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SHORT COMMUNICATION

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Molecular identification of *Rickettsia* spp. in chigger mites in Taiwan

Chi-Chien Kuo[1] | **Pei-Lung Lee**[2] | **Hsi-Chieh Wang**[2]

[1]Department of Life Science, National Taiwan Normal University, Taipei, Taiwan

[2]Center for Diagnostics and Vaccine Development, Centers for Disease Control, Ministry of Health and Welfare, Taipei, Taiwan

Correspondence
Hsi-Chieh Wang, Center for Diagnostics and Vaccine Development, Centers for Disease Control, Taipei, Taiwan.
Email: sjwang1019@gmail.com

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Abstract

The genus *Rickettsia* is the causative agent of several rickettsial diseases that are primarily transmitted by hard ticks. The occurrence of *Rickettsia* in chigger mites, which are vectors of scrub typhus in the western Pacific region, has been infrequently investigated. We identified *Rickettsia* spp. in chiggers collected from small mammals in six counties of Taiwan. Moreover, by capitalising on parallel *Rickettsia* detections on small mammals and their infested ticks and fleas, we were able to identify *Rickettsia* spp. that suggested more intimate associations with chigger mites. *Rickettsia* detection rates in 318 pools of chiggers were 21.7% and 22.3% when based on the *ompB* and *gltA* gene, respectively. Overall, we identified six (based on the *ompB* gene) and eight (*gltA* gene) *Rickettsia* species. Approximately half of the sequenced species were most similar to *Rickettsia* sp. clone MB74-1 (*ompB* gene) and *Rickettsia* sp. TwKM02 (*gltA* gene). Furthermore, both species were either infrequently or never identified in small mammals, ticks and fleas, which suggests that chigger mites might be the primary host of both rickettsiae. Whether both species are pathogenic to humans remains to be studied. They may also be microbial endosymbionts of chigger mites, with their potential effects on the pathogenicity of the aetiologic agent of scrub typhus deserving further investigations.

KEYWORDS
chiggers, microbiomes, *Rickettsia*, *Rickettsia* sp. clone MB74-1, *Rickettsia* sp. TwKM02, Taiwan, Trombiculidae

INTRODUCTION

The family Rickettsiaceae, which includes the genera *Rickettsia* and *Orientia*, is a group of obligate intracellular bacteria residing in eukaryotes. The genus *Rickettsia* contains several species known to be pathogenic to humans, such as *Rickettsia rickettsii*, which is the causative agent of Rocky Mountain spotted fever. Although most of these pathogenic *Rickettsia* species are transmitted by hard ticks, some are transmitted by fleas (*Rickettsia felis* and *Rickettsia typhi*), lice (*Rickettsia prowazekii*) or mites (*Rickettsia akari*). However, the list of new *Rickettsia* species has been continuously growing due to identifications from many other arthropod groups even leeches (Perlman et al., 2006). These *Rickettsia* species may simply be endosymbionts of invertebrates without causing human diseases (Perlman et al., 2006), or they may ultimately be found to be pathogenic to humans decades after their initial discovery (Parola et al., 2013).

Chigger mites of the family Trombiculidae are well known for their vectoring of *O. tsutsugamushi*—that is, the aetiologic agent of scrub typhus—to humans throughout the western Pacific region. During the life cycle of chigger mites, only the larval stage (commonly called chiggers) is parasitical, while the nymphs and adults are free-living in soils. Different from mites in the family Dermanyssidae, which can transmit *R. akari*, the significance of Trombiculidae chigger mites in vectoring *Rickettsia* has been infrequently evaluated despite the chiggers' ability to bite humans and thus potentially transmit infective pathogens. A number of *Rickettsia* species have been

