CHAPTER 6

General conclusion

Bacterial communities are known to play important roles during the developmental stages of insects, but current knowledge of bacteria associated with the midgut of *Apis dorsata*, the giant Asian honeybee, is limited. Using polymerase chain reaction-denaturing gradient gel electrophoresis analysis (PCR-DGGE) and 16S rRNA sequencing, the aim of this study was to determine the dynamics of bacterial community structure across four *A. dorsata* life stages in different geographical locations. The results reveal that bacterial diversity increased as the bee progressed through larval stage to emerged bee and worker. However, in the pupal stage, no bands identified as bacteria could be observed. Overall, two bacterial phyla (Proteobacteria and Firmicutes) and four classes (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Bacilli) were identified, but the frequency varied among the different stages and locations. The classes of Gammaproteobacteria and Bacilli dominated among larval, emerged bee and worker developmental stages. Bacterial profile variations within individual colonies were far less diverse than variations that emerged among colonies and between geographical locations. Our study findings suggest that social interactions and environment factors could significantly influence bacterial symbiont acquisition in *A. dorsata*.

Another culture-independent approach using a nested PCR-DGGE and 16S rRNA gene sequencing investigated bacterial community structure in the midgut of *A. florea* larva and compared bacterial diversity and distribution among different sampling locations. The result revealed similarities of bacterial community profiles in each individual colony, but differences between colonies from the same and different locations. *A. florea* larvae harbor bacteria belonging to two phyla (Firmicutes and Proteobacteria), five classes (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli, and Clostridia), six genera (*Clostridium*, *Gilliamella*, *Melissococcus*, *Lactobacillus*, *Saccharibacter*, and *Snodgrassella*), and an unknown genus from uncultured bacterial

species. The classes with the highest abundance of bacteria were Alphaproteobacteria (34%), Bacilli (25%), Betaproteobacteria (11%), Gammaproteobacteria (10%), and Clostridia (8%), respectively. Similarly, uncultured bacterial species were identified (12%). Environmental bacterial species, such as *Saccharibacter floricola*, were also found. This is the first study in which sequences closely related to *Melissococcus plutonius*, the causal pathogen responsible for European foulbrood, have been identified in Thai *A. florea* larvae.

Common bacterial communities in both *A. dorsata* and *A. florea* were phylum Proteobacteria and Firmicutes including class Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Bacilli. *Gilliamella apicola*, *Lactobacilus kunkeei* and *Saccharibacter floricola* were shared among these two honey bee larvae. Bacterial profiles were homogeneous among colonies, but heterogeneous within the different colonies and locations, likely highlighting environmental factors that might influence the persistence of bacteria in the midgut.

Studying *A. dorsata* worker foraging flight activity here is the first report of the observation in a tropical environment. The first flight began shortly after dawn but before actual sunrise. The number of foraging bees increased after the first flight in the early morning to a peak in the late morning dropping sharply thereafter. The average temperature at flight initiation was 23°C. The diurnal flight activity was temperature-independent but certainly dependent on ambient daylight. The time of first flight is probably related to abundant superior food and the active flight activity in early morning should correlate with the food source availability. The diurnal foraging was more confined to the early morning period in the newly established colonies than the last phrase colonies. The change in foraging behavior could well present a change in colony composition.

Overall, this thesis provides important clues that shed light on the survival of the native Asian honey bees by studying the bacteria in their midguts and foraging behavior. Additionally, it would be interesting to evaluate the impacts of environment

factors. Further studies, new advanced sequencing technologies are needed to clarify the quality and quantity of microorganisms in wild honey bee guts.