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Short Communication

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A new record of fleas from nilgai antelope in southern Texas and fleas from other wildlife

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Wildlife are hosts of ectoparasites, such as fleas and ticks that may transmit human and animal pathogens. Little is known about the ecology of many ectoparasite species native to southern Texas, or their role in pathogen maintenance and transmission. Much attention has been given to the role of nonnative nilgai antelope as cattle fever tick hosts and agents of dispersal, but little attention has been given to other ectoparasites that may utilize nilgai antelope as hosts. As southern Texas is a hot-spot for flea-borne (murine) typhus, it is important to examine flea species presence, abundance, and host use in this region. Fleas were opportunistically collected during wildlife depredation activities, from hunter-harvested animals, or during handling in the course of other research activities in several southern Texas counties. A total of 9 wildlife species were sampled, from which 3 flea species were identified. A total of 83 *Pulex porcinus* (Jordan and Rothschild) were collected from nilgai, coyotes, bobcats, javelina, feral swine, and a black-tailed jackrabbit. In total, 9 *Euhoplopsyllus glacialis affinis* (Baker) were collected from cottontail rabbits, and 1 *Echidnophaga gallinacea* (Westwood) was collected from a raccoon. To our knowledge, this represents the first report of fleas from nilgai antelope. *Pulex porcinus*, although often considered a specialist species, was collected from a wide range of hosts, including 2 (nilgai antelope and black-tailed jackrabbit) that represent new host records for this species. The role of *P. porcinus* as a pathogen vector is unknown, but its apparent abundance in this region warrants further investigation.

Key words: flea, nilgai, wildlife, Texas, javelina

Introduction

Nilgai (*Boselaphus tragocamelus* Pallas) antelope is an invasive Indian antelope that was introduced to Texas, USA, between the 1920s and 1930s (Sheffield et al. 1971, Sheffield 1983). They are hosts of the southern cattle fever tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) in southern Texas (Goolsby et al. 2017). Since their initial introduction, free-ranging nilgai antelope populations have expanded across much of southern Texas, causing concern over their potential contribution to the spread of *R.* (*B.*) *microplus* (Lohmeyer et al., 2018) in their invasive range within southern Texas. Nilgai are long-distance dispersers (Foley et al. 2017) with the potential to move ectoparasites (such as fleas or ticks) or pathogens long distances. Most current studies involving nilgai in southern Texas are focused on nilgai ecology, or nilgai as hosts of cattle fever ticks and their potential to act as a reservoir for bovine babesiosis. Here, we consider nilgai and cattle fever ticks as a model for the potential of nilgai to spread fleas and flea-borne pathogens in southern Texas.

Studies on the ecology of fleas in the Rio Grande Valley (RGV) are few, with most focusing on flea-borne typhus, caused by *Rickettsia typhi* (Azad et al. 1997, Blanton et al. 2016) and primarily older literature available with regard to basic flea ecology (Eads 1951, Samuel and Trainer 1970). Human cases of flea-borne typhus in the RGV are among the highest in the United States (Irons et al. 1952, Anstead 2021, Wang et al. 2022). One of the most commonly studied fleas in southern Texas is the cat flea (*Ctenocephalides felis* Bouché), a common vector of the agent of flea-borne typhus (Azad et al. 1997, Blanton et al. 2016, 2019). However, many other species of fleas exist in southern Texas (Randolph et al. 1946) and many are of potential importance to wildlife and/or human health (Ewing and Fox 1943, Hubbard 1947). The existing literature for the flea

fauna in Texas and the literature regarding fleas on ungulates in Texas is in need of update, especially with the introduction of exotic ungulates for hunting into the state. Although fleas on ungulates in North America are uncommon (McCauley et al. 2008), some reports exist of the flea *Pulex porcinus* (Jordan and Rothschild), colloquially known as the javelina flea, infesting white-tail deer (*Odocoileus virginianus* Zimmerman) in southern Texas (Samuel and Trainer 1970). At the time of this writing, the authors have seen no records of fleas from nilgai in the United States or in their native range. Here, we report the first finding of fleas from nilgai and the abundance and composition of fleas from some other wildlife sampled in southern Texas.

