Exocrine glands in Erotylidae (Coleoptera, Cucujoidea): chemical ecology, morphology and evolution

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Summary

In most insect orders chemical defence is highly important and a multiplicity of partly spectacular defence mechanisms were described in the last years. It is well known that members of the Erotylidae show a particularly rich equipment of exocrine compound glands. However, morphology and ultrastructure as well as the chemistry of the secretions of these compound glands remain unexplored so far.

The cosmopolitan Erotylidae is assigned to the superfamily Cucujoidea (Clavicornia) of the Coleoptera-Cucujiformia and comprises about 3500 described species in 258 genera. Today the family includes both the phytophagous species of the former Languriidae and the mycophagous species of the former Erotylidae s. str. (now ranked as the subfamily Erotylinae). The adult beetles, as well as their larvae, are bounded to different bracket fungi or live under the bark. Most species are striking in appearance, frequently in combination with conspicuous patters of stripes, spots or rings. The present contribution deals with species of this coleopteran family and concerns altogether five different subject areas:

(1) Morphological details of the internal structure and ultrastructure of the compound glands were examined in exemplar species of the family (*Tritoma bipustulata*, *Triplax scutellaris*) for the first time (SEM, TEM). Each compound gland consists of a central excretory duct and numerous identical gland units. These gland units are composed of two different cells, whereof one forms a cuticular ductule. Thus the glands belong to class III as defined by Noirot & Quennedey (1974, 1991). Furthermore, the glands offered two structural features (lateral appendix, the spongious wall of the ductus), which were previously not known from compound glands of beetles.

(2) Hitherto hardly known was the ability of reflex bleeding in these species. The phenomenon is reported, for instance, from the closely related families Coccinellidae and Endomychidae. However, the hemolymph is not, like in the mentioned taxa, released from the joints of the leg, but from the abdominal tip. The chemistry of the reflex blood as well as the discharged secretion of the pronotal glands was examined by GC-MS for the first time. Biological effects of the identified compounds of both secretions were evaluated in bioassays and agar diffusion tests.

(3) Furthermore, a study on the role of emitted fungal volatile compounds in recognition of the hostfungus by mycophagous beetles was conducted (GC-EAD, EAG). Beside the two erotylid-species (*Tritoma bipustulata* and *Dacne bipustulata*), mycophagous species of the families Tenebrionidae and Ciidae were included in this study. The scents of young as well as aged fungi were tested. The results imply that the species are able to discriminate between fungi of different ages as well as the degree of colonization.

(4) Due to the multiplicity of different exocrine compound glands in Erotylidae (within the angles, as well as along the lateral margin of the pronotum, on the prosternal and mesoventral intercoxal processes, anteromesal to the compound eyes, on the subgenal braces, and rarely on the mentum), a comparative analysis on the occurrence of compound glands was carried out.

47 species were included in this analysis. The results were mapped on an existing phylogeny of the family and other phylogenetic hypotheses were discussed. Several glandular characters support the monophyly of the Erotylidae, Erotylinae as well as some tribes of the latter subfamily. Also the postulated position of *Languria bicolor* (Languriinae) within the Erotylinae is confirmed by glandular characters.

(5) Finally, it was possible to identify *Brachyserphus parvulus* (Proctotrupidae) as a parasitoid of *T. bipustulata*. Members of this group of Hymenoptera are endoparasites in larvae of numerous families of the Coleoptera, Diptera and Lepidoptera. *B. parvulus* was hitherto known from species of Nitidulidae, Melandryidae, Phalacridae as well as the erotylid genus *Triplax*.

Zusammenfassung

Chemische Abwehr ist innerhalb der meisten Insektenordnungen von großer Bedeutung und eine Vielzahl von zum Teil spektakulären Abwehrmechanismen konnte in den letzten Jahren beschrieben werden. Dass Vertreter der Erotylidae (Pilzkäfer) eine Vielzahl von exocrinen Komplexdrüsen aufweisen ist seit langem bekannt. Die Morphologie und Ultrastruktur, sowie die Chemie der abgegebenen Sekrete dieser Komplexdrüsen wurde bis dato allerdings nicht eingehend untersucht.

Die kosmopolitisch verbreiteten Erotylidae gehören zu der Überfamilie der Cucujoidea (Clavicornia) innerhalb der Teilordnung Cucujiformia und umfassen etwa 3500 Arten in 258 Gattungen. Die Familie schließt heute sowohl die phytophagen Vertreter der früheren Languriidae, als auch die mycophagen Arten der früheren Erotylidae s.str. (jetzt als Unterfamilie Erotylinae) ein. Die adulten Käfer, wie auch die Larven, sind an verschiedene Baumpilze gebunden, leben aber auch unter verpilzten Rinden. Viele Arten der Familie sind zudem sehr auffällig in ihrer Färbung, oft in Verbindung mit Streifen, Punkten oder Ringen. Die vorliegende Arbeit behandelt hauptsächlich Vertreter dieser Käferfamilie und umfasst insgesamt fünf verschiedene thematische Gebiete:

(1) Morphologische Einzelheiten zur Struktur und Ultrastruktur der Komplexdrüsen wurden erstmals an exemplarischen Vertretern der Familie (*Tritoma bipustulata*, *Triplax scutellaris*) untersucht (REM, TEM). Eine Komplexdrüse besteht dabei aus zahlreichen identischen Drüseneinheiten, welche in einen langen, zentralen Ausführkanal münden. Eine einzelne Drüseneinheit wiederum besteht aus zwei verschiedenen Zellen, wovon eine einen kutikulären Ductus ausbildet. Daher sind die Drüsen der untersuchten Arten zu Klasse III nach Noirot & Quennedey (1974, 1991) zu rechnen. Außerdem weisen die Komplexdrüsen zwei strukturelle Besonderheiten (lateraler Appendix, die schwammartige Wand des Ductus) auf, welche bisher nicht von Komplexdrüsen anderer Käfer bekannt waren.

(2) Bisher kaum bekannt war, dass die Arten, wie auch beispielsweise die nahverwandten Coccinellidae und Endomychidae, die Fähigkeit des Reflexblutens zeigen. Die Hämolymphe wird dabei nicht, wie bei den erwähnten Taxa, über die Gelenke der Beine, sondern über die Spitze des Abdomens abgegeben. Die Chemie des Reflexblutes, wie auch des Sekrets der pronatalen Drüsen wurde erstmals mittels GC-MS untersucht. Anschließend wurden die hier identifizierten flüchtigen Inhaltsstoffe beider Sekrete in Biotests und Agardiffusionstests auf ihre biologische Wirkung hin untersucht.

(3) Weiterhin wurde die Rolle flüchtiger, von Pilzen abgegebener Duftstoffe bei der Wirtsfindung durch mycophage Käfer analysiert (GC-EAD, EAG). Dies schloss, neben zwei Arten der Erotylidae (*Tritoma bipustulata* und *Dacne bipustulata*), auch mycophage Arten der Familien Tenebrionidae und Ciidae ein. In den hier durchgeführten Untersuchungen wurden sowohl Duftkomponenten junger als auch älterer Pilze getestet. Aus den Ergebnissen lässt sich schließen, dass die untersuchten Arten in der Lage sind zwischen Pilzen verschiedenen Alters als auch verschiedener Stufen der Besiedlung zu unterscheiden.

(4) Aufgrund der Vielzahl der verschiedenen exocrinen Komplexdrüsen innerhalb der Erotylidae (in den Ecken, sowie entlang der lateralen Seiten des Pronotums, auf dem prosternalen und mesoventralen Fortsatz, anteromesal zu den Komplexaugen, auf den subgenalen Leisten und selten auf dem Mentum), wurde eine vergleichende Untersuchung zur Verteilung der Komplexdrüsen durchgeführt. In diese Untersuchung wurden 47 Arten der Erotylidae einbezogen. Die Ergebnisse wurden auf eine bestehende Stammbaumhypothese der Familie „gemappt" und weitere phylogenetische Hypothesen wurden diskutiert. Mehrere Drüsenmerkmale unterstützen dabei sowohl die Monophylie der Erotylidae, der Erotylinae sowie einiger Triben innerhalb dieser Unterfamilie. Auch die postulierte Stellung von *Languria bicolor* (Languriinae) innerhalb der Erotylinae konnte anhand von Drüsenmerkmalen untermauert werden.

(5) Schlussendlich wurde im Rahmen der Arbeit die Zehrwespe *Brachyserphus parvulus* (Proctotrupidae) als Parasitoid von *T. bipustulata* identifiziert. Vertreter dieser Hymenopterengruppe leben endoparasitisch in Larven zahlreicher Familien der Coleoptera, Diptera und Lepidoptera. *B. parvulus* war bisher aus Arten der Nitidulidae, Melandryidae, Phalacridae sowie der Erotylidengattung *Triplax* bekannt.

Introduction

Insects constitute about 75% of all animal species (Laurent et al. 2005). Several reasons may explain their ecological success, e.g., a high fecundity rate, a remarkable adaptation to different environments and climatic conditions as well as the evolution of specialized structures (e.g., mandibles, ovipositors, wings), and for some groups the existence of highly organized societies. Also the development of extremely diversified and sophisticated communication systems plays a prominent role in their ecological success.

The most species-rich order within the Insecta is the holometabolic Coleoptera, which comprises about 360 000 described species (Beutel 2005). It is assumed that about 10% of the estimated actual amount is recognised (Francke & Dettner 2005). Apart from open oceans, they have colonized nearly all terrestrial as well as limnic habitats. Some species expand to brackish water, and others live even in tarns of splash water near the seashore (e.g. *Ochthebius*, Hydraenidae; Klausnitzer 2005). Their body size ranges from very small (some Ptiliidae show a body length below 0.1 mm) to gigantic (some Scarabaeidae and Cerambycidae are up to 15 - 20 cm big; Klausnitzer 2005).

The earliest fossils attributed to the order Coleoptera were dated to the Lower Permian (about 290 million years before present; Klausnitzer 2005). These fossils were found in the today's Czech Republic and the Ural Mountains in the west of Russia (Lawrence & Newton 1982). The elytra of these oldest representatives († Protocoleoptera) still offer relics of the primal wing venation and overlap the abdomen laterally as well as at the rear (Klausnitzer 2005).

The Coleoptera are divided in four subgroups in the following branching pattern: (Archostemata + (Adephaga + (Myxophaga + Polyphaga))) (Beutel & Haas 2000). Cladistical analyses with extant taxa disclosed following autapomorphies of the order: Presence of elytral epipleura, abdominal sclerites closely jointed, reduction of 8 thoracic muscles, reduction of the first abdomional sternite as well as invagination of the terminal abdominal segment (Beutel & Haas 2000). The majority of the species belongs to the Polyphaga (about 90 % of all beetles), which is classified in five infraorders (Staphyliformia, Scarabaeifiormia, Elateriformia, Bostrichiformnia, Cucujiformia). The enormous radiation of the Polyphaga, in particular the Chrysomeloidea and Curculionoidea (both belonging to Cucujiformia) is surely correlated with the evolution of angiosperms in the Cretaceous (about 145 – 65 million years before present).

The present contribution deals with the coleopteran family Erotylidae (pleasing fungus beetles). Several taxonomic and systematic studies on this family were provided in the past. These literatures will be presented shortly in the concerning chapter. However, ecological, chemical and morphological studies are lacking so far. Since members of this family exhibit numerous glands all over their body and the species are striking in appearance, it seems interesting which chemicals respectively chemical properties are inherent in the glandular secretion and also whether the sporadic distribution of some of these glands is caused by a

particular way of life. Indeed, most species of Erotylidae possess well-developed wings and are considered as good flyers, but their strict mycophagous lifestyle makes questions on host recognition and perception of particular natural products exciting. The thesis comprises these chemical and ecological aspects. The morphology of the glands, which is hitherto unknown, appears also quite interesting in this context. These different topics will be outlined in the following and afterwards the results will be presented in the synopsis. For most investigations in this thesis specimens of *Tritoma bipustulata* were used (Fig. 1); of the scattered distributed erotylid-species, one of the most abundant in Central Europe. With its two basal red spots on the black elytra and the three-segmented antennal club, this fungivorous species is easily to identify (Vogt 1967).

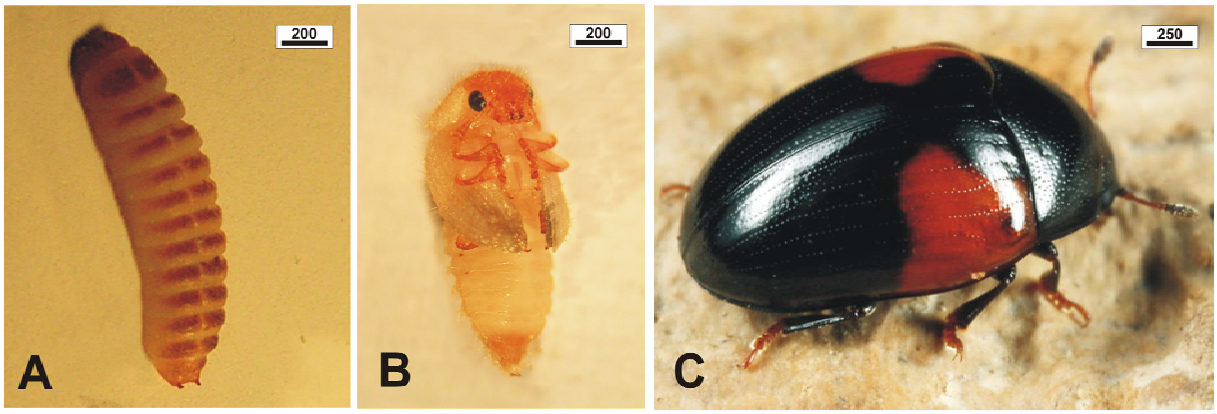

Fig. 1. Developmental stages of *Tritoma bipustulata*. (A) Last larval instar. (B) Pupae shortly before eclosion. (C) Fully coloured adult beetle. Scales in µm.

Systematics of Erotylidae

The cosmopolitan Erotylidae is assigned to the superfamily Cucujoidea (Clavicornia) of the Coleoptera-Cucujiformia. It comprises about 3500 described species (including members of both former families "Languriidae" and Erotylidae s.str.) in approximately 258 genera (Leschen et al. 2010). Most species occur in tropical and subtropical regions; for Germany 16 species are reported. However, 8 of these species were found only before 1950 or the announcement seems doubtful (Köhler & Klausnitzer 1998).

Current morphological and molecular studies on the phylogeny of Erotylidae indicate that the former "Languriidae" is paraphyletic with respect to the former "Erotylidae" (Węgrzynowicz 2002; Leschen 2003; Robertson et al. 2004). The former separation of these two groups was primarily based on their different biology, with "Erotylidae" being mycophagous and "Languriidae" being phytophagous. Leschen (2003) proposed a new classification of the family Erotylidae, where Erotylinae (= Erotylidae in the old, limited sense) stands beside five other subfamilies (together with the former Languriidae); subfamilies of the former Erotylidae are now ranked as tribes of Erotylinae.

Erotylidae is certainly a subgroup of the cucujiform beetles. The monophyly of Cucujiformia is supported by a number of autapomorphies (e.g., Klausnitzer 2005; Lawrence & Newton 1982) and also by the extensive molecular study of Hunt et al. (2007). Phylogenetic relationships within Cucujiformia are widely unresolved, and the Cucujoidea, which comprises mostly detritus- and fungus-associated species, are not likely to be a monophyletic group (Buder et al. 2008; Hunt et al. 2007). The placement of Erotylidae in "Cucujoidea" must be seen on this background. Various families were regarded as the closest relatives of Erotylidae in the past: members of the cerylonid series (Alexiidae, Endomychidae; Crowson 1955; Sen Gupta & Crowson 1971), certain "lower Cucujoidea" (Cryptophagidae, Propalticidae; Sen Gupta & Crowson 1969, 1971; McHugh 1997; Leschen 1996) as well as Phloeostichidae, Lamingtoniidae (Leschen 2003) and Biphyllidae (Sen Gupta & Crowson 1971; Leschen 2003). The molecular studies by Hunt et al. (2007) suggest either Monotomidae, Helotidae, and Protocucujidae or a clade comprising Laemophloeidae, Phalacridae, Propalticidae, and Cucujidae as the closest relatives of Erotylidae.
Compound integumentary glands are widespread in Coleoptera and it is well known that the Erotylidae show a particularly rich equipment of compound exocrine glands. Glands can occur within the angles of the pronotum, as well as along the lateral margin, on the pro- and mesosternal intercoxal processes, on the head anteromesal to the compound eyes, on the subgenal brace, and rarely on the mentum. However, despite this manifold occurrence of such glands over the erotylid beetles´ body, their spatial consideration in the previous literature is quite sparse. 38 species (incl. members of the former Languriidae) were examined in a comparative analysis of gland occurrence and 9 species were added from the literature. The results were mapped on an existing phylogeny of the Erotylidae (Węgrzynowicz 2002) and other phylogenetic hypotheses were discussed. Species of Cryptophagidae, Biphyllidae, and the former Languriidae were used as outgrouptaxa.

Question: Are there phylogentically informative characters in the distribution of exocrine glands in Erotylidae, and how evolution of exocrine glands took place within the family?

Morphology of compound glands in Erotylidae

Noirot & Quennedey (1974, 1991) defined three classes of insect gland cells with respect to the cuticle and the way of egress of secretion. In class I, the cell is simply covered by the cuticle, and the secretion must cross this barrier. The cuticle above the gland was secreted by the gland cells themselves. Scattered class I cells have rarely been described at the ultrastructural level, and were previously reported from the coccinellid *Semiadalia undecimnotata* (Barbier et al. 1992) and from exocrine glands of the pyrrhocorid species

Dysdercus cingulatus and *D. fasciatus* (Lawrence & Staddon 1975; Farine 1988). In both other classes, the gland cell is not in contact with the cuticle, which is furthermore not secreted by the gland cell. In class II, the gland cell is surrounded by differentiated epidermal cells. As in class I, the secretion must cross the cuticle after transfer into the modified epidermal cells. This type is typically found in sternal glands of termites, except for the primitive genus *Mastotermes*, where they are lacking (Quennedey 1978). Finally, in class III, a cuticular ductule penetrates the gland cell. The ductule passes a canal cell and is in continuity with the cuticle. In the simple case, a single gland cell is in contact with one canal cell, but the system is sometimes complicated by the presence of an additional cell between the other two. Isolated class III units are generally found in the epidermis.

Ectodermal (integumentary) glands occur in two different organisational levels: simple and compound. Simple glands consist of a single gland unit opening individually on the body surface and a gland unit is composed of a few specialised cells - one or more being secretory. This is the case for dermal glands observed in many orders of insects. In compound glands numerous gland units are combined upon a common outlet duct, which may additionally form a reservoir (Noirot & Quennedey 1974, 1991).

However, comparative data on the morphology and ultrastructure throughout taxa are scarce. Among the Cucujiformia, the coccinellid *Semiadalia undecimnotata* has compound glands scattered over the head capsule, mouthparts, thorax and abdomen (Barbier et al. 1992). Most tenebrionid beetles have a pair of large reservoirs in the abdomen (Tschinkel 1975), which originate from the membrane behind the seventh visible sternite. Anthicid and meloid beetles possess a large, paired mesothoracic gland opening ventrally in a slightly depressed area (Hemp & Dettner 1997; Morgan 1968; Berrioz-Oritz 1985). Ciidae (Buder at al. 2008) and some Erotylidae (Węgrzynowicz 2002) bear a gland associated with a hairy tuft on the first visible male abdominal sternite (such a structure was also reported from species of Buprestidae and Dermestidae; Węgrzynowicz 2002). In Chrysomelidae compound glands have been observed in several subfamilies (Pasteels et al. 1989); members of Chrysomelinae, Criocerinae, and some Galerucinae have morphologically similar glands in similar positions, mostly along the lateral and cranial margins of the pronotum.

Question: What is the morphology and ultrastructure of exocrine glands in examplar species of Erotylidae?

Chemical ecology of Erotylidae

All organisms are chemosensitive, and are also the source of substances to which others can potentially respond. In the course of evolution this potential for interaction has been thoroughly exploited, and organisms depend on an exchange of chemical cues with other organisms in their environment. When this exchange is between members of the same species, the mediating substances are called pheromones (Eisner & Meinwald 1966). These compounds are important in regulation of courtship and other social activities in animals. In 1970, Brown et al. introduced the term kairomone to describe "a transspecifical chemical messenger; the adaptive benefit of which falls on the recipient rather than on the emitter". Such compounds are for instance floral scent compounds, which guide the pollinator to the host plant (foraging kairomones). Allomones, a third class of trans- or interspecific chemical messengers is beneficial for the emitter (Brown et al. 1970). In plants and animals they mainly serve as defensive chemicals for protection against predators, herbivores or parasites.

Extensive investigations on chemical defence and secondary compounds were carried out, and the knowledge as well as the published literature has grown significantly during the past decades. However, the diversity of defensive chemicals produced by the insects themselves or through other organisms is amazing, and numerous remarkable compounds were identified. The often aposematic coloured Coccinellidae as well as the related Endomychidae show reflex bleeding and offer a large spectrum of repellent and bitter alkaloids, pyrazines and lactones (Dettner 1987; Daloze et al. 1994; Laurent et al. 2005). Macrocyclic lactones are also typical components of cucujid beetles, which have been given the trivial name cucujolides (Oehlschlager et al. 1987, 1988). Most species of the usually nocturnal tenebrionids have large abdominal defensive glands, which produce mainly quinoic mixtures in admixture with diverse alkenes (Tschinkel 1975; Dettner 1987). For species of Nitidulidae rather stereotypic structures like methyl- and ethyl-branched aliphatic hydrocarbons with three or four (*E*)-configured conjugated double bonds were reported (Bartelt 1999). The chemically unique monoterpene anhydrid cantharidin is reported for both Oedemeridae and Meloidae. This hemolymph toxin represents a powerful vesicant, insecticide, and feeding deterrent (Dettner 1987). Reviews on chemical defence of beetles and certain cucujid taxa are given by Tschinkel 1975 (Tenebrionidae); Pasteels et al. 1988, 1989, 1994 (Chrysomelidae); Daloze et al. 1994 (Coccinellidae); Dettner 1987; Francke & Dettner 2005, and Laurent et al. 2005.

Both the chemistry of the glands in Erotylidae and their ecological role has remained unexplored so far. McHugh (1997), in his work on the morphology of *Megalodacne heros*, presented an initial insight into the morphology of such a gland of an erotyline species, and he reported the secretion to be a clear odorous fluid. Further observations on the chemistry are lacking so far.

Question: How does the chemical defensive system of an erotylid beetle work and which biological properties are inherent?

Host recognition in Erotylidae

For many beetles and other arthropods, fungi and dead wood material are natural and crucial resources for nutrition, oviposition and shelter in a forest landscape (Scheerpeltz & Höfler 1948, Benick 1952, Lawrence 1989). Fungi concentrate valuable nutrients about 10 times higher than the wood they grow on (Martin 1979, Jonsell & Nordlander 2004). Fungal tissue is a richer source of protein and poorer source of carbohydrate than fruits, and is a less concentrated source of all types of the major classes of nutrients than seeds or nuts (Hodgman et al. 1959). Therefore, fungal tissue more closely resembles foliage than other types of tissue derived from higher plants, such as fruits, seeds, or wood.

About half of the recognized beetle families are primarily mycophagous or feed on plant material which has been substantially altered by the action of fungal enzymes, although only about 25 families of Coleoptera are mycophagous in the strict sense (Lawrence 1989). Collembola, Coleoptera, Diptera and Acarina are the orders of arthropods most frequently collected from woody fungi (Martin 1979).

Larvae and adults of the erotylid subfamily Erotylinae (the remaining subfamilies comprise the former Languriidae, which are phytophagous) are exclusively associated with various Polyporales and related higher fungi (Leschen 2003; Robertson et al. 2004). Examination of gut contents of *Tritoma bipustulata* revealed that all developmental stages feed on fungal hyphae and spores. A summary on the fungal hosts of erotylid species of the Nearctic is given by Skelley et al. (1991); Hawkeswood et al. (1997) summarized the fungal hosts of the Australian species.

In this context, volatile organic compounds (VOCs) might play an important role in host recognition and selection for mycophagous species. Earlier studies indicate that insects use these olfactory signals in two different ways while searching for fruiting bodies or dead wood material. Insects are either able to perceive volatile compounds emitted by their hosts or they colonize the host by following the attractive volatiles (pheromones) released by a so called pioneer individual that selected the host. Pacioni et al. (1991) demonstrated by a trapping-experiment that Coleoptera (Leiodidae, Staphylinidae), Diptera and Lepidoptera were attracted by dimethyl sulphide, a component of the aroma of truffles (*Tuber* spp.). Also cisids associated with *Formitopsis pinicola* (Polyporales) recognised the host odor during the flight and were attracted in significant numbers to baited traps (Jonsell & Nordlander 1995). Electrophysiological and behavioural analyses of *Cis boleti* (Ciidae) revealed that this fungivorous species is attracted by 1-octene-3-ol and other host fungal volatiles (Thakoew et al. 2008); 1-octene-3-ol is known as a typical mushroom alcohol and is the most

characteristic fungal VOC. Similar analyses with the wood-breeding scolytid *Trypodendron domesticus* and the syntopic *Hylecoetus dermestoides* (Lymexylonidae) revealed a high number of substances (e.g. 2-methoxy-phenol, 1,2-dimethoxybenzene, 2-methyl-1-butanol) used as semiochemicals in host selection (Holighaus & Schütz 2006); such interactions enhance the success of colonisation for the mentioned insects. Random searching of a pioneer individuum, representing a second strategy in host finding, was observed for the tobacco beetle *Lasioderma serricorne* (Anobiidae; Levinson & Levinson 1987) and is also suggested for *Dorcatoma puctulata* and *D. robusta* (both Anobiidae; Jonsell & Nordlander 1995). In these cases the pioneer individual attracts conspecifics by emitting pheromones (similar to Scolytidae).

Question: Do volatile chemical compounds play a role as key attraction factors in recognition and host selection in selected species of Erotylidae, Ciidae and Tenebrionidae? Which compounds are involved in host recognition?

Synopsis

Systematics of Erotylidae

The occurrence of the various glands is described for 46 species of Erotylidae. The glandular characters are mapped on a previously published erotylid phylogeny (Węgrzynowicz 2002) and phylogenetic implications are discussed

In the sample of Erotylidae the following compound glands were found (Fig. 2; manuscript I): **(1)** Pores of periocular glands are located on the frons anteromesal to the compound eye, if present always as one pair. **(2)** Pores of subgenal glands are located on the subgenal braces, if present always as one pair. **(3)** Pores of pronotal glands occur on the lateral margins of the pronotum. If they are present, they include always one pair of pores each at the anterior and posterior corners of the pronotum; additional pores along the lateral pronotal edges in between can be present in varying numbers (1–19 per side). **(4)** Pores of prosternal glands are located on the ventral surface of the prosternal process, if present always as one pair. **(5)** Pores of mesosternal glands are located on the ventral surface of the mesosternal process, if present always as one pair. **(6)** Pores of mental glands are located at the base of the mentum, if present always as one pair.

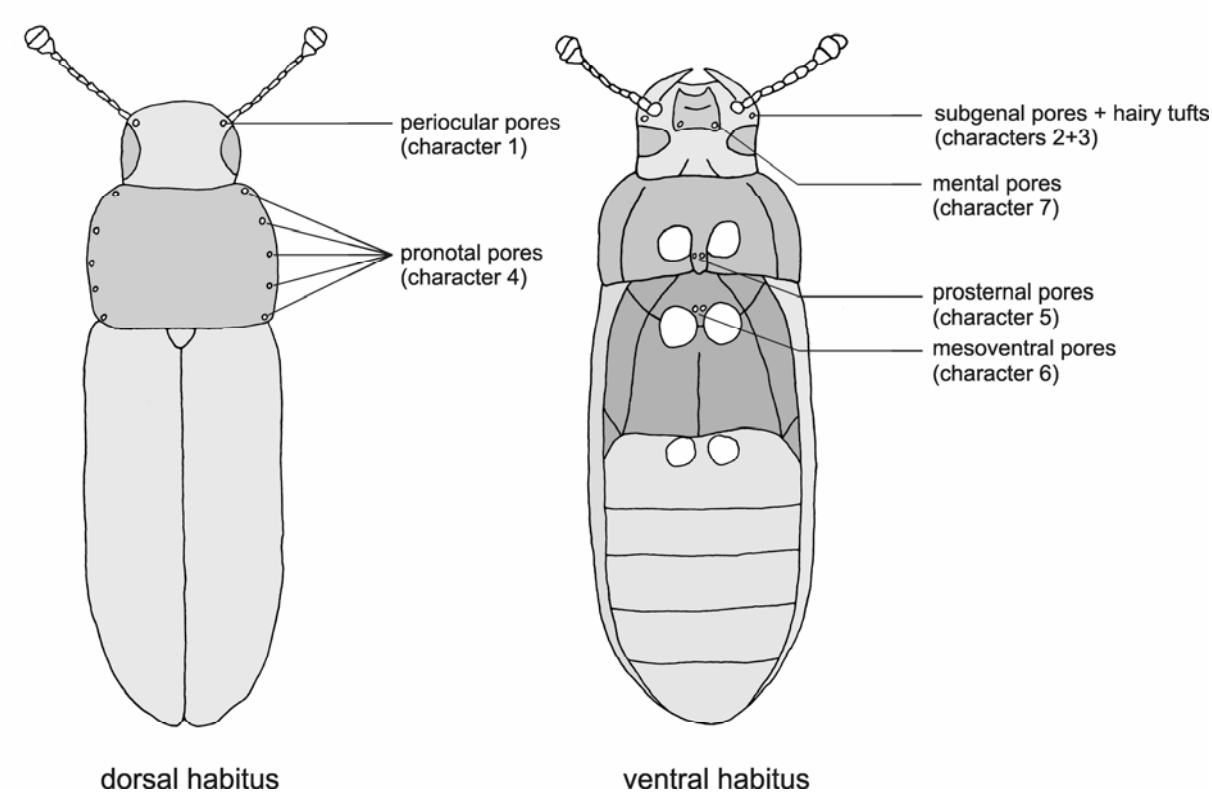

Fig. 2. Schematic drawing indicating the positions of the particular glandular characters. Number of characters correspond to manuscript I.

Each examined specimen of Erotylidae was found to possess pores in at least one of the above mentioned body parts, and the same is true for the "languriid" taxa (*Languria* and *Toramus*; see also Węgrzynowicz, 2002). A complete lack of pores is true for the other "languriid" taxa studied herein (*Setariola, Camptocarpus, Langurites, Tetraphala*). For the outgroup taxa (47 genera of languriid Erotylidae; see Leschen, 2003) severeal pores were detected, but their distribution is ambiguous in most cases. The two non-erotylid species here studied had no pores at all (*Biphyllus lunulatus* and *Cryptophagus lycoperdi*).

Usually the glandular pores in Erotylinae lack any special modifications for the release or evaporation of the discharged secretion. In some exceptional cases, however, the pores show a groove- or plateau-like extension, or patterns of circular ridge-like elevations of the cuticle. In other cases the area around the pore looks sponge-like (as in the subgenal pores of *Tritoma bipustulata, Triplax russica* and *Pselaphacus nigropunctatus*), and these structures are accompanied by trichomes. Such structures are widespread among fungivorous beetles and enable transfer of spores or fragments of spawn (Węgrzynowicz 2002). For Erotylinae an enhancement of the evaporation of discharged secretions is also conceivable since these trichomes are located beside the subgenal pores.

The currently most elaborate hypotheses on Erotylidae phylogeny are that of Robertson et al. (2004) based on DNA-sequences (18S and 28S rDNA sequences), that of Węgrzynowicz (2002; Fig. 3A) and Leschen (2003) based on morphology; the latter treat mainly the former languriid subfamilies. There are some pivotal differences between these hypotheses (manuscript I):

(1) Robertson et al. (2004) find the languriid taxon Cryptophilinae (*Toramus*) subordinate in the Erotylinae-Tritomini, but no morphological characters have been proposed in support of this relationship (Węgrzynowicz, 2002; Robertson et al., 2004). In Węgrzynowicz (2002) as well as in Leschen (2003) Cryptophilinae are placed outside of the Erotylinae, associated with other languriid lineages. Apart from that difference, basal relationships are Dacnini + (Languriinae + remaining Erotylinae) in both trees. In Leschen (2003) the Languriinae are also placed outside of Erotylinae, but nested within the Loberinae.

(2) The Erotylinae-Tritomini are paraphyletic in all hypotheses, but while in Robertson et al. (2004) they are paraphyletic only with regard to Erotylini (and Cryptophilinae), the Encaustini and Megalodacnini are additionally nested in Tritomini in the hypothesis of Węgrzynowicz (2002) and Leschen (2003), where Erotylini, Encaustini, and Megalodacnini together form a clade.

(3) *Coptengis*, formerly assigned to Dacnini, is placed in the Encaustini according to Robertson et al. (2004), but in the Megalodacnini according to Węgrzynowicz (2002).

(4) Each of the tribes Erotylini, Encaustini, and Megalodacnini is monophyletic in all hypotheses (except for the case of *Coptengis*), but only in Węgrzynowicz (2002) and Leschen

(2003) the three tribes together form a clade with *Pselaphacus nigropunctatus* (Tritomini) being the sister taxon to this clade in Węgrzynowicz (2002).

The taxon sample used herein shows much overlap with that in Węgrzynowicz (2002). Gland characters were mapped on that previously published erotylid phylogeny, with character transformations placed on the tree in the most parsimonious way (Fig. 3A, additional taxa studied herein are supplemented).

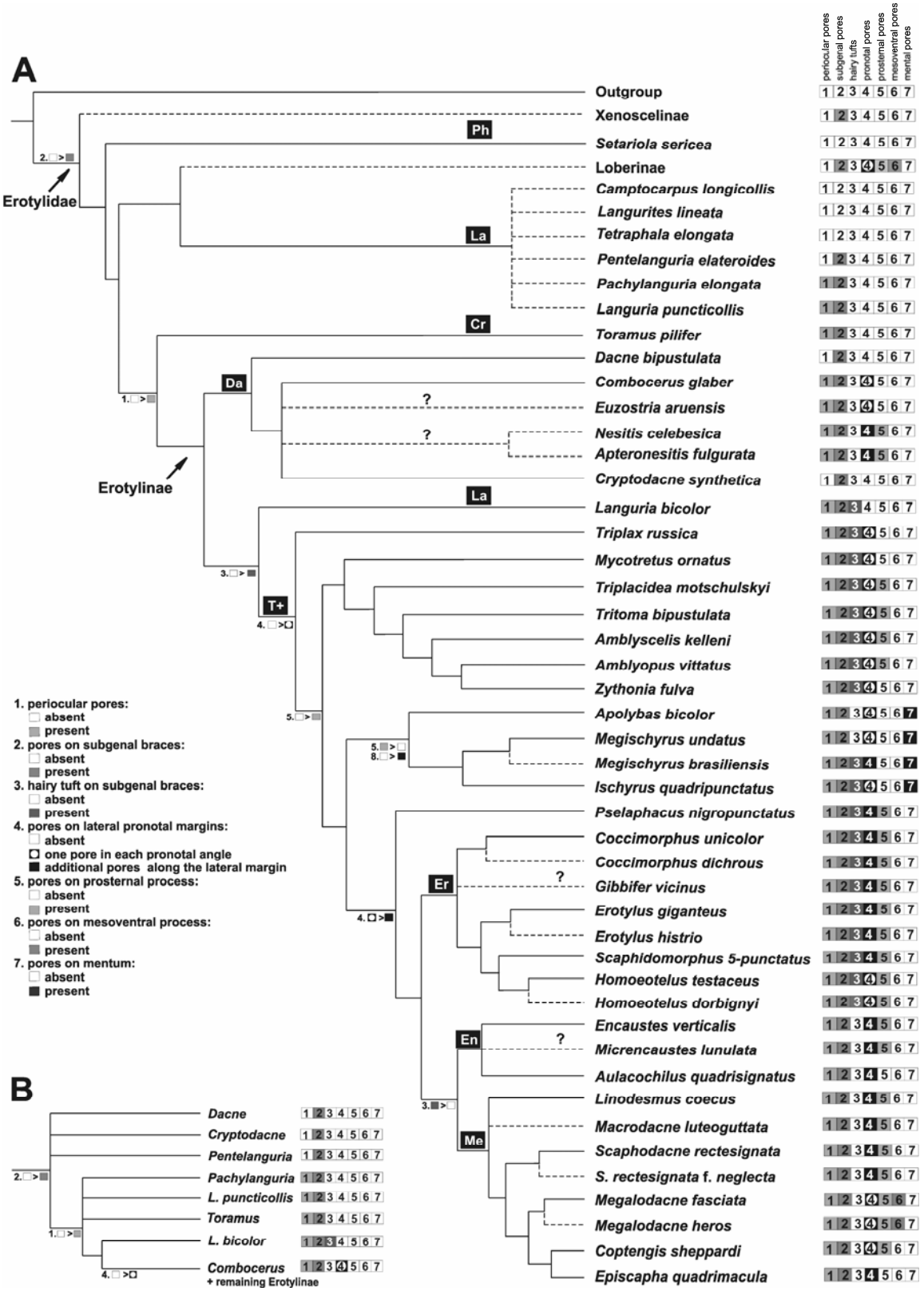

Fig. 3. (A) Characters of pores mapped on a phylogeny of the Erotylidae based on 85 morphological characters (after Węgrzynowicz 2002; species added herein indicated by dashed lines). (B) Hypothesis for the basal splitting events in Erotylidae derived from gland characters. Cr – Cryptophilinae; Da – Dacnini; En – Encaustini; Er – Erotylini; La – Languriinae; Me – Megalodacnini; Ph – Pharaxonothinae; T+ – Tritomini + remaining Erotylinae.

Basal relationships in Erotylinae. The Erotylidae in this broad sense are supported by the presence of subgenal pores (Leschen, 2003). In the sample here studied the presence of periocular pores supports the monophyly of the Erotylinae including *Toramus* (Cryptophilinae) and *Languria bicolor* (Languriinae). The other languriine species studied herein (*Languria puncticollis* and *Pachylanguria*) also had these pores, suggesting a related position to *L. bicolor* derived from glandular characters (Fig. 3A). However, there are some points of ambiguity at the base of the tree that mainly concern the absence or presence of particular glands in species of the former Languriidae and various Dacnini.

Based on gland characters alone, *Dacne bipustulata, Cryptodacne synthetica* and the languriid *Pentelanguria*, which only have subgenal pores, would appear as sister group to a clade comprising *Toramus* and all other Erotylinae including *Pachylanguria* as well as both examined *Languria* species; *Pachylanguria, L. puncticollis* and even *Toramus*, which additionally have periocular pores, would be the next branch of the erotyline tree, and *L. bicolor*, which also has trichomes associated with the subgenal pores would follow. The remaining Erotylinae form a clade weakly supported by the presence of pores in the corners of the pronotum, since these pores are absent in *Brachypterosa* (which represents the sister taxon to all Erotylinae) as well as in *Dacne* and *Cryprodacne* (Dacnini)).

This relationship *Dacne* + *Cryptodacne* + *Pentelanguria* + (*Toramus* + *Pachylanguria* + *L. puncticollis* + (*L. bicolor* + *Combocerus* and remaining Erotylinae)), shown in Fig. 3B, would be incongruent with the monophyly of Dacnini proposed by Węgrzynowicz (2002). Nonetheless, in the latter hypothesis Dacnini is poorly supported by apomorphies: the mesepisterna are fused to the mesepimera far in front of the meso-metathoracic suture inside the coxal cavities, and cross-veins r3 and r4 approach or touch one another. Thus, there are hardly any counter-arguments against the relationships suggested by gland characters. As mentioned above, both Węgrzynowicz (2002) and Robertson et al. (2004) found that *Coptengis* has to be removed from Dacnini. According to the gland characters here studied, *Nesitis, Apteronesitis, Euzostria* and *Combocerus* are also highly unlikely to be related to any of the other genera assigned to Dacnini, and even if limited to *Dacne* and *Cryptodacne*, Dacnini may be paraphyletic.

Whereas a position of *Languria bicolor* (Languriinae) inside the Erotylinae (Węgrzynowicz, 2002; Robertson et al., 2004) is clearly confirmed by the presence of periocular pores and trichomes next to the subgenal pores. *Pachylanguria*, *L. puncticollis* and *Toramus* lack these

trichomes; the other Languriinae here examined, *Tetraphala elongata*, *Langurites lineata* and *Camptocarpus longicollis*, lack all gland pores. This suggests that these taxa are placed outside Erotylinae, and Languriinae is non-monophyletic. Leschen´s (2003) arguments for monophyletic Languriinae are the presence of submesocoxal lines, of an apical pit of the spermatheca, and of a wedge cell in the wing venation. In sum, the monophyly of Languriinae appears at least debatable.

Apical relationships in Erotylinae. The clade comprising the Tritomini (paraphyletic), Encaustini, Erotylini, and Megalodacnini (and Cryptophilinae in Robertson et al., 2004) likely has a groundplan set of pores that includes pores in the corners of the pronotum as well as periocular and subgenal pores, the latter associated with a tuft of trichomes.

One character conflict at the base of Tritomini concerns the distribution of prosternal pores (character 5 in Fig. 3A). Węgrzynowicz´s (2002) clade comprising Tritomini (under exclusion of *Triplax russica*), Erotylini, Encaustini, and Megalodacnini is ambiguously supported by the presence of these pores (Fig. 3A, where the "accelerated" version of character transformation is mapped). Considering the basal dichotomies within this clade, prosternal pores are consistently absent in the clades *Mycotretus* and *Apolybas* + *Ischyrus* + *Megischyrus*, while they are present in the respective sister clades *Triplacidea* + *Tritoma* + *Amblyscelis* + *Amblyopus* + *Zythonia* (absent in the latter genus) and *Pselaphacus* + Erotylini + Encaustini + Megalodacnini (with a few scattered absences appearing as secondary). Therefore, gland characters would rather suggest that the two latter clades form a monophyletic group.

Also problematic is the presence of the pronotal pores in the clade *Apolybas* + *Ischyrus* + *Megischyrus* (character 4 in Fig. 3A). While *Apolybas*, *Megischyrus undatus* and *Ischyrus quadripunctatus* (as well as three other examined species of this genus; *I. scriptus, I. femoralis, I. flavitarsis*) had pores only in the pronotal corners, the closely related species *M. brasilensis* (as well as three other examined species of this genus; *M. semipunctatus, M. decempunctatus, M. discipennis*) had one additional pore at the lateral pronotal margin. Since *M. undatus* was unfortunately not available for this study, it remains open whether this finding is defective. If applied, the genus *Megischyrus* more likely belongs to Erotylini, Encaustini, or Megalodacnini.

However, the just mentioned clade is supported by another gland apomorphy: the presence of pores on the mentum (character 7 in Fig. 3A). This character was found in all examined members of the clade and seems to be unique among Erotylidae. However, their occurrence is not stable since some *Ischyrus* species lack them (Węgrzynowicz, 2002).

