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Flea surveillance on wild mammals in northern region of Xinjiang, northwestern China

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ABSTRACT

Flea distribution in northern region of Xinjiang Uygur Autonomous Region (XUAR) and fluctuations of the annual fleas index in Alataw Pass were investigated. During a 4-year (2015–2018) study, 5789 fleas were collected directly from 15 mammals at eight counties in northern XUAR of northwestern China. Nineteen flea species, belonging to sixteen genera and seven families, were further confirmed by four genetic markers (*18S rDNA*, *28S rDNA*, *COI* and *COII*) after morphological observation. *Pulex irritans* and *Paraceras crispum* parasitizing Asian badgers (*Meles leucurus*) were recorded for the first time. In addition, the fluctuations of the annual fleas index in Alataw Pass were surveyed. *Xenopsylla gerbilli minax*, *Xenopsylla conformis conformis* and *Nosopsyllus laeviceps laeviceps* were highly detected in the warm season while *Paradoxopsyllus repandus*, *Ctenophthalmus dolichus dolichus* and *Coptopsylla lamellifer ardua* were only found in the cold season. These findings extend our knowledge of flea species, distribution and annual fluctuations especially in China-Kazakhstan border.

1. Introduction

Siphonaptera (flea) is a highly specialized holometabolous insect order, and 2575 flea species belonging to 16 families and 238 genera have been described worldwide (Bitam et al., 2010; Lewis, 1998). Fleas distribution extends to all continents (Whiting et al., 2008). Biogeographically, the richest was from the Nearctic, the sparsest taxonomic sampling was from Asia (Whiting et al., 2008). Fleas, as poikilotherm parasites, have no internal control of their body temperatures, and as such depend on their hosts as a transient habitat, and abiotic conditions for survival (Oshaghi et al., 2009). The number of fleas is correlated with the type of host and the cycle of activity. As previous report, it is a certain link between the contact of flea parasitism on the host and the seasonal variation (Krasnov et al., 2005). Some fleas tend to be host specific (restricted or specialist), but others have a wide host range (permissive or opportunistic) (Maleki-Ravasan et al., 2017). The fleas

cause some problems for human beings and animals. Not only their bites cause irritation and allergy dermatitis but fleas are also major vectors transmitting plague, endemic typhus, cat-scratch disease, hy-menolepiasis nana and other important epidemics (Bitam et al., 2010; Higgins et al., 1996).

Xinjiang Uygur Autonomous Region (XUAR), covering 1.66 million square kilometers, and bordering eight countries with a 5600-km long borderline, have distinct features of four seasons especially in northern region. In winter, the average temperature ranges from $-19.96\text{ }^{\circ}\text{C}$ to $2.89\text{ }^{\circ}\text{C}$ while the average temperature ranges from $-12.93\text{ }^{\circ}\text{C}$ to $25.79\text{ }^{\circ}\text{C}$ in summer due to different geographical environment (Tan et al., 2018). In addition, XUAR, there are more than 130 species of wild mammals, which contributes to diversity of flea species. Alataw Pass (ALSK), the second-largest overland port in China, borders with the Republic of Kazakhstan.