Materials and Methods

Observations for this short communication were made while conducting general tick surveillance in Hidalgo, Starr, and Cameron Counties, Texas, USA. Flea collections were opportunistic and were not the focus of animal sampling. Fleas were collected from depredated wildlife, hunter-harvested animals, or live-captured animals handled in the course of sampling for other research efforts from May 2022 to February 2024. In one case, fleas were collected from the clothing of field technicians and from dry ice traps while conducting routine tick surveillance. Dry ice traps consisted of 3.8-l water coolers filled with approximately 1 kg of pelleted dry ice. Depredation was aimed at nuisance animals, such as feral swine (*Sus scrofa* L.), coyotes (*Canis latrans* Say), rabbits (Leporidae), and nilgai. Depredation activities were carried out by USDA-APHIS Wildlife Services personnel under approved protocols. We did not distinguish between cottontail species as both desert cottontails (*Sylvilagus audubonii* Baird) and eastern cottontails (*Sylvilagus floridanus* Allen) are present in sampled areas of southern Texas. Fleas were removed from animals with forceps, placed in 70% ethanol, and stored at room temperature. Live animal handling of nilgai was carried out under the USDA-ARS IACUC-approved protocol #2023-08.

Fleas were identified to species and sex using the keys of Ewing and Fox (1943), Hubbard (1947), and Lewis et al. (1988). Images of male and female voucher specimens were taken with a VHX-7000 digital stereomicroscope (Keyence, Itasca, IL, USA) (Fig. 1). Voucher specimens were then cleared for better visualization of the genitalia. Samples were placed in 10% KOH for 1–2 days, then rinsed sequentially with glacial acetic acid, distilled water, 70% ethanol, 95% ethanol, 100% ethanol twice, xylene, and then mounted on a slide in Canada balsam (Fig. 2). Voucher specimens are stored at the USDA Cattle Fever Tick Research Unit.

Three fleas were selected for barcoding based on putative morphological identification to add currently lacking molecular characterization data to morphology data for selected species. Individual fleas were homogenized in 200 µl of lysis buffer T1 and proteinase K with four 2.8-mm ceramic beads for 2 cycles of 30 s at a speed of 4,000 on a Bead Ruptor 24 (Omni International, Kennesaw, GA, USA). Whole genomic DNA was extracted from the homogenate following the manufacturer's instructions using the NucleoMag Tissue kit (Macherey Nagel, Germany) on an IsoPure Mini (Accuris Instruments, Edison, NJ, USA).