A clade comprising the derived Tritomini *Pselaphacus nigropunctatus* as well as the examined Erotylini, Encaustini, and Megalodacnini is supported by the presence of additional pores (at least one) along the lateral pronotal edge. However, the finding for *P. nigropunctatus* (seven specimens were examined and the arrangement of pores was always the same) differs from that of Węgrzynowicz (2002), who found only pores in the pronotal corners; this may indicate intraspecific variability, which would limit the usefulness of this

character for phylogenetic conclusions. The presence of pores only in the pronotal corners in both examined *Homoeotelus*-species among Erotylini and *Coptengis* as well as both examined *Megalodacne*-species among Megalodacnini is also conflicting, yet this is quite likely to result from secondary loss.

The presence of trichomes on the subgenal braces supports a clade comprising *Languria bicolor*, Tritomini and Erotylini. In the clades Encaustini and Megalodacnini trichomes are always absent while pores are present.

In the sample examined a clade comprising both *Megalodacne* species (Megalodacnini) is supported by the presence of a pair of mesoventral pores, which show identical location in both taxa. In contrast, Leschen (2003) reported mesoventral glands also for a few species of the other subfamilies as well as for all Erotylinae.

In sum, very confusing and hardly interpretable distributions of glandular characters were found in the examined system. The distributions of compound glands fit the present phylogenies (Węgrzynowicz, 2002; Robertson et al., 2004) most notably in the subfamily of Erotylinae. However, there is much homoplasy in the evolution of the glandular equipment, and this mainly concerns the scattered and confused distributions within the languriid subfamilies (especially the periocular pores, prosternal and mesoventral pores). Within the Erotylinae the distributions are quite stable and several clades may be supported by some of these characters. Hence, gland characters alone are hardly qualified to resolving the phylogeny of Erotylidae; at most they are applicable in some apical erotyline clades (see also Tschinkel, 1975; Tschinkel & Doyen, 1980 and Steidle & Dettner, 1993 for gland characters as a phylogenetic tool). Unfortunately, little is known about the biology and ecology of many of the erotylid genera and species, and virtually nothing about the contents and functional role of their various glands. This presently hampers estimations of how plausibly secondary losses of glands could be explained as resulting from changes in life history.

Manuscript I reports the distribution of compound glands in members of Erotylidae as well as their phylogenetic implications. The presence of periocular pores supports the monophyly of the Erotylinae (incl. *Languria bicolor* and *Toramus*). Based on gland characters, *Dacne bipustulata, Cryptodacne synthetica* and the languriid *Pentelanguria*, which only have subgenal pores, would appear as sister group to a clade comprising *Toramus* and all other Erotylinae (incl. *Pachylanguria* as well as both examined *Languria* species). The remaining tribes of Erotylinae form a clade weakly supported by the presence of pores in the corners of the pronotum, and a clade comprising the Tritomini-species *Pselaphacus nigropunctatus* as well as the derived Erotylini, Encaustini, and Megalodacnini is supported by the presence of additional pores along the lateral pronotal margin. The proposed position of *Languria bicolor* inside the Erotylinae is confirmed by glandular characters; the other Languriinae studied herein (*Tetraphala elongata*, *Pentelanguria elateroides*), lack all gland pores. These findings

suggest that these taxa are placed outside Erotylinae and Languriinae results as non-monophyletic.

Morphology of compound glands in Erotylidae

The morphology and ultrastructure of the compound glands in Erotylidae were studied in *Tritoma bipustulata* and *Triplax scutellaris* (manuscript II). The pronotum of both species bears a single distinct pore in each of its four corners. From each pore a long, whitish, pennate gland extends internally, as seen in the opened, macerated pronotum. Each gland has a central excretory duct 300–330 µm long and 16–23 µm wide (near its proximal base), which is usually unbranched; only in a single case a dichotomy was found. This could be a phylogenetically informative character, but might also be correlated with the size of a species. The excretory duct is divided in two regions: The proximal part, about 50 µm long, has a smooth wall and lacks gland units. The much longer distal part has a wrinkled wall and bears numerous identical gland units. Observations using TEM confirm that the wall of the excretory duct is lined with cuticle (about 1 µm thick) on its entire inner surface and is thus likely derived from an epidermal invagination. The outer (lumen-facing) surface of the cuticle is even, while the wrinkling seen on the inner (cell-facing) side of the cuticle is due to strong linear thickenings of the cuticle. A wrinkled cuticular surface of the excretory duct is also found in the abdominal compound glands of many Tenebrionidae (Tschinkel 1975) and Staphylinidae (Dettner 1987). According to Tschinkel (1975), this reflects an ability to expand the reservoir. However, such a mechanism is unlikely for the examined Erotylidae, where wrinkling is not due to folding of the cuticle but to linear thickenings, which would hardly allow any expansion of the cuticle.

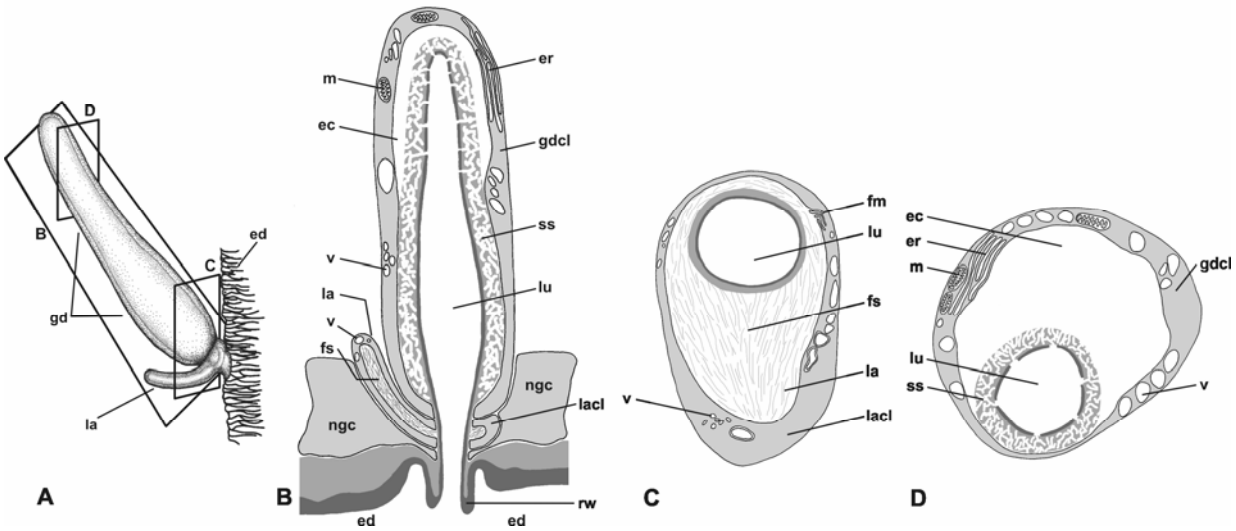

Fig. 4. Schematic drawing of a pronotal gland unit of the pronotal compound gland of *T. bipustulata*. (A) One gland unit upon piece of excretory duct, with sectional planes shown in

B, C, and D. (B) Longitudinal section through gland unit. (C) Cross-section through proximal region of gland unit; lateral appendix directed towards bottom; membrane of the cell (lacl) that encloses the extracellular filamentous structure (fs) is strongly folded near the conducting canal. (D) Cross-section through distal region of gland unit. In B, C, and D cuticle is shown in dark gray (darker outer layer) or medium gray (brighter inner layer); cytoplasm is shown in light gray; continuous black lines are membranes (of cells or cellular organelles such as vesicles and mitochondria). ec – extracellular cavity, ed – excetory duct, er – endoplasmatic reticulum, fm – folding of membrane, gd – glandular ductule, gdcl – glandular ductule cell, la – lateral appendix, lacl – lateral appendix cell, lu – lumen, m – mitochondria, ngc – non-glandular cell, rw – ring wall, ss – spongiose structure, v – vesicle.

Each gland unit consists of a large glandular ductule (gd) 13–15 µm long and a smaller lateral appendix (la) 3–4 µm long (Figs. 4A, B). The presents of a cuticular ductule indicates that the glandular cells belong to class III of Noirot & Quennedey (1974, 1991). The ductule has a fairly narrow base where it originates from the excretory duct. Slightly further distally the ductule is widened; from this part the lateral appendix (la) originates sideward, having a wide base embracing most of the ductule (Figs. 4A, B). The surfaces of the entire appendix and of the ductule distal to its basal constriction are strongly spongiose. A spongiose structuring of the cuticle along the distal part of the canal of a gland unit has apparently not been found before in compound glands of beetles. In many taxa the membrane of the secretory cell, which is facing the extracellular cavity, forms microvilli (e.g. *Tenebrio molitor*; Delachambre 1973; *Semiadalia undecimnotata*, Barbier et al. 1992). Absence of microvilli, as in *T. bipustulata*, however, is also found in the staphylinid *Philontus varians* (Quennedey et al. 2002).
Sections studied by TEM (manuscript II) show that the ductule has a canal with a defined lumen. The cuticle often appears to consist of two layers, which may represent a cuticulin (closer to the epithelium) and an epicuticular layer as specified in some previous contributions (Noirot & Quennedey 1974, 1991, Quennedey 1998). Around the opening of the ductule into the excretory duct the cuticle forms a valve- or ringwall-like structure (Fig. 4B) of varied discreteness. The part of the canal in the proximal half of the ductule is called the conducting canal; in this part the cuticular wall is not perforated. The part of the canal in the distal half is the receiving canal; here the cuticle is penetrated by pores (Fig. 4B). The cuticular pores along the receiving canal open into the cavities inside the above mentioned spongiose layer, and there is thus altogether a labyrinth-like system of penetrations through the cuticle.
Beyond the spongiose cuticular layer a narrow cell was observed (gdcl, Figs. 4B, D), which like a cap encloses the longer distal part of the ductule, proximally almost reaching the base of the lateral appendix. Along one flank of the ductule, the outer membrane of the cell is in contact with the spongiose cuticular layer, but on the other side it is elevated from that layer, whereby a large extracellular cavity (ec, Figs. 4B, D) is present between cell membrane and cuticle: the central extracellular space. Inside the cell numerous mitochondria, tubular

endoplasmatic reticulum as well as an extensive system of vesicles and cisternae are found (Figs. 4B, D). From that, the cell (gdcl) shows clear signs of a secretory activity and thus constitute the terminal cell defined by Noirot & Quennedey (1974, 1991).
The lateral appendix is also enclosed by a single cell (lacl, Figs. 4B, C). At the base of the appendix upon the ductule, this cell, being the canal cell after Noirot & Quennedey (1974, 1991), embraces the widened part of the ductule completely. There is no other, intercalary cell in contact with the gland unit in between these two cells. The nature of what is enclosed by cell lacl of the appendix is enigmatic: It is a homogeneous mass composed of numerous filaments (fs, Fig. 4C), which originate from the cuticular intima of the ductule. The persistence of the appendix after clearing with KOH suggests that the filamentous structure is also cuticular in nature. The cell lacl enclosing the appendix contains numerous vesicles, and its outer membrane facing the filamentous structure shows intense folding in some areas (Fig. 4C). The presence of small vesicles in the canal cell might indicate a secretory function, but this would be in contrast to the usual properties of the canal cell in class III gland units according to Noirot & Quennedey (1974, 1991). In addition, since the cuticular intima of the proximal part of the ductule lacks perforations, there is apparently no open connection between the core of the appendix and the canal of the ductule, so that probably no secretions can be contributed by the appendix.
Proximal to cell lacl, the non-glandular cells covering the excretory duct also ensheathe the most proximal part of the ductule (ngc, Fig. 4B). Most of the pronotal gland is embedded in non-glandular cells, only the most distal portions of the ductules surpass this cell layer and are in contact with the hemolymph space. Furthermore, neither any innervation of the gland or gland units nor any muscle cells associated with the pronotal gland were observed. The regular prothoracic muscles are probably involved in the discharging of the secretion from the glands.
The examined prosternal glands of *T. bipustulata* (manuscript II) are very similar to those of the pronotum but smaller (about 160 µm long). A pennate unbranched gland originates from each of the two prosternal pores. The gland is also composed of an excretory duct blotched with numerous gland units, each including a ductule and an appendix.
Further SEM studies of a single, female specimen of a *Triplax scutellaris* showed the same arrangement and basic structure of the pronotal glands (manuscript II). The unbranched excretory ducts (about 400 µm long) as well as the gland units are slightly larger than in the smaller species *T. bipustulata*.

Thus, manuscript II reports the internal structure and ultrastructure of the pronotal and prosternal glands of *Tritoma bipustulata* as well as the pronotal glands of *Triplax scutellaris*. Each gland consists of a central excretory duct with numerous glandular units. The glandular units correspond to class III gland units defined by Noirot & Quennedey (1974, 1991). Both structural features, the spongious cuticle of the cuticular

ductule as well as the lateral appendix, filled with a filamentous mass, were previously not reported for compound glands of beetles.

Chemical ecology of Erotylidae

Adults of *T. bipustulata* respond to disturbance by emitting secretion from their pronotal glands. A clear odourless fluid is oozing out (when disturbed), spreading over the pronotum and volatilises rapidly. The insects respond only to direct contact stimulation; movement nearby or minute molestations induced no discharge of secretion. However, they are able to emit secretion several times consecutively. Furthermore, when considerably disturbed at the abdominal tip a clear, malodorous and highly volatile secretion was simultaneously discharged from this body region. This secretion disperses over the tergum and the rear of the elytra. Both the detected chemical (GC-MS) and proteinaceous (SDS-PAGE) patterns in the abdominal secretion as well as the hemolymph were entirely equal. Since no glandular structures were detected in this body part, the abdominal secretion was interpreted as reflex bleeding. Small grooves at the abdominal tip, which facilitate the discharge of hemolyphe, and remains of solidified hemolymph, were detected.

The secretion of the pronotal glands was collected by closed loop stripping technique (Boland et al. 1984); compounds were eluted with acetone and analysed by GC-MS. Common methods for collecting the secretion like small pieces of filter paper, capillary tubes or dissection of whole glands were surprisingly not successful. Also the separation of the living animals into sexes for chemical analyses was unfortunately not successful, since the beetles respond with reflex bleeding and contracting the abdomen beneath the elytra. Furthermore, it can not be excluded that some of the detected compounds originate from other glands than the large pronotal ones, since *T. bipustulata* possess additional glands (beside the compound eyes, on the subgenal braces and on the prosternal process; manuscript I). However, these additional glands are very small and release of secretions was never observed by the author. Despite the above mentioned problems, the present contribution displays an interesting insight in the chemical defence of a member of this neglected coleopteran family.

GC-MS analyses of the secretion from the pronotal glands of *T. bipustulata* yielded more than 30 peaks, 10 could be allocated to the beetle's defensive secretion with certainty and the structure of 7 compounds could be confirmed. Minor compounds were identified as six aromatic hydrocarbons, one sesquiterpene, one ketone and two alkanes. The identity of following compounds was determined by injection of authentic substances (Fig. 5A): Benzaldehyde (1), benzyl alcohol (2), benzothiazole (3), anisaldehyde (5), benzophenone (8) as well as the two alkanes tricosane and pentacosane. The main compound was unfortunately not identifiable. EI and CI-mass spectra indicate the presence of an aromatic compound

(strong signals at m/z 79, 91, and 105) with a moderately abundant molecular ion at M = 176. Patterns of fragmentation of this compound resemble EI-spectra of sesquiterpenes with strong signals at m/z 79, 91, 133, and 161. However, instead of clear signals at m/z 105 and 119, m/z 107 and 121 were found. Furthermore, the molecular mass does not correspond with that of sesquiterpenes.

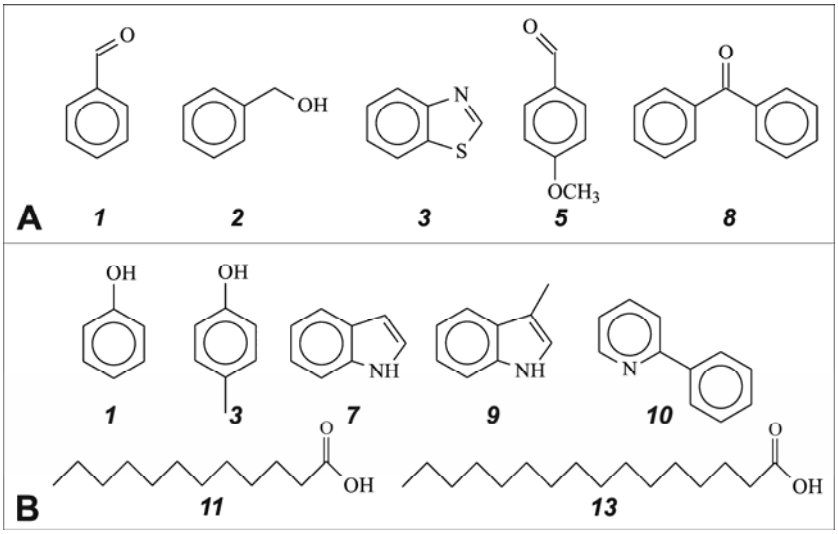

Fig. 5. Structures of compounds identified from (A) the pronotal gland secretion and (B) the hemolymph of *T. bipustulata*.

In the crude extracts of the abdominal reflex bleeding as well as the hemolymph 16 compounds were detected by GC-MS constantly. The main component in the abdominal reflex bleeding was identified as indole (7 in Fig. 5B). Furthermore, the structures of the following compounds were clarified (Fig. 5B): Phenol (1), *p*-cresole (3), 3-methylindole (= skatole; 9), 2-phenylpyridine (10), lauric acid (11), palmitic acid (13) and pentacosane. In the chemical composition of the reflex blood as well as the hemolymph no differences between the sexes were detected.

The defensive and microbiological properties of the detected compounds were evaluated by behavioural tests with *Lasius flavus* as well as agar diffusion tests with two entomopathogenic micro-organisms (*Serratia entomophila*, *Bacillus sphaericus*) and *Escherichia coli*. For the pronotal secretion significant repellent effects were obtained for benzyl alcohol and benzothiazole. The compounds benzaldedyde and benzophenone showed considerable, but not significant repellent effects on *Lasius flavus* ($p = 0.067$ and $p = 0.063$). In the abdominal reflex blood indole as well as 3-methylindole were detected; both compounds showed highly significant repellent effects on the species tested; 2-phenylpyridin was effective by trend ($P = 0.058$). In agar diffusion tests almost all tested compounds had significant antimicrobial properties. Merely for *p*-anisaldehyde, phenol and the two long chained carboxylic acids no significant results were obtained. 3-Methylindole, indole, *p*-cresol and bezothiazole had the strongest effects on the three entomopathogenic micro-organisms. It should be mentioned that

none of the tested compounds effected such large zones of inhibition like the glycylcycline antibiotic Tygacil®, which was also tested.
Functionally the discharged pronotal secretion had rather antimicrobial properties than being an effective deterrent against arthropods. The chemical properties can be correlated with the beetle's mycophagous way of life. The lucent and clean adults live cryptic within the host fungus and while feeding merely the abdominal tip is visible from the outside. From that, it seems beneficial to emit an antimicrobial secretion from the glands of the pronotum and to possess an effective chemical weapon against predators at the exposed abdominal tip. The presence of malodorous compounds in the abdominal secretion might also irritate fungus-feeding mammals. In European forests many ground squirrels and microtine rodents are extensively mycophagous (Johnson 1996), and as most fungus-feeding nonprimate mammals are colour-blind (Martin 1979), the scent may serve as the aposematic signal associated by vertebrates with poisons.
A pheromonal function seems rather unlikely, since the pronotal glands are equally developed in both sexes (manuscript II); preliminary intraspecific behavioural tests revealed no response to the secretion at all. Chûjô (1969) described a pair of stridulatory files on the vertex of the head in males of Japanese species of *Dacne*, and also other species of the family possess these sound-producing organs (Arrow 1925). The front border of the pronotum forms a sharp ridge, corresponding in position to the stridulatory files on the vertex (Ohya 1996). The mentioned ridge may scrape the pair of files to produce chirps. Since only males exhibit this apparatus, sound might play an important role in courtship behaviour in erotylid species. Later, Ohya (2001) demonstrated the significance of sound for intraspecific communication.

Manuscript III deals with the chemical defensive system of *T. bipustulata* and reports the ability of abdominal reflex bleeding for members of the family Erotylidae for the first time. The detected compounds in the discharged glandular secretion and the abdominal reflex blood had rather antimicrobial properties than being effective deterrents against other arthropods. The cleanliness of the beetles might result from these chemical properties of the secretions. Moreover, since the small species of Erotylidae live cryptic within the host fungus and while feeding merely the abdominal tip is visible from the outside it seems beneficial to release malodorous compounds at this exposed part of the body.

Host recognition in Erotylidae

Larvae and adults of the erotylid subfamily Erotylinae (the remaining subfamilies comprise the former Languriidae, which are phytophagous) are exclusively associated with various Polyporales and related higher fungi (Leschen 2003, Robertson et al. 2004); they are used as nutrition as well as breeding substrate. However, the role of volatile compounds (VOCs) involved in host recognition and selection is poorly investigated. In the present study chromatography-mass spectrometry (GC-MS), gas chromatography-electroantennographic detection (GC-EAD) and electroantennography (EAG) were chosen to identify VOCs potentially used in host selection in two European species of Erotylidae (*Tritoma bipustulata*, *Dacne bipustulata*) associated mainly with the fungus *Trametes vericolor* (Polyporaceae). Additional investigations involved the cisid species *Sulcacis affinis* associated with the same fungus and the tenebrionid species *Diaperis boleti*, mainly found on *Fomitopsis pinicola* and *Laetiporus sulphureus* (Scheerpeltz & Höfler 1948, Benick 1952; manuscript IV). *T. versicolor* is a common wood-rotting fungus on woody debris and stumps of deciduous trees, especially on beech, birch, poplar and willow (Guevara et al. 2000a). Fruiting bodies are annual and typically occur in a relatively early phase of decay succession (3-7 years; Hintikka 1993, Komonen & Kouki 2005). The fungus forms clusters which remain attached to wood for one or two years after they died. During this time the fruiting bodies may be entirely consumed by insects.

15 compounds in noticeable amounts were detected in GC-MS analysis of the scent of fresh *Trametes versicolor*. Sesquiterpenes dominated the eluting volatiles; main compounds were δ-cadinene, β-guaiene and isoledene (Fig. 6). Previously described compounds like 3-octanol or linalool were present in traces; 1-octene-3-ol was surprisingly absent (37% in *T. gibbosa,* Thakoew et al. 2008). This latter compound constitutes the typical fungal odor and was previously described from the scent of numerous fungi (Gross et al. 1989, Fäldt et al. 1999, Wu et al. 2005, Ziegenbein et al. 2006, Thakoew et al. 2008). It was found that 6 of these 15 compounds elicited reproducible antennal signals in tested fungus-feeding arthropods. Both examined erotylid-species responded to isoledene and δ-cadinene (Fig. 6); in *Tritoma bipustulata* as well as in *Dacne bipustulata* the sexes reacted equally. *Diaperis boleti* also detected both above mentioned compounds; in addition β-guaiene, γ-patchoulene (Fig. 6) and an unidentified minor sesquiterpene caused antennal responses in both sexes. The examined specimens of *Sulcacis affinis* perceived merely the minor compound γ-cadinene (Fig. 6). In two out of five analyses β-guaiene caused a weak antennal signal; however the obtained response was not consistent. Also in *S. affinis* no differences between the sexes were found.

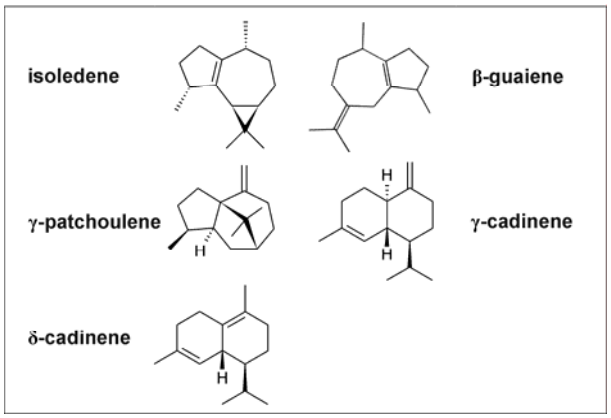

Fig. 6. Perceived fungal volatiles discovered in GC-MS/EAD experiments.

The previously described fungal alcohol 1-octene-3-ol is assumed to be a key cue for host finding in species of Ciidae (Fäldt et al. 1999, Wu et al. 2005, Ziegenbein et al. 2006, Thakoew et al. 2008). Despite the fact, that it was not detected in the scent of *T. versicolor*, a racemate was tested in behavioural tests and EAG. Attraction of the compound to the tested beetles was statistically significant. In the case of *T. bipustulata* the attracting effect of 1-octene-3-ol was stronger than the effect of the eluted scent sample of *T. versicolor* (P = 0.058). In *S. affinis* the attracting effect of 1-octene-3-ol was comparable to fresh fungal material as well as the scent sample. Dose-response curves of the racemate (dilution 10^{-5} – 10^{-1} in paraffin oil) for *S. affinis* males and females revealed significant differences with respect to the sexes and the doses (male, $F(7,39) = 32.057$, $p < 0.001$; female, $F(7,39) = 27.549$, $p < 0.001$). Among the ranges of the tested doses, the males yielding 3 stages of dose discrimination at 10^{-5} – 10^{-3}, 10^{-2} and 10^{-1}. In contrast, the females yielding only 2 stages of discrimination at 10^{-5} –10^{-3} and 10^{-2} – 10^{-1}.

Since cisids were found in much higher numbers in the fungi than erotylids, and several generations may occur in the same fruiting body before they disperse for a new one (Lawrence 1973) the strong attracting effect may result from a higher mobility of Erotylidae which disperse earlier for a new habitat then the tiny *S. affinis* and other cisids. In that case, *T. bipustulata* would be able to detect young unsettled fruiting bodies easier. Furthermore, it was reported that some species of Ciidae were predominantly found in young fruiting bodies (e.g. *Octotemnus glabriculus*) other in fully developed ones (e.g. *C. boleti*; Guevara et al. 2000b); such cases of resource partitioning are likely controlled by variations in release rates and composition of VOCs.

In the analysis of Thakoew et al. (2008) the scent of *Trametes gibbosa* was dominated by compounds like alcohols, ketones, acids, aldehydes and aromatics; only a single sesquiterpene alcohol (α-bisabolol) was present in the scent sample. Most abundant compounds in these analyses were alcohols comprising 49% of the scent. In contrast to the mentioned analysis (Thakoew et al. 2008), the scent of the examined *T. versicolor* was exclusively composed of sesquiterpenes in noticeable amounts. This is in accord with the results of Holighaus & Schütz (2006), who found that after degradation of lignin and cell

structures only sesquiterpenes were present in scent bouquet. Moreover, analyses of different colonization stages of *T. gibbosa* revealed, that the release rate of 1-octene-3-ol was much higher in minimally colonized fruiting bodies (up to 20 times higher) than in highly colonized ones (Thakoew et al. 2008). This finding is most probably due to the fact that in highly colonized fruiting bodies the tissues releasing these volatiles are already consumed or damaged; further studies on release rates of 1-octene-3-ol report comparable variation depending on fungal age and on the season (Fäldt et al. 1999, Wu et al. 2005). The absence of 1-octene-3-ol in the present analysis might, therefore, be due to the fact that the examined fungus was senescent and highly colonized by species of Erotylidae and Ciidae.
For the fungivore Ciidae it has been hypothesized that 2 main fractions of fungal volatiles may play an important role in host selection: C_8-compounds and terpenoids (Fäldt et al. 1999, Guevara et al. 2000a). Thakoew et al. (2008) demonstrated 1-octene-3-ol to be a key cue for host finding in *Cis boleti*, and the present EAG experiments as well as the behavioural tests showed also statistically significant attraction to the racemate of authentic 1-octene-3-ol, fresh *T. versicolor* and to the eluted odor sample in all species tested.
Thakoew et al. (2008) demonstrated that fungi produce individually variable chemical profiles depending from the age (Thakoew et al. 2006). The present data indicate that the examined species of Erotylidae, Ciidae and Tenebrionidae are able to perceive C_8-compounds (present in rather young fruiting bodies) as well as sesquiterpenes (present in senescent fruiting bodies) present in the scent bouquet of their host fungus. The role of sesquiterpenes is rather speculative, however, specific profiles of volatiles or individual compounds which appear at a later developmental stage might enable the beetles to discriminate between quality and age of the nutrition and breeding substrate and, furthermore, the developmental stage and the degree of colonization.

The role of volatile organic compounds in host selection and recognition in different mycophagous beetles is examined and reported in manuscript IV. The scent bouquet of *T. versicolor* was dominated by different sesquiterpenes. The examined species of Erotylidae, Ciidae and Tenebrionidae were able to perceive some of these compounds and in addition several previously described fungal C_8-compounds like alcohols and aromatics. The ability to perceive these different compounds, emitted at different fungal developmental stages, might enable the beetles to discriminate between fungals quality, age and degree of colonization.

While rearing *T. bipustulata* in the laboratory dead larvae of this species with a ventrally adherent pupa were found. After hatching (about 5-6 days after pupation), these insects were determined as *Brachyserphus parvulus* (Proctotrupidae; Pschorn-Walcher 1971; Fig. 7B).

The Proctotrupidae represents a small group of parasitic wasps which is assigned to the superfamily Proctotrupoidea. From 320 described species (Dathe 2003), merely 35 are known from Europe. The tiny species of this family (3 – 5 mm) are characterised by a strongly reduced and faded wing venation. Conspiciouos is the way of pupation in this family. The larva of the wasp leaves the host partly through the posterior ventral intersegmental membranes. They pupate while being inserted in the dead host larva (Fig. 7A; Pschorn-Walcher 1971, Early & Dugdale 1994).

The genus *Brachyserphus* comprises 10 mostly holarctic distributed species (Townes & Townes 1981, Williams et al. 1992). The distribution area of this species reaches from Central Europe, Scandinavia and Great Britain to the north of Japan (Pschorn-Walcher 1971). Hitherto known hosts of *B. parvulus* are *Meligethes aeneus* and *M. viridescens* (Nitidulidae) from France and Great Britain (Osborne 1960, Pschorn-Walcher 1971), as well as larvae of Serropalpidae and Phalacridae (Nixon 1938). *B. parvulus* parasitises also larvae of the erotylid-genus *Triplax* in Lower Austria (Pschorn-Walcher 1971). This is the first report of *T. bipustulata* as new host species for *B. pravulus*.

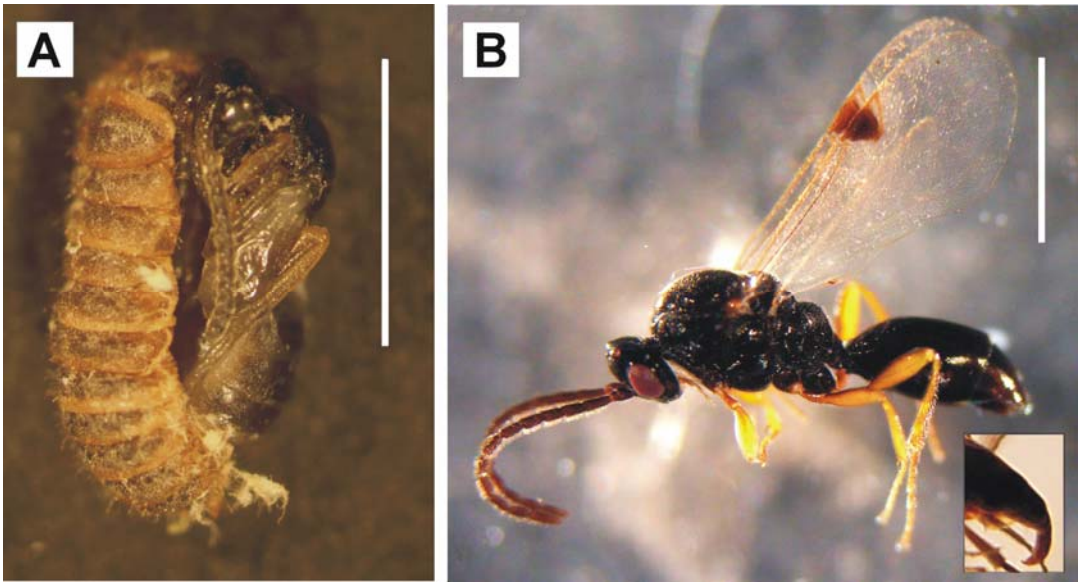

Fig. 7. *Brachyserphus parvulus*. (A) Pupa of *B. parvulus* adherent at a dead larva of *T. bipustulata*. The pupa is interconnected to the posterior ventral side. Scale: 2 mm. (B) Hatched male of *B. parvulus*. Insertion: skean-like ovipositor of the females. Scale: 1mm.

Manuscript V reports *Tritoma bipustulata* as a new host species for the parasitic wasp *Brachyserphus parvulus* (Procrotupidae). This species is hitherto known from larvae of several coleopteran families as soon as from larvae of the erotylid species *Triplax russica*.

Literature

Arrow, G.J., 1925. Coleoptera. Clavicornia. Erotylidae, Languriidae, and Endomychidae. – In: Shipley, A.E., Scott, A. (eds.). The fauna of British India, including Ceylon and Burma. Taylor and Francis, London: XVI + 416 pp.

Barbier, R., Ferran, A., Le Lannic, J., Allo, M.-R., 1992. Morphology and ultrastructure of integumentary glands of *Semiadalia undecimnotata* Schn. (Coleoptera: Coccinellidae). International Journal of Insect Morphology and Embryology 21(3): 223-234.

Bartelt, R.J., 1999. Sap beetles. – In: Hardie, J., Minks, A.K. (eds.). Pheromones of non-lepidopteran insects associated with agricultural plants. CABI Publishing, Oxford, UK: 69-89.

Benick, L., 1952. Pilzkäfer und Käferpilze: Ökologische und statistische Untersuchungen. Acta Zoologica Fennica 70: 1-250.

Berrioz-Ortiz, A., 1985. The presence of mesothoracic glands in *Epicauta segmenta* (Coleoptera: Meloidae) and other blister beetles. Journal of the Canadian Entomological Society 58: 179-181.

Beutel, R.G., 2005. 26. Ordnung: Coleoptera, Käfer; excl. part: Polyphaga. – In: Dathe, H.H. (ed.). Lehrbuch der Speziellen Zoologie, Band I, 5. Teil: Insecta. 2nd edn. Spektrum Akademischer Verlag; Heidelberg, Berlin: 426-452.

Beutel, R.G., Haas, F., 2000. Phylogenetic relationships of the suborders of Coleoptera (Insecta). Cladistics 16: 103-141.

Boland, W., Ney, P., Jaenicke, L., Gassmann, G., 1984. A 'closed-loop-stripping' technique as a versatile tool for metabolic studies of volatiles. – In: Schreier, P. (ed.). Analysis of Volatiles. Walter de Gruyter, Berlin, pp. 371-373.

Buder, G., Grossmann, C., Hundsdoerfer, A., Klass, K.-D., 2008. A contribution to the phylogeny of the Ciidae and its relationships with other cucujoid and tenebrionoid beetles (Coleoptera: Cucujiformia). Arthropod Systematics and Phylogeny 66: 165-190.

Brown Jr., W.L., Eisner, T., Witthaker, R.H., 1970. Allomones and Kairomones: Transspecific chemical messengers. Bioscience 20(1): 21-22.

Chûjô, M., 1969. Erotylidae (Insecta: Coleoptera) Fauna Japonica. Academic Press of Japan, Tokyo.

Crowson, R.A., 1955. The Natural Classification of the Families of Coleoptera. London, Nathaniel Lloyd and Co., 187 pp.

Daloze, D., Braekman, J.-C., Pasteels, J.M., 1994. Ladybirds defence alkaloids: structural, chemotaxonomic and biosynthetic aspects (Col.: Coccinellidae). Chemoecology 5(3/4): 173-183.

Dathe, H.H., 2003. 31. Ordnung Hymenoptera, Hautflügler. – In: Dathe, H.H. & Gruner, H.-E. (eds.). Lehrbuch der speziellen Zoologie, Band I: Wirbellose Tiere, 5. Teil: Insecta. Spektrum Akademischer Verlag, Heidelberg, Berlin: 585-651.

Delachambre, J., 1973. L´ultrastructure des glandes dermiques de *Tenebrio molitor* L. (Insecta, Coleoptera). Tissue and Cell 5: 243-257.

Dettner, K., 1987. Chemosystematics and evolution of beetle chemical defenses. Annual Review of Entomology 32: 17-48.

Early, J.W., Dugdale, J.S., 1994. *Fustiserphus* (Hymenoptera: Proctotrupidae) parasitises Lepidoptera in leaf litter in New Zealand. New Zealand Journal of Zoology 21: 249-252.

Eisner, T., Meinwald, J., 1966. Defensive secretions of arthropods. Science 153: 1341-1350.

Fäldt, J., Jonsell, M., Nordlander, G., Borg-Karlson, A.-K., 1999. Volatiles of bracket fungi *Fomitopsis pinicola* and *Fomes fomentarius* and their functions as insect attractants. Journal of Chemical Ecology 25(3): 567-590.

Farine, J.P., 1988. The exocrine glands of *Dysdercus cingulatus* (Heteroptera, Pyrrhocoridae): Morphology and function of adults´ gland. Annals of the Society of Entomology of France 24: 241-256.

Francke, W., Dettner, K., 2005. Chemical signalling in beetles. Topics in Current Chemistry 240: 85-166.

Gross, B., Gallois, A., Spinnler, H.-E., Langlois, D., 1989. Volatile compounds produced by the ligninolytic fungus *Phlebia radiata* Fr. (Basidiomycetes) and influence of the strain specificity on the odorous profile. Journal of Biotechnology 10: 303-308.

Guevara, R., Rayner, A.D.M., Reynolds, S.E., 2000a. Effects of fungivory by two specialist ciid beetles (*Octotemnus glabriculus* and *Cis boleti*) on the reproductive fitness of their host fungus, *Coriolus versicolor*. New Phytologist 145(1): 137-144.

Guevara, R., Hutcheson, K.A., Mee, A.C., Rayner, A.D.M., Reynolds, S.E., 2000b. Resource partitioning of the host fungus *Coriolus versicolor* by two ciid beetles: the role of odour compounds and host ageing. Oikos 91: 184-194.

Hawkeswood, T.J., Turner, J.R., Wells, R.W., 1997. A new fungal host for *Episcaphula australis* (Boisduval), *E. pictipennis* Crotch and *Thallis compta* Errichson, with a review of the fungal host records for the Australian Erotylidae (Coleoptera). Mauritiana 16: 307-312.

Hemp, C., Dettner, K., 1997. Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera: Anthicidae). Entomologia Generalis 22(2): 97-108.

Hintikka, V., 1993. Occurrence of edible fungi and other macromycetes on tree stumps over a sixteen-year period. Acta Botanica Fennica 149: 11-17.

Hodgman, C.D., Weast, R.C., Shankland, R.S., Selby, S.M., 1959. Handbook of chemistry and physics. Chemical Rubber Publishing Co., Cleveland.

Holighaus, G., Schütz, S., 2006. Odors of wood decay as semiochemicals for *Trypodendron domesticum* L. (Col., Scolytidae). Mitteilungen der deutschen Gesellschaft für allgemeine und angewandte Entomologie 15: 161-165.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., St John, O., Wild, R., Hammond, P.M., Ahrens, D., Balke, M., Caterino, M.S., Gómez-Zurita, J., Ribera, I., Barraclough, T.G., Bocakova, M., Bocak, L., Vogler, A.P., 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science 318: 1913-1916.

Johnson, C.N., 1996. Interactions between mammals and ectomycorrhizal fungi. Tree 11(12): 503-507.

Jonsell, M., Nordlander, G., 1995. Field attraction of Coleoptera to odors of the wood-decaying polypores *Formitopsis pinicola* and *Fomes fomentarius*. Annales Zoologici Fennici 32: 391-402.

Jonsell, M., Nordlander, G., 2004. Host selection patterns in insects breeding in bracket fungi. Ecological Entomology 29: 697-705.

Klausnitzer, B., 2005. 26. Ordnung: Coleoptera, Käfer; part: Polyphaga. – In: Dathe, H.H. (ed.). Lehrbuch der Speziellen Zoologie, Band I, 5. Teil: Insecta. 2nd edn. Spektrum Akademischer Verlag; Heidelberg, Berlin: 452-526.

Köhler, F., Klausnitzer, B. (eds.), 1998. Verzeichnis der Käfer Deutschlands. Entomologische Nachrichten und Berichte (Dresden) Beiheft 4: 1-185.

Komonen, A., Kouki, J., 2005. Occurence and abundance of fungus-dwelling beetles (Ciidae) in boreal forests and clearcuts: habitat associations at two spatial scales. Animal Biodiversity and Conservation 28(2): 137-147.

Laurent, P., Braekman, J.-C., Daloze, D., 2005. Insect chemical defense. Topics in Current Chemistry 240: 167-229.

Lawrence, J.F., 1973. Host preference in ciid beetles (Coleoptera: Ciidae) inhabiting the fruiting bodies of basidiomycetes in North America. Bulletin of the Museum of comparative Zoology 145(3): 163-212.

Lawrence, J.F., 1989. Mycophagy in the Coleoptera: Feeding strategies and morphological adaptations. – In: Wilding, N., Collins, N.M., Hammond, P.M., Webber, J.F. (eds.). Insect–Fungus Interactions. Academic Press, London: 1-23.

Lawrence, J.F., Newton, A.F., 1982. Evolution and classification of beetles. Annual Review of Ecology and Systematics 13: 261-290.

Lawrence, P.A., Staddon, B.W., 1975. Peculiarities of the epidermal gland system of the cotton stainer *Dysdercus fasciatus* Signoret (Heteroptera: Pyrrhocoridae). Journal of Entomology (A) 49: 121-130.

Leschen, R.A.B., 1996. Phylogeny and revision of the genera of Cryptophagidae (Coleoptera: Cucujoidea). Kansas Science Bulletin 55: 549-634.

Leschen, R.A.B., 2003. Erotylidae (Insecta: Coleoptera: Cucujoidea): phylogeny and review. Fauna of New Zealand 47: 108 pp.

Leschen R. A. B., Skelley P. A.& McHugh J. V., 2010. 10.7. Erotylidae Leach, 1815. – In R.A.B. Leschen, R.G. Beutel & J. Lawrence (eds.): Coleoptera, Beetles, vol. 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). Handbuch der Zoologie/Handbook of Zoology IV/39. Walter de Gruyter, Berlin, New York: 311–319.

Levinson, H.Z., Levinson, A.R., 1987. Pheromone biology of the tabacco beetle (*Lasioderma serricorne* F., Anobiidae) with notes on the pheromone antagonism between 4S, 6S, 7S- and 4S, 6S, 7R-serricorin. Journal of applied Entomology 103: 217-240.

Martin, M.M., 1979. Biochemical implications of insect mycophagy. Biological Review, 54: 1-21.

McHugh, J.V., Marshall, C.J., Fawcett, F.L., 1997. A study of adult morphology in *Megalodacne heros* (Say) (Coleoptera: Erotylidae). Transactions of the American Entomological Society 123(4), 167-223.

Morgan, A., 1968. The occurrence of a pair of exocrine glands in the metathorax of *Lytta nutalli* (Coleoptera: Meloidae). Canadian Journal of Zoology 46: 915-917.

Nixon, G.E.J., 1938. A preliminary revision of the British Proctotrupinae (Hym., Proctotrupoidea). Transactions of the royal entomological Society London 87: 431-465.

