Chemoecology (2019) 29:171–178
https://doi.org/10.1007/s00049-019-00286-0

CHEMOECOLOGY

ORIGINAL ARTICLE

3-Methyl-1-(methylthio)-2-butene: a component in the foul-smelling defensive secretion of two *Ceroglossus* species (Coleoptera: Carabidae)

Sihang Xu[1] · Ramu Errabeli[1] · Kipling Will[2] · Elizabeth Arias[3] · Athula B. Attygalle[1]

Received: 6 May 2019 / Accepted: 3 July 2019 / Published online: 11 July 2019

Abstract

A sulfur-containing compound causes the foul smell of the defensive pygidial gland fluid of *Ceroglossus buqueti* Laporte and *Ceroglossus magellanicus* Gehin. This compound was identified as 3-methyl-1-(methylthio)-2-butene by a comparison of its mass spectrum, and chromatographic properties with those of a chemically synthesized standard. Although a few sulfur compounds are known from insect secretions, this sulfur-bearing isoprene derivative has not been characterized previously from any arthropod source. Additional components in the defensive section include acetic, propanoic, isobutyric, butanoic, methacrylic, ethacrylic, tiglic and benzoic acids, and 11-tricosene. Samples prepared from specimens of *Ceroglossus chilensis* Eschscholtz showed methacrylic, ethacrylic, and other acids. However, 3-methyl-1-(methylthio)-2-butene was not detected in the defensive fluid of *C. chilensis.*

Keywords Defensive secretion · Methacrylic acid · Tiglic acid · *Ceroglossus buqueti* and *Ceroglossus magellanicus* · *Ceroglossus chilensis* · 3-Methyl-1-(methylthio)-2-butene · Methyl prenyl sulfide · Sulfur-containing natural products

Introduction

The extraordinary success of beetles in terrestrial ecosystems, to a certain extent, has been attributed to their diverse defense systems (Meinwald and Eisner 1995). Among the beetles, Adephaga, the second largest suborder in Coleoptera, is a group that has been well investigated. Studies of over 500 species of Adephaga show that their secretions are complex mixtures of rather simple polar and nonpolar organic compounds (Blum 1981; Dettner 1987; Will et al. 2000). Most compounds found among the several hundreds of defensive allomones that have been characterized from insects are small-molecular-weight aliphatic and aromatic hydrocarbons, acids, alcohols, esters, carbonyl compounds and nitrogen-containing heterocyclic compounds (Butovsky 1992; Blum 1981; Attygalle and Morgan 1984). Although many mammals are well-known to deploy many sulfur-containing volatiles effectively as allomones (Verts 1967; Andersen and Bernstein 1975; Wood 1990; Zhang et al. 2002), only very few sulfur compounds have been characterized from arthropods.

The chemical composition information of defensive secretions is valuable for scientists to make biosynthetic, systematic and phylogenetic predictions (Moore and Brown 1979; Dettner 1985, 1987; Will 2000; Will et al. 2000). With this in mind, we have investigated the volatile constituents in pygidial glands of three species from the previously uninvestigated carabid beetle tribe Ceroglossini: *Ceroglossus buqueti* (Laporte 1834) *Ceroglossus magellanicus* (Géhin, 1885) and *Ceroglossus chilensis* (Eschscholtz 1829) and found the presence of a previously unreported sulfur-containing compound in two of the species. We interpret our results in terms of what is known of their phylogeny, ecology and patterns of variation of these three species.

Communicated by Günther Raspotnig.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00049-019-00286-0) contains supplementary material, which is available to authorized users.

✉ Athula B. Attygalle
athula.attygalle@stevens.edu

[1] Department of Chemistry and Chemical Biology, Center for Mass Spectrometry, Stevens Institute of Technology, Hoboken, NJ 07030, USA

[2] Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720, USA

[3] Essig Museum of Entomology, University of California, Berkeley, CA 94720, USA

Experimental

Chemicals

Sodium thiomethoxide and 3,3-dimethylallyl bromide were purchased from Sigma-Aldrich Chemical Company (Saint Louis, MO, USA). Ethacrylic acid was available from previous studies (Attygalle et al. 2004, 2007). All other chemicals were available in our collection of synthetic materials, or purchased from Sigma-Aldrich Chemical Company (St. Louis, MO).

Insects

Specimens of *C. buqueti* and *C. magellanicus* were collected from Valdivian rainforest rich in Southern Beech (Nothofagaceae) and Olivillo (Aextoxicaceae) in the vicinity of Villarrica, Flor del Lago, Chile. Specimens of *C. chilensis* were collected from Coigüe (*Nothofagus dombeyi* (Mirb.) Oerst.) riparian habitat in the vicinity of Curico, Chile (Table 2). Live beetles were kept for a few days on locally acquired leaf litter and fed sliced apple and banana. Beetles were anesthetized by chilling for approximately seven minutes in a − 20 °C freezer. Once anesthetized, their abdomens were removed and pygidial gland reservoirs were excised by following sampling methods described previously (Will et al. 2000). Glands, placed in hexane (80 µL) in small vials were shipped to Hoboken for chemical analysis. The remaining bodies were prepared as vouchers that were pinned and placed in the collection of Essig Museum of Entomology, Berkeley, CA.

