The Relationship between Parasitic Fleas and Small Mammals in Household of Western Yunnan Province, China

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Abstract

The Yunnan Province has one of the most serious outbreaks of the plague epidemic in China. Small mammals and fleas are risk factors for the occurrence of plague in commensal plague foci. Understanding the relationship between parasitic fleas and small mammals will help control fleas and prevent the onset of the plague. Four hundred and twenty-one small mammals, belonging to 9 species, were captured. Of these, 170 small mammals (40.4%) were infested with fleas. A total of 992 parasitic fleas (including 5 species) was collected. The number of *Leptopsylla Segnis* and *Xenopsylla Cheopis* was 91.0%. The final multiple hurdle negative binomial regression model showed that when compared with *Rattus Tanezumi*, the probability of flea infestation on *Mus musculus* and other host species decreased from 58% to 99%, while the infestation with fleas from other host species increased 4.7 fold. The probability of flea prevalence in adult hosts increased by 74%, while the number of fleas decreased by 76%. The number of flea infestations in small male mammals increased by 62%. The number of fleas in small mammals weighing more than 59 grams has been multiplied by about 4. *Rattus Tanezumi* is the predominant species in households in West Yunnan Province, while *Leptopsylla Segnis* and *Xenopsylla Cheopis* are dominant parasitic fleas. There is a strong relationship between the abundance of parasitic fleas and the characteristics of small mammals (e.g. Species, age, sex, and body weight).

Keywords: Small Mammal; Parasitic Flea; Household; Western Yunnan Province.

Introduction

Plague is a zoonotic bacterial infectious disease transmitted by fleas with reservoirs in rodent populations, in which the parasitic flea plays a potentially fundamental role as bridge vectors to transmit the bacteria (*Yersinia pestis*) to animals and humans [1]. The potential role of fleas as carriers and host-supported local reservoirs can help explain the persistence of plague [2].

The plague has played a huge role in human history and is still prevalent in most parts of China. In China, animal plague is reported almost every year, and occasional human plagues occur [3, 4]. Transmission to humans sometimes occurs through contact with fleas that have fed on an infected small mammal or by skinning infected small mammals (e.g. Marmot) [5-7]. The province of Yunnan is one of the most serious plague epidemic foci in China [8,9], and especially the most active in commensal rodent plague foci [2]. The province of Yunnan is characterized by complex natural conditions, an obvious vertical climate, consisting of embedded microhabitats that create a series of ecological niches, which may increase the risk of establishing vectors and specific hosts that carry pathogens.

Fleas are obligatory hematophagous insects and rodents are the most common host of fleas, although they can appear in other types of mammals and birds [10, 11]. At different stages of the life cycle, fleas are located in the hosts or in the host's nest. In most cases, the imago flea is usually parasitic on the host and until the end of its life cycle, differentiating with the larvae. When leaving a pupa, a new flea of imago finds a host because the reproduction of the flea is not possible without the feeding of blood [12]. Therefore, it is likely to handle flea infestations on live hosts. The parasites get their food and other biological supplies from their hosts. Consequently, they can affect the host directly by reducing host resources, and indirectly by provoking behavioral responses [13, 14] or immunological responses [1, 15]. Fleas with high density and richness not only favor the bacteria plague, but also spread widely among the hosts, but also suppress the immune response of the hosts, thus improving the susceptibility of the hosts [16, 17], which is vital for the maintenance of the plague [18, 19].

The abundance of hosts is an important factor that affects the distribution and abundance of parasites. In terms of the reservoir, some individuals, populations or host species are characterized by a higher level of parasite infestation than others [20]. It is well known that there is a great variation in the abundance of a parasite among the different host species. The host with the highest abundance of parasites is commonly defined as the main host, while those with lower abundance of parasites are defined as auxiliary hosts [20, 21].

In this study, we attempt to describe the distribution of small parasitic mammals and fleas and reveal the effect of small mammals on the abundance of parasites in homes in the western province of Yunnan. The information on the factors that affect the flea abundance in hosts is essential to provide evidence-based recommendations on flea control and to implement plague control and prevention programs in natural plague foci.

