

# *Ixodes chilensis* Kohls, 1956 (Acari: Ixodida: Ixodidae): re-description of the female, description of the nymph, and phylogenetic position inferred from mitochondrial DNA sequences of the 16S rRNA gene

Daniel González-Acuña · María N. Saracho-Bottero · Gonzalo Ossa · Alberto A. Guglielmonne · Santiago Nava

Received: 31 May 2018 / Accepted: 6 August 2018  
© Springer Nature B.V. 2018

**Abstract** This paper provides a re-description of the female and a description of the nymph of *Ixodes chilensis* Kohls, 1956. Additionally, the phylogenetic position of the species of *Ixodes* Latreille, 1796 belonging to the subgenus *Pholeoixodes* Schulze, 1942 was analysed and discussed based on a phylogenetic pattern obtained with mitochondrial DNA sequences of the 16S rRNA gene. The diagnostic characters unique for the female of *I. chilensis* are a combination of coxae I–IV of legs with one external minute triangular spur each (barely perceptible) and lacking internal spur, tarsus I with a subapical dorsal hump, basis capituli subrectangular with sinuous posterior margin, hypostome rounded apically, cornua

and auriculae absent, scapulae pointed, palps short, porose areas rounded separated by an interporose area wider than the diameter of one area, scutum with posterior margin straight and surface in the anterolateral field rugose, with punctations larger in the posterior field. The nymph of *I. chilensis* is characterised by the presence of scutum with posterior margin nearly straight, lateral carinae absent, basis capituli subrectangular in shape with posterior margin nearly straight, small auriculae as lateral ridges, palps short, hypostome rounded apically, and coxae I–IV of legs with just one external small triangular spur each (barely perceptible) and lacking internal spurs. Analysis of 16S sequences showed that *I. chilensis* form a well-supported clade with the following species with a wide geographical distribution but mostly established in the Palaearctic region and none from the Neotropics: *I. simplex* Neumann, 1906; *I. arboricola* Schulze & Schlottke, 1929; *I. lividus* Koch, 1844; *I. canisuga* Johnston, 1849; *I. vespertilionis* Koch, 1844; *I. ariadnae* Hornok, 2014; *I. collaris* Hornok, 2016; and *I. kangdingensis* Gou, Sun, Xu & Durden, 2017. The phylogenetic analysis also has demonstrated that the subgenus *Pholeoixodes* Schulze, 1942 is not monophyletic. The species considered as belonging to this subgenus were grouped in two different clades which did not have a well-supported common node defining monophyly.

---

This article is part of the Topical Collection Arthropoda.

---

D. González-Acuña  
Departamento de Ciencias Pecuarias, Facultad de Ciencias Veterinarias, Universidad de Concepción, Av. Vicente Méndez 595, CP 3780000 Chillán, Chile

M. N. Saracho-Bottero · A. A. Guglielmonne · S. Nava (✉)  
Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, and Consejo Nacional de Investigaciones Científicas y Técnicas, CC 22, CP 2300 Rafaela, Santa Fe, Argentina  
e-mail: nava.santiago@inta.gov.ar

G. Ossa  
ConserBat EIRL, Camino a la Balsa s/n,  
CP 3860000 San Fabián, Chile

## Introduction

The genus *Ixodes* Latreille, 1796 (Acari: Ixodidae) is represented by nine species in Chile (Nava et al., 2017). Of these, *Ixodes chilensis* Kohls, 1956 is known from the holotype, an engorged female with the distal portion of the hypostome broken, collected from an unknown host at Angol, in 1929, as described by Kohls (1956). Tagle (1971) speculated that the female of *I. chilensis* was collected from a horse, but there is no evidence to support this statement.

*Ixodes chilensis* is placed within the subgenus *Pholeoixodes* Schulze, 1942, by Clifford et al. (1973) and Filippova (1977). Guglielmone et al. (2017) stated that most tick workers support the subgeneric position of *Pholeoixodes*, but Camicas & Morel (1977) elevated *Pholeoixodes* to the generic rank, and, accordingly, Camicas et al. (1998) treated this species as a member of the genus *Pholeoixodes*. Clifford et al. (1973) stated that *I. chilensis*, *Ixodes taglei* Kohls, 1969, *Ixodes nuttalli* Lahille, 1913 and *Ixodes rubidus* Neumann, 1901 are the Neotropical members of *Pholeoixodes*, but Camicas et al. (1998) excluded *I. nuttalli* and *I. taglei* from *Pholeoixodes*. Filippova (1957) erected the genus *Ixodiopsis* Filippova, 1957 but neither Clifford (1973) nor Camicas et al. (1998) treated it as valid and included all species of *Ixodiopsis* in *Pholeoixodes*. However, Robbins & Keirans (1992) soundly defended the validity of the subgenus *Ixodiopsis*. Recently, Hornok et al. (2017) presented evidence that challenges the monophyletic status of the subgenus *Pholeoixodes*. This controversial situation will be further treated in the discussion section.

One of us (GO) conducted a survey of ticks of bats in the Valdivian rainforest from Chile, where females and nymphs of the genus *Ixodes* were collected. Morphological and molecular studies were carried out for species identification, but also to assess the phylogenetic position of the *Ixodes* spp. from southern South America, compatible with *I. chilensis*.

