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Additional observations on the morphology and hosts of *Ixodes stilesi* Neumann, 1911 (Acari: Ixodidae)

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Abstract

Three females and a nymph of *Ixodes stilesi* were collected by dragging ground vegetation in a southern beech (*Nothofagus*) forest at San Martin (39° 38'S, 73° 75'W), Valdivia Province, Los Ríos Region (Region XIV), Chile, 20 April 2005 and 1 April 2006; a second nymph was removed from the long-tailed pygmy rice rat or colilargo, *Oligoryzomys longicaudatus* (Bennett), same locality, 20 April 2005. The 16S rDNA sequence for one of the *I. stilesi* females showed 100% agreement with the sequence for this species deposited in GenBank. The morphology of our nymphal specimens agrees with a recently published description of the nymph, but our females of *I. stilesi* differ from the redescription of a partly engorged female in four respects: 1) cornua prominent and triangular instead of small, rectangular and blunt; 2) hypostome pointed instead of rounded apically; 3) genital aperture situated between coxae III and IV instead of between coxae II and III; 4) anal groove horseshoe shaped instead of U shaped. In the female described earlier, the cornua and hypostomal apex were likely damaged when the specimen was removed from its host, while differences in the position of the genital aperture and shape of the anal groove may be due to the effects of engorgement on the integument. This is the first record of *I. stilesi* from *O. longicaudatus*.

Key words: Ixodes stilesi, morphology, female, nymph, Oligoryzomys longicaudatus, Chile

Introduction

Ixodes stilesi Neumann, 1911, is known to parasitize the southern pudu, *Pudu puda* (Molina) (Artiodactyla: Cervidae), in Chile (Guglielmone *et al.* 2006). The female of this tick species was originally briefly described by Neumann (1910) under the name *Ixodes elegans*. However, Neumann (1911) recognized that this name was preoccupied by *I. elegans* Guérin-Méneville, 1843, a synonym of *Amblyomma variegatum* (Fabricius, 1794), and changed the name to *I. stilesi*. Recently, Guglielmone *et al.* (2006) redescribed the female and, for the first time, described the male and nymph of *I. stilesi* using specimens collected from *P. puda* in Chile's Region VIII. Subsequent examination of additional Chilean specimens has compelled us to modify the female redescription in order to facilitate its diagnosis. Herein we present new interpretations of some female morphological features, with further data on the hosts and distribution of *I. stilesi*.

Materials and methods

Specimens of *I. stilesi* were collected by dragging ground vegetation in a southern beech (*Nothofagus*) forest, yielding: 1 female, San Martín (39° 38'S, 73° 75'W), Valdivia Province, Los Ríos Region (Region XIV), Chile, 20 April 2005, coll. Claudio Hernández; 2 females and 1 nymph, same data except 1 April 2006. Additionally, 1 nymph was removed from the long-tailed pygmy rice rat or colilargo, *Oligoryzomys longicaudatus* (Bennett) (Rodentia: Cricetidae: Sigmodontinae), at the same locality, 20 April 2005. Ticks were examined by stereoscopic and scanning electron microscopy, and one female was used for DNA extraction and polymerase chain reaction (PCR) amplification to obtain 16S rDNA sequences for comparison with the DNA sequences of other Neotropical *Ixodes*, as described by Guglielmone *et al.* (2006). All specimens have been deposited in the Laboratorio de Zoología, Departamento de Ciencias Pecuarias, Universidad de Concepción, Chillán, Chile.

Results

The 16S rDNA sequence for one of the females of *I. stilesi* collected during this study (GenBank accession number EF362757) shows 100% agreement with the sequence obtained from a female analyzed earlier (GenBank DQ061292; Guglielmone *et al.* 2006). The morphology of both nymphs also corresponds with the description of this stage in Guglielmone *et al.* (2006). However, our latest female specimens differ from the redescription in four key respects: 1) the cornua are prominent and triangular instead of small, rectangular and blunt (Figures 1A and 1B); 2) the hypostome is pointed apically, rather than rounded (Figures 2A and 2B); 3) the genital aperture is situated between coxae III and IV instead of between coxae II and III (Figures 3A and 3B); and 4) the anal groove (not shown) is horseshoe shaped rather than U shaped, *i.e.*, the arms of the groove converge slightly as they approach the posterior body margin.

