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CHEMICAL PROFILES OF SCENT GLAND SECRETIONS IN THE CYPHOPHTHALMID OPILIONID HARVESTMEN, *Siro duricorius* AND *S. exilis*

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Abstract—Gas chromatographic–mass spectrometric analyses of the scent gland secretions of *Siro duricorius* and *S. exilis* (Opiliones, Cyphophthalmi, Sironidae) revealed a set of 24 components, comprising a series of saturated and unsaturated methyl ketones (C11–C15) and four naphthoquinones. Whereas the scent gland secretions of *S. duricorius*, collected in Austria, and *S. exilis* from USA were qualitatively nearly indistinguishable (with the exception of acetophenone that was specific to *S. duricorius*), they distinctly differed in their relative quantitative compositions: major components of the secretion of *S. duricorius* were 7-tridecen-2-one, tridecan-2-one, undecan-2-one, 1,4-naphthoquinone, 6-methyl-1,4-naphthoquinone (tentatively identified only), and 4-chloro-1,2-naphthoquinone. In contrast, in *S. exilis* a compound tentatively identified as 6-methyl-4-chloro-1,2-naphthoquinone was present in large amounts (in *S. duricorius* a trace component), whereas undecan-2-one only occurred in minor quantities. Secretion profiles of juveniles and adults (both sexes) of each species showed high correspondence.

This is the first report on the chemistry of scent gland secretions of the opilionid suborder Cyphophthalmi. 4-Chloro-1,2-naphthoquinone was identified as a new exocrine product of arthropods, whereas 1,4-naphthoquinone and the tentatively identified 6-methyl-1,4-naphthoquinone are known constituents of exocrine secretions from one species of palpatorid opilionids, *Phalangium opilio*. In contrast, all ketones identified were new for opilionid scent glands, although similar ketones are characteristic of scent gland secretions of palpatorid genera *Leiobunum* and *Hadrobunus*. With regard to the near-basic position of Cyphophthalmi in currently proposed phylogenetic

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trees of Opiliones, naphthoquinones and ketones from *Siro* may represent the condition ancestral to the (derived) naphthoquinone- and ketone-rich secretions in phalangid Palpatores.

Key Words—*Siro*, Cyphophthalmi, Opiliones, scent glands, naphthoquinones, methyl ketones.

INTRODUCTION

The arachnid order Opiliones (also known as harvestmen or daddy longlegs) is estimated to comprise about 5,000 species (Shear, 1982). All possess large scent glands in the prosoma (Juberthie, 1976; Martens, 1978). These glands constitute paired hollow sacs that are surrounded by secretory tissue, each sac opening to the body surface via one single pore at either of the lateral margins of the cephalothorax (Juberthie, 1961; Clawson, 1988). Glandular openings may be developed as oblique slits (Acosta et al., 1993) or, as is the case in the opilionid suborder Cyphophthalmi, they may be located atop conspicuous tubercles, so-called ozophores that dorsolaterally protrude from the carapace (Juberthie, 1961). With regard to biological roles, opilionid scent glands are considered to serve mainly for chemical defense: their secretions, discharged as fine sprays or administered by so-called "leg-dabbing" behavior, have been shown to deter ants and other predatory invertebrates (Juberthie, 1976; Martens, 1978; Holmberg, 1986). In addition, pheromonal roles, such as for territorial marking, have been suggested (Juberthie et al., 1991), and recently, the scent gland secretion of a laniatorid opilionid has been reported to possess alarm pheromonal properties (Machado et al., 2002).