## Materials and methods

Six bats (Chiroptera: Vespertilionidae), five *Histiotus magellanicus* (Philippi) and one *Myotis chiloensis* (Waterhouse), were captured on 12 May 2005 with mist nets in Oncol (39°39'49.89"S, 73°18'46.77"W, Los Rios region, at the Valdivian Forest Province of

the Andean region *sensu* Morrone (2006). The locality is dominated by young trees *Drimys winteri* and old trees *Nothofagus dombeyi*, in a dense matrix of Quila scrublands (*Chusquea* spp.) (Luebert & Pliscoff, 2006).

Seven ticks (one female and six nymphs) belonging to the genus *Ixodes* were collected on two *H. magellanicus*, and deposited in the Tick Collection of Facultad de Ciencias Veterinarias, Universidad de Concepción, Chile. The conspecificity of the female and nymphs was determined by comparison of mitochondrial DNA sequences of the 16S rRNA gene.

Ticks were cleaned with ultrasound (20 kHz) using distilled water and commercial detergent in a proportion of 9:1. All specimens were measured using a Leica DM 1000 optical microscope. All measurements in millimetres and are given as the range followed by the mean in parentheses. Scanning electron photomicrographs were taken at the Servicio de Microscopía Electrónica, Museo de La Plata, Universidad Nacional de La Plata, Argentina, using a JEOL/JSM 6360 LV<sup>®</sup> Digital Scanning Microscope.

DNA was extracted from the female and one nymph and processed using a polymerase chain reaction (PCR) following the methodology described by Mangold et al. (1998) and a 410 bp fragment of the mitochondrial 16S rDNA was amplified using the primers designed by Mangold et al. (1998). Sequences were aligned together with sequences for *Ixodes* spp. (including those belonging to the subgenus *Pholeoixodes*) available on GenBank, using the BioEdit sequence alignment editor (Hall, 1999) with the CLUSTAL W program (Thompson et al. 1994). Phylogenetic relationships were assessed in terms of maximum likelihood method (ML). Best-fitting substitution models were determined with the Bayesian information criterion using the ML model test implemented in MEGA 5 (Tamura et al., 2011). The number of variable nucleotide positions between sequences was used to calculate pairwise estimates of percent sequence divergence. The phylogenetic tree was generated with the GTR model and a discrete gamma distribution with invariant sites (GTR+G+I). Support for the topologies was tested by bootstrapping over 1000 replications.

## Results

The morphological congruence of the female and nymphs collected from *H. magellanicus* indicates that they belong to the same species of *Ixodes*, a fact further reinforced by identical 16S DNA sequences obtained from the female and a nymph (see below). The morphology of the female tick matches the description of *I. chilensis* in Kohls (1956, figure 9, pp. 648–649) and is compatible with the morphology of the type-specimen we have examined (deposited in the United States National Tick Collection, Institute for Coastal Plain Science, Georgia Southern University, USA, under the accession number USNMMENT00714114), although this author report a scutum larger than in the specimen described in this work, and a straight posterior border of the basis capituli. Kohls (1956) also stated that the coxae I–IV bear no spurs; nevertheless, his figure 9B shows an indication of the minute external spur in coxae III–IV. We believe that these apparent disagreements about coxae spurring and posterior margin of basis capituli is due to difficulties to observe morphological characteristics of the fully engorged holotype and the impossibility to obtain scanning electron microscopy images. Otherwise, the female tick collected from *H. magellanicus* shows the peculiar characters of *I. chilensis* in the original description of Kohls (1956). Additionally, localities where the holotype of *I. chilensis* and the new specimens were collected are in the same ecoregion. Therefore, we conclude that the female and nymphs examined by us belong to *I. chilensis*.

### Family Ixodidae Murray, 1877

#### Genus *Ixodes* Latreille, 1796

#### *Ixodes chilensis* Kohls, 1956

*Host*: *Histiopus magellanicus* (Philippi) (Chiroptera: Vespertilionidae).

*Locality*: Oncol (39°39′49.89″S, 73°18′46.77″W), Los Rios region, Chile.

*Voucher material*: Tick Collection of the Facultad de Ciencias Veterinarias, Universidad de Concepción, Chile (accession number Hi.ma-Ix.ch.CDCA 68.1–68.7).

*Comparative material examined*: Type-specimen of *I. chilensis* (female) from the United States National