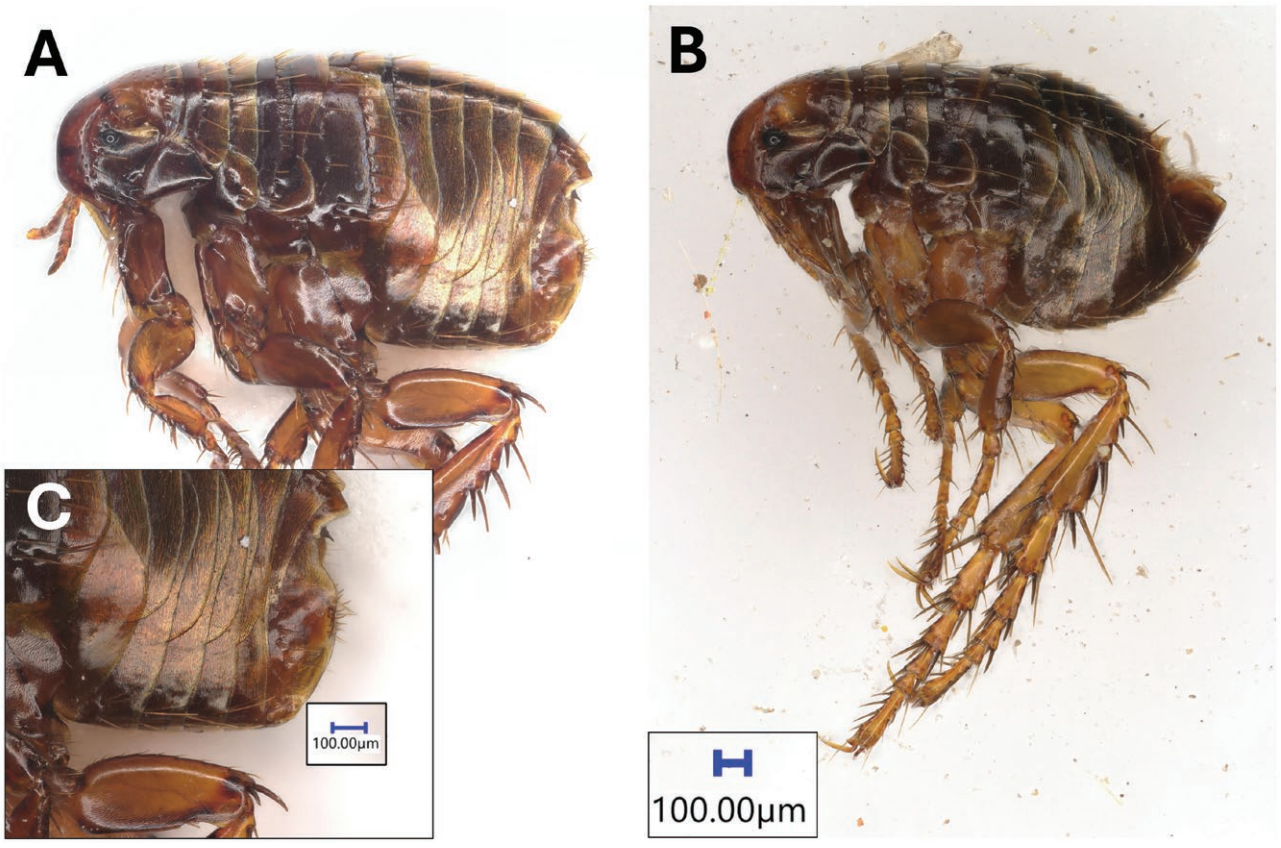

Fig. 1. Images of *Pulex porcinus* specimens. A) Female specimen (150× magnification). B) Male specimen (200× magnification). C) Female posterior region (200× magnification). All Images were taken with an E100 lens on a VHX-7000 digital stereomicroscope (Keyence). Of note are the true morphological spines of the genal comb and rounded frontal margin of the head.