Gas chromatography–mass spectrometry

Ceroglossus extracts were analyzed on two GC–MS instruments. The first instrument was a Shimadzu GC-17A gas chromatograph coupled to a Shimadzu QPMS-5050 mass spectrometer. A fused-silica capillary column (30 m × 0.25 mm) coated with ZB-WAXplus (100% polyethylene glycol; 0.25 µm film thickness) (Phenomenex, Torrance, CA, USA) was used. The oven program started with an initial hold time of 3 min at 40 °C. Then, the oven temperature was increased at a rate 15 °C/min to 240 °C and held for 3 min. The second instrument was an Agilent HP 6890 Series II gas chromatograph (GC) linked to a Hewlett-Packard (HP) 5973 Mass Selective Detector (MSD). Analyses were performed using an Agilent J&W capillary column coated with DB-1 (100% dimethylpolysiloxane, 30 m × 0.25 mm ID, and film thickness: 1 µm) (Agilent, Santa Clara, CA, USA). The initial oven temperature (40 °C) was kept for 2 min and raised to 280 °C at a rate of 20 °C/min and held for 7.5 min.

For all GC–MS experiments, helium was used as the carrier gas at a flow rate of 1 mL/min. Electron ionization (70 eV) spectra were recorded at a rate of 3.5 acquisitions per second. Extracts were introduced by the splitless injection technique. The injector and Transferline temperatures were 250 and 290 °C, respectively.

To determine the double-bond position of tricosene, a sample of *C. buqueti* secretion (5 µL) was mixed with a solution of I_2 (2 µL) in ether (5%), followed by dimethyl disulfide (1 µL DMDS). The mixture was left at room temperature overnight and decolorized with a minimum amount of aqueous $Na_2S_2O_3$ solution. The upper hexane/ether layer was removed and analyzed by GC–MS.

Synthesis of 3-methyl-1-(methylthio)-2-butene

A solution of 3,3-dimethylallyl bromide (prenyl bromide, 20.0 mg, 0.14 mmol) in THF (0.1 mL) was added dropwise to a suspension of sodium thiomethoxide (14.0 mg, 0.2 mmol) in THF (0.25 mL) at 0 °C (Šiaučiulis et al. 2018). The reaction mixture was allowed to warm up to room temperature, and stirred for 24 h. The reaction was quenched by adding ice-cold water (0.5 mL). The mixture was stirred for another 10 min and extracted with diethyl ether (2 × 2.0 mL). The combined organic extracts were dried over $MgSO_4$. The solvent was removed slowly in vacuo by mild warming, and the desired compound was obtained in near quantitative yield (95%) as a foul-smelling pale-yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ; 5.23 (m, 1H), 3.10 (d, J = 8 Hz, 2H), 2.03 (s, 3H), 1.75 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ; 130.0, 115.2, 26.1, 20.4, 12.5, 9.3.

Results and discussion

GC–MS analysis of the solvent extracts made from excised pygidial glands of *C. buqueti*, *C. magellanicus* and *C. chilensis* indicated the secretion to be a mixture of low-molecular-weight carboxylic acids and hydrocarbons. From the mass spectral and GC retention time data, it was evident that most of these compounds were similar to those found in many other species of carabid beetles (Moore and Brown 1979; Dettner 1985, 1987; Will 2000; Will et al. 2000; Attygalle et al. 2004) (Fig. 1, Table 1). However, the mass spectrum of one component that eluted very early in the chromatogram in *C. buqueti* (Peak 1, Fig. 1) and *C. magellanicus* was very different from any other previously identified compounds.

The mass spectrum of this component showed a peak at *m/z* 116 which was recognized as the molecular ion