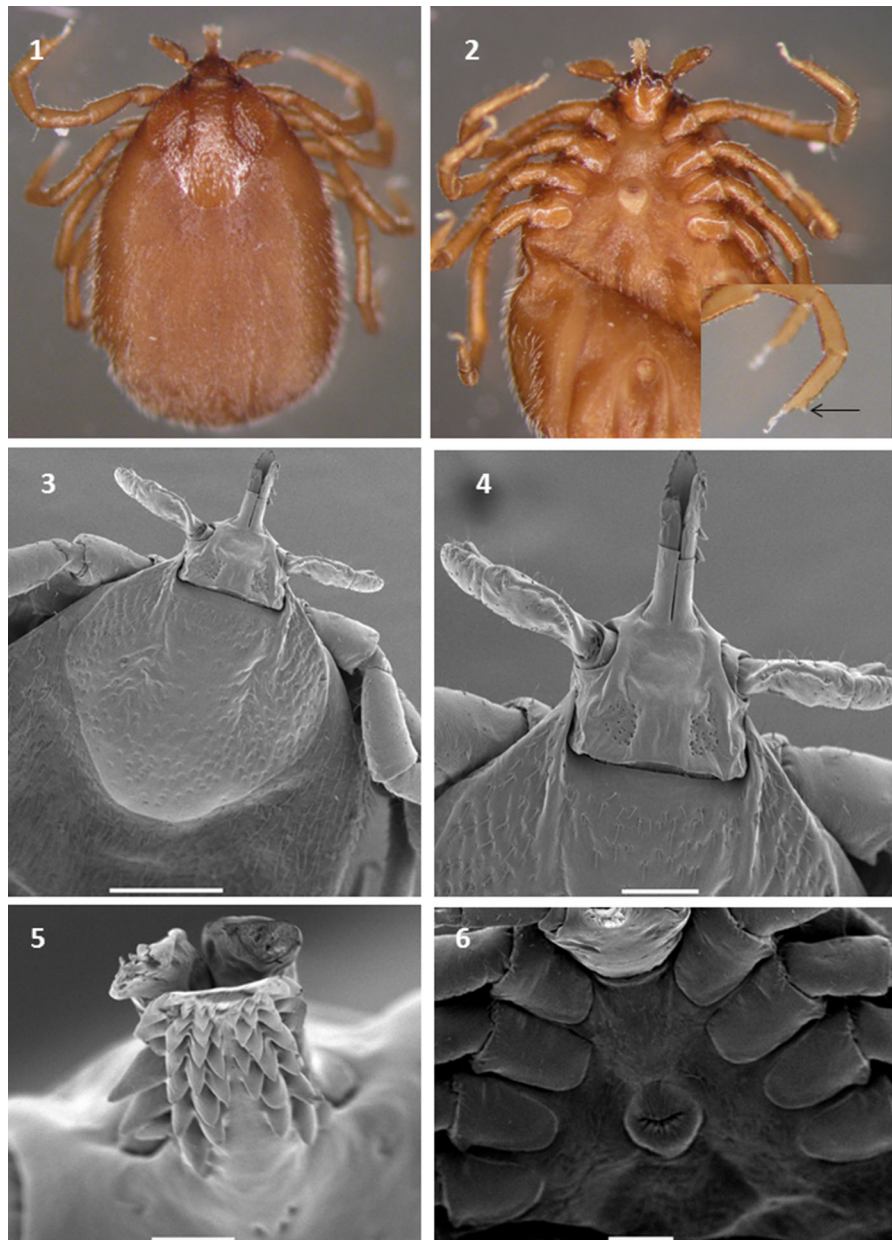
Tick Collection, Institute for Coastal Plain Science, Georgia Southern University, USA (accession number USNMMENT00714114).

*Representative DNA sequences*: Mitochondrial 16S rRNA gene, GenBank accession number MH730656.

#### Description (Figs. 1–10)

*Female* [Re-description based on one semi-engorged specimen; Figs. 1–6.] Body (Figs. 1–3) oval in outline; notum with moderately long, whitish setae, more numerous on dorsal and lateral surface, scattered on ventral surface; genital aperture located between coxae II and III. Scutum (Figs. 1, 3) slightly longer than wide, length 1.15, width 1.10, inornate; surface of anterolateral field rugose; punctuations conspicuous, larger in the posterior field; posterior margin straight; setae on the anterior and lateral margins present; lateral carinae absent; scapulae pointed; cervical grooves shallow, converging anteriorly then diverging as shallow depressions. Basis capituli (Figs. 3, 4) subrectangular, posterior margin sinuous; cornua absent; porose areas rounded (0.08 in diameter) with indistinct borders, separated by interporose area 0.12 wide. Palpi (Fig. 4) short, length 0.46, width 0.15, article II length 0.32, article III length 0.11; suture between articles II and III conspicuous; article IV recessed within ventrolateral surface of article III. Hypostome (Fig. 5) rounded apically, dental formula 3/3 in the anterior third, then 2/2 to base; lateral denticles larger than median. Spiracular plate sub-circular in outline. Legs (Figs. 2, 6): coxae I–IV with one external minute triangular spur each, internal spurs absent, trochanters without spurs; tarsus I length 0.55, tarsus I width 0.17; tarsus I with a subapical dorsal hump.