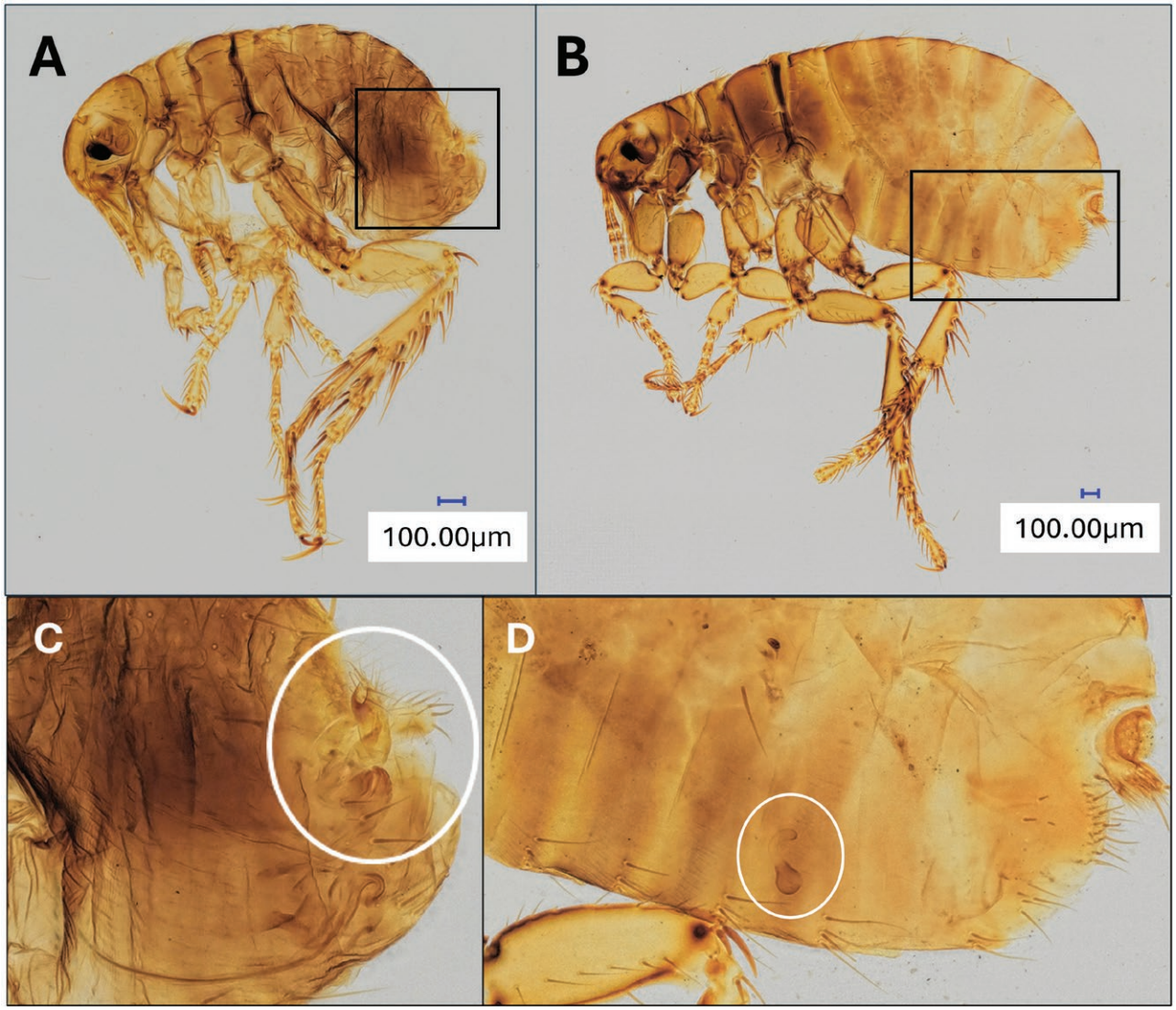

Fig. 2. Images of cleared specimens of *Pulex porcinus*. A) Male specimen. The black rectangle indicates the magnified region of the accompanying inset. B) Female specimen with a black rectangle indicating the magnified region of the accompanying inset. C) Magnified inset from Fig. 2A demonstrating the clasper, sternite IX, and paramere of the male. D) Magnified inset from Fig. 2B demonstrating the spermatheca (white circle) of the female. Images were taken with an E100 objective at 300× on a VHX-7000 (Keyence).

Universal primers were used to amplify *cox1* (LCO1490—5′ GGTCAACAAATCATAAAGATATTGG 3′, HCO2198—5′ TAAAC TTCAGGGTGACCAAAAAATCA 3′) and 12S (T2A—5′ AATGAG AGCGACGGGCGATGT 3′, T1B—5′ AAACTAGGATTAGATA CCCT 3′) gene regions (Folmer et al. 1994, Beati and Keirans 2001) for comparison to existing data from related *Pulex* species. The 25-µl PCR mix was composed of 12.5 µl of 2× Platinum II PCR Mastermix (Invitrogen, Carlsbad, CA, USA), 0.75 µl of each of the forward and reverse primers (0.3 µM reaction concentration), 10 µl of molecular grade water, and 1 µl of DNA template. All PCRs were prepared on a Myra liquid handler (Bio Molecular Systems, Upper Coomera, Australia) and run on a VeritiPro thermal cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA). The 12S thermal cycling reaction conditions were as described in those of Beati and Keirans (2001). The *cox*1 thermal cycling conditions followed the protocol of Lv et al. (2014).

PCR products for *cox1* and 12S amplifications were purified with an ExoSap-IT kit (Thermo Fisher Scientific Inc.) and sent for Sanger sequencing (Retrogen, San Diego, CA, USA). Sequence quality was assessed by their Q-scores with Geneious Prime software (v2024.0). Resulting consensus sequences were submitted to the NCBI Genbank database.

Results

A total of 132 animals were sampled: 65 nilgai, 34 feral swine, 11 coyotes, 8 black-tailed jackrabbits (*Lepus californicus* Gray), 4 raccoons (*Procyon lotor* (L.)), 3 javelina (*Pecari tajacu* L.), 3 opossums (*Didelphis virginiana* Kerr), 3 cottontail rabbits (*Sylvilagus* spp.), and 1 bobcat (*Lynx rufous* Schreber). We found a total of 93 fleas of 3 species, *P. porcinus* ($N = 83$), *Euhoplospyllus glacialis affinis* (Baker) ($N = 9$), and *Echidnophaga gallinacea* (Westwood) ($N = 1$). *Pulex porcinus* was the most common species, being found on nilgai, a bobcat, feral swine, javelina, a black-tailed jackrabbit, coyotes, and CO_2 traps. *Euhoplospyllus glacialis affinis* was found on cottontail rabbits and the 1 *E. gallinacea* was found on a raccoon (Table 1).

Table 1. Flea species collected from depredated, hunter-harvested, and live captured wildlife and dry ice traps in southern Texas, May 2022—February 2024.

