

## Structure and chemical analysis of the metathoracic scent glands of *Carpocoris fuscispinus* (Boheman, 1851) (Heteroptera: Pentatomidae) from Turkey

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Received: 17.05.2011

**Abstract:** Metathoracic scent glands (MTGs) of *Carpocoris fuscispinus* (Boheman, 1851) (Heteroptera: Pentatomidae) were studied in adult females and males. Structures of the glands were investigated using scanning electron microscopy, and the volatile fraction was analyzed by gas chromatography-mass spectrometry. MTGs of *C. fuscispinus* have many features in common: a large, median reservoir and paired lateral glands opening laterally at each anterior margin forming evaporation areas composed of mushroom-like structures, which are diagnostic for species-level taxonomy. In the MTGs of *C. fuscispinus*, 24 and 21 compounds were detected in females and males, respectively. Most of the chemical compounds are qualitatively similar in each male and female but differ in quantity. In the MTG analysis of females, *n*-tridecane (57.76%) was most abundant, and bromoacetic acid decyl ester (0.04%) was least abundant. In males, *n*-tridecane (49.26%) was most abundant, and 2,3-dihydroxypropyl ester (0.06%) was least abundant in all samples.

**Key words:** Morphology, chemical analysis, scent glands, volatile compounds, Heteroptera

### Türkiye'den *Carpocoris fuscispinus* (Boheman, 1851) (Heteroptera: Pentatomidae)'un metatorasik koku bezlerinin yapısı ve kimyasal analizi

**Özet:** *Carpocoris fuscispinus* (Boheman, 1851) (Heteroptera: Pentatomidae)'un metatorasik koku bezleri (MTGs) dişi ve erkek bireylerinde çalışılmıştır. Bez yapısı taramalı elektron mikroskobu kullanılarak incelenmiş ve uçucu maddeler ise gaz kromatografi-kütle spektrometride analiz edilmiştir. *Carpocoris fuscispinus*'un MTGs yapısı genel olarak bir çok özelliğe sahip olup bunlar geniş, ortada bir rezervuar ve her biri anterior bölgede laterale doğru açılan çift halde lateral bezlerdir. Türün taksonomisini belirleyen mantar benzeri yapılar buharlaşma alanını oluşturmaktadır. *C. fuscispinus*'un MTG'sinde dişi ve erkek bireylerde sırasıyla yirmi dört ve yirmi bir madde tespit edilmiştir. Her bir dişi ve erkek bireylerde kimyasal maddelerin çoğu benzerdir fakat miktar olarak farklılık göstermektedir. *C. fuscispinus* dişilerinin MTGs analizlerinde en fazla bulunan *n*-tridecane (% 57,76) en az bromoacetic acid decyl ester (% 0,04)'dir. Erkek bireylerinde ise diğer kimyasallara göre en fazla olan *n*-tridecane (% 49,26) en az 2,3-dihydroxypropyl ester (% 0,06)'dir.

**Anahtar sözcükler:** Morfoloji, kimyasal analiz, koku bezi, uçucu maddeler, Heteroptera

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## Introduction

Adult metapleural scent glands are considered an autapomorphy for Heteroptera (Hemiptera) within Insecta. Heteroptera species are known as stink bugs because they usually retaliate by discharging volatile secretions from their metathoracic scent glands (MTGs) when they are disturbed or molested. The functional substitution of the metapleural glands in adults was assumed in many previous studies (Nagnan et al., 1994; Davidova-Vilimova et al., 2000; Zarbin et al., 2000). Krall et al. (1999), Ho and Millar (2001), and Roth (1961) summarized the scent gland secretion functions in Heteroptera such as chemical defense against microbial, vertebrate, and invertebrate enemies. The secretions can control other functions as well in aggregation or alarm behavior (Davidova-Vilimova and Podoubsky, 1999).

