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The human flea *Pulex irritans* (Siphonaptera: Pulicidae) in northwestern Argentina, with an investigation of *Bartonella* and *Rickettsia* spp.

La pulga humana Pulex irritans *(Siphonaptera: Pulicidae) en el noroeste argentino, una investigación de* Bartonella *y* Rickettsia *spp.*

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Abstract

Pulex irritans is the only cosmopolitan flea species and the most studied one within the genus *Pulex*. It has importance in public health since it commonly parasitizes humans causing dermatitis, and it has been also implicated in the transmission of bacterial pathogens. *Pulex irritans* has been confused with the closely related *Pulex simulans* species for years. Herein, *Pulex* specimens collected from a Pampas fox and a Chacoan peccary from northwestern Argentina were identified by comparison with type specimens. In addition, the presence of *Bartonella* spp. and *Rickettsia* spp. was investigated using PCR assays. Our results provided characters of diagnostic importance to identify *P. irritans*, which include the shape of sternite VII in the females, and of the aedeagal sclerite, clasper and crochet in the males. Besides, we report for the first time *P. irritans* parasitizing a peccary. This finding reinforces the hypothesis of the origin of this flea associated with this mammal, and then colonizing humans and domestic mammals. There was no evidence of *Bartonella* or *Rickettsia* DNA in the analyzed fleas. This information even if negative may be considered relevant for *P. irritans* from Argentina.

Keywords: Flea; Siphonaptera; *Pulex*; Bacteria; Pathogens; Argentina

Resumen

Pulex irritans es la única especie cosmopolita y la más estudiada dentro del género *Pulex*. Tiene importancia en la salud pública ya que comúnmente parasita a los seres humanos causando dermatitis y también ha sido implicada en la transmisión de patógenos bacterianos. *Pulex irritans* se ha confundido con la especie cercana *Pulex simulans* durante años. En este sentido, se identificaron los especímenes de *Pulex* recolectados de un zorro pampeano y un pecarí del

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Chaco del noroeste de la Argentina por comparación con los ejemplares tipo. Además, se investigó la presencia de *Bartonella* spp. y *Rickettsia* spp. utilizando ensayos de PCR. Nuestros resultados aportaron caracteres de importancia diagnóstica para identificar a *P. irritans*, que incluyen la forma del esternito VII en las hembras y del esclerito aedeagal, clasper y crochet en los machos. Además, se reporta por primera vez a *P. irritans* parasitando un pecarí. Este hallazgo refuerza la hipótesis del origen de esta pulga asociada con este mamífero y luego coloniza humanos y mamíferos domésticos. No hubo evidencia de ADN de *Bartonella* ni de *Rickettsia* en las pulgas analizadas. Esta información, si bien negativa, puede ser considerada relevante para *P. irritans* de Argentina.

Palabras clave: Pulga; Siphonaptera; *Pulex*; Bacterias; Patógenos; Argentina

Introduction

Fleas (Siphonaptera) are holometabolous, laterally flattened, wingless insects, highly specialized to ectoparasitic life. Male and female adults are obligate hematophagous ectoparasites of mammals and birds. Fleas are important in public health as parasites, and as vectors of pathogens (Bitam et al., 2010; Marshall, 1981). About 2,574 species of fleas belonging to 16 families and 238 genera have been described, but only a minority lives in close association with humans (Lewis, 1999). The genus *Pulex* Linnaeus, 1758 (Pulicidae) contains 7 recognized species. Within this genus, *Pulex irritans* Linnaeus, 1758 is the most studied species and the only one which occurs in Europe (Hopla, 1980). This flea was originally described on the basis of specimens collected from humans from Uppsala, Sweden, and consequently called "the human flea". Subsequently, *Pulex simulans* Baker, 1895 was described on the basis of specimens collected from America, and the author remarked that, although distinct, this species was morphologically very close and easily confused with *P. irritans*. However, *P. simulans* was again considered a synonym of *P. irritans* (Jordan & Rothschild, 1908), and it was in 1958 when *P. simulans* was considered a valid species since the morphological differences in the aedeagal sclerite and crochet in males of the 2 species were reported (Smit, 1958). However, in America *P. irritans* and *P. simulans* have been confused for years. Possibly these fleas continue to be confused up to the present. The original description of *P. irritans* included specimens from Europe and America, and probably specimens of *P. simulans* erroneously confused with *P. irritans* (Smit, 1958). Since the type specimen of *P. irritans* was lost, a male neotype from Budapest, Hungary, deposited in the Rothschild Collection of fleas, was designated (Smit, 1958). Confusion between close species of *Pulex* continues and a revision in order to enable a correct identification of *P. irritans* and *P. simulans* is necessary.

