PCR reactions were performed in a thermal cycler (ProFlexTM Base 32 × 3; Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) in a final reaction volume of 25 µl (12.5 µl DreamTaq Green PCR Master Mix [Thermo Fisher Scientific], 1 µl of each primer (0.4 µM), 8.5 µl of ultra-pure water and 2 µl template DNA. The PCR products were stained with GelRed® (Biotum, Tehran, Iran), separated by electrophoresis in 2% agarose gels and then visualized using an ENDURO™ GDS UV transilluminator (Labnet International, Edison, NJ, USA). Amplicons with bands of the expected size were purified and Sanger-sequenced at Macrogen (Seoul, South Korea).

Assembly and sequence analyses

Amplicon sequences were quality-checked and edited with Geneious Prime® version (v) 2021.2.2 (www.geneious.com) to generate consensus sequences. Base calls with Phred values ≥ 20 were considered suitable for the analyses [35, 36]. The BLAST® tool (https://blast.ncbi.nlm.nih.gov) was employed to compare obtained nucleotide sequences and identify orthologous sequences.

Phylogenetic analyses

Orthologous sequences downloaded from GenBank (https://www.ncbi.nlm.nih.gov) and consensus sequences were used to build alignments with the MAFFT multiple sequence alignment program using default parameters [37]. The alignments were subsequently trimmed and filtered with Block Mapping and Gathering with Entropy (BMGE) using default parameters to map informative regions for phylogenetics inferences [38].

Table 1 Primers and thermal conditions used for PCR detection and genetic characterization of *Anaplasma* and ticks

Organisms	Gene	PCR	Primer	Sequence	T_o (°C)	Expected length (in bp)	Reference
Mammals	*GAPDH*	Conventional	gapdh F	CCTTCATTGACCTCAACTACAT	52	400	[25]
			gapdh R	CCAAAGTTGTCATGGATGACC			
Ticks	Mitochondrial 16S rRNA (*rrs*)	Conventional	16S + 1	CCGGTCTCAACTCAGATCAAGT		460	[26]
			16S − 1	GCTCAATGATTTTTTAAATTGCTGT			
Anaplasma	16S rRNA (*rrs*)	Conventional	EC9	TACCTTGTTACGACTT	48	1300	[27]
			EC12A	TGATCCTGGCTCAGAACGAACG			[28]
		Nested	A17a	GCGGCAAGCCTCCCACAT	54	1200	[29]
			IS58-1345r	CACCAGCTTCGAGTTAAACC			
	groEl	Conventional	HS1a	AYTGGGCTGGTAYTGAAAT	47	1614	[30]
			GroEl_2R	CGTTCTTACTAGGAACATCAAC			[31]
		Nested	Gro677F	ATTACTCAGAGTGCTTCTCARTG	53	942	[32]
			GroEL_rev2	GCCGACTTTTAGTACAGCAA			[33]
		Heminested	GroEl_2F	TGTAAAGGCGCCTGGTTTCG	55	772	[31]
			GroEl_2R	CGTTCTTACTAGGAACATCAAC			[31]
	gltA	Conventional	F4b	CCAGGCTTTATGTCAACTGC	55	800	[34]
			R1b	CGATGACCAAAACCCAT			
		Nested	EHR-CS136F	TTYATGTCYACTGCTGCKTG	55	650	
			EHR-CS778R	GCNCCMCCATGMGCTGG			

GAPDH Glyceraldehyde-3-phosphate dehydrogenase, *gltA* citrate synthase gene, *groEL* heat-shock operon

Phylogenetic trees were constructed with the Bayesian inference (BI [39, 40]) and maximum-likelihood (ML [41]) methods in MrBayes v 3.2.6 [42] and IQ-TREE v 1.6.12 [43], respectively. As protein-coding genes present different nucleotide exchange rates (heterogeneity) at the first, second and third codon positions [42, 44], datasets were partitioned into the three codon positions (position-1, position-2 and position-3) [42, 44–46]. Then, the Model Finder command "TESTNEWONLYMERGE -mrate G" was implemented to select the best-fit evolutionary models and best-partition scheme for protein-coding gene datasets [47]. The ML best evolutionary models for non-coding genes were calculated using the ModelFinder command "-m TESTNEWONLY -mrate G" [47]. We used rapid hill-climbing and stochastic disturbance methods with 1000 ultrafast bootstrapping pseudo-replicates to evaluate the inferred tree robustness. Bootstrap values < 70%, 70–94% and ≥ 95% were considered non-significant, medium and solid statistical support [48], respectively.