MTGs, Brindley's glands, and ventral glands are described in insects: MTGs and Brindley's glands are situated in the metathoracic region (Kálin and Barret, 1975; Schofield and Upton, 1978; Santos-Mallet and De Souza, 1990). MTGs are present in most hemipterans and are usually found in the third thorax region. The adult scent gland complex in both males and females consists of a median ventral metathoracic scent reservoir, which is orange-yellow, and paired colorless lateral glands, sometimes called accessory glands. The lateral glands discharge through ducts into the reservoir, which also receives secretions from the gland cells that form the epithelium. The glands open to the exterior on the ventral surface (Zarbin et al., 2000).

*Carpocoris fuscispinus* is a cosmopolitan species and feeds on Umbelliferae, Gramineae, and Fabaceae (Hoberlandt, 1955). They are one of the most destructive wheat pests in Turkey. *C. fuscispinus* is found at low densities almost everywhere. If the population levels grow in the host, they can become a significant pest. This insect is common in central Turkey and reaches a length of 8-9 mm; it can be collected from early spring to early autumn. Chemical analyses were done on most stink bugs (Ho and Millar, 2001; Djozan et al., 2005). However, there are few studies on the structure of the MTGs (Santos-Mallet and De Souza, 1990; Durak and Kalender, 2007a, 2007b, 2007c).

In this study, the external morphology of MTGs in *C. fuscispinus* is described by scanning electron

microscopy (SEM), and volatiles released from MTG extracts are analyzed by gas chromatography-mass spectrometry (GC-MS). Further, chemical composition of the MTG volatiles and identifying compounds that elicit physiological responses were characterized and then compared with other species of Heteroptera for secretion of toxins, irritants, and repellents and alarm and attractant pheromones.

## Materials and methods

### Insects

Adult *C. fuscispinus* (15 females and males) were collected from various Fabaceae species in Ayaş (39°92'N, 32°85'E; 910 m), Ankara, Turkey, from June to September 2010. Insects were reared in the laboratory and maintained in transparent containers (20 × 20 × 8 cm) on fresh host plants (*Astragalus* leaves) at 26 °C and 70% relative humidity with 16L:8D photoperiodic conditions until dissection.

### Scanning electron microscopy

In order to study the morphology of *C. fuscispinus* MTGs, SEM was used. To prevent insect discharge of gland contents, bugs were anesthetized with CO<sub>2</sub> and then killed by freezing. Insects were dissected and their thoracic regions were stored in saline in a Petri dish (0.7% NaCl + 0.3% KCl; Santos-Mallet and De Souza, 1990). The tergites were removed, and the MTGs (reservoir and glands) were fixed for 3 h on 3% glutaraldehyde (0.1 M sodium phosphate buffer, pH 7.2). After washing in fresh buffer, the MTGs were postfixed with 1% osmium tetroxide (0.1 M sodium phosphate buffer), dehydrated in a graded ethanol series, dried using 1,1,1,3,3,3-hexamethyldisilazane (HMDS), mounted on studs, and sputter-coated with gold. The observations were made with a JEOL JSM 6060 scanning electron microscope.

### Chemical analyses

Analyses of *C. fuscispinus* MTGs were done for each male and female (15 female and male). The MTGs were removed and immersed in approximately 100 µL of analytical grade *n*-hexane distilled from calcium hydride (CaH<sub>2</sub>) and stored at -20 °C for posterior analyses. Extracts were analyzed (approximately 2 µL of the extract) by splitless coupled GC-MS (Agilent 6890 series) fitted with an HP-5 MS column (30 m × 0.25 mm i.d. × 0.25 µm film) and interfaced to an Agilent 5973 mass selective detector (electron impact

ionization, 106 eV). The GC temperature program for the column oven was 50 °C/2 min and then 5 °C/min to 250 °C, with injector and transfer line temperatures of 250 °C and 280 °C, respectively. Helium was used as the carrier gas (Zarbin et al., 2000).

Compounds were preliminary identified by GC-MS, and identifications were confirmed by comparison of the retention times (RTs) and mass spectra with those of authentic samples. The relative amount of each compound was determined from the area under GC peaks, and identifications were confirmed by comparison of RTs and mass spectra with those of commercial standards. The molecular structures of compounds were determined by a comparison of the recorded mass spectra with the reference spectra of the NIST and WILEY library and coinjections of the extracts and synthetic compounds on the 2 columns. The relative proportions of the compounds in the extracts were obtained by integration of the GC-peak areas.