The relationship between *Pulex irritans* and humans could suggest a long history of association (Marshall, 1981). Currently, *P. irritans* has a cosmopolitan distribution, probably due to human transport. However, the genus *Pulex* is of American origin (Hopla, 1980). *Pulex irritans* has been recovered from archaeological sediments in Viking York (England), Dublin (Ireland) and abandoned farm sites in Norse Greenland (Iceland), but the origins of the flea appear to be Central to South American, where several congeners are known. The probable routes by which the species reached Western Europe are discussed and resolved in favor of a Beringian and Asiatic one, at any time during the postglacial era. Although this flea is presently relatively promiscuous, initial evolution is likely to have involved a single host, probably a peccary, closely associated with humans (Buckland & Sadler, 1989). Currently, a variety of mammals are known as hosts of *P. irritans*, and because of its close association with domestic mammals such as pigs and dogs, *P. irritans* can bite humans. *Pulex irritans* is able to transmit several zoonotic pathogens, namely the agents of plague, and the flea-borne spotted rickettsiosis (Brouqui & Raoult, 2006), and it has been important in transmitting *Yersinia pestis* from man to man and possibly from domestic animals to man (Hopla, 1980).

Because of its association with humans and domestic animals, and with disease transmission, *P. irritans* has been the most studied species within the genus *Pulex*. However, in Argentina most of the records of this flea were accidental and no morphological studies have been carried out in order to establish a correct identification of the species. In Argentina, *P. irritans* have been mentioned parasitizing wild mammals such as marsupials, carnivores, lagomorphs, rodents and even-toed ungulates, and the role of this flea as vector of pathogens had not been investigated (Lareschi et al., 2016).

The aim of this work is to show the results of the study of *Pulex* specimens from northwestern Argentina which involves a correct identification. In addition, we investigate the presence of bacteria from *Bartonella* and *Rickettsia* genera using molecular biology techniques.

Materials and methods

Forty-nine fleas were recovered from carcasses of a Chacoan peccary *Catagonus wagneri* (Rusconi, 1930)

(Artiodactyla, Tayassuidae) and a Pampas fox *Lycalopex gymnocercus* (Fischer, 1814) (Carnivora, Canidae). Both animals were found dead at Rivadavia Banda Sur (24°11' S, 62°53' W), Salta Province, Argentina. Fleas were preserved in vials with 96% ethanol. All fleas (12 specimens) collected from the Chacoan peccary were mounted for their identification at the microscope. Since fleas from the Pampas fox were more abundant (37 specimens), 12 were prepared for morphological studies whereas the remaining 25 were preserved for molecular studies.

All fleas were identified using a binocular stereoscopic microscope. Twenty-four selected fleas were cleared in KOH, dehydrated in a graduated series of alcohol, diaphanized in eugenol and mounted on slides in Canada balsam for their detailed examination using the compound microscope. Photographs were taken by using a Microscope Olympus BX51 equipped with Photographic Camera Olympus DP71, BX51TF, Tokyo, Japan. Morphology was studied comparing our specimens with the male neotype of *P. irritans* and the female lectotype of *P. simulans*, as well as other specimens of both sexes of these species deposited at the Natural History Museum (NHM), London, U.K. The neotype of *P. irritans* (C. Rothschild Coll. 1923-615) and a male of *P. simulans* (Navarro Coll., Texas. Brit. Mus. 1949-268) (the last although not in very good condition) were photographed by using a dino lite Digital Microscope. Female specimens of both species were not in good enough condition to be photographed. For comparative purposes, specimens of other species of *Pulex* deposited at the NHM were also examined. In addition, figures, keys and descriptions of species of *Pulex* given in Hopkins & Rothschild (1953), Barrera (1955), and Hopla (1980) were also considered. Morphological terminology follows the 2 first authors. Representative specimens of fleas from northwestern Argentina were deposited in the Department of Entomology, Museo de La Plata (MLP), Argentina.

