

### " Bartonella thailandensis": a new genotype of identified from rodents

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### Accepted Manuscript

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#### 26 Abstract

27 Bartonella species, intracellular parasite of erythrocytes and endothelial cells, are 28 zoonotic pathogens of wild and domestic animals including rodents. Many species of rodents 29 are commensally infected with a few *Bartonella* species in Asia. However, there are only few 30 reports on detection of Bartonella in Thailand. Our objective was to detect the presence of 31 Bartonella species in rodents from Thailand. Among 247 rodents captured in five provinces from Thailand we identified *Bartonella* species using molecular methods targeting 3 genes i.e. 32 33 citrate synthase (gltA),  $\beta$ -subunit of the RNA polymerase (rpoB) and cell division protein 34 gene (ftsZ) and the 16-23S rRNA intergenic spacer (ITS). Overall, we found 21 rodents being infected with a Bartonella species including 7 B. coopersplainsensis, 4 B. phoceensis, 6 B. 35 queenslandensis, 1 B. rochalimae, 1 Bartonella sp. RN24BJ and 2 genotypes of a new 36 Bartonella that we propose to give the provisional status "Candidatus Bartonella 37 38 thailandensis". To the best of our knowledge, these Bartonella species have been detected for the first time in Thailand. 39

40 Keywords: Candidatus Bartonella thailandensis, rodent

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#### 51 **1. Introduction**

52 Bartonella species are intracellular parasites of erythrocytes and endothelial cells of 53 several animals including rodents, ruminants, cats and dogs. Bartonella species are considered 54 emerging pathogens in humans. A wide range of mammalian reservoir hosts including rodent 55 and arthropod vectors have been involved in the natural cycle of various *Bartonella* spp. The 56 persistent bacteremia is more readily documented in the primary reservoir species and may 57 occur less frequently or to a much lower level in accidental hosts. In the natural host, clinical 58 manifestation of the infection may be minimal or unrecognized (Chomel et al., 2003). Some 59 Bartonella species such as B. elizabethae, B. grahamii, B. vinsonii subsp. arupensis and B. 60 washoensis have been isolated from humans and linked to a rodent reservoir (Ellis et al., 61 1999; Kosoy et al., 2003). The close relation between rodents and humans throughout the world makes the study of rodent-borne *Bartonella* essential to determine the extent to which 62 63 rodents may serve as a source of human infections (Castle et al., 2004).

Bartonella species associated with animals and fleas have been recovered in Asia 64 65 (Ying et al., 2002; Parola et al., 2003; Castle et al., 2004), Australia (Fournier et al., 2007; 66 Gundi et al. in press) and Europe (Gundi et al., 2004). Different species of mice (Apodemus 67 spp., Mus spp.), rodents (Bandicota spp., Eothenomys spp., Rattus spp.), shrews (Crocidura spp., Sorex spp) and voles (Microtus spp., Clethrionomys spp.) also act as a primary 68 69 vertebrate reservoir of *Bartonella* species that were reported in Asia (Table 1). There are only 70 a few reports on detection of Bartonella in Thailand. Castle et al. (2004) reported a prevalence 71 of 9% in Bandicota indica and Rattus spp. in Chiangrai, Thailand and Parola et al. (2003) 72 identified Bartonella BNfRs in flea (Nosopsyllus fasciatus) collected from Rattus surifer in 73 Kanchanaburi province, on the Thai-Myanmar border. The newly described B. tamiae 74 pathogen was isolated from human patients in Thailand (Kosoy et al., 2008) while B. henselae 75 was recently isolated from biopsy skin lesions of bacillary angiomatosis (Paitoonpong et al.,

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76 2008). The objective of our study was to detect the presence of *Bartonella* species from
77 rodents captured in Thailand by using molecular tools to identify the species.

- 78 2. Materials and methods
- 79 *2.1. Study area*

80 During May and July 2005, rodents were captured in five districts of Thailand near 81 Myanmar (Sangkhlaburi district, Kanchanaburi province; Pobphra district, Tak province), 82 near Laos PDR (Ponepisai district, Nongkhai province) and near Cambodia (Pongnamron 83 district, Chanthaburi province; Sangkha district, Surin province). The localities of study sites 84 were rice paddies, forests and plantations where populations were mainly made up of farmers. 85 Everyday, 200 wire mesh traps were put following rodent signs, rodent burrow, feces 86 footprint or rodent runway from 6 p.m. to 6 a.m. The traps were set up for 5 days in each province. This study was conducted on the animal welfare by the authority of National 87 88 Institute of Health, Thailand.