In last two decades, a broad-range flea surveillance on wild

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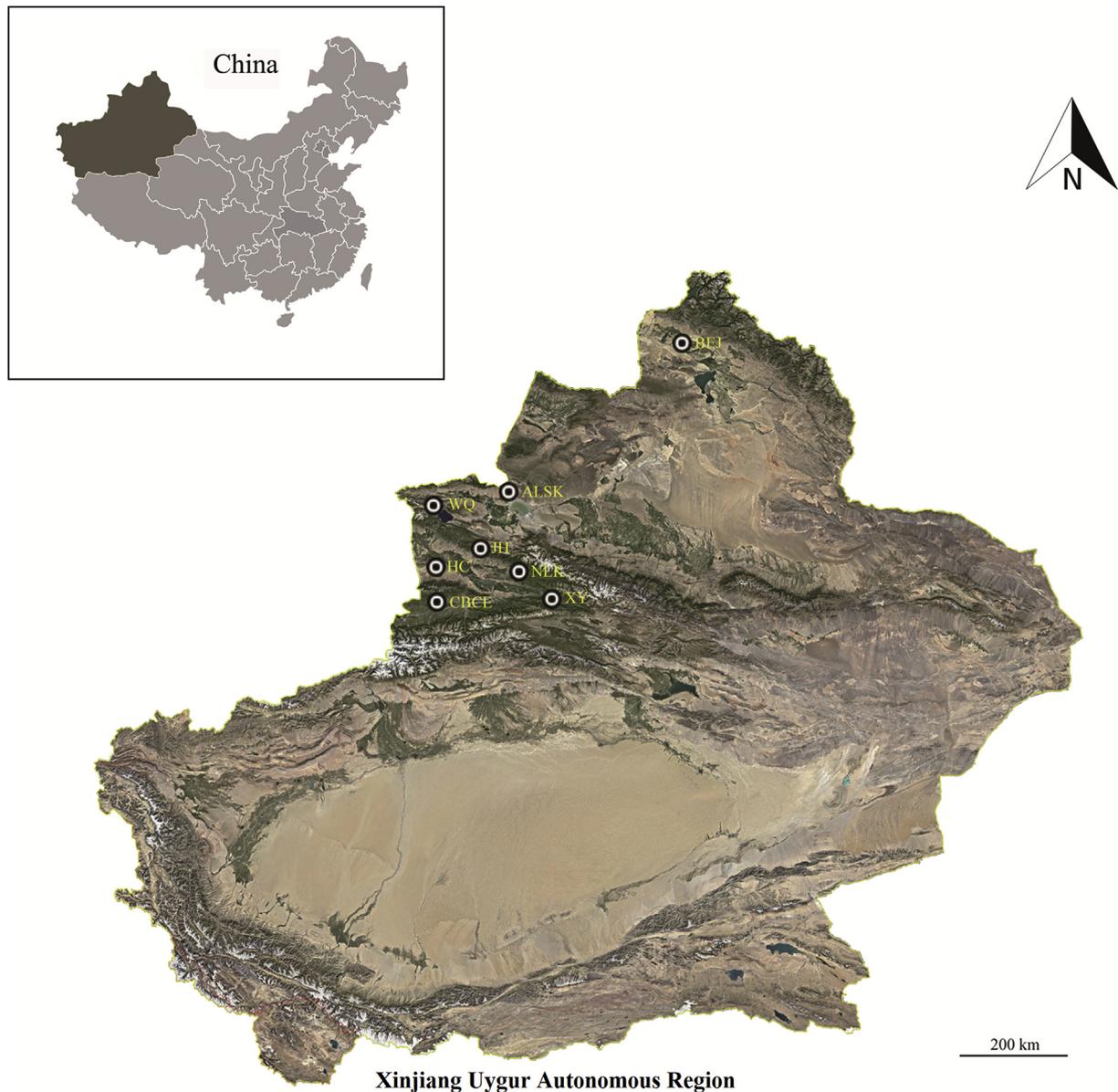


Fig. 1. A map of the study area.

mammals in XUAR is lack. Here flea distribution in northern region of XUAR and fluctuations of the annual fleas index in Alataw Pass were systematically investigated.

2. Materials and methods

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of Animal Ethics Committee of Shihezi University (Approval No. AECSU2015-17) at which the studies were conducted.

2.1. Sampling of mammals and fleas

The wild mammals were captured and the fleas were collected between 2015 and 2018 in eight locations [ALSK, Wenquan County (WQ), Huocheng County (HC), Chabuchar County (CBCE), Jinghe County (JH), Burqin County (BEJ), Xinyuan County (XY) and Nilka County (NLK)] in northern region of XUAR, northwestern China (see Fig. 1). The first four counties and city bordering the Republic of Kazakhstan.

As to the study of fluctuations of the annual fleas index, flea collection was conducted monthly from March 2015 through August 2016 in Alataw Pass.

The gray marmots (*Marmota baibacina*), long-tailed ground squirrels (*Spermophilus undulatus*), the great gerbils (*Rhombomys opimus*) and other less-abundant rodents were captured as previously described (Guo et al., 2015; Aplin et al., 2003). As to marbled polecats (*Vormela peregusna*), Asian badgers (*Meles leucurus*) and common pipistrelle bats (*Pipistrellus Pipistrellus*), their fresh carcasses were treated and submitted for postmortem examination to the XUAR Wildlife Management Office, and then sent to our laboratory for flea sampling. The animal fur was combed thoroughly, using a tooth-brush, over fleas were carefully collected (Krasnov et al., 1997). All the wild mammals were identified through morphological characteristics by experienced zoologists. Parts of mammal species were further molecularly identified by *COI*, *Cytb* and/or *16S rRNA*. This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2015-17).