Twenty-five fleas obtained from *L. gymnocercus* were examined for the presence of *Rickettsia* and *Bartonella* species. Fleas were rinsed in distilled water and dried on sterile filter paper under a laminar-flow hood (Pérez-Martínez et al., 2009). Each flea was crushed with a sterile pestle, and DNA was extracted by lysis with 0.7 M ammonium hydroxide (Rijpkema et al., 1996). Detection of *Bartonella* spp. was tested using PCR primers targeting the RNA polymerase β-subunit-encoding gene (*rpo*B) and the citrate synthase (*gltA*) partial genes as well as the 16S-23S rRNA intergenic region (ITS) (Jensen et al., 2000, Norman et al., 1995; Renesto et al., 2001). *Rickettsia* DNA was tested with 2 PCR assays targeting the rickettsial *gltA* gene (Labruna et al., 2004; Regnery et al., 1991). A positive control consisting of *Bartonella henselae* (DNA extracted from a cat flea —*Ctenocephalides felis*— from La Rioja, Spain), or *Rickettsia slovaca* strain S14ab DNA (obtained from Vero cells inoculated with a *Dermacentor marginatus* tick at the Center of Rickettsiosis and Arthropod Borne Diseases from La Rioja, Spain), and a negative control (sterile water instead of template DNA) were used for each PCR assay.

Results

All 49 fleas were identified as *P. irritans* (Figs. 1-7). Five male specimens (2 from the Chacoan peccary, and 3 from the Pampas fox) and 19 females (10 from the Chacoan peccary, and 9 from the Pampas fox) were studied in more detailed: fleas from both genders presented large, dark and conspicuous eyes, without ventral sinus, falax extending down to the base of antennal fossa, shorter than the wider diameter of the eye, occipital groove not evident, labial palp and lacinia half to three-quarters length of anterior margin of fore coxa, lacinia with weak teeth, thinner at every level than the base of the first segment of the maxillary palps, second and fourth segments of maxillary palp subequal in length (Fig. 1); apex of clypeal apodeme dorsal to the implementation of the preocular seta, genal ctenidium constituted by a single dark tooth; occiput with only 1 strong bristle, with a single ocular bristle and a single bristle near base of maxilla (Fig. 2); with a row of small spines on inside of hind coxa near apex, consisting of 13-16 spines forming a patch (Fig. 3), break of mesocoxa incomplete.

Female (Figs. 1, 4, 5): length: 2.0-3.5 mm (Fig. 1); sternite VII with a sinus and with 4/6 setae on each side the sinus (Fig. 4); spermatheca as in Figure 5. No differences were observed between female fleas from the Chacoan peccary and those from the Pampas fox. In contrast, the lectotype of *P. simulans* presented a greater number of setae on each side the sinus of sternite VII.

Male (Figs. 6-7): length 1.8-2.0 mm; with dorsal aedeagal sclerite (das) relatively long and slender; clasper with processes 1 (P1) very large and completely covering processes 2 (P2) and 3 (P3), ovoid but with postero-distal angle nearly straight; processes 2 and 3 shorter than 1; the crochet (cr) expanded apically, slightly different in the length of the expansion between fleas (shorter —on those from the Chacoan peccary; figure 6— than in those from the Pampas fox; figure 7); proximal arm of sternite IX thin, slightly curved, with parallel margins, but with the apex dilated; distal arm of sternite IX almost as long as the proximal arm, with parallel edges, straight, except in the apical portion is blunt (Fig. 7). Males of *P. irritans* from Argentina were similar to the neotype (Fig. 8). In