Methods

Description of the study area

The western of Yunnan province is located in the central region of the Hengduan mountains of the terrestrial part of the Tibetan plateau, which belongs to the low latitude mountain valley. It is influenced by the enormous difference in latitude and altitude gradient, which is characterized by complex terrain and varied climate [22]. Therefore, it is one of the regions that possess the most abundant animal and plant species in China. There are many minority nationalities, including Yi, Dai, Naxi, Hui, Bai et al. inhabiting this area. The main economic source is planting and economic development is relatively poor. These conditions contribute to the breeding of small mammals and the spread of the plague.

During the period from July 2011 to October 2012, the study was carried out in 800 households in 40 villages, 10 counties, in the western part of Yunnan province (Fig 1). In the study area, the 10 counties have a total area of approximately 41,130 square kilometers and a population of approximately 3.4 million.

Fig 1 here

Small mammals capture and identification

Five live traps were placed in each home for three continuous nights to capture small mammals. The bait was fried ham sausage. Each live trap was verified on the morning of the next day. If a small mammal was caught, a new live trap with fresh bait was replaced by the old live trap and placed in the same place. The species of small mammals, sex and age were identified according to their morphological characteristics. Their weight, body length and tail length were measured.

Parasitic flea collections and identification

The parasitic fleas were harvested by combing from the tail end forward using a toothbrush in a white enamel tray after anesthetizing small mammals with ether. The parasitic fleas were stored in labeled vials containing 75% ethanol. Fleas from each small mammal were kept in separate vials and kept at room temperature. Flea species were identified under a light microscope.

Data analysis

The distribution of small parasitic mammals and fleas was summarized using descriptive statistics. Then the abundance of parasitic fleas of each small mammal was generated. Species, sex, age, weight, body length and tail length of each small mammal were considered potential factors for the abundance of parasitic fleas. For continuous variables, the median was used to classify these variables into binary variables. The relationship between potential factors and a result was explored using the hurdle binomial regression model (HNB) of the regression model under R software 3.02 [23-25]. This regression model was applied to take into account the current data set that shows a count of excess zeros and an overdispersion distribution. It is a two-component model: one is the logistic model adjusting counts against zero, the other is the hurdle binomial model adjusting positive counts. Potential factors (P<0.20) related to small mammals entered the prototype multiple regression model HNB. The final model to predict the factors associated with the parasitic flea abundance was refined using a backward method (α = 0.10 as a criterion of statistical significance). The proportion of Prevalence odds ratio (OR) and abundance ratio (AR) for a parasitic infestation of fleas were calculated based on pieces of literature [26,27].

Results

Distribution and description of small mammals

A total of 12,000 traps was placed in 800 households in 40 villages in 10 counties in western Yunnan Province and 421 small mammals were captured. The overall density of small mammals was 3.51%. Small mammals were divided into 9 species, 6 genera, 2 families and 2 orders (Table 1). *Rattus Tanezumi* is the dominant species and it represents 66.03% (278/421) of the entire sample. Among the 10 counties, the highest number and the smallest number of small mammals were captured in Mangshi (100) and Deqin (16) counties, respectively. The number of small mammals richness was 9. The highest number and lowest number of richness were in Yongren (5), Nanhua (1) and Deqin (1) counties, respectively.

Table 1 Here

In our study, the weight, body length and tail length of 407 small mammals were calculated (for other 14 small mammals there was no calculation due to incomplete data). The mean body weight was 62.93g, standard deviation 42.67g, median 59.31g (range: 7.39-186.89g); the average body length was 22.79cm, the standard deviation of 8.57cm and the median 21.00cm (range: 7.50 to 39.30cm); the average length of the tail was 12.30cm, the standard deviation of 3.76cm and the median 13.30cm (extremes: 1.00 and 20.00cm).

Distribution of parasitic fleas

Of 421 small mammals, 170 small mammals were infested with fleas and their prevalence was 40.38%. A total of 992 parasitic fleas, divided into 5 species, 5 genera, and 3 families, were collected and the flea value was 2.36 (992/421). The highest prevalence of fleas and the highest flea index occurred in Yunxian County (71.79%) and Yulong County (7.33), respectively. Out of 992 fleas, the number of *Xenopsylla cheopis* and *Leptopsylla segnis* was 903, or 91.03%. The proportion of *Xenopsylla cheopis* exceeds 90% in Yunxian and Gengma counties. (Table 2)

Table 2 Here

Among the 9 small mammal species, the prevalence of *Rattus Tanezumi* fleas was highest (53.60%, 149/278). The infestation of *Rattus Tanezumi* by *Xenopsylla cheopis* was particularly high (35.61%, 99/278). *Crocidura* *attenuata* and *Anourosorex squamipes* were not infested with fleas (Table 3).

