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Fleas (Insecta: Siphonaptera) with public health relevance in domestic pigs (Artiodactyla: Suidae) from Argentina

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The presence of fleas can have a great negative impact on the productivity and well-being of the animals, and can contribute towards propagation of causative pathogens of important diseases in animals and humans. Fleas and domestic pigs (*Sus scrofa* Linnaeus, 1758) are a known association. However, knowledge about fleas on pigs in Argentina is scarce. In this country, swine farming has become one of the main components of livestock production. This activity is primarily carried out by smallholder farmers, involving a potential risk to humans of acquiring pathogens. Our study was developed in Buenos Aires province, Argentina, in a region where there is strong swine livestock activity. We provide the first record for Argentina of *Ctenocephalides felis felis* (Bouché, 1835) and *Pulex irritans* Linnaeus, 1758 in domestic pigs, and the first data for the country of parasitological indexes of fleas in pigs. *Ctenocephalides felis felis* and *P. irritans* cause discomfort, nuisance, allergic reactions, and anaemia, and are known as vectors of several pathogens with importance for public health. The high prevalence and abundance of *C. felis felis* and *P. irritans* observed in pigs from Azul, Buenos Aires, are significant, and the absence of them in other studied localities could correspond to environmental characteristics. This paper expands the knowledge about the flea diversity associated with *S. scrofa* in Argentina and contributes to the knowledge of the ecology and biology of two cosmopolitan fleas and with public health relevance. This information is important for future epidemiological studies, as well as to establish prevention and control measures where appropriate.

Fleas (Insecta: Siphonaptera) are a group of obligate hematophagous parasites of mammals and birds with more than 2500 species described worldwide (Krasnov 2008; Whiting *et al*. 2008). The presence of fleas can have a significant impact on populations and communities of their hosts (Marshall 1981; Krasnov *et al*. 2006). The insidious attacks by these parasites cause discomfort, nuisance, allergic reactions, and anaemia. Fleas are also known as vectors of the causative agents of important diseases in animals and humans, such as cat scratch disease (*Bartonella henslae*), Q fever (*Coxiella burnetii*), murine typhus (*Rickettsia typhii*), flea-borne spotted fever (*Rickettsia felis*) and bubonic plague (*Yersinia pestis*) (Linardi & Guimarães 2000; Bitam *et al*. 2010). There is no flea specific to humans, and only a minority of the species descripted are synanthropic. However, several synanthropic fleas are important for public health (e.g. *Pulex irritans, Xenopsylla cheopis, Ctenocephalides felis, Nosopsyllus fasciatus*) and they are cosmopolitan, or with wide ranges of distribution, because these fleas have been dispersed by humans, their livestock and pets (Bitam *et al*. 2010).

In Argentina, the production of the domestic pig, *Sus scrofa*, increased about 80% in the last decade, and has become one of the main components of livestock production (Brunori 2013). The major area of swine farming in the country is Buenos Aires province, where there is a strong interaction between populations of domestic pigs, *Sus scrofa domestica,* and populations of feral pigs, *Sus scrofa scrofa,* which were introduced into the region for hunting (Merino & Carpinetti 2003). The main swine production system in Argentina is the extensive system "to field", where pigs are open-air, in contact with other domestic animals, synanthropic rodents (such as rats) and occasionally wild animals (Lovera *et al*. 2015). In such a system, poor environmental hygiene conditions, precarious facilities and poor sanitary controls result in a higher frequency of parasites in pigs, including fleas. The system "to field" is primarily carried out by small farmers, with a high frequency of contact and interaction between pigs and humans (Brunori 2013). This interaction implies a potential risk to humans of acquiring parasites and pathogens that the pigs carry.

