RESEARCH ARTICLE Lau et al., Microbial Genomics 2023;9:000954 DOI 10.1099/mgen.0.000954



## The dynamics of the microbiome in Ixodidae are shaped by tick ontogeny and pathogens in Sarawak, Malaysian Borneo

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## Abstract

Tick-borne diseases have recently been considered a potential emerging public health threat in Malaysia; however, fundamental studies into tick-borne pathogens and microbiome appear limited. In this study, six tick species (Ixodes granulatus, Haemaphysalis hystricis, Haemaphysalis shimoga, Dermacentor compactus, Dermacentor steini and Dermacentor atrosignatus) collected from two primary forests and an oil palm plantation in Sarawak, Malaysian Borneo, were used for microbiome analysis targeting bacterial 16S rDNA using next-generation sequencing (NGS). In addition, bacterial species were further characterized in conventional PCRs to identify potential pathogens. Sequences generated from NGS were first filtered with the Decontam package in R before subsequent microbial diversity analyses. Alpha and beta analyses revealed that the genus Dermacentor had the highest microbial diversity, and H. shimoga significantly differed in microbial composition from other tick species. Alpha and beta diversities were also significantly different between developmental stages of *H. shimoga*. Furthermore, we observed that some bacterial groups were significantly more abundant in certain tick species and developmental stages of H. shimoga. We tested the relative abundances using pairwise linear discriminant analysis effect size (LEfSe), which also revealed significant microbial composition differences between Borrelia-positive and Borrelia-negative I. granulatus ticks. Finally, pathogenic and potentially pathogenic bacteria circulating in different tick species, such as Rickettsia heilongjiangensis, Ehrlichia sp., Anaplasma sp. and Bartonella spp. were characterized by PCR and sequencing. Moreover, Coxiella and Francisella-like potential symbionts were identified from H. shimoga and D. steini, respectively. More studies are required to unravel the factors associated with the variations observed in this study.

## DATA SUMMARY

Ticks (16S rDNA: LC602422–LC602456 and LC603786); *Anaplasmatacea* (16S rDNA: LC602250–LC602251); *Francisella* (16S rDNA: LC602252–LC602256; *tul4*: LC602776–LC602781); *Rickettsia* (16S rDNA: LC602357–LC602360; *ompA*: LC602733–LC602736; *ompB*: LC602737–LC602740; *gltA*: LC602741–LC602744; *Sca4*: LC602745–LC602748; *htrA*: LC602770–LC602773);

Received 31 March 2022; Accepted 19 December 2022; Published 09 February 2023

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<sup>\*</sup>Correspondence: Toshio Tsubota, tsubota@vetmed.hokudai.ac.jp; Mohamed Abdallah Mohamed Moustafa, mam1205@njms.rutgers.edu Keywords: microbiome; next-generation sequencing; pathogens; Sarawak; ticks.

Abbreviations: ASV, amplicon sequence variant; NGS, next-generation sequencing; PCoA, principal coordinates analysis; TBD, tick-borne disease; TBP, tick-borne pathogen.

Ticks (16S rDNA: LC602422 – LC602456 & LC603786); Anaplasmatacea (16S rDNA: LC602250 – LC602251); Francisella (16S rDNA: LC602252 – LC602256; tul4: LC602776 – LC602781); Rickettsia (16S rDNA: LC602357 – LC602360; ompA: LC602733 – LC602736; ompB: LC602737 – LC602740; gltA: LC602741 – LC602744; Sca4: LC602745 – LC602748; htrA: LC602770 – LC602773); Bartonella (ftsZ: LC602774; gltA: LC602775); Coxiella (23S rDNA: LC602368 – LC602388 ; 16S rDNA: LC602389 – LC602400; dnaK: LC602703 – LC602712; rpaB: LC602713 – LC602732; groEL: LC602749 – LC602769). Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Five supplementary figures and one supplementary table are available with the online version of this article. 000954 © 2023 The Authors