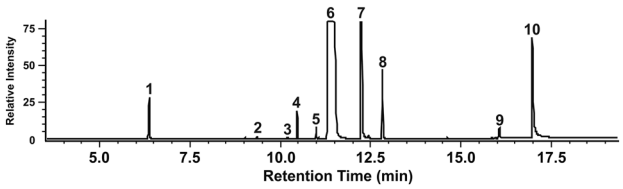


Fig. 1 Reconstructed gas chromatogram recorded from GC–MS analysis of a hexane extract of the defensive secretion of *C. buqueti* (see Table 1 for peak identifications). A fused-silica capillary column (30 m × 0.25 mm) coated with ZB-WAXplus (0.25 µm) was used. The oven temperature was kept at 40 °C for 3 min, raised at a rate of 15 °C/min to 240 °C and held for 3 min

peak (Fig. 2a). The *m/z* 116 peak was accompanied by a small satellite peak at *m/z* 118. The intensity ratio of the *m/z* 118 and 116 peaks indicated that the compound could bear one sulfur atom. Analogously, the intensity ratio of the *m/z* 117 and 116 peaks indicated that the compound bears at least six carbon atoms. Taken in combination, all these results indicated the molecular formula of the compound could be $C_6H_{12}S_1$. A search against all the spectra present for this molecular formula in NIST/EPA/NIH Mass Spectral Library v17 (2017), and Wiley Registry of Mass Spectra (Version 7) indicated that a spectrum that is indistinguishable from that of the natural compound was not found is these databases. The spectrum of the natural compound was similar but not identical to those of 4-methylthio-2-pentene (CAS No, 89534-73-6), 1-methylthio-2-methyl-2-butene (CAS No, 89534-74-7), or 4-methylthio-2-pentene (CAS No, 98534-73-6). Moreover, its mass spectrum showed an intense peak at *m/z* 69 for a loss of 47-Da radical ($\cdot SCH_3$). Assuming that the *m/z* 69 peak represented the prenyl cation, we envisaged and the compound could be a putative isoprenoid (Rizzi 1995), and postulated the natural compound to be 3-methyl-1-(methylthio)-2-butene (CAS No, 5897-45-0). Although the mass spectrum of this compound was not available in literature, its presence has been recognized in synthetic mixtures (Oswald et al. 1962; Roussel Uclaf 1966). To confirm the identification unambiguously, we synthesized the predicted compound starting from 1-bromo-3-methylbut-2-ene (Scheme 1). The mass spectrum (Fig. 2) and chromatographic elution properties (RI = 923 on DB-1, and 1219 on DB-wax coated columns) of the compound synthesized were identical to those of the natural compound.

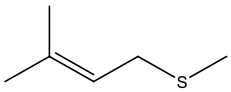
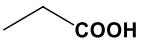
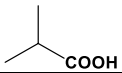
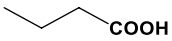
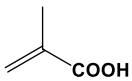
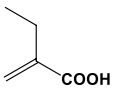
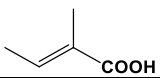
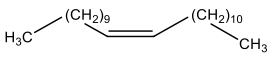
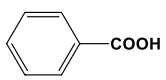
All the other compounds, except tricosene, were identified by comparing mass spectra and chromatographic elution properties with those of synthetic materials. The tricosene isomer was identified by the mass spectrum of its dimethyl disulfide (DMDS) derivative (Attygalle 1998) to be 11-tricosene (Supplementary material, Figure S1). The major component in *C. buqueti* is methacrylic acid (peak number 6; Fig. 1 and Table 2), which has been shown to be biosynthesized from L-valine (Attygalle et al. 1991). The peak number 7 was recognized as that of ethacrylic acid. This acid has not been commonly reported from arthropod secretions (Waterhouse and Wallbank 1967; Moore and Wallbank 1968; Benn et al. 1973). However, we previously identified ethacrylic acid as the most abundant compound in the defensive fluid of *Trachypachus gibbsii* LeConte (Attygalle et al. 2004) and *Pterostichus (Hypherpes) californicus* (Dejean) (Attygalle et al. 2007). It is also seen in carabid beetles related to *Ceroglossus* (see the discussion below).

Although several hundreds of defensive allomones have been characterized from insects, only a very few sulfur-containing volatiles compounds have been identified (Butovsky 1992; Blum 1981; Attygalle and Morgan 1984). However, humans, and many other vertebrates are known to have a highly sensitive sense of smell toward volatile sulfur compounds (Block and Zhuang 2013). Several mammals such as skunks deploy many sulfur-containing volatiles effectively as defensive compounds (Verts 1967; Andersen and Bernstein 1975; Wood 1990; Zhang et al. 2002). We suggest that these carabids may have evolved a way of repelling mammalian predators by adding sulfur compounds to their mixture of defensive compounds, a hypothesis that remains to be tested, beyond our own exposure to the beetles' defensive discharges.

At least for a species of earwigs [*Labidura riparia* (Labiduridae)], it has been documented that, when bitten by *Anolis carolinenesus* lizards, it spits a substance with a fetid odor consisting of dimethyl disulfide and dimethyl trisulfide that causes these insectivores to reject them as prey (Byers 2015). Once a lizard rejects an earwig, it learns not to attack another earwig for several weeks while consuming other prey. Whether the foul-smelling 3-methyl-1-(methylthio)-2-butene acts in a similar manner remains to be tested. It is likely that the acid–hydrocarbon mixture fortified with the sulfur compound is more effective as a deterrent to predators than that with acids alone.

