Ann. Zool. Fennici 51: 285–300 ISSN 0003-455X (print), ISSN 1797-2450 (online)
Helsinki 30 June 2014

Defensive secretions in three ground-beetle species (Insecta: Coleoptera: Carabidae)

Sonja Lečić[1], Srećko Ćurčić[1,*], Ljubodrag Vujisić[2], Božidar Ćurčić[1], Nina Ćurčić[3], Zoran Nikolić[1], Boban Anđelković[2], Slobodan Milosavljević[2], Vele Tešević[2] & Slobodan Makarov[1]

[1]) *Institute of Zoology, University of Belgrade — Faculty of Biology, Studentski Trg 16, 11000 Belgrade, Serbia (*corresponding author's e-mail: srecko@bio.bg.ac.rs)*
[2]) *University of Belgrade — Faculty of Chemistry, Studentski Trg 12–16, 11000 Belgrade, Serbia*
[3]) *Geographical Institute "Jovan Cvijić", Serbian Academy of Sciences and Arts, Đure Jakšića 9, 11000 Belgrade, Serbia*

Received 7 Mar. 2013, final version received 6 July 2013, accepted 1 Aug. 2013

Lečić, S., Ćurčić, S., Vujisić, L., Ćurčić, B., Ćurčić, N., Nikolić, Z., Anđelković, B., Milosavljević, S., Tešević, V. & Makarov, S. 2014: Defensive secretions in three ground-beetle species (Insecta: Coleoptera: Carabidae). — *Ann. Zool. Fennici* 51: 285–300.

The adults of three ground-beetle species were induced to discharge defensive secretions into vials. The secretions were obtained by CH_2Cl_2 extraction. Altogether 11 compounds were identified by GC-MS analysis. *Calosoma sycophanta* possesses 10 defensive compounds, *Carabus ullrichii* seven, while *Abax parallelepipedus* has six compounds. Methacrylic, tiglic and isobutyric acids were present in all samples. The first two organic compounds were predominant in the extracts of *Abax parallelepipedus*. Methacrylic acid and salicylaldehyde were the major compounds in extracts of *Calosoma sycophanta*. Methacrylic and angelic acids were the major components in extracts of *Carabus ullrichii*. Propanoic acid was detected for the first time in the family Carabidae and in all animals. 2-Methyl butyric, angelic and benzoic acids were found for the first time in the subfamily Carabinae. Our finding of butyric acid is its first precise identification in the Carabinae subfamily. 2-Methyl butyric, angelic, crotonic, senecioic and benzoic acids were found for the first time in a European ground-beetle species. The compounds detected in the defensive secretions serve as protection against predators.

Introduction

A great number of beetle species use chemical defense. A variety of defensive glands and compounds exist within the order Coleoptera (Blum 1981). Ground beetles (Carabidae) possess abdominal pygidial glands that produce defensive secretions. Defensive mechanisms in ground beetles based on chemistry have been known for a long time (Schildknecht *et al*. 1964). These are diverse and their secretions vary within the family (Thiele 1977, Dettner 1987). Secretion discharge may be executed in three ways: by oozing, spraying, or crepitation (Thiele 1977, Moore 1979). The main groups of defensive secretions discovered in species of different ground-beetle tribes are as follows: hydrocarbons, aliphatic ketones, saturated esters, formic acid, higher saturated fatty acids, unsaturated carboxylic acids, phenols, aromatic aldehydes (salicylaldehyde), and quinones (Moore 1979, Will *et al.* 2000, Giglio *et al.* 2011). Pygidial gland secretions in ground beetles may serve for defense against predators; in some cases the secretions possess both antimicrobial and antifungal properties, and in certain taxa they represent a type of alarm substance (Whitman *et al.* 1990, Blum 1996).

Defensive secretions have been found in more than 500 ground-beetle species belonging to the subfamilies Anthiinae, Brachininae, Broscinae, Carabinae, Cicindelinae, Dryptinae, Elaphrinae, Harpalinae, Lebiinae, Licininae, Loricerinae, Nebriinae, Omophroninae, Panagaeinae, Paussinae, Platyninae, Pseudomorphinae, Psydrinae, Pterostichinae, Scaritinae, and Trechinae (Pavan 1968, Blum 1981, Blum *et al.* 1981, Kanehisa & Kawazu 1982, Balestrazzi *et al.* 1985, Pearson *et al.* 1988, Kelley & Schilling 1998, Eisner *et al.* 2000, 2001, Will *et al.* 2000, Attygalle *et al.* 2009, Bonacci *et al.* 2011, Holliday *et al.* 2012). If we consider the ground-beetle species inhabiting Europe, data on the compounds included in defensive secretions can be found for 86 species from 17 subfamilies (Table 1). The chemical content of the defensive secretions of ground beetles inhabiting Europe varies within subfamilies. A review of all analyzed species and detected compounds is summarized in Table 1.

The defensive compounds of ground beetles from Europe have not been efficiently studied in the past. Therefore, we chose the following three species for our analysis: *Abax* (*Abax*) *parallelepipedus* (Pterostichinae: Pterostichini; inhabiting Europe and North America), *Calosoma* (*Calosoma*) *sycophanta* (Carabinae: Carabini; inhabiting the entire Holarctic), and *Carabus* (*Eucarabus*) *ullrichii* (Carabinae: Carabini; inhabiting central and eastern Europe and the Balkan Peninsula). Besides, the three species are sympatric during spring and summer in deciduous forests in Serbia and have similar dietary preferences (invertebrate predators). Here their populations are abundant and greater amounts of both male and female specimens can be caught for the analysis. In addition, we wanted to check the validity of previous results on the content of defensive secretions and to search for possible minor components in the secretions.

The objectives of the paper were: (i) to determine the chemical composition of the defensive secretions of three ground-beetle species; (ii) to test for possible intergeneric and interspecific variations of chemical compounds in the investigated and other taxa; and (iii) to search for possible new compounds that have not been registered within the family so far.

Material and methods

Collection and handling of ground beetles

Adult specimens of *Abax parallelepipedus*, *Calosoma sycophanta* and *Carabus ullrichii* were collected by turning over stones and rotten stumps, and from trees during spring (6 June 2012) on Mt. Avala (44°41´25´´N 20°30´51´´E), near Belgrade, central Serbia by S. Ćurčić, Z. Nikolić, and S. Lečić. The ground beetles were stored in the laboratory for several days at 10 °C, in the dark, in plastic boxes, with moist soil and partly decomposed litter taken from the sites of beetle collection. Humidity in the boxes was kept high by spraying water on the soil and litter every day. The insects were fed on mealworms and earthworms. After analysis, beetle individuals were deposited in the collection of the Institute of Zoology, University of Belgrade — Faculty of Biology.

Chemicals

Standards of the most abundant compounds in the defensive secretions of ground beetles (butyric acid, methacrylic acid, salicylaldehyde, angelic acid, senecioic acid, tiglic acid, and benzoic acid) and silylation reagent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane was obtained from Merck (Darmstadt, Germany).

Table 1. Chemicals in defensive secretions of European Carabidae.

Species	Subfamily	Chemicals	Source
Abax (*Abax*) *ovalis*	Pterostichinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al.* (1964, 1968a, 1968c), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Abax (*Abax*) *parallelepipedus*	Pterostichinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al.* (1964, 1968a), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Abax (*Abax*) *parallelus*	Pterostichinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al.* (1964, 1968a, 1968c), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Acinopus sp.	Harpalinae	Formic acid	Pavan (1968)
Agonum (*Agonum*) *marginatum*	Platyninae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Agonum (*Olisares*) *duftschmidi*	Platyninae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Agonum (*Olisares*) *sexpunctatum*	Platyninae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Agonum (*Olisares*) *viduum*	Platyninae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Anchomenus (*Anchomenus*) *dorsalis*	Platyninae	Formic acid; methyl salicylate; *n*-decane; undecane; heneicosane; (*Z*)-9-tricosene; tricosane	Schildknecht *et al.* (1968a, 1968b), Schildknecht (1970), Bonacci *et al.* (2011)
Anisodactylus (*Anisodactylus*) *binotatus*	Harpalinae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Amara (*Amara*) *familiaris*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane; *n*-decane; *n*-tridecane)	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Amara (*Amara*) *similata*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane; *n*-decane; *n*-tridecane)	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Asaphidion flavipes	Trechinae	Salicylaldehyde; *n*-valeric acid	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Aptinus (*Aptinus*) *pyrenaeus*	Brachininae	Quinones	Schildknecht *et al.* (1968a, 1968c)
Badister (*Badister*) *bullatus*	Licininae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Bembidion quadrimaculatum	Trechinae	Aliphatic ketones	Schildknecht *et al.* (1968a, 1968c)
Brachinus sp.	Brachininae	Nitrogen oxides	Pavan (1968)
Brachinus (*Brachinus*) *crepitans*	Brachininae	1,4-Benzoquinone; 2-methyl-1,4-benzoquinone; nitrogen oxides; nitrous acid	Schildknecht and Holoubek (1961), Roth and Eisner (1962), Weatherston (1967), Pavan (1968), Schnepf *et al.* (1969)
Brachinus (*Brachynidius*) *explodens*	Brachininae	1,4-Benzoquinone; 2-methyl-1,4-benzoquinone	Schildknecht and Holoubek (1961), Roth and Eisner (1962), Weatherston (1967), Pavan (1968)
Brachinus (*Brachynidius*) *sclopeta*	Brachininae	1,4-Benzoquinone; 2-methyl-1,4-benzoquinone	Schildknecht and Holoubek (1961), Roth and Eisner (1962), Weatherston (1967), Pavan (1968)
Broscus cephalotes	Broscinae	Isovaleric acid; isobutyric acid	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Calathus sp.	Platyninae	Formic acid	Pavan (1968)

continued

Table 1. Continued.