Table 3 Here

The results of the prototype multiple HNB regression model

Table 4 shows the prototype multiple HNB regression model for the abundance of parasitic fleas. Of the six potential factors compared to *Rattus Tanezumi*, the probability of flea infestation of *Mus musculus* and other host species decreased, while the number of flea infestations of other host species increased. The number of fleas in adult hosts decreased; the number of fleas in small mammals weighing more than 59 grams increased.

Table 4 here

The results of the final multiple HNB regression model

The final model showed that four factors - species, age, sex and body weight of small mammals - are closely associated with the abundance of parasitic flea (Table 5). Compared to *Rattus Tanezumi*, the probability of infestation with *Mus musculus* fleas as well as other host species decreased by 58% to 99%, while the number of flea infestations of the other host species increased by 4.71. The probability of flea prevalence in adult hosts increased by 74%, while the number of fleas decreased by 76%. The number of flea infestations in small male mammals increased by 62%. The number of fleas in small mammals weighing more than 59 grams increased by about 4 times.

Table 5 here

Discussion

In this study, 421 small mammals were captured and *Rattus Tanezumi* was the predominant species. A total of 992 parasitic fleas was collected from small mammals. The number of *Leptopsylla segnis* and *Xenopsylla cheopis* was 91.03%. A close relationship between parasitic flea abundance and small mammal traits (including species, age, body weight, sex) was evident in households in Western Yunnan Province.

The results of our investigation showed that *Rattus Tanezumi* is the predominant species in households in western Yunnan Province. *Xenopsylla cheopis* and *Leptopsylla segnis* are the dominant fleas host as well as the dominant fleas host of *Rattus Tanezumi*. These results correspond to the previous study [28]. In the commensal plague foci of the western Yunnan Province, *Rattus Tanezumi* is the main host species, while *Mus musculus* is the auxiliary host species. Yin et al. [26] have suggested that the abundance of parasitic fleas is related to host species. Our data indicated that *Rattus Tanezumi* was more likely to be infested with fleas than *Mus musculus* and other species. The main host may or may not be the species in which the parasite evolved for the first time, but it's currently home to most individuals in the parasite population [21]. It is therefore believed that *Rattus Tanezumi* is the best species in which the majority of parasites live and the probability of flea infestation on *Mus musculus* as well as other species has decreased compared to *Rattus Tanezumi*. The reason for the increase in the number of fleas in other species could be due to the fact that the number of flea infestations on other species was sufficient, although the number of other host species is lower in other species in our study, like *Apodemus chevrieri*. In addition, the fundamental reasons for the diversity in parasitic abundance between the main host and any auxiliary host are often related to the different successes of exploitation and reproduction of a parasite [29]. Previous studies have shown that the phylogenetic correlation between the main host and the auxiliary host can determine parasitic abundance on its auxiliary hosts, as it should reflect the similarities between host species in terms of physiological, ecological characteristics and immunological [30]. On the other hand, it was found that for flea infestation in small mammals, the abundance of a flea on its auxiliary hosts decreased with increasing phylogenetic distance of these hosts relative to the main host [30]. The mechanisms underlying this model are not yet clear but are supposed to be consistent with the differential performance of a flea on auxiliary hosts, which in turn is correlated with a phylogenetic distance from the auxiliary host to the main host [31].

Recent studies have shown that parasites appear to make favorable choices and decisions for hosts in which their reproductive benefits are maximized [32]. In the meantime, the choice of the host is important, which may contribute to the variation in the abundance of the fleas collected when the potential risk of flea-mediated diseases are assessed. In addition, the variation in the level of infection in host individuals is well known, indicating that some individuals may represent better patches for parasites than other individuals [20]. Namely, reservoirs providing better patches for parasites are usually defined as primary hosts.