Fleas and domestic pigs are a well-known association worldwide (Mullen & Durden 2009; Durden *et al*. 2005). In

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Argentina, there are 127 known species and subspecies of fleas; however, probably due to the scarcity of studies about fleas in pigs, only *Tunga penetrans* has been recorded parasitizing *S. scrofa* (Lareschi *et al*. 2016). Herein, we provide new records of fleas of public health importance in pigs kept under extensive management systems by smallholder farmers in Buenos Aires province, Argentina. Also, we provide the first data for the country, about the parasitological indexes of fleas infesting pigs. This information expands knowledge about the flea fauna associated with *S. scrofa* in Argentina, and may be useful in epidemiological studies of flea-borne diseases in domestic mammals and humans.

As part of a study on ectoparasites of domestic pigs in Buenos Aires province, pigs from farms with the extensive management system "to field" were examined between August 2014 and August 2016. Samplings were made in seven localities: Azul (36°45'03.3"S; 59°53'38.8"W), Mariano Benítez (33º42'43"S; 60º35'16"W), Pinzon (33º59'22"S; 60º45'21"W), Ayerza (33º50'17"S; 60º34'48"W), Fontezuela (33º57'37"S; 60º28'34"W), General Viamonte (34°56′00″S; 61°10′00″W), and Junín (34°34'10"S 60°57'35"W) (Figs. 1A, B). In each farm between 10 and 20 pigs of both sexes and all age classes were viewed, examining the anterior-right flank of the body. Fleas were collected using combs and tweezers and stored in 96% ethanol. At the laboratory, fleas were cleared and softened in 10% KOH, dehydrated in an increasing series of ethanol (80% to 100%), further diaphanized in eugenol, and mounted in Canadian Balsam for study with a compound microscope. We followed the classification of Whiting *et al*. (2008) for the higher taxa. Voucher specimens will be deposited at the Colección de Entomología del Museo de La Plata (MLP, La Plata, Buenos Aires province, Argentina). Flea specimens studied are listed and the data include sex, prevalence [P= (Number of parasitized specimens / Number of examined hosts) × 100], mean abundance (MA=Number of individuals of a parasitic species / Number of examined hosts) and mean intensity (MI= Number of individuals of a parasitic species / Number of parasitized hosts) (Bush *et al*. 1997). For each species and subspecies of fleas identified a brief report is included with comments on their known geographical distribution, known host species in Argentina and health significance. Also, main diagnostic characteristics observed in all specimens studied are mentioned.

