8 Summary

The aim of this thesis is to provide a contribution to the systematics of galerucine leaf beetles *sensu stricto*, which constitute a highly diverse species rich group. However, a phylogenetic tree by means of cladistics is not achieved by now. A traditional, but controversial checklist of known species to a suprageneric classification of five tribes (Galerucini, Luperini, Sermylini, Oidini, and Metacyclini) is more or less arbitrary.

The principle focus of this investigation were species of the tribe Sermylini WILCOX 1965. Several species of the Sermylini bear larval defensive openings on thoracal and abdominal segments. These so far only externally described particular features are considered as defensive glands in literature and constitute a new taxon "glanduliferous galerucine" which are further divided into three groups. However, is was uncertain if these defensive structure really consist of exocrine glands.

Because clear evidence for the presence of glandular cells was lacking, as well as informaton on the composition of the secretion and on the defensive pontential of these structures, larvae of 10 Sermylini species were examined concerning

(1.) morphology: defensive openings were investigated by means of scanning electron microscopy and histological methods;

(2.) behavioural ecology: effects of discharged fluids from defensive openings were studied towards ants as a model predator and towards conspecific larvae concerning alerting properties;

(3.) chemistry: discharged fluids were analysed by means of GC-MS, HPLC-DAD, SDS-PAGE, and solid phase extraction;

(4.) a phylogenetic tree of representative species of Galerucinae *s. str.* based on three molecular markers was worked out. DNA sequences of 13 species of Sermylini, 34 species of species belonging to three other tribes of galerucinae, and 13 chrysomelinae as an outgroup were achieved.

Morphological studies (Chapter 2)

Among 10 Sermylini species investigated two types of defensive openings were distinguished. Type *Agelastica alni*: larvae posess eversible sac-like structures located pairwise dorsolaterally on abdominal segments 1-8. The secretion canal of one glandular cell enters each sac, which might be eversed through increased body pressure and is retracted by attached muscles located apically and laterally. Glands here but also of other parts of the body have

short secretion canals (30 µm). The endapparatus is not morphologically differenciated. Species having this type of defensive opening are: *Agelastica alni*, *A. coerulea*, *Morphosphaera chrysomeloides*, *M. coerulea*, and *M. japonica*. These species were grouped according to number and location of defensive openings and abdominal sclerites.

Type *Sermylassa halensis*: defensive openings lack eversible sacs and exocrine glands in direct relation to openings. Epidermal glands of other locations have long secretion canals (100-120 µm), the end apparatus of which is bifurcated. Besides, these larvae posess conspicous cuticular fibrils which are thought to be part of a muscular insertion. Species having this type of defensive opening are: *Agelasa nigriceps*, *Arthotus niger*, *Hamushia eburata*, *Gallerucida bifasciata*, and *Sermylassa halensis*. *Arthrotus niger* is an exception because it lacks cuticular fibrils. This provides evidence that the so called defensive glands of Galerucinae form two types, one of which clearly lacks glandular cells.

2. Bioassays (Chapter 3 und 4)

Larvae discharge a glutinous fluid from the defensive openings. Even strongly diluted with water it significantly deter ants (*Myrmica rubra*) from feeding. Species tested were: *Agelasa nigriceps*, *Agelasitca alni*, *Agelastica coerulea*, *Gallerucida bifasciata*, and *Sermylassa halensis*.

Gregariously feeding larvae of the alder leaf beetle, *Agelastica alni*, are alerted by volatiles from the discharged defensive fluid. It was suspected that exocrine gland cells which are associated to the defensive openings might be the source of the alarming signal. Bioassays with hemolymph taken from a larva's leg and egg extracts with dichloromethan, respectivly, showed that these fluids contain the alerting compound as well. Therefore exocrine glands associated with defensive openings are not likely to be the source of the alerting signal. However, the alarming effect of the fluid released from defensive openings was significantly higher than effects from volatiles of hemolymph.

3. Chemistry of defensive fluids (Chapter 3 and 4)

It was shown that the discharged defensive fluid consists mainly of hemolymph because it has a similar protein pattern as the hemolymph. Additionally, light microscopy revealed that the discharged fluid contains hemocytes. After fractionization of a watery dilution of defensive fluid of *Agelasa nigriceps*, *Agelastica coerulea*, and *Sermylassa halensis* under bioassay

control the following characterization can be made. Ant detering compounds are highly polar, heat resistant, not volatile, and have a molecular weight under 3 kD.

Volatiles of the defensive fluid and the hemolymph, respectively, of *Agelastica alni* sampled by purge and cold trap were analyzed by GC-MS. Ten compounds could be detected, one of which, (*E*)-4,8-Dimethyl-1,3,7-nonatrien, was present in higher amounts in defensive fluid than in hemolymph. Additionally, traces of a putative methoxyalkylpyrazine were detected in the defensive fluid of *A. alni* only. The role of the exocrine glands associated with the defensive openings in discussed in the light of these new results.

4. Phylogenetic analysis of molecular data

To contribute arguments on Galerucine phylogeny three parts of the beetle genome, Ef1α, 12S rRNA, and 16S rRNA, were sequenced from 51 Galerucine species and 9 chrysomeline species as an outgroup. An intersection data set of sequences of all three molecular markers (34 species) was analysed separately and combined, respectively, using the phylogenetic program POY which implements optimization alignment. Parameters such as cost for alignment gaps as well as tranversion/transition cost ratios were varied. For the combined data the between-data-sets incongruence was calculated as the incongruence length difference (ILD). Parameter sets which gave the lowest ILD measure were chosen. The most-parsimonious tree failed to support monophyly of the tribe Sermylini. Species with larval defensive openings type *A. alni* are clearly separated from species with defensive openings type *S. halensis* which are grouped together. This indicates that larval defensive openings of different types have been evolved convergently. The tribe Luperini as well as the Sermylini are a polyphyletic or paraphyletic taxon. Only the taxon Galerucini is well supported as monophyletic.