

## Fleas of Shrews and Rodents in Rural Lowland Taiwan

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### Abstract

Fleas transmit a variety of pathogens to humans but are relatively understudied in comparison to mosquitoes and ticks, including in Taiwan, where fleas in rural lowlands have never been systematically surveyed. In total, 700 fleas of four species were collected from 1,260 shrews and rodents at nine counties across lowland Taiwan. *Nosopsyllus nicanus* Jordan (Siphonaptera: Ceratophyllidae) and *Xenopsylla cheopis* Rothschild (Siphonaptera: Pulicidae) were the most abundant flea species (79.0 and 14.6% of total fleas, respectively); the former was largely limited to the islets, while the latter was restricted to the Taiwan main island. *Rattus losea* Swinhoe (Rodentia: Muridae) was the most common small mammal species (49.3% of total) and hosted the majority of fleas (88.3% of total). Five *Rickettsia* spp., including *Rickettsia conorii* Brumpt (Rickettsiales: Rickettsiaceae), *Rickettsia felis* Bouyer et al. *Rickettsia japonica* Uchida, *Rickettsia raoultii* Mediannikov, and *Rickettsia rickettsii* Brumpt or closely related species, were identified from 67 individually assayed fleas based on *ompB* and *gltA* genes. *Rickettsia felis*, mainly transmitted by fleas, was detected in one *X. cheopis* in southern Taiwan where a confirmed human case of infection with *R. felis* has been reported. The presence of *R. felis*, along with the other four tick-borne *Rickettsia* spp., demonstrates that a variety of rickettsiae circulate in rural lowland Taiwan and could pose risks to human health.

**Key words:** *Nosopsyllus nicanus*, *Rattus losea*, *Rickettsia felis*, rickettsiae, Taiwan

Fleas (order Siphonaptera) can transmit a variety of pathogens to humans, including *Yersinia pestis* Lehmann & Neumann, which has plagued humans for hundreds of years (Eisen and Gage 2012), and *Rickettsia typhi* Wolbach & Todd, the etiological agent of murine typhus, which has circulated in coastal cities and ports for over a half century (Traub et al. 1978). In addition, *R. felis* Bouyer et al. and *Bartonella henselae* Regnery et al. which cause flea-borne spotted fever and cat scratch disease, respectively, are emerging around the world (Adams et al. 1990, Chomel et al. 1996, Pérez-Osorio et al. 2008). Although humans are continuously afflicted with flea-borne diseases, fleas nevertheless receive relatively less research attention than other disease vectors such as mosquitoes and ticks (Eisen and Gage 2012).

In Taiwan, the species identity of fleas on shrews and rodents has been intensively investigated in the 1960s and 1970s. These studies were implemented mainly in areas of higher elevation (>1,500 m; Jameson and Hsieh 1966, 1967, 1969, 1971; Phillips 1966; Hsieh and Jameson 1971), with only a few investigations being conducted on lowland sites (<500 m; Jameson and Hsieh 1966, Murrell and Cates 1970). More recent research has focused on the cat flea (*Ctenocephalides felis* Bouché) collected from dogs and cats (Hsu et al. 2002, 2011; Tsai et al. 2011) or fleas on shrews and rodents

in airports and seaports (Chien et al. 2012). Therefore, the status of fleas, including those infesting small mammals, in rural lowland environments has been insufficiently investigated. *Stivalius aporus* Jordan & Rothschild (Siphonaptera: Stivaliidae) and *Acropsylla episema* Rothschild (Siphonaptera: Leptopsyllidae) are the primary flea species infesting small mammals in agricultural fields in eastern Taiwan (Kuo et al. 2012); this differs from those collected from small mammals in the ports, which comprised almost exclusively (97.7%) of the oriental rat flea, *Xenopsylla cheopis* Rothschild (Siphonaptera: Pulicidae) (Chien et al. 2012), suggesting that flea fauna might vary with the degree of human disturbance and/or host species (primary rodent hosts differed between Kuo et al. (2012) and Chien et al. (2012)). Flea-borne pathogens are usually associated with specific flea species, such as *R. typhi* with the oriental rat flea or the cat flea (Azad 1990, Blanton et al. 2016), or *R. felis* primarily with the cat flea (Angelakis et al. 2016). Therefore, knowledge on species identity of fleas is both fundamental to and important for the evaluation of human risks for flea-borne diseases.