BI phylogenies were constructed based on nucleotide substitution models selected with the MrBayes command "lset nst = mixed rates = gamma" for the non-coding dataset [42, 49]. On the other hand, the best partition schemes computed by ModelFinder and the MrBayes command "lset = mixed rates = invgamma" were used to calculate the best models for protein-encoding datasets [42, 46, 49]. Two independent tests of 20×10^6 generations and four Markov chain Monte Carlo (MCMC) chains were implemented, sampling trees every 1000 generations and removing the first 25% as burn-in. Tracer v1.7.1 [50] was used to confirm the correlation and effective sample size of the MCMC. Bayesian posterior probabilities (BPP) with values > 0.70 in nodes were considered to indicate strong statistical support [51]. All best-fit models and partitions schemes were selected under the Bayesian Information Criterion (BIC) [52]. Trees were visualized and edited with FigTree v 1.4.1 (http://tree.bio.ed.ac.uk/software/figtree/) and Inkscape v 1.1 (https://inkscape.org/es/). Congruent topologies between ML and BI analyses were used to produce strict consensus trees in Geneious Prime with the Consensus Tree Builder tool, implementing a support threshold of 100%. The consensus phylogram included all monophyletic clades after comparing ML and BI topologies for each dataset.

Genetic distance analyses

To assess the corrected pairwise distance and determine the average sequence divergence within and among ecotypes, an alignment of 936 bp was constructed with default parameters in MAFFT, including 214 *groEL* sequences of *A. phagocytophilum* with > 70% coverage between them, using *Anaplasma odocoilei* and *A. platys* as outgroups. The corrected pairwise distance was assessed using raxmlGUI [53, 54] for RAxML v 8 [55] with the GTR + GAMMA + I substitution model.

Results

Tick identification and blood samples

A total of 26 hard ticks and 55 blood samples were collected from pudues. All ticks were morphologically identified as *I. stilesi* (17 females, 5 males, 4 nymphs). Amplicons of the expected size were obtained for the mt 16S rRNA gene by PCR in 20 of the 26 tick specimens, with negative results obtained for six ticks (4 females, 1 male, 1 nymph), which were subsequently excluded from the analysis. PCR targeting the *GAPDH* gene in pudu blood resulted in amplicons of the expected size, confirming successful DNA extractions in all cases (Table 2).

Anaplasma detection

Anaplasma DNA was amplified in 8/26 (30.8%) *I. stilesi* (1 nymph, 1 male, 6 females) and in 6/55 (10.9%) pudues (Table 2). Eleven identical sequences were obtained for *rrs* (1,212 bp), 12 for *gltA* (722 bp) and 13 for *groEL* (1,286 bp). Pairwise comparisons between generated sequences indicated one genotype for *rrs*, seven genotypes for *gltA* and 11 genotypes for *groEL*. A mitochondrial genotype of 429 bp retrieved for *Anaplasma*-positive ticks (OP750053) was 99.5% (428/430 bp, 100% query cover, 2 gaps, 0 E-value) identical with a previous sequence of *I. stilesi* from Chile (DQ061292) [56].

After BLASTn comparisons, the *rrs* genotype matched with 94.8% identity *A. phagocytophilum* isolate D2_2 (MK814406), detected in *Canis lupus familiaris* from South Africa [57]; the *gltA* genotypes showed an identity ranging from 82.9% to 83.1% with *A. phagocytophilum* strain Sheep (KP861639) detected in an *Ixodes* sp. collected on a Norwegian White Sheep [58]; and the *groEL* genotypes were 91.4–91.8% identical with *A. phagocytophilum* samc001 (LC496077) detected in *Canis lupus familiaris* from Japan [59].

Phylogenies inferred for the three loci positioned *Anaplasma* genotypes retrieved from *I. stilesi* and pudu blood into the *A. phagocytophilum* clade, forming a monophyletic group (Figs. 2, 3, 4). In particular, the *groEL* phylogeny placed our genotypes in an independent clade related to ecotype III of *A. phagocytophilum* [1] (Fig. 4).

