

ECOLOGICAL NICHE MODELING OF *RICKETTSIA*-INFECTED AND -UNINFECTED TICKS IN FOREST EDGES OF THAILAND

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Abstract. *Rickettsia* spp are the causative agents of *rickettsia* infection in humans and animals. In Thailand, many *Rickettsia* spp with unknown pathogenicity have been detected in ticks, but the demographic and geographic distributions of their *Rickettsia* infection status remain largely unknown. Here, an ecological niche model based on maximum entropy method was constructed using a maximum entropy program to determine environmental factors and probability of occurrence and distribution of *Rickettsia*-infected ticks based on 38 environmental raster layers. The ecological niche model predicted that optimal *Rickettsia*-infected tick occurrence was during a season with low precipitation and high temperature and an elevation of <1,400 m. In addition, highest frequency of infected ticks was predicted to be found in along the Thai-Cambodia border at the lower region of northeastern Thailand. The predictions were statistically concordant with data collected from forest edges adjacent to residential areas in 33 remote districts of Thailand between 2008-2011. The study should provide baseline information which should assist in future development of surveillance and prevention programs for tick-borne rickettsioses in humans, especially in remote areas where diagnosis is not available and late responses frequently occur.

Keywords: *Rickettsia* spp, ecological niche modeling, forest edge, Thailand, tick

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INTRODUCTION

Rickettsia causing infections in humans and animals are transmitted by arthropod vectors, such as flea, lice, mite and tick (Aung *et al*, 2014). Abundance of a particular tick vector and prevalence of infected ticks and of their natural hosts that come in contact with humans are factors affecting frequency of tick-borne rickettsioses (Parola and Raoult, 2001). In Thailand, many *rickettsia* species with unknown pathogenicity have been detected in ticks, suggesting a potential public health risk (Parola *et al*, 2003a; Parola *et al*, 2003b; Aung *et al*, 2014; Nooroong *et al*, 2018). When trans-stadial and trans-ovaria passages of *rickettsiae* transmissions are efficiently acquired in a tick species, it serves as a reservoir for this etiologic bacteria and the distribution of rickettsiosis becomes identical to that of its particular tick host (Parola and Raoult, 2001). In natural vertebrate hosts, infection may result in rickettsemia, allowing ticks to become infected and natural cycle to be perpetuated. The life cycles of most tick-borne *rickettsiae*, as well as the demographic and geographic distributions of *Rickettsia*-infected ticks, are largely unknown in Thailand.

Several different approaches have been pursued for identifying the demographic and geographic distributions of vector-borne diseases, such as geographic information system (GIS) mapping and remote sensing

(Thomson and Connor, 2000; Rogers and Randolph, 2003; Eisen *et al*, 2005; Rodgers and Mather, 2006), climate-based modeling (Brownstein *et al*, 2003; Eisen *et al*, 2006; Estrada-Peña and Venzal, 2007) and ecological niche (or species distribution) modeling (ENM) (Peterson *et al*, 2004; Adjemian *et al*, 2006; Foley *et al*, 2008; Neerinckx *et al*, 2008). These methods are also suitable for predictive spatial risk modeling when there is a lack of data, especially in remote areas where access to extensive sampling and species reporting is risky, difficult or prohibited, common challenges in the determination of geographic distribution and range of plants, animals and insect species (Mak *et al*, 2010).

ENM has been developed to infer the environmental requirements of a species (Masuoka *et al*, 2010) and to predict the species distribution (Elith *et al*, 2006). Recent availability of improved global climate, land cover and elevation datasets have increased ENM use for a diversity of applications (Masuoka *et al*, 2009). Furthermore, ENM can be beneficially applied to assess disease risk in remote areas (Pozio, 2000; Peterson and Shaw, 2003; Peterson *et al*, 2006; Moffett *et al*, 2007; Burke *et al*, 2008; Masuoka *et al*, 2009; Masuoka *et al*, 2010).

Here, we report the distribution of ticks and their infection with *Rickettsia* spp in forest edges of 33 remote districts of Thailand. Environmental factors related to their occurrence

were predicted using ENM. This study provides the first preliminary picture and understanding of the geographic distribution and associated environmental factors that potentially impact rural Thai health and a framework for surveillance and prevention programs for tick-borne rickettsioses, especially in remote areas.

MATERIALS AND METHODS

Sample collection and identification

Ticks ($n = 522$) were collected using two methods, namely, flagging/dragging for questing ticks and manually picking off herbaceous plants, from 71 collecting locations at forest edges adjacent to private residential areas in 33 remote districts throughout Thailand during 2008-2011 (Table 1). Geographic coordinates of the collecting sites were obtained using a global positioning system (GPS) device (eTrex Vista HCx, Garmin Ltd, Olathe, KS). Ticks were morphologically identified under a stereomicroscope using standard taxonomic keys (Tanskull and Inlao, 1989; Walker *et al*, 2000) and stored at -20°C until used.