detected in chigger mites in southwestern South Korea, including some that are not known to be transmitted by hard ticks, fleas or Dermanyssidae mites (Choi et al., 2007). In northeastern China, a potentially novel *Rickettsia* species has been identified in *Leptotrombidium scutellare* chiggers (Huang et al., 2017). Moreover, several *Rickettsia* species have been identified in *L. scutellare* in southern Japan (Ogawa et al., 2020). On the Kinmen and Matsu islets of Taiwan, *Rickettsia* sp. TwKM02 and *Rickettsia* sp. TwKM03 were found in *Leptotrombidium deliense* chiggers (Tsui et al., 2007). In this study, which covered most areas of Taiwan and included several assayed chigger species, we aimed to identify *Rickettsia* species and unravel the geographical variation in this understudied arthropod group. Moreover, by capitalising on *Rickettsia* species identification in parallel investigations on small mammal hosts and their associated ticks and fleas (Kuo, Shu, Mu, Lee, et al., 2015; Kuo, Shu, Mu, & Wang, 2015; Wang et al., 2020), we were able to recognise *Rickettsia* species that were more prevalent in chiggers and therefore suggests more intimate associations with chiggers than vertebrates or other arthropod hosts (i.e., ticks and fleas).

MATERIALS AND METHODS

Small mammal trapping and chigger mite collection

From 2006 to 2010, small mammals (shrews and rodents) were trapped in abandoned agricultural fields or villages in rural lowland areas (<500 m in elevation) within three counties each in eastern Taiwan (Yilan, Hualien, Taitung), western Taiwan (Taoyuan, Taichung, Kaoping) and associated islets (Matsu, Kinmen, Penghu) to investigate rickettsia infections in small mammals and their ectoparasites (Kuo, Lee, Chen, & Wang, 2015; Kuo, Shu, Mu, Lee, et al., 2015; Kuo, Shu, Mu, & Wang, 2015; Wang et al., 2020). These nine counties were selected to cover the different regions of Taiwan. In each county, a total of 160 small mammal traps were deployed for at least two seasons and baited with sweet potato covered with peanut butter. Trapped small mammals were euthanised with an overdose of Zoletil 50 (Virbac SA, Carros, France) and collected chiggers were preserved in 70% ethanol and stored at −70°C for subsequent molecular investigation. Since chiggers were uncommon in western Taiwan (Kuo, Lee,