Species	Subfamily	Chemicals	Source
Calathus (*Calathus*) *fuscipes*	Platyninae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c)
Calathus (*Neocalathus*) *melanocephalus*	Platyninae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c)
Callistus lunatus	Licininae	Quinones	Schildknecht *et al.* (1968a, 1968c)
Calosoma (*Calosoma*) *sycophanta*	Carabinae	Salicylaldehyde; methacrylic acid; tiglic acid	Casnati *et al.* (1965), Pavan (1968), Schildknecht *et al.* (1968a), Blum (1981)
Carabus sp.	Carabinae	Butyric acid	Pavan (1968)
Carabus (*Carabus*) *granulatus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al.* (1964, 1968a, 1968c), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Carabus (*Chaetocarabus*) *intricatus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Jacobson (1966)
Carabus (*Chaetocarabus*) *lefebvrei* (pupa)	Carabinae	Pivalic acid; 1,4-benzoquinone; α-pinene; camphene; benzaldehyde; 6-methyl-5-hepten-2-one; β-pinene; 2-carene; α-phellandrene; α-terpinene; orto-cimene; limonene; *cis*-ocimene; *trans*-ocimene; 2-hydroxybenzaldehyde; linalool; nonanal; camphor; 4,8-dimethyl-3,7-nonadien-2-ol; 1-(1-oxobutyl)-1,2-dihydropyridin; 3,4-di(1-butenil)-tetrahydrofuran-2-ol; thymol methyl ether; phenylacetic acid; propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester; propanoic acid, 2-methyl-, 3-hydroxy-4,4-trimethylpenthyl ester; β-elemene; α-santalene; *trans*-α-bergamotene; pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester; dodecanoic acid, 1-methylethyl ester; sclarene	Giglio *et al.* (2009)
Carabus (*Chrysocarabus*) *auronitens*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al.* (1964), Jacobson (1966), Pavan (1968), Schildknecht (1970)
Carabus (*Eucarabus*) *ullrichii*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al.* (1964), Jacobson (1966), Pavan (1968)
Carabus (*Megodontus*) *violaceus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al.* (1964), Jacobson (1966), Pavan (1968)
Carabus (*Mesocarabus*) *problematicus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)

continued

Table 1. Continued.

Species	Subfamily	Chemicals	Source
Carabus (*Morphocarabus*) *scheidleri*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962)
Carabus (*Platycarabus*) *irregularis*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht *et al*. (1964), Jacobson (1966), Pavan (1968)
Carabus (*Procrustes*) *coriaceus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al*. (1964), Jacobson (1966), Pavan (1968)
Carabus (*Tachypus*) *auratus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al*. (1964, 1968a, 1968c), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Carabus (*Tachypus*) *cancellatus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al*. (1964), Pavan (1968), Schildknecht (1970)
Carabus (*Tomocarabus*) *convexus*	Carabinae	Methacrylic acid; tiglic acid	Schildknecht and Weis (1962), Schildknecht *et al*. (1964), Jacobson (1966), Pavan (1968), Schildknecht (1970)
Carterus sp.	Harpalinae	Formic acid	Pavan (1968)
Chlaeniellus tristis	Licininae	*m*-Cresol	Schildknecht *et al*. (1968a, 1968c)
Chlaeniellus vestitus	Licininae	1,4-Benzoquinone; 2-methyl-1,4-benzoquinone; 2,3-dimethyl-1,4-benzoquinone	Schildknecht *et al*. (1968a, 1968c), Balestrazzi *et al*. (1985)
Chlaenius (*Chlaenius*) *festivus*	Licininae	*m*-Cresol	Schildknecht *et al*. (1968a, 1968c)
Chlaenius (*Chlaenius*) *velutinus*	Licininae	*m*-Cresol; 2,5-dimethylphenol; 3,5-dimetylphenol; pentadecane; heptadecane	Balestrazzi *et al*. (1985)
Chlaenius (*Trichochlaenius*) *chrysocephalus*	Licininae	*m*-Cresol	Schildknecht *et al*. (1968a, 1968c)
Clivina (*Clivina*) *fossor*	Scaritinae	Quinones; 1,4-benzoquinone; 2-methyl-1,4-benzoquinone; 2,3-dimethyl-1,4-benzoquinone; 2-methyl-3-metoxy-1,4-benzoquinone	Schildknecht *et al*. (1968a, 1968c), Schildknecht (1970)
Cychrus sp.	Carabinae	Butyric acid	Pavan (1968)
Cychrus caraboides	Carabinae	Methacrylic acid	Jacobson (1966), Claridge (1974)
Diachromus germanus	Harpalinae	Formic acid; alkanes	Schildknecht *et al*. (1968a, 1968c), Blum (1981)
Dicheirotrichus (*Dicheirotrichus*) *obsoletus*	Harpalinae	Formic acid; alkanes	Schildknecht *et al*. (1968a, 1968c), Blum (1981)
Drypta (*Drypta*) *dentata*	Dryptinae	Formic acid; alkanes	Schildknecht *et al*. (1968a, 1968c), Blum (1981)
Elaphrus (*Elaphrus*) *riparius*	Elaphrinae	Isovaleric acid; isobutyric acid	Schildknecht *et al*. (1968a, 1968c), Blum (1981)
Harpalus (*Harpalus*) *atratus*	Harpalinae	Formic acid; alkanes	Schildknecht *et al*. (1968a, 1968c)
Harpalus (*Harpalus*) *dimidiatus*	Harpalinae	Formic acid; alkanes	Schildknecht (1961), Schildknecht *et al*. (1964, 1968a, 1968c), Pavan (1968)
Harpalus (*Harpalus*) *distinguendus*	Harpalinae	Formic acid; alkanes	Schildknecht *et al*. (1968a, 1968c)

continued

Table 1. Continued.

Species	Subfamily	Chemicals	Source
Harpalus (*Harpalus*) *luteicornis*	Harpalinae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c)
Harpalus (*Harpalus*) *tardus*	Harpalinae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c)
Lamprias chlorocephalus	Lebiinae	Formic acid	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Leistus (*Leistus*) *ferrugineus*	Nebriinae	Methacrylic acid; tiglic acid	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Licinus (*Licinus*) *depressus*	Licininae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Limodromus assimilis	Platyninae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Loricera pilicornis	Loricerinae	Isovaleric acid; isobutyric acid	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Nebria (*Eunebria*) *psammodes*	Nebriinae	Methacrylic acid; tiglic acid	Balestrazzi *et al.* (1985)
Nebria (*Paranebria*) *livida*	Nebriinae	Methacrylic acid; tiglic acid	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Notiophilus biguttatus	Nebriinae	Isovaleric acid; isobutyric acid	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Metallina (*Metallina*) *lampros*	Trechinae	Isovaleric acid; isobutyric acid	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Molops elatus	Pterostichinae	Methacrylic acid; tiglic acid	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Ocydromus (*Nepha*) *schmidti*	Trechinae	Salicylaldehyde; *n*-valeric acid	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Ocydromus (*Peryphus*) *andreae*	Trechinae	Isovaleric acid; isobutyric acid	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Odacantha (*Odacantha*) *melanura*	Lebiinae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Omophron limbatum	Omophroninae	Isovaleric acid; isobutyric acid	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Ophonus (*Hesperophonus*) *azureus*	Harpalinae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Panagaeus (*Panagaeus*) *bipustulatus*	Panagaeinae	*m*-Cresol	Schildknecht *et al.* (1968a, 1968c)
Paussus favieri	Paussinae	1,4-Benzoquinone; 2-methyl-1,4-benzoquinone	Schildknecht and Koob (1969)
Poecilus (*Poecilus*) *cupreus*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane; *n*-decane; *n*-tridecane)	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Polistichus connexus	Dryptinae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Pseudoophonus (*Pseudoophonus*) *griseus*	Harpalinae	Formic acid; alkanes	Schildknecht (1961), Schildknecht and Weis (1961), Jacobson (1966), Weatherston (1967), Pavan (1968), Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Pseudoophonus (*Pseudoophonus*) *rufipes*	Harpalinae	Formic acid; alkanes	Schildknecht (1961), Schildknecht and Weis (1961), Jacobson (1966), Weatherston (1967), Pavan (1968), Schildknecht *et al.* (1968a, 1968c), Blum (1981)
Pterostichus (*Adelosia*) *macer*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane; *n*-decane; *n*-tridecane)	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Pterostichus (*Cheporus*) *burmeisteri*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane;	Schildknecht and Weis (1962), Schildknecht *et al.* (1964, 1968a,

continued

Table 1. Continued.