PCR and DNA sequencing protocols

Rickettsia DNA from ticks were extracted using a DNeasy tissue kit (Qiagen, Hilden, Germany). For identification of *Rickettsia* sp, amplicon (343 bp) of a fragment of a *Rickettsia* sp-specific gene encoding a 17 kDa antigen was generated by PCR (Higgins

and Azad, 1995; Roux *et al*, 1997) and directly sequenced (Bionics Co Ltd, Seoul, South Korea). *Rickettsia* sp identification was determined using a BLAST pairwise similarity search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) of reference sequences in GenBank database to confirm the infection of ticks that will further used for modeling the distribution of *Rickettsia*-infected ticks in forest edges of Thailand.

We have confirmed DNA sequence of *Rickettsia* bacteria by BLAST against reference sequences in GenBank database. There are about 60 samples in our studies. For ENM related articles, it is acceptable not to include the accession numbers in the papers and almost all of the published articles about this field do not include the GenBank accession numbers. We usually include the accession numbers in the phylogenetic analysis, single-nucleotide polymorphisms (SNP) or restriction fragment length polymorphism (RFLP) related articles.

Environmental raster layer components

Thirty-eight environmental raster layers obtained from several sources (see below) were initially used in ENM (Phillips *et al*, 2006). Each raster layer was re-sampled to a resolution of 1 km, geo-referenced to study areas, and overlaid upon each other. Climate data used consisted of total precipitation and mean minimum and maximum temperatures from January to December obtained from ground weather

stations monthly measurements and averaged for each month from 1950 to 2000 using WorldClim version 1.4 database (<http://www.worldclim.org>) (Hijmans *et al*, 2005). Global elevation data obtained from WorldClim database were re-sampled to 1 km resolution using NASA Shuttle Radar Topography Mission (SRTM) (<https://www2.jpl.nasa.gov/srtm/>) and then processed to the same projection and scale as the other WorldClim layers. Land cover data derived from GLC 2000 project using FAO Land Cover Classification System (LCCS) were retrieved from Global Land Cover 2000 database produced by the European Commission Joint Research Centre (<https://forobs.jrc.ec.europa.eu/products/glc2000/glc2000.php>) and data hierarchical classifications were converted into the more generalized global land cover classes consisting of 19 land cover classes describing the types of vegetation and densities of cover independent of geo-climatic zone, such as temperate or tropical forest. Land cover was used in its original format at a pixel resolution of 1 km².

ENM procedure

Maximum entropy method in MaxEnt 3.2.1 modeling program (https://biodiversityinformatics.amnh.org/open_source/maxent/) (Phillips *et al*, 2006) was employed to model the distribution of *Rickettsia*-infected ticks in forest edges of Thailand.

This model utilizes a maximum entropy algorithm to analyze values of environmental layers to estimate the range of probability of a species present over a geographic region (Phillips *et al*, 2004; Phillips *et al*, 2006). Twenty five percent occurrence points were used to test accuracy of the model testing points, and the remaining 75% were used to build the model training points. Both training and testing points are randomly selected. Using a jackknife test, the program calculates the importance of each environmental variable in developing a predictive distribution model of the species by measuring training gain of the variables used individually as well as in combination with other variables. If a variable produces a high training gain when used alone, the variable is considered important. A variable is also important if the training gain is low when the variable is removed from the model (Phillips *et al*, 2006). The model is considered accurate when the area under the curve (AUC) is >0.8 and when *p*-value of the minimum training presence is <0.05, indicating that the model is not random (Phillips and Dudik, 2008).

In order to determine the best combination of environmental data for modeling, the model was tested six times using six different sets of input layers, namely, i) bioclimatic layers together with precipitation, mean minimum and maximum temperatures, elevation and land

cover data, ii) bioclimatic layers and elevation data, iii) bioclimatic layers and land cover data, iv) different bioclimatic layers only, v) elevation data only, and vi) land cover data only. The probability of occurrence at the pixels of each *Rickettsia*-infected location have been extracted and examined for selecting suitable combination of input environmental data for MaxEnt model.

Statistical analysis

In order to compare the class of land cover between *Rickettsia*-infected and non-infected tick locations, the pixel values of these two locations were extracted using an ArcGIS program (ESRI, Redlands, CA) (www.esri.com). χ^2 test was used to compared land cover association using a Statistical Package for the Social Sciences (SPSS) version 11.5 (SPSS Inc, Chicago, IL). A p -value <0.05 is considered statistically significant.

RESULTS

Tick samples

Tick samples were collected by flagging, dragging or picking from herbaceous leaves in forest edges adjacent to residential areas located in 33 remote districts throughout Thailand from 2008-2011 (Fig 1A). Ticks ($n = 522$) belonged to 16 species of 6 genera as determined by morphology and DNA sequence of a fragment of *Rickettsia* sp-specific gene coding for

a 17 kDa antigen (Table 1).

Distribution of *Rickettsia*-infected and -uninfected ticks

Amblyomma spp ($n=32$), *Dermacentor* spp ($n = 47$), *Haemaphysalis* spp ($n = 282$), and *Rhipicephalus* spp ($n = 153$), both *Rickettsia*-infected and uninfected, were found in forest edges widely distributed across Thailand, with an abundance of *Rickettsia*-infected ticks in the northeastern and eastern regions along the Thai-Cambodian border, and in the northern region along the Thai-Myanmar and Thai-Lao borders (Figs 1B-E). *Boophilus* sp ($n=7$) (infected and uninfected) and *Hyalomma* sp ($n = 1$) (uninfected) were discovered in one district at the Thai-Myanmar and Thai-Cambodia border respectively (Figs 1F, G).