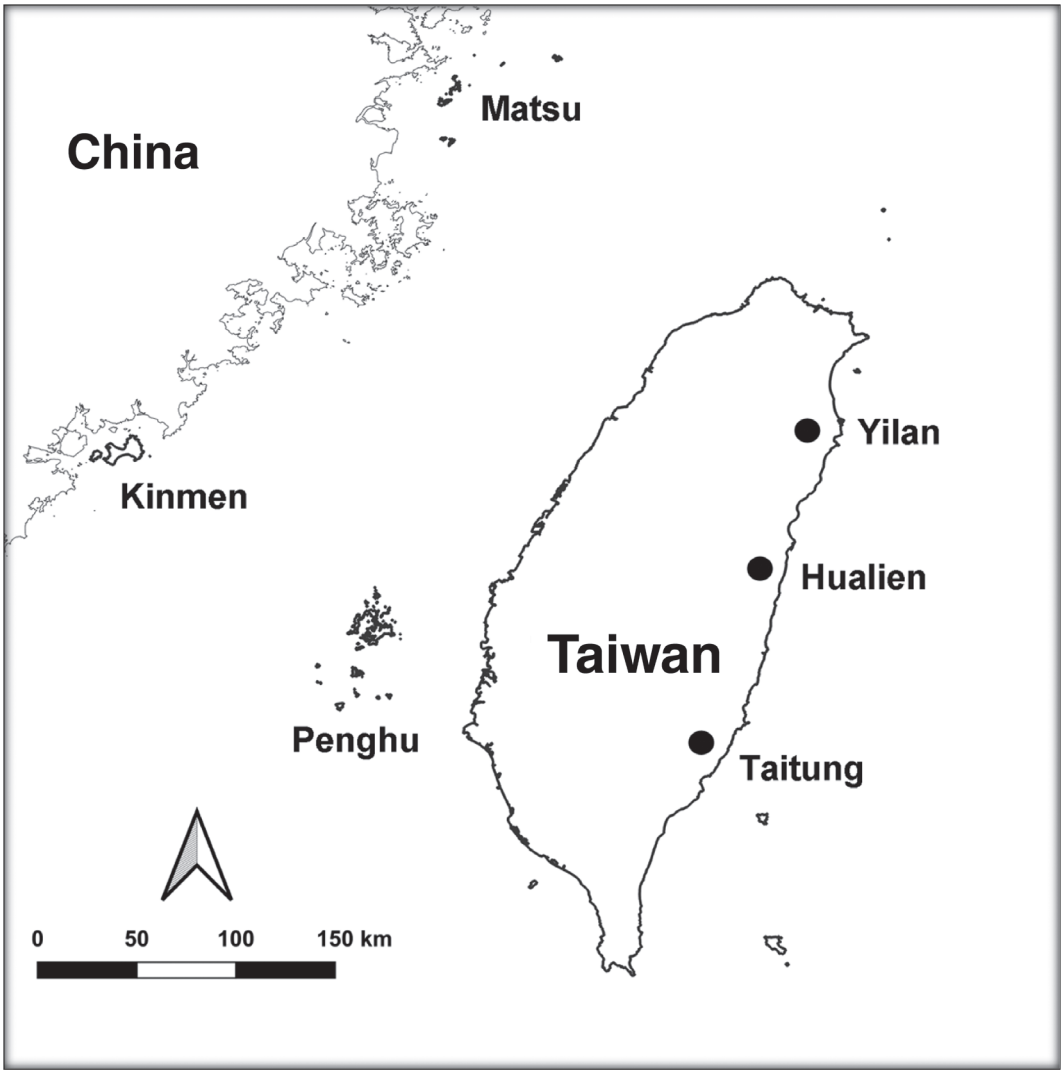

FIGURE 1 Study sites for the detection of *Rickettsia* spp. in chigger mites

Chen, & Wang, 2015), the detection of *Rickettsia* spp. was only performed in eastern Taiwan and associated islets (Figure 1).

Morphological identification of chigger mites

Since the morphological identification of chigger species involves the use of the Berlese fluids that can damage the chigger DNA (thereby inhibiting further *Rickettsia* detection), we were unable to confirm the species of chiggers assayed for *Rickettsia* detection. Thus, species identifications were indirectly inferred from chiggers identified at the species level collected from the same individual mammal host in parallel nationwide investigations of chiggers and *O. tsutsugamushi* infections in Taiwan (Kuo, Lee, Chen, & Wang, 2015). This is plausible because only one very common chigger species typically occurred at each study site or in different seasons at the same study site. Additionally, based on our prior experience, chigger genus can be reliably identified under light microscope based on body shape without the use of slide mounting mediums. We only included the common genus (i.e., *Leptotrombidium*) and excluded uncommon genera (e.g., *Eutrombicula* or *Walchia*) in *Rickettsia* detection because it was difficult to gather 100 chiggers (pooled for pathogen detection, see below) of the same (uncommon) species from the same study site during a single season. To morphologically identify species, chiggers were immersed

TABLE 1 Positivity rate for *Rickettsia* spp. detection and *Rickettsia* spp. or closely related species identified in chigger mites (Acari, Trombiculidae) for each study site in Taiwan from 2006 to 2010