Moreover, because flea-borne rickettsial pathogens remain largely neglected (such as *R. typhi*; Chikeka and Dumler 2015) or are emerging worldwide (such as *R. felis*; Brown and Macaluso

2016), investigating the existence of these rickettsiae in fleas is needed. In Taiwan, *R. felis* or closely related species were identified from *S. aporus* and *A. episema* in the rural fields of eastern Taiwan, although at low prevalence (1.3% for each flea species; Kuo et al. 2012). Both *R. felis* and *R. typhi* or closely related species were also detected in small mammals trapped in rural Taiwan, with a prevalence of 2.9% for *R. felis* and 9.4% for *R. typhi* (Kuo et al. 2015b). Both studies imply that flea-borne rickettsiae might circulate in rural areas, thus warranting an investigation of fleas in rural Taiwan. This is particularly important when the clinical manifestations of flea-borne spotted fever and murine typhus (e.g., high fever, headache, and rash; Parola and Raoult 2006) are the same as those of scrub typhus, which is the most common rickettsial disease in rural Taiwan (Kuo et al. 2011). Assessing the circulation of any potential rickettsia is, therefore, the first step toward elucidating likely disease-causing agent.

In this study, fleas infesting small mammals (shrews and rodents) were surveyed in rural lowland Taiwan and examined for the occurrence of *Rickettsia*, especially *R. felis* and *R. typhi*. Fleas were collected from small mammals across several lowland regions to advance the general understanding of flea fauna in rural Taiwan and geographical variation in dominant flea species. Rodents were particularly targeted because they are the primary hosts of fleas, with about 74% of over 2,000 extant flea species having been recorded on rodents (Whiting et al. 2008). To our knowledge, this research constitutes one of the few intensive studies on fleas of small mammals (>1,000 small mammals) not only in Taiwan (e.g., Chien et al. 2012) but also in East and Southeast Asia (e.g., Kim et al. 2010, Ko et al. 2011, Peng et al. 2015).

## Materials and Methods

### Small Mammal Trapping and Collection of Fleas

From 2006 to 2010, shrews and rodents were trapped in three counties each in eastern Taiwan (Yilan, Hualien, and Taitung), western Taiwan (Taoyuan, Taichung, and Kaoping), and surrounding islets (Matsu, Kinmen, and Penghu) (Fig. 1). In each site, a total of 80 Sherman traps (26.5 × 10.0 × 8.5 cm) and 80 meshed traps (27 × 16 × 13 cm) were deployed in lowland (<500 m) agricultural fields and rural villages for at least two seasons and baited with sweet potato smeared with peanut butter. Trapped small mammals were euthanized with an overdose of Zoletil 50 (Virbac SA, Carros, France), with collected fleas preserved in 70% ethanol and stored at -70°C for subsequent molecular investigation. Fleas were morphologically identified to species following Hopkins and Rothschild (1987) and Liu (1986). Livers, kidneys, and spleens of shrews and rodents were also collected for a parallel study on detection of *Rickettsia* in small mammals (Kuo et al. 2015b). All animal handling procedures were approved by the Taiwan Centers for Disease Control and adhered to Guideline for the Care and Use of Laboratory Animals established by the Taiwan Council of Agriculture.

### Detection of *Rickettsia* spp. in Fleas

A small proportion of fleas was selected for detection of *Rickettsia*; from most sites, we randomly selected 10 fleas each, and included representatives from each flea species recovered in that county. Flea DNA was extracted from individual fleas and purified with a QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany), and then tested individually for the presence of *Rickettsia* with nested polymerase chain reaction (PCR) following Kuo et al. (2015b). This method targeted the gene encoding the 120- to 135-kDa outer membrane



Fig. 1. Study sites for the collection of fleas in Taiwan from 2006 to 2010.