Many plants, particularly those of genus *Allium*, produce sulfur compounds to deter herbivores (Thibout et al. 1996; Ishikawa 1983). On the other hand, sulfur compounds have been implied as chemoattractants for certain insects (Das et al. 2018). Among the sulfur compounds that have been identified from insects are dimethyl disulfide and trimethyl trisulfide from *Eciton burchellii* Westwood (Formicidae) venom glands (Keegans et al. 1993) and *Paltothyreus tarsatus* Fabricius (Formicidae) mandibular glands (Casnati et al. 1967). These two compounds dimethyl disulfide and dimethyl trisulfide, present in the mandibular gland reservoirs of *Megaponera analis* (Latreille) (Formicidae), coordinate raids on

Table 1 Volatile compounds identified from the pygidial grand contents of *C. buqueti* females

Peak No.[a]	Compound (Structure)	Relative Peak area[b]	EI Mass spectrum
1	3-methyl-1-(methylthio)-2-butene 	4.5	118 (1), 117 (2), 116 [$M^{+\bullet}$, 27], 101 (2), 70 (2), 69 (40), 68 (13), 67 (12), 65 (2), 61 (4), 59 (2), 55 (2), 53 (8), 51 (2), 47 (3), 46 (2), 45 (6), 43 (2), 42 (4), 41 (100), 40 (2), 39 (15).
2	acetic acid CH_3COOH	0.2	60 [$M^{+\bullet}$, 55], 45 (93), 44 (6), 43 (100), 42 (15), 41 (4).
3	propanoic acid 	0.1	75 (3), 74 [$M^{+\bullet}$, 83], 73 (59), 58 (2), 57 (38), 56 (24), 55 (22), 53 (2), 47 (5), 46 (9), 45 (100), 44 (4), 43 (4), 42 (8), 40 (2), 39 (2).
4	isobutyric acid 	2.2	88 [$M^{+\bullet}$, 4], 73 (20), 55 (4), 45 (10), 44 (4), 43 (100), 42 (12), 41 (46), 40 (2), 39 (13), 38 (2).
5	butanoic acid 	0.9	88 [$M^{+\bullet}$, 2], 73 (25), 71 (2), 61 (2), 60 (100), 55 (8), 45 (21), 44 (2), 43 (24), 42 (29), 41 (30), 40 (3), 39 (17), 38 (3).
6	methacrylic acid 	100	87 (8), 86 [$M^{+\bullet}$, 100], 71 (4), 69 (27), 68 (17), 58 (9), 57 (8), 55 (2), 53 (5), 45 (31), 44 (6), 43 (16), 42 (21), 41 (96), 40 (51), 39 (90), 38 (28), 37 (16), 36 (2).
7	Ethacrylic acid 	33.2	101 (4), 100 [$M^{+\bullet}$, 60], 86 (2), 85 (39), 83 (8), 82 (37), 81 (5), 73 (4), 72 (13), 71 (4), 60 (4), 58 (5), 57 (12), 56 (19), 55 (100), 54 (40), 53 (31), 52 (4), 51 (10), 50 (9), 49 (2), 46 (2), 45 (38), 44 (5), 43 (18), 42 (5), 41 (42), 40 (10), 39 (75), 38 (8), 37 (4).
8	tiglic acid 	6.7	101 (4), 100 [$M^{+\bullet}$, 86], 85 (23), 83 (8), 82 (17), 81 (2), 73 (2), 72 (3), 71 (2), 60 (2), 58 (2), 57 (7), 56 (11), 55 (100), 54 (31), 53 (25), 52 (4), 51 (11), 50 (10), 49 (2), 45 (18), 44 (3), 43 (15), 42 (3), 41 (25), 40 (4), 39 (52), 38 (5), 37 (3).
9	11-tricosene 	1.3	322 [$M^{+\bullet}$, 6], 139 (7), 125 (15), 124 (5), 120 (6), 112 (7), 111 (29), 110 (6), 98 (12), 97 (58), 96 (15), 95 (6), 85 (19), 84 (19), 83 (61), 82 (19), 81 (11), 79 (5), 71 (33), 70 (32), 69 (56), 68 (14), 67 (20), 61 (14), 58 (5), 57 (77), 56 (39), 55 (81), 54 (21), 53 (5), 47 (5), 45 (17), 44 (7), 43 (100), 42 (13), 41 (72), 39 (6).
10	benzoic acid 	19.2	123 (6), 122 [$M^{+\bullet}$, 82], 106 (8), 105 (100), 94 (2), 78 (7), 77 (78), 76 (7), 75 (4), 74 (8), 73 (2), 66 (2), 65 (3), 63 (2), 53 (3), 52 (10), 51 (49), 50 (28), 49 (3), 47 (2), 45 (4), 39 (14), 38 (11), 37 (5).