Study site	Positivity rate (%) for *ompB* gene	*Rickettsia* spp. detected	Positivity rate (%) for *gltA* gene	*Rickettsia* spp. detected	Presumed chigger species[a] (*L.* = *Leptotrombidium*)
Eastern Taiwan					
Yilan	12.5 (5 out of 40)	*R. rhipicephali* (2)	25.0 (10 out of 40)	*R. conorii* (1); *R. raoultii* (1); *Rickettsia* sp. IG-1 (2); *R. typhi* (1)	*L. deliense*
Hualien	32.4 (23 out of 71)	*R. conorii* (4); *Rickettsia* sp. clone MB74-1 (17); *Rickettsia* sp. IG-1 (1)	33.8 (24 out of 71)	*R. conorii* (4); Candidatus *R. jingxinensis* (1); *Rickettsia* sp. TwKM02 (11); *R. typhi* (1)	*L. deliense*; *L. imphalum*
Taitung	28.9 (13 out of 45)	*R. conorii* (1); *Rickettsia* sp. clone MB74-1 (10); *Rickettsia* sp. IG-1 (1)	26.7 (12 out of 45)	*Rickettsia* sp. IG-1 (1); *Rickettsia* sp. TwKM02 (10); *R. typhi* (1)	*L. deliense*
Islets					
Matsu	34.0 (17 out of 50)	*R. conorii* (12); *Rickettsia* sp. clone MB115-1 (3)	24.0 (12 out of 50)	*R. conorii* (2); *Rickettsia* sp. clone MG73-6 (1); *Rickettsia* sp. clone MG91-2 (4); *Rickettsia* sp. TwKM02 (5)	*L. deliense*; *L. pallidum*
Kinmen	13.7 (6 out of 44)	*R. felis* (1); *Rickettsia* sp. clone MB74-1 (5)	9.1 (4 out of 44)	*Rickettsia* sp. TwKM02 (3); *R. typhi* (1)	*L. deliense*; *L. scutellare*
Penghu	7.4 (5 out of 68)	*R. conorii* (1); *R. felis* (1); *Rickettsia* sp. clone MB74-1 (2); *Rickettsia* sp. IG-1 (1)	13.2 (9 out of 68)	*R. conorii* (2); Candidatus *R. jingxinensis* (1); *Rickettsia* sp. IG-1 (5); *R. typhi* (1)	*L. deliense*
Overall	21.7 (69 out of 318)	*R. conorii* (18); *R. felis* (2); *R. rhipicephali* (2); *Rickettsia* sp. clone MB74-1 (34); *Rickettsia* sp. clone MB115-1 (3); *Rickettsia* sp. IG-1 (3)	22.3 (71 out of 318)	*R. conorii* (9); Candidatus *R. jingxinensis* (2); *R. raoultii* (1); *Rickettsia* sp. clone MG73-6 (1); *Rickettsia* sp. clone MG91-2 (4); *Rickettsia* sp. IG-1 (8); *Rickettsia* sp. TwKM02 (29); *R. typhi* (5)	

[a]Identifying chigger species involves damaging the chigger DNA (thus inhibits further molecular study), so we were unable to confirm species of the chiggers assayed for *Rickettsia* detection and species was inferred from other species-identified chiggers collected from the same mammal host implemented in Kuo, Lee, Chen, & Wang (2015).

in deionised water 2–3 times (30 min each) to replace ethanol, slide-mounted in Berlese fluid (Asco Laboratories, Manchester, UK), and identified to species following published keys (Li et al., 1997; Wang & Yu, 1992) under a light microscope.

Detection of *Rickettsia* spp. in chigger mites

Chiggers were pooled for *Rickettsia* detection, largely 100 chiggers collected from a single host individual or combined from two host individuals of the same species at the same study site during the same sampling season. *Rickettsia* detection was performed via nested polymerase chain reaction (PCR) following Kuo, Shu, Mu, & Wang (2015) that targeted the genes encoding the 120- to 135-kDa outer membrane 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′; 426 bp) 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′; 338 bp). Nucleic acids were extracted as pooled samples. Laboratory *R. rickettsii* DNA and DEPC-treated water were used as positive and negative controls, respectively. PCR products in positive samples were purified using a QIAquick Gel Extraction Kit and then sequenced twice in each direction. DNA nucleotide sequences were assessed using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov) to determine their similarity to known *Rickettsia* spp.

RESULTS

A total of 318 pools of chiggers were assayed for the presence of *Rickettsia*. According to the previous collections from the same sites (Kuo, Lee, Chen, & Wang, 2015), assayed chiggers could primarily belong to *L. deliense* (197 pools) except for *Leptotrombidium pallidum* (30) on the Matsu islet and *L. scutellare* (20) on the Kinmen islet, which both occurred during the winter (*L. deliense* occurred during the summer on both islets) along with a mixture of *L. deliense* and *Leptotrombidium imphalum* (71) in Hualien throughout the year (Table 1). The chiggers were predominately collected from the rat *Rattus losea* (275 pools), except for some *Rattus tanezumi* individuals (26) in Taitung and a few *Bandicota indica* (15), *Rattus exulans* (1) and *Apodemus agrarius* (1) in Hualien.