*Nymph* [Based on 6 specimens; measurements based on 3 unengorged specimens; Figs. 7–10.] Body (Figs. 7, 8) length from apices of hypostome to posterior margin 1.10–1.20 (1.13), breadth at level posterior to spiracular plates 0.64–0.68 (0.66); outline oval; notum with moderately long, whitish setae, numerous on dorsal and lateral surface, scattered on ventral surface. Scutum (Fig. 7) length 0.40–0.60 (0.50), width 0.50–0.50 (0.50), inornate; posterior margin nearly straight; lateral carinae absent; scapulae rounded; cervical grooves shallow, converging anteriorly then diverging as shallow depressions; few and

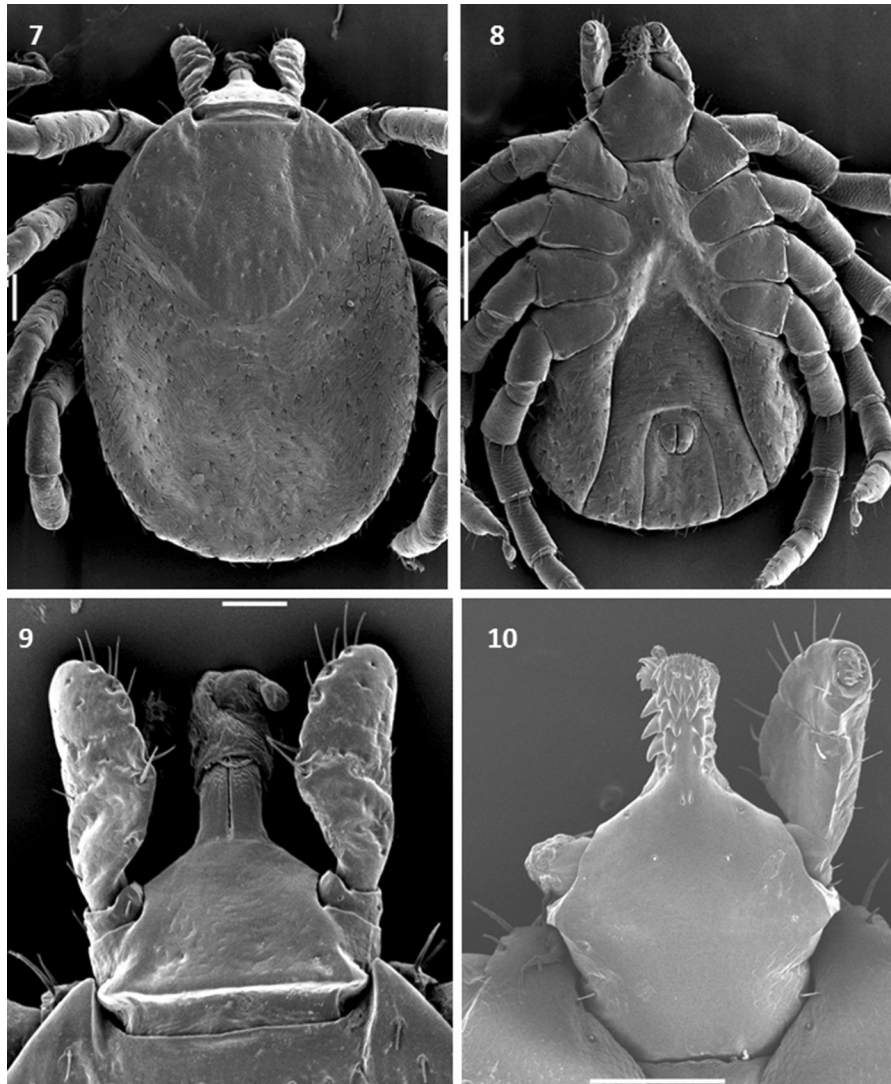


**Figs. 1–6** *Ixodes chilensis*, female. 1 Body, dorsal view; 2 Body, ventral view (arrow indicates subapical dorsal hump of tarsus I); 3 Capitulum (dorsal view) and scutum; 4 Capitulum and palps, dorsal view; 5 Hypostome; 6 Coxae. Scale-bars: 3, 500  $\mu\text{m}$ ; 4, 6, 200  $\mu\text{m}$ ; 5, 50  $\mu\text{m}$

scattered setae present. Basis capituli (Fig. 9) sub-rectangular, posterior margin nearly straight; cornua absent; small auriculae as lateral ridges. Palpi (Fig. 10) length 0.20–0.22 (0.20), width 0.11–0.12 (0.11), article II length 0.1–0.1 (0.1), article III length 0.07–0.07 (0.07); suture between articles II and III

conspicuous. Hypostome (Fig. 10) short, rounded apically, length from apex of hypostome to Ph1 setae 0.12–0.13 (0.12); dental formula 3/3 for the first row, then 2/2 nearly to base; lateral denticles larger than medians. *Spiracular plate* sub-circular in outline. Legs (Fig. 8): coxae I–IV with just one external small