Noirot, C., Quennedey, A., 1974. Fine structure of insect epidermal glands. Annual Review of Entomology 19: 61-80.

Noirot, C., Quennedey, A., 1991. Glands, gland cells, glandular units: Some comments on terminology and classification. Annales de la Société Entomologique de France 27(2): 123-128.

Oehlschlager, A.C., King, G.G.S., Pierce Jr., H.D., Pierce, A.M., Slessor, K.M., Millar, J.G., Borden, J.H., 1987. Chirality of macrolide pheromones of grain beetles in the genera *Oryzaephilus* and *Cryptolestes* and its implications for species specificity. Journal of Chemical Ecology 13(6): 1543-1554.

Oehlschlager, A.C., Pierce, A.M., Pierce Jr., H.D., Borden, J.H., 1988. Chemical communication in cucujid grain beetles. Journal of Chemical Ecology 14(11): 2071-2098.

Ohya, E., 1996. External structure of the putative stridulatory apparatus of the fungus beetles, *Dacne japonica* and *D. picta* (Coleoptera: Erotylidae). Applied Entomology and Zoology 31(2): 312-325.

Ohya, E., 2001. Aggressive and courtship chirps of sympatric pleasing fungus beetles, *Dacne japonica* and *D. picta* (Coleoptera: Erotylidae). Entomological Science 4(3): 287-290.

Osborne, P., 1960. Observations on the natural enemies of *Meligethes aeneus* (F.) and *M. viridescens* (F.) (Coleoptera: Nitidulidae). Parasitology 50: 91-110.

Pacioni, G., Bologna, M.A., Laurenzi, M., 1991. Insect attraction by *Tuber*: a chemical explanation. Mycological Research 95(12): 1359-1363.

Pasteels, J.M., Braekman, J.-C., Daloze, D., 1988. Chemical defense in the Chrysomelidae. – In: Jolivet, P., Petitpierre, E., Hsiao, T.H. (eds.). Biology of Chrysomelidae. Kluwer Academic Publishers, Dordrecht, Netherlands: 233-255.

Pasteels, J.M., Rowell-Rahier, M., Braekman, J.C., Daloze, D., Duffey, S., 1989. Evolution of exocrine chemical defense in leaf beetles (Coleoptera: Chrysomelidae). Experientia 45: 295-300.

Pasteels, J.M., Rowell-Rahier, M., Braekman, J.-C., Daloze D., 1994. Chemical defence of adult leaf beetles updated. – In: Jolivet, P.H., Cox, M.L., Petipierre, E. (eds.). Novel aspects of the biology of Chrysomelidae. Kluwer Academic Publishers, Dordrecht, Netherlands: 289-301.

Pschorn-Walcher, H., 1971. Hymenoptera: Heloridae et Proctotrupidae. Schweizerische Entomologische Gesellschaft.

Quennedey, A., 1978. Les glandes exocrines des termites. Ultrastructure comparée des glandes sternales et frontales. Dissertation, Université de Dijon: 254 pp.

Quennedey, A., 1998. Insect epidermal gland cells: Ultrastructure and Morphogenesis. – In: F.W. Harrison & M. Locke (eds.). Microscopic Anatomy of Invertebrates Vol. 11A. Wiley-Liss Inc.: 177-207.

Quennedey, A., Drugmand, D., Deligne, J., 2002. Morphology and ultrastructure of paired prototergal glands in the adult rove beetle *Philonthus varians* (Coleoptera: Staphylinidae). Arthropod Structure and Development 31: 173-183.

Robertson, J.A., McHugh, J.V., Whiting, M.F., 2004. A molecular phylogenetic analysis of the pleasing fungus beetles (Coleoptera: Erotylidae): evolution of colour patterns, gregariousness and mycophagy. Systematic Entomology 29: 173-187.

Scheerpeltz, O., Höfler, K., 1948. Käfer und Pilze. Verlag für Jugend und Volk, Wien.

Sen Gupta, T., Crowson, R.A., 1969. On a new family of Clavicornia (Coleoptera) and a new genus of Languriidae. Proceedings of the Royal Entomological Society London (B) 38: 125-131.

Sen Gupta, T., Crowson, R.A., 1971. A review of classification of the family Languriidae (Coleoptera: Clavicornia) and the place of Languriidae in the natural system of Clavicornia. Memoirs of the Zoological Survey of India 15: 1-42.

Skelley, P.E., Goodrich, M.A., Leschen, R.A.B., 1991. Fungal host records of the Erotylidae (Coleoptera: Cucujoidea) of America north of Mexico. Entomological News 102: 57-72.

Steidle, J.L.M., Dettner, K., 1993. Chemistry and morphology of the tergal gland of freeliving adult Aleocharinae (Coleoptera: Staphylinidae) and its phylogenetic significance. Systematic Entomology 18: 149-168.

Thakoew, P., Weißbecker, B., Schütz, S., 2006. Volatile organic compounds emitted from fungal-rotting beech (*Fagus sylvatica*). Mitteilungen der deutschen Gesellschaft für allgemeine und angewandte Entomologie 15: 157-160.

Thakoew, P., Angeli, S., Weißbecker, B., Schütz, S., 2008. Antennal and behavioral responses of *Cis boleti* to fungal odor of *Trametes gibbosa*. Chemical Senses 33: 379-387.

Townes, H., Townes, M., 1981. A revision of the Serphidae (Hymenoptera). Memoirs of the American Entomological Institute 32, Ann. Arbor, Mich..

Tschinkel, W.R., 1975. A comparative study of the chemical defensive system of tenebrionid beetles. 3. Morphology of the glands. Journal of Morphology 145: 355-370.

Tschinkel, W.R., Doyen, J.T., 1980. Comparative anatomy of the defensive glands, ovipositors and female genital tubes of tenebrionid beetles (Coleoptera). International Journal of Morphology and Embryology 9: 321-368.

Vogt, H., 1967. 54. Familie: Erotylidae. – In: Freude, H., Harde, K.W., Lohse, G.A. (Ed.). Die Käfer Mitteleuropas, Band 7. Goecke & Evers Verlag, Krefeld: 104-109.

Węgrzynowicz, P., 2002. Morphology, phylogeny and classification of the family Erotylidae based on adult characters (Coleoptera: Cucujoidea). Genus 13(4): 435-504.

Wheeler, W.C., Whiting, M., Wheeler, Q.D., Carpenter, J.M., 2001. The phylogeny of the extant hexapod orders. Cladistics 17: 113-169.

Williams, R.N., Fickle, D.S., Galford, J.R., 1992. Biological studies of *Brachyserphus abruptus* (Hym.: Proctotrupidae), a nitidulid parasite. Entomophaga 37(1): 91-98.

Wu, S., Zorn, H., Krings, U., Berger, R.G., 2005. Characteristic volatiles from young and aged fruiting bodies of wild *Polyporus sulfureus* (Bull.: Fr.) Fr.. Journal of Agricultural and Food Chemistry 53: 4524-4528.

Ziegenbein, F.C., Hanssen, H.-P., König, W.A., 2006. Chemical constituents of the essential oils of three wood-rotting fungi. Flavour and Fragrance Journal 21: 813-816.

Record of contributions to this thesis

Manuscript I:

The distribution and evolution of exocrine glands in Erotylidae (Insecta: Coleoptera)

Kai Drilling, Konrad Dettner & Klaus-Dieter Klass

European Journal of Entomology – will be submitted soon.

The examination of the glandular distributions, the preparing of the drawings and plates and also the writing of the manuscript were conducted by me.

Percental portion: 90%

Manuscript II:

Morphology of the pronotal compound glands in *Tritoma bipustulata* (Coleoptera: Erotylidae)

Kai Drilling, Konrad Dettner & Klaus-Dieter Klass

Organism, Diversity and Evolution – in press.

The required species for this examination were collected and determined by myself. Also the preparations for the SEM- and TEM-studies, the preparing of the drawings and plates as well as the writing of the manuscript was done by me.

Percental portion: 90%

Manuscript III:

First insights in the chemical defensive system of the erotylid beetle, *Tritoma bipustulata*

Kai Drilling & Konrad Dettner

Chemoecology – submitted.

The analyses (several methods for extracting the glandular secretions were tested) and evaluation of the secretions respectively the resulting data as well as the performance and statistical evaluation of the behavioural and microbiological tests were conducted by me. Furthermore, I wrote the manuscript.

Percental portion: 95%

Manuscript IV:

Electrophysiological responses of four fungivorous coleoptera to volatiles of *Trametes versicolor*: implications for host selection

Kai Drilling & Konrad Dettner

Chemoecology 19: 109-115 (2009).

All species required for this study were collected and determined by myself. Furthermore, I conducted the electroantennographic and massspectrometric analyses as well as the behavioural tests and their statistical evaluation. The manuscript was written by me.

Percental portion: 95%

Manuscript V:

***Tritoma bipustulata* FABRICIUS, 1775 (Coleoptera: Erotylidae): eine neue Wirtsart für *Brachyserphus parvulus* (NEES, 1834) (Hymenoptera: Proctotrupidae)**

Kai Drilling

Entomologische Nachrichten und Berichte 53(2): 126-127 (2009).

The collection and determination of the species as well as the literature research and the preparation of the manuscript were conducted by me.

Percental portion: 100%

Manuscript I
European Journal of Entomology – will be submitted soon.

The distribution and evolution of exocrine compound glands in Erotylidae (Insecta: Coleoptera)

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Abstract. Members of the family Erotylidae and especially of the erotylid subfamily Erotylinae possess a whole arsenal of compound integumentary glands. Their external pores are located in several parts of the body, mainly in the corners and along the lateral margins of the pronotum, beside the compound eyes (periocular pores), on the subgenal braces (subocular pores), on the abdominal ventrites, and more rarely on the prosternal and mesoventral intercoxal processes, the mentum and the mandibels. The occurrence of the various glands is described for 38 species of Erotylidae (including the former Languriidae), and data from the literature are included for 9 further species and 2 subfamilies. In some phylogenetically crucial cases, the glandular nature was verified by internal inspection (search for glandular ducts), and in some critical species or genera an extended sample was studied. Gland characters and their phylogenetic implications are discussed and mapped on a previously published erotylid phylogeny.

Key words. Languriidae; dermal glands; phylogeny; SEM.

1. INTRODUCTION

The cosmopolitan Erotylidae comprises about 3500 described species in ca. 258 genera (Leschen et al., 2010), which are classified into six subfamilies: the Cryptophilinae, Pharaxonothinae, Languriinae, Xenoscelinae, and Loberinae were previously comprised as a separate family, "Languriidae"; and the Erotylinae were ranked as a family until some years ago. Recent morphology-based phylogenetic studies indicate that the former "Languriidae" is paraphyletic with respect to Erotylinae (Węgrzynowicz, 2002; Leschen, 2003; Leschen & Buckley, 2007), with Cryptophilinae being the sister group of Erotylinae. On the other hand, the so far only extensive molecular-based phylogenetic study of Erotylidae suggests the two languriid taxa sampled therein, Languriinae and Cryptophilinae, to be nested in Erotylinae (Robertson et al., 2004, see Fig. 1 herein). The Erotylinae are further classified into 5 tribes: Dacnini, Tritomini, Erotylini, Encaustini, and Megalodacnini (Węgrzynowicz, 2002; Leschen, 2003; Leschen et al., 2010).

Erotylidae is certainly a subgroup of the cucujiform beetles, which are supported as monophyletic by a number of autapomorphies (e.g., Klausnitzer, 2005; Lawrence & Newton, 1982; Leschen & Ślipiński, 2010) and also by the extensive molecular study of Hunt et al. (2007). Within the Cucujiformia the Erotylidae are generally assigned to the Cucujoidea. However, basal phylogenetic relationships in Cucujiformia are widely unresolved, the Cucujoidea are unlikely to be a monophyletic group (see Buder et al., 2008; Hunt et al., 2007), and the phylogenetic relationships of Erotylidae to other cucujiform taxa are unclear. Various families were regarded as the closest relatives of Erotylidae in the past: members of the cerylonid series of Cucujoidea (Alexiidae, Endomychidae; Crowson, 1955; Sen Gupta & Crowson, 1971), certain "lower Cucujoidea" (Cryptophagidae, Propalticidae; Sen Gupta & Crowson, 1969, 1971; McHugh et al., 1997; Leschen, 1996, 2003) as well as Phloeostichidae, Lamingtoniidae (Leschen, 2003), and Biphyllidae (Sen Gupta & Crowson, 1971; Leschen, 2003). The molecular studies by Hunt et al. (2007) suggest either Monotomidae, Helotidae, and Protocucujidae as the closest relatives of Erotylidae (supporting fig. S1 therein: Bayesian analysis), or a clade comprising Laemophloeidae, Phalacridae, Propalticidae, and Cucujidae (supporting fig. S4 therein: Parsimony analysis).

It is well known that the Erotylidae show a particularly rich equipment of compound exocrine glands. Gland pores can occur in the corners and along the lateral margins of the pronotum, on the prosternal and mesoventral intercoxal processes, on the head anteromesal to the compound eyes, on the subgenal brace, on the abdominal ventrites, and rarely on the mentum and the manibels. However, despite this manifold occurrence of such glands over the beetles´ body, their consideration in the previous literature is quite sparse. The distribution of gland pores was included in the phylogenetic studies of Węgrzynowicz (2002; mostly on the erotyline tribes) and Leschen (2003; mostly on the former languriid subfamilies). A detailed

morphological and histological study of the pronotal glands in one erotylid species, *Tritoma bipustulata*, was recently published by Drilling et al. (in press).

Since not all Erotylinae have glands in all the locations mentioned above, and since identically positioned glands sporadically also occur within the languriid taxa, a comparative analysis of gland occurrence across erotylid genera could further illuminate phylogenetic relationships in Erotylidae as well as evolution of various glands in the family. The main scope of this contribution is the further exploration of the distribution of gland pores over the body of Erotylidae for phylogenetically informative characters. As compared to previous studies, we specifically included additional languriid taxa and additional members of the erotyline; mainly the "basal" erotyline tribe Dacnini. Especially in these taxa our results show some striking differences to those in the major foregoing studies of Węgrzynowicz (2002) and Leschen (2003).

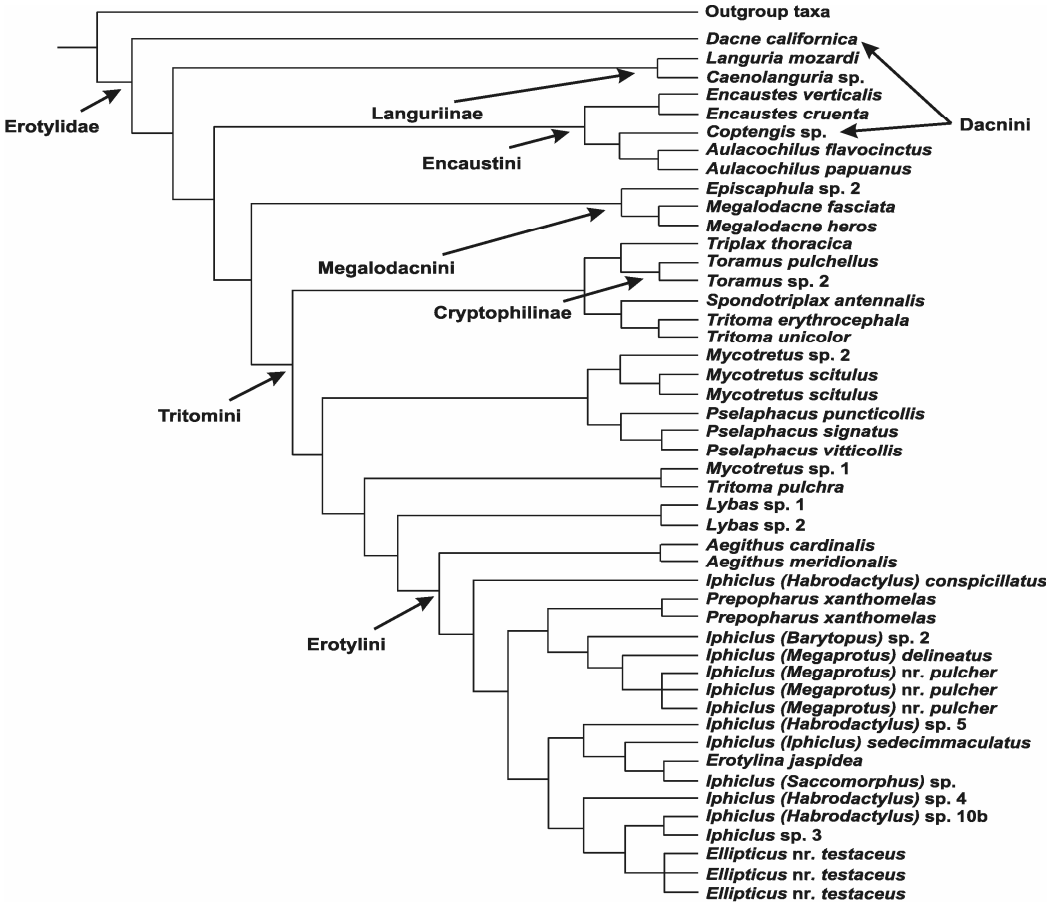

Fig. 1. Erotylid phylogeny by Robertson et al. (2004), based on 18S and 28S rDNA sequences. The two included "languriid" subgroups are subordinate in two different positions within Erotylinae: *Languria* + *Caenolanguria* (Languriinae) and *Toramus* (Cryptophilinae); the erotyline *Dacne* is sister to a clade comprising these "languriids" and the other Erotylinae.

2. MATERIAL AND METHODS

2.1. Examined taxa

The generic and suprageneric classification of Erotylidae follows Leschen (2003) and Leschen et al. (2010). Of the "languriid" subfamilies, the Pharaxonothinae, the Cryptophilinae, and Languriinae are represented in this study, but not the Xenoscelinae and Loberinae. Of the Erotylinae tribes, all five – Dacnini, Tritomini, Erotylini, Encaustini, and Megalodacnini – are represented.

For the Erotylinae, species level systematics follows the catalogs of Chûjô and Chûjô (1988, 1989, 1990: Palaearctic, Oriental, and Australian faunas, respectively) and Alvarenga (1994: Neotropical fauna), and the surveys of Delkeskamp (1981: Ethiopian fauna) and Boyle (1956: Nearctic fauna). For the "languriids" only a genus-level catalog (Leschen & Węgrzynowicz, 1998) and an issue about the Nearctic species (Lawrence & Vaurie, 1983) are available.

The 38 Erotylidae species here studied are listed in Table 1; 29 of these were also studied by Węgrzynowicz (2002) (marked with "w" in Table 1, column 3). 9 additional species only studied by Węgrzynowicz (2002), differing results for 4 species as well as results for 2 subfamilies reported by Leschen (2003) are also included in Table 1 (marked with "W" or "L" in column 4) and in our discussions. An expanded sample of one particular species or of several congeneric species was examined in the following cases:

(1) In cases where our results differed from those in Węgrzynowicz (2002; Table 1), we examined several specimens from different localities to test for intraspecific variability; this concerns six species: *Toramus pilifer*, 3x Taraz (Kazakhstan), 2x Margilan (Uzbekistan); *Dacne bipustulata*, 2x Saxon Switzerland (Germany), 2x Trenčin (Slovakia), 2x from near Berlin (Germany); *Triplax russica*, 2x Magdeburg (Germany), 2x Saxon Switzerland (Germany), 2x Kadaň (Czech Republic); *Tritoma bipustulata*, 4x Saxon Switzerland (Germany), 6x Bayreuth (Germany), 5x Trenčin (Slovakia); *Pselaphacus nigropunctatus* (species identification confirmed by P. Węgrzynowicz), 2x Peru, 3x São Paulo de Olivença (Brazil), 2x Bogota (Colombia); *Megalodacne fasciata*, 2x Texas (USA), 2x Chicago (USA), 1x New Orleans (USA).

(2) In the single case where Węgrzynowicz´s (2002) and our results differ for species from the same genus we studied the gland pores concerned in an expanded sample of several congeneric species; this applies to *Megischyrus brasiliensis* versus *M. undatus* in Table 1: we studied *M. brasiliensis*, 2x Brasilia (Brazil), 2x Espírito Santo (Brazil); *M. semipunctatus*, 1x Brasilia (Brazil), 2x Santa Catharina (Brazil); *M. decempunctatus*, 3x Peru; *M. discipennis*, 1x Santa Catharina (Brazil), 1x Chiriqui (Panama) (*M. undatus* was not available to us).

(3) The same as in (2) was done in some cases crucial for phylogenetic conclusions; this applies to **(a)** *Ischyrus quadripunctatus*, 2x Manaos (Brazil); *I. scriptus*, 2x Rio de Janeiro (Brazil), 1x Joinville (Brazil); *I. femoralis*, 2x Rio de Janeiro (Brazil), 1x Peru; *I. flavitarsis*,

3x Cuba; **(b)** *Pselaphacus nigropunctatus*, 4x São Paulo de Olivença (Brazil), 2x Peru, 1x Bogota (Colombia); *P. giganteus*, 3x Cayenne (French Guiana); *P. rubricatus*, 2x Peru, 1x Amazonas (Brazil); *P. puncticollis*, 2x Rio Grande do Sul (Brazilia), 1x Paraguay.
Members of all languriid subfamilies were chosen as outgroup taxa and data were taken from literature (Leschen, 2003).

Table 1. List of taxa studied (column 2) with systematic assignment (column 1), occurrence of various glands (as a character matrix; see chapter 3 for character definitions) and number of specimens examined (column 4; "W" = data exclusively from Węgrzynowicz 2002, "L" = data exclusively from Leschen 2003). Taxa also studied by Węgrzynowicz (2002) marked with "w" in column 3. Entry of more than one number indicates differences between our results (first entry) and those of Węgrzynowicz (2002) (second entry) or Leschen (2003) (second entry, bold). ~ = character is polymorphic; * = the glandular nature of particular pores has been checked by internal examination, i.e. observation of a glandular duct. Genus and species names according to Chûjô and Chûjô (1988, 1989, 1990), Delkeskamp (1981) and Alvarenga (1994), higher-level systematic assignment according to Leschen and Węgrzynowicz (1998) and Leschen (2003).

Systematic Assignment	Species	Lit.	No.	C1	C2	C3	C4	C5	C6	C7
Biphyllidae	*Biphyllus lunulatus* Fabricius, 1787	w	3	0	0	0	0	0	0	0
Cryptophagidae	*Cryptophagus lycoperdi* Scopoli, 1763		3	0	0	0	0	0	0	0
Erotylidae, Xenoscelinae			L	**0**	**1**	**?**	**0**	**0**	**0**	**?**
Erotylidae, Pharaxonothinae	*Setariola sericea* Mulsant, 1863	w	3/L	0	0/1	0	0/1	0/1	0/1	0/?
Erotylidae, Loberinae			L	**0**	**1**	**?**	**1**	**~**	**1**	**?**
Erotylidae, Cryptophilinae	*Cryptophilus integer* Heer, 1841	w	W/L	0	0/1	0	0/1	0	0/1	0
Erotylidae, Cryptophilinae	*Toramus pilifer* Reitter, 1885	w	5/L	1	1/0	0	0/~	0	0	0
Erotylidae, Languriinae	*Languria bicolor* Fabricius, 1798	w	2/L	1*/**0**	1	1	0	0	0/1	0/?
Erotylidae, Languriinae	*Languria pucticollis* Say, 1823		2	1*	1	0	0	0	0	0
Erotylidae, Languriinae	*Pachylanguria elongata* Fabricius, 1801		2	1	1	0	0	0	0	0
Erotylidae, Languriinae	*Tetraphala (Tetralanguria) elongata* Fabricius, 1801		3	0	0	0	0	0	0	0
Erotylidae, Languriinae	*Camptocarpus longicollis* Motschulsky, 1860		2	0	0	0	0	0	0	0
Erotylidae, Languriinae	*Langurites lineata* Laporte, 1832		2	0	0	0	0	0	0	0
Erotylidae, Languriinae	*Pentelanguria elateroides* Crotch, ????		4	0	1	0	0	0	0	0
Erotylidae, Erotylinae, Dacnini	*Dacne bipustulata* Thunberg, 1781	w	6	0	1/0	0	0	0	0	0
Erotylidae, Erotylinae, Dacnini	*Combocerus glaber* Schaller, 1783	w	2	1	1	0	1	0	0	0
Erotylidae, Erotylinae, Dacnini	*Euzostria aruensis* Gorham, 1888		2	1	1	0	1	0	0	0
Erotylidae, Erotylinae, Dacnini	*Nesitis celebesica* Heller, 1926		2	1	1	0	2*	1	0	0
Erotylidae, Erotylinae, Dacnini	*Apteronesitis fulgurata* Arrow, 1928		2	1	1	0	2	1	0	0
Erotylidae, Erotylinae, Dacnini	*Cryptodacne synthetica* Sharp, 1878	w	W	0	1	0	0	0	0	0
Erotylidae, Erotylinae, Tritomini	*Triplax russica* Linnaeus, 1758	w	6	1	1	1/0	1*	0	0	0
Erotylidae, Erotylinae, Tritomini	*Mycotretus ornatus* Duponchel, 1825	w	2	1	1	1	1	0	0	0
Erotylidae, Erotylinae, Tritomini	*Triplacidea motschulskyi* Bedel, 1872	w	W	1	1	1	1	1	0	0
Erotylidae, Erotylinae, Tritomini	*Tritoma bipustulata* Fabricius, 1775	w	15	1	1	1/0	1*	1*	0	0
Erotylidae, Erotylinae, Tritomini	*Amblyscelis kelleni* Gorham, 1888	w	W	1	1	1	1	1	0	0
Erotylidae, Erotylinae, Tritomini	*Amblyopus vittatus* Olivier, 1807	w	2	1	1	1	1	1	0	0
Erotylidae, Erotylinae, Tritomini	*Zythonia fulva* Westwood, 1874	w	W	1	1	1	1	0	0	0
Erotylidae, Erotylinae, Tritomini	*Apolybas bicolor* Guèrin-Mèneville, 1841	w	W	1	1	0	1	0	0	1
Erotylidae, Erotylinae, Tritomini	*Megischyrus undatus* Olivier, 1792	w	W	1	1	0	1	0	0	1
Erotylidae, Erotylinae, Tritomini	*Megischyrus brasiliensis* Lacordaire, 1842		4	1	1	1	2	0	0	1
Erotylidae, Erotylinae, Tritomini	*Ischyrus quadripunctatus* Olivier, 1792	w	2	1	1	1	1	0	0	1
Erotylidae, Erotylinae, Tritomini	*Pselaphacus nigropunctatus* Percheron, 1835	w	7	1*	1	1	2*/1	1	0	0
Erotylidae, Erotylinae, Erotylini	*Coccimorphus dichrous* Lacordaire, 1842		4	1	1	1	2	1	0	0
Erotylidae, Erotylinae, Erotylini	*Coccimorphus unicolor* Olivier, 1807	w	4	1	1	1	2	1	0	0
Erotylidae, Erotylinae, Erotylini	*Gibbifer vicinus* Guèrin-Mèneville, 1841		2	1	1	1	2	1	0	0
Erotylidae, Erotylinae, Erotylini	*Erotylus histrio* Linnaeus, 1758		2	1	1	1	2*	1	0	0
Erotylidae, Erotylinae, Erotylini	*Erotylus giganteus* Linnaeus, 1758	w	3	1	1	1	2	1	0	0
Erotylidae, Erotylinae, Erotylini	*Homoeotelus dorbignyi* Guèrin-Mèneville, 1841		4	1	1	1	1	1	0	0
Erotylidae, Erotylinae, Erotylini	*Homoeotelus testaceus* Fabricius, 1775	w	2	1	1	1	1	1	0	0
Erotylidae, Erotylinae, Erotylini	*Scaphidomorphus quinquepunctatus* Fabricius, 1775	w	W	1	1	1	2	1	0	0
Erotylidae, Erotylinae, Encaustini	*Encaustes verticalis* MacLeay, 1825	w	3	1	1	0	2	1	0	0
Erotylidae, Erotylinae, Encaustini	*Aulacochilus quadrisignatus* Guèrin-Mèneville, 1841	w	2	1	1	0	2	0	0	0
Erotylidae, Erotylinae, Encaustini	*Micrencaustes lunulata* MacLeay, 1825		2	1	1	0	2	1	0	0
Erotylidae, Erotylinae, Megalodacnini	*Megalodacne heros* Say, 1823		5	1	1	0	1	1	1	0
Erotylidae, Erotylinae, Megalodacnini	*Megalodacne fasciata* Fabricius, 1777	w	5	1	1	0	1/2	1	1	0
Erotylidae, Erotylinae, Megalodacnini	*Coptengis sheppardi* Pascoe, 1860	w	4	1	1	0	1	1	0	0
Erotylidae, Erotylinae, Megalodacnini	*Episcapha quadrimacula* Wiedemann, 1823	w	4	1	1	0	2	0	0	0
Erotylidae, Erotylinae, Megalodacnini	*Linodesmus coecus* Fabricius, 1777	w	3	1	1	0	2	1	0	0
Erotylidae, Erotylinae, Megalodacnini	*Macrodacne luteoguttata* Crotch, 1876		2	1	1	0	2	1	0	0
Erotylidae, Erotylinae, Megalodacnini	*Scaphodacne rectesignata* Crotch, 1876	w	W	1	1	0	2	1	0	0
Erotylidae, Erotylinae, Megalodacnini	*Scaphodacne rectesignata* f. *neglecta* Heller, 1918		2	1	1	0	2	1	0	0

2.2. Morphological studies, illustrations, and terminology

All morphological studies are based on dried specimens from the Museum of Zoology Dresden; only of *Triplax russica* and *Tritoma bipustulata* some freshly killed specimens were additionally used. The head, pronotum and remaining body were separated and macerated in hot 10% KOH. Afterwards the external cuticular surface was searched for gland openings in ca. 70% ethanol under a stereo-microscope, using magnifications up to 80x. In some exemplary cases the presence of a compound gland was checked for by an inspection of the internal face of the cuticle of some pore area. For SEM studies of structural details of gland pores the respective parts of the exoskeleton were critical point dried (with CO_2 as transitional

medium), coated with gold (Edwards S150B), and examined with a Philips/FEI XL 30 ESEM. Drawings and plates were completed using the graphic computer programmes CorelPhotoPaint vers. 12 and CorelDraw vers. 12. Morphological terminology follows Lawrence et al. (2010).

3. RESULTS

3.1. Distribution and morphology of compound gland pores

In our sample of Erotylidae we altogether found the following gland pores (mostly only based on external inspection of the cuticle): **(1)** Pores of periocular glands are located on the frons anteromesal to the compound eye and posterior to the antenna base, if present always as one pair (Figs. 4D, E, 5F; figs. 4, 8 in Węgrzynowicz, 2002). **(2)** Pores of subgenal glands are located on the subgenal braces (ventrolaterally on the head; subocular pores), if present always as one pair (Figs. 4B, F, H, 5D, H, K; figs. 5, 9 in Węgrzynowicz, 2002). **(3)** Pores of pronotal glands occur on the lateral margins of the pronotum (Fig. 3). If they are present, they include always one pair of pores each at the anterior and posterior corners of the pronotum; additional pores along the lateral pronotal edges in between can be present in varying numbers (1–19 per side). The pores at the anterior and posterior corners can be placed at different levels with regard to the lateral edge of the pronotum; accordingly, they are either visible from dorsally, or not. **(4)** Pores of prosternal glands are located on the ventral surface of the prosternal process, if present always as one pair (Figs. 5G, M; figs. 82, 83 in Węgrzynowicz, 2002). **(5)** Pores of mesoventral glands are located on the ventral surface of the mesoventral process, if present always as one pair (Fig. 5M). **(6)** Pores of mental glands are located at the base of the mentum, if present always as one pair (Fig. 5K). The distribution of the previously reported abdominal pores (fig. 15 in Leschen, 2003) was not examined in this study.

Each examined specimen of Erotylidae was found to possess pores in at least one of the above mentioned body parts, and the same is true for the "languriid" taxa (*Languria* and *Toramus*; see also Węgrzynowicz, 2002). A complete lack of pores is true for the other "languriid" taxa studied herein (*Setariola, Camptocarpus, Langurites, Tetraphala*). For the outgroup taxa (47 genera of languriid Erotylidae; see Leschen, 2003) severeal pores were detected, but their distribution is ambiguous in most cases. The two non-erotylid species here studied had no pores at all (Table 1).

Usually the gland pores in Erotylidae lack any special modifications for the release or evaporation of the discharged secretion. In some exceptional cases, however, the pores show a groove- or plateau-like extension, such as the pores at the lateral pronotal margin in *Aulacochilus quadrisignatus* (Fig. 2A) and the subgenal pores of *Encaustes verticalis* (Fig. 2B). These pores in *E. verticalis* (Fig. 2B) and the periocular pores in *Megischyrus*

brasiliensis (Fig. 2C) show a pattern of near-circular ridge-like elevations of the cuticle. In other cases the area around the pore looks sponge-like; this is true for the subgenal pores of many taxa (e.g. *Tritoma bipustulata*, *Triplax russica* and *Pselaphacus nigropunctatus* in Figs. 2E, D, 5H). In many erotylids the sponge-like gland pores are accompanied by groups of trichomes, which might enhance the evaporation of the secretion; such trichomes are frequently found associated with the pores on the subgenal brace.

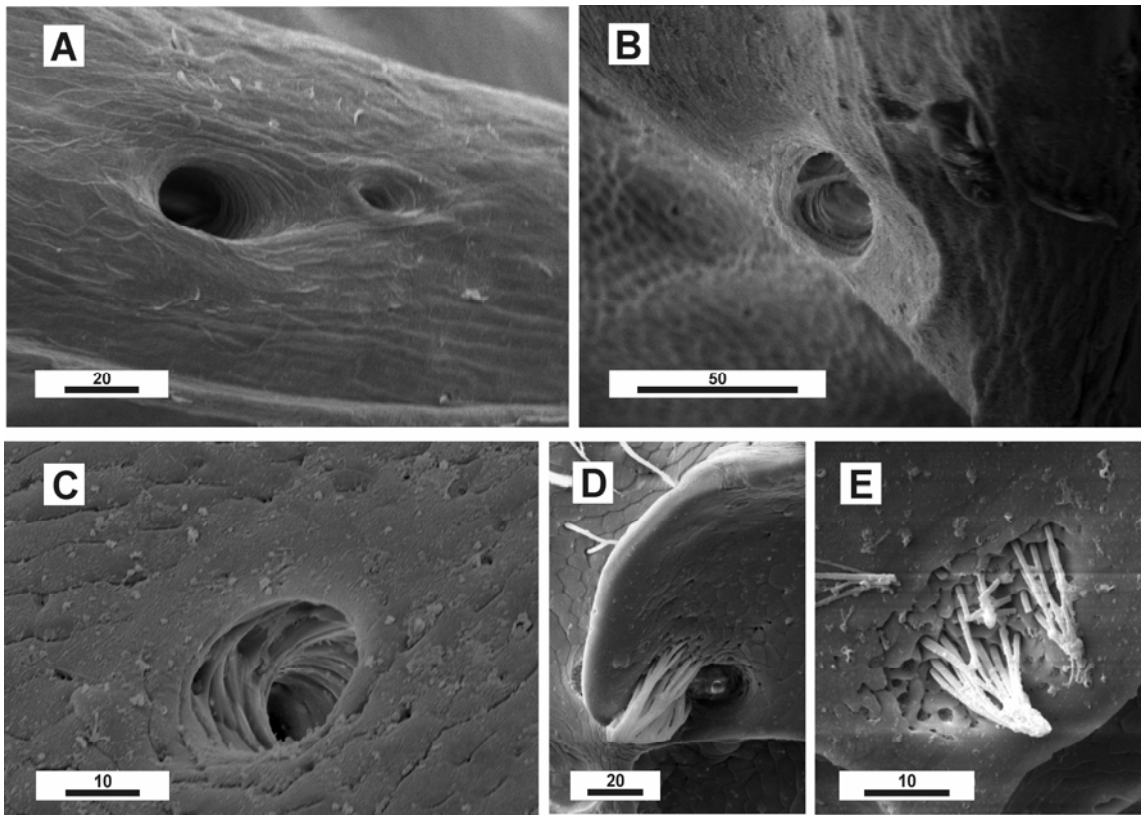

Fig. 2. Modified gland pores in Erotylinae. (A) Pore on left anterior corner of pronotum in *Aulacochilus quadrisignatus*, with groove-like extension (the smaller additional pore is one of those along the lateral pronotal edge; the anterior pronotal corner would follow at the left side). (B) Pore on subgenal brace of *Encaustes verticalis*, with plateau-like adjacent area. (C) Periocular pore of *Megischyrus brasiliensis*, with folded and wrinkled external part of outlet duct. (D) Pore on subgenal brace of *Triplax russica*, associated with a group of trichomes. (E) Pore on subgenal brace of *Tritoma bipustulata*, appearing sponge-like and associated with trichomes. Scales in μm.

Based on the occurrence of particular gland pores and of trichomes associated with the subgenal brace pores we define the following characters C1–C7. The distribution of character states across the taxa of our sample (and the erotylids additionally studied by Węgrzynowicz, 2002 and Leschen, 2003) is given in Table 1.

C1 Pores beside compound eyes: [0] absent, [1] present as one pair.

C2 Pores on subgenal brace: [0] absent, [1] present as one pair.

C3 Group of trichomes associated with pores on subgenal brace: [0] absent, [1] present.

C4 Pores on lateral pronotal margins: [0] absent, [1] only one in each anterior and posterior corner, [2] additional ones along the lateral margin.

C5 Pores on prosternal process: [0] absent, [1] present as one pair.

C6 Pores on mesoventral process: [0] absent, [1] present as one pair.

C7 Pores on mentum: [0] absent, [1] present as one pair.

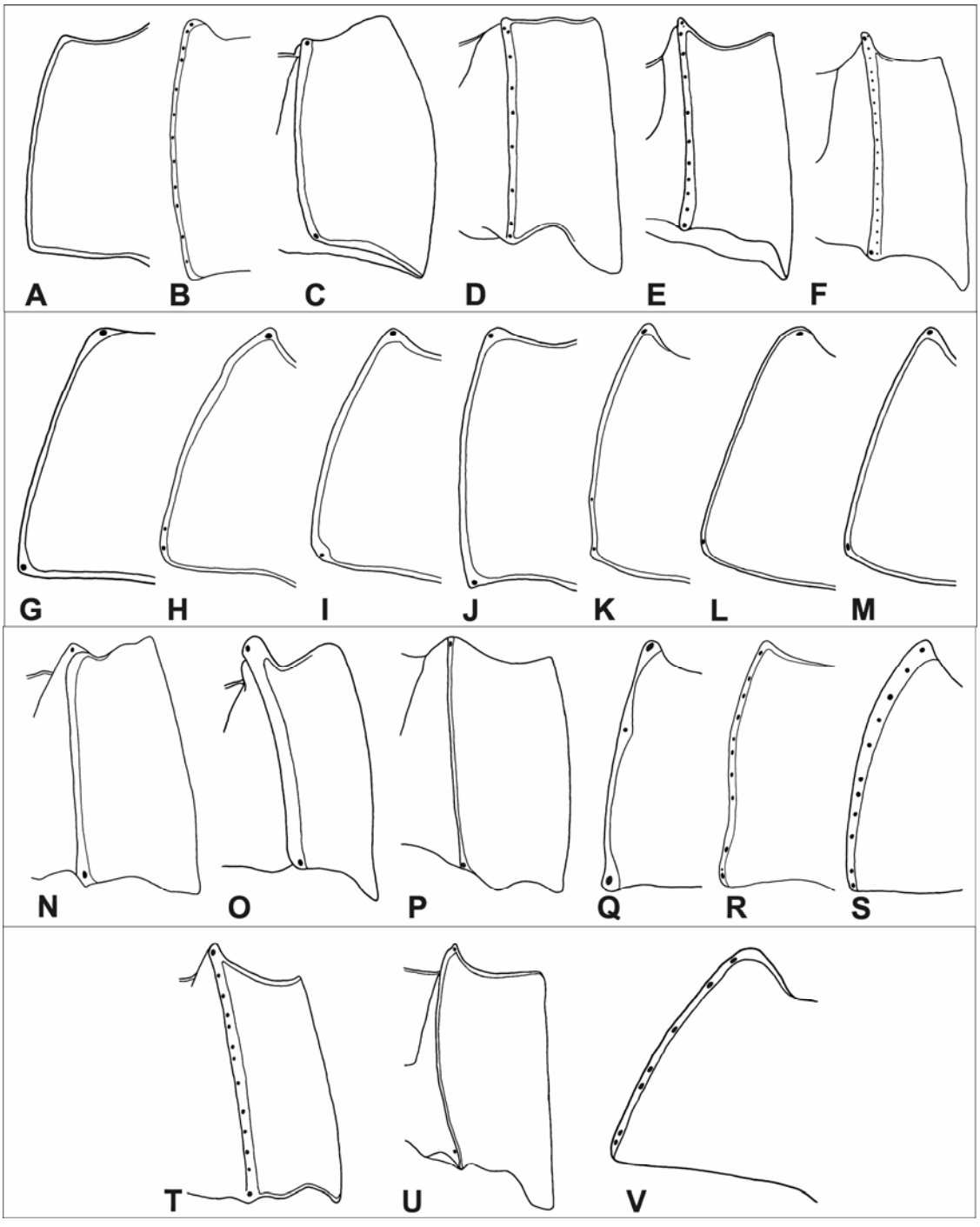

Fig. 3. Distribution of gland openings on left lateral edge of pronotum in Erotylinae species; left margin of pronotum shown in lateral (C–F, N–P, T, U) or dorsal (A, B, G–M, Q–S, V) view, anterior at top. (A–C) Dacnini: (A) *Dacne bipustulata*, (B) *Nesitis celebesica*, (C) *Combocerus glaber*; (D–F) Encaustini: (D) *Encaustes verticalis*, (E) *Aulacochilus quadrisignatus*, (F) *Micrencaustes lunulata*; (G–M) Tritomini: (G) *Tritoma bipustulata*, (H) *Megischyrus brasiliensis*, (I) *Amblyopus vittatus*, (J) *Triplax russica*, (K) *Pselaphacus nigropunctatus*, (L) *Ischyrus quadripunctatus*, (M) *Mycotretus ornatus*; (N–S) Megalodacnini: (N) *Megalodacne heros*, (O) *Megalodacne fasciata*, (P) *Coptengis sheppardi*, (Q) *Episcapha quadrimacula*, (R) *Linodesmus coecus*, (S) *Scaphodacne rectesignatus* f. *neglecta*; (T–V) Erotylini: (T) *Erotylus histrio*, (U) *Homoeotelus dorbignyi*, (V) *Coccimorphus dichrous*.

Setariola sericea. No gland pores are present (Fig. 4A; same in Węgrzynowicz, 2002). Leschen (2003) reported subgenal pores, pronotal pores, prosternal and mesoventral pores for this species; these pores were not detected in our analyses.

Cryptophilus interger. No gland pores are present (data from Węgrzynowicz, 2002). However, Leschen (2003) reported subgenal pores, pronotal pores, and mesoventral pores for this species.