The positivity rate for the presence of *Rickettsia* in chiggers was 21.7% when based on the *ompB* gene. Geographically, Hualien and Taitung in eastern Taiwan and Matsu islet had a higher positivity rate (Table 1). A total of six *Rickettsia* or closely related species were identified. More than half of the sequences (54.8%; 34 out of 62 successful sequencings) were most similar to *Rickettsia* sp. clone MB74-1 (97.3%–98.1% similarity). *Rickettsia* sp. clone MB74-1 was identified in Hualien and Taitung counties in eastern Taiwan and the Kinmen and Penghu islets. Notably, it was particularly common in Hualien (17 detections) and Taitung (10 detections) (Table 1). This rickettsia was presumably detected in *L. deliense* (14 detections), *L. scutellare* (3) and a mixture of *L. deliense* and *L. imphalum* (17). The species most closely related to *Rickettsia conorii* (99.2%–100% similarity) were also common (29.0%, 18 out of 62 sequences) and detected in Hualien, Taitung, Matsu and Penghu, with the most detections occurring in Matsu (12 detections) (Table 1). *R. conorii* was presumably identified in *L. pallidum* (12 detections), *L. deliense* (2) and a mixture of *L. deliense* and *L. imphalum* (4). Other less common *Rickettsia* species also included species resembling *R. felis* (99.2%–100% similarity), *Rickettsia rhipicephali* (98.6%–98.9% similarity), *Rickettsia* sp. clone MB115-1 (97.1% similarity) and *Rickettsia* sp. IG-1 (99.5%–100% similarity).

On the other hand, the positivity rate for *Rickettsia* was 22.3% when based on the *gltA* gene. The positivity rate was highest in Hualien. Overall, we identified eight *Rickettsia* or closely related species. *Rickettsia* sp. TwKM02 or related species (98.7%–100% similarity) accounted for nearly half of the identified species (49.2%; 29 out of 59). *Rickettsia* sp. TwKM02 was detected in Hualien, Taitung, Matsu and Kinmen, being especially frequent in Hualien (11 detections) and Kinmen (10 detections) (Table 1). This rickettsia was presumably detected in *L. deliense* (15 detections), *L. pallidum* (2), *L. scutellare* (1) and a mixture of *L. deliense* and *L. imphalum* (11). Species closely related to *R. conorii* (99.3%–100% similarity) and *Rickettsia* sp. IG-1 (99.0%–100% similarity) were also common (15.3%, 9 out of 59; 13.6%, 8 out of 59). *R. conorii* and *Rickettsia* sp. IG-1 were found in four and three counties, respectively, particularly in Hualien (four detections) and Penghu (five detections) (Table 1). Except for two and four *R. conorii* detections that were presumably identified in *L. pallidum* and a mixture of *L. deliense* and *L. imphalum*, respectively, the others were presumably detected in *L. deliense*. The remaining less common *Rickettsia* included species that were mostly related to Candidatus *Rickettsia jingxinensis* (99.3%–99.7% similarity), *Rickettsia raoultii* (100% similarity), *Rickettsia* sp. clone MG73-6 (98.0% similarity), *Rickettsia* sp. clone MG91-2 (98.0% similarity) and *Rickettsia typhi* (96.7%–100% similarity).

DISCUSSION

A total of six and eight *Rickettsia* species were identified in chiggers when based on the *ompB* and *gltA* genes, respectively. Approximately half of the successfully sequenced species showed the highest similarity to *Rickettsia* sp. clone MB74-1 (for the *ompB* gene) and *Rickettsia* sp. TwKM02 (*gltA* gene). Moreover, both species have been detected in four locations and more than one chigger species, demonstrating the widespread and general occurrence of both species. In contrast, *Rickettsia* sp. clone MB74-1 has never been identified in parallel *Rickettsia* investigations on small mammal hosts and their associated ticks and fleas (Table 2); *Rickettsia* sp. TwKM02 has been detected in small mammals (Table 2), but only in one location (Yilan in eastern Taiwan)

TABLE 2 Presence (+) or absence (−) of *Rickettsia* spp. or closely related species identified in small mammal hosts and associated ectoparasites in Taiwan from 2006 to 2010

	Chigger mite[a]	Hard tick[b]	Flea[c]	Mammal host[d]
R. australis	−	+	−	−
R. conorii	+	+	+	+
R. felis	+	+	+	+
R. japonica	−	+	+	+
R. raoultii	+	−	+	+
R. rhipicephali	+	−	−	−
R. rickettsii	−	+	+	+
R. typhi	+	+	−	+
Rickettsia sp. clone MB74-1	+	−	−	−
Rickettsia sp. clone MB115-1	+	−	−	−
Rickettsia sp. clone MG73-6	+	−	−	−
Rickettsia sp. clone MG91-2	+	−	−	−
Rickettsia sp. IG-1	+	+	−	+
Rickettsia sp. TwKM01	−	+	−	+
Rickettsia sp. TwKM02	+	−	−	+
Candidatus *R. jingxinensis*	+	−	−	−