For the *groEL* gene, the average sequence divergence calculated within ecotypes was always less than the average sequence divergence calculated among them, including the ecotype characterized in this study (Table 3). Collectively, the genetic evidence provided by

Table 2 Sampled and *Anaplasma*-positive animals with the geographical coordinates of provenance

Species	Provenance	Locality[a]	Geographical coordinates (latitude, longitude)[b]	*Anaplasma phagocytophilum*[c]
Pudu puda	Continent	Cerefas Universidad San Sebastián, Puerto Montt (1)	− 41.469628, − 72.907159	1/3
	Island	Queilén (2)	− 42.885721, − 73.468359	0/3
	Island	Degañ (3)	− 42.145274, − 73.720717	0/1
	Island	Pauldeo (4)	− 41.908360, − 73.891784	0/2
	Island	Mocopulli (5)	− 42.336344, − 73.706289	0/2
	Island	Tehuaco (6)	− 42.372438, − 73.657162	0/1
	Continent	Calbuco (7)	− 41.677865, − 73.201237	0/1
	Continent	Contao (8)	− 41.803322, − 72.719169	0/2
	Continent	Ensenada (9)	− 41.213838, − 72.545666	0/1
	Continent	Peñol Bajo (10)	− 41.598174, − 73.498427	0/1
	Island	Centro de Conservación Chiloé Silvestre (11)	− 41.839786, − 73.936015	0/1
	Island	Lago Tarahuín (12)	− 42.714684, − 73.788520	0/1
	Island	Chonchi (13)	− 42.625050, − 73.774028	0/3
	Island	Chauman (14)	− 41.797195, − 73.951494	0/2
	Island	Mechaico (15)	− 41.926147, − 73.809907	1/1
	Island	Butalcura (16)	− 42.252443, − 73.736915	1/1
	Island	Quellón (17)	− 43.116902, − 73.613887	0/4
	Island	Chepu (18)	− 42.041574, − 73.973976	1/1
	Island	Castro (19)	− 42.480140, − 73.762413	0/3
	Island	Quichitúe (20)	− 42.026219, − 73.793279	0/1
	Island	Guapilacuy (21)	− 41.839390, − 73.871975	0/1
	Island	Lechagua (22)	− 41.879088, − 73.891482	0/1
	Continent	Caleta Puelche (23)	− 41.742766, − 72.648612	0/1
	Island	Dalcahue (24)	− 42.377552, − 73.651920	1/3
	Island	Caulin (25)	− 41.819313, − 73.610747	0/1
	Island	Hueihue (26)	− 41.880609, − 73.837354	0/1
	Island	Quemchi (27)	− 42.144713, − 73.478056	0/2
	Island	Puntra (28)	− 42.119891, − 73.816342	1/1
	Island	Sector Naval—Faro Corona (29)	− 41.820747, − 73.929428	0/2
	Island	El Quilar (30)	− 41.926431, − 73.616454	0/1
	Island	Lliuco (31)	− 42.042983, − 73.514278	0/2
	Continent	Osorno (32)	− 40.576192, − 73.114948	0/1
	Island	Ancud (33)	− 41.867489, − 73.827690	0/1
	Continent	Los Lagos (34)	− 41.858622, − 72.073451	0/1
	Island	Quiquel (35)	− 42.354553, − 73.579767	0/1
	Total no. positive specimens			*6/55*
Ixodes stilesi	Island	Centro de Conservación Chiloé Silvestre (11)	− 41.839786, − 73.936015	*8/20*

[a] Numbers in parentheses correlate to the sites shown in Fig. 1

[b] Presented in pure numeric format

[c] Number of positive specimens/number of specimens tested

our study points to the finding of a fifth *A. phagocytophilum* ecotype, for which the name *A. phagocytophilum* strain "Patagonia" is proposed. GenBank accession numbers generated in this study are available in Additional file 1: Tables S1, S2).

Discussion

Tick-borne bacteria, including *A. phagocytophilum*, are geographically expanding, probably due to climate change and anthropogenic landscape perturbation, both factors that favor the spread of their vectors synergically [13, 60]. Although *A. phagocytophilum* was previously