Opilionid scent glands not only represent sources of diverse natural products with interesting biological functions, but also may prove useful in chemosystematic studies. Several authors have already alluded to the chemotaxonomic potential of these secretions (Roach et al., 1980; Ekpa et al., 1985), particularly as the taxonomy of this group is in a state of flux (Shultz and Regier, 2001; Giribet et al., 2002). In fact, in Laniatores, benzoquinones and phenols seem to characterize the scent gland secretions of the superfamily Gonyleptoidea (Eisner et al., 1971, 1977; Roach et al., 1980; Duffield et al., 1981; Gnaspini and Cavalheiro, 1998) whereas from one representative of the laniatorid superfamily Travunioidea, terpenes, bornyl esters, and nitrogen-containing products have been reported (Ekpa et al., 1984). In Palpatores, acyclic compounds have been found in representatives of the genera *Leiobunum* and *Hadrobunus*, and naphthoquinones have been found in scent glands of *Phalangium* (overview in Ekpa et al., 1985). In contrast, nothing is known about the scent gland chemistry of the mitelike representatives of the third classical suborder of Opiliones, the Cyphophthalmi. This small group of Opiliones, so far

comprising about 115 species (Giribet and Boyer, 2002), holds a key position in the phylogenetic systematics of Opiliones, possibly representing the most basal opilionid group (e.g., Giribet et al., 2002).

In the present paper, to address this gap in opilionid scent gland chemistry, we investigated the scent gland secretions of two species of cyphophthalmid opilionids from two continents, *Siro duricorius* (mainly distributed in Southeastern Europe) and *S. exilis* (from the United States).

METHODS AND MATERIALS

Specimen Collection. *S. duricorius* (Joseph, 1868) was collected from soil samples at two different localities in Austria. Collection site I was South Carinthia, near Waidischbach (= first collection: 30 adult and 7 juvenile individuals). Collection site II was South Carinthia, Sattnitz (= second and third collection: 27 adults, 9 juveniles and 38 adults, 3 juveniles, respectively). *Siro exilis* Hoffman (1963) was collected from two soil samples from West Virginia, USA (= fourth and fifth collection: 12 adults, 1 juvenile and 14 adults, 8 juveniles, respectively).

Sample Preparation. Scent gland secretions were either directly collected from ozophores or indirectly by whole-body extraction. For direct sampling from ozophores, a specimen was grasped by a leg with forceps, which usually led to the immediate extrusion of a yellowish or brownish droplet of scent gland secretion. The droplet was absorbed on a small piece of filter paper (2 × 2 mm), then extracted for 5 min in hexane (100 μl). Alternatively, whole specimens were extracted in 100 μl of hexane for about 15 min, expelling their secretions directly into the solvent. Extracts were separated from filter paper pieces or bodies, respectively, and frozen until analysis. Each extract contained the exudate from one individual.

Chemical Analysis. Aliquots of crude extracts, in most cases 1 μl, were analyzed by gas chromatography–mass spectrometry (GC-MS), using a Fisions MD 800 GC-MS (Thermo-Quest, Vienna, Austria). The GC column (ZB-5, 30 m × 0.25 mm × 0.25 μm film thickness, Phenomenex via HPLC Service, Vienna, Austria) was directly connected to the ion source of the MS. The splitless Grob injector was held at 260°C with helium carrier gas (1.5 ml/min). The electron impact (EI) ion source of the MS was kept at 200°C and the transfer line at 310°C. The following temperature program was used: initial temperature 50°C/1 min, 10°C/min to 200°C, then 15°C/min to 300°C and hold for 5 min. Dimethyldisulfide (DMDS) derivatives, to determine the positions of double bonds, were prepared according to Vincenti et al. (1987). Methyloxime derivatives of the DMDS-derivatized alkanones were prepared by concentrating 20 μl of the solution containing the DMDS adducts under a stream of N_2

and then adding 50 μl of *O*-methylhydroxylamine hydrochloride (Pierce Biotechnology Inc., Rockford, IL, USA) in pyridine (2%, w/w). The mixture was heated at 75°C for 1 hr. After cooling, 0.5 ml of water was added and the methyloximes were extracted with hexane (2 ml). The solvent was removed under nitrogen and the residue was dissolved in 100 μl ethyl acetate for GC-MS analysis.