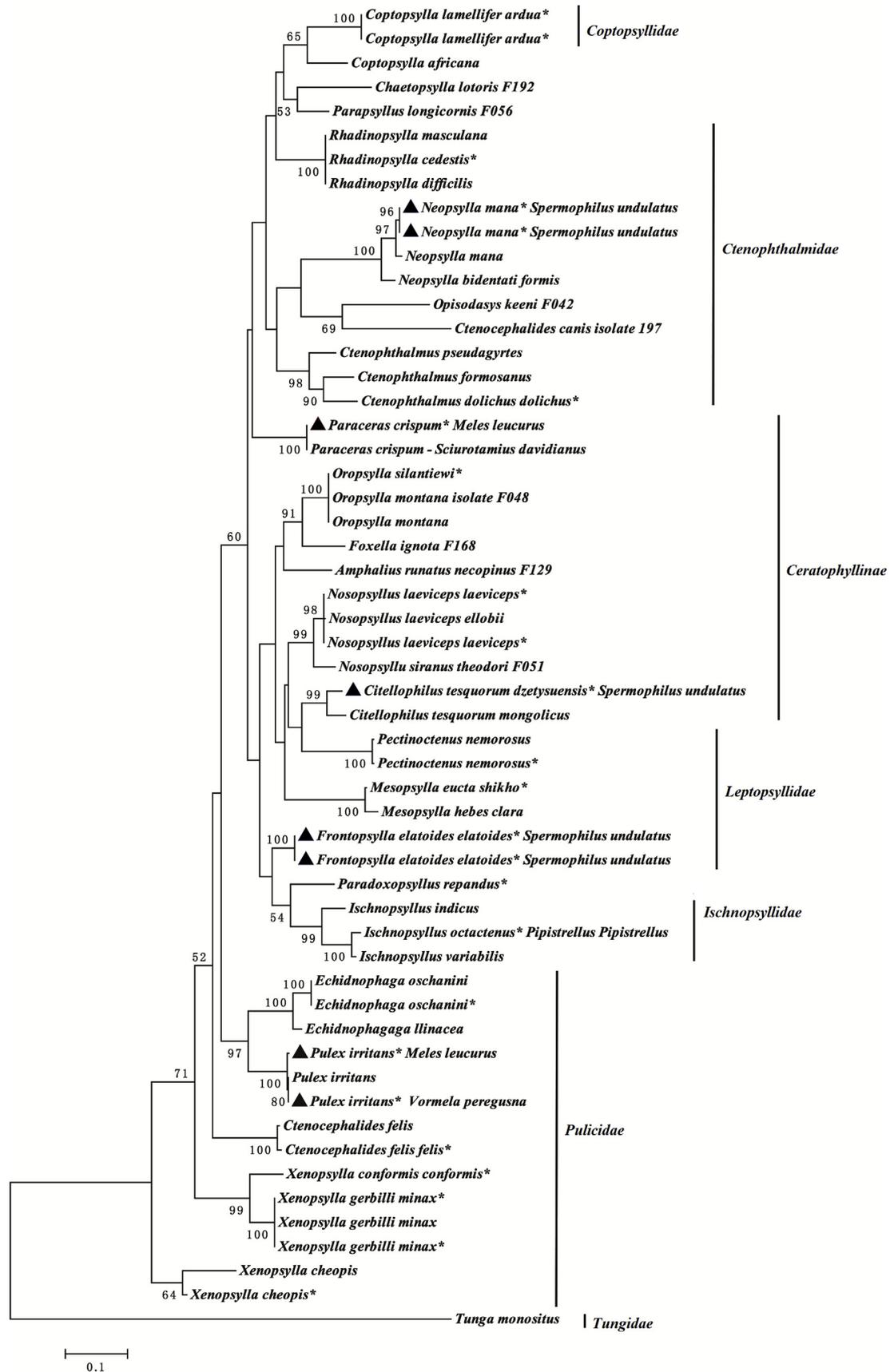


Fig. 2. Phylogenetic tree of the 18S–28S–COI–COII concatenated sequence of fleas (▲) from XUAR obtained in this study and sequences from nineteen fleas species retrieved from the GenBank database. The tree was constructed on the basis of neighbor-joining (NJ; 1000 bootstrap replicates) and maximum-likelihood (ML; 1000 bootstrap replicates) analyses using MEGA6. The scale bar represents the inferred substitutions per nucleotide site. The relative support for clades in the tree was produced from the NJ and ML analyses.