(*E*)-2-octenal (Aldrich Chemical Co.); (*E*)-2-nonanal *n*-dodecane, *n*-undecane, (*Z*)-cyclodecene, *n*-tetracosane, and octadecanol (Fluka Chemical Co.); and (*E*)-2-hexenal, *n*-tridecane, *n*-tetradecane, *n*-pentadecane, standards, and *n*-hexane (Merck Chemical Co.) were used.

## Results

### SEM results

Adult MTGs of *C. fuscispinus* were located ventrally in the metathorax, with lateral ostia between the meso- and metathoracic legs; they have a well developed bag-like reservoir and paired glands located in the lateral areas of the reservoir (Figure 1a). Lateral glands have many tubular structures (Figure 1b). In *C. fuscispinus*, the ostioles are triangular (Figure 1c). A long, wide, and groove-like structure extends downwards from the ostioles [ostiolar duct (OD) in Figure 1c]. The ostiole, the ostiolar groove, and their

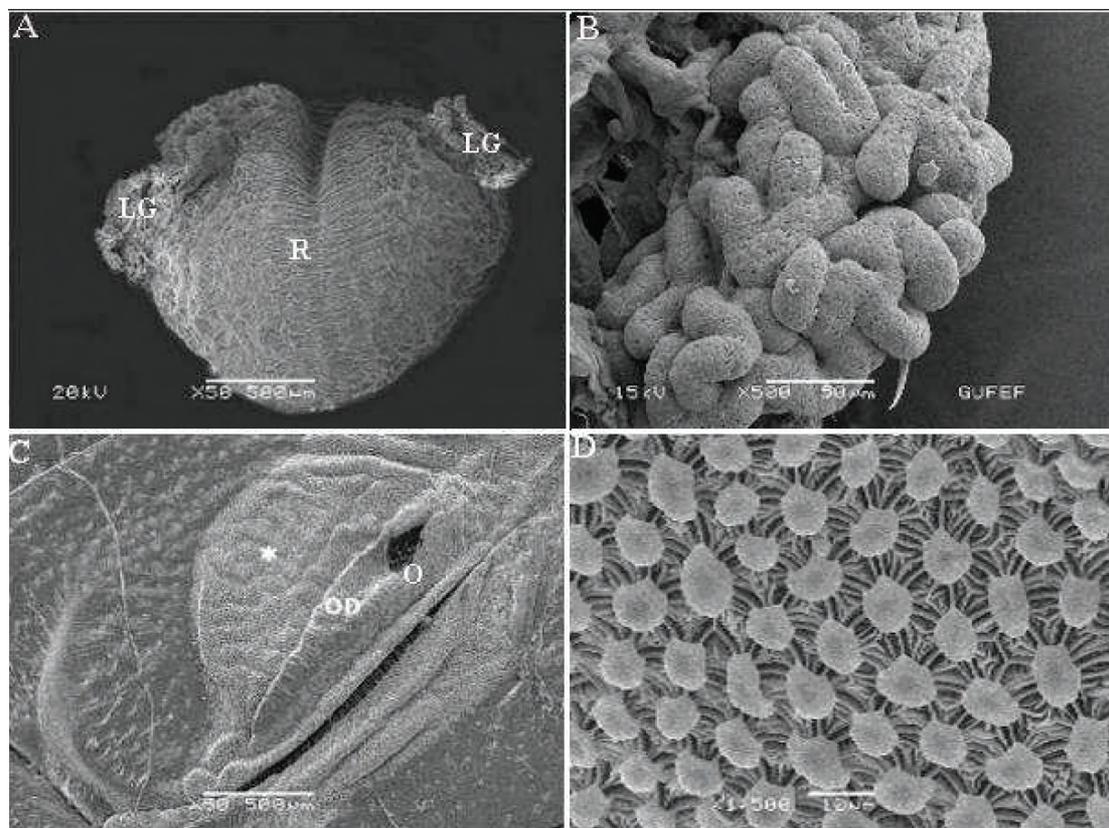


Figure 1. Scanning electron micrograph of the MTGs of *C. fuscispinus*. **A**) MTGs with a well-developed bag-like reservoir (R) with the paired glands (LG) laterally located; scale bar = 500 µm. **B**) Multitubular lateral glands of MTGs; scale bar = 500 µm. **C**) The evaporation area, with a well-formed ostiole (O), the groove-like ostiolar duct (OD), and evaporation areas (ë); scale bar = 500 µm. **D**) Detail of evaporation area, with many mushroom-like structures; scale bar = 10 µm.

surroundings are collectively called the evaporation area. The MTG secretions exit from the ostiole and spread out in this region through the ostiolar groove and into the rest of the evaporation area, which features mushroom-like structures (Figure 1d). The polygonal mushroom-like structures, which are slightly concave in the center, have irregular projections. The mushroom-like structures are linked to each other through cuticular ridges and numerous trabecules found under the ridges (Figure 1d). No differences