Toramus pilifer. One pair each of periocular pores and subgenal pores (Figs. 4D, B); for absence of pronotal pores see Figs. 4C. Our findings differ from Węgrzynowicz (2002), who reported no gland pores for this species. However, Leschen (2003) reported periocular pores as absent. We examined five specimens by SEM and found always the same arrangement of gland pores (Table 1).

Tetraphala elongata. No gland pores are present.

Camptocarpus longicollis. No gland pores are present.

Langurites lineata. No gland pores are present.

Pentelanguria elateroides. One pair of subgenal pores.

Languria bicolor. One pair each of periocular pores (Fig. 4E, G) and subgenal pores (Fig. 4F), the area around the latter bears a tuft of trichomes (same in Węgrzynowicz, 2002). Leschen (2003) reported periocular pores as absent as well as pronotal pores and mesoventral pores as present; these pores were not detected in our analyses.

Languria puncticollis. One pair each of periocular pores and subgenal pores.

Pachylanguria elongata. One pair each of periocular pores and subgenal pores (Fig. 4H).

***Dacne bipustulata*.** One pair of subgenal pores (Fig. 5D). Our findings differ from Węgrzynowicz (2002), who reported no gland pores for this species. We examined six specimens by SEM and found always the same arrangement of gland pores (Table 1).

Combocerus glaber. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3C), anterior ones not visible from dorsal. One pair each of periocular pores and subgenal pores (same in Węgrzynowicz, 2002).

Euzostria aruensis. Pronotal glands represented by a single pore in each anterior and posterior corner, pores visible from dorsal. One pair each of periocular pores and subgenal pores.

Nesitis celebesica. Pronotal glands represented by 12 pores per side, distributed over the whole lateral margin of the pronotum (Figs. 3B; 5E). One pair each of periocular pores (Fig. 5F), prosternal pores (Fig. 5G) and subgenal pores.

Apteronesitis fulgurata. Pronotal glands represented by 4 pores per side, distributed over the lateral margin of the pronotum. One pair each of periocular pores, prosternal pores and subgenal pores.

Cryptodacne synthetica. One pair of subgenal pores (data from Węgrzynowicz, 2002).

Triplax russica. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3J). One pair each of periocular pores and subgenal pores. The area around the subgenal pores appears sponge-like and bears additional trichomes (Fig. 2D). Our finding of additional trichomes differs from Węgrzynowicz (2002), who found such trichomes absent in this species. We examined six specimens by SEM and always found the trichomes (Table 1).

Mycotretus ornatus. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3M). One pair of periocular pores and subgenal pores, the respective area bears a tuft of trichomes (same in Węgrzynowicz, 2002).

Triplacidea motschulskyi. Pronotal glands represented by a single pore in each anterior and posterior corner. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes pores (data from Węgrzynowicz, 2002).

Tritoma bipustulata. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3G). One pair each of periocular pores, subgenal pores and prosternal pores. The area around the subgenal pores appears sponge-like and bears additional trichomes (Fig. 2E). Our finding of additional trichomes differs from Węgrzynowicz (2002), who found such trichomes absent in this species. We examined 15 specimens by SEM and found this character always as present (Table 1).

Amblyscelis kelleni. Pronotal glands represented by a single pore in each anterior and posterior corner. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes pores (data from Węgrzynowicz, 2002).

Amblyopus vittatus. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3I). One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes (same in Węgrzynowicz, 2002).

Zythonia fulva. Pronotal glands represented by a single pore in each anterior and posterior corner. One pair of periocular pores and subgenal pores, the respective area bears a tuft of trichomes (data from Węgrzynowicz, 2002).

Apolybas bicolor. Pronotal glands represented by a single pore in each anterior and posterior corner. One pair each of periocular pores, mental pores and subgenal pores (data from Węgrzynowicz, 2002).

***Megischyrus undatus*.** Pronotal glands represented by a single pore in each anterior and posterior corner. One pair each of periocular pores, mental pores and subgenal pores (data from Węgrzynowicz, 2002).

Megischyrus brasiliensis. Pronotal glands represented by a single pore in each anterior and posterior corner plus a single one at the lateral margin (Figs. 3H, 5L). One pair each of periocular pores, mental pores and subgenal pores (Fig. 5K), the respective area bears a tuft of trichomes. Periocular pores with folded and wrinkled cuticular sculpture (Fig. 2C). Since the results differed between the two *Megischyrus*-species we examined further representatives of this genus (*M. semipunctatus, M. decempunctatus, M. discipennis*). In all cases we found the same arrangement of pronotal pores like in *M. brasiliensis*.

Ischyrus quadripunctatus. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3L). One pair each of periocular pores, mental pores and subgenal pores, the respective area bears a tuft of trichomes (same in Węgrzynowicz, 2002). Because of the phylogenetic position of *Ischyrus* as well as the presence of an additional pronotal pore in the closely related *M. brasiliensis* (also in other species of *Megischyrus*) we examined further representatives of this genus (*I. scriptus, I. femoralis, I. flavitarsis*). In all cases we found the same arrangement of pronotal pores like in *I. quadripunctatus*.

Pselaphacus nigropunctatus. Pronotal glands represented by a single pore in each anterior and posterior corner plus a single one at the lateral margin (Figs. 3K, 5I, J). One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes (Fig. 5H). Our findings differ from Węgrzynowicz (2002), who only reported a single pore in each anterior and posterior corner of the pronotum. We examined seven specimens by SEM and found always an additional pore in between (Table 1). Because of the phylogenetic position of *Pselaphacus* (sistergroup to Erotylini + Encasutini + Megalodacnini) we examined further representatives of this genus (*P. giganteus, P. rubriculus, P. puncticollis*). In all cases we found the same arrangement of pronotal pores like in *P. nigropunctatus*.

Coccimorphus dichrous. Pronotal glands represented by 7 pores per side, distributed over the whole lateral margin of the pronotum (Fig. 3V). One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes.

***Coccimorphus unicolor*.** Pronotal glands represented by 7 pores per side, distributed over the whole lateral margin of the pronotum. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes (same in Węgrzynowicz, 2002).

Gibbifer vicinus. Pronotal glands represented by 12 pores per side, distributed over the whole lateral margin of the pronotum. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes.

***Erotylus giganteus*.** Pronotal glands represented by 9 pores per side, distributed over the whole lateral margin of the pronotum. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes (same in Węgrzynowicz, 2002).

Erotylus histrio. Pronotal glands represented by 13 pores per side, distributed over the whole lateral margin of the pronotum (Fig. 3T), pores not visible from dorsal. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes.

Homoeotelus dorbignyi. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3U), pores not visible from dorsal. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes.

Homoeotelus testaceus. Pronotal glands represented by a single pore in each anterior and posterior corner. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes (same in Węgrzynowicz, 2002).

Scaphidomorphus quinquepunctatus. Pronotal glands represented by several pores per side, distributed over the lateral margin of the pronotum. One pair each of periocular pores, prosternal pores and subgenal pores, the respective area bears a tuft of trichomes (data from Węgrzynowicz, 2002).

Encaustes verticalis. Pronotal glands represented by 9 pores per side, distributed over the whole lateral margin of the pronotum (Fig. 3D), pores not visible from dorsal. One pair each of periocular pores, subgenal pores and prosternal pores. Subgenal pores have a plateau-like adjacent area (Fig. 2B) (same in Węgrzynowicz, 2002).

Aulacochilus quadrisignatus. Pronotal glands represented by 11 pores per side, distributed over the whole lateral margin of the pronotum (Fig. 3E), pores not visible from dorsal. Pronotal pores within the anterior corner have a groove-like extension (Fig. 2A). One pair each of periocular pores and subgenal pores (same in Węgrzynowicz, 2002).

Micrencaustes lunulata. Pronotal glands on each side represented by two larger pores within the anterior and the posterior corner and 19 tiny pores distributed over the whole lateral margin (Fig. 3F), pores not visible from dorsal. One pair each of periocular pores, subgenal pores and prosternal pores.

Megalodacne heros. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3N), pores not visible from dorsal. One pair each of periocular pores, subgenal pores, prosternal pores, and mesoventral pores.

Megalodacne fasciata. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3O), anterior ones not visible from dorsal. One pair each of periocular pores, subgenal pores, prosternal pores, and mesoventral pores (Fig. 5M). Our findings differ from Węgrzynowicz (2002), who reported numerous pores to occur along the lateral margin of the pronotum. We examined five specimens by SEM and found always only a single pore in each anterior and posterior pronotal corner (Table 1).

Coptengis sheppardi. Pronotal glands represented by a single pore in each anterior and posterior corner (Fig. 3P), pores not visible from dorsal. One pair of periocular pores, subgenal pores, and prosternal pores (same in Węgrzynowicz, 2002).

Episcapha quadrimacula. Pronotal glands represented by three pores per side, one each in the anterior and posterior corners and one in the posterior half of the lateral margin (Fig. 3Q).

One pair of periocular pores and subgenal pores (same in Węgrzynowicz, 2002; Fig. 5N demonstrates the absence of the prosternal and mesoventral pores).

Linodesmus coecus. Pronotal glands represented by 11 pores per side, distributed over the whole lateral margin of the pronotum (Fig. 3R). One pair each of periocular pores, subgenal pores, and prosternal pores (same in Węgrzynowicz, 2002).

Macrodacne luteoguttata. Pronotal glands represented by 7 pores per side, distributed over the whole lateral margin of the pronotum, pores partly visible from dorsal. One pair each of periocular pores, subgenal pores and prosternal pores.

Scaphodacne rectesignata. Pronotal glands represented by 7 pores per side, distributed over the whole lateral margin of the pronotum. One pair each of periocular pores, subgenal pores and prosternal pores (data from Węgrzynowicz, 2002).

Scaphodacne rectesignata* f. *neglecta. Pronotal glands represented by 11 pores per side, distributed over the whole lateral margin of the pronotum (Fig. 3S). One pair each of periocular pores, subgenal pores and prosternal pores.

In some of the sampled Erotylidae we examined the inner surface of the exoskeleton in order to verify the presence of a compound gland that opens through the pore here considered a gland pore. This was especially done in cases crucial for phylogenetic conclusions. Pores confirmed as glandular in this way are supplied with an asterisk* in Table 1.

Languria bicolor. Periocular pores continue internally into elongated compound glands (Fig. 4G).

Nesitis celebesica. Pores along the lateral margin of the pronotum continue internally into compound glands with a bottle-brush-like structure.

Triplax russica. Pores in the anterior and posterior corner of the pronotum continue internally into compound glands with a bottle-brush-like structure (see Drilling et al., in press).

Tritoma bipustulata. Pores in the anterior and posterior corner of the pronotum as well as of the prosternal process continue internally into compound glands with a bottle-brush-like structure (glands studied in detail in Drilling et al., in press).

Megischyrus brasiliensis. Both the pore in the posterior pronotal corner and that on the posterior part of the lateral pronotal margin continue internally into compound glands with a bottle-brush-like structure (Fig. 5L, arrow).

Pselaphacus nigropunctatus. Same as for foregoing species (Fig. 5J, arrow); both glands are branch out.

Erotylus histrio. Pores along the lateral margin of the pronotum continue internally into small compound glands with a bottle-brush-like structure.

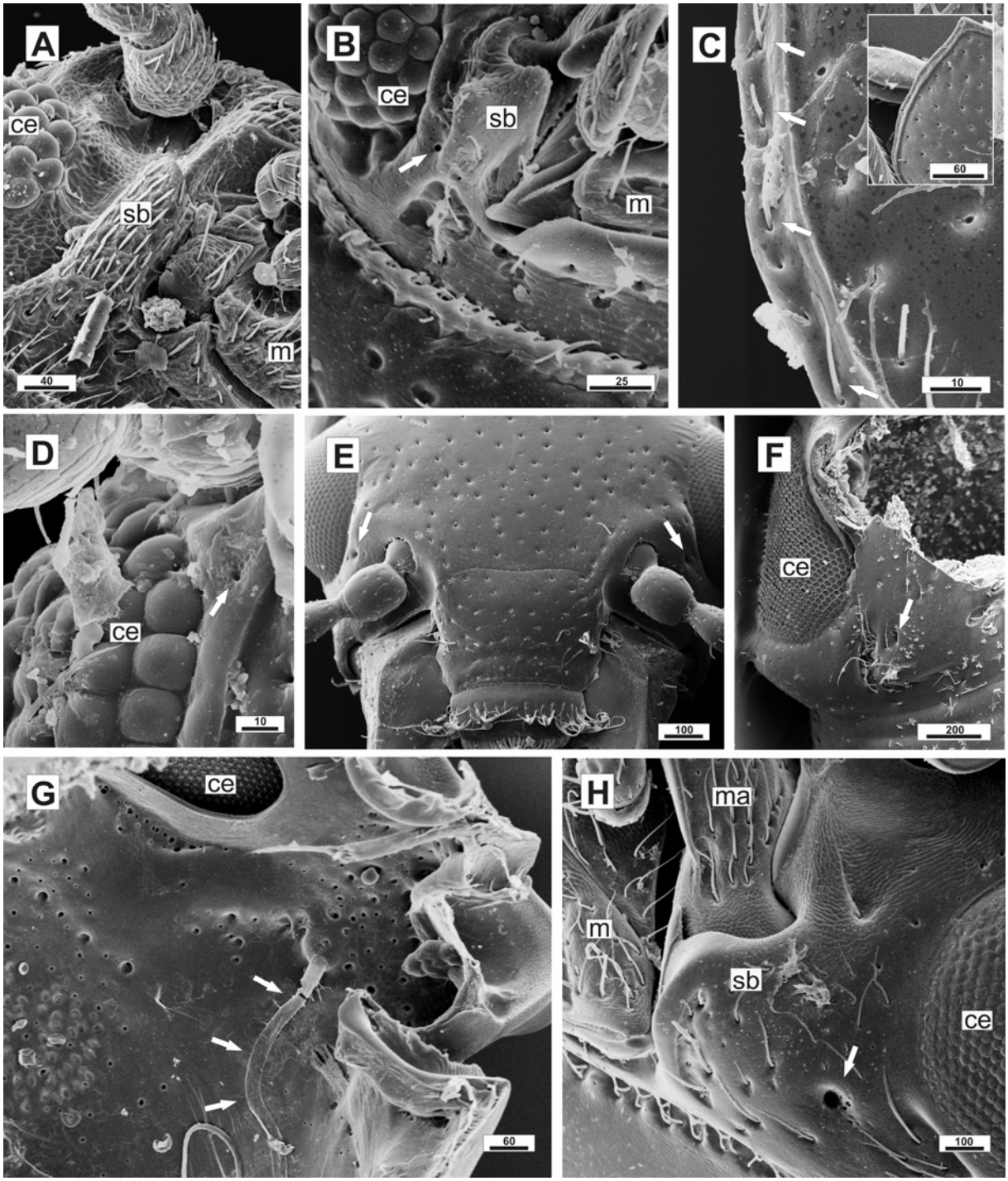

Fig. 4. SEM pictures of typically glandpore-bearing areas in languriid Erotylidae (with or without glandular pores). (A) *Setariola sericea*. The subgenal brace bears no glandular pore. (B-D) *Toramus pilifer*. (B) Gland pore on subgenal brace (arrow). (C) Detail of lateral pronotal margin with setae (arrows). Insert: Lateral margin of pronotum, without gland pores (punctures are setal bases, setae mostly broken off). (D) Periocular pores (arrow) near the compound eye (at the left); the area around the pore appears fluted. (E-G) *Languria bicolor*. (E) Head with periocular pores (arrow). (F) Subgenal pore (arrow) beneath a compound eye. (G) Periocular pore continues internally into an elongated compound gland (arrow). (H) *Pentelanguria elateroides*, subgenal pore (arrow). ce – compound eye; m – mentum; ma – mandible; sb – subgenal brace. Scales in μm.

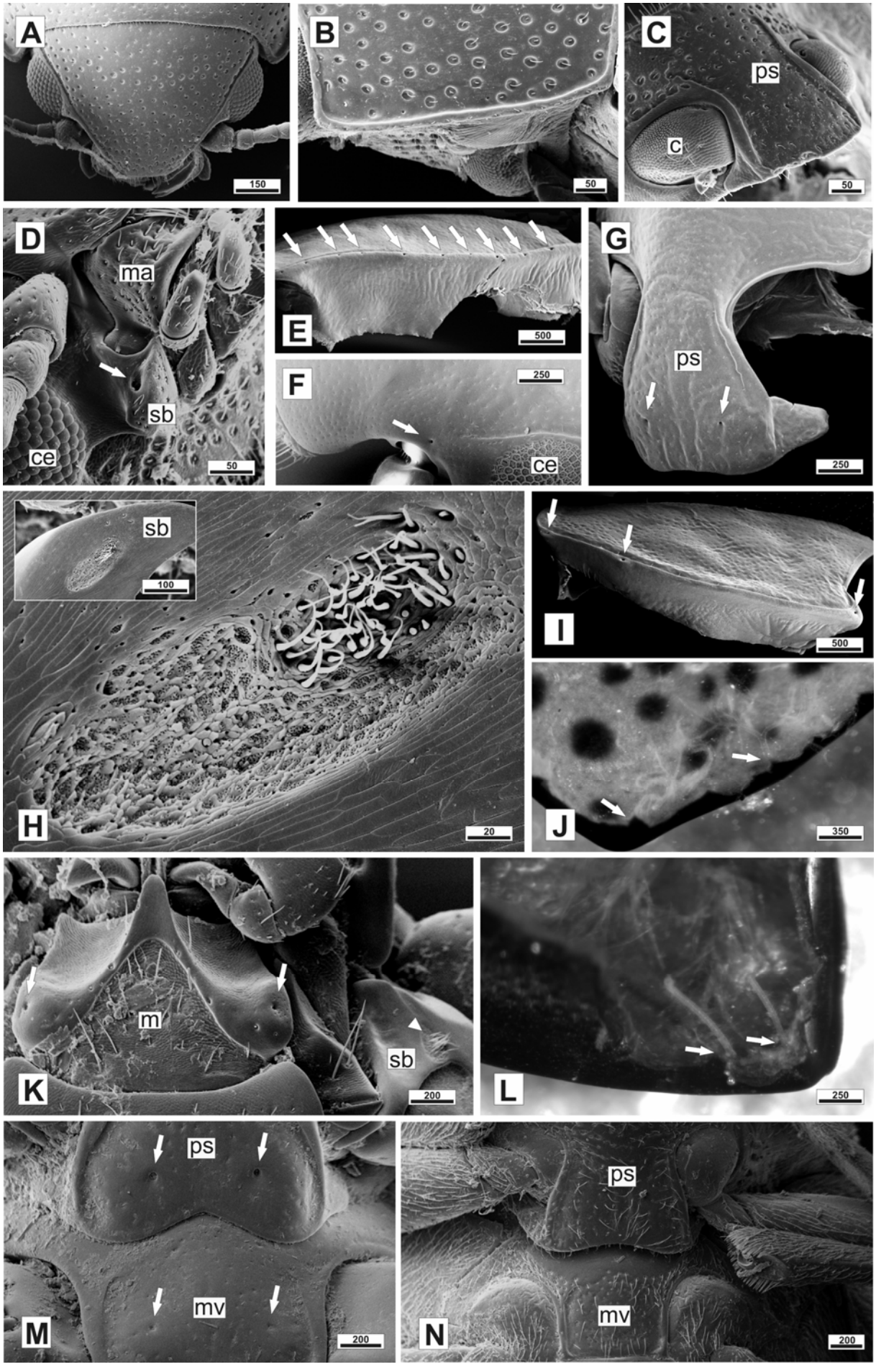
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Fig. 5. SEM pictures and photographs of typically glandpore-bearing areas in erotyline Erotylidae (with or without glandular pores). (A–D) *Dacne bipustulata* exhibits neither (A) periocular pores nor (B) pores in the pronotal corners or (C) at the prosternal process, but there are (D) subgenal pores beneath the compound eye. (E–G) *Nesitis celebesica*: (E) Numerous pores along the lateral pronotal margin (arrows; anterior to the right). (F) A periocular pore. (G) A pair of prosternal pores (arrows). (H–J) *Pselaphacus nigropunctatus*: (H) Detail of a subgenal pore; within the depression numerous tiny pores are placed beside a group of trichomes; insert: overview over the subgenal brace. (I) Three pores (arrows) along the lateral pronotal margin (anterior to the right). (J) The inner surface of the pronotum (photo taken through stereomicroscope); one branched gland each originates from the pronotal corner and from the pronotal margin (arrows; compare Fig. 3K). (K–L) *Megischyrus brasiliensis*: (K) Mental pores (arrows) and a subgenal pore with trichomes (arrowhead). (L) The inner surface of the pronotum (photo taken through stereomicroscope); one branched gland each originates from the pronotal corner and from the pronotal margin (arrows; compare Fig. 3H). (M) Glandular pores on the prosternal and mesoventral processes in *Megalodacne fasciata* (arrows). (N) The closely related *Episcapha quadrimacula* lacks glandular pores on the prosternal and mesoventral processes. c – coxa; ce – compound eye; m – mentum; ma – mandible; mv – mesoventrite; ps – prosternum; sb – subgenal brace. Scales in μm.

4. DISCUSSION

4.1. Previous hypotheses on the phylogeny of Erotylidae

The currently most elaborate hypotheses on Erotylidae phylogeny are that of Robertson et al. (2004) based on DNA-sequences (18S and 28S rDNA sequences; Fig. 1), that of Węgrzynowicz (2002; Fig. 6A) and Leschen (2003) based on morphology; the latter treat mainly the former languriid subfamilies. There are some pivotal differences between these hypotheses:

(1) Robertson et al. (2004) find the languriid taxon Cryptophilinae (*Toramus*) subordinate in the Erotylinae-Tritomini, but no morphological characters have been proposed in support of this relationship (Węgrzynowicz, 2002; Robertson et al., 2004). In Węgrzynowicz (2002) as well as in Leschen (2003) Cryptophilinae are placed outside of the Erotylinae, associated with other languriid lineages. Apart from that difference, basal relationships are Dacnini + (Languriinae + remaining Erotylinae) in both trees (Węgrzynowicz, 2002; Robertson et al., 2004; while *Dacne* is the only true dacnine taxon represented in both trees). In Leschen (2003) the Languriinae are also placed outside of Erotylinae, but nested within the Loberinae (fig. 109 in Leschen, 2003).

(2) The Erotylinae-Tritomini are paraphyletic in all hypotheses, but while in Robertson et al. (2004) they are paraphyletic only with regard to Erotylini (and Cryptophilinae), the Encaustini and Megalodacnini are additionally nested in Tritomini in the hypothesis of Węgrzynowicz (2002) and Leschen (2003), where Erotylini, Encaustini, and Megalodacnini together form a clade.
(3) *Coptengis*, formerly assigned to Dacnini, is placed in the Encaustini according to Robertson et al. (2004; sister to *Aulacochilus*), but in the Megalodacnini according to Węgrzynowicz (2002).
(4) Each of the tribes Erotylini, Encaustini, and Megalodacnini is monophyletic in all hypotheses (except for the case of *Coptengis*), but only in Węgrzynowicz (2002) and Leschen (2003) the three tribes together form a clade, *Pselaphacus nigropunctatus* (Tritomini) being the sister taxon to this clade in Węgrzynowicz (2002).
The taxon sample used herein shows much overlap with that in Węgrzynowicz (2002) (see Table 1). In the discussion of our gland characters we thus mainly refer to his phylogenetic hypothesis (Fig. 6A, where characters are mapped on that tree but additional taxa studied herein are supplemented). The taxon sampling of Robertson et al. (2004) differs strongly; hence it is difficult to devolve the gland characters to their phylogeny (Fig. 1).

4.2. Homology and systematic distribution of compound glands

Erotylidae compared with other cucujiform beetles. Gland pores present on corresponding parts of the body are here considered homologous as long as there is no contradictory evidence from phylogenetic analyses (primary homology hypotheses sensu De Pinna, 1991). Particular morphological differentiations of similarly located pores are viewed as confirming the assumption of homology but are quite rare.
In the various Erotylidae gland pores were found on the lateral edges of the pronotum (either only in the anterior and posterior corners, or additionally in between), beside the eyes, on the subgenal braces, on the mentum, and on the prosternal and mesoventral processes. However, in all Erotylidae only part of this pore set is present, and some lack all these pores (some languriid taxa).
For most cucujiform subgroups no compound glands at all (or respective pores) have been reported, and glands previously reported mostly open in positions completely different from Erotylidae: behind seventh visible abdominal sternite in many Tenebrionidae (Doyen, 1966; Tschinkel, 1975), mesothoracic ventral midline in Meloidae and Anthicidae (Hemp & Dettner, 1997; Morgan, 1968; Berrioz-Oritz, 1985), on the labium (the cuticle of the labium shows numerous openings of glands, but no further details are provided) and the maxillary palps in *Semiadalia* (Coccinellidae, Barbier et al., 1992), middle of first visible abdominal sternite in Ciidae (Buder at al., 2008; also in a few Erotylinae, but not studied herein).

This altogether means that for none of the gland pores occurring in Erotylidae a homologue is known in any other cucujiform beetles. Consequently, the possession of gland pores would appear as (a set of) autapomorphies evolved within the Erotylidae.

Erotylinae compared with other erotylid subfamilies. Since the subfamily Erotylinae is nested deep inside the erotylid tree, numerous genera of the former languriid subfamilies were used as outgroup taxa (Leschen, 2003). Several glandular characters occur scattered within these subgroups and their occurrence will be discussed in the relating section.

Gland pores on lateral edge of pronotum. Nearly all examined Erotylinae possess these pores; only the two Dacnini species *Dacne bipustulata* (Fig. 3A) and *Cryptodacne synthetica* (Fig. 6A), as well as the examined languriid species, lack them (state [0] of character). In case of the presence of pronotal pores, two cases can be distinguished: There is either only one pore present in each pronotal corner (state [1]), or additional pores are present along the lateral pronotal edge (state [2]). Since most members of the basal Xenoscelinae lack these glandular pores (present in *Protoloberus*; Leschen, 2003), outgroup comparison would suggest that this represents the ancestral state. Within the Pharaxonothinae, Languriinae, and Cryptophilinae this character is polymorphic (Leschen, 2003), but present in all examined species of Loberinae (Leschen, 2003). Unfortunately the character is coded as present or absent (Leschen, 2003); therefore it is not possible to estimate the arrangement for particular species. However, since these glands should be widely distributed within the other erotylid subfamilies secondary losses seem more parsimonious in cases were they are absent than several independent origins.

In Dacnini all three states of this character occur. One pore in each pronotal corner is the predominant condition in the likely paraphyletic Tritomini; only *Megischyrus brasiliensis* (in contrast to *M. undatus*) and *Pselaphacus nigropunctatus* (sister group to the clade comprising Erotylini, Encaustini, and Megalodacnini) have a single additional pore in the posterior part of the lateral edge (Figs. 3H, K, 5I, J, L; in contrast to our result Węgrzynowicz's (2002) found in *Pselaphacus* merely one pore in each pronotal corner, Table 1). Such conditions could represent a transition from pores only in the pronotal corners to numerous pores along the pronotal edge.

In most Erotylini, Encaustini, and Megalodacnini numerous pores along the pronotal edge occur (Fig. 6A) – with only few exceptions: Both examined *Homoeotelus*-species among the Erotylini as well as both *Megalodacne*-species (in contrast to our result Węgrzynowicz (2002) found in *M. fasciata* numerous pores along the pronotal edge; Table 1) and *Coptengis sheppardi* among the Megalodacnini have pores only in the pronotal corners (Fig. 6A); these cases likely represent secondary reductions.

When this character was mapped on the phylogenetic tree of Węgrzynowicz (2002) it became evident that numerous pores represent the most derived state within the Erotylinae (Fig. 6A). However the situation within the Dacnini (all three character states) is still ambiguous based on current hypotheses. Evidently *Nesitis celebesica* (Fig. 5E) and *Apteronesitis fulgurata*,

having numerous pores, more likely belong to Erotylini, Encaustini, or Megalodacnini than to Dacnini (Fig. 6A).

Gland pores on prosternal process. A pair of such pores was found in most examined members of the clade comprising Tritomini (excl. the basal *Triplax bipustulata*), Erotylini, Encaustini, and Megalodacnini (Fig. 6A). Among the other erotylid subfamilies this character is absent in the basal Xenoscelinae, Pharaxonothinae (in contrast to our results, Leschen (2003) reported these glands as present for *Setariola*), Cryptophilinae (present in *Loberoschema*; Leschen, 2003), and Erotylinae-Dacnini (present in *Nesitis* (Fig. 5G) and *Apteronesitis*). However, pores are scattered present in a few species of Loberinae (except for *Telamtoscius* and *Paphezia*) and Languriinae (Leschen, 2003). Since these glands occurred in distantly related species, several independent origins seem likely.

Within the above mentioned erotyline clade several independent losses have apparently occurred; conspicuous cases are the Tritomini clade including *Apolybas bicolor*, *Ischyrus quadripunctatus*, and both examined *Megischyrus* species (Fig. 6A). In addition, these pores are present in the Dacnini species *Nesitis celebesica* and *Apteronesitis fulgurata*, which, however, appear misplaced in Dacnini also according to the foregoing character. For the erotyline tribe Encaustini Leschen (2003) coded this character as absent since only *Aulacochilus* was included in his analyses.

Gland pores on mesoventral process. Among the examined Erotylinae such pores occur only in the two *Megalodacne*-species from Megalodacnini. However, Leschen (2003) found these pores within all tribes of Erotylinae with the exception of Dacnini. Within the other erotylid subfamilies these pores are absent in Xenoscelinae as well as in most species Cryptophilinae (Leschen, 2003). The distribution within Languriinae, Pharaxonothinae and Loberinae is polymorphic (Leschen, 2003). Since these glands occurred in distantly related species, several independent origins seem likely.

Gland pores beside compound eyes (periocular pores). The tiny periocular pores are found in nearly all examined Erotylinae as well as in *Toramus* (Cryptophilinae; Fig. 5B) and *Languria* (Languriinae; Fig. 5F; we found these pores in both examined species of *Languria*; Leschen (2003) coded this character as absent for the genus *Languria*). However, the two Dacnini species *Dacne bipustulata* and *Cryptodacne synthetica* as well as the other erotylid subfamilies (with only a few scattered exceptions) lack them (Leschen, 2003). The scattered distribution of periocular pores among distantly related species of the other erotylid subfamilies indicates multiple independent origins of these pores within Erotylidae.

Gland pores on subgenal brace (subocular pores). A pair of subgenal pores is present in all examined Erotylinae as well as in most languriids studied herein (Table 1). In *Setariola*, *Camptocarpus*, *Langurites* and *Tetraphala* these pores are absent, but for the other erotylid subfamilies Leschen (2003) coded this character as present. However, within the erotyline tribes Tritomini and Encaustini this character is reported as absent respectively polymorphic for Erotylini (Leschen, 2003). The absence of these glands in a few species is likely due to secondary losses, since this character is widely distributed within the family.

The subgenal pores are commonly associated with a group of trichomes (Figs. 2D, E; 5H, K). The trichomes are found in most Tritomini (except *Apolybas bicolor* and *Megischyrus undatus*) and Erotylini. The examined Encaustini and Megalodacnini lack the trichomes (Fig. 6A). In contrast to Węgrzynowicz's (2002) results, we found the Tritomini species *Tritoma bipustulata* and *Triplax russica* to have trichomes on their subgenal braces (Figs. 2D, E, Table 1). This character is not treated in Leschen (2003).

Gland pores on mentum. These are restricted to a Tritomini clade comprising *Apolybas bicolor*, *Megischyrus undatus*, *M. brasiliensis*, and *Ischyrus quadripunctatus* (Fig. 6A). This character is not treated in Leschen (2003).

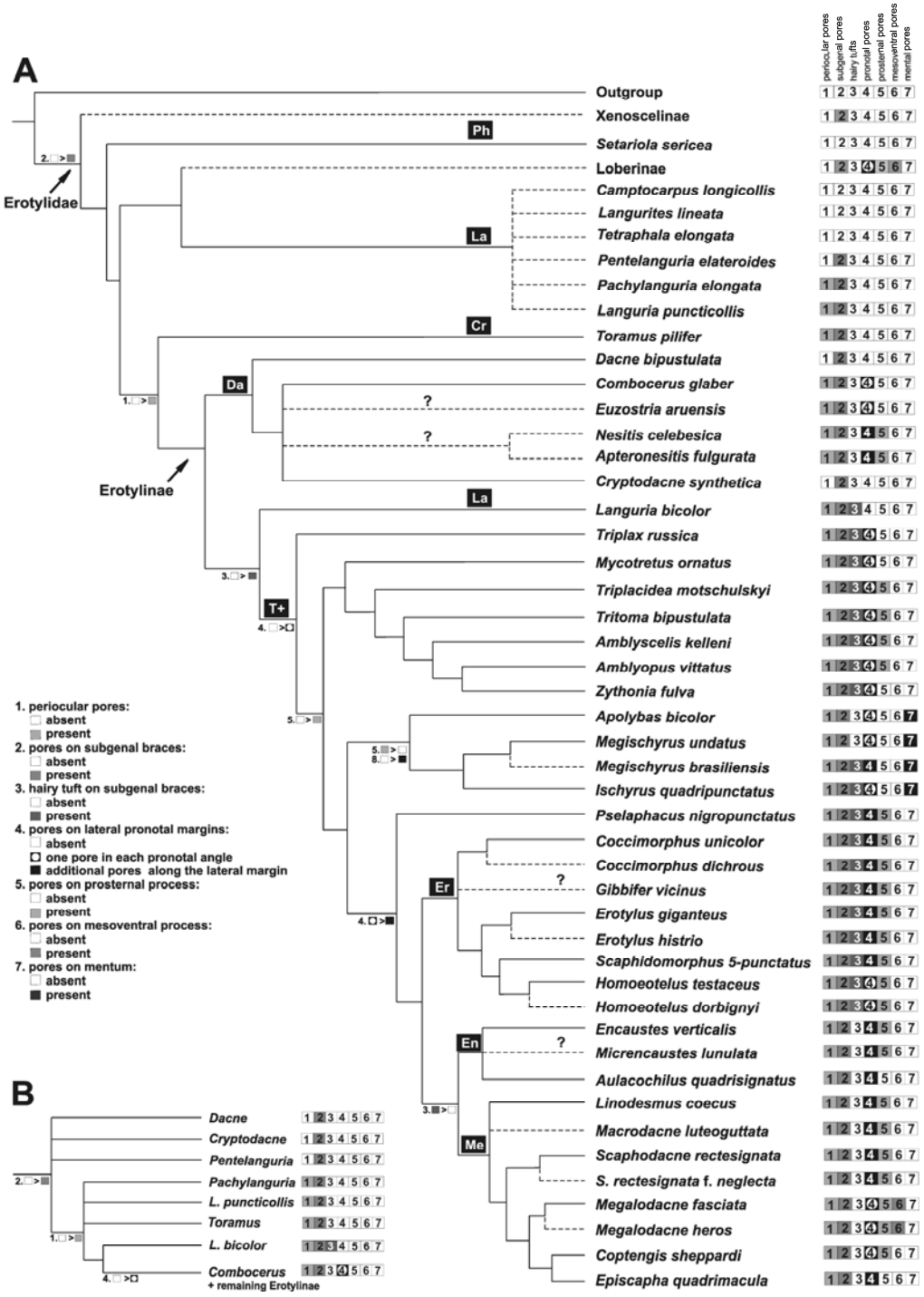

Fig. 6. (A) Characters of pores mapped on a phylogeny of the Erotylidae based on 85 morphological characters (after Węgrzynowicz 2002; species added herein indicated by dashed lines). (B) Hypothesis for the basal splitting events in Erotylidae derived from gland characters. Cr – Cryptophilinae; Da – Dacnini; En – Encaustini; Er – Erotylini; La – Languriinae; Me – Megalodacnini; Ph – Pharaxonothinae; T+ – Tritomini + remaining Erotylinae.

4.3. Phylogenetic implications

In Fig. 6 gland characters are mapped on the phylogenetic tree of Węgrzynowicz (2002), with character transformations placed on the tree in the most parsimonious way. While characters are here essentially interpreted along Węgrzynowicz´s (2002) hypothesis, alternative relationships will also be discussed if there is strong evidence from gland characters.

Basal relationships in Erotylinae. The Erotylidae in this broad sense are supported by the presence of subgenal pores (Leschen, 2003). In our sample the presence of periocular pores supports the monophyly of the Erotylinae including *Toramus* (Cryptophilinae) and *Languria bicolor* (Languriinae). The other languriine species studied herein (*Languria puncticollis* and *Pachylanguria*; both taxa were not included in the previous phylogenetic analyses of Węgrzynowicz (2002), Leschen (2003), and Robertson et al. (2004)) also had these pores, suggesting a related position to *L. bicolor* derived from glandular characters (Fig. 6A). However, there are some points of ambiguity at the base of the tree that mainly concern the absence or presence of particular glands in species of the former Languriidae and various Dacnini.

Based on gland characters alone, *Dacne bipustulata, Cryptodacne synthetica* and the languriid *Pentelanguria*, which only have subgenal pores, would appear as sister group to a clade comprising *Toramus* and all other Erotylinae including *Pachylanguria* as well as both examined *Languria* species; *Pachylanguria, L. puncticollis* and even *Toramus*, which additionally have periocular pores, would be the next branch of the erotyline tree, and *L. bicolor*, which also has trichomes associated with the subgenal pores would follow. The remaining Erotylinae form a clade weakly supported by the presence of pores in the corners of the pronotum, since these pores are absent in *Brachypterosa* (which represents the sister taxon to all Erotylinae; Leschen, 2003) as well as in *Dacne* and *Cryprodacne* (Dacnini)).

This relationship *Dacne* + *Cryptodacne* + *Pentelanguria* + (*Toramus* + *Pachylanguria* + *L. puncticollis* + (*L. bicolor* + *Combocerus* and remaining Erotylinae)), shown in Fig. 6B, would be incongruent with the monophyly of Dacnini proposed by Węgrzynowicz (2002) (Fig. 6A). Nonetheless, in the latter hypothesis Dacnini is poorly supported by apomorphies: the mesepisterna are fused to the mesepimera far in front of the meso-metathoracic suture inside the coxal cavities, and cross-veins r3 and r4 approach or touch one another. Thus, there are hardly any counter-arguments against the relationships suggested by gland characters. As mentioned above, both Węgrzynowicz (2002) and Robertson et al. (2004) found that

Coptengis has to be removed from Dacnini. According to the gland characters here studied, *Nesitis, Apteronesitis, Euzostria* and *Combocerus* are also highly unlikely to be related to any of the other genera assigned to Dacnini, and even if limited to *Dacne* and *Cryptodacne*, Dacnini may be paraphyletic.

Whereas a position of *Languria bicolor* (Languriinae) inside the Erotylinae (Węgrzynowicz, 2002; Robertson et al., 2004) is clearly confirmed by the presence of periocular pores and trichomes next to the subgenal pores. *Pachylanguria*, *L. puncticollis* and *Toramus* lack these trichomes; the other Languriinae (sensu Leschen, 2003) here examined, *Tetraphala elongata, Langurites lineata* and *Camptocarpus longicollis*, lack all gland pores. This suggests that these taxa are placed outside Erotylinae, and Languriinae is non-monophyletic. Unfortunately, neither *Tetraphala* nor *Langurites* or *Camptocarpus* species have been included in the phylogenetic analyses of Węgrzynowicz (2002), Leschen (2003), and Robertson et al. (2004). Leschen´s (2003) arguments for monophyletic Languriinae are the presence of submesocoxal lines, of an apical pit of the spermatheca, and of a wedge cell in the wing venation. In sum, the monophyly of Languriinae appears at least debatable.

Apical relationships in Erotylinae. The clade comprising the Tritomini (paraphyletic), Encaustini, Erotylini, and Megalodacnini (and Cryptophilinae in Robertson et al., 2004) likely has a groundplan set of pores that comprises pores in the corners of the pronotum as well as periocular and subgenal pores, the latter associated with a tuft of trichomes.

One character conflict at the base of Tritomini concerns the distribution of prosternal pores (character 5 in Fig. 6A). Węgrzynowicz´s (2002) clade comprising Tritomini (under exclusion of *Triplax russica*), Erotylini, Encaustini, and Megalodacnini is ambiguously supported by the presence of these pores (Fig. 6A, where the "accelerated" version of character transformation is mapped). Considering the basal dichotomies within this clade, prosternal pores are consistently absent in the clades *Mycotretus* and *Apolybas* + *Ischyrus* + *Megischyrus*, while they are present in the respective sister clades *Triplacidea* + *Tritoma* + *Amblyscelis* + *Amblyopus* + *Zythonia* (absent in the latter genus) and *Pselaphacus* + Erotylini + Encaustini + Megalodacnini (with a few scattered absences appearing as secondary). Therefore, gland characters would rather suggest that the two latter clades form a monophyletic group.

Also problematic is the presence of the pronotal pores in the clade *Apolybas* + *Ischyrus* + *Megischyrus* (character 4 in Fig. 6A). While *Apolybas*, *Megischyrus undatus* and *Ischyrus quadripunctatus* (we examined three further species (see chapter 3.1.) of this genus and found always the same arrangement like in *I. quadripunctatus*) had pores only in the pronotal corners, the closely related species *M. brasilensis* (as well as three other examined species of this genus; see chapter 3.1.) had one additional pore at the lateral pronotal margin. Since *M. undatus* was unfortunately not available for this study, it remains open whether this finding is defective. If applied, the genus *Megischyrus* more likely belongs to Erotylini, Encaustini, or Megalodacnini.

However, the just mentioned clade is supported by another gland apomorphy: the presence of pores on the mentum (Figs. 5K, 6A). This character was found in all examined members of

the clade and seems to be unique among Erotylidae. Admittedly, however, their occurrence is not stable since some *Ischyrus* species lack them (Węgrzynowicz, 2002).

A clade comprising the derived Tritomini *Pselaphacus nigropunctatus* as well as the examined Erotylini, Encaustini, and Megalodacnini is supported by the presence of additional pores (at least one) along the lateral pronotal edge. However, our finding for *P. nigropunctatus* (we examined seven specimens and found always the same arrangement of pores; Figs. 3K, 5I, J) differs from that of Węgrzynowicz (2002), who found only pores in the pronotal corners; this may indicate intraspecific variability, which would limit the usefulness of this character for phylogenetic conclusions. The presence of pores only in the pronotal corners in both examined *Homoeotelus*-species among Erotylini and *Coptengis* as well as both examined *Megalodacne*-species among Megalodacnini is also conflicting, yet this is quite likely to result from secondary loss.

The presence of trichomes on the subgenal braces supports a clade comprising *Languria bicolor*, Tritomini and Erotylini (alternative to the relationships shown in Fig. 6B). Such tufts, located on different parts of the body, are widespread among fungivorous beetles and enable transfer of spores or fragments of spawn (Węgrzynowicz, 2002). For Erotylinae an enhancement of the evaporation of discharged secretions is also conceivable since these trichomes are located near the subgenal pores. In the clades Encaustini and Megalodacnini trichomes are always absent while pores are present.

In our sample a clade comprising both *Megalodacne* species (Megalodacnini) is supported by the presence of a pair of mesoventral pores, which show identical location in both taxa. In contrast, Leschen (2003) reported mesoventral glands also for a few species of the other subfamilies as well as for all Erotylinae (see chapter 4.2.).