Host species	No. sampled	No. infested	Flea species		
			Pulex porcinus	*Euhoplopsyllus glacialis*	*Echidnophaga gallinacea*
Nilgai (*Boselaphus tragocamelus*)	65	3	7	0	0
Coyote (*Canis latrans*)	11	2	2	0	0
Virginia Opossum (*Didelphis virginianus*)	3	0	0	0	0
Black-tailed Jackrabbit (*Lepus californicus*)	8	1	1	0	0
Bobcat (*Lynx rufus*)	1	1	5	0	0
Javelina (*Pecari tajacu*)	3	3	19	0	0
Raccoon (*Procyon lotor*)	4	1	0	0	1
Feral swine (*Sus scrofa*)	34	14	29	0	0
Cottontail spp (*Sylvilagus* spp.)	3	2	0	9	0
CO_2 Traps	-	2	20	0	0

Approximately 21% of sampled animals were infested with fleas; 89% of the collected fleas were *P. porcinus*. Flea collections from CO_2 traps were extremely rare, with fleas being detected from less than 2% of CO_2 traps deployed over the course of this study.

Amplicons for the *cox1* gene were successfully sequenced from 2 of the 3 *P. porcinus* samples, and an amplicon for the 12S gene was successfully sequenced from 1 of the 3 samples. When compared with available sequences in the NCBI database, samples shared 94.85% identity with *Pulex irritans* (L.) 12S sequences (NC_063709), and 89.96% identity with *P. irritans cox1* sequences (LT797467). Gene fragments for the *cox1* and 12S genes for *P. porcinus* were submitted to the NCBI Genbank repository (*cox1*: PP866731, PP866730; 12S: PP864703).

Discussion

Pulex porcinus previously has been reported from white-tailed deer, coyotes, bobcats, and feral swine (Eads 1951, Samuel and Trainer 1970); however, this is the first known record of *P. porcinus* from nilgai, and to our knowledge, the first report of fleas of any species collected from nilgai. The role of nilgai as hosts for native ectoparasite pests and vectors warrants further investigation given their free-ranging status and increasing populations in southern Texas. While the vector competence of *P. porcinus* for common flea-borne pathogens is not known, southern Texas is a hotspot for human cases of flea-borne *Rickettsia* (Pieracci et al. 2017). Additionally, molecular detections have been made of several *Bartonella* species in other *Pulex* species (Yore et al. 2014, López-Pérez et al. 2017). The relationship of *P. porcinus* with pathogens of human and companion animals should be further investigated.

These are the first available gene fragments for the *cox1* and 12S genes for *P. porcinus* and provide additional molecular information on a species with a restricted range in the United States. Generating molecular data on this little-known species is important to linking phylogenetics with traditional taxonomic approaches using morphological characters, such as the presence of true spines of the genal comb that distinguish members of the former genus *Juxtapulex* (Ewing and Fox 1943) for species identification; or in determining potential relationships between fleas and pathogens in pathogen maintenance and transmission in southern Texas ecosystems.

Personal observation of the authors indicates that *P. porcinus* will bite people. The abundance of this flea in wildlife collections in this region and its apparently broad host range highlight the importance of investigating understudied species and suggest a need to further evaluate the relationship of this flea with *Rickettsia* and *Bartonella* in rural southern Texas. Although the overall wildlife sample size herein is small, it is important to update the literature as the landscape changes with the introduction of exotic species and continued expansion of some species such as feral swine which have become ubiquitous in the southern Texas landscape. The introduction of exotic species may complicate the dynamics of wildlife disease transmission in southern Texas by providing additional species to act as reservoir hosts of endemic pathogens or as sources of introduced pathogens.

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Author contributions

Sarah Mays Maestas (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal],

Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Jason Tidwell (Formal analysis [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), John Goolsby (Conceptualization [equal], Investigation [equal], Resources [equal], Writing—review & editing [equal]), and Lauren Maestas (Conceptualization [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal])

References

Anstead GM. 2021. History, rats, fleas, and opossums: II. the decline and resurgence of flea-borne typhus in the United States, 1945–2019. Trop. Med. Infect. Dis. 6(1):2. https://doi.org/10.3390/tropicalmed6010002

Azad AF, Radulovic S, Higgins JA, et al. 1997. Flea-borne rickettsioses: ecologic considerations. Emerg. Infect. Dis. 3(3):319–327. https://doi.org/10.3201/eid0303.970308

Beati L, Keirans JE. 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J. Parasitol. 87(1):32–48. https://doi.org/10.1645/0022-3395(2001)087[0032:AOTSRA]2.0.CO;2

Blanton LS, Idowu BM, Tatsch TN, et al. 2016. Opossums and cat fleas: new insights in the ecology of murine typhus in Galveston, Texas. Am. J. Trop. Med. Hyg. 95(2):457–461. https://doi.org/10.4269/ajtmh.16-0197