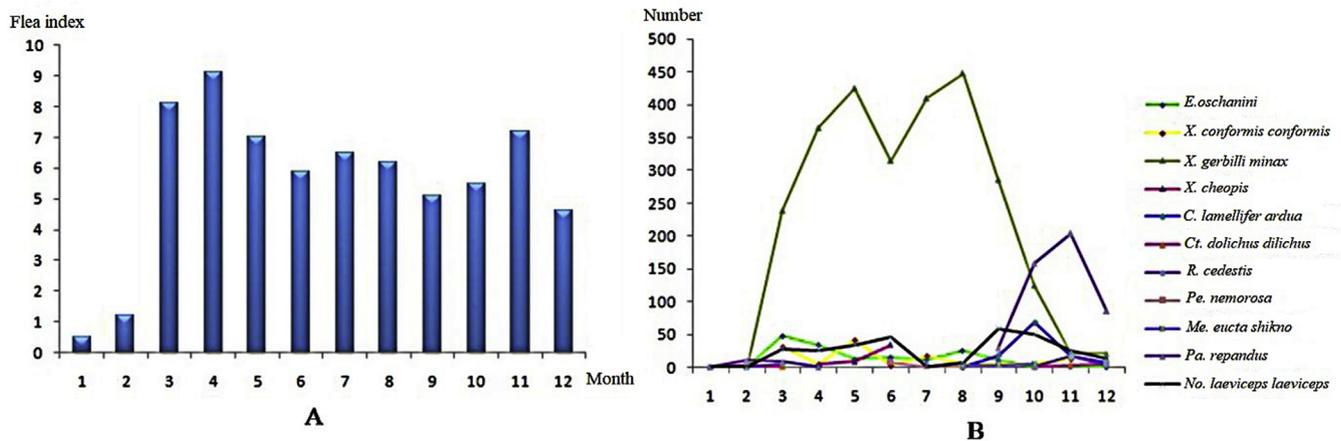


Fig. 3. The fluctuations of the annual fleas index in Alataw Pass, northwestern China.

2.2. Fleas identification

The adult fleas are identified based on the morphological characteristics of their extraordinarily complex genitalia, the presence and distribution of setae and spines (Traub et al., 1978; Dunnet and Mardon, 1991; Hastriter and Whiting, 2003) and other keys. Photographs of representative fleas were captured via Leica stereo microscope M165 C (LEICA M165 C, Germany).

Genomic DNA was extracted from all excised fleas by using the 96 flux automatic nucleic acid extraction instrument with a matching commercial kit (Cell & Tissue Kit, Biotেকে, Beijing, China) according to the manufacturer's instructions, and stored at -20°C . The molecular identification of the fleas was further carried out via four genetic markers [18S ribosomal DNA (18S rDNA, 1150 bp), 28S ribosomal DNA (28S rDNA, 989 bp), cytochrome c oxidase subunit I (COI, 685 bp) and cytochrome c oxidase subunit II (COII, 780 bp)] (Whiting, 2002). The PCR products were purified using the TIAN gel Midi Purification Kit (TIANGEN, Beijing, China) and sequenced by Sangon Biotech Co., Ltd (Shanghai, China).

2.3. Sequence analysis

All sequences obtained in this study were compared with GenBank data using the nucleotide BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>). A phylogenetic tree was constructed using the maximum-likelihood and Neighbor-Joining algorithms implemented in MEGA 6 software (Tamura et al., 2013). The representative sequences of fleas were deposited in GenBank, and the related information was described in Appendix Table 1. In addition, the deposited sequences of wild mammals were shown as below (COI: KT763032-KT763041 and KT613845-KT613848; Cytb: MF106222, KU156716-KU156732 and KU156736-KU156740; and 16S rRNA: KU707935, KT763065-KT763067, KT763069-KT763072 and KT763075-KT763077).

3. Results

A total of 5789 fleas, belonging to 16 genus and 19 species, were collected from 15 wild mammals detected in eight Counties. Four flea species including *Echidnophaga oschanini*, *Xenopsylla conformis conformis*, *Xenopsylla gerbilli minax* and *Nosopsyllus laeviceps laeviceps*, were dominant found on small rodents and carnivore (marbled polecats). Interestingly, *Pulex irritans* was firstly observed both in marbled polecats and Asian badgers while *Paraceras crispum* was the first record in China, and Asian badger is a new host record. Analysis of BLAST and phylogenetic tree confirmed the morphological identification of flea species (Fig. 2).