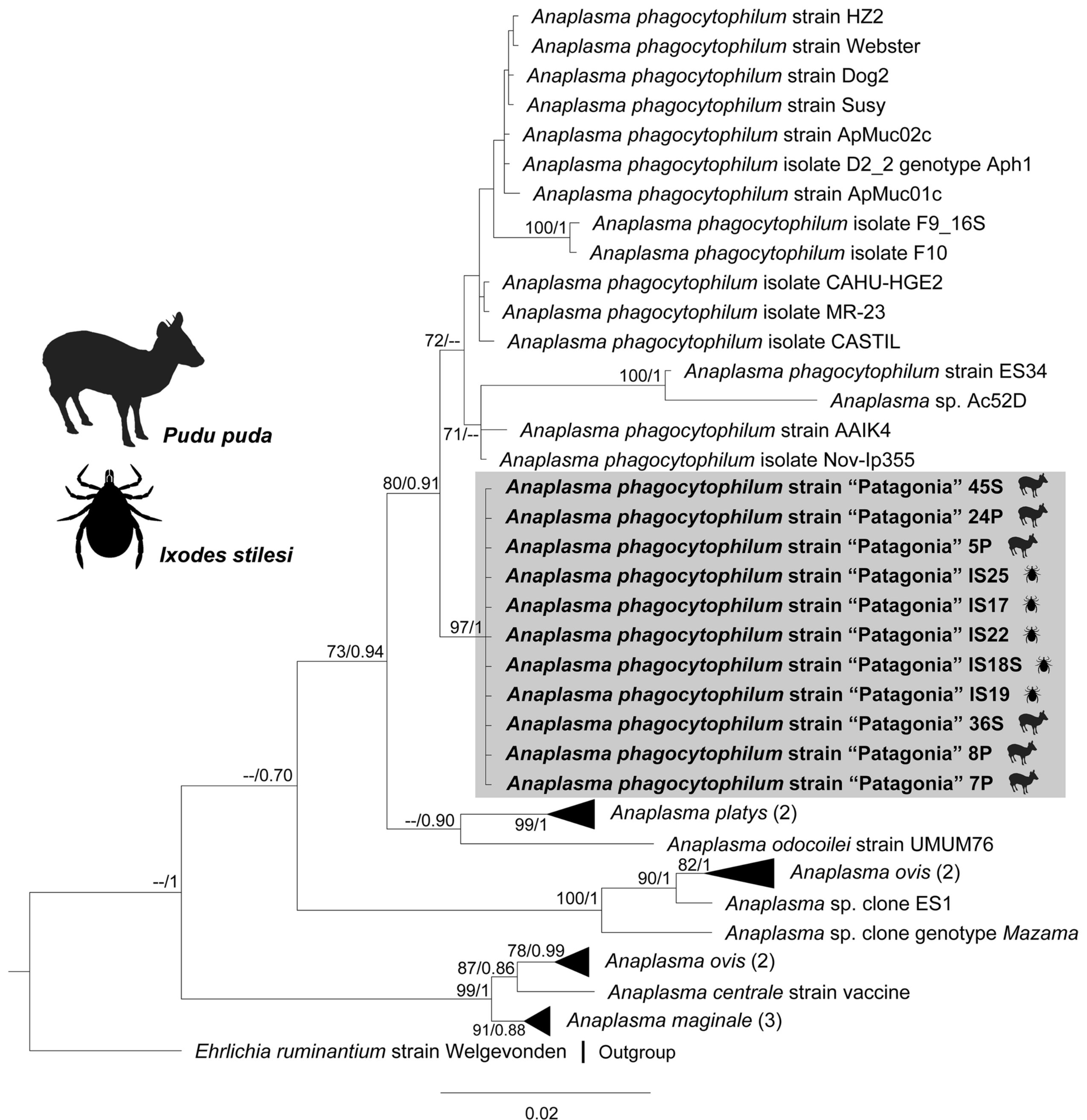

Fig. 2 Maximum likelihood (ML) and Bayesian inference (BI) *rrs* gene consensus tree inferred for a subset of *Anaplasma* spp., using 41 sequences and an alignment of 1,382 bp. Best-fit evolutionary models calculated for the ML and BI methods were TPM3u + F + G4; and M_{90}, M_{177}, M_{85}, M_{152}, M_{179}, M_{117}, M_{195}, respectively. Bootstrap values and Bayesian posterior probabilities (BPP) are indicated above or below each branch. The position of the strain of *Anaplasma phagocytophilum* characterized in the present study is highlighted in a gray box

thought to be a single bacterial species [61], recent phylogenetic reconstructions have revealed a complex of lineages with different pathogeny, geographical distribution, reservoirs and vectors [1]; nevertheless, host range, zoonotic potential and transmission dynamics of this bacterium are still incompletely solved [1, 7, 8, 11].

Based on average divergence of partial *groEL* sequences (Table 3) and strongly supported phylogenies for *rrs*, *gltA*, and *groEL,* in this study we identified a novel genovariant of *A. phagocytophilum* associated with pudues, for which the name "Patagonia" is proposed (Figs. 2, 3, 4). Accordingly, this genovariant has been