In sum, in the examined system we found very confusing distributions, which are difficult to interpret. The distributions of compound glands fit the present phylogenies (Węgrzynowicz, 2002; Robertson et al., 2004) most notably in the subfamily of Erotylinae. However, there is much homoplasy in the evolution of the glandular equipment, and this mainly concerns the scattered and confused distributions within the languriid subfamilies (especially the periocular pores, prosternal and mesoventral pores). Within the Erotylinae the distributions are quite stable and several clades may be supported by some of these characters. Hence, gland characters alone are hardly qualified to resolving the phylogeny of Erotylidae; at most they are applicable in some apical erotyline clades (see also Tschinkel, 1975; Tschinkel & Doyen, 1980 and Steidle & Dettner, 1993 for gland characters as a phylogenetic tool). Unfortunately, little is known about the biology and ecology of many of the erotylid genera and species, and virtually nothing about the contents and functional role of their various glands. This presently hampers estimations of how plausibly secondary losses of glands could be explained as resulting from changes in life history. A large-scale analysis on the structure and ultra structure of such glands would surely provide additional interesting and crucial characters to resolving phylogenetic problems within this family.

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6. REFERENCES

ALVARENGA, M. 1994: Catálogo dos Erotylidae (Coleoptera) Neotropical. *Revista Brasileira de Zoologia* **11**: 1–175.

BARBIER, R., FERRAN, A., LE LANNIC, J. & ALLO, M.-R. 1992: Morphology and ultrastructure of integumentary glands of *Semiadalia undecimnotata* Schn. (Coleoptera: Coccinellidae). *International Journal of Insect Morphology and Embryology* **21**(3): 223–234.

BERRIOZ-ORTIZ, A. 1985: The presence of mesothoracic glands in *Epicauta segmenta* (Coleoptera: Meloidae) and other blister beetles. *Journal of the Canadian Entomological Society* **58**: 179–181.

BOYLE, W. W. 1956: A revision of the Erotylidae of America north of Mexico (Coleoptera). *Bulletin of the American Museum of Natural History* **110**: 61–172.

BUDER, G., GROSSMANN, C., HUNDSDOERFER, A. & KLASS, K.-D. 2008: A contribution to the phylogeny of the Ciidae and its relationships with other cucujoid and tenebrionoid beetles (Coleoptera: Cucujiformia). *Arthropod Systematics and Phylogeny* **66**: 165–190.

CHÛJÔ, M. & CHÛJÔ, M. T. 1988: A catalog of the Erotylidae (Insecta, Coleoptera) from the old world (excl. the Ethiopian region). *Esakia* **26**: 139–185.

CHÛJÔ, M. & CHÛJÔ, M. T. 1989: A catalog of the Erotylidae (Insecta, Coleoptera) from the old world (excl. the Ethiopian region) II. *Esakia* **28**: 75–96.

CHÛJÔ, M. & CHÛJÔ, M. T. 1990: A catalog of the Erotylidae (Insecta, Coleoptera) from the old world (excl. the Ethiopian region) III. *Esakia* **29**: 1–67.

CROWSON, R. A. 1955: The Natural Classification of the Families of Coleoptera. London, Nathaniel Lloyd and Co., 187 pp.

DELKESKAMP, K. 1981: Erotylidae von Afrika und Madagascar. In Wilcox, J.A. (eds.): *Coleopterorum Catalogus Supplementa.* Pars 34. Dr W. Junk Publishers, The Hague, 65 pp.

DE PINNA, M. 1991: Concepts and tests of homology in the cladistic paradigm. *Cladistics* **7**: 367–394.

DOYEN, J. T. 1966: The skeletal anatomy of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Miscellaneous Publications of the Entomological Society of America* **5**(3): 103–150.

DRILLING, K., DETTNER K. & KLASS K.-D. (in press): Morphology of the pronotal compound glands in *Tritoma bipustulata* (Coleoptera: Erotylidae). *Organisms, Diversity & Evolution*. DOI: 10.1007/s13127-010-0008-0

HEMP, C. & DETTNER, K. 1997: Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera: Anthicidae). *Entomologia Generalis* **22**(2): 97–108.

HUNT, T., BERGSTEN, J., LEVKANICOVA, Z., PAPADOPOULOU, A., ST JOHN, O., WILD, R., HAMMOND, P. M., AHRENS, D., BALKE, M., CATERINO, M. S., GÓMEZ-ZURITA, J., RIBERA, I., BARRACLOUGH, T. G., BOCAKOVA, M., BOCAK, L. & VOGLER, A. P. 2007: A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science* **318**: 1913–1916.

KLAUSNITZER, B. 2005: 26. Ordnung: Coleoptera, Käfer; Part: Polyphaga. In DATHE, H.H. (eds.): *Lehrbuch der Speziellen Zoologie*, Band I, 5. Teil: Insecta. 2nd edn. Spektrum Akademischer Verlag; Heidelberg, Berlin, pp. 452–526.

LAWRENCE J., BEUTEL R. G., LESCHEN R. A. B. & ŚLIPIŃSKI A. 2010: 2. Glossary of Morphological Terms. In R. A. B. LESCHEN, R. G. BEUTEL & J. LAWRENCE (eds.): *Coleoptera, Beetles, vol. 2: Morphology and*

Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). Handbuch der Zoologie/Handbook of Zoology IV/39. Walter de Gruyter, Berlin, New York. pp. 9–20.

LAWRENCE, J. F. & NEWTON, A. F. 1982: Evolution and classification of beetles. *Annual Review of Ecology and Systematics* **13**: 261−290.

LAWRENCE, J. F. & VAURIE, P. 1983: A catalog of the Coleoptera of America north of Mexico. Family: Languriidae. United States Department of Agriculture. x +13 pp.

LESCHEN, R. A. B. 1996: Phylogeny and revision of the genera of Cryptophagidae (Coleoptera: Cucujoidea). *Kansas Science Bulletin* **55**: 549–634.

LESCHEN, R. A. B. 2003: Erotylidae (Insecta: Coleoptera: Cucujoidea): phylogeny and review. *Fauna of New Zealand* **47**, 108 pp.

LESCHEN, R. A. B. & BUCKLEY T. R. 2007: Multistate characters and diet shifts: Evolution of Erotylidae (Coleoptera). *Systematic Biology* **56**: 97–112.

LESCHEN R. A. B., SKELLEY P. A.& MCHUGH J. V. 2010: 10.7. Erotylidae Leach, 1815. In R.A.B. LESCHEN, R.G. BEUTEL & J. LAWRENCE (eds.): *Coleoptera, Beetles, vol. 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). Handbuch der Zoologie/Handbook of Zoology IV/39*. Walter de Gruyter, Berlin, New York. pp. 311–319.

LESCHEN R. A. B. & ŚLIPIŃSKI A. 2010: 7. Cucujiformia Lameere, 1938: Introduction, Phylogeny. In R. A. B. LESCHEN, R. G. BEUTEL & J. LAWRENCE (eds.): *Coleoptera, Beetles, vol. 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). Handbuch der Zoologie/Handbook of Zoology IV/39*. Walter de Gruyter, Berlin, New York. pp. 227–228.

LESCHEN, R. A. B. & WĘGRZYNOWICZ, P. 1998: Generic catalogue and taxonomic status of Languriidae (Cucujoidea). *Annales Zoologici* **48**(3/4): 221–243.

MCHUGH, J. V., MARSHALL, C. J. & FAWCETT, F. L. 1997: A study of adult morphology in *Megalodacne heros* (Say) (Coleoptera: Erotylidae). *Transactions of the American Entomological Society*, 123(4): 167-223.

MORGAN, A. 1968: The occurance of a pair of exocrine glands in the metathorax of *Lytta nutalli* (Coleoptera: Meloidae). *Canadian Journal of Zoology* **46**: 915–917.

PASTEELS, J. M., ROWELL-RAHIER, M., BRAEKMAN, J. C., DALOZE, D. & DUFFEY, S. 1989: Evolution of exocrine chemical defense in leaf beetles (Coleoptera: Chrysomelidae). *Experientia* **45**: 295–300.

ROBERTSON, J. A., MCHUGH, J. V. & WHITING, M. F. 2004: A molecular phylogenetic analysis of the pleasing fungus beetles (Coleoptera: Erotylidae): evolution of colour patterns, gregariousness and mycophagy. *Systematic Entomology* **29**: 173–187.

SEN GUPTA, T. & CROWSON, R. A. 1969: On a new family of Clavicornia (Coleoptera) and a new genus of Languriidae. *Proceedings of the Royal Entomological Society London (B)* **38**: 125–131.

SEN GUPTA, T. & CROWSON, R. A. 1971: A review of classification of the family Languriidae (Coleoptera: Clavicornia) and the place of Languriidae in the natural system of Clavicornia. *Memoirs of the Zoological Survey of India* **15**: 1–42.

STEIDLE, J. L. M. & DETTNER, K. 1993: Chemistry and morphology of the tergal gland of freeliving adult Aleocharinae (Coleoptera: Staphylinidae) and its phylogenetic significance. *Systematic Entomology* **18**: 149-168.

TSCHINKEL, W. R. 1975: A comparative study of the chemical defensive system of tenebrionid beetles. 3. Morphology of the glands. *Journal of Morphology* **145**: 355–370.

TSCHINKEL, W. R. & DOYEN, J. T. 1980: Comparative anatomy of the defensive glands, ovipositors and female genital tubes of tenebrionid beetles (Coleoptera). *International Journal of Morphology and Embryology* **9**: 321-368.

WĘGRZYNOWICZ, P. 2002: Morphology, phylogeny and classification of the family Erotylidae based on adult characters (Coleoptera: Cucujoidea). *Genus* **13**(4): 435–504.

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Morphology of the pronotal compound glands in *Tritoma bipustulata* (Coleoptera: Erotylidae)

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Morphology of the pronotal compound glands in *Tritoma bipustulata* (Coleoptera: Erotylidae)

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Abstract Members of the cucujiform family Erotylidae possess a whole arsenal of compound integumentary glands. Structural details of the glands of the pronotum of *Tritoma bipustulata* and *Triplax scutellaris* are provided for the first time. These glands, which open in the posterior and anterior pronotal corners, bear, upon a long, usually unbranched excretory duct, numerous identical gland units, each comprising a central cuticular canal surrounded by a proximal canal cell and a distal secretory cell. The canal cell forms a lateral appendix filled with a filamentous mass probably consisting of cuticle, and the cuticle inside the secretory cell is strongly spongiose—both structural features previously not known for compound glands of beetles. Additional data are provided for compound glands of the prosternal process and for simple (dermal) glands of the pronotum. A combined defense plus anti-microbial function of the compound glands is tentatively proposed.

Keywords Dermal glands · Gland unit · Gland pore · Pronotum · Prosternum · SEM · TEM

Abbreviations

ec	Extracellular cavity
ed	Excretory duct of compound gland
edcu	Cuticular intima of excretory duct
er	Endoplasmatic reticulum
fs	Filamentous structure forming core of lateral appendix
gd	Glandular ductule of gland unit
gdc	Constriction of glandular ductule
gdcl	Cell enclosing glandular ductule (secretory cell)
la	Lateral appendix of gland unit
lacl	Cell enclosing lateral appendix (canal cell)
lu	Lumen of glandular ductule or canal
m	Mitochondrion
mgdcl	Membrane of cell enclosing glandular ductule
mlacl	Membrane of cell enclosing lateral appendix
ngc	Non-glandular cell
rw	Ringwall around orifice of glandular ductule
ss	Spongiose structure of cuticular intima of glandular canal
v	Vesicle

Introduction

Erotylidae is distributed worldwide and comprises about 3200 described species in more than 280 genera (Węgrzynowicz 2002; Leschen 2003). These figures refer to a revised concept of Erotylidae, which also includes the languriids: Recent morphology- and molecular-based phylogenetic studies have shown that the former ‘Languriidae’ is paraphyletic with respect to the former ‘Erotylidae’ (Węgrzynowicz 2002; Leschen 2003; Robertson et al. 2004), and the latter is now ranked as a subfamily ‘Erotylinae’ of an expanded Erotylidae. The former separation of these two groups was primarily based on their different biology, with ‘Erotylidae’ being mycophagous and ‘Languriidae’ being phytophagous. Erotylidae belongs to the Coleoptera-

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Cucujiformia and has traditionally been assigned to its subgroup Cucujoidea (Clavicornia). Cucujiformia is supported as monophyletic by a number of autapomorphies (e.g. Klausnitzer 2005; Lawrence and Newton 1982) and also by the extensive molecular study of Hunt et al. (2007). However, phylogenetic relationships within Cucujiformia are widely unresolved, and Cucujoidea is very unlikely to be a monophyletic group (see Buder et al. 2008; Hunt et al. 2007).

Beetles and other insects have ectodermal (integumentary) glands of two different organisational levels: simple and compound. Simple (dermal) glands consist of a single gland unit opening individually on the body surface; a gland unit is composed of a few specialised cells, one or more being secretory. In compound glands several or numerous such gland units are combined upon a common outlet duct, which may additionally form a reservoir. The external opening of a compound gland is usually a fairly large pore visible through a stereo-microscope.

Compound integumentary glands are widespread in Coleoptera; they occur in very different positions and produce different secretions. However, data on their fine structure and occurrence throughout taxa are scarce. Among the Cucujiformia, the coccinellid *Semiadalia undecimnotata* has compound glands scattered over the head capsule, mouthparts, thorax and abdomen (Barbier et al. 1992). Most Tenebrionidae have a pair of large reservoirs in the abdomen (Tschinkel 1975), which originate from the membrane behind the seventh visible sternite. Anthicid and meloid beetles possess a large, paired mesothoracic gland opening ventrally in a slightly depressed area (Hemp and Dettner 1997; Morgan 1968; Berrioz-Ortiz 1985). Ciidae (Buder et al. 2008: Fig. 2) and some Erotylidae (*Coccimorphus*, *Erotylus*; Węgrzynowicz 2002) bear a gland associated with a hairy tuft on the first visible male abdominal sternite. In Chrysomelidae compound glands have been observed in several subfamilies (Pasteels et al. 1989); Chrysomelinae, Criocerinae, and some Galerucinae have morphologically similar glands in similar positions, mostly along the lateral and cranial margins of the pronotum.

It is well known that the Erotylidae show a particularly rich equipment of compound exocrine glands. Gland pores can occur along the lateral margin of the pronotum, on the prosternal and mesoventral intercoxal processes, on the head anteromesal to the compound eyes, on the subgenal brace, and rarely on the mentum and the mandibles. However, despite this manifold occurrence of such glands over the beetles´ body, their consideration in the previous literature is quite sparse. The distribution of the glandular pores was included in the phylogenetic studies of Węgrzynowicz (2002) and Leschen (2003). McHugh et al. (1997), in his work on the morphology of *Megalodacne heros*, presented an initial insight into the morphology of such a gland of an erotyline species, and he reported the secretion to be a clear odorous fluid. Further observations on the internal morphology as well as data on the ultrastructure of the glands are lacking. The biochemistry of the glands has also remained unexplored, nor is anything known about the ecological role of the glands in Erotylidae.

The present contribution provides a detailed study of the internal structure and ultrastructure of glands in examplar species of Erotylidae. We focused on the pronotal compound glands of *Tritoma bipustulata*, whose external openings are situated at the four corners of the pronotum, and studied these using SEM and TEM. In addition, we provide data on the homologous glands in *Triplax scutellaris*, on prosternal compound glands in *T. bipustulata*, and on dermal glands on the pronotum of *T. bipustulata*.

Material and methods

Specimens

Freshly killed specimens were available from *Tritoma bipustulata* (botanical garden of the University of Bayreuth, Germany, 49°55′27,46″N 11°35′14,53″E, 357 m asl; several males and females) and *Triplax scutellaris* (Bagnéres-de-Luchon, France, 42°46′02,43″N 0°38′35,66″E, 1064 m asl; one female).

Morphological studies, illustrations, and terminology

For SEM studies of internal structures the glands and surrounding integument of freshly killed specimens were dissected and macerated in 10% KOH for one day, then rinsed in H_2O and dehydrated with ethanol (25%, 50%, 75%, 100%, 30 min each). The single specimen of *Triplax scutellaris* was dissected and subsequently cleaned in 2 M aqueous solution of HCl for one day, then rinsed and dehydrated as described above (Quennedey et al. 2002). Afterwards the glands were transferred to acetone (first acetone : ethanol = 1:1, then pure acetone two times). All preparations for SEM were critical point dried (with CO_2 as transitional medium), coated with gold (Edwards S150B), and examined with a Philips/FEI XL 30 ESEM.

For serial sectioning and TEM studies the glands of freshly killed *Tritoma bipustulata* (2 ♂♂, 2 ♀♀) were dissected. Samples were fixed for 16 h in cold 2,5% glutaraldehyde in 0,1 M cacodylatebuffer (pH 7,3) and then transferred to 2% agarose. Afterwards samples were postfixed for 2 h in osmium tetroxide and for 90 min in uranylacetate; after dehydration, samples were embedded in Epon. The obtained resin blocks were trimmed (Leica EM Trim) and slices of about 50 nm were made using an

ultramicrotome (Leica Ultracut UCT). These slices were fixed on holders and stained with 2% uranylacetate and lead citrate; for examination a transmission electron microscope Zeiss EM 902A was used.

Drawings and plates were completed using the graphic computer programmes CorelPhotoPaint vers. 12 and CorelDraw vers. 12. The abbreviations used in the illustrations are also used in the text. In the terminology of glandular elements we mainly follow Noirot and Quennedey (1974, 1991).

Morphological orientations: For the internal structures here under consideration, we use 'proximal' for a location closer to the external origin and 'distal' for one further remote from the external origin. To address the two surfaces of the cuticle we use 'outer' for the one facing the outer world (here usually the lumen of some invagination), and 'inner' for the one facing the interior of the body (usually in contact with epidermal cells). This use of 'inner' and 'outer' is applied to cuticulised body wall areas no matter whether they are level, evaginated, or invaginated. Note that in case of invaginations (e.g. tubes) the outer surface of the cuticle is - seen from the perspective of the entire tube - located internal to the inner surface. We expand this use of 'inner' and 'outer' to structures lying beneath the cuticle, such as cell membranes.

Results

Structure of compound glands

The glands were examined initially by stereo-microscopy and then studied by scanning and transmission electron microscopy in *Tritoma bipustulata* (both sexes) and *Triplax scutellaris* (female). No differences between sexes were found in the former species.

In *Tritoma bipustulata* the pronotum bears a single distinct pore in each of its four corners (one indicated by arrow in Fig. 1b). From each pore a long, whitish (as seen in the stereo-microscope), pennate gland extends internally, as seen in the opened, macerated pronotum (Fig. 1a; see Fig. 1b for spatial correlation of pore and gland).

Each gland has a central excretory duct 300–330 μm long and 16–23 μm wide (near its proximal base), which is usually unbranched (Fig. 1a); only in a single case we found a dichotomy (Fig. 1c). The excretory duct is divided in two regions: The proximal part, about 50 μm long, has a smooth wall and lacks gland units. The much longer distal part has a wrinkled wall and bears numerous identical gland units (Figs. 1d, e). Observations using TEM confirm that the wall of the excretory duct is lined with cuticle (about 1 μm thick) on its entire inner surface (Fig. 2a) and is thus likely derived from an epidermal invagination. The outer (lumen-facing) surface of the cuticle is even, while the wrinkling seen on the inner (cell-facing) side of the cuticle (Fig. 1d) is due to strong linear thickenings of the cuticle (Figs. 2a, b).

Each gland unit consists of a large glandular ductule (gd) 13–15 μm long and a smaller lateral appendix (la) 3–4 μm long (Figs. 1d, e). Gland units were studied by SEM (Figs. 1d–g; macerated condition, i.e. only cuticle retained) and TEM (Figs. 2, 3; tissue retained) and are shown diagrammatically in Fig. 5.

Considering only the cuticular components seen in the macerations (Figs. 1d–g), both parts (gd and la) have a slender appearance and hardly narrow towards their distal ends. The ductule has a fairly narrow base where it originates from the excretory duct; ductules are easily, and apparently in a regular way, detached from the excretory duct along a ring-shaped rupture in this area (see scars along midline of duct ed in Fig. 1d). Slightly further distally the ductule is widened; from this part the lateral appendix (la) originates sideward, having a wide base embracing most of the ductule (arrows in Figs. 1d, f). Shortly distal to the origin of the appendix, the ductule has a distinct constriction (gdc in Fig. 1f, about 0,4 μm in diameter) and then becomes wider. The surfaces of the entire appendix and of the ductule distal to its constriction gdc are strongly spongiose (Figs. 1f, g); this likely represents the inner (cell-facing) surface of the cuticle in these macerated preparations.

Sections studied by TEM (Figs. 2, 3) show that the ductule has a canal with a defined lumen. The canal is lined with cuticle, which, however, is thinner than that along the excretory duct (Figs. 2b, 5b). The cuticle often appears to consist of two layers, which may represent a cuticulin (closer to the epithelium) and an epicuticular layer as specified in some previous contributions (Noirot and Quennedey 1974, 1991; Quennedey 1998). Around the opening of the ductule into the excretory duct the cuticle forms a valve- or ringwall-like structure (rw in Figs. 2b, 5b) of varied discreteness. The part of the canal in the proximal half of the ductule is called the conducting canal; in this part the cuticular wall is not perforated. The part of the canal in the distal half is the receiving canal; here the cuticle is penetrated by pores (white arrowheads in Fig. 3d; Fig. 5b). Distal to the constriction of the ductule (gdc), the cuticle is very thick and spongiose (ss in Figs. 2b, d, 3a, d); this causes the widening of the ductule seen in Fig. 1f, while the lumen of the ductule is not distinctly widened in this area. The cuticular pores along the receiving canal open into the cavities inside the spongiose layer, and there is thus altogether a labyrinth-like system of penetrations through the cuticle. Beyond the spongiose cuticular layer a narrow cell was observed (gdcl, Figs. 2b, c, 3a, d, 5b, d), which like a cap encloses the longer distal part of the ductule, proximally almost reaching the base of the lateral appendix. Along one flank of the ductule, the outer membrane of the

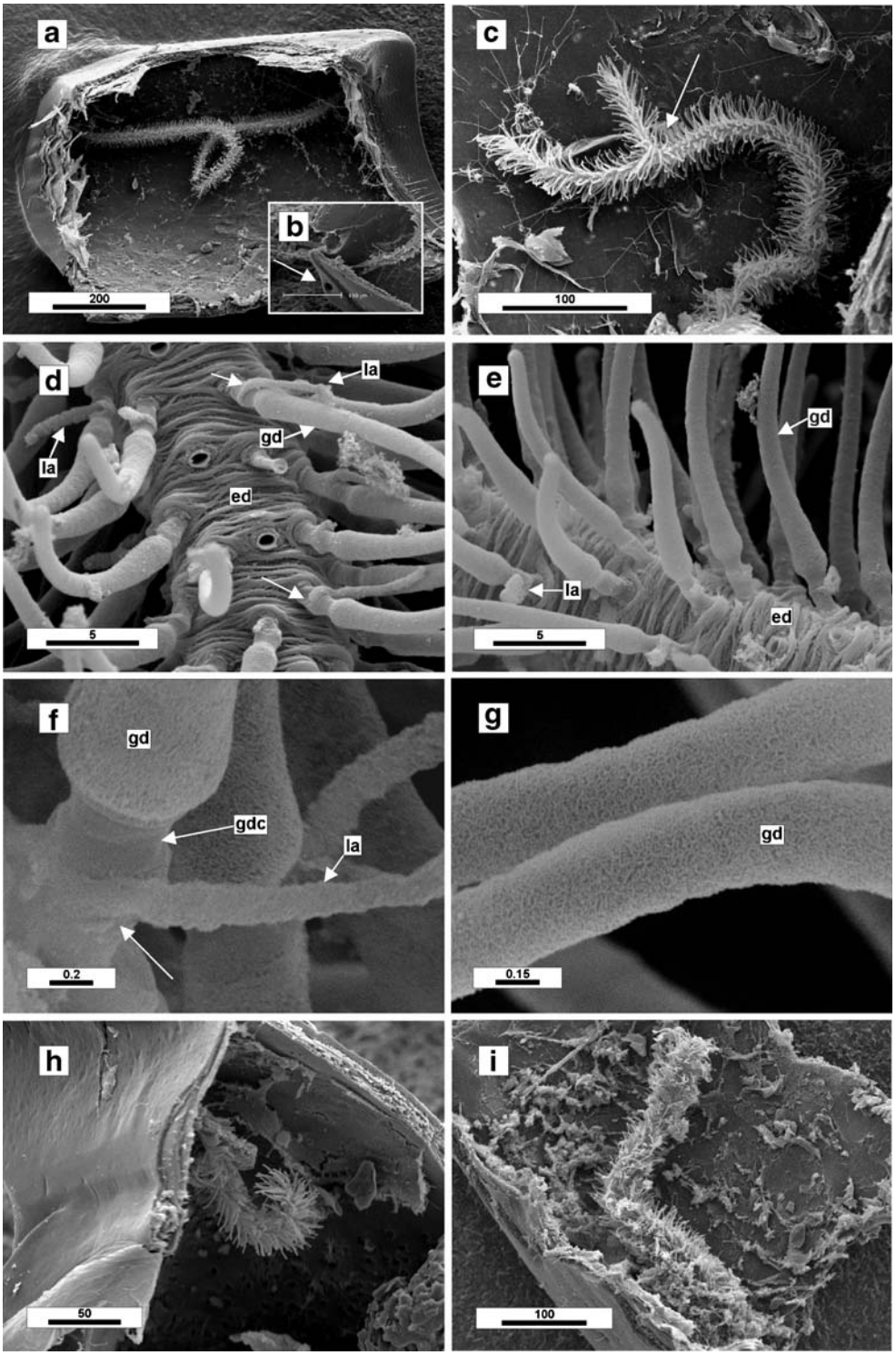

Fig. 1 SEM pictures of compound glands of two Erotylinae species. **a**–**g** Pronotal glands of *Tritoma bipustulata* after treatment with KOH. **a** Ventral view of inside of right half of pronotum, anterior corner of pronotum to the right. **b** Position of gland opening corresponds with position of externally visible pore (*arrow*). **c** Overall view of a branched gland (*arrow*). **d**, **e** Piece of main excretory duct (*ed*) of gland bearing glandular ductules (*gd*) and smaller lateral appendices (*la*) originating from base of glandular ductules (*unlabeled arrows*). **f** Base of a glandular ductule (*gd*), with constriction (*gdc*) and origin of lateral appendix (*la*); the cuticular filaments of lateral appendix embrace the base (*unlabeled arrow*). **g** Pieces of two glandular ductules (*gd*), showing their spongiose surface. **h** Prosternal gland of *T. bipustulata*. **i** Overall view of pronotal gland of *Triplax scutellaris*. Scale bars in μm

cell is in contact with the spongiose cuticular layer, but on the other side it is elevated from that layer, whereby a large extracellular cavity (ec in Figs. 2c, 3a, 5b, d) is present between cell membrane and cuticle: the central extracellular space. Inside the cell numerous mitochondria, tubular endoplasmatic reticulum as well as an extensive system of vesicles and cisternae are found (Figs. 2d, 3c, e, 5d).

The lateral appendix is also enclosed by a single cell (lacl, Figs. 2b, 3b, 5b, c). At the base of the appendix upon the ductule, this cell embraces the widened part of the ductule completely (Figs. 2b, 3b, 5b, c); this embracing portion is the small right part of the cell in Fig. 5b, and the small left part in Fig. 2b. Distally the membrane of cell lacl is in contact with the membrane of cell gdcl of the ductule

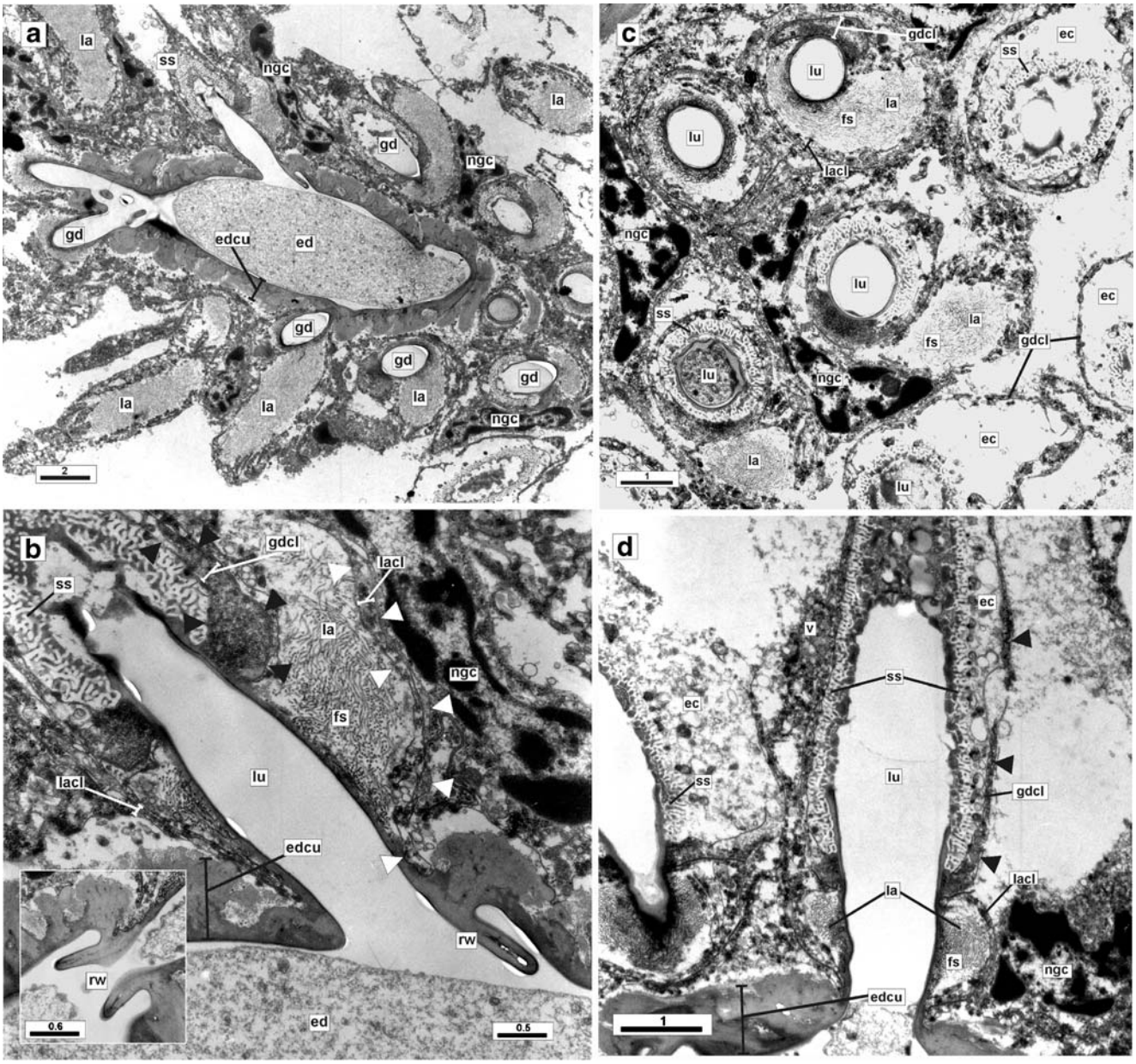

Fig. 2 TEM serial sections of pronotal gland of *Tritoma bipustulata*. **a** Overall view of sectioned gland, cut nearly perpendicular to axis of excretory duct (*ed*, filled), which is surrounded by several gland units (glandular ductules, *gd*, and lateral appendices, *la*). **b** Proximal part of glandular ductule with opening into excretory duct, sectioned approximately along axis of ductule and along axis of lateral appendix; the extracellular filamentous structure (*fs*) forming the core of the lateral appendix is enclosed by a narrow cell (lacl; delimiting membrane indicated by white arrowheads), which contains numerous vesicles; further distally (*left upper part*) a second cell (*gdcl*; delimiting membrane indicated by *black arrowheads*) encloses the conducting canal of the glandular ductule. The conducting canal has a lumen (*lu*) lined by cuticle (its distal part forming a spongiose layer, *ss*). Insert: Detail of opening of a glandular ductule into excretory duct. **c** Several glandular ductules (*gd*) sectioned nearly perpendicular to their axes at different levels, with distal secretory cell (*gdcl*), extracellular cavity (*ec*), and receiving canal with porous, spongiose cuticle; non-glandular (*ngc*) cells between sectioned ductules. **d** Proximal portion of a glandular unit sectioned approximately along axis of glandular ductule but perpendicular to axis of lateral appendix (of which only the narrow parts embracing the glandular ductule are seen); extracellular cavity of glandular ductule enclosed by the narrow secretory cell (*gdcl*; *black arrowheads*). Scale bars in µm

(Figs. 2b, d, 5b), i.e., there is no other, intercalary cell in contact with the gland unit in between these to cells. The nature of what is enclosed by cell lacl of the appendix—i.e., of the spongiose matter constituting the 'lateral appendix la' in the SEM pictures after maceration, Fig. 1f—is enigmatic: It is a homogeneous mass composed of numerous filaments (around fs in Figs. 2b, 3b, f, 5c), which originate from the cuticular intima of the ductule (Figs. 2b, d, 3b, f). The

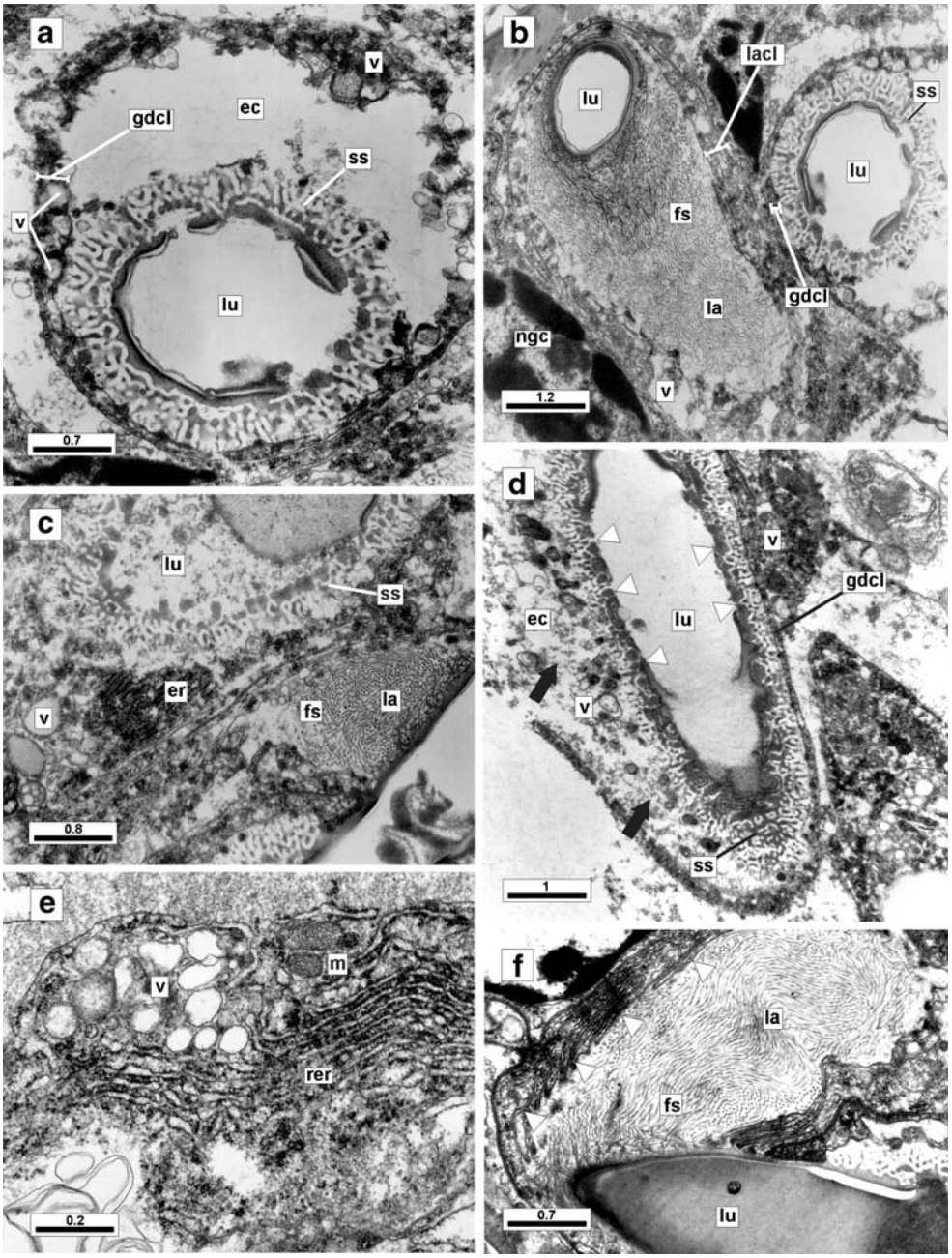

Fig. 3 TEM serial sections of a gland unit of the pronotal gland of *Tritoma bipustulata*. **a** Detail of glandular ductule in region of receiving canal, cut perpendicular to axis of glandular ductule; canal surrounded by a narrow dense and a wide spongiose layer (*ss*) of the cuticle, and the secretory cell (*gdcl*); extracellular cavity (*ec*) in upper part. **b** Detail of glandular ductule in region of lateral appendix, cut perpendicular to axis of glandular ductule; extracellular filamentous structure (*fs*) enclosed by a narrow vesicle-containing cell (*lacl*). **c** Glandular ductule cut perpendicular to its axis in region of receiving canal, with dense and spongiose (*ss*) layers of cuticle, extracellular cavity, and secretory cell (*gdcl*); endoplasmatic reticulum (*er*) and numerous vesicles (*v*) inside cell gdcl. **d** Glandular ductule cut nearly longitudinally in region of receiving canal; with pores (*white arrowheads*) in the dense and the spongiose (*ss*) cuticular layers of canal wall, and strands of secretion (*black arrows*) in extracellular cavity. **e** Vesicles, rough endoplasmatic reticulum, and mitochondria in a secretory cell. **f** Partly strongly folded membrane (*white arrowheads*) of cell (*lacl*) enclosing the filamentous structure *fs*. Scale bars in μm

persistence of the appendix—or rather of its filamentous mass (fs)—after clearing with KOH suggests that the filamentous structure is also cuticular in nature. The cell lacl enclosing the appendix contains numerous vesicles (Figs. 3b, c, d, f, 5c), and its outer membrane facing the filamentous structure shows intense folding in some areas (Fig. 3f).

Proximal to cell lacl, the non-glandular cells covering the excretory duct also ensheathe the most proximal part of the ductule (ngc in Figs. 2d, 5b). Additional non-glandular cells were observed between the gland units (ngc in Figs. 2b, c). Figure 2a shows that most of the pronotal gland is embedded in non-glandular cells, only the most distal portions of the ductules surpass this cell layer and are in contact with the hemolymph space. Furthermore, neither any innervation of the gland or gland units nor any muscle cells associated with the pronotal gland were observed (Fig. 2a).

SEM studies of a single, female specimen of a *Triplax scutellaris* showed the same arrangement and basic structure of the pronotal glands (Fig. 1i). The unbranched excretory duct (about 400 μm long) as well as the gland units are slightly larger than in the smaller species *T. bipustulata*.

The examined prosternal glands of *T. bipustulata* (Fig. 1h) are very similar to those of the pronotum but smaller (about 160 μm long). A pennate unbranched gland

originates from each of the two prosternal pores. The gland is also composed of an excretory duct blotched with numerous gland units, each including a ductule and an appendix.

Structure of pronotal dermal glands

In *Tritoma bipustulata* we additionally examined the whole internal surface of the pronotum, where we found numerous tubules of dermal glands distributed over the whole area (Fig. 4); we studied these glands by SEM after maceration with KOH, hence only their cuticular components. Each dermal gland consists of a single tubule, and depending on its shape four types are recognized. All have a long, slender proximal part, likely the conducting canal, and are abruptly widened further distally; this dilated part is likely a receiving canal coated with glandular cell(s) in the unmacerated condition. Two types of glands are characterized by a smooth and moderately wide dilatation (Figs. 4a, b); one of these bears an additional narrow distal part (Fig. 4a; tubule ca. 55–70 μm long), which is lacking in the other one (Fig. 4b; tubule ca. 30–33 μm long). The two other types (Fig. 4c) are characterized by a rough dilatation, which is shorter but wider than in the two former types. One of these types has a narrow appendage distal to the dilatation (tubule ca. 42–47 μm long), while the other type lacks this appendage (tubule ca. 20–25 μm long).

Discussion

Structure and function of compound glands

Concerning compound glands in Erotylidae, the description of the overall morphology of pronotal glands in *Megalodacne heros* (McHugh et al. 1997) is the only data avilable for comparison with our results. The glands of *M. heros* show a similar pennate structure as we found in *Tritoma bipustulata* and *Triplax scutellaris*. However, while the pronotal and prosternal glands in *T. bipustulata* and *T. scutellaris* most usually have an unbranched excretory duct, the pronotal glands of *M. heros* show multiple branching. This could be a phylogenetically informative character, but might be correlated with the size of a species.

Data concerning the fine structure of integumentary compound glands in adult cucujiform Coleoptera are available for *Tenebrio molitor* (Tenebrionidae; Delachambre 1973), *Semiadalia undecimnotata* (Coccinellidae; Barbier et al. 1992) as well as *Leptinotarsa decemlineata* (Chrysomelidae; Deroe and Pasteels 1977) and other leaf beetles (Deroe and Pasteels 1982). In addition, there are contributions on glands in cucujiform larvae (e.g. Bünnige and Hilker 2005).

A wrinkled cuticular surface of the excretory duct, as in *T. bipustulata* and *T. scutellaris*, is also found in the abdominal compound glands of many Tenebrionidae (Tschinkel 1975) and Staphylinidae (Dettner 1987; unpaired glands between first and second visible abdominal sternites). According to Tschinkel (1975), this reflects an ability to expand the reservoir. However, such a mechanism is unlikely for our Erotylidae, where wrinkling is not due to folding of the cuticle but to linear thickenings (the outer surface of the cuticle of the excretory duct is smooth, Fig. 2b), which would hardly allow for any expansion of the cuticle.

A gland unit of *T. bipustulata* contains a cuticular canal and thus belongs to class III of Noirot and Quennedey (1974, 1991). Class III gland units usually consist of three cells arranged in succession from proximal to distal: canal cell, intercalary cell, and sectretory (= terminal) cell. However, in many cases the unit is made up of only two cells, a non-secretory canal cell and a secretory terminal cell (Noirot and Quennedey 1991). The condition in *T. bipustulata* conforms with the latter arrangement (Fig. 5b): the cell around the lateral appendix (lacl) is a canal cell, and the one capping the glandular ductule (gdcl) is the secretory/terminal cell. We note that the canal cell (lacl) only encloses a short, far proximal part of the canal, while the secretory cell (gdcl) coats the much longer distal portion.