between *C. fuscispinus* males and females in the structure of MTGs were found. MTGs have irregular projections and intrusions on their surface.

### GC-MS results

Analyses of the MTGs of *C. fuscispinus* were done separately for each sex (15 females and males). While 24 different compounds were detected for females, 21 different compounds were detected for males (Table). The quantitative and qualitative

Table. Percentages of compounds detected by GC-MS in metathoracic scent secretion of females and males of *Carpocoris fuscispinus*; n.d. = not detected.

Group	Chemical compounds	<i>C. fuscispinus</i>	
		Female %	Male %
ALKANES	Cyclohexane	0.32	7.49
	<i>n</i> -Undecane	0.25	0.19
	<i>n</i> -Dodecane	3.28	2.00
	Heptylcyclohexane	0.60	n.d.
	<i>n</i> -Tridecane	57.76	49.26
	<i>n</i> -Tetradecane	0.17	0.94
	<i>n</i> -Pentadecane	1.17	1.76
	<i>n</i> -Tetracosane	n.d.	1.50
	<i>n</i> -Octacosane	n.d.	0.52
ALKENES	( <i>Z</i> )-Cyclodecene	0.19	0.67
	( <i>Z</i> )-9-Tricosene	0.78	n.d.
ALDEHYDES	( <i>E</i> )-2-Hexanal	0.07	3.15
	( <i>E</i> )-2-Octenal	1.98	1.51
	( <i>E</i> )-2-Nonenal	0.07	n.d.
	( <i>E,Z</i> )-2,6-Nonadienal	2.15	0.15
ESTERS	Methoxyacetic acid tridecyl ester	0.10	1.51
	Pentafluoropropionic acid undecyl ester	0.05	n.d.
	Bromoacetic acid decyl ester	0.04	n.d.
	Butyric acid hexadecyl ester	0.05	n.d.
	Linoleic acid ethyl ester	0.15	n.d.
	2,3-Dihydroxypropyl ester	0.07	0.06
	Acetic acid undec-2-enyl ester	n.d.	11.46
	11-Octadecynoic acid methyl ester	n.d.	0.07
ACIDS	( <i>Z</i> )-13-Tetradecen-1-ol acetate	n.d.	0.14
	<i>n</i> -Hexadecanoic acid	0.35	n.d.
	( <i>Z,Z</i> )-9,12-Octadecadienoic acid	1.26	0.15
ALCOHOL	2-Cyclohexen-1-ol	30.26	15.64
	1-Tridecanol	0.49	n.d.
	Octadecanol	0.09	n.d.
	( <i>E</i> )-2-Hexen-1-ol	n.d.	1.69
	( <i>E</i> )-2-Decen-1-ol	n.d.	2.63