ENM test

ENM is based on a null hypothesis, *ie*, test points are predicted to be no better than a random prediction using various thresholds. Accuracy of an ENM was determined by comparing eight different combinations of environmental variable inputs, including a single data input, which demonstrated three most accurate models were combination of all environmental layers, combination of bioclimatic and elevation layers, and combination of bioclimatic and land cover layers, with the latter also having the highest area under curve (Table 2).

Table 1

Rickettsia infection of tick species collected from forest edges adjacent to residential properties located in 33 remote districts of Thailand (2008-2011)

Tick genus/species	Number infected (%)	Stage
<i>Amblyomma</i> sp (unidentified)	7 (14)	Adult
<i>A. helvolum</i>	1 (100)	Adult
<i>A. integrum</i>	2 (100)	Adult
<i>A. testudinarium</i>	3 (67)	Nymph, adult
<i>A. varanense</i>	19 (37)	Adult
<i>Boophilus</i> (now known as subgenus of genus <i>Rhipicephalus</i>) sp (unidentified)	7 (57)	Adult
<i>Dermacentor</i> sp (unidentified)	17 (53)	Nymph, adult
<i>D. atrosignatus</i>	9 (0)	Adult
<i>D. auratus</i>	21 (0)	Nymph, adult
<i>Haemaphysalis</i> sp (unidentified)	148 (8)	Larvae, adult
<i>H. bispinosa</i>	23 (0)	Adult
<i>H. darjeeling</i>	1 (0)	Adult
<i>H. hystrix</i>	6 (0)	Adult
<i>H. lagrangei</i>	78 (4)	Adult
<i>H. obesa</i>	10 (0)	Adult
<i>H. semermis</i>	5 (0)	Adult
<i>H. shimoga</i>	11 (0)	Adult
<i>Hyalomma</i> sp (unidentified)	1 (0)	Adult
<i>Rhipicephalus</i> sp (unidentified)	43 (49)	Adult
<i>R. haemaphysaloides</i>	3 (0)	Adult
<i>R. microplus</i>	31 (0)	Nymph, adult
<i>R. sanguineus</i>	76 (10)	Adult

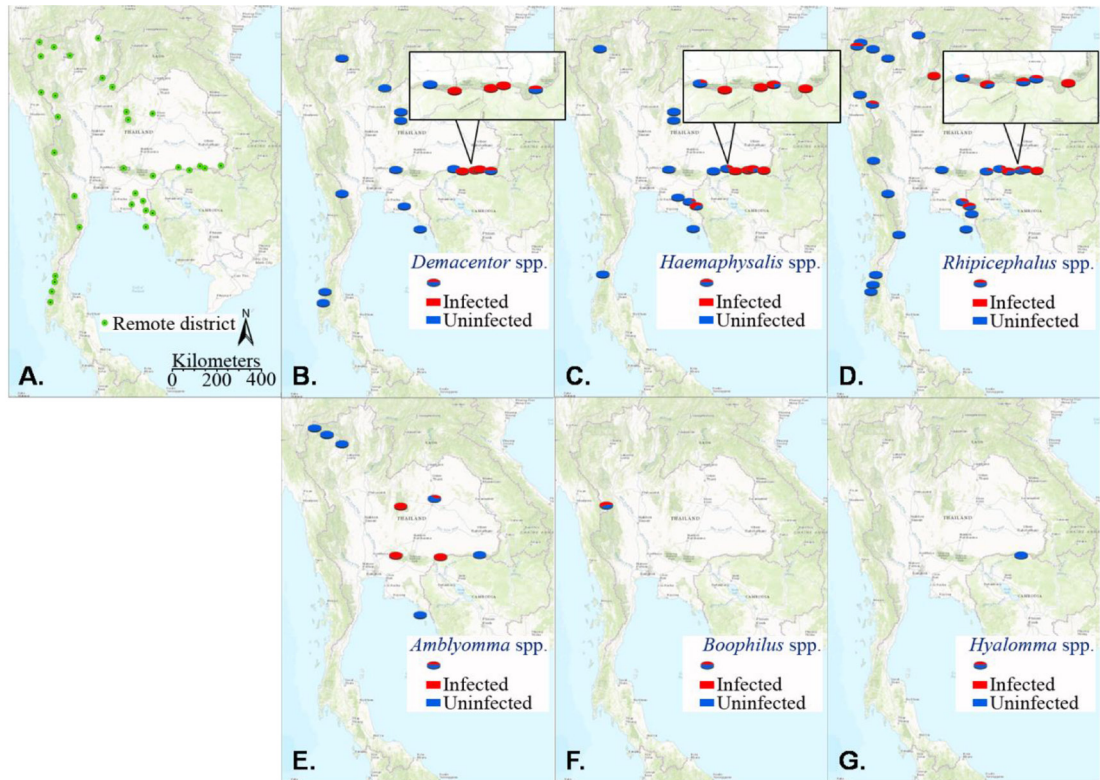


Fig 1 - Sites of *Rickettsia*-infected and -uninfected ticks according to genera in forest edges adjacent to residential properties located in 33 remote districts of Thailand (2008-2011)

A: Location of districts; B: *Dermacentor* spp; C: *Haemaphysalis* spp; D: *Rhipicephalus* spp; E: *Amblyomma* spp; F: *Boophilus* sp; G: *Hyalomma* sp

Red and blue areas represent sites of *Rickettsia*-infected and -uninfected ticks respectively. Green area indicates forest.