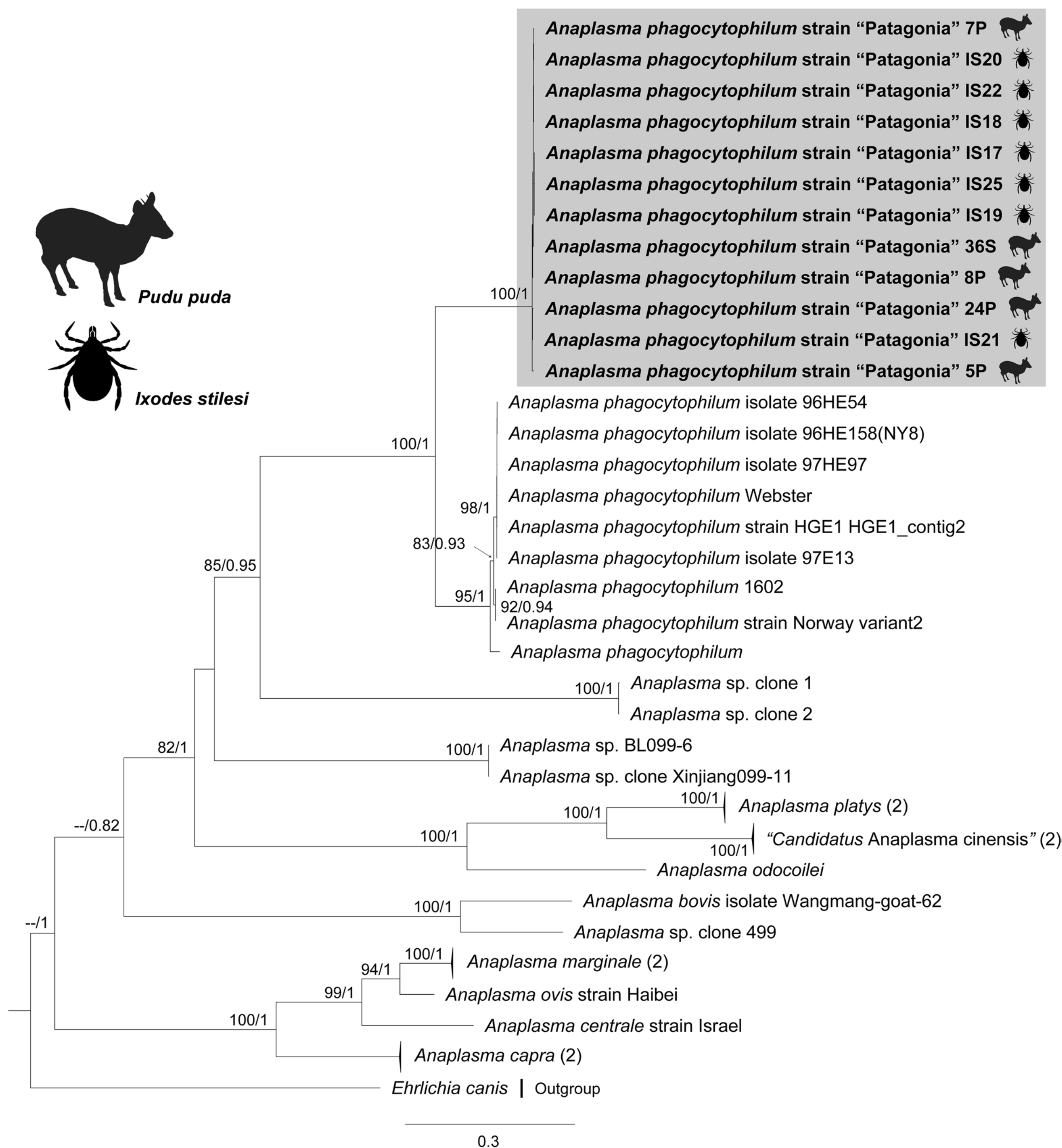

Fig. 3 ML and BI consensus tree inferred for a subset of *Anaplasma* spp., using 40 sequences of the *gltA* gene and an alignment of 1152 bp. Best-fit evolutionary models calculated for the ML and BI methods were GTR + F + I + G4 (position-1), GTR + F + G4 (position-2), HKY + F + I + G4 (position-3); and M_{64}, M_{175}, M_{173}, M_{25}, M_{171}, M_{50}, M_{125} (position-1); M_{80}, M_{135}, M_{164}, M_{166}, M_{145} (position-2); M_{90}, M_{177}, M_{152}, M_{183}, M_{136} (position-3), respectively. Bootstrap values and BPP are indicated above or below each branch. The position of the strain of *A. phagocytophilum* characterized in the present study is highlighted in a gray box

designated as the ecotype V (cluster 8) of *A. phagocytophilum*, which constitutes the first ecotype of this species complex described for South America. Variants of *A. phagocytophilum* are adapted to different hosts and vector species, therefore configuring different enzootic cycles [1, 13]. The fact that *A. phagocytophilum* strain "Patagonia" conforms an additional ecotype suggests that