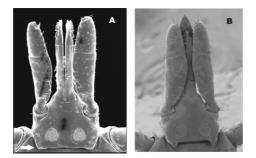


FIGURE 1. *Ixodes stilesi* female. A, Capitulum, dorsal view, from redescription of a partially fed specimen (Guglielmone *et al.* 2006). Arrow indicates probable breakage at apex of cornu; B, Capitulum, dorsal view, unfed specimen. Figure 1A reproduced with permission of Springer Science and Business Media.

Examination by electron microscopy has convinced us that the seeming absence of prominent cornua and the apparently rounded hypostomal apex originally reported by Guglielmone *et al.* (2006) for the female of *I. stilesi* resulted from damage to the specimen used for redescription. This is particularly evident on the left side of the basis capituli, where the affected cornu (Figure 1A) appears to have a jagged edge, as if the distal portion had been broken off. The differences in the position of

the genital aperture and the shape of the anal groove between the redescribed female and those collected in Valdivia Province likely stem from stretching of the integument associated with feeding by the former specimen. Although we have not seen the female specimens that were the basis both of Neumann's (1910) original description and the redescription of this stage by Nuttall and Warburton (1911), it seems likely that they were at least partly fed (two are described as "replete"), having been collected from *P. puda* rather than by dragging.

The nymph of *I. stilesi* collected from *O. longicaudatus* constitutes the first record of this species from a host other than *P. puda*.

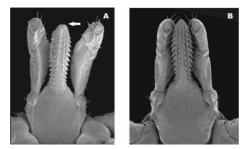


FIGURE 2. *Ixodes stilesi* female. A, Capitulum, ventral view, from redescription of a partially fed specimen (Guglielmone *et al.* 2006). Arrow indicates probable breakage at apex of hypostome; B, Capitulum, ventral view, unfed specimen. Figure 2A reproduced with permission of Springer Science and Business Media.

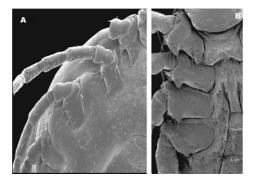


FIGURE 3. *Ixodes stilesi* female. 3A, Coxae and genital aperture, from redescription of a partially fed specimen (Guglielmone *et al.* 2006); B, Coxae and genital aperture, unfed specimen. Figure 3A reproduced with permission of Springer Science and Business Media.

Discussion

When describing tick specimens, it is clearly preferable to rely on well-sclerotized structures, which are less subject to damage or distortion. However, minor damage may escape notice, as in the recent redescription of a partly engorged female of *I. stilesi* by Guglielmone *et al.* (2006), where the apices of the cornua and hypostome were probably lost as the tick was being removed from its host, leading to an incorrect interpretation of this species' morphology. This situation went unnoticed until unfed females with 100% agreement in 16S rDNA sequences and a close resemblance to *I. stilesi* were collected. Historically, many tick species have been described from partly or fully engorged specimens, but unfed examples, when available, should always be used for comparison. Errors may

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also arise when describing integumentary structures that are prone to distortion during feeding or growth. In the present case, unfed females of *I. stilesi* were found to differ from a fed female in the position of the genital aperture and shape of the anal groove, but this problem is not unique. For example, key characters of the larva of *Argas keiransi* Estrada-Peña, Venzal & González-Acuña, 2003, vary greatly between fed or unfed specimens (Estrada-Peña *et al.* 2003, 2006). Such plastic features should be analyzed with caution.

The shape of the cornua in females of *I. stilesi* appears to be similar to that of the rodent parasite *I. nuttalli* Lahille, 1913, as described by Nuttall (1916); however, in *I. stilesi* the porose areas are rounded and separated by the width of one area, whereas in *I. nuttalli* the porose areas are triangular and separated by less than the width of one area. As well, in *I. nuttalli* the apex of the hypostome appears not to be as pointed as in *I. stilesi*.