Jackknife test

A jackknife test evaluates the contribution of each environmental variable by comparison training gains of models constructed with single variables (Phillips *et al*, 2006).

Test of importance of each environmental variable (elevation (altitude), precipitation, land cover, and minimum and maximum temperatures) showed that low regularized gain variables, namely, average precipitation and maximum

Table 2

Maximum entropy model accuracy using different combinations of environmental variable inputs

Environmental variable input	AUC training points accuracy	AUC test points accuracy	Minimum training presence <i>p</i> -value
All environmental layers	0.989	0.945	<0.001
Bioclimatic and elevation layers	0.986	0.947	<0.001
Bioclimatic and land cover layers	0.983	0.967	<0.001
Precipitation layers only	0.959	0.930	0.003
Maximum temperature layers only	0.912	0.903	0.002
Minimum temperature layers only	0.925	0.925	0.024
Elevation layers only	0.849	0.745	0.020
Land cover layers only	0.865	0.818	0.001

AUC: area under the curve

temperature, were observed during the summer/rainy seasons (April-September) while higher regularized training gains were obtained during the dry season (October-March) (Fig 2). In contrast, regularized gain of minimum temperature was higher than the two above mentioned variables during the summer/rainy seasons, April-September. When tested alone annual precipitation had the highest regularized training gain in December, with the strongest predictive ability of any variable. On the other hand, omission of elevation (altitude) in the test resulted in the largest decrease in regularized training gain, indicating this parameter contributed the most to

the model. Thus, the three variables making the most contributions were elevation (altitude), annual precipitation and annual maximum temperature, helping to explain the common outbreak of tick-borne infections during dry (November-February) and summer (March-April) in Thailand (<https://www.tmd.go.th/en/>).

Factors affecting probability of *Rickettsia*-infected tick occurrence

1. Elevation, precipitation, and maximum and minimum temperatures

Graphs of environmental variables versus probability of *Rickettsia*-infected

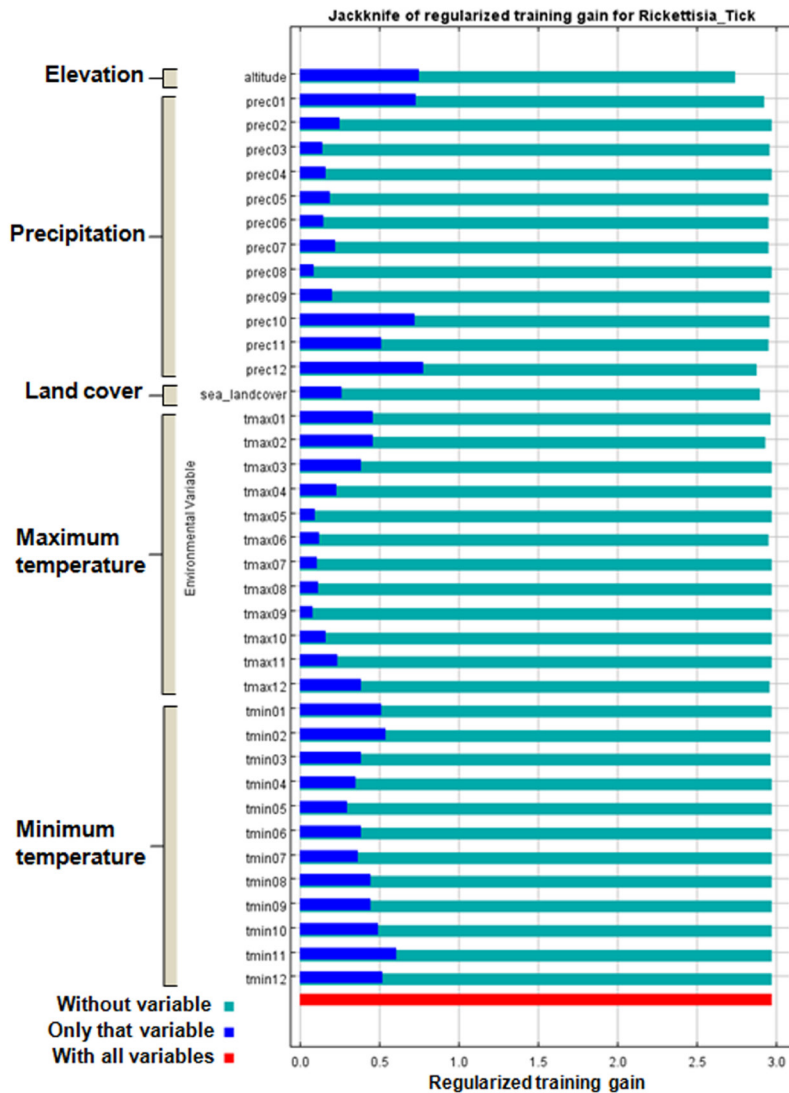


Fig 2 - Jackknife of regularized training gain for variables affecting *Rickettsia*-infected tick occurrence

Red bar represents training gain achieved by a model using all variables. Dark blue bar represents training gain achieved in the model using a single variable. Aqua bar represents training gain achieved when that particular variable is not present in the model.