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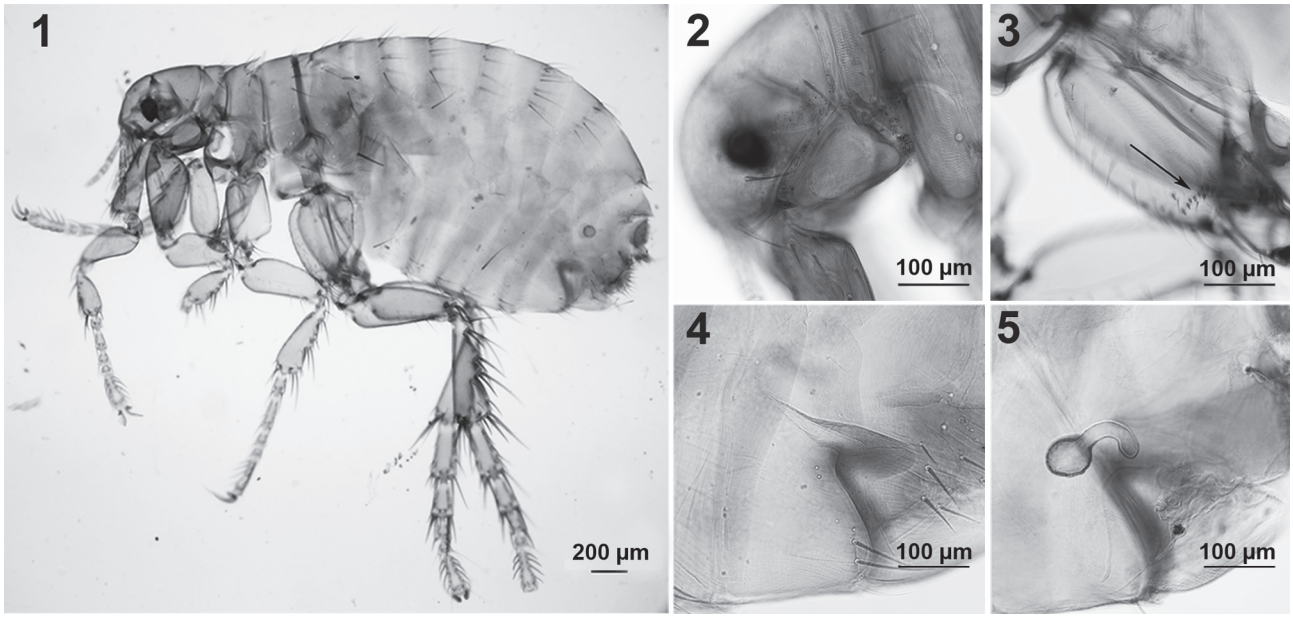
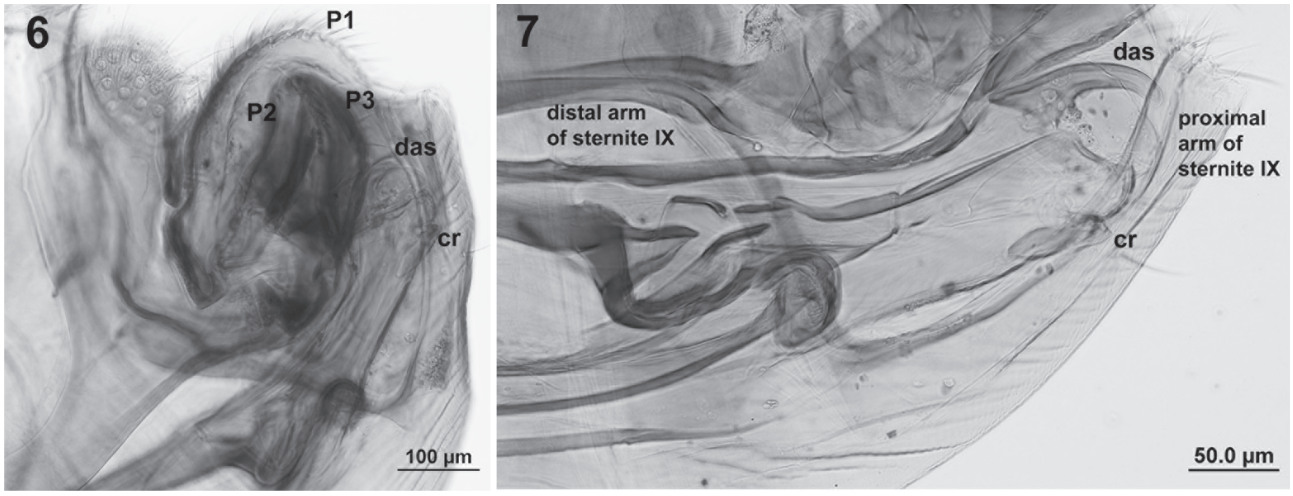


Figures 1-5. Female of *Pulex irritans* from the Chacoan peccary. 1, General view; 2, details of the head; 3, details of a row of small spines forming a patch on inside of the hind coxa; 4, detail of sternite VII with a sinus; 5, detail of the spermatheca.