89 2.2. Samples preparation

Each animal was identified before being euthanasied. Blood samples were collected, separated by centrifugation and total genomic DNA were extracted using NucleoSpin Blood Kit (Macherey-Nagel GmbH, Germany) and kept at -20°C. Samples were handled under sterile conditions to avoid the risk of cross-contamination.

94 *2.3. Samples analysis* 

95 The DNA samples were screened for the presence of *Bartonella* spp. by the use of a 96 real-time quantitative PCR targeting the 23S rRNA gene with Taqman probe. The primers and 97 probe using in this study were: Barto23SF, 5'-GAGTAAGTGGTAGCGGAGCG-3'; 98 Barto23SR, 5'-GCCGACTCACCCTGCTCAG-3' Barto23Sprobe, 6-FAMand 99 GAGAGCTCCTGGAGGTATCGGAAG-TAMRA; size of the PCR product was 172 bp. 100 Positive samples at screening were further studied by PCR amplification and DNA

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101 sequencing of 3 genes including citrate synthase (gltA) (Birtles and Raoult. 1996),  $\beta$ -subunit 102 of the RNA polymerase (*rpoB*) (Renesto et al., 2001) and cell division protein gene (*ftsZ*) 103 (Zeaiter et al., 2002) and 16S-23S rRNA intergenic spacer (ITS). Sequencing of PCR 104 products were carried out using the BigDye Terminator Cycle sequencing Ready Reaction 105 (ABI PRISM, Applied Biosystems) and sequences obtained were compared to those available 106 in GenBank. Phylogenetic relationships among the studied Bartonellae were inferred from 107 sequence alignment from concatenated gene sequences using the maximum parsimony and 108 neighbor-joining methods within the MEGA version 4.1 software package.

109 **3. Results** 

110 *3.1. Rodent species and prevalence of Bartonella infection* 

111 A total of 247 rodent samples representing nine species within three different genera 112 were collected and identified during the study including 2 species of bandicoot rats 113 (*Bandicota* spp.), mouse (*Mus* spp.) and 6 species of rats (*Rattus* spp.) (Table 1).

Overall, the prevalence of *Bartonella* was 12.7% (17 of 134) in the provinces on the border of Cambodia, 3.7% (2 of 54) in the provinces on the border of Myanmar and 3.4% (2 of 59) in the province on the border of Laos PDR as determined using our *Bartonella* genus RT-PCR. *Bartonella* DNA was detected in 6 rodent species: *B. savilei, M. cervicolor, R. berdmorei, R. exulans, R. rattus* and *R. surifer* (Table 1).

119 3.2. Bartonella identification

Using standard PCR and sequencing of all four loci: *gltA*, *rpoB*, *ftsZ* and ITS, we identified the presence of 7 *B. coopersplainsensis*, 4 *B. phoceensis*, 6 *B. queenslandensis*, 1 *B. rochalimae*, 1 *Bartonella* sp. RN24BJ previously identified from *R. norvegicus* and 2 genotypes of a new *Bartonella* in *R. surifer*. The percentage similarity of DNA sequences from known *Bartonella* species ranged from 96 to 97% on *gltA*, 96 to 97% on *ftsZ* and 96 to 98% on *rpoB*. Among known validated species, the two new species shared 90.1 to 90.2%

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homology with *B. phoceensis* for partial *rpoB*, 90.3 to 90.9% homology with *B. washoensis*for *ftsZ* and 92.0 to 92.6% homology with *B. grahamii* for *gltA* gene. The GenBank accession
numbers for this new species are as follows: FJ411481 (*ftsZ*), FJ411482 (*gltA*), FJ411483
(*rpoB*), FJ411484 (ITS) and FJ411485 (ITS).

According to the current knowledge, newly encountered *Bartonella* strains should be considered a new species if a 327-bp *gltA* fragment and a 825-bp *rpoB* fragment share <96.0% and <95.4% sequences similarity with those of the validated species as in the current case. We propose for this new *Bartonella* species the provisional name "*Candidatus* B. thailandensis" since it was first detected in rodents from Thailand. Figure 2 shows the phylogenetic position of this new species among members of the genus *Bartonella* based on comparisons of concatenated sequences of 4 genes.