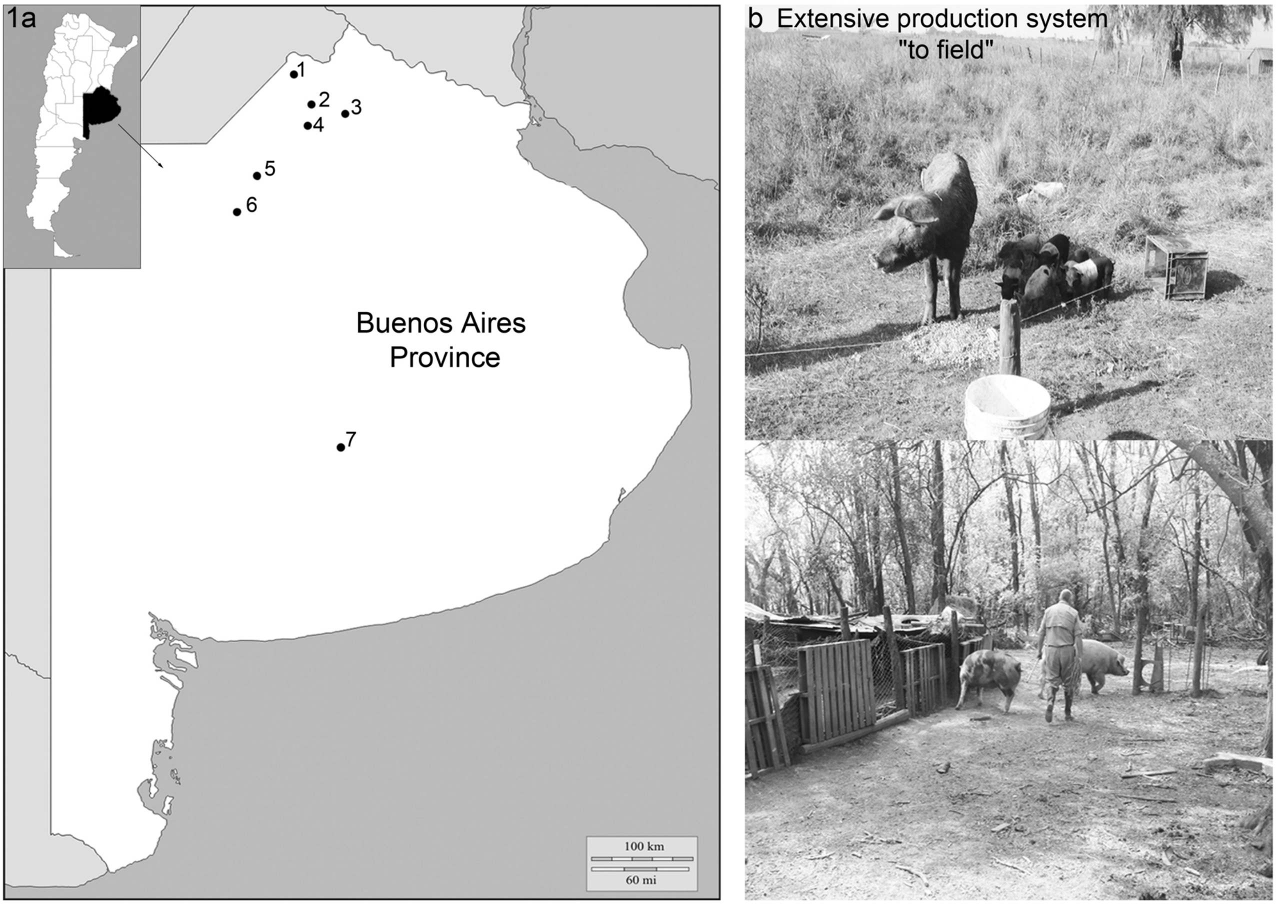

FIGURE 1. Locations and pig production. A) Geographical locations of the studied farms: 1—Mariano Benítez (33º42'43"S; 60º35'16"W), 2—Ayerza (33º50'17"S; 60º34'48"W), 3—Fontezuela (33º57'37"S; 60º28'34"W), 4—Pinzon (33º59'22"S; 60º45'21"W), 5—Junín (34°34'10"S 60°57'35"W), 6—General Viamonte (34°56′00″S; 61°10′00″W), and 7—Azul (36°45'03.3"S; 59°53'38.8"W). B) Illustrative images of extensive production system "to field", in farms from Buenos Aires Province.

In total, 120 pigs from 8 farms were examined. Only pigs from Azul (n= 30) were parasitized by fleas (n= 38). Fleas were identified as follows.

Family Pulicidae Billberg, 1820
Subfamily Pulicinae Tiraboschi, 1904
Pulex irritans Linnaeus, 1758

Type host and locality. "Man"; Sweden, Suecia.

Geographic range. Cosmopolitan. In Argentina (Fig. 2): provinces of Catamarca, Córdoba, Chubut, Formosa, La Rioja, Mendoza, Neuquén, Río Negro, Salta, San Luis, Santiago del Estero, and Tucumán. In Buenos Aires province: Ajó (General Lavalle), Bahía Blanca, Bonifacio, Cañada Mariano, La Plata, and Los Yngleses (Lareschi *et al*. 2016).