protein B (*ompB*; outer primer pair: *ompB* OF, 5'-GTA ACC GGA AGT AAT CGT TTC GTA A-3'; *ompB* OR, 5'-GCT TTA TAA CCA GCT AAA CCA CC-3'; inner primer pair: *ompB* SFG IF, 5'-GTT TAA TAC GTG CTG CTA ACC AA-3'; *ompB* SFG/TG IR, 5'-GGT TTG GCC CAT ATA CCA TAA G-3'; *ompB* TG IF, 5'-AAG ATC CTT CTG ATG TTG CAA CA-3') and citrate synthase (*gltA*; outer primer pair: RpCS.877p, 5'-GGG GGC CTG CTC ACG GCG G-3'; RpCS.1258n, 5'-AAT GCA AAA AGT ACA GTG AAC A-3'; inner primer pair: RpCS.896, 5'-GGC TAA TGA AGC AGT GAT AA-3'; RpCS.1233n, 5'-GCG ACG GTA TAC CCA TAG C-3'). Laboratory *Rickettsia rickettsii* Brumpt antigen and PBS solution were used as positive and negative controls, respectively. The PCR products were separated by electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and identified under UV fluorescence. Samples were deemed positive for the presence of *Rickettsia* when either the *ompB* or *gltA* gene was detected. To further identify the *Rickettsia* spp. in positive samples, PCR products were purified with a QIAquick Gel Extraction Kit and then sequenced once in each direction. DNA nucleotide sequences were assessed with the Basic Local Alignment Search Tool (<http://www.ncbi.nlm.nih.gov>) for any resemblance to known *Rickettsia* spp. reference sequences in GenBank.

### Statistical Analyses

We compared flea load (mean fleas per host individual) among host species and study sites with negative binomial generalized linear model to account for overdispersion of data, and significant difference was evaluated based on the 95% Wald CI. All procedures were implemented in SPSS Statistics version 19.0 (IBM Corp.).

## Results

### Flea Infestations on Small Mammals

We collected 700 fleas comprising four species from 1,260 small mammals belonging to 11 species (Tables 1). *Rattus losea* Swinhoe

was the most common small mammal species (49.3% of total traps), followed by *Suncus murinus* L. (22.1%) and *Mus caroli* Bonhote (11.5%); the other eight species each constituted <5% of all captures (Table 1). *R. losea* was also loaded with most of the collected fleas (88.3% of total fleas), and hosted all four flea species, while *Apodemus agrarius* Pallas had the highest prevalence of infestation (29.2%; proportion of infested individuals), although this rodent species hosted only a small number of fleas (1.6%) (Table 1). Flea load (fleas per host individual) varied significantly among host species (negative binomial generalized linear model,  $P < 0.001$ ), with *R. losea* having the highest flea load of  $1.0 \pm 0.11$  (mean  $\pm$  SE; Table 1), which was significantly higher than the other host species, except for *A. agrarius* and *Rattus norvegicus* Berkenhout (all  $P < 0.05$ ).

More than three quarters of the fleas belonged to *Nosopsyllus nicanus* Jordan (Siphonaptera: Ceratophyllidae) (79.0% of total); the remaining fleas comprised *X. cheopis* (14.6%), *A. episema* (3.9%), and *S. aporus* (2.6%). Even with the large number of *N. nicanus*, it was found only in three small mammal species, in contrast to *X. cheopis*, which was collected from six species. Despite their limited abundance, *A. episema* and *S. aporus* was each associated with four species (Table 1). Flea species varied in geographical distribution, whereas *N. nicanus* was distributed mainly in the islets, *X. cheopis* occurred primarily in the main island of Taiwan, and *A. episema* and *S. aporus* were limited to western and eastern Taiwan, respectively (Table 2). Flea load on the primary host, *R. losea*, also varied significantly among study sites ( $P < 0.001$ ): flea load was significantly higher in Matsu islet ( $3.18 \pm 0.58$ ; Table 2) and Kinmen islet ( $1.88 \pm 0.29$ ) than the other study sites (all  $P < 0.05$ ).

### Detection of *Rickettsia* in Fleas

In total, 67 fleas were subjected to PCR in order to detect presence of *Rickettsia*, including 5 *A. episema*, 33 *N. nicanus*, 8 *S. aporus*, and 21 *X. cheopis* (Table 3). Overall prevalence was 20.9 and 16.4% when based on the *ompB* and *gltA* genes, respectively, but there was large variation in prevalence among flea species and study sites, with the highest rate occurring in *N. nicanus* and the Matsu islet (Table 3).