Note: Species was identified based on the *ompB* or *gltA* gene, and was assigned presence when detected in one of the genes.
[a]This study.
[b]Kuo, Shu, Mu, Lee, et al. (2015).
[c]Wang et al. (2020).
[d]Kuo, Shu, Mu, & Wang (2015).

and with low frequency (2.2%, 4 out of 184 sequences) (Kuo, Shu, Mu, Lee, et al., 2015; Kuo, Shu, Mu, & Wang, 2015; Wang et al., 2020). Therefore, it is very plausible that the species closely related to *Rickettsia* sp. clone MB74-1 and *Rickettsia* sp. TwKM02 have more intimate associations with chigger mites than other host groups. That is, chigger mites might be the primary hosts of both rickettsiae.

Before this study, *Rickettsia* sp. clone MB74-1 had only been identified in chiggers (species not reported) in southwestern South Korea (Choi et al., 2007). The species sequenced in this study showed some dissimilarity to the *Rickettsia* sp. clone MB74-1 detected in South Korea (97.3%–98.1% similarity). The next mostly closely related species is *Rickettsia australis*, but with high dissimilarity (93.5% similarity to CP003338.1). *Rickettsia* sp. TwKM02 was firstly identified in *L. deliense* in the Kinmen and Matsu islets of Taiwan (Tsui et al., 2007) and showed the closest similarity to *R. australis* (98.4% similarity to CP003338.1). Species closely related to *Rickettsia* sp. TwKM02 have also been commonly found in chiggers in southwestern South Korea, with similarities ranging from 97.7% to 99.3% (Choi et al., 2007); the sequences obtained from this study were comparatively more similar to *Rickettsia* sp. TwKM02 (98.7%–100% similarity). Since *Rickettsia* sp. clone MB74-1 and *Rickettsia* sp. TwKM02 were identified based on different genes, there is a possibility that both belong to the same *Rickettsia* species. Among the 34 *Rickettsia* sp. clone MB74-1 and 29 *Rickettsia* sp. TwKM02 detections in this study, most (23 detections) were identified from the same chigger pools. This suggests that both may indeed be the same species. In addition to recognising *Rickettsia* sp. TwKM02 based on the *gltA* gene, Tsui et al. (2007) also provided partial sequence of the *ompB* gene for this species (EF364045). However, the different primers used (primers for the *ompB* gene were not provided in Tsui et al., 2007) may lead to the unsuccessful BLAST sequence analysis of partial *ompB* gene sequences of *Rickettsia* sp. clone MB74-1 and *Rickettsia* sp. TwKM02. Complete sequencing of the *ompB* gene of *Rickettsia* sp. clone MB74-1 can help resolve the issue.

Until now, most *Rickettsia* spp. identified in chiggers have the highest homology with *R. australis*, the agent of Queensland tick typhus. This includes not only *Rickettsia* sp. clone MB74-1 and *Rickettsia* sp. TwKM02 but also the novel species identified in northeastern China (Huang et al., 2017) and most species identified in southwestern South Korea (Choi et al., 2007). *Rickettsia* sp. clone MB115-1, *Rickettsia* sp. clone MG73-6, and *Rickettsia* sp. clone MG91-2—which were identified in this study—were also most closely related to *R. australis* (Choi et al., 2007). Some *Rickettsia* spp. identified in chiggers are most closely related to *R. akari* (Choi et al., 2007; Ogawa et al., 2020). From a phylogenetic perspective, tick-transmitted *R. australis* (Stewart et al., 2017) clusters with mite-transmitted *R. akari* (Weinert et al., 2009). Although both *R. akari* and *R. australis* belong to the spotted fever group (SFG) rickettsiae—which are predominately transmitted by hard ticks—*R. akari* is the only exception and is transmitted by mites instead. Additionally, *R. australis* has a distinctive *rompA* gene from other known members of SFG rickettsiae (Stenos &

Walker, 2000). Together, these results suggest that this pair of species is not ordinary SFG rickettsiae. Such speculation is reinforced by the fact that several *Rickettsia* spp. identified in chigger mites are closely related to *R. australis* and *R. akari*. That is, the cluster containing *R. akari*, *R. australis* and chigger-transmitted *Rickettsia* spp. might be distinctive among primarily tick-transmitted SFG rickettsiae. Therefore, a better understanding of *Rickettsia* in chigger mites and their phylogenetic association with other known *Rickettsia* spp. will help shed light on the evolution of SFG rickettsiae, especially their evolving associations with arthropods in addition to ticks.