Other known hosts for Argentina. Mammalia-Artiodactyla—*Mazama americana, Mazama gouazoubira.* Didelphimorphia—Didelphidae—*Didelphis albiventris, D. aurita*, *Didelphis* sp. Carnivora—Canidae—*Canis familiaris, Lycalopex griseus, L. gymnocercus*. Felidae—*Puma concolor*. Mephitidae—*Conepatus humboldtii*, *C. chinga*. Mustelidae—*Galictis cuja*. Procyonidae—*Nasua nasua*. Lagomorpha—*Lepus europeus*, *Lepus* sp. Rodentia—Caviidae—*Dolichotis patagonum*, *Microcavia australis*. Chinchillidae—*Lagidium viscacia*, *Lagostomus maximus*. Ctenomyidae—*Ctenomys* sp. Cricetidae—*Graomys griseoflavus*. Muridae—*Mus musculus* (Lareschi *et al*. 2016).

Specimens examined. Azul, Buenos Aires province, 10 females (MLP JS1_1, MLP JS2_1, MLP JS3_1, MLP JS3_2, MLP JS6_1, MLP JS7_1, MLP JS8_1, MLP JS10_1, MLP JS12, MLP JS14_1); 7 males (MLP JS1_2, MLP JS4, MLP JS5, MLP JS9, MLP JS11_1, MLP JS13_1, MLP JS13_2).

parasitological indexes. $n_{(\text{fleas anterior-right flank})}=17$; $n_{(\text{pigs})} = 30$; $n_{(\text{parasitized pigs})} = 14$; prevalence (anterior-right flank) = 46.7%; mean abundance (anterior-right flank) = 0.5; mean intensity (anterior-right flank) = 1.2.

Remarks. *Pulex irritans* is characterized by the following morphological characters: Frons smoothly rounded, much higher than long; without a tubercle. Internal incrassation of frons hardly projecting inwards from margin of frons. Antennal club asymmetrical. Eye large, dark, subangulate ventrally; with single ocular setae. Occipital region with only one visible setae. Labial palpus stiff, more than a half to length of fore coxa; with four segment and anterior side more strongly sclerotized than posterior. Mandibles not enlarged, much shorter than maxillary palpus. Metanotum longer than tergite I. Pleural rod of mesothorax absent. Hind coxa with a row of small spiniforms on inside, near apex, forming a patch. Male with fixed process of clasper broad, very large and covering the other two processes of clasper. Female with fore margin of sternite VII sclerotized. Sternite VIII truncate, very pale and poorly sclerotized. Tergite VIII with a numerous setae on apex. Bulga of spermatheca subglobular, hila longer, almost twice the length of the bulga.

Pulex spp. are known to feed on pigs (*Pulex irritans*, *Pulex porcinus* and *Pulex simulans*). Piggeries often become the source of acute human infections after the pigs have been taken to market (Durden *et al*. 2005; Mullen & Durden 2009). Particularly, *P. irritans* is mistakenly called the human flea, although it attacks a wide variety of mammals, including domesticated animals and wildlife. Infestations in humans can reach tremendous levels, particularly when farmers share their dwellings with their livestock, or hold these animals in corrals or buildings adjacent to their homes (Bitam *et al*. 2010). This flea is a recognized vector of pathogens causing salmonella, bubonic plague, tularemia and murine typhus (Mullen & Durden 2009).

Ctenocephalides felis felis (Bouché, 1835)

Type host and locality. "Housecat"; Type locality not indicated.

Geographic range. Cosmopolitan. In Argentina the distribution probably includes all the country; however, these are the published records (Fig. 2): Chaco, La Pampa, La Rioja, Santiago del Estero, and Tucumán. In Buenos Aires province: Los Yngleses and Ajó (General Lavalle) (Lareschi et al. 2016).

Other known hosts for Argentina. Mammalia-Artiodactyla-Cervidae—*M. americana, M. nana.* Didelphimorphia-Didelphidae—*D. albiventris, Lutreolina crassicaudata, Didelphis* sp. Carnivora-Canidae—*C. familiaris, L. griseus, L. gymnocercus.* Felidae—*Felis catus, P. concolor.* Procyonidae—*N. nasua. Rodentia*—Cricetidae—*G. griseoflavus.* Muridae—*Rattus* sp. Primates—Hominidae-*Homo sapiens* (Lareschi *et al.* 2016).

