

Widespread *Rickettsia* spp. Infections in Ticks (Acari: Ixodoidea) in Taiwan

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J. Med. Entomol. 52(5): 1096–1102 (2015); DOI: 10.1093/jme/tjv083

ABSTRACT Ticks are second to mosquitoes as the most important disease vectors, and recent decades have witnessed the emergence of many novel tick-borne rickettsial diseases, but systematic surveys of ticks and tick-borne rickettsioses are generally lacking in Asia. We collected and identified ticks from small mammal hosts between 2006 and 2010 in different parts of Taiwan. *Rickettsia* spp. infections in ticks were identified by targeting *ompB* and *gltA* genes with nested polymerase chain reaction. In total, 2,732 ticks were collected from 1,356 small mammals. *Rhipicephalus haemaphysaloides* Supino (51.8% of total ticks), *Haemaphysalis bandicota* Hoogstraal & Kohls (28.0%), and *Ixodes granulatus* Supino (20.0%) were the most common tick species, and *Rattus losea* Swinhoe (44.7% of total ticks) and *Bandicota indica* Bechstein (39.9%) were the primary hosts. The average *Rickettsia* infective rate in 329 assayed ticks was 31.9% and eight *Rickettsia* spp. or closely related species were identified. This study shows that rickettsiae-infected ticks are widespread in Taiwan, with a high diversity of *Rickettsia* spp. circulating in the ticks. Because notifiable rickettsial diseases in Taiwan only include mite-borne scrub typhus and flea-borne murine typhus, more studies are warranted for a better understanding of the real extent of human risks to rickettsioses in Taiwan.

KEY WORDS *Rickettsia*, mammal host, rickettsiosis, Taiwan, tick

Ticks are second to mosquitoes as the most important disease vectors and are capable of transmitting a variety of pathogens, including viruses, bacteria, protozoa, and helminthes, to humans and animals (Parola and Raoult 2001, de la Fuente et al. 2008). In fact, ticks transmit the greatest diversity of pathogens among all arthropod vectors (Ahmed et al. 2007). Moreover, recent decades have witnessed the emergence of many tick-borne diseases, including several novel *Rickettsia* species that are pathogenic to humans, such as *Rickettsia africae*, *Rickettsia japonica*, *Rickettsia helvetica*, *Rickettsia honei*, and *Rickettsia slovaca* (Parola and Raoult 2001). Unsurprisingly, ticks and tick-borne diseases are currently under intensive scrutiny to reveal their real significance for human health. However, systematic surveys of ticks and tick-borne diseases are generally lacking in Asia, including the Southeast Asia (Ahmed et al. 2007, Petney et al. 2007).

In Taiwan, ticks have mostly been surveyed sporadically and limited to a few places or species, leaving the

relative occurrence of various tick species around Taiwan unknown. For instance, Hoogstraal and colleagues studied the hosts and geographic distributions of a couple of tick species, including *Argas robertsi* Hoogstraal, Kaiser & Kohls, *Dermacentor taiwanensis* Sugimoto, *Haemaphysalis bandicota* Hoogstraal & Kohls, and *Haemaphysalis mageshimaensis* Saito & Hoogstraal (Hoogstraal and Kohls 1965; Hoogstraal and Santana 1974; Hoogstraal et al. 1974, 1986). Robbins (1996) described the occurrence and host of *Aponomma varanensis* Supino, and recently, Kuo et al. (2011) identified the hosts of *Rhipicephalus haemaphysaloides* Supino and Tsai et al. (2012) reported the hosts and collection sites of several tick species. Checklists of tick species in Taiwan have also been catalogued by Robbins (2005), Chen et al. (2010), and Shih and Chao (2011).