Species with the highest similarity to *R. conorii*, *R. felis*, *R. raoultii*, *R. rhipicephali*, *Rickettsia* sp. IG-1, *R. typhi* and Candidatus *R. jingxinensis* were also detected in chiggers, despite some having high dissimilarity to known species (e.g., 96.7% similarity to *R. typhi*). Other than *R. felis* and *R. typhi*, which are vectored by fleas, the remaining species are transmitted by or identified in hard ticks. Since chiggers were collected from small mammals in this study, the rickettsiae detected in chiggers might originate from ingested animal tissues instead of natural infections. Indeed, most of the *Rickettsia* spp. (except for *R. rhipicephali* and Candidatus *R. jingxinensis*) have been found in small mammals or their associated fleas and ticks (Table 2).

Geographical variation in *Rickettsia* detection rate has been observed for both the *ompB* (7.4%–34.0%) and *gltA* (9.1%–33.8%) genes. Spatially, there was no significantly positive correlation between the detection rate of both genes ($r_s = 0.43$, $p > 0.05$, Spearman's rank correlation). Nor was the detection rate of either gene associated with the *Rickettsia* detection rate on mammal hosts (both $p > 0.05$, data from Kuo, Shu, Mu, & Wang, 2015), chigger infestation rate or chigger load on rodent hosts (all $p > 0.05$, data from Kuo, Lee, Chen, & Wang, 2015) (correlative analyses were not implemented for *Rickettsia* detection rate on ticks and fleas due to lack of data for some counties). Overall, *Rickettsia* detection rates in chigger mites for both the *ompB* and *gltA* genes were higher in Hualien (eastern Taiwan). The *Rickettsia* detection rate on mammal hosts was also the highest in Hualien (100%, Kuo, Shu, Mu, & Wang, 2015); however, this was not the case for *Rickettsia* infections in ticks and fleas (Kuo, Shu, Mu, Lee, et al., 2015; Wang et al., 2020). The chigger infestation rate and load on rodent hosts were also not highest in Hualien (Kuo, Lee, Chen, & Wang, 2015). While the reason for the higher *Rickettsia* detection rate in chigger mites in Hualien is unknown, it suggests that an abundance of chigger mite-associated *Rickettsia* species circulate in this part of Taiwan and should be a target area for further surveillance.

Our study demonstrates that some species—particularly those showing the highest similarity to *Rickettsia* sp. clone MB74-1 and *Rickettsia* sp. TwKM02—might have closer associations with chigger mites than other arthropod groups. However, it should be emphasised that the relatively short nucleotides (*ompB*: 426 bp; *gltA*: 338 bp) sequenced in this study prevented us from further constructing stable and reliable phylogenetic trees. Therefore, this study may be deemed as a starting point for the further understanding *Rickettsia* infection in chigger mites in Taiwan at best. Additional studies aiming to sequence more complete sequences of both genes should be pursued to determine the evolutionary relationship of *Rickettsia* species in this group of important mites. In addition, whether or not *Rickettsia* sp. clone MB74-1 and *Rickettsia* sp. TwKM02 are pathogenic to humans remains to be studied. On the other hand, both species may be microbial endosymbionts of chigger mites. Studies on ticks have demonstrated that the acquisition of some pathogens can be determined by their microbiomes (Narasimhan et al., 2021). Therefore, whether or not symbiotic bacteria will affect pathogenicity or transovarial transmission of *O. tsutsugamushi* in chigger mites deserves further investigations.

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CONFLICT OF INTEREST

We declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

CCK and HCW conceived and designed the study; PLE and HCW collected the data; CCK analyzed the data; CCK and HCW wrote the first draft of the manuscript. All authors contributed to the final draft of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Chi-Chien Kuo https://orcid.org/0000-0002-1892-6712

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