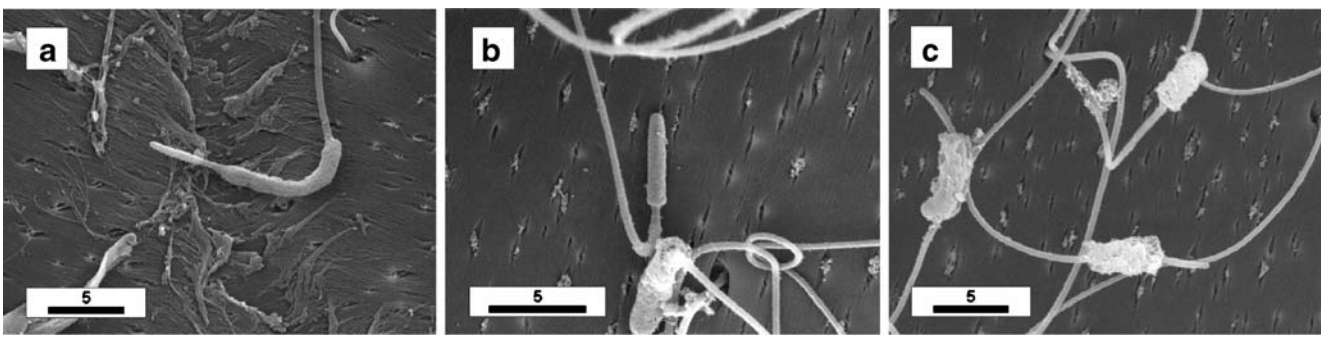

Fig. 4 SEM pictures showing tubules of dermal glands of four different types found in *Tritoma bipustulata* (after treatment with KOH). **a** Tubule with smooth dilatation followed distally by a narrow part. **b** Tubule with smooth dilatation and without a narrow part further distally. **c** Tubules with rough dilatation, either followed distally by a narrow part (*lower tubule*), or lacking this narrow part (*two upper tubules*). Scale bars in μm

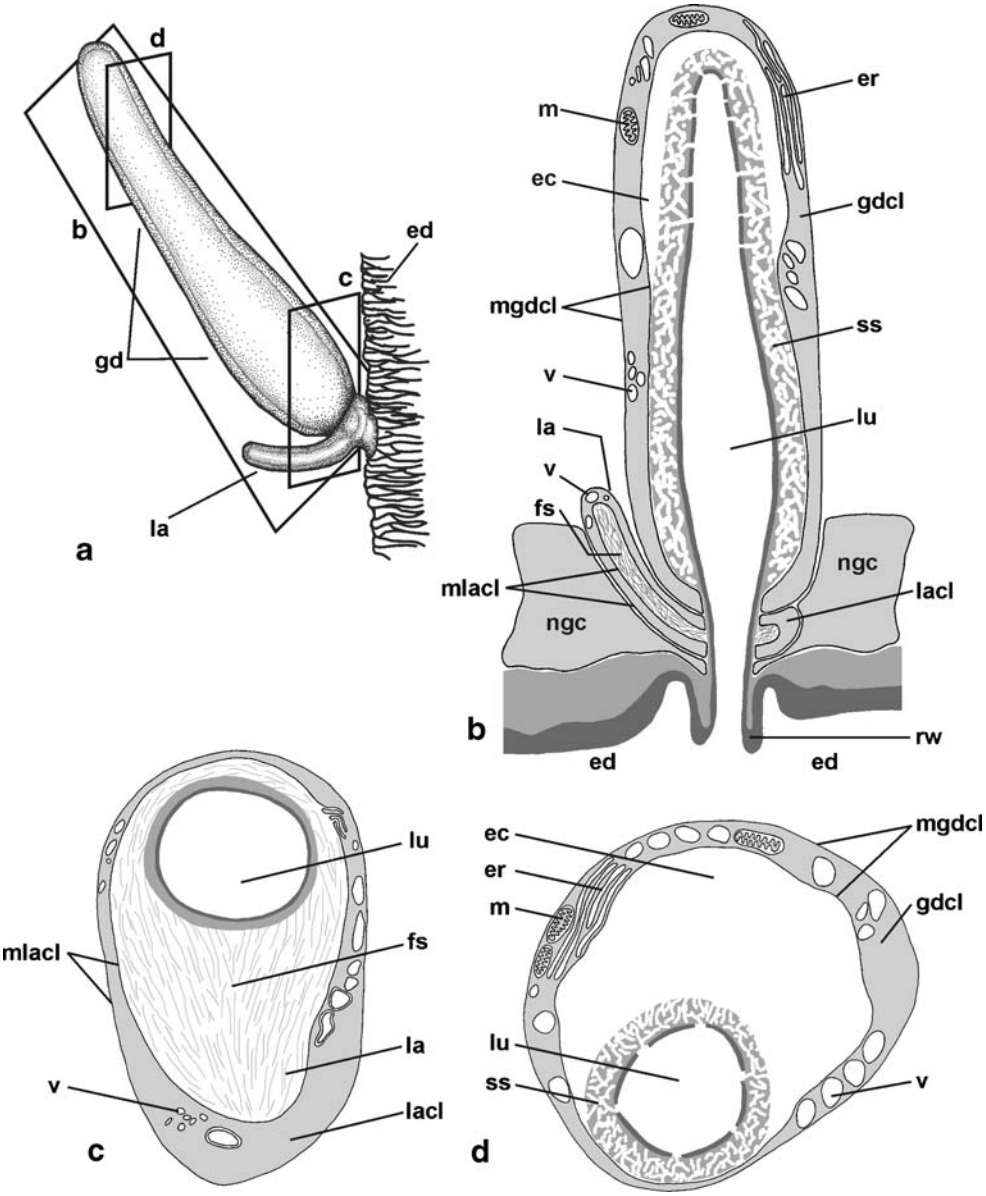

Fig. 5 Schematic drawing of a gland unit of the pronotal compound gland of *T. bipustulata*. **a** One gland unit upon excretory duct, with sectional planes shown in b, c, and d. **b** Longitudinal section through gland unit (compare Figs. 2b, d, 3d). **c** Cross-section through proximal region of gland unit; lateral appendix directed towards bottom (compare Figs. 2c, 3b, f). **d** Cross-section through distal region of gland unit (compare Figs. 2c, 3a). In b, c, and d, cuticle in *dark gray* (*darker outer layer*) or *medium gray* (*brighter inner layer*); cytoplasm in *light gray*; continuous *black lines* are membranes (of cells or cellular organelles)

In *T. bipustulata* the distal part of the canal of a glandular ductule clearly appears as a receiving canal due to the structure of its cuticle, which includes large pores in the dense superficial layer and a labyrinth of narrower cavities in the spongiose deeper layer (ss in Figs. 2b, d; Figs. 5b, d). Pores and cavities together establish numerous small open connections between the canal and the extracellular cavity. In addition, the cell coating this receiving canal (gdcl) shows clear signs of a secretory activity: It is rich in mitochondria and endoplasmatic reticulum (but see next section), and it includes a great amount of vesicles and cisternae. A spongiose structuring of the cuticle along the distal part of the canal of a gland unit has apparently not been found before in compound glands of beetles. In many taxa the membrane of the secretory cell facing the extracellular cavity forms microvilli (e.g. *Tenebrio molitor*, Delachambre 1973; *Semiadalia undecimnotata*, Barbier et al. 1992). Absence of microvilli, as in *T. bipustulata*, however, is also found in the staphylinid *Philontus varians* (Quennedey et al. 2002).

The extracellular filamentous structure (Figs. 2d, 3b, f, 5c) of the lateral appendix in the *T. bipustulata* gland unit is most likely derived from secretion by the canal cell, which completely coats this structure. Furthermore, as it stands maceration by KOH and as its filaments originate from the portion of the canal cuticle lying in the territory of the canal cell, it appears to be some cuticular secretion. A structure of this kind has apparently not been reported previously for glands of beetles or other insects. The function of the filamentous structure and even of the entire appendix is enigmatic: In contrast to the ductule, there is no true lumen inside the appendix—apart from narrow gaps between the

filaments and between the folds formed by some parts of the outer cell membrane (Fig. 3f). In addition, since the cuticular intima of the proximal part of the ductule lacks perforations (Fig. 2b), there is apparently no open connection between the core of the appendix and the canal of the ductule, so that probably no secretions can be contributed by the appendix. On the other hand, the conspicuous presence of small vesicles in the canal cell might indicate a secretory function. This would be in contrast to the usual properties of the canal cell in class III gland units according to Noirot and Quennedey (1974, 1991).

Musculature closely associated with insect epidermal glands were found, for instance, in *Agrypnus murinus* (Elateridae; Dettner and Beran 2000) and *Semiadalia undecimnotata* (Coccinellidae; Barbier et al. 1992). An innervation of secretory cells has been rarely reported; the salivary gland of *Periplaneta americana* (Whitehead 1971) is an example. In *T. bipustulata* neither particular gland muscles nor an innervation of secretory cells were here observed. The regular prothoracic muscles are probably involved in the discharging of the secretion from the glands.

Among the compound glands occurring in cucujiform beetles, those of Chrysomelinae are here of particular interest, since like in Erotylinae some glands open along the pronotal edges. In the chrysomeline *Leptinotarsa decemlineata* (studied by Deroe and Pasteels 1977), however, the internal structure of the glands differs strongly from that in Erotylidae. While in the erotylids here studied the gland units are seated upon an unbranched excretory duct in a pennate pattern (Fig. 1a), the excretory duct of *L. decemlineata*, at some distance from its opening, suddenly undergoes dense and rich branching, each branch leading to a gland unit. Nonetheless, considering the multibranched condition of the pronotal gland in *Megalodacne heros* (see above), this difference may not appear too striking. In *L. decemlineata* a gland unit has, like in *T. bipustulata*, cuticlar canals capped by secretory cells and thus also belongs to class III of Noirot and Quennedey (1974, 1991). Otherwise, however, the composition of a unit is very different: it includes a triramous cuticular canal, each ramus capped by a secretory cell. Other cells, like canal or intercalary cells were not observed. The three secretory cells of a gland unit are of two different types (one larger cell, C1; two smaller cells, C2). In the smaller ones microvilli surround the distal part of the cuticular canal, which are conspicuous when the cell is devoid of vacuoles. These structural differences between the chrysomeline and the erotylids make homology of the pronotal glands unlikely.

Ecological role of compound glands

The ecological function of the various glands in Erotylidae is unknown, and of course different glands may produce different secretions and have different functions. Since the glands, as far as studied herein, are similar and equally developed in both sexes, they are altogether no good candidates for the production of sexual pheromones. Yet it cannot be excluded that the secretions include compounds promoting aggregation independent of sex. Our initial chemical examinations of pronotal gland secretions in *Tritoma bipustulata* revealed that no proteinaceous compounds are included (despite the presence of endoplasmatic reticulum in the secretory cells, Figs. 3c, e). Instead, there are numerous compounds that are generally assumed to have antimicrobial properties, and others that would be suited for chemical defense against arthropods (K. Drilling, work in progress). This and the fact that many Erotylidae exhibit striking colours, frequently in combination with conspicuous patterns of stripes, spots, or rings, suggest that glands in Erotylidae might have a combined anti-microbial and defensive function.

Occurrence and structure of dermal glands

On the inner side of the pronotum of *Tritoma bipustulata* we found four different types of dermal glands with a cuticular canal (Fig. 4) probably surrounded by a single gland unit. Similar dermal glands are known from many insects (and may be ubiquitous), such as Dictoyptera (e.g. Brossut and Sreng 1980; Quennedey 1969), Mecoptera (Crossley and Waterhouse 1969), Hemiptera (e.g. Farine 1987, 1988), and Coleoptera (e.g. Staphylinidae: Kellner and Dettner 1992; Chrysomelidae: Hilker et al. 1992; Tenebrionidae: Delachambre 1973; Scarabaeidae: Plout-Sigwalt 1991). They are usually distributed in large numbers over larger areas of the body wall, but these areas can differ among insect taxa. Nonetheless, often only the areas studied may be different ones: Not in all beetle taxa represented in these studies the pronotum was searched for such glands—just as in the present study only the pronotum was searched. Different structural types of dermal glands are discernable in almost every examined taxon. Thus, features concerning the structure and spatial distribution of dermal glands could provide a new interesting character system for phylogenetic work in Coleoptera and also in Erotylidae.

In Scarabaeidae dermal glands are scattered over the abdominal sternites. There is an evolutionary trend towards a grouping and increasing differentiation of the gland units, and a formation of reservoirs (Plout-Sigwalt 1991); this may show how compound glands can evolve from a dense vestiture of simple dermal glands. This is of particular interest with regard to the rich equipment with compound glands in Erotylidae. Forthcoming studies on this topic could examine whether in taxa related to Erotylidae—or in Erotylidae that lack particular gland pores present in others—

there is a particularly dense placement of dermal glands in those body areas where (other) Erotylinae have compound glands. On the other hand, the cuticular structure of all dermal gland types we found on the pronotum of *T. bipustulata* is quite different from that of the gland units in the pronotal compound gland of this species. This is somewhat in conflict with the assumption of such an evolutionary origin of compound glands in Erotylidae.

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References

Barbier, R., Ferran, A., Le Lannic, J., & Allo, M.-R. (1992). Morphology and ultrastructure of integumentary glands of *Semiadalia undecimnotata* Schn. (Coleoptera: Coccinellidae). *International Journal of Insect Morphology and Embryology, 21*(3), 223–234.

Berrioz-Ortiz, A. (1985). The presence of mesothoracic glands in *Epicauta segmenta* (Coleoptera: Meloidae) and other blister beetles. *Journal of the Canadian Entomological Society, 58*, 179–181.

Brossut, R., & Sreng, L. (1980). Ultrastructure comparée des glandes exocrines abdominales des Blattaria (Dictyoptera). *International Journal of Insect Morphology and Embryology, 9*, 199–213.

Buder, G., Grossmann, C., Hundsdoerfer, A., & Klass, K.-D. (2008). A contribution to the phylogeny of the Ciidae and its relationships with other cucujoid and tenebrionoid beetles (Coleoptera: Cucujiformia). *Arthropod Systematics and Phylogeny, 66*, 165–190.

Bünnige, M., & Hilker, M. (2005). Do "glanduliferous" larvae of Galerucinae (Coleoptera: Chrysomelidae) possess defensive glands? A scanning electron microscopic study. *Zoomorphology, 124*, 111–119.

Crossley, A. C. S., & Waterhouse, D. F. (1969). The ultrastructure of a pheromone secreting gland in a male scorpion fly *Harpobittacus australis* (Bittacidae, Mecoptera). *Tissue and Cell, 1*, 273–294.

Delachambre, J. (1973). L´ultrastructure des glandes dermiques de *Tenebrio molitor* L. (Insecta, Coleoptera). *Tissue and Cell, 5*, 243–257.

Deroe, C., & Pasteels, J. M. (1977). Defensive mechanisms against predation in the Colorado beetle (*Leptinotarsa decemlineata*, Say). *Archives de Biologie, Bruxelles, 88*, 289–304.

Deroe, C., & Pasteels, J. M. (1982). Distribution of adult defense glands in chrysomelids (Coleoptera: Chrysomelidae) and its significance in the evolution of defense mechanisms within the family. *Journal of Chemical Ecology, 8*(1), 67–82.

Dettner, K. (1987). Chemosystematics and evolution of beetle chemical defenses. *Annual Review of Entomology, 32*, 17–48.

Dettner, K., & Beran, A. (2000). Chemical defense of the fetid smelling click beetle *Agrypnus murinus* (Coleoptera: Elateridae). *Entomologia Generalis, 25*(1), 27–32.

Farine, J. P. (1987). The exocrine glands of *Dysdercus cingulatus* (Heteroptera, Pyrrhocoridae): morphology and function of nymphal glands. *Journal of Morphology, 194*, 195–207.

Farine, J. P. (1988). The exocrine glands of *Dysdercus cingulatus* F. (Heteroptera, Pyrrhocoridae): morphology and function of adults´ gland. *Annales de la Société Entomologique de France (NS), 24*, 241–256.

Hemp, C., & Dettner, K. (1997). Morphology and chemistry of mesothoracic glands in anthicid beetles (Coleoptera: Anthicidae). *Entomologia Generalis, 22*(2), 97–108.

Hilker, M., Daloze, D., & Pasteels, J. M. (1992). Cardenolide glycosides from the adults and eggs of *Chrysolina fuliginosa* (Coleoptera: Chrysomelidae). *Experientia, 48*, 1023–1027.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., St John, O., Wild, R., et al. (2007). A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science, 318*, 1913–1916.

Kellner, R. L. L., & Dettner, K. (1992). Comparative morphology of abdominal glands in Paederinae (Coleoptera: Staphylinidae). *International Journal of Insect Morphology and Embryology, 21*(2), 117–135.

Klausnitzer, B. (2005). 26. Ordnung: Coleoptera, Käfer; Part: Polyphaga. In H. H. Dathe (Ed.), *Lehrbuch der Speziellen Zoologie, Band I, 5. Teil: Insecta* (Vol. 2, pp. 452–526). Heidelberg: Spektrum Akademischer Verlag.

Lawrence, J. F., & Newton, A. F. (1982). Evolution and classification of beetles. *Annual Review of Ecology and Systematics, 13*, 261–290.

Leschen, R. A. B. (2003). Erotylidae (Insecta: Coleoptera: Cucujoidea): phylogeny and review. *Fauna of New Zealand, 47*, 1–108.

McHugh, J. V., Marshall, C. J., & Fawcett, F. L. (1997). A study of adult morphology in *Megalodacne heros* (Say) (Coleoptera: Erotylidae). *Transactions of the American Entomological Society, 123*(4), 167–223.

Morgan, A. (1968). The occurrence of a pair of exocrine glands in the metathorax of *Lytta nutalli* (Coleoptera: Meloidae). *Canadian Journal of Zoology, 46*, 915–917.

Noirot, C., & Quennedey, A. (1974). Fine structure of insect epidermal glands. *Annual Review of Entomology, 19*, 61–80.

Noirot, C., & Quennedey, A. (1991). Glands, gland cells, glandular units: some comments on terminology and classification. *Annales de la Société Entomologique de France, 27*(2), 123–128.

Pasteels, J. M., Rowell-Rahier, M., Braekman, J. C., Daloze, D., & Duffey, S. (1989). Evolution of exocrine chemical defense in leaf beetles (Coleoptera: Chrysomelidae). *Experientia, 45*, 295–300.

Plout-Sigwalt, D. (1991). Le systéme glandulaire abdominal des Coleoptéres coprophages Scarabaeidae: ses tendances èvolutives et ses relations avec la nidification. *Annales de la Société Entomologique de France (NS), 27*, 205–229.

Quennedey, A. (1969). Innervation de type neurosécréteur dans le glande sternale de *Kalotermes flavicollis* (Isoptera). Etude ultrastructurale. *Journal of Insect Physiology, 15*, 1807–1814.

Quennedey, A. (1998). Insect epidermal gland cells: Ultrastructure and Morphogenesis. In F. W. Harrison & M. Locke (Eds.), *Microscopic anatomy of invertebrates* (Vol. 11A, pp. 177–207). London: Wiley-Liss Inc.

Quennedey, A., Drugmand, D., & Deligne, J. (2002). Morphology and ultrastructure of paired prototergal glands in the adult rove beetle *Philonthus varians* (Coleoptera: Staphylinidae). *Arthropod Structure and Development, 31*, 173–183.

Robertson, J. A., McHugh, J. V., & Whiting, M. F. (2004). A molecular phylogenetic analysis of the pleasing fungus beetles (Coleoptera: Erotylidae): evolution of colour patterns, gregariousness and mycophagy. *Systematic Entomology, 29*, 173–187.

Tschinkel, W. R. (1975). A comparative study of the chemical defensive system of tenebrionid beetles. 3. Morphology of the glands. *Journal of Morphology, 145*, 355–370.

Węgrzynowicz, P. (2002). Morphology, phylogeny and classification of the family Erotylidae based on adult characters (Coleoptera: Cucujoidea). *Genus, 13*(4), 435–504.

Whitehead, A. T. (1971). The innervation of the salivary gland in the American cockroach: light and electron microscopic observations. *Journal of Morphology, 135*, 483–505.

Manuscript III
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First insights in the chemical defensive system of the erotylid beetle, *Tritoma bipustulata*

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Abstract. The present study gives first insights in the chemical defensive system of the erotylid beetle *Tritoma bipustulata*, and reports the previously unknown ability of abdominal reflex bleeding in this coleopteran family. The defensive chemistry of the pronotal glands, the abdominal reflex blood as well as the hemolymph were analysed by GC-MS. The secretions were dominated by aromatic hydrocarbons; furthermore we detected alkenes, ketones, organic acids as well as a single sesquiterpene. The majority of the detected compounds had strong antimicrobial properties in microbiological assays with entomopathogenic micro-organisms. In behavioural tests only benzyl alcohol, benzothioazole, indole and 3-methylindole, detected in the abdominal reflex blood, were significantly deterrent to ants.

Key words. Erotylidae, gland, reflex bleeding, repellent, *Lasius flavus*.

1. Introduction

Many Coleoptera possess various types of glands which secrete repellent, irritating or alluring chemicals. These chemicals are used in intraspecific relationships or in encounters with potential predators (Roth and Eisner 1962, Dettner 1987, Laurent et al. 2005a, Francke and Dettner 2005). The defence systems of several coleopteran families have been the subject of intensive research in the last years (e.g. Staphylinidae; Klinger and Maschwitz 1977, Dettner 1991, Steidle and Dettner 1992, Tenebrionidae; Tschinkel 1975, Chrysomelidae; Pasteels et al. 1988, 1989, 1994; Coccinellidae; Daloze et al. 1994), while others were rather unnoticed. One of these disregarded families is the Erotylidae. The cosmopolitan family is assigned to the superfamily Cucujoidea (Clavicornia) of the Coleoptera-Cucujiformia and comprises about 3500 described species (including members of the former family Languriidae) in approximately 258 genera (Leschen et al. 2010). Leschen (2003) proposed a new classification of the family Erotylidae, where Erotylinae (= Erotylidae in the old, limited sense) stands beside five other subfamilies (together the former Languriidae); subfamilies of the former Erotylidae are now ranked as tribes of Erotylinae. The former separation of these two groups was primarily based on their different biology, with "Erotylidae" being mycophagous and "Languriidae" being phytophagous.

Apart from optical, acoustical, and tactile cues, transfer of information by volatile compounds plays a pivotal role in the communication between species. The presence of a particularly rich equipment of compound glands is well known for members of the family Erotylidae. These glands occur within the pronotal angles respectively along the lateral margin of the pronotum, on the prosternal and mesoventral intercoxal processes, on the head anteromesal to the compound eyes, on the subgenal brace and rarely on the mentum (similar to Histeridae and Chrysomelidae). Despite this manifold occurrence of compound glands as soon as the fact that most erotylid species exhibit striking colours, frequently in combination with conspicuous patterns of stripes, spots or rings their consideration and chemical examination in the previous literature is quite sparse or rather absent.

Extensive investigations on chemical defence and secondary compounds (pheromones) were carried out, and the knowledge as well as the published literature has grown significantly during the past decades. Reviews on chemical defence of beetles and certain cucujoid taxa are given by Tschinkel 1975 (Tenebrionidae), Pasteels et al. 1988, 1989, 1994 (Chrysomelidae), Daloze et al. 1994 (Coccinellidae), Dettner 1987, Francke and Dettner 2005, and Laurent et al. 2005. However, the diversity of defensive chemicals produced by the insects themselves or through other organisms is amazing, and numerous remarkable compounds were identified. The often aposematic coloured Coccinellidae as well as the related Endomychidae show reflex bleeding and offer a large spectrum of repellent and bitter alkaloids, pyrazines and lactones (Dettner 1987; Daloze et al. 1994; Laurent et al. 2005). Macrocyclic lactones are also typical components of cucujid beetles, which have been given the trivial name cucujolides (Oehlschlager et al. 1987, 1988). Most species of the usually nocturnal tenebrionids have large abdominal defensive glands, which produce mainly quinoic mixtures in admixture with diverse alkenes (Tschinkel 1975; Dettner 1987). For species of Nitidulidae rather stereotypic structures like methyl- and ethyl-branched aliphatic hydrocarbons

with three or four (*E*)-configured conjugated double bonds were reported (Bartelt 1999). The chemically unique monoterpene anhydrid cantharidin is reported for both Oedemeridae and Meloidae. This hemolymph toxin represents a powerful vesicant, insecticide, and feeding deterrent (Dettner 1987).

In the present study we were focused on *Tritoma bipustulata*. Although erotylid-species are infrequently found, *T. bipustulata* is one of the most abundant erotylid-species in central Europe. With two basal red spots on the black elytra and the three-segmented antennal club, this fungivorous species is easily to identify (Vogt 1967). Larvae and adults prefer fruiting bodies of *Polyporus* spp., *Lenzites* spp. and *Daedalea* spp. on reclined rottened wood of *Fagus* spp. and *Quercus* spp. (Koch 1989). The contribution provides first insights in the chemical defensive system of *T. bipustulata*, an examplar species of Erotylinae. Furthermore, we report the previously undescribed ability and chemistry of the abdominal reflex bleeding in the same species. We focused on the secretion of the large pronotal glands, whose external openings are situated at the four corners of the pronotum. In behavioural and microbiological tests we demonstrated the repellency and irritancy of several detected substances to ants and entomopathogenic micro-organisms.

2. Material and Methods

Specimens

Specimens of *Tritoma bipustulata* (FABRICIUS, 1775) were collected, along with the fungus *Trametes versicolor*, between May and June 2007 in botanical garden of the University of Bayreuth (49°55´25,66´´N, 11°34`59,28´´E, 350 m). Beetles were maintained in our laboratory (20°C; LD 16/8 cycle) in caged groups on their host fungus. The animals mated, oviposited and coexisted at all developmental stages on *T. versicolor*. They were given at least two weeks of undisturbed confinement before being used for chemical purposes.

Chemical analyses

Different methods for collecting the secretion have been used to obtain suitable results. The glandular secretions of *Tritoma bipustulata* were collected by using the closed loop stripping technique (Boland et al. 1984). 45 beetles were enclosed by an oven bag (Neoten®, 30 x 30 cm) and were irritated frequently by shaking and slight pressure. Emitted volatiles were trapped in an adsorbent tube filled with 20 mg of a 1:1 mixture of Tenax-TA 60-80 and Carbotrap 20-40. The air was exhausted from the bag, passing the adsorbent, by a membrane pump (G12/01 EB, Rietschle Thomas, Puchheim, Germany). Volatiles were eluted with 80 µl of acetone (SupraSolv, Merck KGaA, Germany); obtained samples were frozen (- 25 °C) for chemical analyses. An analysis of the empty oven bag was carried out to estimate possible contaminations.

For analysis of the abdominal secretion the beetles were irritated at the abdominal region (by forceps) and the oozing secretion was collected by using capillary tubes (volume 1µl, Hirschmann Laborgeräte, Eberstadt). For comparative analysis with the hemolymphe, the forelegs of living

specimens were dissected and emergent hemolymph was collected by using the capillary tubes. These were inserted into the GC/MS directly.

EI and CI mass spectra were obtained on a Finnigan MAT GCQ (gas chromatograph with combined mass spectrometer) operated at 70 eV using a DB-5 column (5% phenyl polysiloxane; length 25 m, i.d. 0.25 mm, film thickness 0.25 µm, SGE). MS in the CI mode was performed with methane as reactant gas. The oven temperature was kept at 50°C for 2 min, and raised at 10°C min^{-1} to 250 °C in all analyses. Component identification was carried out using the NIST 02 mass spectral data base, or MassFinder 3, and confirmed by comparison of retention times of authentic standards or by comparison of retention indices and mass spectra from literature (NIST08).

Morphological studies

For SEM studies of abdominal structures freshly killed specimens were used; for this several specimens were transferred to liquid nitrogen before and after discharging the reflex blood. All preparations for SEM were dried, coated with gold (Edwards S150B), and examined with a Philips/FEI XL 30 ESEM.

SDS-PAGE

SDS-PAGE analysis was performed with the Phast system (Pharmica, Freiburg Germany) using high-density gels according to the manufacturer's instructions. Protein bands were stained with Coomassie Blue.

Behavioural assays

To evaluate the biological significance of the glandular compounds we performed a two-choice bioassay with *Lasius flavus*. Ants were collected from several sites near Bayreuth. Colony fragments composed of groups of 200-300 workers were housed in plastic boxes (40 x 40 cm), the walls were treated with teflon (Hostaflon®, Hoechst AG) to prevent escape. Ants were fed on honey water, freshly killed insects, and artificial diet; they starved about 5 days before the behavioural tests were conducted.

Compounds for behavioural assays were solved in UHT-milk (+ 1.5g saccharose/100ml) due to low water solubility of some compounds. A dilution of 0.1 mg/ml was tested; for control pure milk (+ saccharose) was used. The test solution as well as the control were applied on a microscope slide (20 µl each) and were placed in the colony. After contact with the test solution or the control, ants were counted and removed from the colony. A single test took 5 minutes; 10 recurrences were conducted.

Microbiological analyses

The microbiological properties of the detected compounds were tested by agar diffusion tests. For this purpose we used two entomopathogenic micro-organisms (*Serratia entomophila*, *Bacillus sphaericus*) and *Escherichia coli*. Besides *E. coli*, all bacteria were obtained from the "German Collection of Micro-organisms and Cell Cultures" (DSMZ; Tab. 1). The micro-organisms were

inoculated in tryptic soy broth (Merck KgaA, Darmstadt) and incubated at 130 rpm and 28 °C for 24 h. When the suspension reached the turbidity of the 0.5 McFarland standard (Andrews 2005) 100 µl were plated on petri dishes with 20 ml tryptic soy agar. The petri dishes were split into four equal sized sectors and a drug-impregnated disc (concentration of 1.5 mg / ml) was placed on each sector. Subsequently the plates were incubated for 18 h at 30 °C. Tygacil®, a glycylcycline antibiotic that inhibits the protein translation in bacteria was used as control to judge the size of the inhibition zone. Tygacil® discs were supplied with a concentration of 15 µg / disc. The diameters of the zones of inhibition were measured to the nearest whole millimeter using a piece of scale paper held to the petri dish bottom.

Tab. 1. Bacteria used for agar diffusion tests.

Bacteria	Strain
Escherichia coli	K12
Serratia entomophila	DSM 12358
Bacillus sphaericus	DSM 1867

Statistical analyses

Statistical analyses were performed in SPSS for Windows version 13.0 (SPSS Inc., Chicago, USA); graphics were made in SigmaPlot for Windows version 10.0, graph package (Systat Software Inc., Point Richmond, CA, USA). Distributions of data as well as homogeneity of variances were checked by Shapiro-Wilk test and Levene test. Variances of data were homogenous but predominantly not normally distributed. Hence we performed the non-parametric Mann-Whitney-U-test (error level of 5%; Dytham 2001).

3. Results

The defense

Adults respond to disturbance by emitting secretion from their pronotal glands. A clear odourless fluid is oozing out (when disturbed), spreading over the pronotum and volatilises rapidly. The insects respond only to direct contact stimulation; movement nearby or minute molestations induced no discharge of secretion. However, beetles are able to emit secretion several times consecutively.

Furthermore, when considerably disturbed at the abdominal tip a clear, malodorous and highly volatile secretion was simultaneously discharged from this body region. This secretion disperses over the tergum and the rear of the elytra. Both the detected chemical (GC-MS) and proteinaceous (SDS-PAGE) patterns in the abdominal secretion as well as in the hemolymph were entirely equal (Figs. 3A, B; 4). Despite extensive examinations, no glandular structures were detected in this body part. Regrettably it was not possible to demonstrate sutures or weak areas within the intersegmental membranes, responsible for the release of the blood. However, we found small grooves at the abdominal tip, which facilitate the discharge of hemolymph (Fig. 1A); also remains

of solidified hemolymph were detected (Fig. 1B). On the basis of the above mentioned results, we interpreted the abdominal secretion as reflex bleeding.

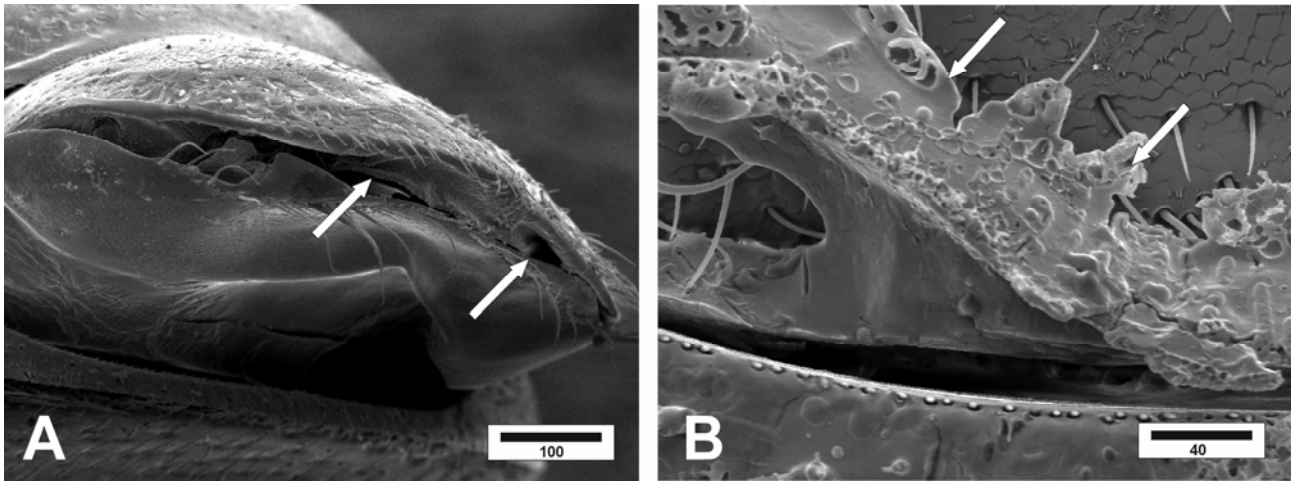


Fig. 1. Position at the abdominal tip of *Tritoma bipustulata*, where the reflex blood is released. (A) Small grooves (arrows) at the last tergite enhance the discharge of the abdominal reflex bleeding. (B) Remains of solidified secretion at the abdominal tip (arrows) after the release of reflex blood. Scales in μm.

Chemistry

Although GC-MS analyses of the secretion from the pronotal glands of *T. bipustulata* yielded more than 30 peaks, 10 could be allocated to the beetles defensive secretion with certainty and the structure of 7 compounds could be confirmed (*1* to *10*, Fig. 2 and Tab. 2, compound numbers in italics). A comparison with the eluted compounds of the oven bag showed a lot of contamination (B, C and S in Fig. 2). Minor compounds were identified as six aromatic hydrocarbons (*1* to *5*, *7*), one sesquiterpene (*6*), one ketone (*8*) and two alkanes (*9*, *10*). The identity of following compounds was determined by injection of authentic substances (Tab. 2; Fig. 8A): Benzaldehyde (*1*), benzyl alcohol (*2*), benzothiazole (*3*), anisaldehyde (*5*), benzophenone (*8*) as well as the two alkanes tricosane (*9*) and pentacosane (*10*). The main compound (*7*) was unfortunately not identifiable so far. EI and CI-mass spectra indicate the presence of an aromatic compound (strong signals at m/z 79, 91, and 105) with a moderately abundant molecular ion at M = 176 (Fig. 7). Typical fragments are m/z 55(24), 67(60), 79(71), 91(base peak), 93(97), 105(46), 107(92), 121(68), 133(89), 147(2), 151(36), 161(33) and 176(3). A SDS-PAGE analysis of the pronotal secretion revealed no proteins at all (column 3 in Fig. 4).

In the crude extracts of the abdominal reflex bleeding as well as the tested hemolymph 16 compounds were detected by GC-MS constantly (*1* to *16*, Figs. 3A, B and Tab. 3, compound numbers in italics). The increase of the TIC in Figs. 3A, B seems to be caused by the high amounts of proteins and sugars present in the sample. The main component in the abdominal reflex blood was identified as indole (*7*, Figs. 3A, 8B). Furthermore, we were able to clarify the structures of the following compounds (Tab. 3; Fig. 8B): Phenol (*1*), *p*-cresole (*3*), 3-methylindole (= skatole; *9*), 2-phenylpyridine (*10*), lauric acid (*11*), palmitic acid (*13*) and pentacosane (*16*). In the

chemical composition of the reflex blood as well as the hemolymph no differences between the sexes were detected.

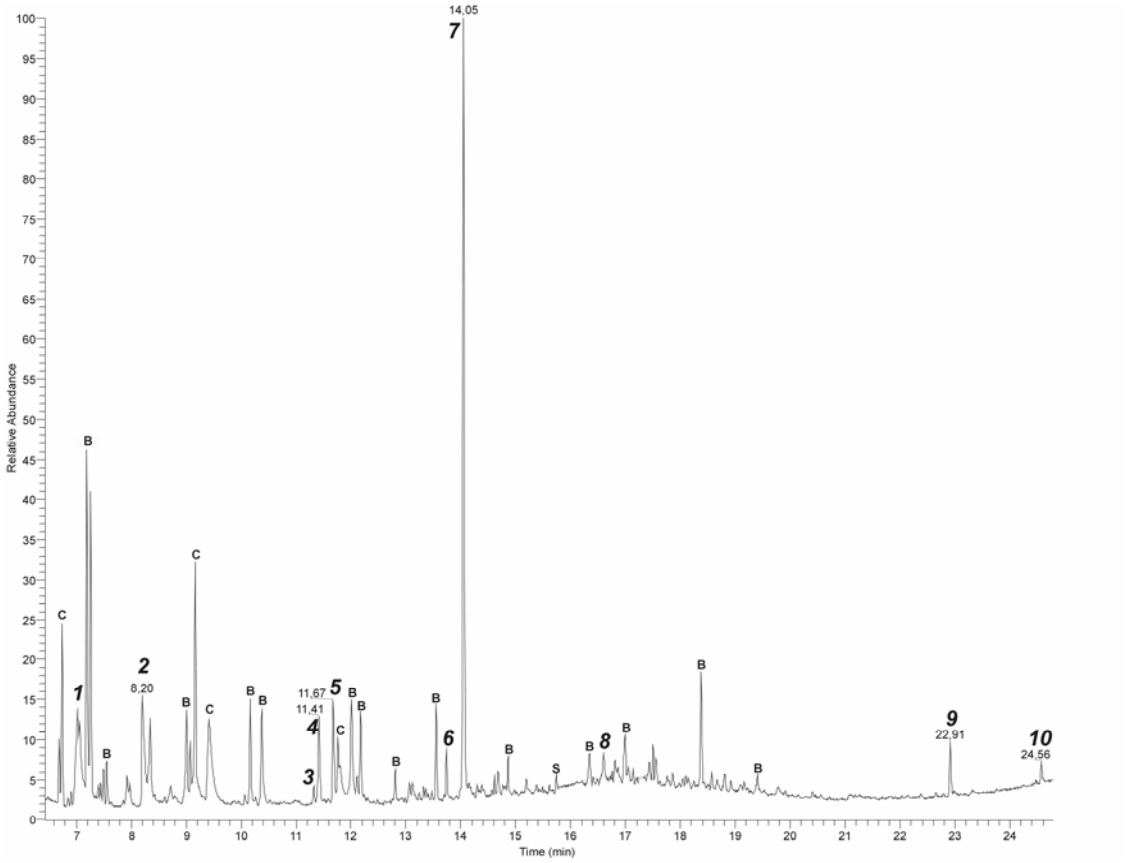


Fig. 2. Total ion chromatogram (TIC) of the pronotal secretion of 45 adult *T. bipustulata*. Analytical conditions are described in the text. B = compound also present in the sample of the oven bag; C = column; S = phthalates; numbering of compounds according to Tab. 2.

Tab. 2. Compounds identified in the pronotal secretion of *T. bipustulata*.

No.	RI	RI (Lit.)	Compound	MW	Structure class
1	980	960	**Benzaldehyde**	106	Aromatic hydrocarbon
2	1057	1051	**Benzyl alcohol**	108	Aromatic hydrocarbon
3	1261	1246	**Benzothiazole**	135	Aromatic hydrocarbon
4	1268	/	Unknown [a]	150	Aromatic hydrocarbon
5	1285	1262	***p*-Anisaldehyde**	136	Aromatic hydrocarbon
6	1437	/	Unknown [b]	204	Sesquiterpene
7	1460	/	Unknown [c]	176	Aromatic hydrocarbon
8	1669	1655	**Benzophenone**	182	Ketone
9	2300	2300	**Tricosane**	324	Alkane
10	2500	2500	**Pentacosane**	352	Alkane

[a] mass spectral data of the unknown compound 4: 54(35), 77(37), 79(38), 91(100), 93(86), 105(15), 106(23), 107(36), 108(21), 135(8), 150(3); [b] mass spectral data of the unknown compound 6: 51(8), 65(13), 67(27), 77(32), 78(9), 79(82), 91(100), 93(44), 95(21), 105(79), 107(33), 119(48), 120(15), 133(46), 134(17), 135(17), 147(28), 161(67), 162(14), 175(12), 189(21), 204(9); [c] mass spectral data of the unknown compound 7: 51(10), 53(10), 55(24), 65(23), 67(60), 77(37), 79(71), 80(13), 81(31), 91(100), 93(97), 94(16), 95(16), 105(46), 107(92), 108(15), 109(30), 119(13), 121(68), 133(84), 134(11), 136(29), 151(36), 161(33), 176(3).

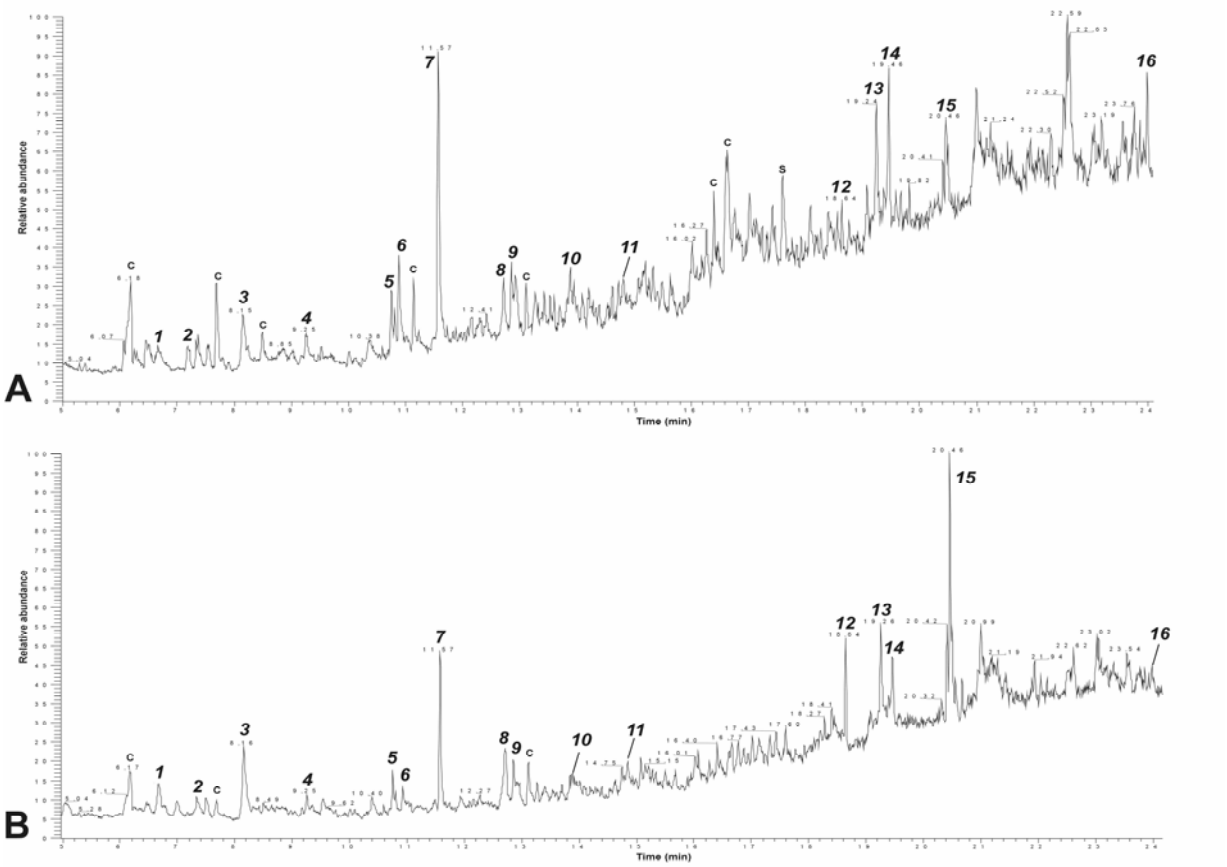


Fig. 3. Total ion chromatogram (TIC) of (A) abdominal reflex bleeding from three males and (B) hemolymph from a cutted leg of a single male of *T. bipustulata*. Analytical conditions are described in the text. C = column; S = phthalates; numbering of compound according to Tab. 3.