Similarly, information on tick-borne rickettsioses in Taiwan remains sparse. *Rickettsia* sp. TwKM01 was detected in the tick *R. haemaphysaloides* in eastern Taiwan (Hualien) and an islet near Taiwan (Kinmen) and *Rickettsia* sp. TwKM03 was identified in the tick *Ixodes granulatus* Supino in another islet of Taiwan (Matsu; Tsui et al. 2007). *Rickettsia* sp. IG-1 was isolated in *I. granulatus* of eastern Taiwan (Taitung; Tsai et al. 2008a) and *Rickettsia rhipicephali* was identified in *Rhipicephalus sanguineus* Latreille and *R. haemaphysaloides* in central Taiwan (Taichung; Hsu et al. 2011). On the other hand, rickettsial diseases presumably transmitted by ticks were also identified in small mammals and other arthropod vectors in Taiwan, calling for a further surveillance of rickettsiae in co-occurring

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Fig. 1. Study sites for the collection of ticks from small mammal hosts in Taiwan during 2006–2010.

ticks. *Rickettsia japonica*, *Rickettsia conorii*, and *Rickettsia* sp. TwKM01 or closely related species were detected in fleas in Hualien (Kuo et al. 2012) and species most similar to *R. japonica*, *R. conorii*, *Rickettsia raultii*, *Rickettsia rickettsii*, *Rickettsia* sp. IG-1, and *Rickettsia* sp. TwKM01 were detected in small mammals in various study sites of Taiwan (Kuo et al. 2015).

Identification of circulating tick-borne rickettsiae is helpful for the recognition of definitive etiologic agents when many suspected rickettsial diseases in Taiwan cannot be verified (Kuo et al. 2015). In this study, we systematically surveyed ticks and associated *Rickettsia* spp. infections in different regions of Taiwan to unravel the general status and geographical variation in tick fauna and tick-borne *Rickettsia* infections. We capitalized on small mammals for the collection of ticks because small mammals, especially rodents, are the main hosts of ticks (Durden 2006). To date, this is the first systematic surveillance of ticks and tick-borne rickettsiae in Taiwan.

Materials and Methods

Small Mammal Trapping and Collection of Ticks. The study was part of a research project (Kuo et al. 2015) to investigate *Rickettsia* infections in small mammal hosts and associated ectoparasites in Taiwan. From 2006 to 2010, small mammals were trapped in eastern (Yilan, Hualien, Taitung), western (Taoyuan, Taichung, Kaoping), and main islets near Taiwan (Matsu, Kinmen, Penghu). These nine study sites covered different parts of Taiwan (Fig. 1). At each site, 80 Sherman traps (26.5 by 10 by 8.5 cm) and 80 Taiwan-made rodent traps (27 by 16 by 13 cm) were deployed and baited with sweet potato covered with peanut butter. Each site

was surveyed for four consecutive nights and surveyed at least twice. Each night, traps were set up at different locations within the same study site to maximize the trapping coverage. Trapped small mammals (shrews and rodents) were placed in clean nylon mesh bags, anesthetized with an overdose of Zoletil 50 (Virbac SA, Carros, France), and ticks collected, preserved in 70% ethanol, and stored at -70°C for subsequent *Rickettsia* detection. Ticks were morphologically identified to species under a stereomicroscope (Leica MZ12) following Yamaguti et al. (1971), Teng and Jiang (1991), and Baker (1999). Unrecognized ticks were confirmed with molecular methods following Beati and Keirans (2001). All the trapping and animal handling procedures fulfilled the regulations of Taiwan legislations.

Detection of *Rickettsia* spp. in Ticks. Ticks were detected for the presence of *Rickettsia* spp. individually following the method of Kuo et al. (2015). Briefly, we targeted the 120- to 135-kDa surface antigen (*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* genes: 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') with nested polymerase chain reaction (PCR). The length of the *ompB* gene amplified was 250–426 bp and the *gltA* gene was 338 bp. Ticks were deemed positive for *Rickettsia* infections when either the *ompB* or *gltA* gene was detected. The PCR products were separated by electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and identified under UV fluorescence. The PCR products were purified with a QIAquick Gel Extraction Kit (Qiagen, Venlo, Limburg, Netherlands) and then sequenced twice in each direction. DNA nucleotide sequences were assessed with the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov, accessed 15 January 2011) for any resemblance to known *Rickettsia* spp.