compositions of these compounds differ by sex. In the females of *C. fuscispinus*, the following substances were found: 7 types of alkanes (cyclohexane, *n*-undecane, *n*-dodecane, heptylcyclohexane, *n*-tridecane, *n*-tetradecane, and *n*-pentadecane), 2 types of alkenes [(*Z*)-cyclodecene and (*Z*)-9-tricosene], 4 types of aldehydes [(*E*)-2-hexanal, (*E*)-2-octenal, (*E*)-2-nonenal, and (*E,Z*)-2,6-nonadienal], 6 types of esters (methoxyacetic acid tridecyl ester, pentafluoropropionic acid undecyl ester, bromoacetic acid decyl ester, butyric acid hexadecyl ester, linoleic acid ethyl ester, and 2,3-dihydroxypropyl ester), 2 types of acids [*n*-hexadecanoic acid and (*Z,Z*)-9,12-octadecadienoic acid], and 3 types of alcohols (2-cyclohexen-1-ol, 1-tridecanol, and octadecanol). In the analysis of MTGs of females of *C. fuscispinus*, *n*-tridecane (57.76%) was most abundant and bromoacetic acid decyl ester (0.04%) was least abundant (Figure 2 and Table).

In the males of *C. fuscispinus*, the following substances were found: 8 types of alkanes (cyclohexane, *n*-undecane, *n*-dodecane, *n*-tridecane, *n*-tetradecane, *n*-pentadecane, *n*-tetracosane, and *n*-octacosane), 1 type of alkene [(*Z*)-cyclodecene], 3 types of aldehydes [(*E*)-2-hexanal, (*E*)-2-octenal, and (*E,Z*)-2,6-nonadienal], 5 types of esters (methoxyacetic acid tridecyl ester, 2,3-dihydroxypropyl ester, acetic

acid undec-2-enyl ester, 11-octadecynoic acid methyl ester, and (*Z*)-13-tetradecen-1-ol acetate), 1 type of acid [(*Z,Z*)-9,12-octadecadienoic acid], and 3 types of alcohols (2-cyclohexen-1-ol, (*E*)-2-hexen-1-ol, and (*E*)-2-decen-1-ol). In the analyses of MTGs of males of *C. fuscispinus*, *n*-tridecane (49.26%) was most abundant and 2,3-dihydroxypropyl ester (0.06%) was least abundant (Figure 3 and Table).

## Discussion

Most adult male and female hemipteran insects, or stink bugs, possess a well-developed metathoracic scent gland system (Carayon, 1971; Staddon, 1979; Aldrich, 1988; Durak and Kalender, 2009a, 2009b). In well-fed *C. fuscispinus* bugs (15 males and females), the MTGs extend into the abdominal cavity up to the second segment; in starved bugs, the aspect was milky and shriveled, and they were only located in the thorax.

The structure of the ostiole differs between species (Davidova-Vilimova et al., 1999; Eger and Baranowski, 2002). Ostiole structure of MTGs is often used in systematic keys and diagnosis (Kamaluddin and Ahmad, 1988). In *C. fuscispinus*, the MTG ostiole has an elongate peritreme (ostiole duct) with mushroom-like surfaces of evaporation. These