Five *Rickettsia* or closely related species were identified, including *Rickettsia conorii* Brumpt (100% similarity to reference sequence AE006914), *Rickettsia felis* URRWXC12 Bouyer et al. (100% to CP000053), *Rickettsia japonica* Uchida (100% to CP000053), *Rickettsia raoultii* Mediannikov (99.0% to JQ792105), and *Rickettsia rickettsii* Brumpt (99.7% to CP018914; 99.7–100% to CP006009; Table 4). *Rickettsia felis* was identified in an *X. cheopis* in Kaoping. Once again, *N. nicanus* was detected as having the highest number of *Rickettsia* spp. (four), whereas geographical variation in the number of *Rickettsia* spp. was relatively small (range: 1–2 species; Table 4).

### Discussion

In this study, we found that in rural lowland Taiwan, *R. losea* was the most common small mammal flea host, and *N. nicanus* was the most abundant flea species. In addition, a total of five *Rickettsia* spp. were identified, including *R. felis*, an emerging zoonotic flea-borne pathogen.

Past studies have revealed *X. cheopis* to be the primary flea species on shrews and rodents (mainly commensal *R. norvegicus* and *S. murinus*) in the airports and seaports of Taiwan (Chien et al. 2012). *Xenopsylla cheopis* was also the most common species infesting commensal *Rattus tanezumi* Temminck (*R. rattus tanezumi*) in Taipei (Murrell and Cates 1970). On the other hand, Jameson and Hsieh (1966) noted that *Leptopsylla segnis* Schönherr was abundant on commensal shrews and rodents (*R. norvegicus*, *R. tanezumi*, and *S. murinus*) in the Penghu islet but was rare on the Taiwan main island. Furthermore, flea species in higher elevation (>1,500 m) areas (Jameson and Hsieh 1966, 1967, 1969, 1971; Hsieh and Jameson 1971) were different from those in lowland (<500 m) areas as shown in the preceding text (Jameson and Hsieh 1966, Murrell and Cates 1970, Chien et al. 2012). In this study, we found instead *N. nicanus* to be the most dominant species infesting small mammals in rural lowland Taiwan, although it should be noted that *N. nicanus* was collected predominantly from *R. losea*

**Table 1.** Total number of captures of each shrew and rodent species at nine study sites in Taiwan from 2006 to 2010, as well as prevalence of flea infestation (proportion of infested individuals), flea load (fleas per host individual), total fleas collected, and flea species identified

Mammal species	Number of mammals (% of total)	Prevalence of flea infestation (%)	Flea load (mean per animal $\pm$ SE)	Number of fleas (% of total)	Flea species (number of fleas)
<b>Shrew</b>					
<i>Suncus murinus</i>	278 (22.1%)	4.7	$0.09 \pm 0.04$	26 (3.7%)	<i>A. episema</i> (5); <i>N. nicanus</i> (8); <i>S. aporus</i> (1); <i>X. cheopis</i> (12)
<b>Rodent</b>					
<i>Apodemus agrarius</i>	24 (1.9%)	29.2	$0.46 \pm 0.22$	11 (1.6%)	<i>S. aporus</i> (11)
<i>Bandicota indica</i>	56 (4.4%)	5.4	$0.29 \pm 0.22$	16 (2.3%)	<i>A. episema</i> (12); <i>X. cheopis</i> (4)
<i>Callosciurus erythraeus</i>	2 (0.2%)	0	0	0 (0%)	
<i>Mus caroli</i>	145 (11.5%)	4.8	$0.10 \pm 0.05$	14 (2.0%)	<i>A. episema</i> (8); <i>S. aporus</i> (2); <i>X. cheopis</i> (4)
<i>Mus musculus</i>	48 (3.8%)	2.1	$0.02 \pm 0.02$	1 (0.1%)	<i>N. nicanus</i> (1)
<i>Niviventer coninga</i>	2 (0.2%)	0	0	0 (0%)	
<i>Rattus exulans</i>	25 (2.0%)	12.0	$0.24 \pm 0.15$	6 (0.9%)	<i>X. cheopis</i> (6)
<i>Rattus losea</i>	621 (49.3%)	23.5	$1.0 \pm 0.11$	618 (88.3%)	<i>A. episema</i> (2); <i>N. nicanus</i> (544); <i>S. aporus</i> (4); <i>X. cheopis</i> (68)
<i>Rattus norvegicus</i>	11 (0.9%)	18.2	$0.73 \pm 0.56$	8 (1.1%)	<i>X. cheopis</i> (8)
<i>Rattus tanezumi</i>	48 (3.8%)	0	0	0 (0%)	
Total	1260	14.4	$0.56 \pm 0.06$	700	<i>A. episema</i> (27); <i>N. nicanus</i> (553); <i>S. aporus</i> (18); <i>X. cheopis</i> (102)

**Table 2.** Total number of fleas collected from shrews and rodents, and flea load (fleas per host individual) on the primary host *Rattus losea* at each study site of Taiwan from 2006 to 2010.