Differences in flea infestation due to the age of the host may be influenced by differences in parasite aggregation. We tested the effect of age of small mammals on parasitic fleas and found that the probability of flea prevalence in adult hosts increased as the number decreased. This phenomenon has generally been linked to host immune responses and parasite-host associations. Firstly, it is known that immune defenses often deteriorate with increasing age [33], so that in smaller mammals the decline of antiparasitic defense has been reported with increasing age [34]. These hosts have different defenses that can affect the number of blood a flea can acquire [35]. Fleas were found to take more blood from juveniles and older animals than from sub-adult and adult animals [36]. The degree of deterioration of immune function with age may, however, be affected by environmental conditions (e.g. environmental stress) [37] and may also differ between males and females (due to faster aging of men) [38]. Secondly, differential parasite abundance in hosts belonging to different age cohorts has been reported for various host and parasite taxa [39-41]. However, the influence of host age on the distribution pattern of parasite abundance differs among different host-parasite associations. In some host-parasite associations, the abundance of parasites increases with the age of the host [39], while in other associations it increases or decreases in the youngest and oldest hosts compared to hosts of median age (called "adult hosts")[40]. In fact, acquired resistance against parasites may be lower in young or old hosts than in median-age hosts. Young hosts may simply not have the time to develop parasite resistance, while older hosts may lose their ability to resist parasites due to immunosenescence [33, 42, 43]. As a result, hosts in the youngest and the oldest cohorts would have better habitat for parasites, so the relationship between parasite abundance and host age would be convex. If the negative effect of heavy parasite loads causes mortality mainly in young instead of old hosts, the abundance of parasites will increase with the age of the host. In all cases, fleas performed better in younger and older hosts than in middle-aged hosts.

In addition, the effect of the age of the host was strongly influenced by the effect of the sex of the host. In particular, from the point of view of resource acquisition (i.e. The size of the blood meal), there has been an improvement in the quality of cohorts of young and old among female, but not in male hosts, while, in the context of resource processing (digestion of blood), some trends in age-dependent host quality has been observed in male but not in female hosts [35]. In other words, the results of the Liberman study suggest that the age of the host does not predict unequivocally whether it is more or less beneficial for a flea [36].

Similarly, the sex of the host was also a determining factor influencing the abundance of fleas. Previous studies have shown the effect of host sex in flea reproduction [44,45]. In this study, we studied the consequences of host sex on flea abundance. Finally, we observed an increase in the number of flea infestations in small male mammals compared to females. The effect of host sex on parasite performance is essential to understanding the mechanisms of male-biased parasitism.

Under normal conditions, two non-mutually exclusive mechanisms are invoked to explain the variation in the number of fleas observed. Male hosts are characterized by higher mobility and poorer immunological defense than female. In general, fleas feed faster, take relatively more blood and digest more quickly when feeding on male rather than female, although the pattern of blood digestion related to the sex of the host depends on external conditions (relative humidity). In addition, fleas produced more eggs exploiting male hosts than female hosts [44]. This would lead to a better ability of parasites exploiting male rather than female because of the immunosuppressive effects of androgens, which would lead to fleas favoring male [45]. It is suggested that, in many cases, male hosts represent better patches for parasites than females. Thus, it can be seen that more flea prefers to stay in male hosts than in females. Consequently, a greater infestation of a male host than a female host in terms of abundance, prevalence and species richness of parasites, this has been reported for a wide variety of parasite and host taxa [46], although higher levels of parasite infection have been reported in some mammalian female [47].

The body weight of the hosts could be considered as an additional indicator of the number of fleas assembled. The results of this study showed that the number of fleas increased in small mammals weighing more than 59 grams. Hawlena [48] noted that the number of fleas, mainly *Xenopsylla cheopis* and *Xenopsylla Astia* (Siphonaptera: Pulicidae), caught in the wild (Rodentia: Muridae) increased with the weight of the host (and thus its age) at an optimum before decreasing. In practice, the body weight of the host increases with age. As mentioned above, the probability of flea infestation on the host increases with the age of the host. Therefore, the number of fleas would increase with the body weight of the host.

Associations between the aggregation of fleas and the abundance of their hosts vary according to different factors. This study confirmed that the variation in the number of fleas was due to different factors in the host.