Prec: precipitation; *Rickettsia_Tick*: *Rickettsia*-infected ticks; sea_landcover: Southeast Asia land cover; tmax: maximum temperature; tmin: minimum temperature.

Numbers 01-12 following variables represent month: 01 being January, and so on.

ticks occurrence demonstrated the following: (i) Probability of occurrence increased with rise in elevation (altitude), but declined at elevation >250 m until zero occurrence at elevation >1,400 m (Fig 3A); (ii) When precipitation was tested alone, annual precipitation in December had the highest regularized training gain (Fig 2) and probability of occurrence

during this month covered a very narrow precipitation range (0-100 mm) with a peak at 10 mm and zero probability outside this range (Fig 3B); (iii) Similarly, as the highest regularized training gain of maximum (tmax) and minimum (tmin) temperature was obtained in February and March respectively (Fig 2), probability of occurrence in the former month began

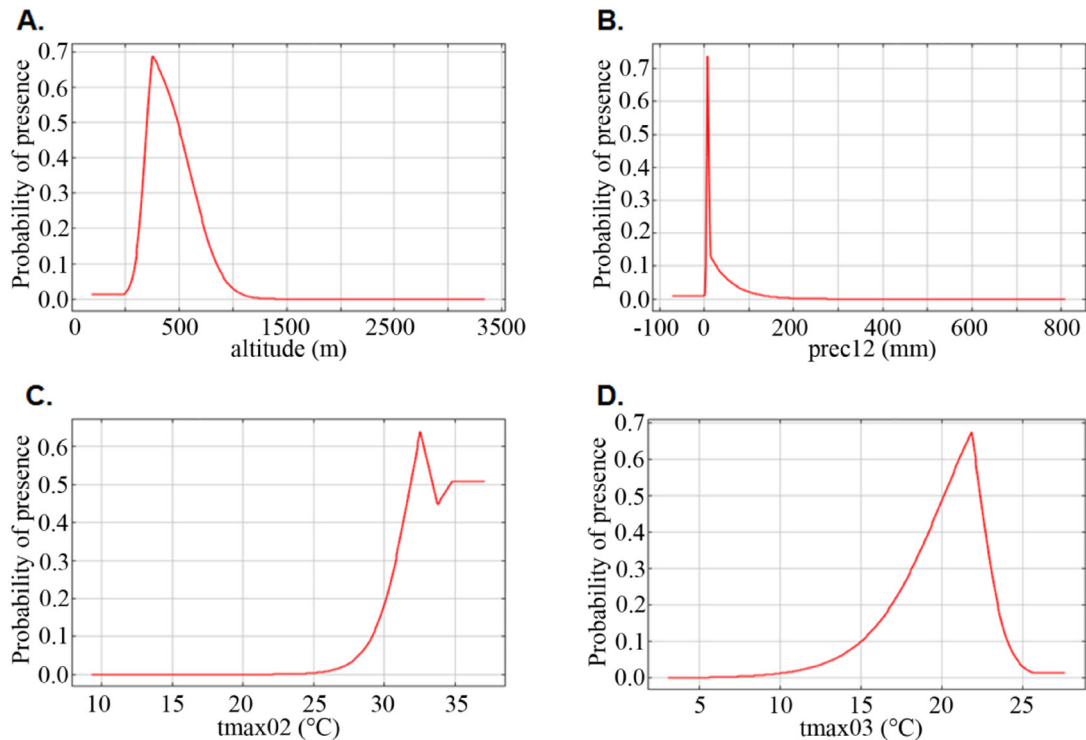


Fig 3 - Predicted probability of *Rickettsia*-infected ticks versus selected variables

A: Elevation (altitude); B: Precipitation in December (prec12); C: Maximum temperature in February (tmax02); D: Minimum temperature in March (tmin03); *Rickettsia*_tick, *Rickettsia*-infected ticks

to rise from zero at t_{max} of 27.5°C, peaked at 32.5°C, dropped and then leveled at $\geq 35.0^\circ\text{C}$ with a probability of 0.5 (Fig 3C), while in March probability of occurrence rose at 13.5°C, reached a peak at 22.0°C and then declined to zero at $\geq 25.5^\circ\text{C}$ (Fig 3D).

2. Land cover

Although there are 19 classes of land cover describing types of vegetation and density of cover, only eight land cover classes are observed in forest edges and can be categorized into two main types of vegetation, namely, forest land and cultivated crop land (<https://forobs.jrc.ec.europa.eu/products/glc2000/glc2000.php>). Of the 71 collecting sites, 55 (77%) were mainly mosaics and shrub covers, with evergreen and deciduous areas, while the other 16 sites were cultivated and managed crop land (Table 3). Occurrence of *Rickettsia*-infected and -uninfected ticks is significantly higher in forest land compared to cultivated crop land ($\chi^2 = 11.2$, 284.8; p -value = 0.001 and <0.001 respectively). Among there are eight vegetation classes in forest land, 44-60% *Rickettsia*-infected ticks in Thailand were detected in four land classes (Classes 1, 3, 6 and 7) (Table 3).