Study site	Flea species				Overall	Flea load on <i>Rattus losea</i> mean per animal $\pm$ SE ( <i>n</i> of <i>R. losea</i> )
	<i>Acropsylla episema</i>	<i>Nosopsyllus nicanus</i>	<i>Stivalius aporus</i>	<i>Xenopsylla cheopis</i>		
Eastern Taiwan						
Yilan	0	0	0	0	0	0 (91)
Hualien	0	0	15	7	22	0.11 $\pm$ 0.07 (19)
Taitung	0	0	3	3	6	0.26 $\pm$ 0.15 (19)
Western Taiwan						
Taoyuan	0	6	0	7	13	0.11 $\pm$ 0.04 (116)
Taichung	0	0	0	20	20	0.42 $\pm$ 0.25 (33)
Kaoping	27	0	0	65	92	1.05 $\pm$ 0.26 (44)
Islets						
Matsu	0	184	0	0	184	3.18 $\pm$ 0.58 (57)
Kinmen	0	356	0	0	356	1.88 $\pm$ 0.29 (186)
Penghu	0	7	0	0	7	0.13 $\pm$ 0.07 (56)
Overall	27	553	18	102	700	1.0 $\pm$ 0.11 (621)

**Table 3.** Prevalence for detection of *Rickettsia* spp. in fleas for each study site in Taiwan from 2006 to 2010.

Study site	Prevalence (%) for <i>ompB</i> gene/ <i>gltA</i> gene (sample size)				Overall
	<i>Acropsylla episema</i>	<i>Nosopsyllus nicanus</i>	<i>Stivalius aporus</i>	<i>Xenopsylla cheopis</i>	
Eastern Taiwan					
Yilan	–	–	–	–	–
Hualien	–	–	0%/25% (8)	0%/0% (2)	0%/20% (10)
Taitung	–	–	–	–	–
Western Taiwan					
Taoyuan	–	33.3%/0% (6)	–	50%/0% (4)	40%/0% (10)
Taichung	–	–	–	0%/0% (10)	0%/0% (10)
Kaoping	0%/0% (5)	–	–	40%/0% (5)	20%/0% (10)
Islets					
Matsu	–	70%/80% (10)	–	–	70%/80% (10)
Kinmen	–	0%/10% (10)	–	–	0%/10% (10)
Penghu	–	14.3%/0% (7)	–	–	14.3%/0% (7)
Overall	0%/0% (5)	30.3%/27.3% (33)	0%/25% (8)	19.0%/0% (21)	20.9%/16.4% (67)

(544/553 = 98.4%) and dominance of this flea species might be an artifact of overrepresentation of *R. losea* in our trapping effort, particularly in the Kinmen islet.

However, a remarkable geographical variation was observed in this study: *N. nicanus* occurred predominantly in the islets, whereas *X. cheopis* was found only in the main island of Taiwan (Table 1), despite the fact that these two flea species were collected from the same host (*R. losea*) in a rural environment. An integration of this study and results from Chien et al. (2012)—both studies overlapping greatly in the surveillance period (2006–2010, 2004–2011, respectively)—concluded that *X. cheopis* was the most common species infesting small mammals both in the ports and in the rural areas of the main island, particularly in Kaoping and Taichung of western Taiwan; for the Matsu and Kinmen islets, however, although *X. cheopis* was still the most abundant species in the ports, *N. nicanus* dominated in the rural environment. On the other hand, because Penghu islet was not studied by Chien et al. (2012), the species composition of fleas in the ports of Penghu was unknown. Nonetheless, based on the commensal characteristics of species of small mammals reported by Jameson and Hsieh (1966), it is likely that in Penghu, *L. segnis* may be common in a more urban environment (based on Jameson and Hsieh 1966), whereas *N. nicanus*

may be common in a more rural environment (this study), although significant environmental change in Penghu since the publication of Jameson and Hsieh (1966) might have affected the flea fauna, too.