Specimens examined. Azul, Buenos Aires province, 13 females (MLP JS1_3, MLP JS1_4, MLP JS1_5, MLP JS3_3, MLP JS6_2, MLP JS7_2, MLP JS7_3, MLP JS10_2, MLP JS10_3, MLP JS13_3, MLP JS14_2, MLP JS15_1, MLP JS15_3); 8 males (MLP JS1_6, MLP JS1_7, MLP JS6_3, MLP JS6_4, MLP JS7_4, MLP JS7_5, MLP JS13_4, MLP JS15_2).

parasitological indexes. $n_{(\text{fleas anterior-right flank})} = 21$; $n_{(\text{pigs})} = 30$; $n_{(\text{parasitized pigs})} = 8$; prevalence (anterior-right flank) = 27%; Mean abundance (anterior-right flank) = 0.7; mean intensity (anterior-right flank)= 2.6

Remarks. *Ctenocephalides felis felis* is characterized by the following morphological characters: Head longer; frons oblique and strongly curved. Genal comb horizontal, with 8 spines each side, first spine of genal comb about same length as second. Eye large, dark. Occiput bearing 1 long bristle above middle of antennal fossa. Labial palpus with five segments; anterior margin thickened, well sclerotized. Fore tarsal segment V of male with only 2 sub-apical spiniform setae. Metepisternum with 1 or 2 long setae. Male with sternite VIII enlarged, enclosing most of tail end posteriorly. Fixed process of clasper with divided apical portion. Movable process of clasper arising from fixed process proper. Manubrium of clasper little dilated apically. Female without small setae above antennal fossa. Spermatheca with sub-globular bulga and hila longer and with end curved.

The cat flea, *C. felis felis*, is extremely common on cats and dogs in many temperate and tropical regions, but it also infests rats and other wildlife. It represents the great majority of fleas in human homes (Durden *et al*. 2005; Bitam *et al*. 2010). This flea is a recognized vector of *Rickettsia felis* in several countries, including Argentina (Brown & Macaluso 2016; Nava *et al*. 2008). Infection with this agent in humans produces a disease known as flea-borne spotted fever (or cat flea typhus). This disease is considered an emergent global threat to human health, with cases likely underestimated due to similarities in clinical signs with other febrile illnesses (e.g. fever, rash, headache, and myalgia) and limited access to appropriate laboratory tests (Brown & Macaluso 2016). Moreover, this flea species can be naturally infected with *Rickettsia typhi,* the causative agent of murine typhus, a zoonotic disease that circulates in rodents via the oriental rat flea. It is also a vector of *Bartonella henselae,* the causative agent of Cat scratch disease (Bitam *et al*. 2010).

This study represents the first records of *C. felis felis* and *P. irritans* parasitizing *S. scrofa* in Argentina. From an epidemiological point of view *C. felis felis* and *P. irritans* are vectors of several pathogens in various regions of the world, including Argentina (Nava *et al*. 2008; Bitam *et al*. 2010). Both species of fleas are considered synanthropic and cosmopolitan, being able to disperse pathogens to new regions and hosts (Bitam *et al*. 2010). As noted by several authors, despite their cosmopolitanism, distribution of these fleas is not uniform. Instead, they are distributed in patches characterized by host and environmental conditions that are favourable for each given species (see Traub 1980; Beaucournu & Menier 1998; Beaucournu & Pascal 1998 in Krasnov 2008). Our results agree with these authors, because considering that the management conditions (e.g. infrastructure, sanitary controls) of all farms examined in this study were similar, fleas were found only in farms in central Buenos Aires province (Azul locality); in the north of the province these parasites were not found. Parasitological index values obtained in this study (tested only for an area of the host's body, see Materials and Methods) suggest that the presence of both flea species in Azul is not accidental. These results contribute to the knowledge of the biology of these fleas whose presence could respond to environmental characteristics. In most countries of the world there is constant concern over flea control, although the incidence of flea-borne diseases is much greater than is generally recognized by physicians and health authorities. Fleas, as hosts for a wide range of largely understudied pathogens, are no exception, and flea-borne diseases may re-emerge in epidemic form. Examples of this are the changing ecology of murine typhus, the finding of *Rickettsia* spp. in new hosts, and the finding of fleas on new hosts or in geographical areas previously unreported in the literature. The past decades have seen a dramatic change in geographic and host ranges of many vector-borne pathogens and the diseases they cause. This process is often driven by climate change and the destruction of wild habitats due to human behaviour modifications (Bitam *et al.* 2010). Agricultural and livestock development is one of the factors of emergence and re-emergence of diseases, as a common pathway in altering the environment (Jones *et al*. 2013).