#### 137 **4. Discussion**

138 We present in this study the molecular detection of Bartonella species detected in 139 rodents from Thailand. Although blood culture is more sensitive and warranted in order to 140 obtain isolates, this was not possible in this study and should be done in the future. We have 141 used the current molecular criteria previously published for detection, identification and 142 discrimination of bacteria of the genus Bartonella (La Scola et al., 2003). These are only few 143 reports on detection of Bartonellae in animals from Thailand (Parola et al., 2003; Castle et al., 144 2004). The overall percentages of infected rodents in our study (8.5%) was similar to that of 145 Castle et al. (2004) who found a prevalence of 9% in rodents (B. indica, R. rattus and R. 146 losea) trapped from Chiangrai province in northern part of Thailand (Castle et al., 2004). 147 However, the Bartonella species detected in the present study were clearly different from 148 Castle et al. (2004) since these species have never been reported in Thailand before. In this 149 previous report, phylogenetic analysis of Bartonella species isolated in rodents indicates that 150 isolates from *B. indica* represent a new *Bartonella* species and those from *Rattus* spp. were

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151 closely related to B. elizabethae and B. grahamii (Castle et al., 2004). However, we have 152 detected in the present study for the first time other *Bartonella* species previously detected in 153 other studies including B. phoceensis (previously isolated from R. norvegicus in the city of 154 Marseille, France) (Gundi et al., 2004), B. rochalimae (previously isolated in a woman with 155 fever, bacteremia and splenomegaly in Peru) (Eremeeva et al., 2007), B. coopersplainsensis 156 and B. queenslandensis (previously isolated in Australian rats, Gundi et al., in press) and 157 Bartonella sp. RN24BJ (previously detected in R. norvegicus in China, Li et al, unpublished 158 data). In this study, we have found *B. phoceensis* from 4 rodents (2 samples *R. rattus* of which 159 1 from Kanchanaburi and 1 from Nongkhai provinces; 2 samples R. surifer from Surin 160 provinces) as previously reported in Asia (Ying et al., 2002; Inoue et al., 2008; Lin et al., 161 2008). However we have not detected in our study either the newly described B. tamiae 162 pathogen isolated from human patients from Khon Kaen province, Thailand (Kosoy et al., 163 2008) or B. henselae isolated from a case of bacillary angiomatosis (Paitoonpong et al., 2008). 164 On the other hands, Parola et al. (2003) identified a new genotype of Bartonella spp. 165 (Bartonella BNfRs) from a Nosopsyllus fasciatus specimen collected on a rat (R. surifer) from 166 the Thai-Myanmar border that was not detected in our study. Conversely, phylogenetic 167 analysis in of concatenated sequences this study indicates that two rodents (R. surifer6 and R. 168 surifer12) were likely infected with a new genotype of Bartonella that we propose to give the 169 provisional status "Candidatus B. thailandensis" according to Murray and Stackerbrandt 170 (1995) guidelines. According to the current molecular criteria from La Scola et al. (2003), our 171 sequences obtained from these 2 rodents fulfilled the criteria for a new Bartonella species (La 172 Scola et al., 2003).

The epidemiological importance of rodent-borne *Bartonella* spp. as a cause of disease in animals and humans is emerging, as suggested for a novel rodent *Bartonella*-associated febrile illness in the rural southwestern USA, based on serological evidence (Boulouis et al.,

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176 2005; Iralu et al., 2006). At present, only a limited number of human infections caused by 177 rodent-borne Bartonella species have been reported such as B. elizabethae, B. grahamii, B. 178 vinsonii subsp. arupensis, B. washoensis. Further studies are required to determine if rodents 179 could be another natural reservoir of *Bartonella* species i.e. *B. rochalimae* identified from *R.* 180 norvegicus in Taiwan (Lin et al., 2008). Further investigations are warranted in order to 181 isolate these new Bartonella species and to determine if they can cause any clinical 182 manifestations related to unknown diseases in Thailand. However, previous experience has 183 shown that Bartonella species firstly detected or isolated in animals or arthropods could be 184 later involved in humans. This was the case for example with *B. alsatica* firstly isolated from wild rabbits (Heller et al., 1999) and later identified as human pathogens in a heart valve of a 185 186 patient with endocarditis (Raoult et al., 2006) and in a patient with cat scratch disease 187 (Angelakis et al., 2008).