Results

Tick Infestations Among Small Mammal Hosts and Study Sites. From 2006 to 2010, 1,356 small mammals were trapped in nine study sites of Taiwan, including one species of shrew (*Suncus murinus* L.) and nine species of rodents (including one squirrel species *Callosciurus erythraeus* Pallas). Among them, *Rattus losea* Swinhoe was the most abundant (58.4% of total captures), followed by *S. murinus* (20.7%), and *Mus caroli* Bonhote (10.3%; Table 1). These three species accounted for ~90% of total captures.

From these small mammals, we collected 2,732 ticks. Prevalence of tick infestations varied among small mammal species, with a greater proportion of *Bandicota indica* Bechstein (48.2%) than *Apodemus agrarius*

Table 1. Prevalence (%) and average loads of ticks among small mammal hosts in Taiwan during 2006–2010

Host species	No. of captures (% of total)	Prevalence (%) of ticks	Mean no. of ticks per host (\pm SE) ^a	Total ticks (% of all)
Shrews				
<i>Suncus murinus</i>	281 (20.7)	13.5	1.0 \pm 0.3	290 (10.6)
Rodents				
<i>Apodemus agrarius</i>	24 (1.8)	25.0	0.5 \pm 0.2	12 (0.4)
<i>Bandicota indica</i>	56 (4.1)	48.2	19.5 \pm 5.1	1,091 (39.9)
<i>Callosciurus erythraeus</i>	3 (0.2)	33.3	2.3 \pm 2.3	7 (0.3)
<i>Mus caroli</i>	140 (10.3)	7.9	0.6 \pm 0.4	90 (3.3)
<i>Mus musculus</i>	21 (1.5)	0	0	0 (0)
<i>Niviventer coxingi</i>	2 (0.1)	50.0	2.0 \pm 2.0	4 (0.1)
<i>Rattus exulans</i>	25 (1.8)	0	0	0 (0)
<i>Rattus losea</i>	792 (58.4)	23.7	1.5 \pm 0.2	1,222 (44.7)
<i>Rattus norvegicus</i>	12 (0.9)	16.7	1.3 \pm 1.0	16 (0.6)
Total	1,356	20.2	2.0 \pm 0.3	2,732

^a Mean number of ticks per host is calculated across all captures, not just those animals that harbored ticks.

Table 2. Total number of ticks of each species recovered from small mammal hosts in Taiwan during 2006–2010

Host species	<i>Ixodes granulatus</i>	<i>Rhipicephalus haemaphysaloides</i>	<i>Haemaphysalis bandicota</i>	<i>Haemaphysali doenitzi</i>	<i>Haemaphysalis spp.</i>	<i>Amblyomma spp.</i>	Total ticks
Shrews							
<i>Suncus murinus</i>	181	108	1	0	0	0	290
Rodents							
<i>Apodemus agrarius</i>	1	10	0	0	1	0	12
<i>Bandicota indica</i>	13	335	741	0	0	2	1,091
<i>Callosciurus erythraeus</i>	7	0	0	0	0	0	7
<i>Mus caroli</i>	3	86	1	0	0	0	90
<i>Mus musculus</i>	0	0	0	0	0	0	0
<i>Niviventer coxingi</i>	4	0	0	0	0	0	4
<i>Rattus exulans</i>	0	0	0	0	0	0	0
<i>Rattus losea</i>	326	870	21	2	3	0	1,222
<i>Rattus norvegicus</i>	11	5	0	0	0	0	16
Total (% of all)	546 (20.0)	1,414 (51.8)	764 (28.0)	2 (0.1)	4 (0.1)	2 (0.1)	2,732

Pallas (25.0%) or *R. losea* (23.7%), disregarding the very few captures of *C. erythraeus* and *Niviventer coxingi* Swinhoe (Table 1). Similarly, tick loads were higher in *B. indica* (mean = 19.5 \pm 5.1 [SE], n = 56) than the other host species (Table 1). However, because of the higher population size of *R. losea*, more ticks were recovered from this rodent species (44.7% of total ticks) than *B. indica* (39.9%) and *S. murinus* (10.6%; Table 1). These three species hosted >95% of total ticks.