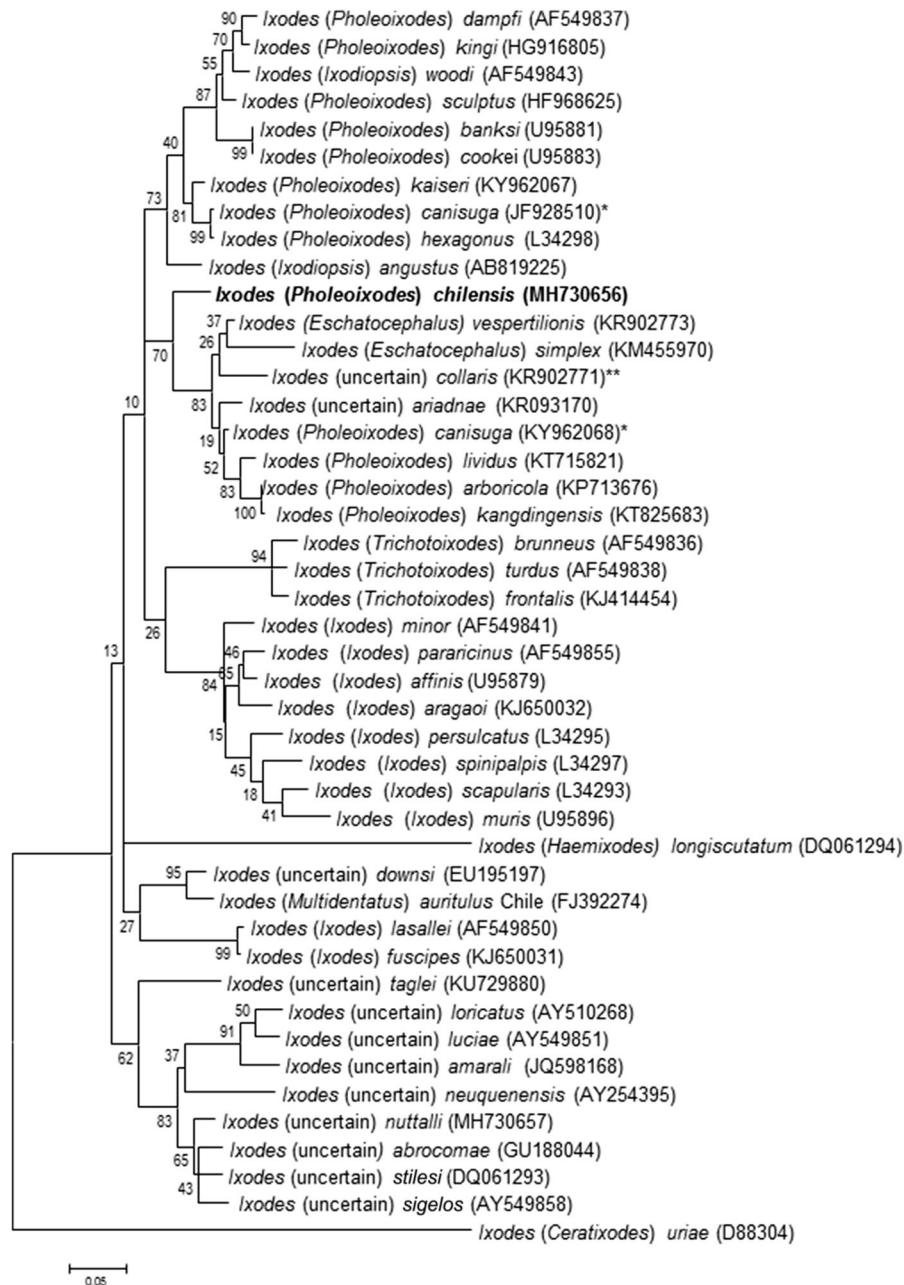
**Figs. 7–10** *Ixodes chilensis*, nymph. 7, Body, dorsal view; 8, Body, ventral view; 9, Capitulum, dorsal view; 10, Hypostome. Scale-bars: 7, 10, 100  $\mu\text{m}$ ; 8, 200  $\mu\text{m}$ ; 9, 50  $\mu\text{m}$

triangular spur each, internal spurs on coxae absent; trochanters without spurs; tarsus I length 0.20–0.20 (0.20); tarsus I width 0.06–0.07 (0.07).

### Phylogenetic analysis

Sequences of the 16S rRNA gene of the female and nymph of *I. chilensis* described above were identical (GenBank accession number MH730656). The phylogenetic position of *I. chilensis* according to the tree obtained with the 16S sequences of *Ixodes* spp. is

depicted in Fig. 11. *Ixodes chilensis* is closely related to four Palearctic species usually included within the subgenus *Pholeoixodes* (*Ixodes arboricola* Schulze & Schlottke, 1929; *I. lividus* Koch, 1844; *I. canisuga* Johnston, 1849; and *I. kangdingensis* Gou, Sun, Xu & Durden, 2017) but also to two species of the subgenus *Eschatocephalus* Frauenfeld, 1853 (*I. simplex* Neumann, 1906 and *I. vespertilionis* Koch, 1844), plus two bat tick species with undetermined subgeneric status (*I. ariadnae* Hornok, 2014 and *I. collaris* Hornok, 2016). These species formed a well-supported clade. The remaining species of the subgenus *Pholeoixodes*



**Fig. 11** Maximum-likelihood tree constructed from and 16S rDNA sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets. \*See the points addressed in the discussion on the reliability of the sequences for *I. canisuga* deposited in the GenBank database, and problems related to subgeneric affiliations. \*\*This sequence is deposited in the GenBank database as belonging to *I. vespertilionis*, but it corresponds in fact to *I. collaris* (see Hornok et al., 2016). The subgenus of *I. collaris* is undetermined

(along with members of the subgenus *Ixodiopsis*) included in the phylogenetic analysis, i.e. *I. hexagonus* Leach, 1815, *I. angustus* Neumann, 1899, *I. sculptus* Neumann, 1904, *I. banksi* Bishopp, 1911, *I. cookei*

Packard 1869, *I. woodi* Bishopp, 1911, *I. dampfi* Cooley, 1943, *I. kingi* Bishopp, 1911 and *I. kaiseri* Arthur, 1957, formed a different clade which was not related to the clade containing *I. chilensis*, *I.*

*arboricola*, *I. collaris*, *I. lividus*, *I. canisuga*, *I. simplex*, *I. vespertilionis*, *I. kangdingensis* and *I. ariadnae*.