Reference Compounds. Undecan-2-one, dodecan-2-one, tridecan-2-one, 1,4-naphthoquinone, and 2-methyl-1,4-naphthoquinone, were purchased from Aldrich (Vienna, Austria). 4-Chloro-1,2-naphthoquinone was prepared as described by Perumal and Bhatt (1980) and Paquet and Brassard (1989): 800 mg lead tetraacetate were added to a solution of 200 mg of 2,4-dichloro-1-naphthol (Aldrich) in 12 ml benzene. The mixture was stirred under nitrogen for 10 hr at room temperature, then poured into 50 ml of water and extracted with ether. The product was purified using silica gel chromatography and $CHCl_3$:hexane (1:2) as eluent.

Scanning Electron Microscopy. For scanning electron microscopy (SEM), specimens were fixed in glutaraldehyde, dehydrated, and mounted on small dishes before sputtering with gold. Micrographs were prepared at the Research Institute for Electron Microscopy, Technical University of Graz, Austria.

RESULTS

Extraction, Separation, and Identification of Scent Gland Components. GC analyses of scent gland secretions of *S. duricorius* and *S. exilis*, either collected directly after discharge from ozophores (Figure 1) on filter paper pieces or indirectly by the technique of whole-body extraction in hexane, led to the same spectrum of 24 gas chromatographically separable compounds (Figure 2, peaks A–X). Chromatographic and mass spectrometric data for the identification of compounds are summarized in Table 1. Generally, the compounds fell into two chemical groups, ketones and naphthoquinones. Among ketones, a homologous series of 20 saturated to triply unsaturated ketones was present. However, peak A (a minor component exclusively present in the secretion of *S. duricorius*) was identified as acetophenone (M^+ at *m*/*z* 120).

Ten compounds of this series (peaks B, C, D, F, J, K, M, N, Q, and W) appeared to be saturated C11–C15 methyl ketones, showing characteristic mass spectra that generally exhibited a prominent fragment ion at *m*/*z* 58 (arising from McLafferty rearrangement). The compounds displayed molecular ions of weak intensity along with M-15, M-18, M-43, and M-58 fragments (see Table 1). The identities of major components of this series (peak B: undecan-2-one; peak D: dodecan-2-one; peak J: tridecan-2-one) were confirmed by comparison of retention times with those of authentic standards. Peaks C and F, with

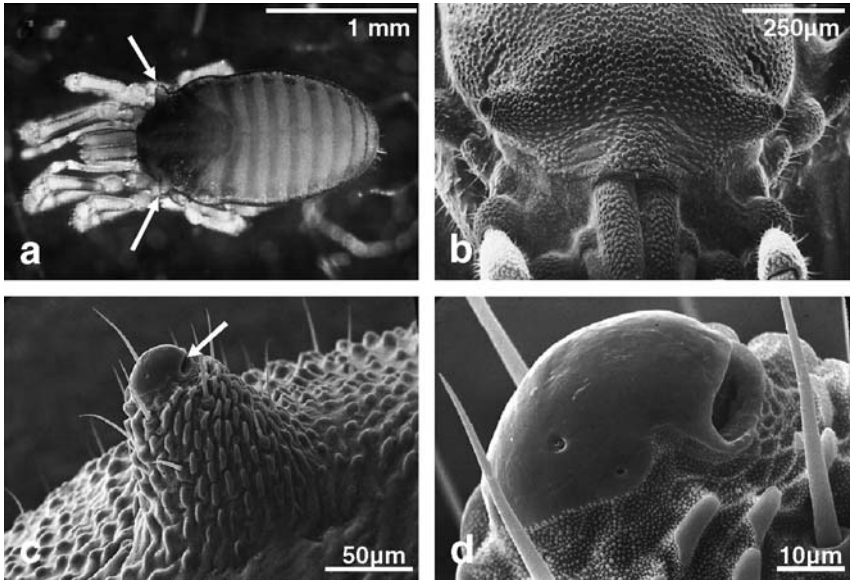

FIG. 1. Topography and external morphology of scent glands in *S. duricorius*. (a) Dorsal view of an adult specimen; arrows point to tubercles of scent glands (ozophores). (b) SEM micrograph of scent gland tubercles; frontal view. (c and d) Details of an ozophore, showing microstructured surface of tubercle and smooth cuticular cap; arrow points to the scent gland orifice.