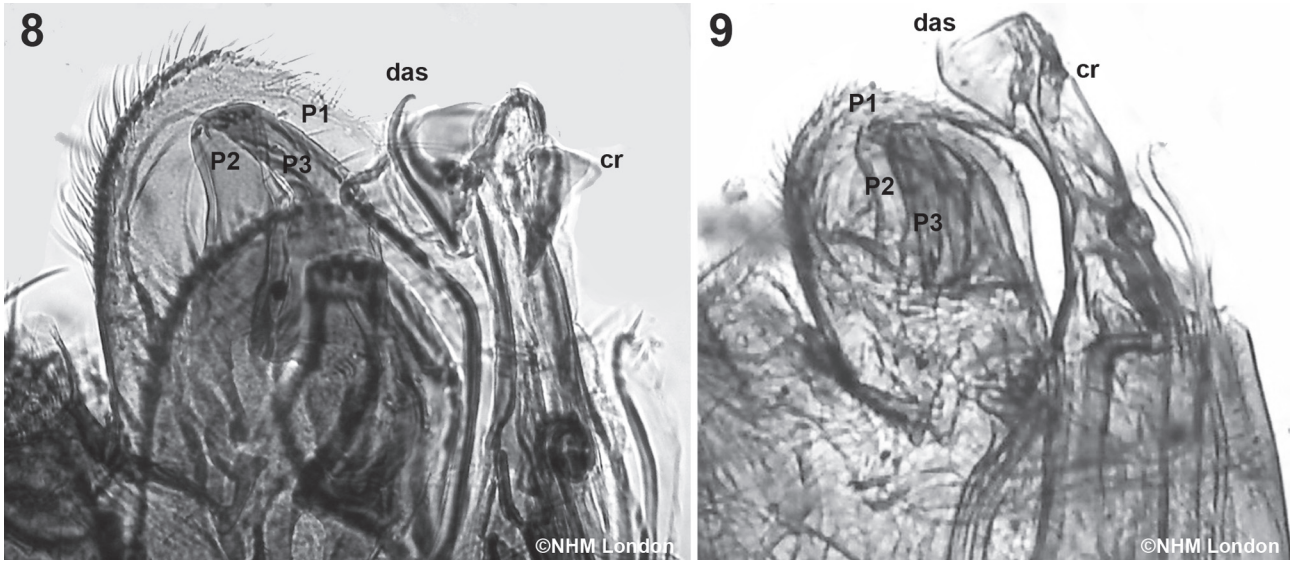
Figures 6-7. Male of *Pulex irritans*: detail of the genitalia and associated segments; 6, flea from the Chacoan peccary, (das) dorsal aedeagal sclerite; (cr) crochet; (P1), (P2) and (P3) processes 1, 2 and 3 of the clasper; 7, flea from the Pampas fox, (cr) crochet.

contrast, they differ with *P. simulans* (Fig. 9) because of the shape of the aedeagal sclerite (das) (broad, although not well visible at the photograph); processes 1 (P1) of the clasper shorter, not covering completely processes 2 (P2) and 3 (P3); and the crochet (cr) almost straight and rodlike, with a very little expansion.

Rickettsia and *Bartonella* DNA was not detected in any flea tested by PCR.

Discussion

Specimens of *P. irritans* and *P. simulans* have been commonly confused in numerous studies because of the difficulty to identify them with certainty (Barrera, 1955). Smit (1958) recognized that there are usually more setae on each side of sternite VII of the females of *P. simulans* than in *P. irritans*. However, some authors (Durden et al.,



Figures 8-9. Male of *Pulex simulans*: 8, neotype, (das) dorsal aedeagal sclerite; (cr) crochet; (P1), (P2) and (P3) processes 1, 2 and 3 of the clasper; 9, (Brit. Mus. 1949-268), (das) dorsal aedeagal sclerite; (cr) crochet; (P1), (P2) and (P3) processes 1, 2 and 3 of the clasper.

2012; Hopla, 1980) do not recognize discriminatory female features for these 2 species. We support that the combination of the following characters obtained from the literature and confirmed by the examination of specimens deposited at the NHM, allows differentiating specimens of *P. irritans* from those of *P. simulans*: number of setae on each side the sinus of sternite VII in females (4-6 in *irritans* vs. 7-9 in *simulans*); shape of aedeagal sclerite (das) (slender in *irritans* vs strong in *simulans*), length of processes 1 (P1) of the clasper (long and covering completely processes 2 and 3 in *irritans* vs short and not covering the other processes in *simulans*), and the presence (in *irritans*) or absence (in *simulans*) of an expansion in the crochet (cr). According with these diagnostic characteristics, we confirmed that fleas from northwestern Argentina herein studied were *P. irritans.* Slight differences in the expansion of the apical part of the aedeagal crochet observed between fleas from the Chacoan peccary and those from the Pampas fox are in accordance with the variation of this structure mentioned in the literature (Hopla, 1980; Smit, 1958).