3. Geographic distribution

Maximum ENM of geographic distribution of *Rickettsia*-infected ticks in forest edges predicted high occurrence along Thai-Cambodia border at the lower northeastern region

of Thailand, and lower occurrence at the Thai-Myanmar border of the northern region and Thai-Cambodia border of the eastern region (Fig 4). There was concordance of the model prediction with actual determination of percent *Rickettsia*-infected ticks at the 71 collection sites in 33 remote districts of 23 provinces between 2008-2011 (Table 4). The most common occurrence of *Rickettsia*-infected ticks was clustered at forest edges in remote districts of Buri Ram, Si Sa Ket, Surin and Ubon Ratchathani Provinces, which are located in border regions of the lower part of northeastern Thailand.

DISCUSSION

We have demonstrated that an optimal maximum ENM was able to predict the geographic distribution of *Rickettsia*-infected ticks in forest edges adjacent to residential properties with significant degree of agreement with data collected from 71 collection sites located in 33 remote districts of 23 provinces of Thailand between 2008-2011. However, the observed *Rickettsia* infection rates in this study may not represent the true infection rate in any given region due to the low sample size obtained from each collection site and limitation of site locations that were restricted to private residential areas adjacent to forest. Nonetheless, the study obtained important insights regarding the distribution of tick genera in forest edges of remote regions, as well as locations with high

Table 3
Number of collection and percent *Rickettsia*-infected tick sites at different classes of land cover

Land cover class (class number*)	Number of collection sites	Percent <i>Rickettsia</i> -infected tick collection sites
Sea (0)	N/A	N/A
Tree cover, evergreen (1)	18	44
Mosaic: tree cover, vegetation or cropland (2)	2	50
Tree cover, deciduous (3)	4	50
Tree cover, regularly flooded mangrove (4)	N/A	N/A
Tree cover, regularly flooded swamp (5)	N/A	N/A
Mosaics and shrub cover dominant, mainly evergreen (6)	15	60
Mosaics and shrub cover dominant, mainly deciduous (7)	18	50
Shrub cover, mainly deciduous (8)	N/A	N/A
Mosaics of cropland/other natural vegetation (shifting cultivation in mountains) (9)	4	0
Herbaceous cover (including alpine grassland) (10)	N/A	N/A
Sparse herbaceous cover >3,000 m (11)	N/A	N/A
Cultivated and managed, non-irrigated (mixed) (12)	7	14
Cultivated and managed, irrigated (flooded, rice farm, shrimp farm) (13)	3	33

Table 3 (cont)

Land cover class (class number*)	Number of collection sites	Percent <i>Rickettsia</i> -infected tick collection sites
Bare areas (rock: lime stone) (14)	N/A	N/A
Snow and ice (15)	N/A	N/A
Artificial surface (16)	N/A	N/A
Water body (17)	N/A	N/A
No data (18)	N/A	N/A

m: meters; N/A: not available

Land cover classification is based on EU Science Hub at <https://forobs.jrc.ec.europa.eu/products/glc2000/glc2000.php>

prevalence of *Rickettsia*-infected ticks. Classification of *Rickettsia* species was limited due to paucity of species-specific gene markers, in particular of species with unknown pathogenicity. Future investigations into the identification of tick species with potential to transmit rickettsiosis is of vital importance in the development of meaningful *Rickettsia* surveillance and prevention programs, particularly in regions with high prevalence of *Rickettsia*-infected ticks.

We recognize that the data used in the MaxEnt analysis are also limited as regards *Rickettsia* spp infecting ticks as the biology and life cycle of *Rickettsia* spp in a broad reservoir of arthropod hosts in Thailand remain largely unknown (Doornbos *et al*, 2013; Sumrandee *et al*, 2014; Sumrandee *et al*, 2016). Furthermore, existence of uninfected ticks in a forest edge does not indicate absolute absence of *Rickettsia* spp in that area, but rather demonstrates that potential host reservoirs of *Rickettsia* have yet to be identified.

In a jackknife test, seasonal variations of *Rickettsia* spp were reported in Lao PDR, where prevalence increases towards December and reaches the highest level in April (Dittrich *et al*, 2015). Unlike tropical regions (Pinheiro *et al*, 2014), maximum prevalence of *Rickettsia*-infected ticks in temperate regions is observed during the spring (May) (Kantsø *et al*, 2010; Padgett *et al*, 2016; Spitalska *et al*, 2016), when the weather is similar