It is concluded that the host is an important factor to consider when comparing parasite flea variation in natural rodent populations. It is necessary that the factors mentioned above be taken into account to control the abundance of parasitic fleas. Fitness related measures should be directly involved, such as reducing the number of small mammals and flea fleas, preventing and controlling flea-borne diseases.

Conclusions

The results of the study show the distribution of small mammals and household parasitic fleas in the western province of Yunnan, China. In addition, a new discovery revealed by the hurdle binomial regression model is that there is a close relationship between the abundance of parasitic fleas and the characteristics of small mammals (e.g. species, age, sex, and body weight). Our study provides direct evidence to explore the relationship between parasitic fleas and the host by the characteristic of instinct and to reveal host structure plays an important role in the allocation of parasitic flea abundance in the ecosystem of the plague. The knowledge of parasitic flea factors will help control and predict the number of parasitic fleas in commensal rodent plague foci and further control the onset of the plague. Although it is relatively limited that the current model predicts the factors affecting the abundance of parasitic fleas. In addition to the characteristics of the host, the number of parasitic fleas may be affected by other potential factors (e.g. environment, climate, economy, human disturbance). Thus, the above factors should be measured and included in the regression model, it would be useful to establish benefits model to predict the abundance of parasitic fleas.

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Figure legend

Figure 1 Location of 10 sampling sites in Western Yunnan Province, China. Ten red dots represent 10 sampling counties and yellow shadow is the acreage of each county. Red five-point star is the location of Kunming city (capital of Yunnan Province).

Table 1 Distribution for small mammals in households of 10 counties in Western Yunnan Province

County	Small mammals			Species and proportion (%) of small mammals								
	Abundance	Density	Richness	*Rattus Tanezumi*	*Mus musculus*	*Rattus norvegicus*	*Rattus Nitidus*	*Suncus Murinus*	*Apodemus Chevrieri*	*Crocidura Attenuata*	*Anoro sorex squamipes*	*Mus caroli*
Yongren	86	7.17	5	4(4.65)	73(84.88)	2(2.33)	6(6.98)	0	0	0	0	1(1.16)
Nanhua	36	3.00	1	36(100.00)	0	0	0	0	0	0	0	0
Xiangyun	41	3.42	3	18(43.90)	0	22(53.66)	0	1(2.44)	0	0	0	0
Yunxian	39	3.25	2	37(94.87)	2(5.13)	0	0	0	0	0	0	0
Gengma	26	2.17	2	25(96.15)	1(3.85)	0	0	0	0	0	0	0
Mangshi	100	8.33	2	94(94.00)	0	0	0	6(6.00)	0	0	0	0
Longyang	28	2.33	3	24(85.71)	0	0	0	0	0	2(7.14)	2(7.14)	0
Lanping	22	1.83	3	17(77.27)	0	4(18.18)	1(4.55)	0	0	0	0	0
Yulong	27	2.25	3	23(85.18)	0	2(7.41))	0	0	2(7.41)	0	0	0
Deqin	16	1.33	1	0	0	0	16(100.00)	0	0	0	0	0
Total	421	3.51	9	278(66.03)	76(18.05)	30(7.13)	23(5.46)	7(1.66)	2(0.48)	2(0.48)	2(0.48)	1(0.24)

[a]Small mammals density = (abundance/ live traps.nights)×100%, the number of live traps.nights each county is 1200

Table 2 Distribution for parasitic fleas in households of 10 counties from Western Yunnan Province