spectra nearly identical to dodecan-2-one and tridecan-2-one) are tentatively proposed as branched-chain isomers of those compounds, with branch points unknown. All other components of this series (four isomeric tetradecanones: peaks K, M, N, Q, and a pentadecanone: peak W) were of minor abundance and were tentatively identified on the basis of their mass spectra (Table 1).

Five components (peaks G, H, O, P, and U) exhibited mass spectra typical of monounsaturated C13–C15 methyl ketones. Peaks G and H, the major components of this series, were identified as isomeric tridecen-2-ones (M^+ at *m*/*z* 196). The double bond positions were determined by derivatization with dimethyldisulfide. The adducts appeared as one large peak at RT = 20.05–20.15 min. This peak obviously consisted of two components, both with a molecular ion at *m*/*z* 290 as expected, but differing in fragmentation patterns. The larger of the two components (thus derived from larger peak H) showed prominent ions at *m*/*z* 159 and *m*/*z* 131, being consistent with either a 5-tridecen-2-one (fragment at *m*/*z* 131 bearing the carbonyl group) or a 7-tridecen-2-one (fragment at *m*/*z* 159 bearing the carbonyl group). In spectra of the DMDS adduct after *O*-methyl-oximation, adding 29 amu to the carbonyl-group-bearing

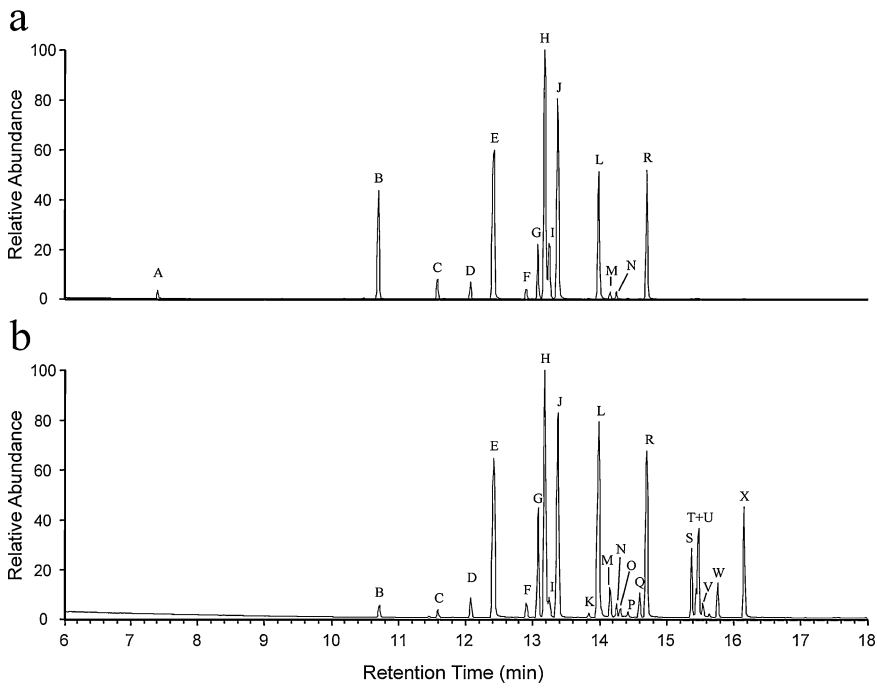

FIG. 2. Gas chromatographic profiles of scent gland secretions of *S. duricorius* (a) and *S. exilis* (b). Peaks marked with an asterisk have been conclusively identified. All other identifications remain tentative. Peak A* (acetophenone), B* (undecan-2-one), C (branched dodecan-2-one), D* (dodecan-2-one), E* (1,4-naphthoquinone), F (branched tridecan-2-one), G* (6-tridecen-2-one), H* (7-tridecen-2-one), I (tridecadienone), J* (tridecan-2-one), K (tetradecan-2-one isomer 1), L (6-methyl-1,4-naphthoquinone), M (tetradecan-2-one isomer 2), N (tetradecan-2-one isomer 3), O (tetradecenone isomer 1), P (tetradecenone isomer 2), Q* (tetradecan-2-one isomer 4), R* (4-chloro-1,2-naphthoquinone), S (pentadecadienone), T (pentadecatrienone), U (pentadecenone), V (unknown), W* (pentadecan-2-one), X (6-methyl-4-chloro-1,2-naphthoquinone).

fragment, the fragment at *m*/*z* 131 remained unaffected whereas the fragment at *m*/*z* 159 disappeared along with the formation of a new fragment at *m*/*z* 188. Thus, the carbonyl group could clearly be classed with the fragment at *m*/*z* 159 in the original DMDS adduct, consistent with the structure of a 7-tridecen-2-one for compound H. The DMDS adduct of the minor compound originating from peak G exhibited a single prominent ion, the base peak, at *m*/*z* 145, indicating a hydrocarbon fragment, and a carbonyl-group-bearing fragment of equal mass. This result is consistent only with the structure of a 6-tridecen-2-one. The remaining monounsaturated ketones (peaks O, P, and U) were present in minor or trace amounts and could only be tentatively identified by their mass spectra