Tab. 3. Compounds identified in the abdominal reflex blood of *T. bipustulata*.

No.	RI	RI (Lit.)	Compound	MW	Structure class
1	999	981	**Phenol**	94	Phenols
2	1034	/	Unknown [a]	109	/
3	1092	1086	***p*-Cresole**	108	Phenols
4	1165	/	Unknown [b]	168	/
5	1264	/	Unknown [c]	182	Aromatic hydrocarbon
6	1273	/	Unknown [d]	147	/
7	1321	1318	**Indole**	117	Aromatic hydrocarbon
8	1404	/	Unknown [e]	143	/
9	1416	1409	**3-Methylindole**	131	Aromatic hydrocarbon
10	1494	1470	**2-Phenylpyridine**	152	Aromatic hydrocarbon
11	1569	1559	**Lauric acid**	200	Carboxylic acid
12	1911	/	Unknown [f]	229	/
13	1971	1966	**Palmitic acid**	256	Carboxylic acid
14	1992	/	Unknown [g]	194	/
15	2094	/	Unknown [h]	264	/
16	2500	2500	**Pentacosane**	352	Alkane

[a] mass spectral data of the unknown compound 2: 55(8), 67(15), 80(13), 82(96), 83(17), 109(100), 110(26); [b] mass spectral data of the unknown compound 4: 58(50), 70(10), 72(18), 84(12), 89(31), 90(44), 98(10), 116(26), 117(46), 154(100), 155(11), 168(27), 169(18); [c] mass spectral data of the unknown compound 5: 58(16), 65(15), 91(100), 92(9), 131(5), 154(6), 168(29), 182(8), 183(4); [d] mass spectral data of the unknown compound 6: 51(5), 77(6), 91(10), 105(5), 117(100), 118(19), 130(21), 131(10), 132(84), 146(17), 147(41); [e] mass spectral data of the unknown compound 8: 56(9), 84(100), 85(5); [f] mass spectral data of the unknown compound 12: no significant data available; [g] mass spectral data of the unknown compound 14: 68(29), 69(9), 70(88), 83(7), 96(24), 97(7), 110(28), 111(6), 124(15), 125(4), 137(9), 138(18), 139(13), 166(38), 186(7), 194(100); [h] mass spectral data of the unknown compound 15: 55(53), 69(34), 70(12), 80(37), 82(26), 83(34), 91(21), 93(13), 94(19), 96(18), 97(28), 98(10), 108(17), 122(67), 123(27), 124(23), 136(100), 137(23), 138(19), 150(40), 151(34), 152(25), 164(21), 165(10), 166(11), 178(31), 179(35), 191(21), 192(259, 193(15), 206(52), 207(34), 217(17), 234(439, 246(15), 247(11), 248(11), 262(5), 263(30).

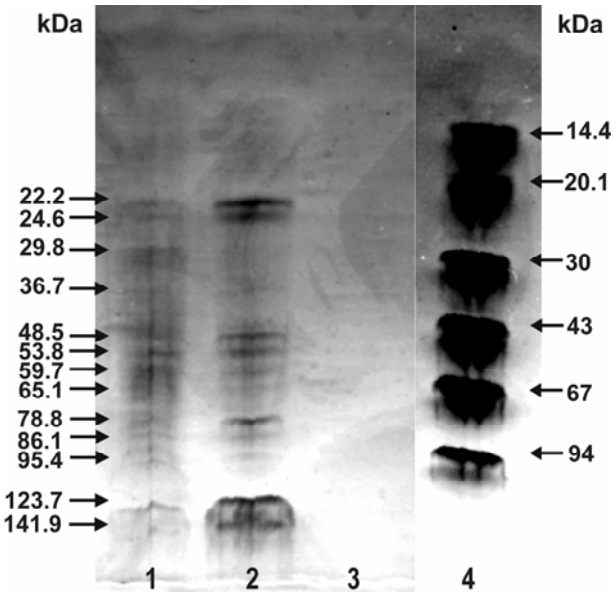


Fig. 4. SDS-PAGE analyses of (1) hemolymph, (2) sample of the abdominal reflex blood, (3) the secretion of the pronotal compound glands, and (4) the internal standard.

Defensive and microbiological properties

To evaluate the defensive and microbiological properties of the detected compounds we performed behavioural as well as agar diffusion tests. For the pronotal secretion significant repellent effects were obtained for benzyl alcohol and benzothiazole (Fig. 5A). The compounds benzaldedyde and benzophenone showed considerable, but not significant repellent effects on *Lasius flavus* ($p = 0.067$ and $p = 0.063$; Fig. 5A). In the abdominal reflex blood indole as well as 3-methylindole were detected; both compounds showed highly significant repellent effects on the ants tested (Fig. 5B); 2-phenylpyridin was effective by trend ($p = 0.058$; Fig. 5B).

In agar diffusion tests almost all tested compounds had significant antimicrobial (Fig. 6). Merely for *p*-anisaldehyde, phenol and the two carboxylic acids no significant results were obtained. 3-Methylindole, indole, *p*-cresol and benzothiazole had the strongest effects on the tested micro-organisms. It should be mentioned that none of the tested compounds induced such large zones of inhibition like the glycylcycline antibiotic Tygacil®, which was used in a lower concentration.

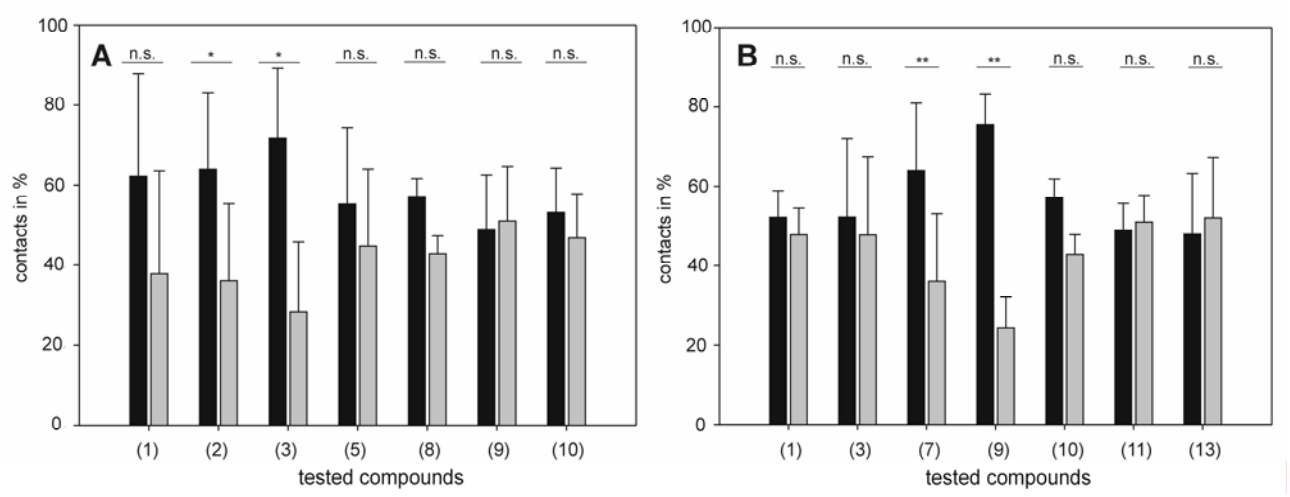
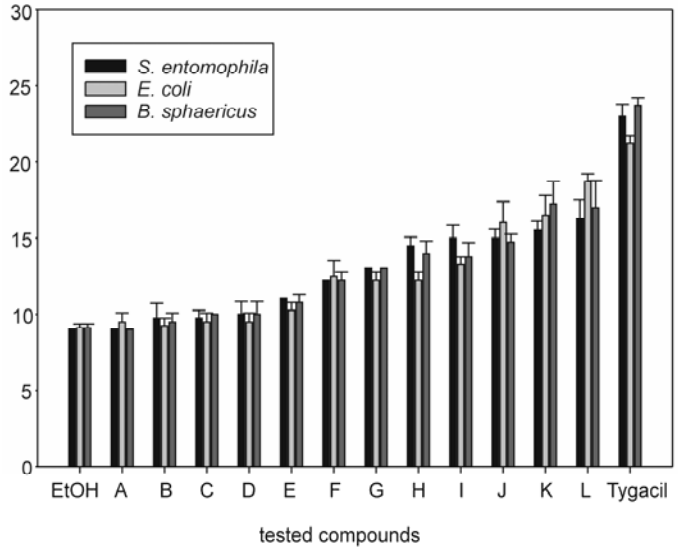


Fig. 5. Two-choice bioassays with *Lasius flavus* on (A) the pronotal secretion and (B) the abdominal reflex bleeding of *T. bipustulata*. n.s. no significant differences ($p > 0.05$); * significant differences ($p < 0.05$); ** highly significant differences ($p < 0.01$); numbers correspond to Figs. 2, 3 and Tabs. 2, 3; black = control, grey = tested compounds.



	S. entomophila	*E. coli*	*B. sphaericus*
EtOH - A	0.317 / n.s.	0.459 / n.s.	0.317 / n.s.
EtOH - B	0.486 / n.s	1.000 / n.s.	0.686 / n.s.
EtOH - C	0.343 / n.s.	0.686 / n.s.	0.114 / n.s.
EtOH - D	0.200 / n.s.	0.686 / n.s.	0.200 / n.s.
EtOH - E	0.011 / *	0.040 / *	0.022 / *
EtOH - F	0.013 / *	0.011 / *	0.011 / *
EtOH - G	0.011 / *	0.015 / *	0.011 / *
EtOH - H	0.029 / *	0.029 / *	0.029 / *
EtOH - I	0.017 / *	0.015 / *	0.017 / *
EtOH - J	0.017 / *	0.017 / *	0.015 / *
EtOH - K	0.017 / *	0.018 / *	0.017 / *
EtOH - L	0.017 / *	0.015 / *	0.018 / *
EtOH - Tygacil	0.017 / *	0.015 / *	0.015 / *

Fig. 6. Agar diffusion tests with tested compounds from the pronotal secretion as well as the abdominal reflex blood. (A) *p*-anisaldehyde (B) phenole (C) lauric acid (D) palmitic acid (E) benzaldehyde (F) 2-phenylpyridine (G) benzyl alcohol (H) benzophenone (I) benzothiazole (J) *p*-cresol (K) indole (L) 3-methylindole. n.s. no significant differences ($p > 0.05$); * significant differences ($p < 0.05$).

4. Discussion

The chemical defensive system of *T. bipustulata* consists of several different glands distributed over the whole body, but the pronotal ones emitted by far the largest amounts of secretion. The secretion was collected by closed loop stripping technique and compounds were eluted with acetone. Common methods for collecting secretions like small pieces of filter paper, capillary tubes or dissection of whole glands were surprisingly not successful, which might be due to the high volatility of the emitted compounds. It should be mentioned, that the concentrations of the emitted compounds had varied, surely depending on the age and the nutritional state of the beetles. Additionally, the used technique for collecting volatiles is not adapted for any quantification. Indeed, the presence of all compounds could be confirmed in later analyses, but the amounts had differed from that in the represented TICs (Figs. 2, 3).

A separation of the living animals into sexes for chemical analyses of the pronotal secretion was unfortunately not successful, since the beetles responded with reflex bleeding and contracting the abdomen beneath the elytra (in analyses of the abdominal reflex bleeding as well as the hemolymph the sexes were determined after obtaining the secretions). Furthermore, we can not exclude that some of the detected compounds originate from other glands than the large pronotal ones, since *T. bipustulata* possess additional glands (Drilling et al., in press). However, these additional glands are very small and release of secretions was never observed by the authors. Despite the above mentioned problems, the present contribution displays an interesting insight in the chemical defence of a member of this neglected coleopteran family.

Tab. 2 lists the chemicals identified in the pronotal secretion of *T. bipustulata*. The most abundant compounds beside the unidentified main compound (*7*) were benzaldehyde (*1*), benzyl alcohole (*2*) and *p*-anisaldehyde (*5*). Benzothiazole (*3*) was present in smaller quantities. The found alkanes tricosane and pentacosane, which do not represent epicuticular lipids, might act as solvents. Such long chained hydrocarbons were previously described from glandular secretions of termites, bees and ants (Blum 1981). Benzyl alcohol was reported from the exocrine secretion of larvae of *Chrysomela lapponica* when feeding on *Salix* (Hilker and Schulz 1994), and also from the secretion of the dorsal abdominal scent gland of some larval pentatomids (Aldrich 1988). Benzothiazole was found in the defensive secretion of *Sipyloidea sipylus* (Phasmatodea; Bouchard et al. 1997). Both, benzyl alcohol and benzothiazole had significant deterrent effects on *Lasius flavus* in our behavioural experiments. The defensive secretion of *S. sipylus*, additionally containing diethyl ether, acetic acid, benzaldehyde, and limonene, showed even potential as a repellent against *Rattus norwegicus* (Bouchard et al. 1997). Benzaldehyde was also found in the pygidial glands of some carabid species, in the secretion of several chrysomelid larvae and in a few species of Hymenoptera (Blum et al. 1969; Blum 1981). In addition, this compound was proven to be a particularly effective insecticide against *Drosophila* flies (Dettner et al. 1992). In our behavioural experiments the repellence was not significant, but benzaldehyde had a strong negative effect by trend (Fig. 5A). The aromatic *p*-anisaldehyde was previously reported from

tracheal gland secretions of *Leucophaea maderae* (Blattodea; Brossut 1983) and is also known as a common floral scent compound (Knudsen et al. 1993).

The presence of benzophenone (*8*) in the pronotal secretion appears conspicuous. This compound was not detected in blanks of the oven bag, and has not previously been reported in any insect species. Benzophenone is used as a photoprobe and this compound protects fragrance and dye in products like perfumes or soaps against UV radiation (Dormán and Prestwich 1994). Also the origin of the unidentified sesquiterpene (*6*) appears ambiguous, although this compound was present in nearly all analyses of the pronotal secretion of *T. bipustulata*. Since the odour of the senescent host fungus *Trametes versicolor* is dominated by sesquiterpenes (Drilling and Dettner 2009), it is conceivable that this sesquiterpene represents a contamination with fungal material while collecting volatiles by the closed loop stripping technique. However, the appropriate sesquiterpene was not present in the former analyses of Drilling and Dettner (2009), but fungi produce individually variable age-depending chemical profiles (Thakoew et al. 2006). From that perspective, compound *6* might represent a volatile compound present at a later developmental stage of the host fungus.

The mass spectroscopic data of the hitherto unidentified main compound (*7*) indicate the presence of an aromatic compound with a molecular mass of m/z 176 (Fig. 7). The pattern of fragmentation resembles EI-spectra of sesquiterpenes with strong signals at m/z 79, 91, 133, and 161. However, instead of clear signals at m/z 105 and 119 we found m/z 107 and 121 (Fig. 7), and also the molecular mass do not correspond with that of sesquiterpenes. Unfortunately, the chemical nature of compound *7*, representing the main compound in the shown TIC (Fig. 2), remains unknown for the present.

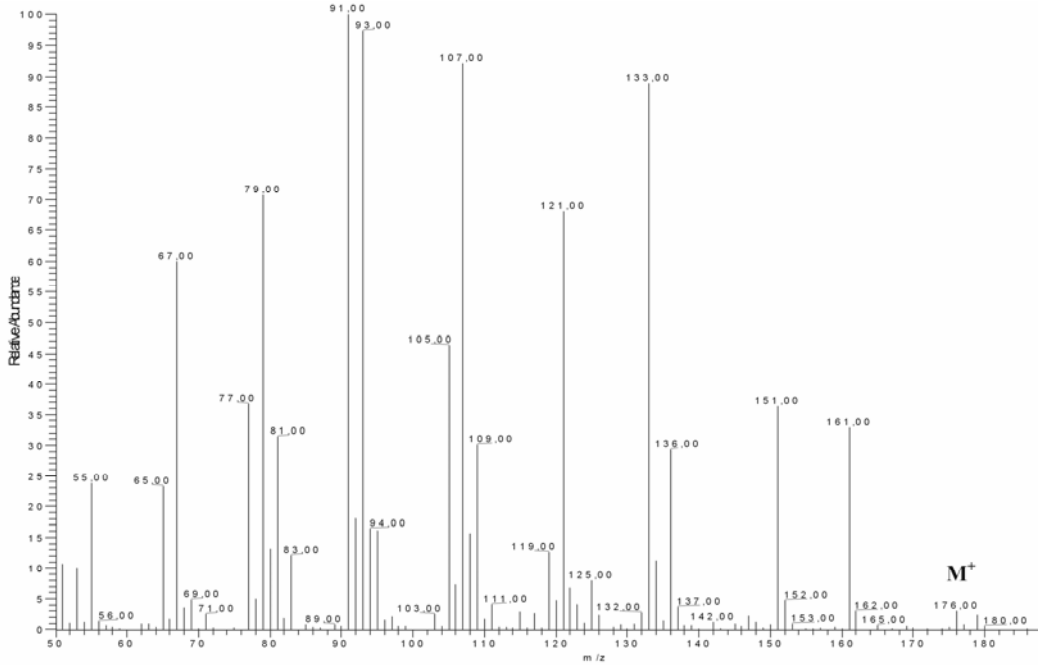


Fig. 7. EI-mass spectrum of the unidentified main compound 7. For details see text.

Many arthropods respond to molestation by discharging blood respectively hemolymph from intersegmental membranes of the legs or from specialized weak spots in the cuticle along the elytra or the antennal sockets (e.g. *Photinus pyralis*, Lampyridae; Blum and Sannasi 1974). The phenomenon is described for different insect groups like Plecoptera (Benfield 1974), Orthoptera (Blum 1981), Hemiptera (Peck 2000), and Coleoptera (Blum 1981). Among the cucujiform beetles the families Meloidae (Carrel and Eisner 1974), Chrysomelidae (Wallace and Blum 1971; Matsuda 1982), Endomychidae (Laurent et al. 2005b), and Coccinellidae (McIndoo 1916, de Jong et al. 1991) show the ability of autohaemorrhage. For species of the family Erotylidae merely a short note on *Callischyrus cyanopterus* is given by Węgrzynowicz (2002). He reported the excretion of droplets of orange-coloured hemolymph of acrid smell from their knee-joints. Reflex bleeding from the abdominal tip is reported for the first time, but is considered as an adaptation to the mycophagous way of life. The small species of Erotylidae live cryptic within the host fungus and while feeding merely the abdominal tip is visible from the outside. Hence, it seems beneficial to possess an effective chemical weapon against predators at this exposed part of the body.

The chemicals identified in the discharged blood as well as the examined hemolymph of *T. bipustulata* are listed in Tab. 3. The amounts of the compounds varied with respect to the obtained portion of blood or hemolymph (by capillary tubes). Main compound in this secretion was the heterocyclic indole (*7*; Fig. 3), which contributes to the malodorous odour of the discharged blood. Indole has been reported to be present in huge quantities in the scarab beetle *Holotrichia consanguinea*, in the pygidial glands of a few ants, the paired glands in the abdomen of the trichopteran *Pycnopsyche scabripennis*, and as an exocrine substance on the wings and body of *Pieris rapae crucivora* (Jackson et al. 1990; Leal 1997). Moreover there are many references to indole as an odorous substance in plants, in fish, essential oils, and for humans it apparently contributes to the aroma of certain types of tea, Gorgonzola cheese, and cooked shrimps (Jackson et al. 1990). Likewise involved in the malodorous odour are 3-methylindole (*9*) and *p*-cresol (*3*). The former compound is known from animal faeces as well as species of Trichoptera, Neuroptera, and Hymenoptera (Blum 1981, Jackson et al. 1990); the later is described from glandular secretions of a few Callipodida and Orthoptera (Blum 1981). In nature indole is often accompanied by 3-methylindole (Jackson et al. 1990). The highly volatile phenol, detected in the abdominal reflex blood, is also widely scattered, and known from species of millipedes and opilionids to grasshoppers and beetles (Morgan 2004). Thereby phenols should provide protection upwards against predators and downwards against micro-organisms (Martin 1979). However, in our behavioural analyses, phenol and *p*-cresol were not effective (Fig. 5B). In microbiological assays merely *p*-cresol effected the growth of the tested micro-organisms significantly (Fig. 6J); phenol had no noticeable effect (Fig. 6B).

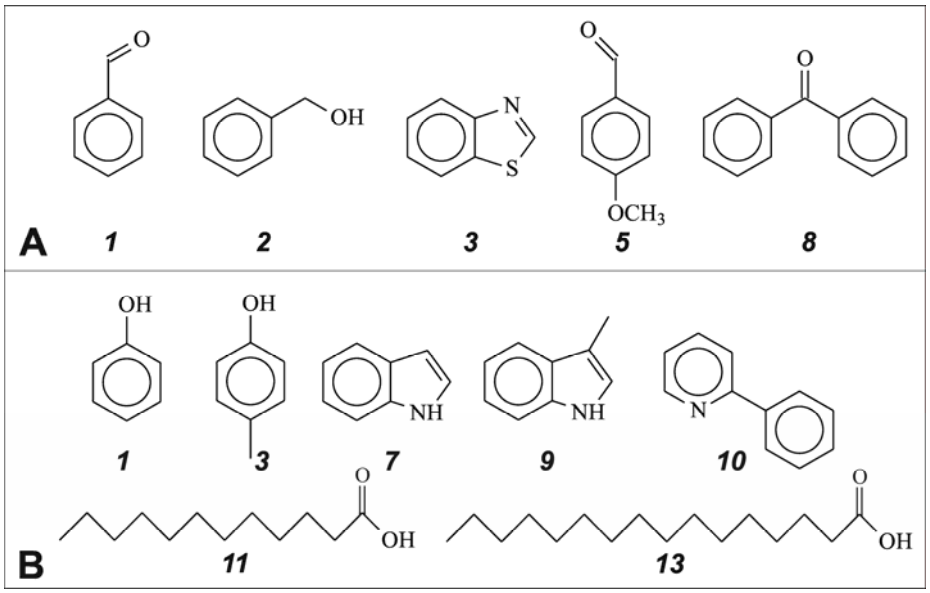


Fig. 8. Structures of compounds identified from (A) the pronotal gland secretion and (B) the hemolymph of *T. bipustulata*. Numbers in (A) correspond to compounds in Tab. 2. Numbers in (B) correspond to compounds in Tab. 3.

In sum, both examined secretions were dominated by aromatic and highly volatile compounds of rather low molecular weights. A wide variety of secondary metabolites, including terpenes, furans, phenols, quinones, complex nitrogen heterocyclics and peptides occur in fungi (Birkinshaw 1965). Many of these substances are surely potentially harmful to any insect which may consume them. Fungi are also sources of several classes of phenol-oxidizing enzymes, including tyrosinase, laccase, and peroxidase (Martin 1979). These enzymes are involved in the metabolism of phenolic compounds (Martin 1979), and the large number of aromatic substances might be explainable by the action of these ingested enzymes. Indole and 3-methylindole appear to owe their biosynthetic origin to the action of a tryptophanase on the essential amino acid tryptophan, which is made only by plants and micro-organisms (Leal 1997; Morgan 2004). Most other detected compounds have no comparable natural amino acid precursor and must be ingested or produced by *T. bipustulata* in some other way.

Functionally the abdominal reflex blood has both antimicrobial and deterrent properties whereas the discharged pronotal secretion appear rather antimicrobial than being an effective deterrent against arthropods (Figs. 5, 6). The chemical properties might be corralatable with the beetle's mycophagous way of live. The lucent adults live cryptic within the host fungus and while feeding merely the abdominal tips are visible from the outside. Hence it seems beneficial to emit an antimicrobial secretion from the pronotal glands (protection against various micro-organisms) and to discharge malodorous compounds from the exposed abdominal tip (protection against predators). The presence of these compounds in the abdominal secretion might also irritate fungus-feeding mammals. In European forests many ground squirrels and microtine rodents are extensively mycophagous (Johnson 1996), and as most fungus-feeding nonprimate mammals are colour-blind (Martin 1979), the scent may serve as the aposematic signal associated by vertebrates with poisons.

A pheromonal function seems rather unlikely, since the pronotal glands are equally developed in both sexes (Drilling et al., in press); preliminary intraspecific behavioural tests revealed no response to the secretion at all (data not shown). Chûjô (1969) described a pair of stridulatory files on the vertex of the head in males of Japanese species of *Dacne*, and also most other species of the family possess these sound-producing organs (Arrow 1925). The anterior margin of the pronotum forms a sharp ridge, corresponding in position to the stridulatory files on the vertex (Ohya 1996). The ridge may scrape the pair of files to produce chirps. Since only males exhibit this apparatus, sound might play an important role in courtship behaviour in erotylid species. Later, Ohya (2001) was able to demonstrate the significance of sound for intraspecific communication. Indeed, further investigations on the biology and chemical defence of erotylid species are needed to illuminate the ecological relationships within this neglected family.

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6. References

Aldrich JR (1988) Chemical ecology of the Heteroptera. Annual Review of Entomology 33: 211-238

Andrews JM (2005) BSAC standardized disc susceptibility testing method (version 4). Journal of antimicrobial Chemotherapy 56: 60-76

Arrow GJ (1925) Coleoptera. Clavicornia. Erotylidae, Languriidae, and Endomychidae. – In: Shipley AE, Scott A (eds.). The fauna of British India, including Ceylon and Burma. Taylor and Francis, London: XVI + 416 pp

Bartelt RJ (1999) Sap beetles. – In: Hardie J, Minks AK (eds.). Pheromones of non-lepidopteran insects associated with agricultural plants. CABI Publishing, Oxford, UK: 69-89

Benfield EF (1974) Autohemorrhage in two Stoneflies (Plecoptera) and its effectiveness as a defense mechanism. Annals of the Entomological Society of America 67(5): 739-742

Birkinshaw JH (1964) Chemical constituents of the fungal cell. 2. Special chemical products. – In: Ainsworth GC, Sussman AS (eds.). The Fungi, Vol. I, Academic Press, New York

Blum MS (1969) Benzaldehyde: Defensive secretion of a harvester ant. Comparative Biochemistry and Physiology 29: 461-465

Blum MS (1981) Chemical defenses of Arthropods. Academic Press, Toronto, 562 pp

Blum MS, Sannasi A (1974) Reflex bleeding in the lampyrid *Photinus pyralis*: defensive function. Journal of Insect Physiology 20: 451-460

Boland W, Ney P, Jaenicke L, Gassmann G (1984) A 'closed-loop-stripping' technique as a versatile tool for metabolic studies of volatiles. – In: Schreier P (ed.) Analysis of Volatiles Berlin. Walter de Gruyter: 371–373

Bouchard P, Hsiung CC, Yaylayan VA (1997) Chemical analysis of defense secretions of *Sipylodea sipylus* and their potential use as repellents against rats. Journal of Chemical Ecology 23(8): 2049-2057

Brossut R (1983) Allomonal secretions in Cockroaches. Journal of Chemical Ecology 9(1): 143-158

Carrel JE, Eisner T (1974) Cantharidin: Potent Feeding Deterrent to Insects. Science 183: 755-757

Chûjô M (1969) Erotylidae (Insecta: Coleoptera) Fauna Japonica. Academic Press of Japan, Tokyo

Daloze D, Braekman J-C, Pasteels JM (1994) Ladybirds defence alkaloids: structural, chemotaxonomic and biosynthetic aspects (Col.: Coccinellidae). Chemoecology 5(3/4): 173-183

de Jong PW, Holloway GJ, Brakefield PM, de Vos H (1991) Chemical defence in ladybird beetles (Coccinellidae). II. Amount of reflex fluid, the alkaloid adaline and individual variation in defence in 2-spot ladybirds (*Adalia bipunctata*). Chemoecology 2: 15-19

Dettner K (1987) Chemosystematics and evolution of beetle chemical defenses. Annual Review of Entomology 32: 17-48

Dettner K (1991) Chemische Abwehrmechanismen bei Kurzflüglern (Coleoptera: Staphylinidae). Jahresberichte des naturwissenschaftlichen Vereins in Wuppertal 44: 50-58

Dettner K, Fettköther R, Ansteeg O, Deml R, Liepert C, Petersen B, Haslinger E, Francke W (1992) Insecticidal fumigants from defensive glands of insects – a fumigant test with adults of *Drosophila melanogaster*. Journal of Applied Entomology 113: 128-137

Dormán G, Prestwich GD (1994) Benzophenone photophores in biochemistry. Biochemistry 33(19): 5661-5673

Drilling K, Dettner K (2009) Electrophysiological responses of four fungivorous coleoptera to volatiles of *Trametes versicolor*: implications for host selection. Chemoecology 19(2): 109-115

Drilling K, Dettner K, Klass K.-D (in press) Morphology of the pronotal compound glands in *Tritoma bipustulata* (Coleoptera: Erotylidae). Organisms, Diversity and Evolution. DOI: 10.1007/s13127-010-0008-0

Dytham C (2001) Choosing and using statistics: A biologist's guide. Blackwell Science, London, 218 pp

Francke W, Dettner K (2005) Chemical signalling in beetles. Topics in Current Chemistry 240: 85-166

Hilker M, Schulz F (1994) Composition of larval secretion of *Chrysomela lapponica* (Coleoptera, Chrysomelidae) and its dependence on host plant. Journal of Chemical Ecology 20(5): 1075-1093

Jackson BD, Keegans SJ, Morgan ED (1990) Trail pheromone of the ant *Tetramorium meridionale*. Naturwissenschaften 77: 294-296

Johnson CN (1996) Interactions between mammals and ectomycorrhizal fungi. Tree 11(12): 503-507

Klinger R, Maschwitz U (1977) The defensive gland of omaliinae (Coleoptera: Staphylinidae). Journal of Chemical Ecology 3(4): 401-410.

Knudsen JT, Tolisten L, Bergstrom LG (1993) Floral scent – A checklist of volatile compounds isolated by headspace techniques. Phytochemistry 33: 253-280

Koch K (1989) Die Käfer Mitteleuropas, Ökologie, Band 2. – Goecke and Evers Verlag, Krefeld

Laurent P, Braekman J-C, Daloze D (2005a) Insect chemical defense. Topics in Current Chemistry 240: 167-229

Laurent P, Daloze D, Braekman J-C, Pasteels JM (2005b) Stenotarsol, a new terpenoid from *Stenotarsus subtilis* (Coleoptera: Endomychidae). Tetrahedron Letters 46: 931-932

Leal WS (1997) Evolution of sex pheromone communication in plant-feeding scarab beetles. – In: Cardè R, Minks AK (eds.) Insect pheromone research: New Directions. New York: Chapman and Hall

Leschen RAB (2003) Erotylidae (Insecta: Coleoptera: Cucujoidea): phylogeny and review. Fauna of New Zealand 47: 108 pp

Leschen RAB, Skelley PA, McHugh JV (2010) 10.7. Erotylidae Leach, 1815. – In: Leschen RAB, Beutel RG, Lawrence J (eds.) Coleoptera, Beetles, vol. 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia partim). Handbuch der Zoologie/Handbook of Zoology IV/39. Walter de Gruyter, Berlin, New York: 311–319

Matsuda K (1982) Reflex Bleeding in *Gallerucida nigromaculata* BALY (Coleoptera: Chrysomelidae). Applied Entomology and Zoology 17(2): 277-278

McIndoo NE (1916)The reflex "bleeding" of the cocclinellid beetle, *Epilachna borealis*. Annals of the Entomological Society of America 9(2): 201-223

Morgan ED (2004) Biosynthesis in insects. The Royal Society of Chemistry, Cambridge, UK

Oehlschlager AC, King GGS, Pierce Jr. HD, Pierce AM, Slessor KM, Millar JG, Borden JH (1987) Chirality of macrolide pheromones of grain beetles in the genera *Oryzaephilus* and *Cryptolestes* and its implications for species specificity. Journal of Chemical Ecology 13(6): 1543-1554

Oehlschlager AC, Pierce AM, Pierce Jr. HD, Borden JH (1988) Chemical communication in cucujid grain beetles. Journal of Chemical Ecology 14(11): 2071-2098

Ohya E (1996) External structure of the putative stridulatory apparatus of the fungus beetles, *Dacne japonica* and *D. picta* (Coleoptera: Erotylidae). Applied Entomology and Zoology 31(2): 312-325

Ohya E (2001) Aggressive and courtship chirps of sympatric pleasing fungus beetles, *Dacne japonica* and *D. picta* (Coleoptera: Erotylidae). Entomological Science 4(3): 287-290

Pasteels JM, Braekman J-C, Daloze D (1988) Chemical defense in the Chrysomelidae. – In: Jolivet P, Petitpierre E, Hsiao TH (eds.) Biology of Chrysomelidae. Kluwer Academic Publishers, Netherlands

Pasteels JM, Rowell-Rahier M, Braekman J-C, Daloze D (1989) Evolution of exocrine chemical defense in leaf beetles (Coleoptera: Chrysomelidae). Experientia 45: 295-300

Pasteels JM, Rowell-Rahier M, Braekman J-C, Daloze D (1994) Chemical defence of adult leaf beetles updated. – In: Jolivet PH, Cox ML, Petipierre E (eds.) Novel aspects of the biology of Chrysomelidae. Kluwer Academic Publishers, Netherlands

Peck DC (2000) Reflex bleeding in Froghoppers (Homoptera: Cercopidae): Variation in behaviour and taxonomic distribution. Annals of the Entomological Society of America 93(5): 1186-1194

Pierce AM, Pierce Jr, HD, Oehlschlager AC, Czyzewska E, Borden HJ (1987) Influence of pheromone chirality on response by *Oryzaephilus surinamensis* and *Oryzaephilus mercator* (Coleoptera: Cucujidae). Journal of Chemical Ecology 13(6): 1525-1542

Roth LM, Eisner T (1962) Chemical defenses of arthropods. Annual Review of Entomology 7: 107-136

Steidle JLM, Dettner K (1996) Chemistry and morphology of the tergal gland of freeliving adult Aleocharinae (Coleoptera: Staphylinidae) and its phylogenetic significance. Systematic Entomology 18: 149-168

Thakoew P, Weißbecker B, Schütz S (2006) Volatile organic compounds emitted from fungal-rotting beech (*Fagus sylvatica*). Mitteilungen der deutschen Gesellschaft für allgemeine und angewandte Entomologie 15:157-160

Tschinkel WR (1975) A comparative study of the chemical defensive system of tenebrionid beetles: Chemistry of the secretions. Journal of Insect Physiology 21: 753-783

Vogt H (1967) 54. Familie: Erotylidae. – In: Freude H, Harde KW, Lohse GA (eds.) Die Käfer Mitteleuropas, Band 7. – Goecke and Evers Verlag, Krefeld: 104-109

Wallace JB, Blum MS (1971) Reflex bleeding: A highly refined defensive mechanism in *Diabrotica* larvae (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America 64(5): 1021-1024

Węgrzynowicz P (2002) Morphology, phylogeny and classification of the family Erotylidae based on adult characters (Coleoptera: Cucujoidea). Genus 13(4): 435-504

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Electrophysiological responses of four fungivorous coleoptera to volatiles of *Trametes versicolor*: implications for host selection

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CHEMOECOLOGY

SHORT COMMUNICATION

Electrophysiological responses of four fungivorous coleoptera to volatiles of *Trametes versicolor*: implications for host selection

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Abstract Fungi of the genus *Trametes* are known as important wood decomposers and are colonized by various species of Coleoptera and other arthropods. The aim of the present study was to investigate the importance of volatile chemical compounds as key attraction factors in recognition and host selection by species of Erotylidae (*Dacne bipustulata*, *Tritoma bipustulata*) as well as Cisidae (*Sulcacis affinis*) and Tenebrionidae (*Diaperis boleti*). Volatiles from freshly collected *Trametes versicolor* were collected by headspace sampling technique and identified by combined gas chromatography–mass spectrometry (GC–MS). To evaluate the biological significance of the volatiles we performed behavioural tests and recorded antennal responses of the fungus-inhabiting species by gas chromatography with electroantennographic detection (GC-EAD). The scent of *T. versicolor* was found to be dominated by sesquiterpenes; in GC-EAD 6 of these compounds elicited reproducible antennal signals in the tested species. Highly significant attraction effects to the fungus, the obtained odour samples and previously described fungal C_8-compounds were observed in behavioural tests. The possibility to detect these chemical compounds as a key cue for host selection implicate that beetles are able to discriminate between fungi of different age as well as different stages of colonization.

Keywords Erotylidae · Cisidae · Tenebrionidae · GC-EAD · Sesquiterpenes

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Introduction

For many beetles and other arthropods, fungi and dead wood material are natural and crucial resources for nutrition in a forest landscape (Scheerpeltz and Höfler 1948; Benick 1952; Lawrence 1989). It has been estimated that as many as 25 beetle families directly feed on fungi (Lawrence 1989). In this context, volatile organic compounds (VOCs) might play an important role in host recognition and selection. Earlier studies indicate that insects use these olfactory signals in various ways while searching for fruiting bodies or dead wood material. Insects are either able to perceive volatile compounds emitted by their hosts or they colonize the host by following the attractive volatiles (pheromones) released by a so called pioneer individual that selected the host.

Pacioni et al. (1991) demonstrated by a trapping experiment that Coleoptera (Leiodidae, Staphylinidae), Diptera and Lepidoptera were attracted by dimethyl sulphide, a component of the aroma of truffles (*Tuber* spp.). Also cisids associated with *Formitopsis pinicola* (Polyporales) recognized the host odour during flight and were attracted in significant numbers to baited traps (Jonsell and Nordlander 1995). Electrophysiological and behavioural analyses of *Cis boleti* (Cisidae) revealed that this species is attracted by 1-octene-3-ol and other host fungal volatiles (Thakoew et al. 2008); 1-octene-3-ol is known as a typical mushroom alcohol and is the most characteristic fungal VOC. Similar analyses with the wood-breeding scolytid *Trypodendron domesticus* and the syntopic *Hylecoetus dermestoides* (Lymexylonidae) revealed a large number of substances (e.g. 2-methoxyphenol, 1,2-dimethoxybenzene, 2-methyl-1-butanol) used as semiochemicals in host selection (Holighaus and Schütz 2006); such interactions enhance the success of colonisation for the mentioned insects. Random searching of a pioneer individuum, representing an alternative strategy in host

finding, was observed for the tobacco beetle, *Lasioderma serricorne* (Anobiidae; Levinson and Levinson 1987), and is also suggested for *Dorcatoma puctulata* and *D. robusta* (both Anobiidae; Jonsell and Nordlander 1995). In these cases the pioneer individual attract conspecifics by emitting pheromones (similar to bark beetles).

Larvae and adults of the erotylid subfamily Erotylinae (the remaining subfamilies comprise the former Languriidae, which are phytophagous) and members of Cisidae are exclusively associated with various Polyporales and related higher fungi (Leschen 2003; Robertson et al. 2004; Lawrence 1973); they are used as nutrition as well as breeding substrate. The role of volatile compounds involved in host recognition and selection is poorly investigated. In our study we chose coupled gas chromatography–mass spectrometry (GC–MS), gas chromatography with electroantennographic detection (GC-EAD) and electroantennography (EAG) to identify VOCs potentially used in host selection in two European species of Erotylidae (*Tritoma bipustulata*, *Dacne bipustulata*) associated mainly with the fungus *Trametes vericolor* (Polyporaceae). Additional investigations involved the cisid species *Sulcacis affinis* associated with the same fungus and the tenebrionid species *Diaperis boleti*, mainly found on *Fomitopsis pinicola* and *Laetiporus sulphureus* (Scheerpeltz and Höfler 1948; Benick 1952). *T. versicolor* is a common wood-rotting fungus on woody debris and stumps of deciduous trees, especially on beech, birch, poplar and willow (Guevara et al. 2000b). Fruiting bodies are annual and typically occur in a relatively early phase of decay succession (3–7 years; Hintikka 1993; Komonen and Kouki 2005). The fungus forms clusters which remains attached to wood for 1 or 2 years after they died. During this time the fruiting bodies may be entirely consumed by insects.

Materials and methods

Insects

Specimens of Erotylidae [*Dacne bipustulata* (Thunberg, 1781), *Tritoma bipustulata* (Fabricius, 1775)] and Cisidae [*Sulcacis affinis* (Gyllenhal, 1827)] were collected from *Trametes versicolor* between May and June 2007 in the botanical garden of the University of Bayreuth (49°55′25.66″N, 11°34′59.28″E, 350 m). Specimens of *Diaperis boleti* (Linnaeus, 1758) (Tenebriondae), were collected from *Fomitopsis pinicola* in September 2008, at Bienenwald near Karlsruhe (49°01′17.43″N, 8°10′14.17″E, 132 m).

Volatile collection

Volatiles from fresh *Trametes versicolor* (total fresh weight of 116 g) were collected by using the closed loop stripping technique (Boland et al. 1984). The fungus was enclosed by an oven bag (Neoten®, 30 × 30 cm), and emitted volatiles were trapped in an adsorbent tube filled with 20 mg of a 1:1 mixture of Tenax-TA 60–80 and Carbotrap 20–40. A membrane pump (G12/01 EB, Rietschle Thomas, Puchheim, Germany) was used to draw the air from the bag over the adsorbent. Volatiles were eluted with 80 µl of acetone (SupraSolv, Merck KGaA, Germany) to get samples of volatiles for chemical and electrophysiological analyses (see below).

GC-MS analyses, GC-EAD and EAG

Electrophysiological analyses of the samples containing fungal volatiles were performed with a GC-EAD system consisting of a gas chromatograph (Vega 6000 Series 2, Carlo Erba, Rodano, Italy) equipped with a flame ionization detector (FID) linked to an EAD setup (heated transfer line, 2-channel USB acquisition controller, Syntech, Hilversum, The Netherlands). Samples (1 µl each) were injected splitless at 60°C, followed by opening the split vent after 1 min and heating the oven at a rate of 10°C/min to 200°C. The final temperature was held for 5 min. A ZB-5 column was used for the analyses (30 m, internal diameter 0.32 mm, film thickness 0.25 µm, Phenomenex Corp., Torrance, CA, US). The column was split at the end by the four arm flow splitter GRAPHPACK 3D/2 (Gerstel, Mülheim, Germany) into two deactivated capillaries (length 50 cm, i.d. 0.32 mm) leading to the FID and to the EAD setup. Makeup gas (He, 16 ml/min) was introduced through the fourth arm of the splitter. For the runnings, an excised antenna was mounted between glass micropipette electrodes filled with insect Ringer's solution (8.0 g/l NaCl, 0.4 g/l KCl, 0.4 g/l $CaCl_2$). The electrodes were connected to silver wires.