These ticks belonged to six taxonomic groups, among which only six ticks can be identified to generic level (Table 2). Common tick species, starting from the more abundant, included *R. haemaphysaloides* (51.8% of total ticks), *H. bandicota* (28.0%), and *I. granulatus* (20.0%). The other tick species each comprised 0.1% of the total ticks (Table 2). However, *I. granulatus* could be found on more host species (eight species) than *R. haemaphysaloides* (six) and *H. bandicota* (four). *H. bandicota* were primarily collected from the host *B. indica*, while *I. granulatus* mainly from *R. losea* and *S. murinus*, and *R. haemaphysaloides* principally from *R. losea* and *B. indica* (Table 2).

Tick infestations varied among study sites, with more ticks recovered at Taichung (41.5% of total ticks) than at Kinmen (40.8%) or Taoyuan (10.4%). In comparison,

we collected none or very few ticks in Yilan (0%) and Penghu (0.1%; Table 3). Similar to the pattern in host generality, *I. granulatus* were collected in more study sites (seven) than *R. haemaphysaloides* (five) and *H. bandicota* (four; Table 3).

Rickettsia spp. Among Tick Species and Study Sites. In total, 329 ticks were individually detected for *Rickettsia* infections based on the presence of *ompB* and *gltA* genes. Overall, the mean positivity rate was 31.9%. Among tick species, *H. bandicota* had high positivity rate (97.3%), *R. haemaphysaloides* and *I. granulatus* had moderate positivity rates (39.7 and 13.2%, respectively), while no *Rickettsia* was detected in *Haemaphysali doenitzi* (Table 4). Geographically, the positivity rate was higher in Kaoping and Penghu (both for 100%), Taitung (64.3%), and Taichung (57.8%; Table 4).

We have successfully sequenced 49 samples, and have identified eight *Rickettsia* spp. or closely related species, including *Rickettsia australis*, *R. conorii*, *Rickettsia felis*, *R. japonica*, *R. rickettsii*, *Rickettsia* sp. IG-1, *Rickettsia* sp. TwKM01, and *Rickettsia typhi*. Except for *R. australis*, *R. felis*, and *R. typhi*, the other five *Rickettsia* species were commonly detected in the ticks (seven or more detections; Table 5). The tick *I. granulatus* harbored more *Rickettsia* species (seven) than

Table 3. Total number of ticks of each species collected from small mammals at different study sites in Taiwan during 2006–2010

Study site	<i>Ixodes granulatus</i>	<i>Rhipicephalus haemaphysaloides</i>	<i>Haemaphysalis bandicota</i>	<i>Haemaphysalis doenitzi</i>	<i>Haemaphysalis</i> spp.	<i>Amblyomma</i> spp.	Total ticks (% of all)
Yilan	0	0	0	0	0	0	0 (0)
Hualien	12	7	0	0	2	0	21 (0.8)
Taitung	65	26	0	0	0	0	91 (3.3)
Taoyuan	118	105	60	0	0	0	283 (10.4)
Taichung	43	444	646	0	0	2	1,135 (41.5)
Kaoping	0	0	51	0	0	0	51 (1.9)
Matsu	34	0	0	0	0	0	34 (1.2)
Kinmen	271	832	7	2	2	0	1,114 (40.8)
Penghu	3	0	0	0	0	0	3 (0.1)
Total	546	1,414	746	2	4	2	2,732

Table 4. Positivity rates (%) of *Rickettsia* detection in ticks collected from small mammals at different study sites in Taiwan during 2006–2010

Study site	<i>Ixodes granulatus</i>	<i>Rhipicephalus haemaphysaloides</i>	<i>Haemaphysalis bandicota</i>	<i>Haemaphysalis doenitzi</i>	Total
Yilan	–	–	–	–	–
Hualien	10.0 (1 of 10) ^a	–	–	–	10.0 (1 of 10)
Taitung	64.3 (9 of 14)	–	–	–	64.3 (9 of 14)
Taoyuan	0 (0 of 10)	–	–	–	0 (0 of 10)
Taichung	0 (0 of 11)	0 (0 of 7)	96.3 (26 of 27)	–	57.8 (26 of 45)
Kaoping	–	–	100 (10 of 10)	–	100 (10 of 10)
Matsu	21.9 (7 of 32)	–	–	–	21.9 (7 of 32)
Kinmen	4.2 (4 of 95)	42.2 (46 of 109)	–	0 (0 of 2)	24.3 (50 of 206)
Penghu	100.0 (2 of 2)	–	–	–	100.0 (2 of 2)
Total	13.2 (23 of 174)	39.7 (46 of 116)	97.3 (36 of 37)	0 (0 of 2)	31.9 (105 of 329)