Species	Subfamily	Chemicals	Source
		n-decane; *n*-tridecane)	1968c), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Pterostichus (*Feronidius*) *melas*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane; *n*-decane; *n*-tridecane)	Schildknecht *et al.* (1968a, 1968c), Schildknecht (1970), Blum (1981)
Pterostichus (*Morphnosoma*) *melanarius*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane; *n*-decane; *n*-tridecane)	Schildknecht and Weis (1962), Schildknecht *et al.* (1964, 1968a, 1968c), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Pterostichus (*Oreophilus*) *externepunctatus*	Pterostichinae	Isobutyric acid; methacrylic acid; tiglic acid; undecane; tridecane	Balestrazzi *et al.* (1985)
Pterostichus (*Platysma*) *niger*	Pterostichinae	Methacrylic acid; tiglic acid; alkanes (*n*-undecane; *n*-decane; *n*-tridecane)	Schildknecht and Weis (1962), Schildknecht *et al.* (1964, 1968a, 1968c), Jacobson (1966), Pavan (1968), Schildknecht (1970), Blum (1981)
Stenolophus (*Stenolophus*) *mixtus*	Harpalinae	Formic acid; alkanes	Schildknecht *et al.* (1968a, 1968c), Blum (1981)

Instrumentation

An Agilent 7890A GC system equipped with a 5975C inert XL EI/CI mass selective detector (MSD) and a flame ionization detector (FID) connected by capillary flow technology through a 2-way splitter were used for analyses. Two different capillary columns were used: non-polar HP-5 MS (30 m length, 0.25 mm inside diam., 0.25 μm film thickness) and polar INNOWax (30 m length, 0.32 mm inside diam., 0.25 μm film thickness), both from Agilent Technologies, Santa Clara, CA, USA.

Chemical extraction

Gas chromatography-mass spectrometry (GC-MS) sampling was performed in the laboratory at room temperature. Ten males of each of the three species were milked into a 12-ml glass vial with dichloromethane (0.5 ml) for 10 min, while ten females of each of the three species were treated in the same way into a second vial. The beetles were forced to discharge defensive secretions by pinching the legs with pins from time to time during milking (Eisner *et al*. 1963a). To eliminate the effects of composition-altering oxidation and degradation of compounds, a portion of the extracts was subjected to gas chromatography-mass spectrometric (GC-MS) analysis immediately after preparation.

Chemical derivatization (silylation)

The dichloromethane extracts of all species were derivatized with BSTFA for GC and GC-MS analyses (Knapp 1979, Zaikin & Halket 2009). Silylation was done in order to check for the possible presence of non-volatile compounds. It was performed with 250 μl of dichloromethane extract of the ground beetles and 100 μl of silylation reagent in a 2 ml-GC vial at 60 °C for 30 min. After cooling at room temperature, the samples were ready for GC and GC-MS analyses on non-polar HP-5 MSI column.

Chemical analyses

The GC and GC-MS analyses were performed in splitless mode. For all analyses, the injection volume was 1 μl, the injector temperature 240 °C, and the transfer line temperature 280 °C. The carrier gas (He) flow rate was 1.6 ml min^{-1}

at 40 °C (constant pressure mode), whereas the column temperature was programmed to increase linearly in a range of 40–315 °C at a rate of 10 °C min^{-1}, with an initial 1-min and a final 6.5-min hold for analyses on the HP-5 MSI column. The analyses on the INNOWax column were performed at a helium flow rate of 2.5 ml min^{-1} at 40 °C (constant pressure mode). The oven temperature was programmed to increase linearly in a range of 40–240 °C at a rate of 10 °C min^{-1}, with a final 10-min hold. The FID detector temperature was 300 °C. EI mass spectra (70 eV) were acquired in the m/z range of 35–550, and the ion source temperature was 230 °C for all analyses.

The library search and mass spectral deconvolution and extraction were performed using NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software, ver. 2.70. We used retention index (RI) calibration data analysis parameters at a 'strong' level with a 10% penalty for compounds without an RI. Retention indices were determined experimentally by the method of van Den Dool and Kratz (1963). This method is based on the retention times of *n*-alkanes, which were injected after the sample under the same chromatographic conditions. The search was performed against our own library containing 4951 spectra, and the commercially available NIST11 and Willey07 libraries containing approximately 500 000 spectra (identifications supported by data in Table 2 and Fig. 1). In addition, the most abundant peaks in the chromatograms of dichloromethane extracts of the ground beetles were compared to authentic standards.

Relative percentages of the identified compounds were computed from the corresponding GC-FID peak areas from chromatograms obtained on INNOWax column, while the analyses of defensive secretions and derivatization on HP-5 MSI column were done for confirmation of the identified compounds and for the search of potential semi-volatile and non-volatile compounds.

Results

Eleven compounds were identified in the dichloromethane extracts of the defensive secretions of the three analyzed ground-beetle species on polar INNOWax column (Table 2, Figs. 1 and 2). The extracts from different ground-beetle species exhibited different GC patterns, which was confirmed by the analyses performed on non-polar HP-5 MSI column (GC-FID and GC-MS patterns were the same in each of the analyzed

Table 2. Chemical composition of the defensive secretions in three ground-beetle species analyzed by GC-FID and GC-MS.

Peak	t_R (min)[a]	RI[a]	Compound	Relative abundance (%)[b]		
				Abax parallelepipedus	*Calosoma sycophanta*	*Carabus ullrichii*
1	11.0	1549	Propanoic acid	T[c]	T[c]	–
2	11.4	1576	Isobutyric acid	0.4	0.3	0.3
3	12.2	1637	Butyric acid	–	1.5	0.2
4	12.7	1680	2-Methyl butyric acid	–	0.1	0.2
5	13.0	1698	Methacrylic acid	76.5	44.8	78.7
6	13.1	1710	Salicylaldehyde	–	42.7	–
7	14.0	1784	Angelic acid	–	–	17.7
8	14.1	1788	Crotonic acid	0.1	0.1	–
9	14.4	1816	Senecioic acid	0.1	2.1	–
10	14.9	1859	Tiglic acid	22.9	5.6	2.5
11	21.4	2461	Benzoic acid	–	2.8	0.4

[a] Obtained from GC-MS data on INNOWax capillary column.
[b] Obtained from GC-FID peak areas on INNOWax capillary column.
[c] Trace (less than 0.1%).