[a]Peak numbers refer to chromatographic peaks in Fig. 1

[b]Integrated peak areas from the chromatogram presented in Fig. 1

termites (Longhurst et al. 1979), and trigger the rescue behavior in nestmates when these ants are injured (Frank et al. 2017). Sulfur compounds are known only from a very small number of adephagan taxa [e.g., *Amphizoa,* (Dettner 1990)] including an unidentified sulfur compound in *Trachypachus inermis* Motschulsky (formerly known as *T. holmbergi*) (Attygalle et al. 2004). The sulfur-bearing isoprene derivative, 3-methyl-1-(methylthio)-2-butene, we identified in *C. buqueti* and *C. magellanicus* has not been characterized previously from any arthropod source. Most

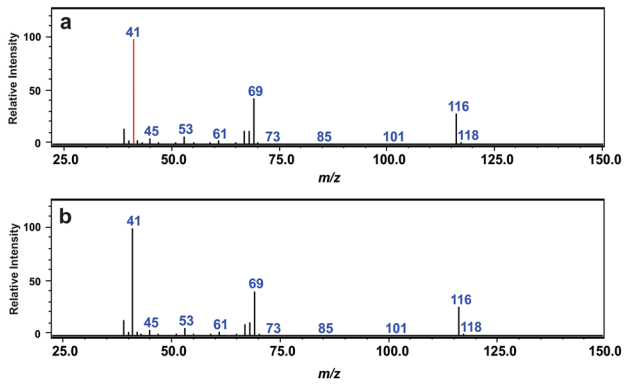
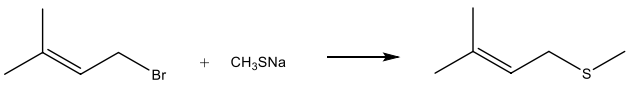


Fig. 2 Electron ionization mass spectrum (70 eV) of the sulfur compound of *C. buqueti* (**a**), and that of synthetic 3-methyl-1-(methylthio)-2-butene (**b**)



Scheme 1 Chemical synthesis of 3-methyl-1-(methylthio)-2-butene from prenyl bromide

constituents in arthropod exocrine secretions are biosynthesized by linking acetate units by the polyketide pathway (Morgan 2004). Thus, 3-methyl-1-(methylthio)-2-butene is biosynthetically an interesting compound because it is a prenyl derivative, which is deemed to be made via the isoprenoid pathway. Although 3-methyl-1-(methylthio)-2-butene has been found as a flavor component in the essential oil of hops (Seaton et al. 1981; Moir et al. 1980), and sometime it is used as a chemical synthon (Michelot et al. 1977), this compound has not been reported from any animal source previously.

The genus *Ceroglossus*, frequently ranked as a tribe of Carabidae, is phylogenetically placed in the subfamily Carabinae together with the tribes Pamborini, Carabini, and Cychrini (Jiroux 2006; Prüser and Mossakowski 1998; Su et al. 2004). While there is some debate regarding the relationships among these taxa (Bousquet 2012) and at what taxonomic levels they should be recognized, the group as a whole has a long standing status with little doubt about its monophyly. A sample of about 50 species in these carabine tribes have been examined for their pygidial gland secretions (Dazzini-Valcurone and Pavan. 1980; Blum 1981; Kanehisa and Kawazu 1982; Giglio et al. 2009; Lecic et al. 2014; Attygalle and Will unpublished). All species of carabids so far examined contain methacrylic acid, the large majority also producing tiglic acid, with scattered occurrences of ethacrylic acid, acetic acid, and other secondary compounds. Within the subfamily, species in the genus *Calosoma* stand out as the only beetles to produce salicylaldehyde (Eisner et al. 1963; Blum 1981). The three *Ceroglossus* species examined here have an admix of pygidial compounds entirely consistent with other Carabinae taxa except for the novel occurrence of 3-methyl-1-(methylthio)-2-butene. This sulfur-bearing compound was detected in multiple samples, including samples from both sexes of *C. buqueti* and *C. magellanicus* but was not seen in any sample from *C. chilensis* (Table 2).