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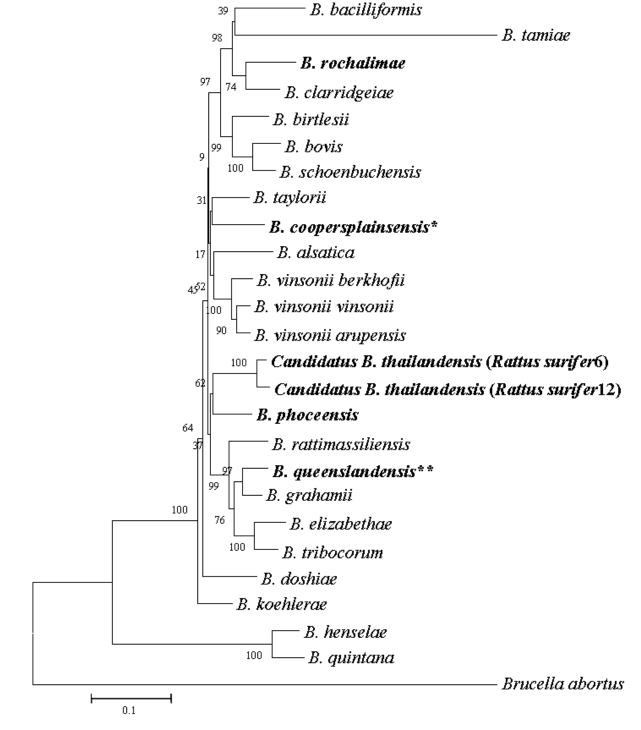
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- 1 Figure 1. Neighbor-Joining concatenated tree of *rpoB*, *gltA*, ITS and *ftsZ* genes on Thai rodent
- 2 samples with known species of *Bartonella*.



GenBank accession numbers: *rpoB* (EU111792\*, EU111791\*\*); *gltA* (EU111803\*,
EU111802\*\*); ITS (EU111770\*, EU111769\*\*); *ftsZ* (EU111781\*, EU111780\*\*)

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## ACCEPTED MANUSCRIP

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Rodent species	Province neighboring Cambodia		Province neighboring Myanmar		Province neighboring Laos PDR	Total (Percentage
	Chanthaburi	Surin	Kanchanaburi	Tak	Nongkhai	of rodent
	No. of tested/positive	No. of tested/positive	No. of tested/positive	No. of tested/positive	No. of tested/positive	infections)
	(Bartonella infection)	(Bartonella infection)	(Bartonella infection)	(Bartonella infection)	(Bartonella infection)	
Bandicota indica	6/0	4/0	8/0	1/0	1/0	20/0 (0)
Bandicota savilei	26/2	3/0	0/0	0/0	4/0	33/2 (6.06)
	(B. queenslandensis {1},					
	B. rochalimae {1})					
Mus cervicolor	0/0	0/0	0/0	2/1	0/0	2/1 (50)
				(B. queenslandensis {1})		
Rattus berdmorei	16/5	4/0	0/0	0/0	0/0	20/5 (25)
	(B. coopersplainsensis {4},					
	B. queenslandensis {1})					
Rattus exulans	1/0	1/0	1/0	21/0	45/1	69/1 (1.45)
					(Bartonella sp. RN24BJ {1})	
Rattus fluvescens	7/0	0/0	0/0	1/0	0/0	8/0 (0)
Rattus losea	2/0	5/0	4/0	0/0	2/0	13/0 (0)
Rattus rattus	24/2	3/0	12/1	3/0	7/1	49/4 (8.16)
	(B. coopersplainsensis {2})		(B. phoceensis {1})		(B. phoceensis {1})	
Rattus surifer	2/0	30/8	1/0	0/0	0/0	33/8 (24.24)
		(B. coopersplainsensis {1},				
	B. queenslandensis {3},					
	B. phoceensis {2},					
		Candidatus B. thailandensis {2})				
Total (Percentage	84/9	50/8	26/1	28/1	59/2	
of infection)	(10.71)	(16)	(3.85)	(3.57)	(3.39)	

#### Table 1. Percentages of Bartonella infections in rodents within three neighboring provinces in Thailand, during May to June, 2005.