^a Positivity rate (number of positive samples/number of all samples).

Table 5. *Rickettsia* spp. or closely related species detected in ticks collected from small mammals at different study sites in Taiwan during 2006–2010

Study site	<i>Haemaphysalis bandicota</i>	<i>Ixodes granulatus</i>	<i>Rhipicephalus haemaphysaloides</i>	Total
Yilan	–	–	–	–
Hualien	–	<i>Rickettsia</i> sp. IG-1 (1) ^a ; <i>R. typhi</i> (1)	–	<i>Rickettsia</i> sp. IG-1 (1); <i>R. typhi</i> (1)
Taitung	–	<i>R. conorii</i> (1); <i>Rickettsia</i> sp. IG-1 (8)	–	<i>R. conorii</i> (1); <i>Rickettsia</i> sp. IG-1 (8)
Taoyuan	–	–	–	–
Taichung	–	–	–	–
Kaoping	<i>R. japonica</i> (7)	–	–	<i>R. japonica</i> (7)
Matsu	–	<i>R. australis</i> (1); <i>R. conorii</i> (4); <i>R. rickettsii</i> (3); <i>R. typhi</i> (1)	–	<i>R. australis</i> (1); <i>R. conorii</i> (4); <i>R. rickettsii</i> (3); <i>R. typhi</i> (1)
Kinmen	–	<i>R. felis</i> (1); <i>R. rickettsii</i> (1); <i>Rickettsia</i> sp. TwKM01 (1)	<i>R. conorii</i> (1); <i>R. rickettsii</i> (4); <i>Rickettsia</i> sp. TwKM01 (10); <i>R. typhi</i> (1)	<i>R. conorii</i> (1); <i>R. felis</i> (1); <i>R. rickettsii</i> (5); <i>Rickettsia</i> sp. TwKM01 (11); <i>R. typhi</i> (1)
Penghu	–	<i>R. conorii</i> (1); <i>Rickettsia</i> sp. TwKM01 (1); <i>R. typhi</i> (1)	–	<i>R. conorii</i> (1); <i>R. typhi</i> (1); <i>Rickettsia</i> sp. TwKM01 (1)
Total	<i>R. japonica</i> (7)	<i>R. australis</i> (1); <i>R. conorii</i> (6); <i>R. felis</i> (1); <i>R. rickettsii</i> (4); <i>Rickettsia</i> sp. IG-1 (9); <i>Rickettsia</i> sp. TwKM01 (2); <i>R. typhi</i> (3)	<i>R. conorii</i> (1); <i>R. rickettsii</i> (4); <i>Rickettsia</i> sp. TwKM01 (10); <i>R. typhi</i> (1)	<i>R. australis</i> (1); <i>R. conorii</i> (7); <i>R. felis</i> (1); <i>R. japonica</i> (7); <i>R. rickettsii</i> (8); <i>Rickettsia</i> sp. IG-1 (9); <i>Rickettsia</i> sp. TwKM01 (12); <i>R. typhi</i> (4)

^a *Rickettsia* species or closely related species detected (number of detections, a tick positive for *ompB* or *gltA* genes was counted as one detection and >1 *Rickettsia* species might be identified in the same tick).

R. haemaphysaloides (four), while only *R. japonica* was detected in *H. bandicota* (Table 5). Among the nine study sites, there were more *Rickettsia* species found in Kinmen (five species), than in Matsu (four) or Penghu (three; Table 5).