Pulex irritans is the only species of the genus reported from Argentina and was previously mentioned parasitizing *L. gymnocercus* in the country without more information about the locality (Hopkins & Rothschild, 1953). Besides, *P. irritans* was reported from Salta Province associated with *Puma concolor* (Linnaeus, 1771) (Carnivora, Felidae) in captivity (Hopkins & Rothschild, 1953). The Chacoan peccary, also known as tagua, was first described in 1930 based on fossils and it was originally thought to be an extinct species (Rusconi, 1930). In 1971, it was discovered to still be alive in the Argentinean Chaco region, in the Province of Salta. The species was well known to the native people, but it took a while for scientists to acknowledge its existence (Mayer & Wetzel, 1986). The Chacoan peccary is one of the 3 living species of the family Tayassuidae, all of them found in Argentina (Gasparini et al., 2006). Peccaries entered North America from Eurasia and then extended their range into South America during the Great American Biotic Interchange. The peccaries had great taxonomic diversity and wide geographic distribution in the past. However, in the modern fauna, peccaries have a lower generic and specific diversity, and they are distributed in the American continent from the southwestern USA to North-Central Argentina (Gasparini, 2013). Even though peccaries are suggested to be the original hosts of *P. irritans* (Buckland & Sadler, 1989), there is no evidence of this parasite-host association. In Argentina, and among Artiodactyla, *P. irritans* was collected only from brocket deer (*Mazama* spp.) (Lareschi et al., 2016). In contrast, collared peccaries (*Pecari tajacu* [Linnaeus, 1758]) from Texas were found parasitized with *Pulex porcinus* Jordan & Rothschild, 1923 (Samuel & Low, 1969).

Pulex irritans is the only cosmopolitan flea in the genus *Pulex*, and this species parasitizes a variety of mammals including guinea pigs, domestic dogs, cats, rats, domestic pigs and goats (Bitam et al., 2010; Hopla, 1980;

Lareschi et al., 2016). *Pulex irritans* is a very aggressive flea and its infestations can reach tremendous levels, particularly when farmers are in contact with corrals or buildings adjacent to their homes (Bitman et al., 2010). In addition, *P. irritans* is involved in the natural cycle of different *Bartonella* species. Currently *Bartonella* species have been recognized associated with mammalian hosts and 11 species have been implicated in human diseases (Bitam et al., 2010). A *Bartonella* species, closely related to *Bartonella rochalimae*, was found in pools of *P. irritans* from foxes in the Iberian Peninsula. This bacterium is implicated as an agent of human diseases, and carnivores were confirmed as major reservoirs for *Bartonella* spp. in Spain (Márquez et al., 2009). Moreover, the identification of *B. rochalimae* in *P. irritans* from dogs in Chile supported the possibility that this flea could be a vector of this human pathogen (Pérez-Martínez et al., 2009). *Bartonella quintana* was also detected in *P. irritans* (Rolain et al., 2005). *Bartonella quintana* is a re-emerging pathogen mainly among homeless in the USA and Europe, which causes infections ranging from asymptomatic to severe illness, but classical symptoms correspond to an acute febrile illness, often accompanied by severe headache and pain in the long bones of the legs (Bitam et al., 2010). In addition, *P. irritans* is an intermediate host of some nematodes and platyhelminths (Hopla, 1980). *Pulex irritans* is also involved in the transmission of *Rickettsia felis*, which causes the flea-borne spotted rickettsiosis in humans (Brouqui & Raoult, 2006). Although few confirmed human cases have been reported, probably because they were misdiagnosed, this infection occurs worldwide (Bitam et al., 2010). In our study, *Rickettsia* and *Bartonella* DNA was not detected in any flea from the fox tested by PCR. In this sense, the results obtained are not representative of what could occur in other regions or other host species. However, since it is the first survey of these pathogens in specimens of *P. irritans* collected in Argentina, we believe that the information, although restricted and negative, may be considered relevant.

Buckland and Sadler (1989) propose that initial evolution of *P. irritans* may have involved a single host, probably a peccary. Our results report for the first time *P. irritans* parasitizing a peccary. In this sense, our results are new and may reinforce the hypothesis about the origin of *P. irritans* (Buckland & Sadler, 1989). In addition, we mentioned for the first time *P. irritans* in Salta Province associated with free ranging wild animals. Fleas from the Pampas fox were negative for *Bartonella* and *Rickettsia* DNA by PCR. More studies considering a bigger sample of fleas may be necessary for detection of the pathogens. The role of *P. irritans* as a human parasite as well as its association with the transmission of diseases implicated in public health support the necessity of new studies of this flea in Argentina, which may involve new locations and host species, and a correct identification of specimens.

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