There is an inconsistency with the two 16S rDNA sequences of *I. canisuga* (both obtained from ticks collected in Germany; GenBank accession numbers JF928511 and KY962045) because they were grouped in different clades. One of the sequences (KY962045) is grouped together with *I. chilensis*, *I. lividus*, *I. simplex*, *I. vespertilionis*, *I. ariadnae*, *I. arboricola*, *I. collaris* and *I. kangdingensis*, but the second (JF928511) is placed in the clade formed by *I. hexagonus*, *I. angustus*, *I. sculptus*, *I. banksi*, *I. cookei*, *I. woodi*, *I. dampfi*, *I. kingi* and *I. kaiseri*.

The pairwise genetic divergence of *I. chilensis* compared with *I. arboricola*, *I. lividus*, *I. canisuga*, *I. simplex*, *I. vespertilionis*, *I. collaris*, *I. kangdingensis* and *I. ariadnae* ranges between 7.5–9%. The genetic divergence between *I. chilensis* and the remaining *Ixodes* spp. included in the analysis is greater than 9%; this comparison also includes *Ixodes* spp. present in Chile (*I. sigelos* Keirans, Clifford & Corwin, 1976; *I. stilesi* Neumann, 1911; *I. neuquenensis* Ringuélet, 1947; *I. abrocomae* Lahille, 1916; *I. taglei* Kohls, 1969; *I. auritulus* Neumann, 1904; and *I. uriae* White, 1852).

## Discussion

The diagnostic characters unique for the female of *I. chilensis* are a combination of coxae I–IV of legs with one external minute triangular spur each (barely perceptible) and lacking an internal spur; tarsus I with a subapical dorsal hump; basis capituli subrectangular with sinuous posterior margin; hypostome rounded apically; cornua and auriculae absent; scapulae pointed; palps short; porose areas rounded, separated by an interporose area wider than the diameter of one area; scutum with posterior margin straight and surface in the anterolateral field rugose, with punctations larger in the posterior field. The nymph of *I. chilensis* is characterised by the presence of scutum with posterior margin nearly straight; lateral carinae absent; basis capituli subrectangular with posterior margin nearly straight; small auriculae as lateral ridges; palps short; hypostome rounded apically; and coxae I–IV of legs with just one external small

triangular spur each (barely perceptible) and lacking internal spurs.

The combination of the morphological characters shown above makes it possible to differentiate the female and nymph of *I. chilensis* from the remaining members of the genus *Ixodes* present in South America, including those distributed in Chile. Additionally, the phylogenetic analysis clearly showed that *I. chilensis* represents an independent lineage among the *Ixodes* spp. with South American geographical distribution.

Analysis of 16S sequences showed that *I. chilensis* form a well-supported clade with the following species of wide geographical distribution but mostly established in the Palaearctic region and none from the Neotropics: *I. arboricola*, *I. ariadnae*, *I. canisuga*, *I. collaris*, *I. kangdingensis*, *I. lividus*, *I. simplex* and *I. vespertilionis* (see Fig. 11) (Guglielmone et al., 2014; Hornok et al., 2014; Guo et al., 2017). The only other Neotropical species consistently recognised as a member of the subgenus *Pholeioxodes* is *I. rubidus*, but unfortunately there are no 16S sequences of this species available on GenBank to determine its phylogenetic relationship with the species included in the present analysis. Based on morphology, the female and nymph of *I. chilensis* can be easily separated from the female and nymph of *I. rubidus* by the internal spur of coxa I, which is present in *I. rubidus* and absent in *I. chilensis*.

The phylogenetic analysis demonstrated that the subgenus *Pholeioxodes* is not monophyletic. The species considered as belonging to this subgenus were grouped in two different clades which did not have a well-supported common node defining monophyly (Fig. 1). These results are in agreement with the results of Hornok et al. (2017), who concluded that subgenus *Pholeioxodes* is not monophyletic after performing phylogenetic analyses with mitochondrial sequences of the 16S rRNA and *cox1* genes. The inconsistencies of the subgeneric division of *Ixodes* have been discussed for species of the Australasia (Klompen, 1999; Heath & Palma, 2017), the Neotropical (Guglielmone et al., 2006) and Palaearctic zoogeographic regions, as shown for *Pholeioxodes* discussed here. Additionally, Camicas et al. (1998) proposed the subgenus *Amerixodes* to contain nine Neotropical species with rather different morphological characters, but *Amerixodes* lacks diagnostic criterion; consequently it was regarded as a *nomen*

*nudum* by Marques et al. (2004). A reappraisal of *Ixodes* subgenera is needed; however, this type of study will represent a hard task considering that the genus contains 252 species (Guglielmone et al., 2015). In the meantime, assignment of subgeneric status for species of *Ixodes* should be done cautiously because several subgeneric affiliations are, in fact, provisionally valid leaving open the possibility of changes as new information is gathered.