as isomeric tetradecenones and a pentadecenone, respectively (Table 1). Positions of double bonds in these compounds remained undetermined.

Another four components of this series (peaks I, S, T, and V), all of them of minor abundance, were tentatively identified as further homologous C13 and C15 ketones, their spectra indicating double or triple unsaturation. Peak V may possibly represent a mixture of doubly and triply unsaturated C15 ketones (presumed molecular ions at *m*/*z* 220 and *m*/*z* 222).

Naphthoquinones. A second, chemically distinct group of compounds was represented by naphthoquinones (peaks E, L, R, and X). Peak E was identified as 1,4-naphthoquinone by comparisons with an authentic sample. Peak L appeared to be a methylated homolog of 1,4-naphthoquinone, exhibiting a molecular ion at *m*/*z* 172 (base peak), and showing the loss of a methyl group at *m*/*z* 157, together with double neutral loss of CO leading to ions at *m*/*z* 144 and *m*/*z* 116, and loss of CO and COH (fragment at *m*/*z* 115). Prominent ions at *m*/*z* 118, *m*/*z* 90, and *m*/*z* 89 were interpreted to arise from (1) loss of CO and C_2H_2, (2) double loss of CO and C_2H_2, and (3) loss of CO, COH, and C_2H_2, respectively. The spectrum and gas chromatographic retention time, however, were different from authentic 2-methyl-1,4-naphthoquinone (menadione), only leaving the possibilities of positions 5 or 6 for the methyl group. The compound was tentatively identified as 6-methyl-1,4-naphthoquinone by its good correspondence to a literature mass spectrum (from the NIST mass spectral database).

Peak R exhibited the typical isotopic pattern of a monochlorinated compound, indicated by M^+ at *m*/*z* 192 (84%) and an isotopic M+2 peak at *m*/*z* 194 (27%), corresponding to a relative intensity of 32% of the molecular ion. Furthermore, the compound showed sequential loss of two CO fragments from *m*/*z* 192 and 194, leading to twin peaks at *m*/*z* 164 and 166 and to peaks at *m*/*z* 136 and 138, respectively. Also, ions due to loss of Cl (*m*/*z* 157), Cl and CO (*m*/*z* 129, base peak), Cl and double loss of CO (*m*/*z* 101) were observed. The compound was conclusively identified by matches with an authentic standard of 4-chloro-1,2-naphthoquinone.