Considering that in the studied region in this work a strong swine livestock activity is carried out, with a high frequency of contact between pigs and humans, the presence of two fleas with sanitary importance in domestic pigs involves a potential risk to humans to acquire pathogens. Also, these findings represent a risk factor for humans for other two main reasons: 1) in pig farms of Argentina rodents (mainly rats and mice) are very common (Lovera *et al*. 2015), and are recognized reservoirs of several of the zoonotic pathogens transmitted by fleas (Linardi & Guimarães 2000; Bitam *et al*. 2010); 2) there is strong interaction between domestic and wild pigs in the province of Buenos Aires, presenting an opportunity for pathogens, previously eradicated from domestic populations of pigs, to be re-introduced into those specific pathogen-free populations (Cooper *et al*. 2010). Feral pig infectious disease research is focused on the threat that feral pigs could contaminate clean domestic herds and is based on the previously known parasites and pathogens of domestic pigs and their long established disease cycles. Some bacterial and parasitic pathogens of feral pigs that can be potentially transmitted to humans and cause disease are bubonic plague and tularemia, and emerging viral diseases of high impact on human health, such as influenza and Hepatitis E (HEV) (Graves 1984; Mullen & Durden 2009). A few epidemiological studies of feral pigs conducted in Argentina have detected the transmission of hemoparasites as *Babesia* spp., *Anaplasma* spp., and *Mycoplasma suis*, whose vectors could be haematophagous ectoparasites, to domestic pigs (Scioscia *et al*. 2011).