Regarding the inconsistency in the phylogenetic position of *I. canisuga* (see results and Fig. 11), the sequence deposited in GenBank with the number KY962045, published by Hornok et al. (2017), is considered here a *bona fide* sequence of *I. canisuga* because the morphological diagnosis of the ticks from which DNA was obtained is explicit (see Hornok et al., 2017). On the contrary, the study of Karger et al. (2012) who generated the sequence of *I. canisuga* (GenBank: JF928511) shows ambiguity in relation to the accuracy of the identification of this tick species, because the 16S sequences of *I. canisuga* obtained by Karger et al. (2012) show 100% homology to 16S sequences of *I. hexagonus* from GenBank (see page 85 and table 2 of Karger et al., 2012). But it is obvious that this problematic issue cannot be resolved here, and further analyses should be performed to determine the reliability of all sequences of *I. canisuga* available on GenBank.

The species of *Ixodes* to which *I. chilensis* is phylogenetically related, use bats, birds and carnivorous mammals as principal hosts (Guglielmone et al., 2014; Hornok et al., 2014; Guo et al., 2017). To date, the only known host for both female and nymphs of *I. chilensis* is the bat *H. magellanicus*. The findings of *Ixodes* ticks on bats in the Neotropical region are scarce. Kohls (1957) has reported larvae of *Ixodes downsi* Kohls, 1957 on *Anoura geoffroyi* Gray in Trinidad & Tobago, and Jones et al. (1972) have recorded larvae of *Ixodes* sp. on *Desmodus rotundus* (E. Geoffroy) and *Sturnira lilium* (E. Geoffroy) in Venezuela. Also, Webb & Loomis (1977) mentioned the finding *Ixodes* sp. in *S. lilium*, *Sturnira ludovici* Anthony and *Artibeus jamaicensis* Leach in Venezuela with no details of tick stage and locality. However, it is important to consider that *H. magellanicus* uses tree cavities as roosting sites, where different bird species also nest (Altamirano et al., 2017); for that reason future records of *I. chilensis* on birds should be expected.

**Acknowledgements** We appreciate the support given by the National Forest Corporation, CONAF, as well as by the Agricultural and Livestock Service, SAG, of Chile. This research was funded by FONDECYT Project 1130948 and 1170972 (Chile). We acknowledge the financial support of INTA, Asociación Cooperadora INTA Rafaela and CONICET (Argentina) to AAG and SN. We are very grateful to Lorenza Beati (United States National Tick Collection, Statesboro, Georgia) for providing the type-specimen.

**Funding** FONDECYT Project 1130948 and 1170972 (Chile). INTA and Asociación Cooperadora INTA Rafaela (Argentina).

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable institutional, national and international guidelines for the care and use of animals were followed.

#### References

- Altamirano, T. A., Ibarra, J. T., Novoa, F., Vermehren, A., Martín, K., & Bonacic, C. (2017). Roosting records in tree cavities by a forest-dwelling bat species (*Histiotus magellanicus*) in Andean temperate ecosystems of southern Chile. *Bosque*, 38, 421–425.
- Camicas, J. L., & Morel, P. C. (1977). Position systématique et classification des tiques (Acarida: Ixodida). *Acarologia*, 18, 410–420.
- Camicas, J. L., Hervy, J. P., Adam, F., & Morel, P. C. (1998). *Les tiques du monde. Nomenclature, stades décrits, hôtes, répartition* (Acarida, Ixodida). Paris: Orstom, 233 pp.
- Clifford, C. M., Sonenshine, D. E., Keirans, J. E., & Kohls, G. M. (1973). Systematics of the subfamily Ixodinae (Acarina: Ixodidae). 1. The subgenera of *Ixodes*. *Annals of the Entomological Society of America*, 66, 489–500.
- Filippova, N. A. (1957). *Ixodes stromi*, new species of tick and its systematic position in Ixodinae. *Zoologicheskij Zhurnal*, 36, 864–869 (In Russian).
- Filippova, N. A. (1977). Ixodid ticks (Ixodinae). In: *Fauna USSR New Series*, 4 (4). Moscow-Leningrad: Nauka. 316 pp (In Russian).
- Guglielmone, A. A., Venzal, J. M., González Acuña, D., Nava, S., Hinojosa, A., & Mangold, A. J. (2006). The phylogenetic position of *Ixodes stilesi* Neumann, 1911 (Acari: Ixodidae): morphological and preliminary molecular evidences from 16S rDNA sequences. *Systematic Parasitology*, 65, 1–11.
- Guglielmone, A. A., Robbins, R. G., Apanaskevich, D. A., Petney, T. N., Estrada-Peña, A., & Horak, I. (2014). *The hard ticks of the world*. Dordrecht: Springer, 738 pp.
- Guglielmone, A. A., Sánchez, M. E., Franco, L. G., Nava, S., Rueda, L. M., & Robbins, R. G. (2015). Hard ticks (Acari: Ixodida: Ixodidae): a non-profit open-access web portal for original descriptions of tick species (valid and invalid),