Peak X also exhibited the isotopic pattern of a mono-chlorinated compound, showing a molecular ion at *m*/*z* 206 (72%) and M+2 at *m*/*z* 208 (22%; corresponding to 31% of M^+). The compound appeared to be a methylated homolog of 4-chloro-1,2-naphthoquinone, exhibiting the loss of a methyl group (twin peaks at *m*/*z* 191 and 193) and again showing repeated decarbonylation and loss of Cl (–CO: *m*/*z* 178 and 180; –CO–CO: *m*/*z* 150 and 152; –Cl: *m*/*z* 171; –Cl and –CO: *m*/*z* 143, base peak). Thus, if peak L is indeed 6-methyl-1,4-naphthoquinone, the compound might be 6-methyl-4-chloro-1,2-naphthoquinone on the basis of mass spectral data.

Secretion Patterns of S. duricorius and S. exilis. In all samples investigated, scent gland secretion profiles of *S. duricorius* and *S. exilis* each showed

TABLE 1. ANALYTICAL DATA TO SCENT GLAND SECRETION COMPONENTS OF *S. duricorius* AND *S. exilis*

Peak no.	Retention time (min)	EI fragmentation (relative intensity)	Identified as[a]
A	7.46	120 (30), 105 (88), 77 (100), 51 (49), 43 (21)	Acetophenone
B	10.72	170 (4), 155 (2), 127 (2), 112 (4), 110 (3), 96 (2), 95 (2), 86 (4), 85 (6), 71 (27), 58 (54), 57 (24), 43 (100), 41 (49)	Undecan-2-one
C	11.59	184 (3), 169 (1), 126 (2), 85 (10), 71 (29), 58 (57), 57 (9), 43 (100), 41 (29)	Dodecan-2-one (branched)
D	12.09	184 (5), 169 (2), 141 (1), 126 (5), 124 (4), 85 (11), 71 (30), 58 (62), 57 (12), 55 (15), 43 (100), 41 (31)	Dodecan-2-one
E	12.43	159 (M+1, 11.5), 158 (M+, 100), 130 (73), 104 (86), 102 (94), 77 (10), 76 (90), 75 (25), 74 (58), 66 (14), 51 (21), 50 (71)	1,4-Naphthoquinone
F	12.95	198 (3), 183 (1), 140 (1), 85 (8), 71 (31), 58 (60), 57 (22), 43 (100), 41 (30)	Tridecan-2-one (branched)
G	13.08	196 (2), 181 (1), 178 (2), 153 (1), 138 (11), 125 (7), 110 (23), 109 (12), 96 (29), 95 (15), 82 (26), 81 (35), 79 (14), 68 (22), 67 (29), 58 (6), 55 (23), 54 (34), 43 (100), 41 (51)	6-Tridecen-2-one
H	13.18	196 (1), 178 (2), 138 (5), 125 (9), 111 (8), 110 (8), 109 (5), 97 (12), 96 (14), 95 (9), 93 (6), 82 (16), 81 (24), 79 (21), 71 (34), 69 (15), 68 (15), 67 (25), 58 (9), 55 (25), 54 (16), 43 (100), 41 (43)	7-Tridecen-2-one
I	13.28	194 (2), 179 (2), 176 (1), 151 (8), 136 (10), 112 (13), 107 (10), 105 (10), 95 (25), 93 (16), 91 (19), 82 (15), 81 (28), 80 (25), 79 (52), 77 (18), 71 (6), 68 (13), 67 (46), 65 (6), 58 (2), 55 (15), 53 (12), 43 (100), 41 (47)	Tridecadienone (tentative)
J	13.38	198 (4), 183 (2), 180 (1), 155 (1), 140 (4), 138 (3), 127 (2), 111 (3), 110 (2), 97 (4), 96 (6), 95 (3), 85 (10), 71 (35), 58 (70), 57 (14), 55 (16), 43 (100), 41 (36)	Tridecan-2-one
K	13.89	212 (1), 197 (1), 169 (1), 154 (3), 152 (1), 125 (2), 111 (3), 109 (3), 96 (8), 95 (5), 86 (12), 85 (18), 71 (13), 67 (12), 58 (15), 57 (53), 56 (35), 43 (100), 41 (88)	Tetradecan-2-one (isomer 1, tentative)
L	14.00	173 (M+1; 13), 172 (M+; 100), 157 (10), 144 (41), 129 (2), 118 (59), 116 (55), 115 (65), 90 (43), 89 (98), 64 (18), 63 (33), 51 (9), 50 (9)	6-Methyl-1,4-naphthoquinone