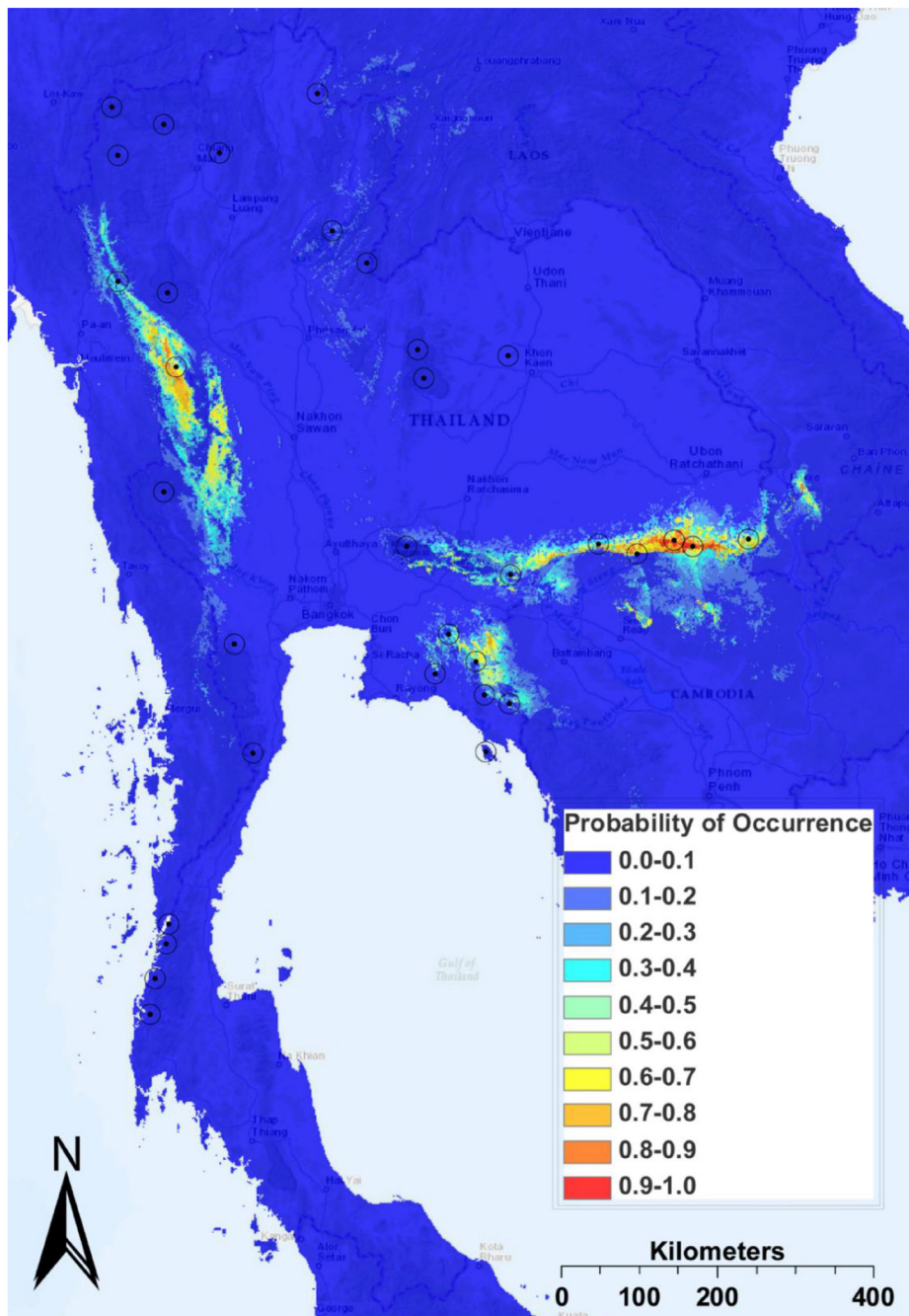


Fig 4 - Maximum entropy model of probability of *Rickettsia*-infected tick occurrence in forest edges adjacent to residential properties located in 33 remote districts of Thailand

Table 4

Locations and numbers of land covers and occurrence probability of *Rickettsia*-infected ticks in forest edges adjacent to residential properties located in 33 remote districts of Thailand (2008-2011)

District	Province	Region	Land cover class number*	Occurrence probability
Tha Ta Kiap	Chachoengsao	Eastern	1	0.051
Soi Dao	Chanthaburi	Eastern	1	0.250-0.289
Pong Nam Ron	Chanthaburi	Eastern	3	0.440
Khao Chamao	Rayong	Eastern	12	0.012
Bo Rai	Trat	Eastern	9	0.001-0.102
Ko Chang	Trat	Eastern	1	0
Nong Bua Daeng	Chaiyaphum	Northeastern	1	0.002
Ban Kruat	Buri Ram	Northeastern	7, 12	0.029-0.733
Nong Ruea	Khon Kaen	Northeastern	13	0.001
Pak Chong	Nakhon Ratchasima	Northeastern	1	0.006-0.097
Kantharalak	Si Sa Ket	Northeastern	6, 7	0.761-0.937
Khun Han	Si Sa Ket	Northeastern	1, 6, 7	0.682-0.935
Phu Sing	Si Sa Ket	Northeastern	2, 7, 12	0.567-0.834
Kap Choeng	Surin	Northeastern	2, 6, 7, 9	0.276-0.838
Nam Yuen	Ubon Ratchathani	Northeastern	6	0.484-0.682
Doi Saket	Chiang Mai	Northern	7	0.005
Thoeng	Chiang Rai	Northern	9	0.145

Table 4 (cont)