Oligoryzomys longicaudatus occurs in a variety of habitats in the central and southern Andes of Argentina and Chile, ranging eastward in Argentina to coastal Buenos Aires Province. It is chiefly a granivorous mouse that commonly invades human habitations, where it is known to be a reservoir of hantavirus (Gallardo & Mercado 1999; Nowak 1999; Padula et al. 2004; Musser & Carleton 2005). The discovery of a rodent host for *I. stilesi* is interesting because analyses of 16S rDNA sequences by Guglielmone et al. (2006) have indicated that this species is phylogenetically close to I. neuquenensis Ringuelet, 1947, and I. sigelos Keirans, Clifford & Corwin, 1976, ticks of uncertain subgeneric status that hitherto were not known to parasitize related hosts - I. neuquenensis feeds on the monito del monte, Dromiciops gliroides Thomas (Microbiotheria: Microbiotheriidae), a semiarboreal marsupial, and I. sigelos parasitizes rodents, while all previously known specimens of I. stilesi have been collected from cervids (Guglielmone et al. 2006). Thus, the discovery of I. stilesi on O. longicaudatus serves as an ecological link between two of these tick taxa. However, the importance of this finding should not be exaggerated. Studies of species relationships in the Southern Cone of South America are hampered by scarce paleontological data, particularly with respect to Pleistocene glaciation, relative to evidence from the Northern Hemisphere (cf., Robbins & Keirans 1992). The effects of glaciation in the Southern Cone were probably magnified by the smaller area of this region and its dissected physiography, with consequent reduction of biotic refugia. It may well be that I. stilesi, I. neuquenensis and I.sigelos are distantly related but relict species that will never fit comfortably into conventional subgeneric classifications.

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References

- Estrada-Peña, A., Venzal, J.M., González-Acuña, D. & Guglielmone, A.A. (2003) Argas (Persicargas) keiransi n. sp. (Acari: Argasidae), a parasite of the chimango, Milvago c. chimango (Aves: Falconiformes) in Chile. Journal of Medical Entomology, 40, 766–769.
- Estrada-Peña, A., Venzal, J.M., González-Acuña, D., Mangold, A.J. & Guglielmone, A.A. (2006) Notes on New World *Persicargas* ticks (Acari: Argasidae) with description of female *Argas* (*P.*) *keiransi*. *Journal* of *Medical Entomology*, 43, 801–809.

Gallardo, M.H. & Mercado, C.L. (1999) Mast seeding of bamboo shrubs and mouse outbreaks in southern

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Chile. Mastozoología Neotropical, 6, 103–111.

- 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.
- Musser, G.G. & Carleton, M.D. (2005) Superfamily Muroidea. In: Wilson, D.E. & Reeder, D.M. (eds.) Mammal Species of the World: a Taxonomic and Geographic Reference, 3rd edition, vol. 2. Baltimore, The Johns Hopkins University Press. pp. 894–1531.
- Neumann, L.G. (1910) Sur quelques espèces d'Ixodidae nouvelles ou insuffisament connues. Annales des Sciences Naturelles Zoologie 9 Serie, 12, 161–176.
- Neumann, L.G. (1911) Note rectificative à propos de deux espèces d'Ixodinae. *Archives de Parasitologie*, 14, 415.
- Nowak, R.M. (1999) Walker's Mammals of the World, 6th ed. Baltimore, Johns Hopkins University Press. lxxx + 1936pp.
- Nuttall, G.H.F. (1916) Notes on ticks. IV. Relating to the genus *Ixodes* and including a description of three new species and two new varieties. *Parasitology*, 8, 294–337.
- Nuttall, G.H.F. & Warburton, C. (1911) *Ticks. A Monograph of the Ixodoidea. Part II. Ixodidae.* London, Cambridge at the University Press. pp. xix + 105–348.
- Padula, P., Figueroa, R., Navarrete, M., Pizarro, E., Cadiz, R., Bellomo, C., Jofre, C., Zaror, L., Rodriguez, E. & Murua, R. (2004) Transmission study of Andes hantavirus infection in wild sigmodontine rodents. *Journal of Virology*, 78, 11972–11979.
- Robbins, R.G. & Keirans, J.E. (1992) Systematics and Ecology of the Subgenus Ixodiopsis (Acari: Ixodidae: Ixodes). Thomas Say Foundation Monograph XIV. Lanham, Maryland, Entomological Society of America. viii + 159 pp.

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