County	No. of small mammals	No. of small mammals with flea infection	Flea prevalence (%)[a]	No. of fleas	Flea index[b]	Richness	Parasitic flea species and proportions				
							Xenopsylla Cheopis	*Leptopsylla Segnis*	*Ctenocephalides felis felis*	*Monopychllusanisus*	*Pulexirritans*
Yongren	86	6	6.98	83	0.97	3	71(85.54)	11(13.26)	0	1(1.20)	0
Nanhua	36	12	33.33	86	2.39	4	1(1.16)	61(70.93)	8(9.3)	0	16(18.6)
Xiangyun	41	21	51.22	146	3.56	2	3(2.05)	143(97.95)	0	0	0
Yunxian	39	28	71.79	97	2.49	2	92(94.85)	5(5.15)	0	0	0
Gengma	26	12	46.15	42	1.62	2	38(90.48)	0	0	4(9.52)	0
Mangshi	100	56	56.00	250	2.50	3	208(83.20)	34(13.60)	0	8(3.20)	0
Longyang	28	11	39.29	41	1.46	4	16(39.02)	23(56.09)	1(2.44)	1(2.44)	0
Lanping	22	9	40.91	49	2.23	2	0	45(91.84)	0	4(8.16)	0
Yulong	27	15	55.56	198	7.33	3	0	152(76.77)	35(17.68)	11(5.56)	0
Deqin	16	0	0	0	0	0	0	0	0	0	0
Total	421	170	40.38	992	2.36	5	429(43.25)	474(47.78)	44(4.44)	29(2.92)	16(1.61)

[a]Flea prevalence = (No. of small mammals with flea infection/ No. of small mammals)×100%

[b]Flea index = No. of fleas/ No. of small mammals

Table 3 Flea infection for different small mammal species in households of Western Yunnan Province

small mammal species	No. of small mammals	No. of small mammals with flea infection	Flea prevalence (%)[a]	No. of fleas	Flea index[b]	No. of small mammals with *Xenopsylla cheopis*	*Xenopsylla cheopis* prevalence (%)
Rattus Tanezumi	278	149	53.60	737	2.65	99	35.61
Mus musculus	76	1	1.32	1	0.01	1	1.32
Rattus norvegicus	30	12	40.00	120	4.00	2	6.67
Rattus nitidus	23	2	8.70	4	0.17	1	4.35
Suncus murinus	7	3	42.86	31	4.43	2	28.57
Apodemus chevrieri	2	2	100	58	29.00	0	0
Crocidura attenuata	2	0	0	0	0	0	0
Anourosorex squamipes	2	0	0	0	0	0	0
Mus caroli	1	1	100	41	41	1	1
Total	421	170	40.38	992	2.36	106	25.18

[a]Flea prevalence = (No. of small mammals with flea infection/ No. of small mammals)×100%

[b]Flea index = No. of fleas/ No. of small mammals

Table 4 The results of the prototype multiple HNB regression model for the abundance of parasitic fleas