District	Province	Region	Land cover class number*	Occurrence probability
Khun Yuam	Mae Hong Son	Northern	13	0.003
Muang Mae Hong Son, (Huai Pha Sub-district)	Mae Hong Son	Northern	3	0.014
Pai	Mae Hong Son	Northern	6	0.001
Nam Nao	Phetchabun	Northern	7	0.007
Chat Trakan	Phitsanulok	Northern	12	0.008
Mae Ramat	Tak	Northern	3, 12	0.182-0.591
Phop Phra	Tak	Northern	6	0.541
Tha Song Yang	Tak	Northern	7	0.429
Ban Khok	Uttaradit	Northern	7	0.070
Thong Pha Phum	Kanchanaburi	Western	7	0.001-0.002
Muang Prachuap Khiri Khan (Khlong Wan Sub-district)	Prachuap Khiri Khan	Western	6	0.005
Suan Phueng	Ratchaburi	Western	6, 13	0.005-0.075
Khuraburi	Phangnga	Southern	1	0
La-Un	Ranong	Southern	1	0
Muang Ranong (Bang Rin Sub-district)	Ranong	Southern	1, 12	0
Suk Samran	Ranong	Southern	1	0

*From Table 3

to the dry season of tropical regions, such as Thailand. In addition, a decrease in probability of infected tick occurrence was observed in seasons of high precipitation. Estrada-Peña *et al* (2008) found that cyclic changes in both temperature and rainfall drive periodic changes in the distribution of ticks, and that high temperatures during the hot season constitute an influential environmental factor for occurrence of *Rickettsia*-infected ticks but with limitation to the permissible temperature (Estrada-Peña *et al*, 2006; St John *et al*, 2016). Burtis *et al* (2016) reported the questing behavior of *Ixodes scapularis* is affected by summer weather, but other strong demographic effects are not found. Our surveillance was limited to regions of forest edges in remote districts of the country. Both *Rickettsia*-harboring and non-harboring tick species have been reported to be widely distributed throughout Thailand (Kollars *et al*, 2001; Parola *et al*, 2003a; Cornet *et al*, 2009; Doornbos *et al*, 2013; Malaisri *et al*, 2015; Sumrandee *et al*, 2016). In order to improve the predictive power of ENM, future sampling in a wider choice of locations should provide a more accurate picture of the distribution of *Rickettsia*-infected ticks in the country. The ecological characteristics of tick vectors have been found to influence epidemiology and clinical aspects of tick-borne diseases (Parola and Raoult, 2001; Parola *et al*, 2005).

Lack of data, especially in remote risk areas where access to extensive human sampling and species reporting are difficult or prohibited, is a common challenge in determining the geographic distribution and range of plants, animals and insect species (Mak *et al*, 2010). Thus, ENM or species distribution modeling can be beneficially applied for assessing disease risk in these remote areas (Pozio, 2000; Peterson and Shaw, 2003; Peterson *et al*, 2006; Moffett *et al*, 2007; Burke *et al*, 2008; Masuoka *et al*, 2009; Masuoka *et al*, 2010). The results of such models can then be used to focus on pertinent areas in future collection of field samples, as well as to verify or refute species habitat models (Mak *et al*, 2010). In our study, ENM had predicted prevalence of *Rickettsia*-infected ticks in forest edges of remote districts throughout Thailand, showing areas of high potential of occurrence as well as of influential environmental factors. This study provides values of predictive modeling for monitoring the geographic distribution of *Rickettsia*-tick interaction that will encourage further insight into the possibility of misdiagnosed spotted fever group (SFG) as well as into the evaluation of fevers of unknown origin in remote areas adjacent to forest of Thailand, and the identification of geographic ranges can facilitate epidemiological studies and efforts to implement health protection and disease prevention. ENM is a powerful tool, especially in

Thailand where biology of *Rickettsia* spp remains under-studied and where many limitations of *rickettsia* diagnosis exist.

In summary, our study reveals the usefulness of ecological niche (or species distribution) modeling to predict potential influential environmental factors related to *Rickettsia*-infected tick occurrence, namely, season with low precipitation and high temperature and elevation of <1,400 m. An understanding of the potential distribution of *Rickettsia*-infected ticks and environmental factors provides preliminary information regarding areas of forest edges where *Rickettsia*-infected ticks may be found with potential impact on rural Thai health, which is of importance as regards to future epidemiology of rickettsioses, which requires good surveillance and early response to achieve health protection and disease prevention, especially in remote areas of Thailand where early diagnosis is not available and late response may occur. The results will help to generate information in the country regarding locations of forest edge areas with presence of *Rickettsia*-infected ticks, which should be useful in assisting future surveillance and prevention programs of tick-borne rickettsioses. Further investigations should be conducted to update information of *Rickettsia*-infected ticks and ecological niche modeling.

ACKNOWLEDGEMENTS

The research was supported by Mahidol University and Center of Excellence on Biodiversity (BDC), Office of Higher Education Commission (BDC-PG3-163005). The authors thank all previous and present lab members of the Tick Research Laboratory, Biodiversity Research Cluster, Department of Biology, Faculty of Science, Mahidol University for assistance in field work and helpful cooperation, and Ms Penny Masuoka, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA, for assistance in use of GIS.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare that no conflicts of interest.

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