- dubious and uncertain names, and selected nomina nuda. <http://rafaela.inta.gov.ar/nombresgarrapatas>.
- Guglielmone, A. A., Petney, T. P., Mastropaolo, M., & Robbins, R. G. (2017). Genera, subgenera, species and subspecies of hard ticks (Acari: Ixodidae) described, named, renamed or given new rank by Paul Schulze (1887–1949) and their current status. *Zootaxa*, 4325, 1–66.
- Guo, T., Sun, Y., Xu, G., & Durden, L. A. (2017). *Ixodes kandingensis* (Acari: Ixodidae), a new species from the Siberian weasel, *Mustela sibirica* (Carnivora: Mustelidae) in China. *Parasitology Open*, 3, 1–8.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Heath, A. C. G., & Palma, R. L. (2017). A new species of tick (Acari: Ixodidae) from seabirds in New Zealand and Australia, previously misidentified as *Ixodes eudyptidis*. *Zootaxa*, 4324, 285–314.
- Hornok, S., Kontschán, J., Kováts, D., Kovács, R., Angyal, D., Gorföl, T., et al. (2014). Bat ticks revisited: *Ixodes ariadnae* sp. nov. and allopatric genotypes of *I. vesperilionis* in caves of Hungary. *Parasites & Vectors*, 7, 202.
- Hornok, S., Gorföl, T., Estók, P., Tu, V. T., & Kontschán, J. (2016). Description of a new tick species, *Ixodes collaris* n. sp. (Acari: Ixodidae), from bats (Chiroptera: Hipposideridae, Rhinolophidae) in Vietnam. *Parasites & Vectors*, 9, 332.
- Hornok, S., Sándor, A. D., Beck, R., Farkas, R., Beati, L., Kontschán, J., et al. (2017). Contributions to the phylogeny of *Ixodes* (*Pholeoixodes*) *canisuga*, *I. (Ph.) kaiseri*, *I. (Ph.) hexagonus* and a simple pictorial key for the identification of their females. *Parasites & Vectors*, 10, 545.
- Jones, E. K., Clifford, C. M., Keirans, J. E., & Kohls, G. M. (1972). The ticks of Venezuela (Acarina: Ixodoidea) with a key to the species of *Amblyomma* in the Western Hemisphere. *Brigham Young University Science Bulletin Biological Series*, 17, 1–40.
- Karger, A., Kampen, H., Bettin, B., Dautel, H., Ziller, M., Hoffmann, B., et al. (2012). Species determination and characterization of developmental stages of ticks by whole-animal matrix-assisted laser desorption/ionization mass spectrometry. *Ticks and Tick-borne Diseases*, 3, 78–89.
- Klompen, J. S. H. (1999). Phylogenetic relationship in the family Ixodidae with emphasis on the genus *Ixodes* (Parasitiformes: Ixodidae). In: Needham, G. R., Mitchell, R., Horn, D. J. & Welbourn, W. C. (Eds) *Acarology IX: Symposia*. Columbus: Ohio Biological Survey, pp. 349–354.
- Kohls, G. M. (1956). Eight new species of *Ixodes* from Central and South America (Acarina: Ixodidae). *Journal of Parasitology*, 42, 636–649.
- Kohls, G. M. (1957). *Ixodes downsi*, a new species of tick from a cave in Trinidad, British West Indies (Acarina-Ixodidae). *Proceedings of the Entomological Society of Washington*, 59, 257–264.
- Luebert, F., & Plissock, P. (2006). *Sinopsis bioclimática y vegetacional de Chile*. Santiago: Editorial Universitaria, 310 pp.
- Mangold, A. J., Bargues, M. D., & Mas-Coma, S. (1998). Mitochondrial 16S rRNA sequences and phylogenetic relationships of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). *Parasitology Research*, 84, 478–484.
- Morrone, J. J. (2006). Biogeographic areas and transition zones of Latin American and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. *Annual Review of Entomology*, 51, 467–494.
- Marques, S., Barros-Battesti, D. M., Onofrio, V. C., Famadas, K. M., Faccini, J. L. H., & Keirans, J. E. (2004). Redescription of larva, nymph and adults of *Ixodes (I.) loricatus* Neumann, 1899 (Acari: Ixodidae) based on light and scanning electron microscopy. *Systematic Parasitology*, 59, 135–146.
- Nava, S., Venzal, J. M., González-Acuña, D., Martins, T. F., & Guglielmone, A. A. (2017). *Ticks of the Southern Cone of America: Diagnosis, Distribution and Hosts with Taxonomy, Ecology and Sanitary Importance*. London: Elsevier, Academic Press, 348 pp.
- Robbins, R. G., & Keirans, J. E. (1992). Systematics and ecology of the subgenus *Ixodiopsis* (Acari: Ixodidae: *Ixodes*). *Thomas Say Foundation Monographs*, 14, 1–159.
- Tagle, I. (1971). Ixodoidea en Chile. *Boletín Chileno de Parasitología*, 26, 46–49.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.
- Thompson, J. D., Higgins, D., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.
- Webb, J. P., & Loomis, R. B. (1977). Ectoparasites. In: Baker, R. J., Jone, J. K. & Carter, D. C (Eds) *Biology of bats of the New World family Phyllostomatidae. Part II*. Special Publications, The Museum, Texas Tech University 13. Lubbock: Texas Tech Press, pp. 57–120