			(tentative identification)
M	14.15	212 (3), 197 (1), 194 (1), 169 (1), 154 (2), 152 (2), 127 (2), 109 (3), 96 (7), 85 (10), 71 (33), 69 (9), 58 (57), 57 (13), 55 (16), 43 (100), 41 (31)	Tetradecan-2-one (isomer 2, tentative)
N	14.26	212 (3), 197 (1), 194 (1), 183 (1), 154 (1), 125 (6), 109 (4), 96 (11), 85 (10), 83 (10), 71 (37), 58 (57), 57 (18), 55 (21), 43 (100), 41 (39)	Tetradecan-2-one (isomer 3, tentative)
O	14.32	210 (2), 192 (2), 152 (12), 125 (10), 124 (22), 110 (16), 109 (16), 96 (30), 95 (24), 82 (42), 81 (53), 79 (18), 71 (14), 68 (49), 67 (83), 55 (36), 54 (77), 43 (100), 41 (47)	Tetradecenone (isomer 1, tentative)
P	14.44	210 (1), 195 (1), 182 (1), 152 (3), 139 (14), 125 (6), 96 (7) 95 (12), 82 (12), 81 (12), 79 (8), 71 (25), 67 (23), 58 (11), 57 (7), 55 (18), 54 (17), 43 (100), 41 (24)	Tetradecenone (isomer 2, tentative)
Q	14.61	212 (3), 197 (1), 183 (1), 169 (1), 154 (3), 152 (2), 96 (6), 95 (4), 85 (8), 71 (43), 59 (35), 58 (100), 57 (11), 55 (20), 43 (91), 41 (28)	Tetradecan-2-one (isomer 4)
R	14.70	194 (27), 192 (84), 166 (5), 164 (18), 157 (50), 138 (4), 136 (15), 129 (100), 104 (35), 101 (49), 76 (51), 75 (48), 74 (53), 50 (53)	4-Chloro-1,2-naphthoquinone
S	15.38	222 (1), 207 (1), 204 (1), 179 (3), 164 (10), 95 (14), 94 (15), 93 (25), 91 (19), 81 (23), 80 (44), 79 (100), 77 (20), 67 (44), 55 (15), 54 (14), 43 (71), 41 (29)	Pentadecadienone (tentative)
T	15.44	220 (1), 205 (1), 202 (1), 177 (2), 173 (7), 162 (13), 159 (6), 133 (14), 131 (8), 119 (21), 117 (17), 108 (24), 106 (40), 105 (41), 94 (25), 93 (91), 91 (94), 80 (32), 79 (100), 78 (53), 77 (56), 65 (9)	Pentadecatrienone (tentative)
U	15.49	224 (2), 209 (1), 206 (1), 166 (8), 138 (12), 125 (10), 110 (11), 109 (14), 96 (41), 95 (29), 82 (41), 81 (54), 79 (19), 69 (24), 68 (48), 67 (62), 55 (25), 54 (51), 43 (100), 41 (44)	Pentadecenone (tentative)
V	15.55	Data not affirmative	Unknown
W	15.77	226 (2), 211 (1), 208 (1), 168 (2), 96 (6), 95 (4), 85 (11), 71 (38), 59 (32), 58 (100), 55 (17), 43 (71), 41 (22)	Pentadecan-2-one
X	16.15	208 (22), 206 (72), 193 (4), 191 (10), 180 (6), 178 (22), 171 (58), 150 (14), 143 (100), 118 (22), 115 (67), 113 (9), 90 (22), 89 (74), 75 (13), 73 (16), 63 (36)	6-Methyl-4-chloro-1,2-naphthoquinone (tentative)