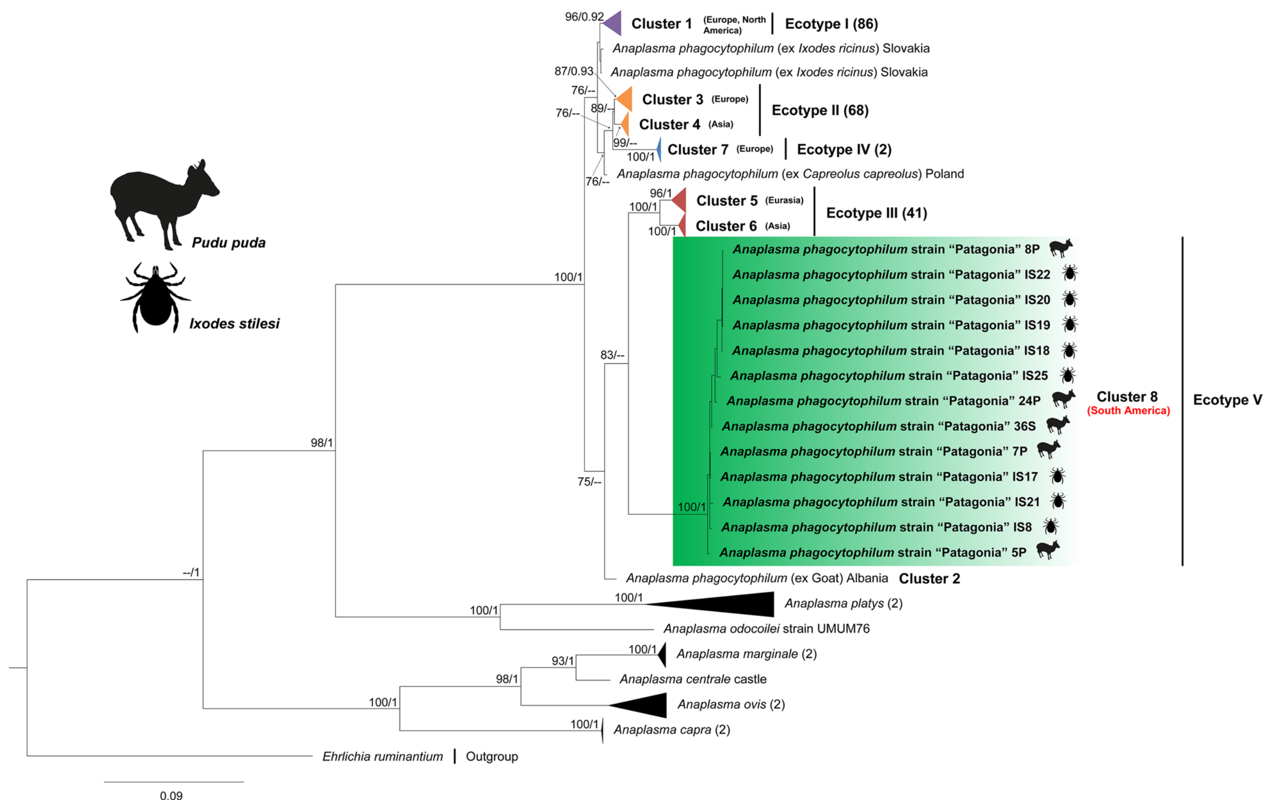

Fig. 4 ML and BI consensus tree inferred for a subset of *Anaplasma* spp., using 226 sequences of the *groEL* gene, and an alignment length of 1224 bp. Best-fit evolutionary models calculated for the ML and BI methods were TIM + F + G4 (position-1); TN + F + G4 (position-2); and K3Pu + F + G4 (position-3); and M_{45}, M_{136}, M_{142}, M_{130}, M_{139}, M_{185} (position-1); M_{81}, M_{40} (position-2); M_{15}, M_{50}, M_{85}, M_{122}, M_{90} (position-3), respectively. Bootstrap values and BPP are indicated above or below each branch. Colors for ecotypes I, II, III and IV were assigned according to Jaarsma et al. [8]. The position of the strain of *A. phagocytophilum* characterized in the present study is highlighted in a green box

Table 3 Average sequence divergence within ecotypes and among ecotypes calculated on the basis of corrected pairwise distances for a subset of *A. phagocytophilum groEl* gene sequences (936 bp)

Ecotype	Ecotype				
	I	II	III	IV	V
I	*0.006007*				
II	0.020384	*0.006410*			
III	0.068461	0.060800	*0.010504*		
IV	0.040475	0.034799	0.065253	*0.000001*	
V	0.090030	0.080672	0.080836	0.079975	*0.003386*

Values highlighted in italics are average sequence divergence within ecotypes

the eco-epidemiology of this novel strain differs from those of the northern latitudes.