This raises the intriguing possibility that the chemical blend may be specific to particular lineages or species within the genus. *Ceroglossus* has only nine recognized species, all restricted to Chile and southwestern Argentina, but > 72 subspecific names, based for the most part on color (Jiroux 1996, 2006), have been proposed. All beetles in the genus are very boldly metallic in color (Fig. 3) and populations have been shown to have sympatric color convergence (Okamoto et al. 2001, Muñoz-Ramírez 2015) with color patterns linked to geography and not species specific. Studies also found some sex-linked and habitat-mediated morphological differences in populations of *C. chilensis* (Benítez et al. 2011). The defensive chemical data presented here include significant difference in compounds, with the presence or absence of 3-methyl-1-(methylthio)-2-butene, suggesting, with the color and morphological variation found in various previous studies, a rather extraordinary evolutionary history for these beetles, which perhaps includes aposematic signaling and chemical defense as major drivers. While our sample covers a third of the species in this small genus, it cannot address some intriguing questions that are raised. How prevalent is 3-methyl-1-(methylthio)-2-butene in the genus and does its distribution correlate with phylogeny, color morphs, populations, predators, or none of these? What sort of life history or behavioral aspects might have led to the production of this sulfur compound? Rather, few details are known about the behaviors of these beetles. All are nocturnally active, though occasionally seen during the day. They are active predators and scavengers of other invertebrates, fallen fruits, and tree sap (K. Will and E. Arias pers. observations). They spray their defensive compounds readily and in copious amounts when disturbed or handled. None of these attributes are unique to this group. The primary predators of these beetles are not known. While small mammals, birds, and spiders are likely candidates for predation on *Ceroglossus* beetles, we have no direct evidence of the efficacy of *Ceroglossus*' sulfur-laden defensive spray on these predators. Our own observations of the smell in the field strongly indicate it would be a powerful repellent.

Table 2 Comparative sample[a] and analytical[b] data.

Species/sex	Voucher# and source location	Remarks	3-Methyl-1-(methylthio)-2-butene	Acetic acid	Propanoic acid	Isobutyric acid	*n*-Butanoic acid	Isovaleric acid	Methacrylic acid	Ethacrylic acid	Tiglic acid	11-Tricosene	Benzoic acid
C. magellanicus ♂	EMEC1138204 1.4 km east of Punta Falsa	Single gland side not recorded	++						++++++	++++			+
C. magellanicus ♂	EMEC1138205 Valdivian Coastal Reserve	Single gland side not recorded	++			+	+		++++++	+++			+
C. buqueti ♂	EMEC1138203 1.4 km east of Punta Falsa	Single gland side not recorded	++	+		+	+		++++++	+++++			+
C. buqueti ♀	EMEC1138206 Villarrica, Flor del Lago	Left gland	++	+	+	++	+	+	++++++	++++	+++	+++	+++
		Right gland, poor sample							++	+			
C. buqueti ♂	EMEC1138207 Villarrica, Flor del Lago	Both glands, poor sample	+						++	+			
C. chilensis ♂	EMEC1138208 Curico	Left gland							+	+			
		Right gland					++		+++	+++		+	+
C. chilensis ♀	EMEC1138209 Curico	Left gland				+	+		++++++	++++	+	+	++
		Right gland				+	+	+	++++++	++++	+	+	++

[a]Voucher numbers refer to the samples placed in the collection of Essig Museum of Entomology, Berkeley, CA

[b]Semiquantitative analytical data ("++++++" or "+" means a major or a minor component, respectively

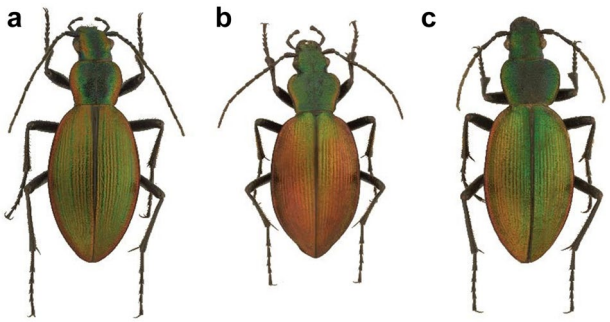


Fig. 3 Chilean *Ceroglossus b*eetles. **a** *Ceroglossus magellanicus*, **b** *Ceroglossus buqueti*, and **c** *Ceroglossus chilensis*. Original images by Mackenzie flight, CC BY-CC 3.0

Acknowledgements We thank Pablo Wagner from Flor del Lago for his assistance with *Ceroglossus* specimen collections. We acknowledge financial support from the Natural Science Foundation (NSF) Grants 1556898 (ABA) and 1556957 (KW).

References

Andersen K, Bernstein DT (1975) Some chemical constituents of the scent of the striped skunk (*Memphitis memphitis*). J Chem Ecol 1:493–499

Attygalle AB (1998) Microchemical techniques. In: Millar J, Haynes K (eds) Methods in chemical ecology. Chapman and Hall, London, pp 207–281

Attygalle AB, Morgan ED (1984) Chemicals from the glands of ants. Chem Soc Reviews 13:245–278

Attygalle AB, Meinwald J, Eisner T (1991) Biosynthesis of methacrylic and isobutyric acids in a carabid beetle, *Scarites subterraneus*. Tetrahedron Lett 32:4489–4852