Similarly, we found a habitat difference in small mammal species composition: *R. losea* and *R. norvegicus* were the most dominant species in the rural environment (Table 1) and ports (Chien et al. 2012), respectively. The fact that *X. cheopis* was common across different habitats in western Taiwan, despite harboring distinct host species (*R. losea* vs. *R. norvegicus*) demonstrates that *X. cheopis* is adaptable in its host use, which is corroborated in this study by its identification in most small mammal species (Table 1). Because *X. cheopis* is the primary vector of murine typhus (Azad 1990), its widespread occurrence in western Taiwan implies a higher chance of human susceptibility to murine typhus in this part of Taiwan. Indeed, most human incidences of murine typhus from 2000 to 2014 occurred in western Taiwan, especially surrounding Kaoping and Taichung (Kuo et al. 2017).

In comparison, in islets such as Kinmen, *X. cheopis* was replaced with *N. nicanus* when transiting from seaports to inland areas. The Kinmen seaports were unique among seaports of Taiwan in that *R. losea* was, by contrast, more abundant than *R. norvegicus* (Chien et al. 2012). Habitat difference in flea fauna in Kinmen, thus,

**Table 4.** *Rickettsia* spp. or closely related species identified in fleas for each study site in Taiwan from 2006 to 2010

Study site	<i>Rickettsia</i> spp. or closely related species based on <i>ompB</i> gene/ <i>gltA</i> gene (number of detections; % identity to sequence on GenBank)				
	<i>Acropsylla episema</i>	<i>Nosopsyllus nicanus</i>	<i>Stivalius aporus</i>	<i>Xenopsylla cheopis</i>	Overall
Eastern Taiwan					
Yilan	–	–	–	–	–
Hualien	–	–	–// <i>R. rickettsii</i> (1; 99.7%)	–	–// <i>R. rickettsii</i> (1)
Taitung	–	–	–	–	–
Western Taiwan					
Taoyuan	–	<i>R. japonica</i> (2; 99.7%)//–	–	<i>R. japonica</i> (2; 99.7%–100%)//–	<i>R. japonica</i> (4)//–
Taichung	–	–	–	–	–
Kaoping	–	–	–	<i>R. felis</i> (1; 100%); <i>R. rickettsii</i> (1; 100%)//–	<i>R. felis</i> (1); <i>R. rickettsii</i> (1)//–
Islets					
Matsu	–	<i>R. conorii</i> (5; 99.7%–100%); <i>R. rickettsii</i> (1; 99.7%)// <i>R. rickettsii</i> (7; 99.7%–100%)	–	–	<i>R. conorii</i> (5); <i>R. rickettsii</i> (1)// <i>R. rickettsii</i> (7)
Kinmen	–	–// <i>R. rickettsii</i> (1; 99.7%)	–	–	–// <i>R. rickettsii</i> (1)
Penghu	–	<i>R. raoultii</i> (1; 99.0%)//–	–	–	<i>R. raoultii</i> (1)//–
Overall	–	<i>R. conorii</i> (5); <i>R. japonica</i> (2); <i>R. raoultii</i> (1); <i>R. rickettsii</i> (1)// <i>R. rickettsii</i> (8)	–// <i>R. rickettsii</i> (1)	<i>R. felis</i> (1); <i>R. japonica</i> (2); <i>R. rickettsii</i> (1)//–	<i>R. conorii</i> (5); <i>R. felis</i> (1); <i>R. japonica</i> (4); <i>R. raoultii</i> (1); <i>R. rickettsii</i> (2)// <i>R. rickettsii</i> (9)

cannot be explained by the difference in host species as both environments were dominated by *R. losea*. Why Kinmen harbored a high abundance of *N. nicanus* is unclear, as ecological studies on most Taiwanese fleas are lacking. Kinmen and Matsu, two counties with the highest abundance of *N. nicanus* (Table 2), are geographically much closer to mainland China than to the Taiwan main island; additionally, the winter temperature in Kinmen and Matsu is lower than in the other study sites (Fig. 1a, in Kuo et al. 2015a). The importance of such geographical and ecological factors (e.g., temperature) in determining flea fauna needs further investigation.