Cervids such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), white-tailed deer (*Odocoileus virginianus*), fallow deer (*Dama dama*), sika deer (*Cervus nippon*) and their associated ticks (*I. ricinus* and *I. scapularis*) are implicated in the maintenance of endemic cycles of some *A. phagocytophilum* variants (e.g. Ap-V1, B, J, S, W) in northern latitudes [13, 62–65]. In contrast, previous knowledge on *A. phagocytophilum* in South American deer species is vague, limited only to Brazil, and does not support its classification within any ecotype. For example, in their study on the brown brocket deer (*Mazama gouazoubira*), Silveira et al. [15] could not discriminate whether *A. phagocytophilum* or *A. platys* caused the infection using PCR and sequencing protocols. However, a posterior survey revealed that *A. phagocytophilum* would be circulating in brown brocket deer [23]. On the other hand, exposure to *A. phagocytophilum* in Brazilian marsh deer (*Blastocerus dichotomus*) has been reported using indirect immunofluorescence assays [14]. As far as we know, our study is the first multigenic detection of *A. phagocytophilum* DNA in pudu and *I. stilesi*.

Records of *A. phagocytophilum* in South American mammals include rodents (*Cavia* sp. and *Calomys cerqueirai*), peccary (*Tayassu pecari* and *Pecari tajacu*), sloths (*Bradypus tridactylus*) and coati (*Nasua nasua*) [17, 66, 67]. However, due to the use of short fragments

of the *rrs* and *groEL* genes for identification, it is difficult to state whether the *Anaplasma* DNA detected in these mammals corresponded to *A. phagocytophilum* or not. While reports of *A. phagocytophilum* on South American cervids are few, other *Anaplasma* spp. have been recorded in deer in Brazil, such as *Anaplasma bovis* and *Anaplasma* sp. in red brocket deer (*Mazama americana*); *A. bovis*, *Anaplasma marginale* and *A. platys* in marsh deer; and *A. marginale* in brown brocket deer [14–17]. Likewise, the records in South America include *A. platys*, *Anaplasma odocoilei*, *A. marginale* and "*Candidatus* Anaplasma boleense" in marsh deer in Argentina [19], and *Anaplasma* sp. Mazama genotype in brown brocket deer in Uruguay [18].

In Chile, evidence of *A. phagocytophilum* is incipient. Indeed, infection by this bacterium has been reported in horses [68]. However, these results deserve further investigation, since the use of *A. phagocytophilum*-specific primers did not yield positive reactions, and the occurrence of a vector in the area where positive animals were detected is unknown. Further reports of *Anaplasma* spp. in Chile include *A. platys* in dogs, Andean foxes (*Lycalopex culpaeus*), the South American gray fox (*Lycalopex griseus*) [69] and hard ticks (*Rhipicephalus sanguineus* sensu lato). An *Anaplasma*-like agent has also been detected in seabird soft ticks (*Ornithodoros spheniscus*) [70]. Moreover, serological evidence of exposure to *Anasplasma* sp. has been recorded in dogs [71] and humans [71–74]. Our results thus expand current knowledge on vertebrate hosts of *A. phagocytophilum* in the continent.

There is no standardized approach for investigating the genetic diversity and population structure of *Anaplasma* species. Although the *rrs*, *gltA* and *groEL* markers used in this study are currently the most appropriate loci for the genetic characterization of *Anaplasma* spp. [1], *rrs* and *groEL* are conserved and do not have sufficient resolution to segregate some groups when short fragments are analyzed, even in different species of the genus. Therefore, the sequenced fragments must be long enough [1, 6]. Based on the above argument, our phylogenetic analyses did not include sequences shorter than 600 bp.

