Summary

The insects are immense element of the present-day terrestrial ecosystems. Evolution of herbivory is one of the main factors which has played an important role in the rapid diversification of insects. The angiosperm radiations in the Cretaceous were followed by the bursts of angiosperm-feeding insects, which have been driven by coevolutionary interactions with angiosperms. In contrast, the bryophytes (liverworts, hornworts and mosses), the earliest-branching clades of land plants, display lower species diversity than the angiosperms and apparently less interactive with animals. However, bryophyte-feeding commenced in the Middle Devonian and presently some insect lineages have obligate associations with specific groups of bryophytes. The diversity and evolution of extant bryophyte–herbivore associations would be a key to understand how the bryophytes have associated with insects during land plant evolution. In the present study, I have pursued diversity and evolutionary processes of two early-diverging lineages of Lepidoptera and Diptera, Micropterigidae and Rhagionidae, which have been associated with the bryophytes for some time.

In Chapter 2 and 3, I targeted the earliest-diverging moth lineage, Micropterigidae, of which larvae feed on liverworts. The Lepidoptera represent one of the most successful radiations of plant-feeding insects, which predominantly took place within angiosperms beginning in the Cretaceous. Angiosperm colonization is thought to underlie the evolutionary success of the Lepidoptera. However, the micropterigid moths have achieved a modest diversity in Japan, although they have unassociated with the angiosperms.

In chapter 2, I explored causes and processes of diversification of the Japanese micropterigid moths by performing molecular phylogenetic analysis and extensive ecological surveying. Phylogenetic analysis recovered a monophyletic group of approximately 25 East Asian endemic species that fed exclusively on the liverwort *Conocephalum conicum*, suggesting that speciation by geographical isolation has been the major process shaping the diversity of Japanese Micropterigidae. To our knowledge, this is the largest radiation of herbivorous insects that does not accompany any apparent niche differentiation. This study suggests that significance of non-ecological speciation during the diversification of the Lepidoptera is commonly underestimated.

In Chapter 3, I further investigated the fauna of Micropterigidae in Japan. I described four new species belonging to *Issikiomartyria* (Micropterigidae) newly discovered from the northeastern part of Japan. Also, a new genus was described, based on two new species inhabited the Southern Alps of Japan. *Issikiomartyria* represents one of the most extensive diversity ever found in the northeastern part of Japan, and their migration history was inferred.

In the subsequent four chapters (Chapter 4-7), I focused on diversity and evolutionary process of Spaniinae (Rhagionidae), which have obligate associations with some bryophyte groups. In Chapter 4, I reported the larval thallus-mining habit of *Litoleptis*, a small genus of Rhagionidae, of which taxonomic position has been uncertain due to the anomalous morphology and the scarcity of the specimens. I described six new species of *Litoleptis* from Japan. All the species described in the present study were thallus-miners of liverworts belonging to Ayatoniaceae and Conocephalaceae (Marchantiopsida: Marchantiophyta). Each fly species mined thalli of only one of the following genera: *Conocephalum*, *Reboulia*, and *Asterella*. The descriptions of the Japanese *Litoleptis* species expanded the concept of this genus. In this study, the female genital morphology of *Litoleptis* was examined for the first time, and it strengthened the current placement of *Litoleptis* as a member of Spaniinae.

In Chapter 5, I elucidated life history, biology, and morphological evolution of the bryophyte-feeders in three genera of Spaniinae (*Spania*, *Litoleptis*, *Ptiolina*). Moreover, I traced changes of the larval morphology associated with evolution of bryophyte-feeding by molecular phylogenetic analysis. *Spania* and *Litoleptis* (thallus-miners of thallose liverworts) shared a toothed form of apical mandibular sclerite with an orifice on its dorsal surface, which contrasted to those of the other members of Rhagionidae possessing a blade-like mandibular hook with an adoral groove; whereas, *Ptiolina* (stem borer of mosses) exhibited a weak groove on the adoral surface of mandible and highly sclerotized maxilla with toothed projections. Based on the larval feeding behavior of the thallus-miners, it was inferred that the toothed mandibles with the dorsal orifice facilitate scraping plant tissue and then imbibing it with a great deal of the sap. A phylogeny indicated that the bryophyte-feeding genera formed a clade with *Spaniopsis* and was sister to *Symphoromyia*, which presumably are detritivores. This study indicated that the loss or reduction of adoral mandibular groove and mandibular brush was coincident with the evolution of bryophyte-feeding, and it is subsequently followed by the occurrence of dorsal mandibular orifice and the loss of creeping welts accompanying the evolution of thallus-mining.

In Chapter 6, I assessed diversity and evolutionary pattern of host-plant use in thallus-miners of Spaniinae, unveiling unknown diversity of *Spania* in Japan. *Spania* consisted of more than 14 morphological species, and these species formed respective monophyly in COI phylogeny. The phylogeny suggested that the thallus-mining rhagionids represented high host-plant conservatism and most speciation events within *Spania* took place on Pelliaceae (Marchantiophyta). Therefore, this study illustrated that the host-plant shifts did not play major role in the diversification, as in the case of the Japanese micropterigid moths.

In Chapter 7, I investigated when and from what feeding habit bryophyte-feeding have originated in Spaniinae by performing divergence time estimation. The evolutionary history of bryophytes is scarcely known, and less understood is their biotic relationships with arthropods during their 400 million-year-long history. Rhagionidae is a family that had its heyday during the Early Jurassic. A variety of larval feeding habits of Rhagionidae enables us to infer the ancestral feeding habit preceding bryophyte-feeding. The fossil-calibrated tree showed that the rhagionids commenced the bryophyte-feeding during Early Cretaceous, and it was followed by the evolution of thallus-mining, in contemporaneous with the bursts of liverworts. This is the first certifiable and the oldest case of long-standing association between bryophytes and the plesiomorphic lineage of brachyceran flies.

Lastly, in Chapter 8, I placed the present study into broader context of ecological and evolutionary studies of plant–arthropod associations and raised some directions for further study. This study highlighted the diversity and evolution of bryophyte-feeders, which have been one of the least understood aspects of plant–herbivore interactions. This study suggests that some bryophyte–herbivore associations may have persisted from the Early Cretaceous to the present, providing a wealth of opportunities for reconstructing the ecological interactions in deep time. Also, the present study demonstrates the obligate associations between early-diverging insects and liverworts. The high host specificity and host conservatism during rhagionid evolution may be attributable to liverwort chemistry; thus, understanding for defense syndromes of bryophytes and responded host-plant specificity of bryophyte-feeders would be important. Two lineages of liverwort-feeders displayed the extreme cases of allopatric speciation without host-plant shifts, suggesting that the factors based both on plants and insects should be examined for understanding the mechanisms underlying diversification of phytophagous insects.