Discussion

In this study, we reported the primary small mammal host and tick species in Taiwan as well as the prevalence and identity of *Rickettsia* spp. in these ticks. The current study clearly demonstrates that

rickettsiae-infected ticks are widespread in Taiwan, with the circulation of a high diversity of *Rickettsia* spp. in ticks.

We found that *R. losea* and *B. indica* were the main small mammal hosts for ticks in Taiwan. Past studies on the relative role of Taiwanese vertebrates as hosts of ticks are very limited; instead, most have focused on specific host species or simply provide checklists of tick occurrence on various hosts. For instance, *Rhipicephalus micropuls* Canestrini was the main tick infesting cattle (Tsai et al. 2011a) and *R. sanguineus* the primary tick on shelter dogs in Taiwan (Tsai et al. 2011c). In eastern Taiwan (Hualien), *A. agrarius* was identified as the primary host of ticks, followed by *R. losea* (Kuo et al. 2011). Nevertheless, occurrence of *A. agrarius* was limited mainly to Hualien in contrast to the widespread existence of *R. losea* in Taiwan (this study, data not shown). Thus, it is expected that, island wide, *R. losea* is the principal host of ticks in Taiwan. In comparison, while *B. indica* was also widespread in Taiwan (trapped in six of the nine study sites), their population sizes were relatively small (Table 1). The high overall tick abundance in this species is due to their high tick loads (mean = 19.5, Table 1). *B. indica* is the largest murine species in Taiwan. Typically, larger host species are more tolerant of parasite infestation because of larger energy reserve, thus hosting more parasites (Olubayo et al. 1993). This may explain the high tick load in *B. indica*. However, a parallel study in Hualien (in eastern Taiwan) showed much lower tick loads in *B. indica* than other smaller host species (*R. losea* and *A. agrarius*; Kuo et al. 2011), indicating that body size alone cannot explain the variation in tick load. *B. indica* was parasitized with a large number of *H. bandicota* (Table 2), which mainly occurred in western Taiwan (Taoyuan, Taichung, and Kaoping) but was not collected in eastern Taiwan, including Hualien (Table 3). The higher tick load in *B. indica* in the current study (which also includes study sites in western Taiwan) compared with our previous study in Hualien (Kuo et al. 2011) might therefore be associated with the occurrence of *H. bandicota* primarily in western Taiwan.

H. bandicota, *I. granulatus*, and *R. haemaphysaloides* were the most common tick species collected from small mammals in Taiwan. *I. granulatus* was particularly general in the host use, widespread in its geographical distribution, and diverse in the *Rickettsia* spp. harbored. Globally, *I. granulatus* also commonly occurs in the Australasian, Oriental, and Palearctic ecoregions, with small mammals as the main hosts (Koloinin 2009, Guglielmone et al. 2014). Because *I. granulatus* also infests humans (Guglielmone et al. 2014), the general occurrence of this species in Taiwan, along with high diversity of rickettsiae it harbors, indicates that *I. granulatus* warrants further study. Indeed, pathogens other than rickettsiae, including *Borrelia burgdorferi* sensu stricto and *Borrelia valaisiana*, have been identified in this species in Taiwan (Huang et al. 2010, Chao et al. 2012). More significantly, *I. granulatus* was recovered from synanthropic small mammal species, including *S. murinus* and *Rattus norvegicus* Berkenhout, suggesting that humans are likely to encounter this species.

Likewise, *R. haemaphysaloides* was widespread in Southeast Asia and is capable of infesting humans (Koloinin 2009, Guglielmone et al. 2014). In Taiwan, *R. haemaphysaloides* was found on domestic cattle (Tsai et al. 2011a, b, 2012), shelter dogs (Hsu et al. 2011; Tsai et al. 2011c, 2012), and also on small mammal species commonly inhabiting in domestic areas (*S. murinus* and *R. norvegicus*, Table 2). Humans thus have a high chance of infestation with *R. haemaphysaloides*. This species had a moderate *Rickettsia* infective rate (39.7%) in this study but pathogenicity of these strains can still not be definitely confirmed. In comparison, *H. bandicota*, while abundant in Taiwan, was relatively limited in host use, geographic distribution, and diversity of *Rickettsia* spp. carried. This species only occurs in a few countries, including Myanmar, Taiwan, Thailand, and Vietnam (Koloinin 2009) and there has been no reported human infestation of *H. bandicota* (Guglielmone et al. 2014). Besides, the hosts of *H. bandicota*, principally *B. indica*, dwell in natural rather than domestic regions. However, the very high *Rickettsia* positivity rate (97.3%) suggests that *H. bandicota* might be important in the enzootic transmission of rickettsiae.