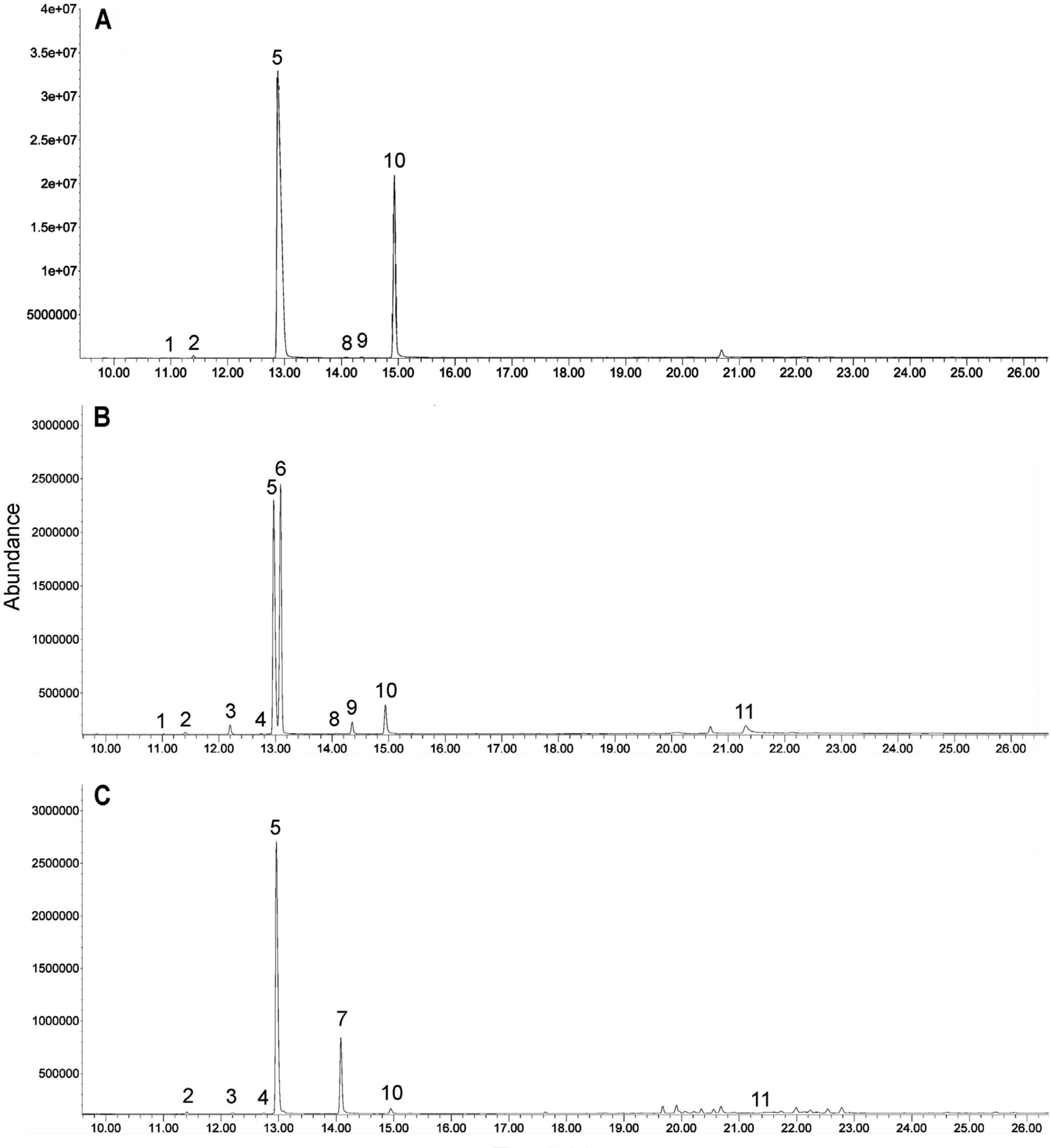

Fig. 1. GC-FID chromatograms of the dichloromethane defensive secretion extracts from adult ground beetles (**A**) *Abax* (*Abax*) *parallelepipedus*, (**B**) *Calosoma* (*Calosoma*) *sycophanta*, and (**C**) *Carabus* (*Eucarabus*) *ullrichii* on INNOWax capillary column. Propanoic acid (peak 1), isobutyric acid (peak 2), butyric acid (peak 3), 2-methyl butyric acid (peak 4), methacrylic acid (peak 5), salicylaldehyde (peak 6), angelic acid (peak 7), crotonic acid (peak 8), senecioic acid (peak 9), tiglic acid (peak 10), and benzoic acid (peak 11).

species) (Table 2 and Fig. 1). All identified organic acids and salicylaldehyde reacted with BSTFA afforded trimethylsilyl derivatives. The extract of *Abax parallelepipedus* was comprised of six compounds (Fig. 1A). The major components were methacrylic and tiglic acids, while the minor ones included isobutyric, crotonic and senecioic acids, as well as a trace amount of propanoic acid. The extract of *Calosoma sycophanta* was composed of 10 compounds (Fig. 1B). The major components were methacrylic acid, salicylaldehyde, and tiglic acid;

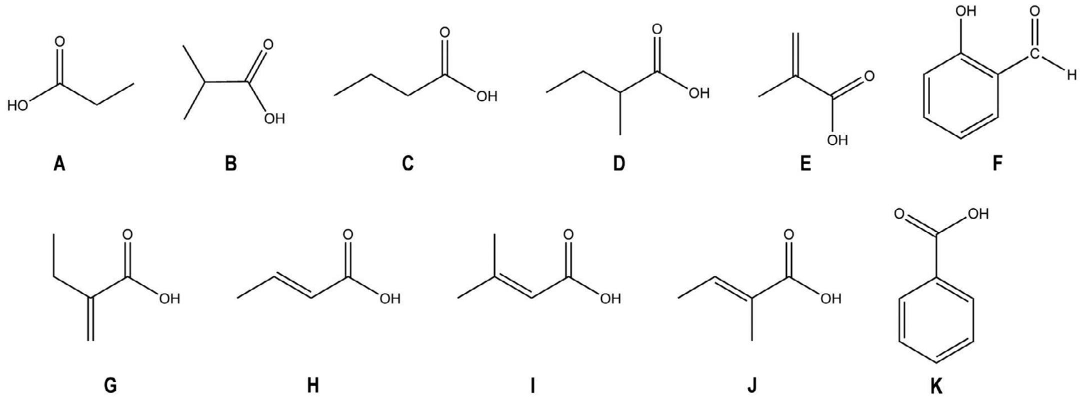

Fig. 2. Compounds isolated from the defensive secretion of the three ground-beetle species analyzed. (**A**) Propanoic acid, (**B**) isobutyric acid, (**C**) butyric acid, (**D**) 2-methyl butyric acid, (**E**) methacrylic acid, (**F**) salicylaldehyde, (**G**) angelic acid, (**H**) crotonic acid, (**I**) senecioic acid, (**J**) tiglic acid, and (**K**) benzoic acid.

minor components were benzoic, senecioic, butyric, isobutyric, 2-methyl butyric and crotonic acids, and a trace amount of propanoic acid. The extract of *Carabus ullrichii* contained seven compounds (Fig. 1C). The major components were methacrylic, angelic, and tiglic acids, while the minor components were benzoic, isobutyric, butyric, and 2-methyl butyric acids.

We confirmed the presence of previously registered compounds in the defensive secretions of the three species (Schildknecht & Weis 1962, Schildknecht *et al.* 1964, Casnati *et al.* 1965, Pavan 1968), but discovered a number of so far unknown compounds. We did not find differences in the amounts of defensive secretion components between the sexes in the three studied species.

Discussion

Methacrylic acid was a major component of the defensive secretions in all three ground-beetle species analyzed. Methacrylic, tiglic, and isobutyric acids were present in all samples. The first two mentioned carboxylic acids are known as one of the most common defensive substances in various ground beetles (mostly within the subfamilies Carabinae and Pterostichinae), along with formic acid, alkanes, and quinones (McCullough & Weinheimer 1966, Moore & Wallbank 1968, McCullough 1969a, 1969b, 1972, Wheeler *et al.* 1970, Benn *et al.* 1973, Scott *et al.* 1975, Kanehisa & Murase 1977, Moore 1979, Kanehisa & Kawazu 1982, Adachi *et al.* 1985, Davidson *et al.* 1989, Attygalle *et al.* 1990, 1991, 2007, Will *et al.* 2000) (Table 1). Regarding other beetles, methacrylic and tiglic acids have been registered in the defensive secretions of certain Trachypachidae (Attygalle *et al.* 2004). As regards other invertebrates, they have been found in the defensive secretions of cockroaches (Brossut 1983). Tiglic acid alone is recognized in the defensive secretions of certain diving and rove beetles (Dettner & Schwinger 1980, Dettner & Reissenweber 1991).

We discovered a trace amount of propanoic acid in the defensive secretions of both males and females of *Abax parallelepipedus* and *Calosoma sycophanta*. This is the first evidence for the presence of this compound in the Carabidae family and animals in general.