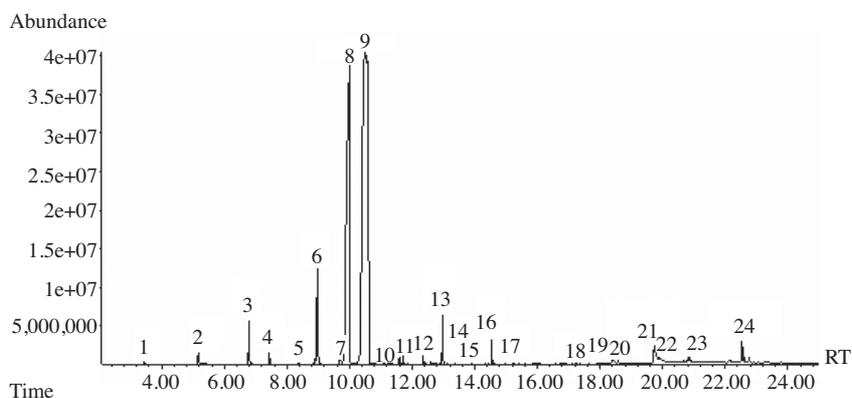


Figure 2. Gas chromatogram of the compounds present in female metathoracic scent glands of *Carpocoris fuscispinus*: 1) (*E*)-2-hexanal, 2) cyclohexane, 3) (*E*)-2-octenal, 4) *n*-undecane, 5) (*E*)-2-nonenal, 6) *n*-dodecane, 7) heptylcyclohexane, 8) 2-cyclohexen-1-ol, 9) *n*-tridecane, 10) methoxyacetic acid tridecyl ester, 11) pentafluoropropionic acid undecyl ester, 12) (*Z*)-cyclodecene, 13) (*E,Z*)-2,6-nonadienal, 14) *n*-tetradecane, 15) bromoacetic acid decyl ester, 16) *n*-pentadecane, 17) 1-tridecanol, 18) butyric acid hexadecyl ester, 19) *n*-hexadecanoic acid, 20) octadecanol, 21) (*Z,Z*)-9,12-octadecadienoic acid, 22) linoleic acid ethyl ester, 23) 2,3-dihydroxypropyl ester, 24) (*Z*)-9-tricosene; RT: retention time in minutes.

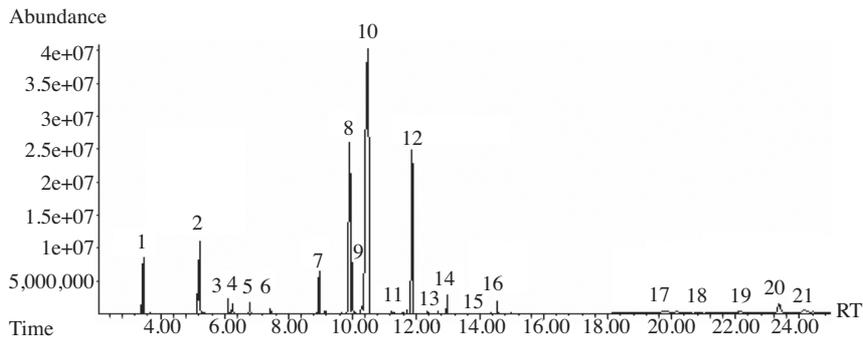


Figure 3. Gas chromatogram of the compounds present in male metathoracic scent glands of *Carpocoris fuscispinus*: 1) (*E*)-2-hexanal, 2) cyclohexane, 3) (*E*)-2-hexen-1-ol, 4) (*Z*)-cyclodecene, 5) (*E*)-2-octenal, 6) *n*-undecane, 7) *n*-dodecane, 8) 2-cyclohexen-1-ol, 9) (*E*)-2-decen-1-ol, 10) *n*-tridecane, 11) *n*-tetradecane, 12) acetic acid undec-2-enyl ester, 13) (*E,Z*)-2,6-nonadienal, 14) *n*-pentadecane, 15) 11-octadecynoic acid methyl ester, 16) methoxyacetic acid tridecyl ester, 17) (*Z,Z*)-9,12-octadecadienoic acid, 18) 2,3-dihydroxypropyl ester, 19) (*Z*)-13-tetradecen-1-ol acetate, 20) *n*-tetracosane, 21) *n*-octacosane; RT: retention time in minutes.

are features that can be used as systematic keys and diagnosis for *C. fuscispinus*; they can also be compared with other species of the same genus in future studies. The evaporation area, i.e. the mushroom-like structure, may show differences between species in the same genus or family (Davidova-Vilimova et al., 2000). As suggested by Carayon (1971), mushroom-like structures and ostiolar grooves are different in the evaporation areas of species belong to *Xylocoris*. Moreover, the ostiolar grooves were observed as long, and the ostioles structures are triangular. In addition, the mushroom-like structures of the evaporation area are polygonal and are connected to each other through numerous trabecules.