To identify the chemical structures of the EAD active compounds, 1 µl of the sample was analysed on a Varian Saturn 2000 mass spectrometer and a Varian 3800 gas chromatograph fitted with a 1079 injector (Varian Inc., Palo Alto, USA). A ZB-5 column (5% phenyl polysiloxane) was used for the analyses (60 m, i.d. 0.25 mm, film thickness 0.25 µm, Phenomenex Corp. Torrance, CA, US); the temperature program was equal to that used at the GC-EAD system. Structure assignments were carried out using the NIST 08 mass spectral data base, or MassFinder 3, and confirmed by comparison with retention times of authentic standards if available; otherwise by comparison of retention index (RI) and mass spectra from the literature (Joulain and König 1998; Adams 2007; NIST 08). These libraries used CpSil5 (Joulain and König 1998) and DB5 (Adams 2007) as stationary phases, however, the polarity of the column used in the context of the present work (ZB-5) is similar to CpSil5 and DB5. Therefore obtained

RI values may slightly differ due to the low polarity of the detected compounds.

Electroantennography (EAG) was employed to obtain a dose–response curve to different dilutions (10^{-5}–10^{-1}) of 1-octene-3-ol in paraffin oil (Uvasol, Merck KgaA, Gemany). For this analysis, a racemate of 1-octene-3-ol was used, and only *S. affinis* was available in sufficient numbers. Pieces of filter paper (2 × 2 cm; Scheicher and Schüll, Germany) were soaked with 20 µl of each dilution or paraffin oil only (control). The filter papers were inserted into a 5 ml syringe (Omnifix, B. Braun Melsungen AG, Germany), and a reproducible stimulus was supplied by puffing 5 ml air over the antenna. The EAG response of the antennae was recorded for each dilution from at least five male and five female beetles. The response to paraffin oil was considered as a negative control and was subtracted from the remaining EAG measurement.

Behavioural assays

To test the attractiveness of the fungus, the collected volatiles, and that of authentic 1-octene-3-ol, a two-choice olfactometer test was carried out. The olfactometer was a glass tube (i.d. 3 cm; length 20 cm) with a 1 cm opening in the middle to release the animals while the ends were closed with a gauze cap. The tube was subdivided into three sections: the intermediate zone, the control zone, and the baited zone. Cone-shaped gauze inserts at the zonal borders acted as weirs to prohibit the return of the animals into the intermediate zone. Some pieces of fresh *T. versicolor* (3 g each), the scent sample (3 µl each) and a racemate of 1-octene-3-ol (3 µl each, dilution 10^{-2} in paraffin oil) were used as lures. The samples of volatiles were applied on filter paper (2 × 2 cm; Schleicher and Schüll, Germany), and the pure solvent was used as a control. To avoid contamination, the lure was placed at the same position while the olfactometer was turned between the tests to eliminate a possible preference for one side. Between the tests, the olfactometer was cleaned with ethanol. Dry runs were conducted to make sure, that the animals dispersed equally inside the tube. This run served as a control for the statistic analysis. The olfactometer was placed in a conditioning cabinet which generated an air stream and kept the temperature at 23–24°C. The bioassays were conducted with *T. bipustulata, S. affinis*, and *D. boleti.* Due to low availability, *D. bipustulata* could not be tested. Experiments with 1-octene-3-ol were carried out with *T. bipustulata* and *S. affinis*, only. A single run took 10 min.

Statistical analysis

Statistical analyses were performed in Statistica 7 (Statsoft Inc., Tulsa, USA; 2004). The net reaction for every single experiment (animals at the bait minus animals at the control) was assessed. Distribution of data as well as homogeneity of variances were checked by Shapiro–Wilk test and Levene test. Variances of data were homogenous but predominantly not normally distributed. Hence we performed the Bonferroni-corrected, non-parametric Mann–Whitney *U* test and used the corrected level of significance $\alpha = \alpha/k = 0.025$, where α is the error level of 5%, and k is the number of possible comparisons ($k = 2$). Significance of EAG results was evaluated by a Scheffè-test post hoc to an analysis of variance.

Results

In GC-MS analysis of the scent of fresh *Trametes versicolor* we detected 15 compounds in noticeable amounts. Sesquiterpenes dominated the eluting volatiles (Fig. 1A; Table 1), main compounds were δ-cadinene, β-guaiene and isoledene. Previously described compounds like 3-octanol or linalool were present in traces (Table 1), 1-octene-3-ol was absent. It was found that 6 of these 15 compounds elicited reproducible antennal signals in tested fungus-feeding arthropods (Figs. 1, 4). Both examined erotylid species responded to isoledene and δ-cadinene (Fig. 1B, C); in *Tritoma bipustulata* as well as in *Dacne bipustulata* the sexes reacted equally (data not shown). *Diaperis boleti* also detected both above mentioned compounds; in addition β-guaiene, γ-patchoulene and an unidentified minor sesquiterpene (15 in Fig. 1E) caused antennal responses in both sexes. The examined specimens of *Sulcacis affinis* were particularly sensitive to the minor compound, γ-cadinene (Fig. 1D). In two out of five analyses, β-guaiene caused a weak antennal signal; however the obtained response was not consistent. Also in *S. affinis* no differences between the sexes were found.

To compare the behaviour mediating activities of fruiting bodies and extracts, two-choice olfactometer tests were carried out. Blanks with *T. bipustulata*, *S. affinis* and *D. boleti* showed that the tested species dispersed equally in the olfactometer (control in Fig. 2). For fresh *T. versicolor* as well as for the headspace collected sample of volatiles of the fruiting body, attracting effects were obtained. The attractivity of the fresh fungus and the scent sample of the fruiting body differed statistically significantly from the blanks (Fig. 2). Moreover, fresh *T. versicolor* compared to extracts of fruiting bodies were similarly attractive to the beetles tested.

The previously described typical fungal alcohol 1-octene-3-ol is assumed to be a key cue for host finding in species of Cisidae (Fäldt et al. 1999; Wu et al. 2005; Ziegenbein et al. 2006; Thakoew et al. 2008); despite the fact, that it was not detected in the scent of *T. versicolor*, a racemate was also tested in behavioural assays and EAG.

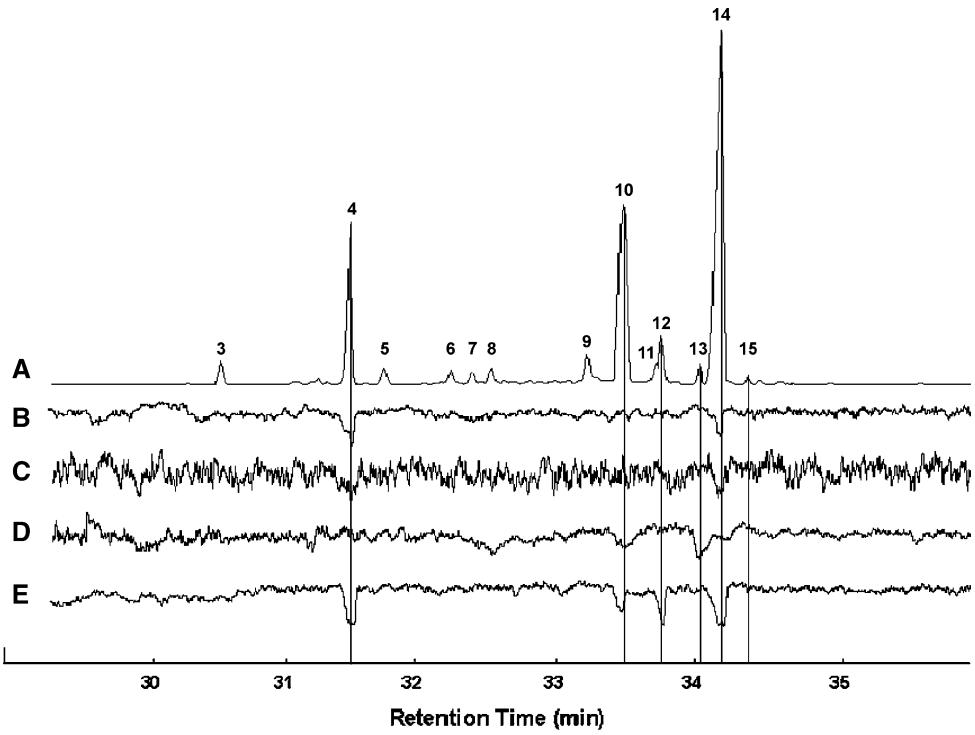

Fig. 1 Antennal responses to volatile compounds from fresh *Trametes versicolor*. *A* Representative flame ionization detector (FID) and electroantennographic detector (EAD) traces of *B Tritoma bipustulata* (female), *C Dacne bipustulata* (female), *D Sulcacis affinis* (female) and *E Diaperis boleti* (female). No differences between the sexes were observed, EAD traces of males not shown. For numbers of compounds see Table 1

Attraction of the compound to the tested beetles was statistically significant (Fig. 2a, b). In the case of *T. bipustulata* the attracting effect of 1-octene-3-ol was stronger than that of the eluted volatiles of *T. versicolor* ($P = 0.058$). In *S. affinis* the attracting effect of 1-octene-3-ol was similar to fresh fungal material as well as to the scent sample (Fig. 2b). Dose–response curves of the racemate (dilution 10^{-5}–10^{-1} in paraffin oil) were recorded for *S. affinis* males and females (Fig. 3), and significant differences with respect to the sexes and the doses were noticed [male $F(7,39) = 32.057$, $P < 0.001$; female $F(7,39) = 27.549$, $P < 0.001$]. Among the ranges of the tested doses, the males yielding three stages of dose discrimination at 10^{-5}–10^{-3}, 10^{-2} and 10^{-1}. In contrast, the females yielding only two stages of discrimination at 10^{-5}–10^{-3} and 10^{-2}–10^{-1} (Fig. 3).

Discussion

For invertebrates as well as vertebrates (Lawrence 1989; Johnson 1996) fungi display an important source for nutrition as they concentrate valuable nutrients about 10 times higher than the wood they grow on (Martin 1979; Jonsell and Nordlander 2004). It was suggested for a long time that host aroma compounds might play an important role during the colonization of fruiting bodies (Lawrence 1973; Jonsell and Nordlander 1995, 2004), but investigations of such interactions were sparse. Field observations with fungi-baited traps revealed that numerous insects, especially Coleoptera (incl. the erotylid species *Triplax russica* and *T. aenea*) and Diptera, were attracted by volatiles emanating from the host fungi or decaying wood, respectively (Jonsell and Nordlander 1995; Jonsson et al. 1997).

The scent of the examined *T. versicolor* was exclusively composed of sesquiterpenes in noticeable amounts (Fig. 1A; Table 1). This is in accord with the results of Holighaus and Schütz (2006), who found that after degradation of lignin and cell structures sesquiterpenes were the only volatiles. The major component was δ-cadinene, followed by β-guaiene, isoledene and γ-patchoulene (Figs. 1A, 4). Some of these compounds have been previously described as fungal or herbal metabolites, or they had been found to be components of essential oils. δ-Cadinene and α-muurolene were reported as the major compounds in cultures of *Lentinus lepideus* (Hanssen 1982), while δ-cadinene occurred also in *Schizophyllum commune* (Ziegenbein et al. 2006) and in a wide variety of essential oil-producing plants (Joulain and König 1998; Kubeczka and Formáček 2002). Isoledene was found as volatile plant constituent in the liverwort *Bazzania trilobata* (Warmers and König 1999) and also in *Ambrosia trifida* (Asteraceae; Wang et al. 2006). Six of these 15 compounds elicited reproducible antennal signals in tested beetles (Figs. 1, 4); isoledene and δ-cadinene were most prominent and were perceivable for both examined erotylid species as well as for *D. boleti*.

Table 1 Volatile constituents of *Trametes versicolor* identified by GC-MS

Compound name and peak no.[a]	RI	RI (Lit.)[b]	EAD activity[c]				
			Tritoma bipustulata	*Dacne bipustulata*	*Sulcacis affinis*	*Diapris boleti*	ID[d]
1. 3-Octanol	1,018	1,012	−	−	−	−	A
2. Linalool	1,109	1,100	−	−	−	−	A
3. α-Cubebene	1,357	1,355	−	−	−	−	A
4. Isoledene	1,400	1,382	++++	++	−	+++	A
5. Sativene	1,416	1,396	−	−	−	−	B
6. β-Caryophyllene	1,438	1,435	−	−	−	−	A
7. Unknown sqt. ($M^+ = 204$)[e]	1,450	/	−	−	−	−	/
8. Unknown sqt. ($M^+ = 204$)[f]	1,457	/	−	−	−	−	/
9. α-Curcumene	1,495	1,483	−	−	−	−	B
10. β-Guaiene	1,502	1,492	−	−	−/+	++	B
11. α-Muurolene	1,515	1,496	−	−	−	−	B
12. γ-Patchoulene	1,517	1,497	−	−	−	++	B
13. γ-Cadinene	1,522	1,515	−	−	+++	−	B
14. δ-Cadinene	1,536	1,527	+++	++	−	+++	A
15. Unknown sqt. ($M^+ = 204$)[g]	1,552	/	−	−	−	+	/

[a] See Fig. 1 for total ion chromatogram

[b] Joulain and König (1998), Adams (2007), NIST 08

[c] + 0.01–0.09 mV, ++ 0.1–0.19 mV, +++ 0.2–0.3 mV, ++++ >0.3 mV

[d] *A* identification by comparison of retention times and mass spectra of synthetic compounds, *B* identification by comparison of retention index and mass spectra from literature (Joulain and König 1998; Adams 2007, NIST 08)

[e] Mass spectral data of unknown sesquiterpene no. 7: 204(13), 189(3), 175(1), 162(17), 161(100), 147(10), 133(23), 120(16), 119(34), 115(6), 106(10), 105(63), 93(16), 92(10), 91(53), 81(23), 79(25), 77(23), 67(10), 41(18), 39(20)

[f] Mass spectral data of the unknown sesquiterpene no. 8: 204(6), 189(6), 175(3), 162(8), 161(31), 148(9), 147(9), 134(16), 133(62), 121(13), 120(50), 119(22), 109(12), 108(4), 107(21), 106(9), 105(35), 95(26), 94 (11), 93(83), 92(18), 91(66), 81(50), 79(47), 77(35), 69(100), 68(15), 67(58), 55(17), 53(24), 41(93), 39(81)

[g] Mass spectral data of the unknown sesquiterpene no. 15: 204(33), 189(3), 175(1), 162(13), 161(72), 147(5), 133(12), 121(16), 120(22), 119(100), 106(11), 105(93), 93(13), 92(12), 91(46), 79(12), 77(17), 65(8), 55(11), 41(19), 39(21)

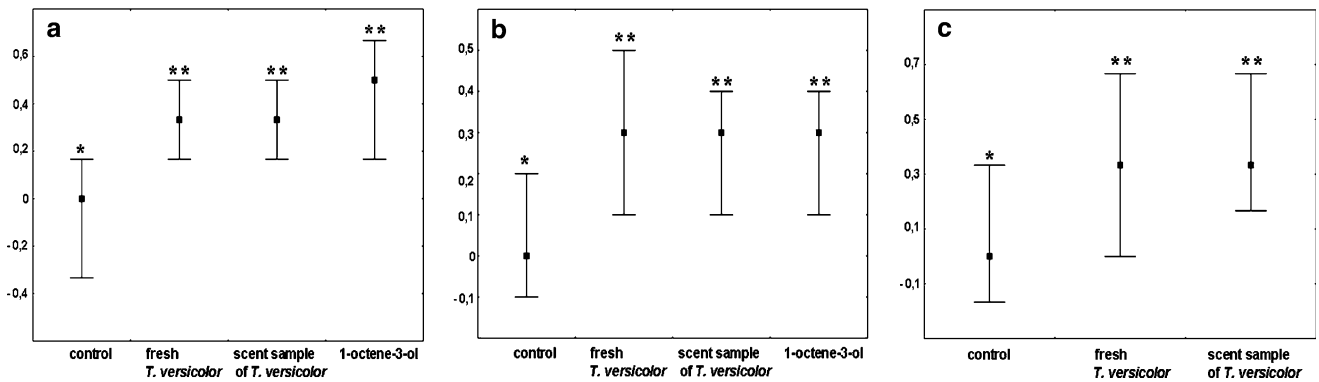

Fig. 2 Attractiveness of fresh *T.* versicolor, the eluted scent sample and a racemate of 1-octene-3-ol on **a** *Tritoma bipustulata*, **b** *Sulcacis affinis* and **c** *Diaperis boleti.* Net reaction of respectively 10 individuals is shown, *asterisks* indicate significant differences between samples, $n = 13$, medians are depicted ±max/min values

In the analysis of Thakoew et al. (2008) the scent of *Trametes gibbosa* was dominated by compounds like alcohols, ketones, acids, aldehydes and aromatics; only a single sesquiterpene alcohol (α-bisabolol) was present in the sample. Most abundant compounds in these analyses were alcohols comprising 49% of the scent. Some of these compounds (e.g. 1-octene-3-ol, 3-octanone) elicited reproducible antennal signals in *Cis boleti* (Thakoew et al. 2008), a specialist in *Trametes* spp. (Fossli and Andersen 1998).

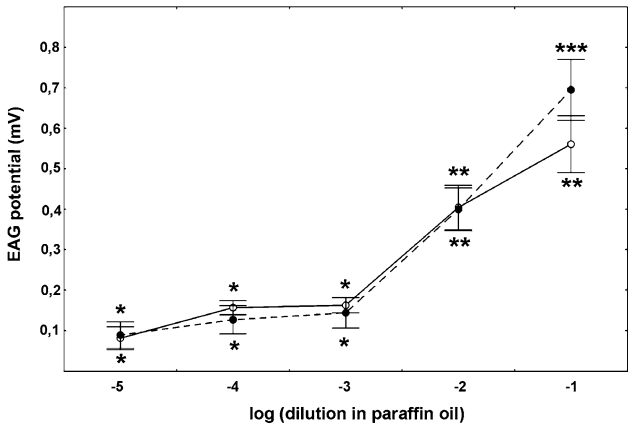

Fig. 3 EAG responses of *Sulcacis affinis* males (*filled circle*) and females (*open circle*) to 1-octene-3-ol. *Asterisks* (*top* male, *bottom* female) indicate statistically significant differences between samples. Means are depicted ±SEs

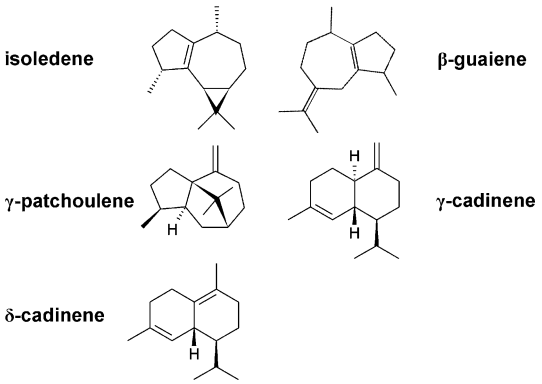

Fig. 4 Perceived substances discovered in GC-MS/EAD experiments

Particularly surprising was the absence of 1-octene-3-ol (37% in *T. gibbosa*) in the present study. This compound constitutes the typical fungal odour and was previously detected in *Laetiporus sulphureus* (Wu et al. 2005), *Fomitopsis pinicola* (Fäldt et al. 1999), *Phlebia radiata* (Gross et al. 1989), *T. gibbosa* (Thakoew et al. 2008) and other fungi (Ziegenbein et al. 2006). However, analyses of different colonization stages of *T. gibbosa* revealed that the release rate of 1-octene-3-ol was much higher in minimally colonized fruiting bodies (up to 20 times higher) than in highly colonized ones (Thakoew et al. 2008). This finding is most probably due to the fact that in highly colonized fruiting bodies the tissues releasing these volatiles are already consumed or damaged. Further studies on release rates of 1-octene-3-ol reported comparable variation depending on fungal age and on the season (Fäldt et al. 1999; Wu et al. 2005). The absence of 1-octene-3-ol in the present analysis might, therefore, be due to the fact that the examined fungus was senescent and highly colonized by species of Erotylidae and Cisidae.

For the fungivore Cisidae it has been hypothesized that two main fractions of fungal volatiles may play an important role in host selection: C_8-compounds and terpenoids (Fäldt et al. 1999; Guevara et al. 2000b). Thakoew et al. (2008) demonstrated 1-octene-3-ol to be a key cue for host finding in *C. boleti*. The enatiomeric composition of this fungal alcohol released by *T. gibbosa* displayed a ratio of 93:7 of the (R)- and (S)-enantiomers (Thakoew et al. 2008). In other bracket fungi species the R:S ratios range from a minimum of 89% to a maximum of 98% of the (R)-enantiomer (Ziegenbein et al. 2006). Although an enantiomeric discrimination was observed in several insect species (Ulland et al. 2006) previous analysis with *C. boleti* revealed no significant differences in responses to either enantiomer in EAG. In behavioural tests the females showed a preference for the (S)-enantiomer (Thakoew et al. 2008: Fig. 2a, b). Hence we tested a racemate of 1-octene-3-ol in EAG experiments as well as behavioural tests to scrutinize its activity (EAG merely with *S. affinis* due to availability). EAG experiments with *S. affinis* demonstrated its ability to perceive 1-octene-3-ol at different doses in males and females (Fig. 3); the behavioural tests showed statistically significant attraction to fresh *T. versicolor*, to the eluted sample of volatiles, and to the racemate of 1-octene-3-ol in all species tested (Fig. 2a, b). For *T. bipustulata* the attracting effect of 1-octene-3-ol was by trend stronger than for the fresh fungus and the collected sample of volatiles ($P = 0.058$; Fig. 2a). The effect was, however, not apparent in the case of *S. affinis*. Since cisids were found in much higher numbers in the fungi than erotylids, and several generations may occur in the same fruiting body before they disperse for a new one (Lawrence 1973), this strong attracting effect may result from a higher mobility of Erotylidae which disperse earlier for a new habitat then the tiny *S. affinis* and other cisids. In that case, *T. bipustulata* would be able to easier detect young unsettled fruiting bodies. Furthermore, it was reported that some species of Cisidae were predominantly found in young fruiting bodies (e.g. *Octotemnus glabriculus*) others in fully developed ones (e.g. *C. boleti*; Guevara et al. 2000a). Such cases of resource partitioning are likely controlled by variations in release rates and composition of VOCs.

Thakoew et al. (2008) suggested that the detected terpenoids do not play a major role in host finding, but demonstrated that fungi produce individually variable age-depending chemical profiles (Thakoew et al. 2006). Our data indicate that the examined species of Erotylidae, Cisidae and Tenebrionidae are able to perceive C_8-compounds (present in rather young fruiting bodies) as well as sesquiterpenes (present in senescent fruiting bodies) present in the scent of their host fungus. The role of sesquiterpenes is rather speculative, however, specific profiles of volatiles or individual

compounds which appear at a later developmental stage might enable the beetles to discriminate between quality and age of the nutrition and breeding substrate and, furthermore, the developmental stage and the degree of colonization. For a better understanding of such interactions and host selection mechanisms, extensive investigations on the profiles of volatiles and their changes during fungal development are needed.

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References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry. Allured Pub Corp. 4th edn

Benick L (1952) Pilzkäfer und Käferpilze: Ökologische und statistische Untersuchungen. Acta Zool Fenn 70:1–250

Boland W, Ney P, Jaenike L, Gassmann L (1984) A "closed-loop-stripping"—technique as a versatile tool for metabolic studies of volatiles. In: Schreier P (ed) Analysis of volatiles. de Gruyter, Berlin, pp 371–380

Fäldt J, Jonsell M, Nordlander G, Borg-Karlson A-K (1999) Volatiles of bracket fungi *Fomitopsis pinicola* and *Fomes fomentarius* and their functions as insect attractants. J Chem Ecol 25(3):567–590

Fossli T-E, Andersen J (1998) Host preference of Ciidae (Coleoptera) on tree-inhabiting fungi in nothern Norway. Entomol Fenn 9:65–78

Gross B, Gallois A, Spinnler H-E, Langlois D (1989) Volatile compounds produced by the ligninolytic fungus *Phlebia radiata* Fr. (Basidiomycetes) and influence of the strain specificity on the odorous profile. J Biotechnol 10:303–308

Guevara R, Hutcheson KA, Mee AC, Rayner ADM, Reynolds SE (2000a) Resource partitioning of the host fungus *Coriolus versicolor* by two ciid beetles: the role of odour compounds and host ageing. Oikos 91:184–194

Guevara R, Rayner ADM, Reynolds SE (2000b) Effects of fungivory by two specialist ciid beetles (*Octotemnus glabriculus* and *Cis boleti*) on the reproductive fitness of their host fungus, *Coriolus versicolor*. New Phytol 145(1):137–144

Hanssen H-P (1982) Sesquiterpene hydrocarbons from *Lentinus lepideus*. Phytochemistry 21(5):1159–1160

Hintikka V (1993) Occurrence of edible fungi and other macromycetes on tree stumps over a sixteen-year period. Acta Bot Fenn 149:11–17

Holighaus G, Schütz S (2006) Odors of wood decay as semiochemicals for *Trypodendron domesticum* L. (Col., Scolytidae). Mitteilungen der deutschen Gesellschaft für allgemeine und angewandte Entomologie 15:161–165

Johnson CN (1996) Interactions between mammals and ectomycorrhizal fungi. Trends Ecol Evol 11(12):503–507

Jonsell M, Nordlander G (1995) Field attraction of Coleoptera to odors of the wood-decaying polypores *Formitopsis pinicola* and *Fomes fomentarius*. Ann Zool Fenn 32:391–402

Jonsell M, Nordlander G (2004) Host selection patterns in insects breeding in bracket fungi. Ecol Entomol 29:697–705

Jonsson M, Nordlander G, Jonsell M (1997) Pheromones affecting flying beetles colonizing the polypores *Fomes fomentarius* and *Fomitopsis pinicola*. Entomol Fenn 8:161–165

Joulain D, König WA (1998) The Atlas of spectral data of sesquiterpene hydrocarbons. E.B, Verlag Hamburg

Komonen A, Kouki J (2005) Occurence and abundance of fungus-dwelling beetles (Ciidae) in boreal forests and clearcuts: habitat associations at two spatial scales. Anim Biodivers Conserv 28(2):137–147

Kubeczka K-H, Formáček V (2002) Essential oils analysis by capillary gas chromatography and carbon-13 NMR spectroscopy, 2nd edn. Wiley, West Sussex England

Lawrence JF (1973) Host preference in ciid beetles (Coleoptera: Ciidae) inhabiting the fruiting bodies of basidiomycetes in north America. Bull Mus Comp Zool 145(3):163–212

Lawrence JF (1989) Mycophagy in the Coleoptera: feeding strategies and morphological adaptations. In: Wilding N, Collins NM, Hammond PM, Webber JF (eds) Insect-Fungus Interactions. Academic Press, London, pp 1–23

Leschen RAB (2003) Erotylidae (Insecta: Coleoptera: Cucujoidea): phylogeny and review. Fauna N Z 47:108

Levinson HZ, Levinson AR (1987) Pheromone biology of the tabacco beetle (*Lasioderma serricorne* F., Anobiidae) with notes on the pheromone antagonism between 4S, 6S, 7S- and 4S, 6S, 7R-serricorin. J Appl Entomol 103:217–240

Martin MM (1979) Biochemical implications of insect mycophagy. Biol Rev 54:1–21

Pacioni G, Bologna MA, Laurenzi M (1991) Insect attraction by *Tuber*: a chemical explanation. Mycol Res 95(12):1359–1363

Robertson JA, McHugh JV, Whiting MF (2004) A molecular phylogenetic analysis of the pleasing fungus beetles (Coleoptera: Erotylidae): evolution of colour patterns, gregariousness and mycophagy. Syst Entomol 29:173–187

Scheerpeltz O, Höfler K (1948) Käfer und Pilze. Verlag für Jugend und Volk, Wien

Thakoew P, Weißbecker B, Schütz S (2006) Volatile organic compounds emitted from fungal-rotting beech (*Fagus sylvatica*). Mitteilungen der deutschen Gesellschaft für allgemeine und angewandte Entomologie 15:157–160

Thakoew P, Angeli S, Weißbecker B, Schütz S (2008) Antennal and behavioral responses of *Cis boleti* to fungal odor of *Trametes gibbosa*. Chem Senses 33:379–387

Ulland S, Ian E, Borg-Karlson A-K, Mustaparta H (2006) Discrimination between enantiomers of linalool by olfactory receptor neurons in the cabbage moth *Mamestra brassicae* (L.). Chem Senses 31:325–334

Wang P, Kong CH, Zhang CX (2006) Chemical composition and antimicrobial activity of the essential oil from *Ambrosia trifida* L. Molecules 11:549–555

Warmers U, König WA (1999) Sesquiterpene constituents of the liverwort *Bazzania trilobata*. Phytochemistry 52:99–104

Wu S, Zorn H, Krings U, Berger RG (2005) Characteristic volatiles from young and aged fruiting bodies of wild *Polyporus sulfureus* (Bull.: Fr.) Fr. J Agric Food Chem 53:4524–4528

Ziegenbein FC, Hanssen H-P, König WA (2006) Chemical constituents of the essential oils of three wood-rotting fungi. Flavour Fragrance J 21:813–816

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Tritoma bipustulata FABRICIUS, 1775 (Coleoptera: Erotylidae): eine neue Wirtsart für *Brachyserphus parvulus* (NEES, 1834) (Hymenoptera: Proctotrupidae)

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FAUNISTISCHE NOTIZEN

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Tritoma bipustulata Fabricius, 1775 (Coleoptera, Erotylidae): eine neue Wirtsart für *Brachyserphus parvulus* (Nees, 1834) (Hymenoptera, Proctotrupidae)

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Die Proctotrupidae sind eine artenarme Familie parasitischer Wespen innerhalb der Superfamilie Proctotrupoidea. Von den weltweit ca. 320 Arten (Dathe 2003) sind 35 aus Europa bekannt. Hier sind allerdings lediglich die Faunen der Britischen Inseln, Skandinaviens, Deutschlands, Tschechiens, der Slowakei sowie Österreichs hinsichtlich der Proctotrupidae einigermaßen vollständig erfasst. Interessanterweise wurden einige Arten lediglich für Nordeuropa nachgewiesen (vermutlich boreale Arten); mediterrane, atlantische oder rein alpine Arten sind dagegen nicht bekannt (Pschorn-Walcher 1971).

Wie ihre Wirte besiedeln Proctotrupidae, welche gemäßigtes, humides Klima bevorzugen (Kolyada & Mostovski 2007), die verschiedensten Biotope. Hauptsächlich sind sie jedoch in Gebieten mit hoher Luftfeuchtigkeit, wie etwa buschreiche Flussufer oder dem Unterwuchs von Wäldern (Pschorn-Walcher 1971) zu finden. Vertreter dieser Gruppe leben endoparasitisch (sowohl solitär als auch gregär) in Larven zahlreicher Familien der Coleoptera (z. B. Carabidae, Elateridae, Nitidulidae). Einige Arten sind jedoch auch aus Larven pilz- und bodenbewohnender Dipteren (Mycetophilidae, Sciaridae), Lepidopteren (Oecophilidae) und sogar Tausendfüßern (Lithobiidae) bekannt (Pschorn-Walcher 1971, Huggert 1979, Kolyada & Mostovski 2007).

Bemerkenswert ist die Art der Verpuppung. Die ausgewachsenen Parasitenlarven verlassen den Wirt nur teilweise. Sie bohren sich durch die ventralen Intersegmentalhäute am posterioren Ende der Wirtsexuvie nach außen und verpuppen sich mit ihrem Hinterende noch in dieser steckend (Abb. 1; Pschorn-Walcher 1971, Early & Dugdale 1994). Die Puppe ist dabei lediglich von einer dünnen, kaum sklerotisierten Membran überzogen. Diese Art der Verpuppung kann z. B. auch bei den nahverwandten Heloridae (parasitieren bei verschiedenen Chrysopidenarten) beobachtet werden (Pschorn-Walcher 1971).

Die Proctotrupidae sind durch ihr stark reduziertes Flügelgeäder (deutliches Pterostigma; meist sehr kurze, geschlossene Radialzelle) charakterisiert, wobei die meisten Adern des Vorderflügels verblasst oder gänzlich verschwunden sind (Abb. 2; Pschorn-Walcher 1971). Die Mehrzahl der Arten weist eine Körperlänge von 3-5 mm auf und ist einfarbig schwarz, Metallglanz tritt nicht auf. Die abgerundeten Seiten des Abdomens, nicht höckerartig vorspringende Antennenbasen sowie die 13-gliedrigen Antennen sind weitere charakteristische Merkmale der einheimischen Vertreter dieser Familie (Pschorn-Walcher 1971).

Die Gattung *Brachyserphus* umfasst weltweit 10 hauptsächlich holarktisch verbreitete Arten (Townes & Townes 1981, Williams et al. 1992); 2 gehören zur europäischen Fauna (*B. laeviceps*, *B. parvulus*; Pschorn-Walcher 1971). Von den übrigen Gattungen der Proctotrupidae ist *Brachyserphus* durch den, vom großen Gastertergit überdachten, Petiolus (dieser von oben daher nicht sichtbar), die senkrechte Kopfstellung (Augen dadurch hochgestellt), einem auffällig großen Pterostigma (nahezu gleichseitig dreieckig; Abb. 2) ohne Radialast und dem deutlich sichtbaren, dolchförmigen Legebohrer der Weibchen (Abb. 2, kleines Bild) zu unterscheiden (Pschorn-Walcher 1971).

Am 24.05.2008 konnten im Bienwald westlich von Karlsruhe (49°01′17.43″N, 8°10′14.17″E, 132m NN) zahlreiche Exemplare von *Tritoma bipustulata* an Trametes versicolor gesammelt werden (leg. K. Drilling). Der Fundort war schattig und durch große Mengen an liegendem Totholz, besonders Buche (Fagus sp.), charakterisiert. Die Fundumstände entsprechen somit der ökologischen Charakterisierung von *T. bipustulata* in Koch (1989): mycetobiont und silvicol, v. a. auf liegendem modernden Stämmen. Die gesammelten Tiere, sowie die nach einiger Zeit auftretenden Larven, wurden im Institut in der Klimakammer (10° C; 12 h Licht, 14 h Dunkelheit) auf *T. versicolor* gehalten. Beim Säubern der Zuchtbehälter fielen abgestorbene Käferlarven mit einer ventral am posterioren Ende anhaftenden Puppe auf (Abb. 1). Nach dem Schlüpfen (ca. 5-6 Tage nach der Verpuppung) wurden diese nach Pschorn-Walcher (1971), sowie Vergleichsmaterial vom Deutschen Entomologischen Institut (Müncheberg) als *Brachyserphus parvulus* bestimmt. Aus dem gesammelten Material konnten insgesamt 4 Tiere (2 ♀♀, 2 ♂♂) gezogen werden.

Das Verbreitungsgebiet von *B. parvulus* erstreckt sich nach Literaturangaben (Pschorn-Walcher 1971; www.faunaeur.org) über ganz Mitteleuropa, Skandinavien und Großbritannien bis nach Nordjapan. Die Flugzeit reicht von Juni bis Oktober, wobei die Art im August besonders abundant auftritt (Pschorn-Walcher 1971). Als Wirte von *B. parvulus* sind bisher *Meligethes aeneus* und *M. viridescens*. (Nitidulidae) aus Frankreich und Großbritannien bekannt (Osborne 1960, Pschorn-Walcher 1971). Nach Angaben von Nixon (1938) parasitiert die Art möglicherweise auch Larven von Melandryidae und Phalacridae. Die genaue Bestimmung der Wirtsarten ist in diesem Fall jedoch ungewiss. In Niederösterreich konnte die Art auch aus Larven von *Triplax* sp. (Erotylidae) gezogen werden (Pschorn-Walcher 1971). Der Nachweis von *B. parvulus* aus Larven von *T. bipustulata* stellt somit die erste Meldung für diese neue Wirtsart dar.

Literatur

Dathe, H. H. (2003): 31. Ordnung Hymenoptera, Hautflügler. S. 585-651. – In: Dathe, H. H. & Gruner, H.-E. (Hrsg.): Lehrbuch der speziellen Zoologie, Band I: Wirbellose Tiere, 5. Teil: Insecta. – Spektrum Akademischer Verlag, Heidelberg, Berlin.

Early, J. W. & Dugdale, J. S. (1994): *Fustiserphus* (Hymenoptera: Proctotrupidae) parasitises Lepidoptera in leaf litter in New Zealand. – New Zealand Journal of Zoology **21**: 249-252.

Huggert, L. (1979): *Cryptoserphus* and Belytinae wasps (Hymenoptera, Proctotrupidae) parasitizing fungus- and soil-inhabiting Diptera. – Notulae Entomologicae **59**: 139-144.

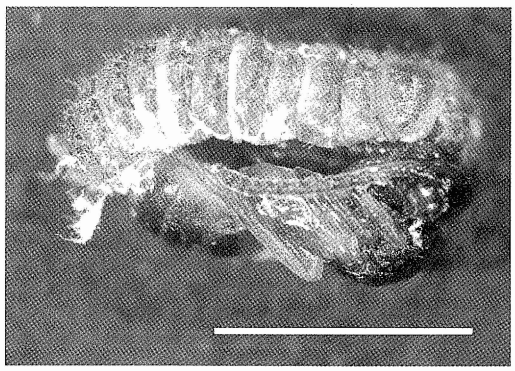

Abb. 1: Puppe von *Brachyserphus parvulus* an der abgestorbenen Larve von *T. bipustulata*. Puppe und Larve bleiben ventral am posterioren Ende miteinander verbunden. Maßstab: 2 mm.

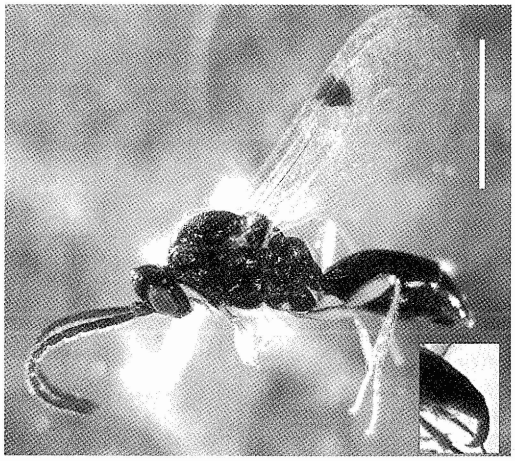

Abb. 2: Geschlüpftes Männchen von *B. parvulus*. Kleines Bild: dolchförmiger Legebohrer der Weibchen. Maßstab: 1 mm.

Koch, K. (1989): Die Käfer Mitteleuropas, Ökologie, Band 2. – Goecke & Evers Verlag, Krefeld.

Koylada, V. A. & Mostovski, M. B. (2007): Revision of Proctotrupidae (Insecta: Hymenoptera) described by Ch. T. Brues from baltic amber. – Zootaxa **1661**: 29-38.

Nixon, G. E. J. (1938): A preliminary revision of the British Proctotrupinae (Hym., Proctotrupoidea). – Transactions of the royal entomological Society London **87**: 431-465.

Osborne, P. (1960): Observations on the natural enemies of *Meligethes aeneus* (F.) and *M. viridescens* (F.) (Coleoptera: Nitidulidae). – Parasitology **50**: 91-110.

Pschorn-Walcher, H. (1971): Hymenoptera: Heloridae et Proctotrupidae. – Schweizerische Entomologische Gesellschaft.

Townes, H. & Townes, M. (1981): A revision of the Serphidae (Hymenoptera). – Memoirs of the American Entomological Institute **32**, Ann Arbor, Michigan.

Williams, R. N., Fickle, D. S. & Galford, J. R. (1992): Biological studies of *Brachyserphus abruptus* (Hym.: Proctotrupidae), a nitidulid parasite. – Entomophaga **37**(1): 91-98.

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List of Publications

Drilling, K., Küchler, S.: Die Wanzenfauna des Ökologisch-Botanischen Gartens der Universität Bayreuth. In prep..

Drilling, K., Klass, K.-D.: Implications on Neopteran phylogeny derived from the sensillar arrangement of scape and pedicel. In prep..

Drilling, K., Klass, K.-D.: The finer cuticular structure of the antenna in *Galloisiana yuasai* (Insecta: Grylloblattodea). In prep..

Drilling, K., Dettner, K., Klass, K.-D.: The distribution and evolution of exocrine glands in Erotylidae (Insecta: Coleoptera). European Journal of Entomology – will be submitted soon.

Drilling, K., Dettner, K.: First insights in the chemical defensive system of the erotylid beetle, *Tritoma bipustulata*. Chemoecology – submitted.

Drilling, K., Klass, K.-D.: The finer cuticular structure of the antennae in Mantophasmatodea (Insecta). Zoologischer Anzeiger – in press.

Küchler, S., **Drilling, K.** (2010): *Corythucha ciliata* SAY, 1832 (Heteroptera, Tingidae): Platanenschädling breitet sich weiter im Dresdner Raum aus. Entomologische Nachrichten und Berichte 54(1), 63.

Drilling, K., Dettner, K., Klass, K.-D. (2010): Morphology of the pronotal compound glands in *Tritoma bipustulata* (Coleoptera: Erotylidae). Organism, Diversity & Evolution, DOI: 10.1007/s13127-010-0008-0.

Drilling, K. (2009): *Tritoma bipustulata* FABRICIUS, 1775 (Coleoptera: Erotylidae): eine neue Wirtsart für *Brachyserphus parvulus* (NEES, 1834) (Hymenoptera: Proctotrupidae). Entomologische Nachrichten und Berichte 53(2), 126-127.

Drilling, K., Dettner, K. (2009): Electrophysiological responses of four fungivorous coleoptera to volatiles of *Trametes versicolor*: implications for host selection. Chemoecology, 19(2), 109-115.

Drilling, K., Klass, K.-D. (2008): *Orsodacne cerasi* (Coleoptera, Orsodacnidae): Neufund für Mecklenburg-Vorpommern. Entomologische Nachrichten und Berichte, 52(3-4), 219-220.

Buder, G., **Drilling, K.**, Klass, K.-D. (2006): *Soronia punctatissima* (ILLIGER, 1794) (Coleoptera, Nitidulidae): Weiterer Fund für Sachsen und männliche Kopulationsorgane. Entomologische Nachrichten und Berichte, 50(4), 242-244.

Drilling, K., Klass, K.-D. (2006): *Lixus subtilis* BOHEMAN, 1836 (Coleoptera, Curculionidae, Lixinae): Neufund für Sachsen. Entomologische Nachrichten und Berichte, 50(3), 165-166.

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Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe. Ferner erkläre ich, dass ich nicht anderweitig mit oder ohne Erfolg versucht habe, diese Dissertation einzureichen. Ich habe keine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden.

Dresden, den 14.04.2010 – Kai Drilling