Isobutyric acid was registered in a smaller number of ground-beetle species belonging to the subfamilies Carabinae (Kanehisa & Murase 1977, Kanehisa & Kawazu 1982, Adachi *et al.* 1985), Broscinae (Kanehisa & Murase 1977, Moore 1979), Elaphrinae, Licininae (Attygale *et al.* 1990, Will *et al.* 2000), Loricerinae, Nebriinae, Omophroninae, Pterostichinae (Kanehisa & Kawazu 1982), Scaritinae (Davidson *et al.* 1989, Attygale *et al.* 1991), and Trechinae (Table 1). Its presence in the defensive secretions of *Abax parallelepipedus* is the first record of its presence in the genus *Abax*, and the second in the tribe Pterostichini. In addition, its presence in the defensive secretions of *Calosoma sycophanta* is the first time it has been found in the genus *Calosoma*. This chemical compound is known in other beetles (Trachypachidae and Staphylinidae) (Dettner & Reissenweber 1991, Attygale *et al*. 2004), as well as in some other insects (Alydidae, Blattidae, Formicidae, Papilionidae, Reduviidae) (Quilico *et al*. 1960, Aldrich & Yonke 1975, Blum 1981, Brossut 1983). The presence of isobutyric acid in the defensive secretions of *Carabus ullrichii* is the first time it has been found in a *Carabus* species inhabiting Europe.

We detected butyric acid in the defensive secretions in the two analyzed species belonging to the tribe Carabini (*Calosoma sycophanta* and *Carabus ullrichii*). This compound had rarely been found in the Carabidae family (seven species belonging to the subfamilies Broscinae, Carabinae, Licininae, and Trechinae) (Pavan 1968, Kanehisa & Murase 1977, Moore 1979, Attygale *et al*. 1990). Pavan (1968) reported the acid from two species belonging to the genera *Carabus* and *Cychrus*, but without precise identification. Ours are the first precise findings in ground beetles of the Carabinae subfamily. Like the previously mentioned compound, this one is already known from representatives of other Coleoptera (rove beetles) (Dettner & Reissenweber 1991) and other insect orders (Dictyoptera, Hemiptera, Hymenoptera, and Lepidoptera) (Aldrich & Yonke 1975, Blum 1981, Brossut 1983, Burger *et al*. 1986, Fortunato *et al*. 2001).

2-Methyl butyric acid has so far been detected in six non-European ground-beetle species from the Licininae, Pterostichinae, and Scaritinae subfamilies (Kanehisa & Kawazu 1982, Davidson *et al*. 1989, Attygale *et al*. 1990, 2007). Its finding in the defensive fluids of *Calosoma sycophanta* and *Carabus ullrichii* demonstrates that it is present in the taxa of the Carabinae subfamily as well (present study). These are its first records in ground beetles inhabiting Europe. Among beetles, it is also found in some Trachypachidae and Staphylinidae (Klinger & Maschwitz 1977, Dettner & Reissenweber 1991, Attygale *et al*. 2004), and in the defensive secretions of other insects (Hemiptera, Lepidoptera, Thysanoptera) (Blum 1981, Chow & Tsai 1987, Suzuki *et al*. 1988).

Salicylaldehyde has mostly been reported from a number of *Calosoma* species (Eisner *et al*. 1963b, Casnati *et al*. 1965, Eisner & Meinwald 1966, McCullough & Weinheimer 1966, Moore & Wallbank 1968, Pavan 1968, Schildknecht *et al*. 1968a, McCullough 1969a, Kanehisa & Murase 1977, Blum 1981), as well as an additional four ground-beetle species belonging to the subfamilies Licininae, Pterostichinae and Trechinae (Schildknecht *et al*. 1968a, 1968c, Schildknecht 1970, Moore 1979, Blum 1981, Will *et al*. 2000). Its occurrence within the genus *Calosoma* (also confirmed by us) supports the view that this feature represents a clear intergeneric difference within the Carabini tribe. Salicylaldehyde is additionally found in certain long-tongued bees and leaf beetles (Hefetz *et al*. 1979, Blum 1981, Cane & Michener 1983).

Angelic acid exists only in the defensive secretions of ground beetles. It has been identified in eight non-European species (seven belonging to the subfamily Scaritinae, and one from the subfamily Pterostichinae) (Moore & Wallbank 1968, Kanehisa & Murase 1977, Davidson *et al*. 1989). We found it in the defensive secretions of *Carabus ullrichii*, which represents its first report in a taxon from the subfamily Carabinae and at the same time in a European species.

Crotonic acid has so far been registered in six non-European ground-beetle species belonging to the Carabinae, Licininae, Pterostichinae, and Scaritinae subfamilies (Moore & Wallbank 1968, Moore 1979, Kanehisa & Kawazu 1982, Adachi *et al*. 1985, Attygale *et al*. 1990). It has not been found in other animals. Its finding in the defensive secretions of *Abax parallelepipedus* is the first from a European species and the second within the tribe Pterostichini, while the finding of this compound in *Calosoma sycophanta* is the second in the tribe Carabini.

Senecioic acid has been detected in a small number of non-European ground-beetle species (seven pterostichine and one each of carabine, broscine, licinine and scaritine species) (Kanehisa & Murase 1977, Moore 1979, Kanehisa & Kawazu 1982, Adachi *et al*. 1985, Davidson *et al*. 1989, Will *et al*. 2000) and two *Bledius* rove beetle species (Steidle & Dettner 1995). Our finding it in the defensive secretions of *Abax parallelepipedus* is the first in the genus *Abax*, as well as from a European species. Its finding in *Calosoma sycophanta* is the second one in the tribe Carabini.

Benzoic acid is found in only four American ground-beetle species — two licinines, and a single cicindeline and pterostichine (Pearson *et al*. 1988, Attygale *et al*. 1990, Will *et al*. 2000). In the present study, we found it in *Calosoma sycophanta* and *Carabus ullrichii*, this being the first finding within the subfamily Carabinae, as well as in ground beetles inhabiting Europe. Benzoic acid has previously been found in diving beetles among beetles (Kuhn *et al*. 1972, Dettner 1977, Newhart & Mumma 1979, Dettner & Schwinger 1980, Classen & Dettner 1983), as well as in millipedes (Barbetta *et al*. 1966, Duffey *et al*. 1977, Blum 1981, Makarov *et al*. 2010) and centipedes (Jones *et al*. 1976) among other invertebrates.

Schildknecht and Weis (1962) published the first data on the defensive secretion in *Abax* (*Abax*) *parallelepipedus*. Two carboxylic acids have so far been found in its defensive secretion (Schildknecht & Weis 1962, Schildknecht *et al*. 1964, 1968a, Schildknecht 1970). These compounds are methacrylic and tiglic acids. Methacrylic acid was the dominant compound (91.4% of the secretion), while tiglic acid was present to a lesser degree (8.5% of the secretion) (Schildknecht & Weis 1962). The same acids were recognized in defensive secretions in two related species: *Abax* (*Abax*) *ovalis* (inhabiting central, southern, eastern Europe, and the Balkans) and *A*. (*A*.) *parallelus* (inhabiting Europe except its northern part), and the ratio was similar to those registered in *A*. (*A*.) *parallelepipedus* (Schildknecht & Weis 1962, Schildknecht *et al*. 1964, 1968a, 1968c, Jacobson 1966, Pavan 1968, Schildknecht 1970, Blum 1981). In the present study, we found the same compounds in the defensive secretions of *A. parallelepipedus*, as well as four carboxylic acids previously unknown to species of the genus *Abax*: isobutyric, crotonic and senecioic acids, and a trace amount of propanoic acid. The high amount of methacrylic acid was in correlation to that presented by Schildknecht and Weis (1962), but somewhat lower (76.5% *vs*. 91.4%) (Table 2). However, the relative percentage of tiglic acid was unexpectedly high as compared with that presented by Schildknecht and Weis (1962) (22.9% *vs*. 8.5%) (Table 2). The ratio of methacrylic/tiglic acid in the defensive secretions in *Abax parallelepipedus* was much lower (about 3.33) than the ratio in *Abax* taxa as measured by previous authors (between 8–11) (Schildknecht & Weis 1962). Additionally, it was unclear which subspecies of *Abax parallelepipedus* was investigated by Schildknecht and Weis (1962) since the two live in Germany [*A. parallelepipedus parallelepipedus* (which was investigated by us) and *A. p. germanus*]. If the latter was studied, perhaps the different ratio of methacrylic/tiglic acid in the defensive secretion could be explained as an additional difference among the subspecies.