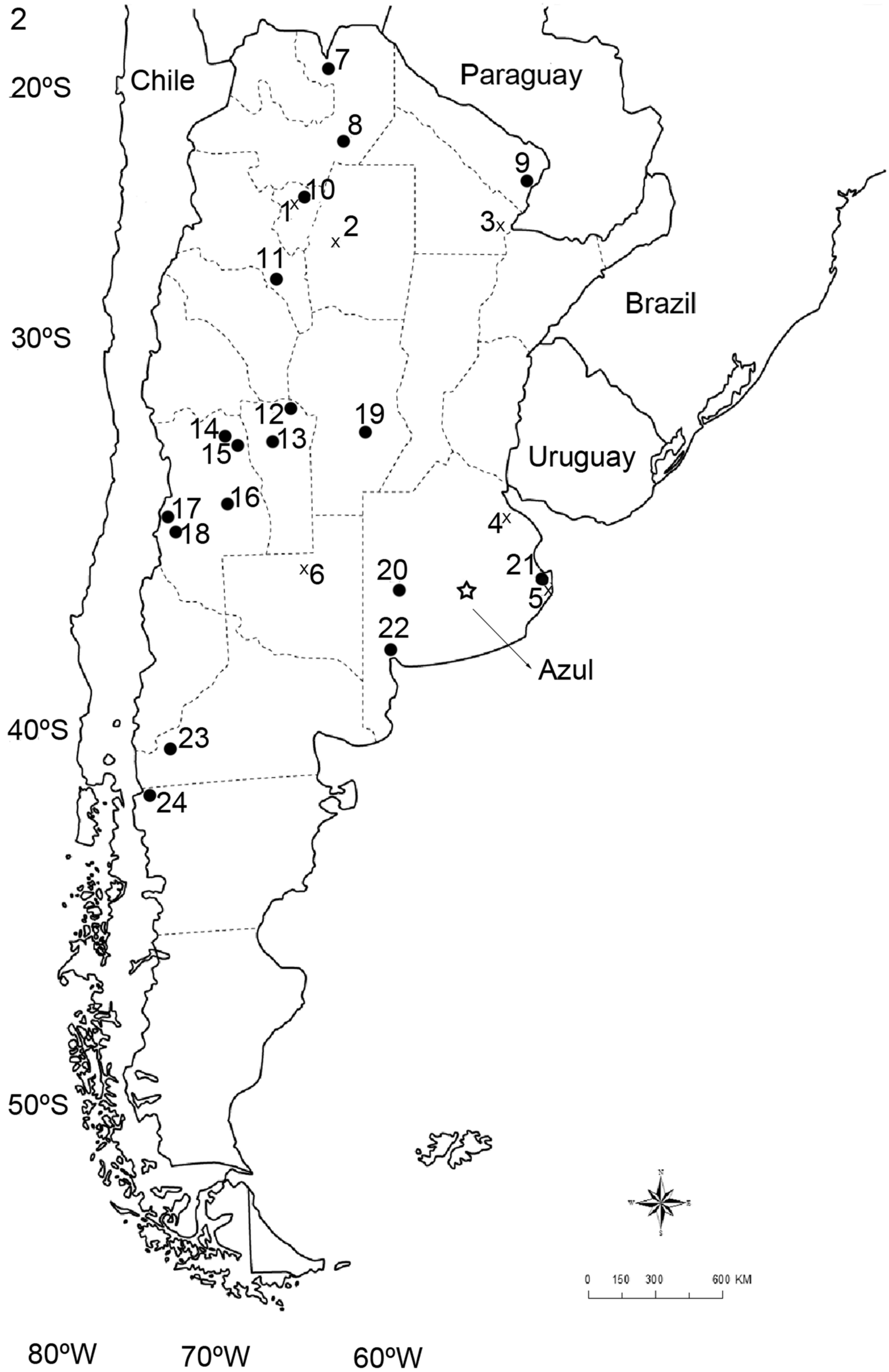

FIGURE 2. Geographical distribution in Argentina of *Ctenocephalides felis felis* (**x**) and *Pulex irritans* (●) (Lareschi *et al.* 2016): 1—Tucumán, 2—Santiago del Estero, 3—Gran Chaco, Chaco, 4—Los Yngleses, Buenos Aires, 5—Ajó, Buenos Aires, 6—La Pampa, 7—Orán (Isla de Cañas), Salta, 8—El Quebrachal, Salta, 9—Formosa, 10—Tucumán, 11—Chumbicha, Catamarca, 12—Quines, San Luis, 13—San Luis capital, 14—Las Catitas, Mendoza, 15—La Paz, Mendoza, 16—San Rafael, Mendoza, 17—Los Molles, Mendoza, 18-Malargüe, Mendoza, 19—Santa Eufemia, Córdoba, 20—Cañada Mariano, Buenos Aires, 21—Ajó (General Lavalle), Buenos Aires, 22—Bahía Blanca, Buenos Aires, 23—Pilcaniyeu, Río Negro, 24—Lago Epuyén, Chubut.

Our results expand knowledge about the flea fauna associated with *S. scrofa,* and enable us to know more about the distribution, biology and ecology of *P. irritans* and *C. felis felis*. This information is relevant for smallholder farmers working on pigs and also for people engaged in all aspects of public health surveillance so they are aware of the distribution of these two flea species and prepared to control them when necessary.

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