In this study, the majority of fleas (88.3%) were collected from *R. losea*, which prefers the rural environment in Taiwan. Our parallel investigation on other arthropod disease vectors in rural lowland Taiwan also found this rodent species to be the primary host of hard ticks (family Ixodidae) and chigger mites (family Trombiculidae), accounting for 44.7 and 76.4% of the collected ectoparasites, respectively (Kuo et al. 2015a,c). Altogether, these studies demonstrate that *R. losea* is the most important small mammal host that helps sustain populations of human-biting disease vectors (fleas, ticks, and chigger mites) in rural lowland Taiwan and therefore when needed, should be the most important target for disease control.

We detected *R. conorii*, *R. felis*, *R. japonica*, *R. raoultii*, and *R. rickettsii* or closely related species in the fleas; however, it should be stressed that only fragments of *ompB* and *gltA* genes were sequenced, so further confirmation is warranted. Except for *R. felis*, the other four species of *Rickettsia* are transmitted mainly by hard ticks (Parola et al. 2013). In Taiwan, *R. felis* or closely related species has been detected in the cat flea (Hsu et al. 2011, Tsai et al. 2011) as well as in *S. aporus* and *A. episema* (Kuo et al. 2012). Our study reveals that *X. cheopis*, which used to be the primary vector of plague in Taiwan (no human cases of plague observed in Taiwan after 1952, McNeill et al. 1968), might also vector *R. felis*, at least in the Kaoping region of southern Taiwan, and this is in accordance with that until now the only confirmed *R. felis* infection human case in Taiwan occurred in Kaoping (Tsai et al. 2008). Also, a

few suspected human cases of *R. felis* infection happened there (Lai et al. 2014). Given that *R. felis* is transmitted mainly by the cat flea (Angelakis et al. 2016), which usually occurs on cats and dogs, and the infection rate of *R. felis* in the cat flea is usually high, including in Taiwan (44.3% in Hsu et al. 2011, 21.4% in Tsai et al. 2011), more studies on extent of cat flea infestation on cats and dogs in rural Taiwan will be valuable for understanding potential spill-over effect on the circulation of *R. felis* among small mammals and their fleas, especially when past studies on the cat flea of dogs and cats have all been conducted in the city of Taipei (Hsu et al. 2002, Hsu et al. 2011, Tsai et al. 2011). Moreover, the fact that most *Rickettsia* spp. are mainly transmitted by hard ticks instead of fleas (Parola et al. 2013) has led us to focus more on ticks as part of our project in detecting *Rickettsia* spp. in disease vectors and their small mammal hosts in Taiwan (Kuo et al. 2015b,c), and explain the limited detection of flea samples in this study (67 fleas). Therefore, more studies devoted to the detection of *Rickettsia* spp. in the fleas of small mammals will also be needed to better estimate the extent of flea-borne rickettsiae circulation in rural Taiwan.

Tick-borne *R. conorii*, *R. japonica*, *R. raoultii*, and *R. rickettsii* or closely related species detected in this study might be ingested when fleas fed on the hosts and do not necessarily indicate the capability of fleas to transmit these pathogens. Indeed, these four tick-borne pathogens or closely related species have been detected in the same small mammals (Kuo et al. 2015b) from which fleas examined in this study were collected. Three of the four pathogens (*R. conorii*, *R. japonica*, and *R. rickettsii* or closely related species) have also been detected in ticks collected from the same small mammal hosts (Kuo et al. 2015c). However, it is still possible that fleas might provide alternative transmission routes for some of these *Rickettsia* species. For example, *R. felis* can also be successfully cultivated in tick and mosquito-derived cell lines despite that fleas are the primary competent vectors (Reif and Macaluso 2009). Therefore, presence of tick-borne rickettsiae still warrants further investigation on the competence of fleas in transmitting *Rickettsia* species. Altogether,

these findings demonstrate that in addition to scrub typhus which is a rickettsial disease as well (*Orientia tsutsugamushi* Tamura et al. as the etiologic agent), a variety of *Rickettsia* spp. that can pose risks to human health also circulate in rural lowland Taiwan. Because rickettsial diseases have similar clinical manifestations, including high fever (Parola and Raoult 2006), better diagnosis in rural patients with febrile illness will be required to assess the relative disease burden of different rickettsial agents.

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