Attygalle AB, Wu X, Ruzicka J, Rao S, Garcia S, Herath K, Meinwald J, Maddison D, Will KW (2004) Defensive Chemicals of two species of *Trachypachus motschulski* (Coleoptera: Trachypachidae). J Chem Ecol 30:577–588

Attygalle AB, Wu X, Will KW (2007) Biosynthesis of tiglic, ethacrylic, and 2-methylbutyric acids in a carabid beetle*, Pterostichus (Hypherpes) californicus*. J Chem Ecol 33:963–970

Benítez H, Briones R, Viviane JV (2011) Intra and inter-population morphological variation of shape and size of the Chilean magnificent beetle, *Ceroglossus Chilensis* in the Baker river basin, Chilean Patagonia. J Insect Sci 11:94–103

Benn MH, Lencucha A, Maxie S, Telang SA (1973) The pygidial defensive secretion of *Carabus taedatus* (Col. Carabidae). J Insect Physiol 19:2173–2176

Block E, Zhuang H (2013) Smelling sulfur: discovery of a sulfur-sensing olfactory receptor that requires copper. In: Biochalcogen chemistry: the biological chemistry of sulfur, selenium, and tellurium; ACS Symposium Series; American Chemical Society: Washington, DC, 1152, pp 1–14

Blum MS (1981) Chemical defenses of arthropods. Academic Press, New York

Bousquet Y (2012) Catalogue of Geadephaga (Coleoptera, Adephaga) of America, North of Mexico. ZooKeys 245:1–1722

Butovsky RO (1992) Sulfur compounds and entomofauna. Agrokhimiya (1):159–168

Byers JA (2015) Earwigs (*Labidura riparia*) mimic rotting-fresh odor to deceive vertebrate predators. Naturwissenschaften 102:1–10

Casnati G, Ricca A, Pavan M (1967) Defensive secretion of the mandibular glands of *Paltothyreus tarsatus*. Chim Ind (Milan, Italy) 49:57–58

Das S, Das S, Sherpa MT, Thakur N (2018) Is sulfur compounds are hidden chemoattractant for insects? Exp Find 25:4. https://doi.org/10.13140/rg.2.2.36581.86243

Dazzini-Valcurone M, Pavan M (1980) Glandole Pigidiali e Secrezioni Difensive Dei Carabidae (Insecta Coleoptera). Pubblicazioni Dell'Istituto Di Entomologia Dell'Universita Di Pavia 12:1–36

Dettner K (1985) Ecological and phylogenetic significance of defensive compounds from pygidial glands of Hydradephaga (Coleoptera). Proc Acad Natl Sci Phil 137:156–171

Dettner K (1987) Chemosystematics and evolution of beetle chemical defenses. Ann Rev Entomol 32:17–48

Dettner K (1990) Chemische Abwehr bei der ursprunglichen Käferfamilie der Amphizoidae—ein Beitrag zur Evolution der Pygidialdruse der Hydradephaga. Mitteilungen der Deutschen Gesellschaft fuer Allgemeine und Angewandte Entomologie 7:519–526

Eisner T, Swithenbank C, Meinwald J (1963) Defense mechanisms of arthropods. VIII. Secretion of salicylaldehyde by a carabid beetle. Ann Entomol Soc Am 56:37–41

Frank ET, Schmitt T, Hovestadt T, Mitesser O, Stiegler J, Linsenmair KE (2017) Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. Sci Adv 3:e1602187

Giglio A, Brandmayr P, Dalpozzo R, Sindona G, Tagarelli A, Talarico F, Brandmayr TZ, Ferrero E (2009) The defensive secretion of *Carabus lefebvrei* Dejean 1826 pupa (Coleoptera, Carabidae): gland ultrastructure and chemical identification. Microsc Res Tech 72:351–361

Ishikawa Y (1983) Insects and secondary compounds of plants. Kagaku to Seibutsu 21:594–601

Jiroux E (1996) Revision du genre Ceroglossus. Collection Systematique, vol 1. Magellanes, Ver-neuil-sur-Seine

Jiroux E (2006) Le genre Ceroglossus. Collection Systematique, vol 14. Magellanes, Verneuil-sur-Seine

Kanehisa K, Kawazu K (1982) Fatty acid components of the defensive substances in acid-secreting carabid beetles. Appl Ent Zool 17:460–466

Keegans SJ, Billen J, Morgan ED, Gokcen OA (1993) Volatile glandular secretions of three species of new world army ants, *Labidus coecus*, and *Labidus praedator*. J Chem Ecol 19:2705–2719

Lecic S, Curcic S, Vujisic L, Curcic B, Curci N, Nikolic Z, Andelkovic B, Milosavljevic S, Teševic V, Makarov S (2014) Defensive secretions in three ground-beetle species (Insecta: Coleoptera: Carabidae). Ann Zool Fennici 51:285–300