After the fluctuations of the annual fleas index in Alataw Pass were described (Fig. 3), the results indicated that flea i) the peak activity of *X. gerbilli minax*, *X. conformis conformis* and *N. laeviceps laeviceps* was in the warm season (April–August), and ii) *Ctenophthalmus dolichus dolichus*, *Rhadinopsylla cecestis* and *Paradoxopsyllus repandus* were captured dominantly in the cold season (from October to next-year April).

4. Discussion

In previous work, The human flea (*P. irritans*) was sampled from South American gray fox (*Lycalopex griseus*), Andean fox (*Lycalopex culpaeus*), red fox, coyote (*Canis latrans*), kit foxes (*Vulpes macrotis*), swift foxes (*Vulpes velox*), burrowing owls, the crested porcupine (*Hystrix cristata*), Beech marten (*Martes foina*), the ground squirrel (*Citellus citellus*), Iberian lynx (*Lynx pardinus*), domestic dog, cat, pig, chicken and dairy goats (Millán et al. 2007, 2018; Víchová et al., 2018; López-Pérez et al., 2018; Sanchez et al., 2018; Zouari et al., 2017; Noden et al., 2017; Rezaei et al., 2016; Graham et al., 2016; Belthoff et al., 2015; Mori et al., 2015; Domínguez-Peñafiel et al., 2011; Uslu et al., 2008; Pence et al., 2004; Ménier, 2003). In this study, *P. irritans* was also collected from marbled polecat (*V. peregusna*) and Asian badger (*M. leucurus*). In addition, *P. crispum* was only collected from rock squirrel (*Sciurotamias davidianus*) (Whiting et al., 2008). Here we firstly sampled it from Asian badger (*M. leucurus*). These findings extend the host profile of *P. irritans* and *P. crispum*. Moreover, *Ischnopsyllus octactenus*, sampled from *P. Pipistrellus* in Huocheng County (XUAR) in 1983, was firstly recorded in China (Li, 1988). Here 28 *I. octactenus* fleas were collected in Chabuchar and Xinyuan counties. As for *P. Pipistrellus* bat widely distributes in Taiwan, Jiangxi, Shaanxi, Yunnan, Sichuan provinces and XUAR, whether its flea species (*I. octactenus*) distributes or not in other provinces exception of XUAR, is still unknown.

Some rodents, such as *Meriones tamariscinus*, *Meriones meridianus* Pallas, *R. opimus*, *S. undulatus*, mainly distribute in Central Asian and northwestern China. However, there is little information on their parasitized flea species. In this study, we found that i) the hosts including *Meriones libycus*, *V. peregusna*, *M. leucurus*, *R. opimus*, *M. tamariscinus* and *M. meridianus* are non-specific for flea species (at least two flea species were collected from these wild animals) after analysis of relationship between the relative abundance of flea species and their hosts (shown in Appendix Fig. 1); and ii) *X. gerbilli minax*, *P. repandus* and *N. laeviceps laeviceps* are constantly found in *M. tamariscinus*, *M. meridianus* Pallas, *R. opimus* and *M. libycus*.

The north region of XUAR is a typical temperate continental drought climate. Gurbantungut Desert, the second largest desert in China, covers 48800 km² and goes through this region. In our previous

study, the annual precipitation and average annual temperature ranged from 108 mm to 3.94 °C to 263 mm and 7.69 °C respectively during 1962–2012 (Wang et al., 2015). Here the flea fauna was systematically surveyed in this special geographical habit. A very different flea fauna (nineteen flea species, belonging to sixteen genera) were built from 15 wild mammals, which might be related to special climate host profile and landscapes.

In addition, the fluctuations of the annual flea index in Alataw Pass were investigated (Fig. 3: A and B). The finding indicates that the peak activity of *X. gerbilli minax*, *X. conformis conformis* and *N. laeviceps laeviceps* were in the warm season (April–August) while *P. repandus*, *C. dolichus dolichus* and *Coptosylla lamellifer ardua* were highly found in the cold season (October–next-year April). In previous work, plague is endemic in *R. opimus*, *S. undulatus* and *M. baibacina* vectored by *X. gerbilli minax*, *O. silantiewi* and *N. laeviceps laeviceps* in warm season (Zhao et al., 2017; Qian et al., 1989). In the future, there is still a hard work to survey flea-borne pathogens and flea-borne diseases both for fleas and their hosts.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2019.11.004>.

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