Previous studies found that the *groEL* gene may delimit lineages (ecotypes, clusters, groups) of *A. phagocytophilum* [1, 7, 8, 11]. Moreover, the discrimination capacity among lineages has improved due to the progressive increase in taxon sampling and the size of the sequences employed in the analyses [1]. Recently, a population study recovered ecotypes I, II, III and IV (mentioned by Jahfari et al. [7] and Jaarsma et al. [8]) as monophyletic but without statistical support for ecotypes I and II [1]. It is worth noting that ecotype IV was designated after including only one sequence in those analyses, and its monophyly was not assessed [1]. In addition, cluster 3 (paraphyletic within ecotype II) lacked statistical support (Electronic Supplementary Material Figure S4. in Rar et al. [1]). Thus, methodological factors, such as the inclusion of an outgroup [10, 75], longer alignments, denser taxon sampling [1, 11] and the application of phylogenetic inferences (BI, ML) [39–41], may circumscribe with higher confidence the monophyly and evolutionary relationships of ecotypes and subclades within *A. phagocytophilum*, as shown in our study.

Applying the above referred methods, ecotypes I, II and IV were depicted as monophyletic lineages with high statistical support (Fig. 4). In particular, ecotype II was only recovered with high support in ML analyses (92% of bootstrap), yet the cluster 3 (Europe) belonging to this ecotype represents a monophyletic group with confident support (0.94/89) (Fig. 4). Our results differ from those of other studies that described these monophyletic groups based on an eco-epidemiological approach without considering systematics [1, 7, 8, 11]. Undoubtedly, ecotype II and cluster 3 represent natural assemblages, but our study shows them now as also phylogenetically supported. Herein described ecotype V was moderately supported in the *groEL*-based ML inference (81% of ultrafast-bootstrap) and closely related to ecotype III (Fig. 4), which is integrated by variants of *A. phagocytophilum* related to small mammals and ticks (Additional file 1: Table S2) [1]. However, the phylogenetic position of the ecotypes should be re-evaluated as new members of the *A. phagocytophilum* complex are discovered.

The presence of *A. phagocytophilum* DNA does not conclusively confirm the role of pudues and *I. stilesi* in the epidemiology of this bacterium or any clinical impact on pudu health. However, the fact that *P. puda* is the sole deer that currently inhabits the areas from which positive animals for this bacterium were recorded [76] strengthens the notion that this cervid could be reservoir of *A. phagocytophilum* strain "Patagonia." In addition, considering the role of *Ixodes* spp. as vectors of *Anaplasma* spp. in the Northern Hemisphere cervids [1], *I. stilesi* and *I. taglei*, two species that commonly parasitize pudues [22], represent potential vectors of *A. phagocytophilum* strain "Patagonia." However, our hypotheses should be tested in experimental studies. Meanwhile, the epidemiological cycle of *A. phagocytophilum* strain "Patagonia" remains unknown.

Conclusions

We report the presence of and ecotype of *A. phagocytophilum* for the first time in South America. The genetic evidence showed conclusively that the *A. phagocytophilum* found in this study is a unique variant, and the name *A. phagocytophilum* strain "Patagonia" is tentatively proposed. The study of the enzootic

cycle of *A. phagocytophilum* strain "Patagonia" is now essential to establish its zoonotic potential and health impact on pudues and further species, such as domestic ruminants. Furthermore, because some variants of *A. phagocytophilum* are infectious agents of public and veterinary health concern, the detection of this bacterium in Chile deserves further attention. Future research should define a standardized approach for genetically characterizing members of *Anaplasma* genus that would afford reliable comparisons, as recommended in Rar et al. [1]. Finally, these findings bring insight into the genetic diversity and ecology of *A. phagocytophilum*.

Abbreviations

BI	Bayesian inference
BLAST	Basic local alignment search tool
gltA	Citrate synthase gene
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase gene
groEL	Heat-shock operon
MAFFT	Multiple alignment using fast Fourier transform
ML	Maximum likelihood
MCMC	Markov chain Monte Carlo
rRNA	Ribosomal ribonucleic acid
rrs	16S rRNA gene

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-023-05657-9.

Additional file 1: Table S1. GenBank accession numbers of the sequences used for *Anaplasma phagocytophilum rrs* and *gltA* phylogenies. Sequences generated in this study are highlighted in bold. **Table S2.** GenBank accession numbers of the sequences used for *Anaplasma phagocytophilum groEL* phylogeny. Sequences generated in this study are highlighted in bold.

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Author contributions
AS, RT, SM-L: material preparation, data collection, analysis, writing of the first draft. AS, RT, SR, JEU, CP-M, JC-S, FV-O, CV-S, DM-A, EH-H, SM-L contributed to the study conception and design and commented on initial versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
GenBank accession numbers generated in this study are available in Additional files 1: Tables S1 and S2.

Declarations

Ethics approval and consent to participate
The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received. Procedures performed in this study were verified and approved by the Bioethics Committee of the School of Veterinary Sciences, Universidad de Concepción (CBE-07-2022).

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no competing interests.

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