Casnati *et al*. (1965) previously investigated the defensive secretion content in *Calosoma* (*Calosoma*) *sycophanta*. They discovered a total of three compounds (two carboxylic acids and a single aldehyde) (Casnati *et al*. 1965, Schildknecht *et al*. 1968a). Salicylaldehyde was the dominant compound (about 60% of the secretion), the content of methacrylic acid was somewhat lower (about 33%), while the quantity of tiglic acid was the lowest (about 3.5% of the secretion) (Casnati *et al*. 1965). The content of the scent fluid was investigated in another related species: *Calosoma* (*Carabosoma*) *parvicollis* (salicylaldehyde; Blum 1981), *C*. (*C*.) *prominens* (salicylaldehyde; Eisner *et al*. 1963b, Eisner & Meinwald 1966), *C*. (*C*.) *marginalis* (salicylaldehyde and methacrylic acid; McCullough & Weinheimer 1966), *C*. (*C*.) *peregrinator* (all inhabiting USA and Mexico) (salicylaldehyde and methacrylic acid; McCullough 1969a, Blum 1981), *C*. (*Callitropa*) *externum* (inhabiting USA and Canada) (salicylaldehyde; McCullough & Weinheimer 1966), *C*. (*C*.) *macrum* (inhabiting USA and Mexico) (salicylaldehyde; Blum 1981), *C*. (*Calosoma*) *schayeri* (from Australia) (salicylaldehyde; Moore & Wallbank 1968), *C*. (*C*.) *scrutator* (inhabiting North America and northern South America) (methacrylic acid; McCullough & Weinheimer 1966), *C*. (*C*.) *oceanicum* (inhabiting Australia and Papua New Guinea) (salicylaldehyde and methacrylic acid; Moore & Wallbank 1968), *C*. (*Campalita*) *chinense* (from eastern Asia) (methacrylic and tiglic acids; Kanehisa & Murase 1977), and *C*. (*Castrida*) *alternans* (from USA and Mexico) (salicylaldehyde; Blum 1981). The presence of salicylaldehyde within the subfamily Carabinae is restricted to members of the genus *Calosoma* only, as confirmed in our study.

We recorded the same compounds as constituents of the defensive secretion. However, we detected an additional seven compounds in *C. sycophanta*. These are the following carboxylic acids: benzoic, senecioic, butyric, isobutyric, 2-methyl butyric and crotonic acids, and a trace amount of propanoic acid. Such a finding strongly supports the possibility that there exist significant chemotaxonomical differences between *C. sycophanta* and related congeners. It is important to note that the ratios of the major constituents registered in this study were somewhat different from those registered by Casnati *et al*. (1965). We measured relative percentages of 44.8% for methacrylic acid (*vs*. ~33% in Casnati *et al*. 1965), 42.7% for salicylaldehyde (*vs*. ~60% in Casnati *et al*. 1965), and 5.6% for tiglic acid (*vs*. ~3.5% in Casnati *et al*. 1965) (Table 2).

The defensive secretions in *Carabus* (*Eucarabus*) *ullrichii* has so far not been investigated in detail. The isolation of two compounds was mentioned, but without any additional data. These two compounds are methacrylic and tiglic acids (Schildknecht & Weis 1962, Schildknecht *et al*. 1964). The same carboxylic acids were identified as allomones in other *Carabus* species from Europe [e.g., *Carabus* (*Carabus*) *granulatus*, *C*. (*Chaetocarabus*) *intricatus*, *C*. (*Chrysocarabus*) *auronitens*, *C*. (*Megodontus*) *violaceus*, *C*. (*Mesocarabus*) *problematicus*, *C*. (*Morphocarabus*) *scheidleri*, *C*. (*Platycarabus*) *irregularis*, *C*. (*Procrustes*) *coriaceus*, *C*. (*Tachypus*) *auratus*, *C*. (*T*.) *cancellatus*, and *C*. (*Tomocarabus*) *convexus*] (Schildknecht & Weis 1962, Schildknecht *et al*. 1964, 1968a, 1968c, Jacobson 1966, Pavan 1968, Schildknecht 1970, Blum 1981). The presence of these carboxylic acids in defensive secretions is characteristic of most of the *Carabus* species investigated throughout the world (Schildknecht & Weis 1962, Schildknecht *et al*. 1964, 1968a, 1968c, Jacobson 1966, Pavan 1968, Schildknecht 1970, Benn *et al*. 1973, Kanehisa & Murase 1977, Blum 1981, Kanehisa & Kawazu 1982, Adachi *et al*. 1985). Our results confirm this fact as well. Butyric acid was found in a single case within the genus (*Carabus* sp.) (Pavan 1968). The chemical analysis of the abdominal gland secretions in *Carabus* (*Chaetocarabus*) *lefebvrei* pupa revealed the presence of a mixture of low molecular weight terpenes, ketones, aldehydes, alcohols, esters and carboxylic acids (Giglio *et al*. 2009). In the present study, we also found methacrylic (as a main constituent) and tiglic acids in the defensive secretions of *Carabus ullrichii*, as well as five new compounds. These are the following carboxylic acids: isobutyric, butyric, 2-methyl butyric, angelic and benzoic acids (Table 2). It is interesting to note that previous authors reported methacrylic and tiglic acids only (without any additional precise data) in the defensive fluids of *Carabus ullrichii* (Schildknecht & Weis 1962, Schildknecht *et al*. 1964, Jacobson 1966, Pavan 1968). They did not find angelic acid, which was present in our sample in a higher amount than tiglic acid. On the other hand, we used individuals of *Carabus ullrichii nastasi*, an endemic subspecies of Serbia, while Schildknecht and Weis (1962) and Schildknecht *et al*. (1964) probably used individuals from Germany belonging to *C. u. ullrichii*, a subspecies inhabiting a wider European area (mostly central and eastern Europe). In this case, the difference in the amount of certain constituents in the defensive secretions may be explained by the different subspecific origin.

Chemicals from the defensive secretions of ground beetles seem to be of considerable taxonomic value at the genus and species levels and probably could be useful in chemotaxonomy (Moore 1979, Dettner 1987, Will *et al*. 2000, Attygalle *et al*. 2007). Our study demonstrates that most differences in the defensive secretions of ground beetles are found among the minor components. Chemical compounds found in the defensive secretions of the three ground-beetle species analyzed (carboxylic acids and an aldehyde) provide protection against putative predators (Thiele 1977, Will *et al*. 2000). The greatest number of defensive compounds (10) was found in *Calosoma sycophanta* (carboxylic acids and an aldehyde), which is more exposed to potential predators than the other two species. This species is huge, arboreal and therefore can be easily noticed. Besides, it has a pronounced metallic body hue of the elytra (structural color), which makes it more visible to predators. A lesser number of defensive compounds (carboxylic acids alone) was recorded in the epigean species analyzed (*Carabus ullrichii* and *Abax parallelepipedus*), which are hiding in leaf-litter and are therefore less visible to potential predators. A somewhat higher number of the compounds (seven) was found in *C. ullrichii*, a huge species with a shiny coppery color (often with green hue) (more visible to predators), while a lesser number of the compounds (six) was recorded in *A. parallelepipedus*, which is smaller and with a black body color (less visible to predators). The validity of our assumptions on evolutionary significance of the differences in chemical content of defensive secretions in the three species analyzed will be confirmed in future studies.

Propanoic acid was detected for the first time within the family Carabidae and in all animals, while 2-methyl butyric, angelic and benzoic acids were found for the first time in the subfamily Carabinae. Our finding of butyric acid is the first precise identification of this compound in the Carabinae subfamily. 2-Methyl butyric, angelic, crotonic, senecioic and benzoic acids were found for the first time in a ground-beetle species inhabiting Europe. Some indications exist that recorded differences in chemical content of defensive secretions of the three ground-beetle species analyzed may be used in chemotaxonomy at species/genus levels. However, previous studies in some cases were performed with an insufficient number of beetle individuals, and gas chromatography was much less precise than nowadays. For this reason, further studies are necessary in order to clarify the differences in chemical composition in additional ground-beetle taxa. Re-analysis of other ground beetles using modern techniques needs to be done to detect the presence/absence of minor constituents in defensive secretions.

Acknowledgments

The study was financially supported by the Serbian Ministry of Education, Science, and Technological Development, Grants 173038, 172053, and 47007. We would like to express our gratitude to two anonymous reviewers, whose useful suggestions significantly improved the manuscript.

References

Adachi, Y., Kanehisa, K. & Tsumuki, H. 1985: Catabolic formation of methacrylic acid and tiglic acid from the branched chain amino acids in the pygidial defensive glands of *Carabus yaconinus* Bates (Coleoptera: Carabidae). — *Applied Entomology and Zoology* 20: 492–493.

Aldrich, J. R. & Yonke, T. R. 1975: Natural products of abdominal and metathoracic scent glands of coreid bugs. — *Annals of the Entomological Society of America* 68: 955–960.

Attygalle, A. B., Eisner, T. & Meinwald, J. 1991: Biosynthesis of methacrylic acid and isobutyric acids in a carabid beetle, *Scarites subterraneus*. — *Tetrahedron Letters* 32: 4849–4852.