Blanton LS, Vohra RF, Fistein L, et al. 2019. Rickettsiae within the fleas of feral cats in Galveston, Texas. Vector Borne Zoonotic Dis. 19(9):647–651. https://doi.org/10.1089/vbz.2018.2402

Eads RB. 1951. A note on the javelina flea *Juxtapulex porcinus*. J. Parasitol. 37(3):328. https://doi.org/10.2307/3273229

Ewing HE, Fox I. 1943. The fleas of North America: classification, identification, and geographic distribution of these injurious and disease-spreading insects (No. 500). Washington, DC: US Department of Agriculture.

Foley AM, Goolsby JA, Ortega S-A, et al. 2017. Movement patterns of nilgai antelope in South Texas: implications for cattle fever tick management. Prev. Vet. Med. 146:166–172. https://doi.org/10.1016/j.prevetmed.2017.08.002

Folmer O, Black M, Hoeh W, et al. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3(5):294–299. https://doi.org/10.1071/ZO9660275

Goolsby JA, Singh NK, Ortega-S A, et al. 2017. Comparison of natural and artificial odor lures for nilgai (Boselaphus tragocamelus) and white-tailed deer (Odocoileus virginianus) in South Texas: Developing treatment for cattle fever tick eradication. Inter. J. Parasitol.: Parasite. Wildlife 6(2):100–107. https://doi.org/10.1016/j.ijppaw.2017.04.006

Hubbard, CA, 1947. Fleas of western North America: Their relation to the public health. Ames (IA): Iowa State College Press.

Irons JV, Eads RB, Johnson CW, et al. 1952. Southwest Texas Q fever studies. J. Parasitol. 38(1):1–5.

Lewis RE, Lewis JH, Maser C. 1988. The fleas of the Pacific Northwest. Corvallis (OR): Oregon State University Press.

Lohmeyer KH, May MA, Thomas DB, et al. 2018. Implication of nilgai antelope (Artiodactyla: Bovidae) in reinfestations of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) in South Texas: a review and update. J. Med. Entomol. 55(3):515–522. https://doi.org/10.1093/jme/tjy004

López-Pérez AM, Osikowicz L, Bai Y, et al. 2017. Prevalence and phylogenetic analysis of Bartonella species of wild carnivores and their fleas in northwestern Mexico. Ecohealth. 14(1):116–129. https://doi.org/10.1007/s10393-017-1216-2

Lv J, Wu S, Zhang Y, et al. 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). Parasit. Vectors. 7(93):1–11. https://doi.org/10.1186/1756-3305-7-93

McCauley DJ, Keesing F, Young T, et al. 2008. Effects of the removal of large herbivores on fleas of small mammals. J. Vector Ecol. 33(2):263–268. https://doi.org/10.3376/1081-1710-33.2.263

Pieracci EG, Evert N, Drexler NA, et al. 2017. Fatal flea-borne typhus in Texas: a retrospective case series, 1985–2015. Am. J. Tropical Med. Hygiene. 96(5):1088.

Randolph NM, Eads RB. 1946. An ectoparasitic survey of mammals from Lavaca County, Texas. Ann. Entomol. Soc. Am. 39(4):597–601. https://doi.org/10.1093/aesa/39.4.597

Samuel WM, Trainer DO. 1970. *Pulex porcinus* Jordan and Rothschild, 1923 (Siphonaptera: Pulicidae), an occasional parasite of the white-tailed deer from the Welder Refuge in Southern Texas. Wildl. Dis. 6(3):182–183.

Sheffield W, Jr. 1983. Food habits of nilgai antelope in Texas. J. Range Manag. 36(3):316. https://doi.org/10.2307/3898478

Sheffield W, Jr., Ables ED, Fall BA. 1971. Geographic and ecologic distribution of nilgai antelope in Texas. J. Wildl. Manage. 35(2):250–257.

Wang L, Rupani A, Grado LA, et al. 2022. Molecular detection of *Rickettsia felis* in fleas of companion animals in East Texas. Am. J. Trop. Med. Hyg. 107(2):416–419. https://doi.org/10.4269/ajtmh.21-0783

Yore K, DiGangi B, Brewer M, et al. 2014. Flea species infesting dogs in Florida and *Bartonella* spp. prevalence rates. Vet. Parasitol. 199(3-4):225–229. https://doi.org/10.1016/j.vetpar.2013.10.017