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博士の専攻分野の名称：博士（感染症学）

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Name

Studies on the Eukaryotic Communities in Ticks
(マダニが保有する真核生物群に関する研究)

< abstract >

Ixodid ticks transmit various pathogens to humans and animals. While acaricidal chemicals are the primary method of tick control, their effectiveness is declining due to acaricide resistance. Alternatively, natural enemies, such as entomopathogenic microbes and predatory arthropods, have been successfully used as biological control agents in crop pest management. However, they remain underexplored for ticks. Additionally, ticks carry diverse non-pathogenic microbes, including symbiotic bacteria for ticks. Despite their importance in tick biology, eukaryotic communities in ticks remain understudied. This dissertation investigates the micro- and macro-eukaryotic organisms associated with ticks to advance understanding of tick biology and contribute to future biological control strategies.

Chapter I introduces novel methods to investigate the eukaryotic microbiome in ticks with high-throughput sequencing. The novel methods were designed to selectively amplify micro-eukaryote genes in tick DNA while suppressing the amplification of the host DNA upon sequencing library preparation. Artificial nucleic acids, PNAs and LNAs, were tested as blockers to suppress the amplification of tick host DNA selectively. Additionally, a non-metazoan-specific PCR, UNonMet, was evaluated. Tests on a tick DNA sample set demonstrated that these approaches significantly increased the micro-eukaryotic sequence enrichment and the alpha diversity. The PNA-based method showed broad taxonomic coverage, including fungi, Alveolata, Rhizaria, and unclassified eukaryotes, while the UNonMet method was particularly effective for fungi and Alveolata.

Chapter II identifies the keystone eukaryotic taxa in ticks using network analyses based on bacterial and eukaryotic microbiome analyses. The PNA-based method developed in Chapter I was applied. Four species of ticks were collected from four distinct regions in Japan. Potentially important taxa for the ticks were determined. Taxa classified as Bivalvulida or Eugregarinorida were abundant. Other taxa classified as Nematozoa and Hypocreales were taxonomically close to entomopathogenic organisms. Finally, *Bromeliothrix* (Ciliophora) in *Haemaphysalis flava*, and *Kurtzmanomyces* (Basidiomycota), unclassified *Brachybasidiaceae* (Basidiomycota), and *Pestalotiopsis* (Sordariomycetes: Ascomycota) in *Haemaphysalis longicornis*, were determined as keystone taxa. These findings propose valuable hypotheses for the ecological roles of these taxa, warranting further study with taxonomy and differential culture.

Chapter III investigates and characterizes a macro-eukaryote, *Ixodiphagus* wasps in Japan. Ticks collected from four distinct regions were fed on rabbits under laboratory conditions to collect adult wasps. Out of 1,933 ticks infested on the rabbits, adult wasps emerged from 15 engorged *Haemaphysalis flava* collected in three distinct regions. The observed wasps were molecularly determined as undescribed species. Additionally, the mitochondrial COI gene was characterized using shotgun sequencing, direct sequencing, and in-fusion cloning methods. Across the samples analyzed, notable intra-individual polymorphisms were detected.

This dissertation introduced the predominant eukaryotes associated with ticks. The technique developed to analyze micro-eukaryotes in ticks and the findings based on that technique offer valuable opportunities to understand the biology of ticks. The macro-eukaryotes, parasitoid wasps of ticks were detected and characterized, which provides a potential biological control agent. Further research is needed to elucidate the roles of these eukaryotic communities and to expand our understanding of their interactions with ticks.