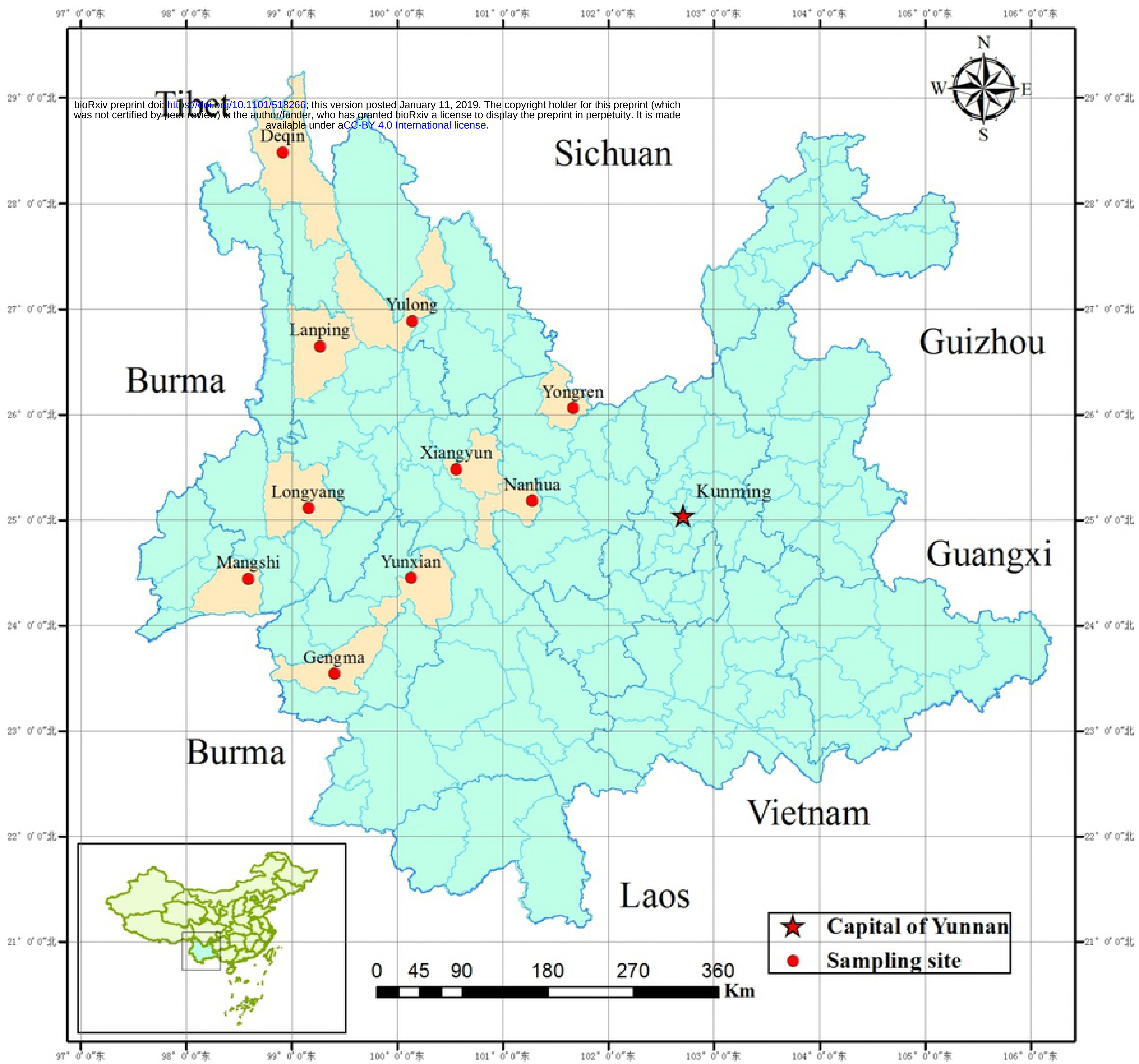
Variables (Factors)	No. of small mammals	Logistic part		Count part	
		OR(95%CI)	*P* value	AR(95%CI)	*P* value
Host species					
Rattus Tanezumi	270	Ref[a]		Ref	
Mus musculus	76	0.01(0,0.08)	0	0(0- +∞)	0.959
others	61	0.41(0.22,0.76)	0.004	4.40(1.70,11.37)	0.002
Age					
immaturity	114	Ref		Ref	
adult	293	1.71(0.67,4.37)	0.265	0.33(0.10,1.10)	0.071
Gender					
female	255	Ref		Ref	
male	152	0.81(0.51,1.29)	0.377	1.47(0.83,2.61)	0.183
Body weight (g)					
≤59	202	Ref		Ref	
>59	205	1.28(0.54,3.04)	0.570	5.03(1.60,15.82)	0.006
Body length(cm)					
≤21	205	Ref		Ref	
>21	202	0.89(0.52,1.52)	0.673	1.33(0.73,2.44)	0.351
Tail length (cm)					
≤13	201	Ref		Ref	
>13	206	0.82(0.39,1.72)	0.603	0.55(0.25,1.22)	0.140

[a]Ref: reference group

Table 5 The results of the final HNB model for the abundance of parasitic fleas

Variables (Factors)	Logistic part		Count part	
	OR(95%CI)	*P* value	AR(95%CI)	*P* value
Host species				
Rattus Tanezumi	Ref[a]		Ref	
Mus musculus	0.01(0,0.07)	0.000	0.00(0- +∞)	0.956
Others	0.42(0.23,0.76)	0.004	5.71(2.21,14.77)	0.000
Age				
Immaturity	Ref		Ref	
Adult	1.74(1.07,2.81)	0.024	0.24(0.07,0.80)	0.020
Gender				
Female			Ref	
Male			1.62(0.92,2.84)	0.093
Body weight (g)				
≤59			Ref	
>59			5.03(1.62,15.64)	0.005

[a] Ref: reference group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Guizhou
Burma
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Xiangyun
Nanhua
Longyang
Kunming
Guangxi
Mangshi
Yunxian
Gengma
Burma
Vietnam
Laos
Capital of Yunnan
Sampling site
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Figure