Attygalle, A. B., Meinwald, J., Liebherr, J. K. & Eisner, T. 1990: Sexual dimorphism in the defensive secretion of a carabid beetle. — *Experientia* 47: 296–299.

Attygalle, A. B., Wu, X., Maddison, D. R. & Will, K. W. 2009: Orange/lemon-scented beetles: opposite enantiomers of limonene as major constituents in the defensive secretion of related carabids. — *Naturwissenschaften* 96: 1443–1449.

Attygalle, A. B., Wu, X., Ruzicka, J., Rao, S., Garcia, S., Herath, K., Meinwald, J., Maddison, D. R. & Will, K. W. 2004: Defensive chemicals of two species of *Trachypachus* Motschulsky. — *Journal of Chemical Ecology* 30: 577–588.

Attygalle, A. B., Wu, X. & Will, K. W. 2007: Biosynthesis of tiglic, ethacrylic and 2-methylbutyric acids in a carabid beetle, *Pterostichus* (*Hypherpes*) *californicus*. — *Journal of Chemical Ecology* 33: 963–970.

Balestrazzi, E., Dazzini, M. L. V., De Bernardi, M., Vidari, G., Vita-Finzi, P. & Mellerio, G. 1985: Morphological and chemical studies on the pygidial defence glands of some Carabidae (Coleoptera). — *Naturwissenschaften* 72: 482–484.

Barbetta, M., Casnati, G. & Pavan, M. 1966: Sulla presenze di D-(+)-mandelonitrile nella secrezione difensiva del miriapode *Gomphodesmus pavani* Dem. — *Memorie della Società entomologica italiana* 45: 169–176.

Benn, M. H., Lencucha, A., Maxie, S. & Telang, S. A. 1973: The pygidial defensive secretion of *Carabus taedatus*. — *Journal of Insect Physiology* 19: 2173–2176.

Blum, M. S. 1981: *Chemical defenses of arthropods*. — Academic Press, New York.

Blum, M. S. 1996: Semiochemical parsimony in the Arthropoda. — *Annual Review of Entomology* 41: 353–374.

Blum, M. S., Jones, T. H., House, G. J. & Tschinkel, W. R. 1981: Defensive secretions of tiger beetles: cyanogenetic basis. — *Comparative Biochemistry and Physiology B* 69: 903–904.

Bonacci, T., Brandmayr, P., Zetto, T., Perrotta, I. D., Guarino, S., Peri, E. & Colazza, S. 2011: Volatile compounds released by disturbed and undisturbed adults of *Anchomenus dorsalis* (Coleoptera, Carabidae, Platynini) and structure of the pygidial gland. — *ZooKeys* 18: 13–25.

Brossut, R. 1983: Allomonal secretions in cockroaches. — *Journal of Chemical Ecology* 9: 143–158.

Burger, B. V., Munro, Z., Roth, L. M., Geertsema, L. H. & Habich, A. 1986: The chemical nature of the adult defensive secretion of the tip wilter, *Elasmopoda valga*. — *Insect Biochemistry* 16: 687–690.

Cane, J. H. & Michener, C. D. 1983: Chemistry and function of mandibular gland products of bees of the genus *Exoneura* (Hymenoptera, Anthophoridae). — *Journal of Chemical Ecology* 9: 1525–1531.

Casnati, G., Pavan, M. & Ricca, A. 1965: Sulla costituzione del veleno dell'insetto *Calosoma sycophanta* L. (Coleoptera Carabidae). — *Annales de la Société entomologique de France (N. S.)* 1: 705–710.

Chow, Y. S. & Tsai, R. S. 1987: Recent studies of insect chemical communications in Taiwan. — *Chinese Journal of Entomology* 7: 73–80.

Claridge, M. F. 1974: Stridulation and defensive behaviour in the ground beetle *Cychrus caraboides* (L.). — *Journal of Entomology* 49A: 7–15.

Classen, R. & Dettner, K. 1983: Pygidial defensive titer and population structure of *Agabus bipustulatus* L. and *Agabus paludosus* F. (Coleoptera, Dytiscidae). — *Journal of Chemical Ecology* 9: 201–209.

Davidson, B. S., Eisner, T., Witz, B. & Meinwald, J. 1989: Defensive secretion of the carabid beetle *Pasimachus subsulcatus*. — *Journal of Chemical Ecology* 15: 1689–1697.

Dettner, K. 1977: Microdetermination of defensive substances of insects. — *Naturwissenschaften* 64: 42.

Dettner, K. 1987: Chemosystematics and evolution of beetle chemical defences. — *Annual Review of Entomology* 32: 17–48.

Dettner, K. & Reissenweber, F. 1991: The defensive secretion of Omaliinae and Proteininae (Coleoptera: Staphylinidae): its chemistry, biological and taxonomic significance. — *Biochemical Systematics and Ecology* 19: 291–303.

Dettner, K. & Schwinger, G. 1980: Defensive substances from pygidial glands of water beetles. — *Biochemical Systematics and Ecology* 8: 89–95.

Duffey, S. S., Blum, M. S., Fales, H. M., Evans, S. L., Roncadori, R. W., Tiemann, D. L. & Nakagawa, Y. 1977: Benzoyl cyanide and mandelonitrile benzoate in the defensive secretions of millipedes. — *Journal of Chemical Ecology* 3: 101–113.

Eisner, T. & Meinwald, J. 1966: Defensive secretions of arthropods. — *Science* 153: 1341–1350.

Eisner, T., Hurst, J. J. & Meinwald, J. 1963a: Defense mechanisms of arthropods. XI. The structure, function, and phenolic secretions of the glands of a chordeumoid millipede and a carabid beetle. — *Psyche* 70: 94–116.

Eisner, T., Swithenbank, C. & Meinwald, J. 1963b: Defense mechanisms of arthropods. VIII. Secretion of salicylaldehyde by a carabid beetle. — *Annals of the Entomological Society of America* 56: 37–41.

Eisner, T., Aneshansley, D. J., Yack, J., Attygalle, A. B. & Eisner, M. 2001: Spray mechanism of crepidogastrine bombardier beetles (Carabidae; Crepidogastrini). — *Chemoecology* 11: 209–219.

Eisner, T., Aneshansley, D. J., Eisner, M., Attygalle, A. B., Alsop, D. W. & Meinwald, J. 2000: Spray mechanism of the most primitive bombardier beetle (*Metrius contractus*). — *Journal of Experimental Biology* 203: 1265–1275.

Fortunato, A., Maile, R., Turillazzi, S., Morgan, E. D., Moneti, G., Jones, G. R. & Pieraccini, G. 2001: Defensive role of secretion of ectal mandibular glands of the wasp *Polistes dominulus*. — *Journal of Chemical Ecology* 27: 569–579.

Giglio, A., Brandmayr, P., Dalpozzo, R., Sindona, G., Tagarelli, A., Talarico, F., Zetto Brandmayr, T. & Ferrero, E. A. 2009: The defensive secretion of *Carabus lefebvrei* Dejean, 1826 pupa (Coleoptera, Carabidae): gland ultrastructure and chemical identification. — *Microscopy Research and Technique* 72: 351–361.

Giglio, A., Brandmayr, P., Talarico, F. & Zetto Brandmayr, T. 2011: Current knowledge on exocrine glands in carabid beetles: structure, function and chemical compounds. — *ZooKeys* 100: 193–201.

Hefetz, A., Batra, S. W. T. & Blum, M. S. 1979: Chemistry of the mandibular gland secretion of the Indian bee, *Pithitis smaragdula*. — *Journal of Chemical Ecology* 5: 753–758.

Holliday, A. E., Holliday, N. J., Mattingly, T. M. & Naccarato, K. M. 2012: Defensive secretions of the carabid beetle *Chlaenius cordicollis*: chemical components and their geographic patterns of variation. — *Journal of Chemical Ecology* 38: 278–286.

Jacobson, M. 1966: Chemical insect attractants and repellents. — *Annual Review of Entomology* 11: 403–422.

Jones, T. H., William, E. C., Meinwald, J., Eisner, H. E. & Eisner, T. 1976: Benzoyl cyanide and mandelonitrile in the cyanogenetic secretion of a centipede. — *Journal of Chemical Ecology* 2: 421–429.

Kanehisa, K. & Kawazu, K. 1982: Fatty acid components of the defensive substances in acid-secreting carabid beetles. — *Applied Entomology and Zoology* 17: 460–466.