Eight *Rickettsia* spp. were identified of 49 samples, revealing a high diversity of rickettsiae circulating in ticks of Taiwan. This agrees with our recent findings that nine *Rickettsia* spp. were detected in small mammal hosts trapped in the same study areas (Kuo et al. 2015). Among the eight *Rickettsia* spp., only *R. australis* (or closely related species) was not identified in its small mammal hosts (Kuo et al. 2015). *R. australis* is vectored by *Ixodes* ticks in Australia and is the etiologic agent for Queensland tick typhus, which is mainly prevalent in the eastern part of Australia (Parola et al. 2013). *R. australis* was later identified in *Ixodes ricinus* in the Netherlands (Van Overbeek et al. 2008), although this finding warrants further validation (Tijssse-Klasen et al. 2010). On the other hand, species similar to *R. australis* have been detected in trombiculid mites, the main arthropod vectors for scrub typhus, in South Korea (Choi et al. 2007). Our study confirms that *R. australis* or a related species, although with limited occurrence (Table 5), also circulates in Taiwan; however, only partial nucleotide fragments were sequenced in this study, so further study is needed for a definitive identification.

In this study, *R. felis* and *R. typhi* were not commonly detected in ticks (Table 5). Both rickettsiae are mainly transmitted by fleas (Eisen and Gage 2012). *R. felis* has been detected in humans in Taiwan (Tsai et al. 2008b, Lai et al. 2014) and murine typhus, caused by *R. typhi*, is a notifiable disease in Taiwan, with 13–47 human cases per year between 2005 and 2014 (online data, Taiwan National Infectious Disease Statistics System, <http://midss.cdc.gov.tw/>, accessed 8 May 2015). Ticks might acquire *R. felis* and *R. typhi* when feeding on vertebrate hosts infected with associated rickettsiae. Indeed, we identified *R. felis* and *R. typhi* or closely related species in co-occurring small mammal hosts (Kuo et al. 2015). In comparison, *R. conorii*, *R. japonica*, *R. rickettsii*, *Rickettsia* sp. IG-1, and *Rickettsia* sp.

TwKM01 were frequently found in ticks of Taiwan (Table 5). These rickettsiae are mainly transmitted by ticks (Parola et al. 2013). We have previously identified the five *Rickettsia* spp. or rickettsiae with similar nucleotide sequences in their vertebrate hosts (Kuo et al. 2015), as well as *R. conorii* and *R. japonica* in fleas of Taiwan (Kuo et al. 2012), suggesting existence of ticks that might vector these rickettsiae in Taiwan. The current findings confirm that ticks in Taiwan can potentially transmit a variety of rickettsioses to humans, particularly when the predominant tick species (*I. granulatus* and *R. haemaphysaloides*) also infest humans.

In Taiwan, notifiable rickettsial diseases include scrub typhus and murine typhus, which are transmitted by *Orientia tsutsugamushi*-infected trombiculid mites and *R. typhi*-infected fleas, respectively. The many *Rickettsia* spp. identified in ticks suggests that humans in Taiwan are susceptible to other rickettsioses not routinely screened for. Truly, the etiologic agents of most suspected rickettsial diseases in Taiwan cannot be confirmed (Kuo et al. 2015). More studies are thus needed for a better understanding of the real extent of human risks to rickettsioses in Taiwan.

Acknowledgment

This study was financially supported by Taiwan Centers for Disease Control, Ministry of Health and Welfare (Grant nos. DOH97-DC-2004, DOH98-DC-2012, and DOH99-DC-2028).

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Received 13 March 2015; accepted 9 June 2015.