Compounds detected in MTGs of male and female *C. fuscispinus* were revealed by other researchers in different amounts and with different behavioral functions from other bugs. MTG secretions of *C. fuscispinus* contained mostly *n*-tridecane and 2-cyclohexen-1-ol. Heptylcyclohexane, (*Z*)-9-tricosene, (*E*)-2-nonenal, pentafluoropropionic acid undecyl ester, bromoacetic acid decyl ester, butyric acid hexadecyl ester, linoleic acid ethyl ester, *n*-hexadecanoic acid, 1-tridecanol, and octadecanol are specific to the female, while *n*-tetracosane, *n*-octacosane, acetic acid undec-2-enyl ester, 11-octadecynoic acid methyl ester, (*Z*)-13-tetradecen-1-ol acetate, (*E*)-2-hexen-1-ol, and (*E*)-2-decen-1-ol are specific to the male. Some of

these compounds are thought to be sex pheromones; sex pheromone blends are generally species-specific (McBrien and Millar, 1999).

Aldehydes, saturated hydrocarbons, acetates, alcohols, terpenes, lactones, ketones, esters, alkenes, acids, and miscellaneous compounds are identified in Pyrrhocoridae species (Farine et al., 1993). In our study, 6 different chemical groups were detected in the chemical analyses of MTGs of *C. fuscispinus*: alkanes, alkenes, aldehydes, esters, acids, and alcohol.

Scent esters are strongly scented and are strong irritants, providing both an easily detected warning signal and a strong defense (Staddon, 1979; Blum, 1981). The esters are usually present in the final secretion only in relatively small amounts. In chemical analyses of *C. fuscispinus*, many esters were detected. Chemical and behavioral analyses showed that aldehydes and hydrocarbons found in the scent glands of a number of Heteroptera species have a dual function. In addition, these compounds may have different effects according to their level of viscosity (Farine et al., 1992). Here, 4 aldehydes and 7 hydrocarbons were identified in the females, and 3 aldehydes and 8 hydrocarbons were identified in the males.

In pentatomids, (*E*)-2-hexenal is as a major component of defensive secretions active against

invertebrate predators such as ants (Aldrich, 1988). It also acts as an alarm pheromone, alerting conspecifics to danger and stimulating dispersal (Lockwood and Story, 1987). A dual role of (*E*)-2-hexenal was found in *Nezara viridula* and some species of Pentatomidae; the compound becomes an attractant at low concentrations and a repellent at high concentrations. In this species, various concentrations of *n*-tridecane cause the same reactions (Farine et al., 1993). In males and females of *C. fuscispinus*, (*E*)-2-hexenal and *n*-tridecane were identified, and this suggests that the compound may have a dual role in *C. fuscispinus*.

*n*-Hexacosane, *n*-tetracosane, *n*-tricosane, and *n*-octacosane are paraffin compounds (Waterhouse and Gilby, 1964); they were not found in the MTGs of every bug. They are called 'odor fixatives' after the more volatile constituents, and they help to penetrate the cuticle of insect enemies and aid in delaying evaporation. These compounds were identified in the male of *C. fuscispinus* and may quickly block the evaporation of scent compounds after their release. That is, paraffin compounds may also be an odor fixative for *C. fuscispinus*. For the male of *C.*

*fuscispinus*, *n*-tetracosane and *n*-octacosane may also be odor fixatives.

Special features of *C. fuscispinus* were pointed out in this paper. The first concerns the structure of the lateral multitubular glands and reservoir. The contents of MTGs are primarily for defense and also play a role as sex pheromones. As the identification of other compounds in other species continues, more data will become available for systematic and phylogenetic analyses. With our present study, we aimed to provide contributions to systematic and phylogenetic research. The composition and structure of MTGs have been identified and quantified in many Heteroptera; the biological function of each compound in scent secretions requires further study. The MTG compounds of *C. fuscispinus* reported in the Table are probably the major contributors to the odors of the Pentatomidae investigated.

### Acknowledgments

We would like to thank Hatice Baş and Özlem Özsaraç for their help in our study.

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