Kanehisa, K. & Murase, M. 1977: Comparative study of the pygidial defensive systems of carabid beetles. — *Applied Entomology and Zoology* 12: 225–235.

Kelley, K. C. & Schilling, A. B. 1998: Quantitative variation in chemical defense within and among subgenera of *Cicindela*. — *Journal of Chemical Ecology* 24: 451–472.

Klinger, R. & Maschwitz, U. 1977: Defense gland of Omaliinae. I. Gross morphology of the gland and identification of the scent of *Eusphalarum longipenne*. — *Journal of Chemical Ecology* 3: 401–410.

Knapp, D. R. 1979: *Handbook of analytical derivatization reactions*. — John Wiley and Sons, New York.

Kuhn, C., Schnepf, E. & Schildknecht, H. 1972: On defense substances of arthropods, the fine structure of the pygidial glands of the water beetle, *Dytiscus marginalis* L. — *Zeitschrift für Zellforschung und mikroskopische Anatomie* 132: 563–576.

Makarov, S. E., Ćurčić, B. P. M., Tešević, V. V., Jadranin, M. B., Vujisić, L. V., Ćurčić, S. B., Mandić, B. M., Sekulić, T. L. & Mitić, B. M. 2010: Defensive secretions in three species of polydesmids (Diplopoda, Polydesmida, Polydesmidae). — *Journal of Chemical Ecology* 36: 978–982.

McCullough, B. T. 1969a: Chemical analysis of the defensive scent fluid produced by the ground beetle *Calosoma peregrinator*. — *Annals of the Entomological Society of America* 62: 1498–1499.

McCullough, B. T. 1969b: Chemical analysis of the scent fluid of *Pasimachus californicus* and *P. duplicatus* (Coleoptera: Carabidae). — *Annals of the Entomological Society of America* 62: 1492.

McCullough, B. T. 1972: Analysis of the defensive scent fluid of *Pasimachus elongatus* (Coleoptera: Carabidae). — *Annals of the Entomological Society of America* 65: 772.

McCullough, T. & Weinheimer, A. J. 1966: Compounds found in the defensive scent fluids of *Calosoma marginalis*, *C. externum*, and *C. scrutator* (Coleoptera: Carabidae). — *Annals of the Entomological Society of America* 59: 410.

Moore, B. P. 1979: Chemical defence in carabids and its bearing on phylogeny. — In: Erwin, T. L., Ball, G. E. & Whitehead, D. R. (eds.), *Carabid beetles: their evolution, natural history and classification*: 193–203. Dr. W. Junk, London.

Moore, B. P. & Wallbank, B. E. 1968: Chemical composition of the defensive secretion in carabid beetles and its importance as a taxonomic character. — *Proceedings of the Royal Entomological Society B* 37: 62–72.

Newhart, A. T. & Mumma, R. O. 1979: Defensive secretions of three species of *Acilius* (Coleoptera: Dytiscidae) and their seasonal variations as determined by high-pressure liquid chromatographic. — *Journal of Chemical Ecology* 5: 643–652.

Pavan, M. 1968: *Defensive secretions of Arthropoda. Final technical report*. — European Research Office, United States Army, Frankfurt am Main.

Pearson, D. L., Blum, M. S., Jones, T. H., Fales, H. M., Gonda, E. & Witte, B. R. 1988: Historical perspective and the interpretation of ecological patterns: defensive compounds of tiger beetles (Coleoptera: Cicindelidae). — *American Naturalist* 132: 404–416.

Quilico, A., Grunanger, P. & Pavan, M. 1960: Sul componente odoroso del veleno del Formicide *Myrmicaria natalensis* Fred. — In: *Proceedings of the XI International Congress of Entomology 3, Vienna, 17-25 August 1960*: 66–68. Organising Committee of the XI International Congress of Entomology, Vienna.

Roth, L. M. & Eisner, T. 1962: Chemical defenses of arthropods. — *Annual Review of Entomology* 7: 107–136.

Schildknecht, H. 1961: Über Insekten- und Pflanzenabwehrstoffe, ihre Isolierung und Aufklärung. — *Angewandte Chemie* 73: 629.

Schildknecht, H. W. 1970: The defensive chemistry of land and water beetles. — *Angewandte Chemie International Edition* 9: 1–9.

Schildknecht, H. & Holoubek, K. 1961: Die Bombardierkäfer und ihre Explosionschemie. V. Mitteilung über Insekten-Abwehrstoffe. — *Angewandte Chemie* 73: 1–7.

Schildknecht, H., Holoubek, K., Weis, K. H. & Krämer, H. 1964: Defensive substances of the arthropods, their isolation and identification. — *Angewandte Chemie International Edition* 3: 73–82.

Schildknecht, H. & Koob, K. 1969: Zur Explosionschemie der Bombardierkäfer. — *Naturwissenschaften* 56: 328.

Schildknecht, H., Maschwitz, U. & Winkler, H. 1968a: Zur Evolution der Carabiden-Wehrdrüsensekrete. Über Arthropoden-Abwehrstoffe XXXII. — *Naturwissenschaften* 55: 112–117.

Schildknecht, H., Winkler, H., Krauss, D. & Maschwitz, U. 1968b: Über das Abwehrsekret von *Idiochroma dorsalis*. Über Arthropoden-Abwehrstoffe XXVIII. — *Zeitschrift für Naturforschung B (Chemical Sciences)* 23: 46.

Schildknecht, H., Winkler, H. & Maschwitz, U. 1968c: Vergleichend chemische Untersuchungen der Inhaltsstoffe der Pygidialwehrblasen von Carabiden. Über Arthropoden-Abwehrstoffe XXXI. — *Zeitschrift für Naturforschung B (Chemical Sciences)* 23: 637.

Schildknecht, H. & Weis, K. H. 1961: Die chemische Natur des Wehrsekretes von *Pseudophonus pubescens* und *Ps. griseus*. VIII. Mitteilung über Insektenabwehrstoffe. — *Zeitschrift für Naturforschung B (Chemical Sciences)* 16: 361–363.

Schildknecht, H. & Weis, K. H. 1962: Die Abwehrstoffe einiger Carabiden insbesondere von *Abax ater*. XII. Mitteilung über Insektenabwehrstoffe. — *Zeitschrift für Naturforschung B (Chemical Sciences)* 17: 439–447.

Schnepf, E., Wenneis, W. & Schildknecht, H. 1969: On the defense substances of arthropods. The chemistry of the bombardier beetle (Coleoptera, Carabidae), IV. The fine structure of the defensive glands of *Brachinus crepitans*. — *Zeitschrift für Zellforschung und mikroskopische Anatomie* 96: 582–599.

Scott, P. D., Hepburn, H. R. & Crewe, R. M. 1975: Pygidial defensive secretions of some carabid beetles. — *Insect Biochemistry* 5: 805–811.

Steidle, J. L. M. & Dettner, K. 1995: The chemistry of the abdominal gland secretion of six species of the rove beetle genus *Bledius*. — *Biochemical Systematics and Ecology* 23: 757–765.

Suzuki, T., Haga, K., Kodama, S., Watanabe, K. & Kuwahara, Y. 1988: Secretion of thrips II. Secretions of three gall-inhabiting thrips (Thysanoptera: Phlaeothripidae). — *Applied Entomology and Zoology* 23: 291–297.

Thiele, H. U. 1977: *Carabid beetles in their environments. A study on habitat selection by adaptations in physiology and behaviour*. — Springer-Verlag, Berlin-Heidelberg.

van Den Dool, H. & Kratz, P. D. 1963: A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. — *Journal of Chromatography* 11: 463–471.

Weatherston, J. 1967: The chemistry of arthropod defensive substances. — *Quarterly Reviews of the Chemical Society* 21: 287–313.

Wheeler, J. W., Chung, R. H., Oh, S. K., Benfield, E. F. & Neff, S. E. 1970: Defensive secretions of cychrine beetles (Coleoptera: Carabidae). — *Annals of the Entomological Society of America* 63: 469–471.

Whitman, W. D., Blum, M. S. & Alsop, D. V. 1990: Allomones: chemicals for defense. — In: Evans, D. L. & Schmidt, O. J. (eds.), *Insect defenses. Adaptive mechanisms and strategies of prey and predators*: 289–351. University of New York Press, Albany.

Will, K. W., Attygalle, A. B. & Herath, K. 2000: New defensive chemical data for ground beetles (Coleoptera: Carabidae): interpretations in a phylogenetic framework. — *Biological Journal of the Linnean Society* 71: 459–481.

Zaikin, V. & Halket, J. 2009: *A handbook of derivatives for mass spectrometry*. — IM Publications, Chichester.