Longhurst C, Baker R, Howse PE (1979) Termite predation by Megaponera foetens (Fab.) (Hymenoptera: Formicidae). Coordination of raids by glandular secretions. J Chem Ecol 5:703–719

Meinwald J, Eisner T (1995) The chemistry of phyletic dominance. In: Meinwald J, Eisner T (eds) Chemical ecology: the chemistry of biotic interaction. National Academy Press, Washington, D.C., pp 29–40

Michelot D, Linstrumelle G, Julia S (1977) Synthesis of 2,5,5-trimethylhepta-2,6-dien-4-one (artemisia ketone). I. Using a rearrangement of an intermediate sulfonium ylide. Syn Commun 7:95–102

Moir M, Gallacher IM, Seaton JC, Suggett A (1980) Terpene methyl sulfides in the essential oil of hops. Chem Ind (London, UK) 15:624–625

Moore BP, Brown WV (1979) Chemical composition of the defensive secretion in *Dyschirius Bonelli* (Coleoptera: Carabidae: Scaritinae) and its taxonomic significance. J Austr Entomol Soc 18:123–125

Moore BP, Wallbank BE (1968) Chemical composition of the defensive secretion in carabid beetles and its importance as a taxonomic character. Proc R Ent Soc Lond (B) 37:62–72

Morgan ED (2004) Biosynthesis in Insects. RSC Publishing, Cambridge

Muñoz-Ramírez C (2015) The phylogenetic position of *Ceroglossus Ochsenii* Germain and *Ceroglossus Guerini* Germain (Coleoptera: Carabidae), two endemic ground beetles from the Valdivian forest of Chile. Revista Chilena de Entomología 40:14–21

Okamoto M, Kashiwai N, Su ZH, Osawa S (2001) Sympatric convergence of the color pattern in the Chilean Ceroglossus ground beetles inferred from sequence comparisons of the mitochondrial ND5 gene. J Mol Evolut 53:530–538

Oswald AA, Griesbaum K, Thaler WA, Hudson BE Jr (1962) Organic sulfur compounds. VIII. Addition of thiols to conjugated diolefins. J Am Chem Soc 84:3897–3904

Prüser F, Mossakowski D (1998) Conflicts in phylogenetic relationships and dispersal history of the supertribe Carabitae (Coleoptera: Carabidae). In: Ball GE, Casale A, Vigna Taglianti A (eds) Phylogeny and classification of Caraboidea (Coleoptera: Adephaga). Proceedings of a symposium (28 August, 1996, Florence, Italy) XX International Congress of Entomology. Museo Regionale di Scienze Naturali, Torino. p 543

Rizzi GP (1995) Formation of sulfur-containing flavor compounds from allylic alcohol precursors. Dev Food Sci 37A:289–302

Roussel Uclaf SA (1966) Cyclic sulfones. Patent NL 6508580

Seaton JC, Suggett A, Moir M (1981) The flavor contribution of sulfur compounds in hops. Tech Q Master Brewers Assoc Am 18:26–30

Šiaučiulis M, Sapmaz S, Pulis AP, Procter DJ (2018) Dual vicinal functionalisation of heterocycles via an interrupted Pummerer coupling/[3,3]-sigmatropic rearrangement cascade. Chem Sci 9:754–759

Su Z-H, Imura Y, Okamoto M, Osawa S (2004) Pattern of phylogenetic diversification of the Cychrini ground beetles in the world as deduced mainly from sequence comparisons of the mitochondrial genes. Gene 326:43–57

Thibout E, Lecomte C, Auger J (1996) Sulfur substances in Allium and insects. Acta Botanica Gallica 143:137–142

Verts BJ (1967) The biology of the striped skunk. University of Illinois Press, Urbana

Waterhouse DF, Wallbank BE (1967) 2-Methylene butanal and related compounds in the defensive scent of Platyzosteria cockroaches. J Insect Physiol 13:1657–1669

Will KW (2000) Systematics and zoogeography of Abaryform genera (Coleoptera: Carabidae: Pterostichini), and a phylogenetic hypothesis for pterostichine genera. PhD dissertation. Cornell University, Ithaca, New York, p 289

Will KW, Attygalle AB, Herath K (2000) New defensive chemical data for ground beetles (Coleoptera: Carabidae): Interpretations in a phylogenetic framework. Biol J Linn Soc 71:459–481

Wood WF (1990) New components in defensive secretion of the striped skunk, *Mephitis mephitis*. J Chem Ecol 16:2057–2065

Zhang J-X, Sun L, Zhang Z-B, Wang Z-W, Chen Y, Wang R (2002) Volatile compounds in anal glands of Siberian weasels (*Mustela sibirica*) and Steppe polecats (*M. eversmanni*). J Chem Ecol 28:1287–1297