[a] Compounds B (undecan-2-one), D (dodecan-2-one), E (1,4-naphthoquinone), J (tetradecan-2-one), and R (4-chloro-1,2-naphthoquinone) were identified by comparison of retention times to authentic samples; all other compounds were tentatively identified on the basis of their mass spectral data.

qualitatively and quantitatively consistent compositions (see Table 2). Qualitatively, profiles of the two species were nearly indistinguishable: *S. duricorius* exhibited a maximum of 24 components (some of the trace components were not detectable in certain individuals of two of the three collections), whereas *S. exilis* consistently showed 23 of these compounds, only lacking acetophenone (peak A). In contrast, profiles of *S. duricorius* and *S. exilis* differed significantly in the relative proportions of secretion components, based on the comparison of relative proportions of peak areas from profiles of 114 samples of *S. duricorius* and 35 samples of *S. exilis*. Major components of the *S. duricorius* secretion of adults were (in order of decreasing abundance) tridecan-2-one (about 20%), 7-tridecen-2-one (about 19%), 1,4-naphthoquinone (about 18%), the tentatively identified 6-methyl-1,4-naphthoquinone (about 12%), undecan-2-one (about 10%), 4-chloro-1,2-naphthoquinone (about 7%), and 6-tridecen-2-one (4%). Other components appeared in minor or trace amounts only (Table 2). Secretion patterns did not show large differences between samples of the three different collections, nor between samples of adults and juveniles. Thus, the secretion profile appeared to be consistent within this species. In juveniles, however, the relative quantitative composition of secretion showed higher variation, leading to higher standard deviations, even within samples of the same collection.

Scent gland profiles of *S. exilis* revealed eight major components (five were also major components in *S. duricorius*: see above) including tridecan-2-one (about 20%), 7-tridecen-2-one (about 15%), 1,4-naphthoquinone (about 14%), the tentatively identified 6-methyl-1,4-naphthoqinone (about 13%), 4-chloro-1,2-naphthoquinone (about 12%), pentadecan-2-one (about 5%), the tentatively identified 6-methyl-4-chloro-1,2-naphthoquinone (about 4%), and 6-tridecen-2-one (about 4%). The same components were also major components of juvenile extracts, although in slightly different proportions (Table 2).

DISCUSSION

Cyphophthalmid Scent Gland Chemistry. We present here results from the first chemical investigation into scent gland secretions of cyphophthalmid opilionids for a European and an American species of Sironidae. All components detected belong to the scent gland secretions of these species, as indicated by direct sampling of secretions from ozophores. Profiles appeared to be consistent within each species, only varying slightly between populations and among developmental stages, representing stable and possibly species-specific characters. However, a common ketone- and naphthoquinone-rich chemistry is obvious. Apart from acetophenone, profiles in *S. exilis* seem to be shifted to larger homologous components such as pentadecanone with regard to the ketone series, or the tentatively identified 6-methyl-4-chloro-1,2-naphthoquinone with

regard to the naphthoquinone series, both of which are only trace components in *S. duricorius*. Even though only two species have been studied here, this kind of scent gland chemistry may be representative for a whole group of Cyphophthalmi, at least for the genus *Siro*, considering that the two study species were from different continents.

All methyl ketones as well as the chloronaphthoquinones found in our investigation represent new compounds for scent gland secretions of Opiliones. However, similar ketones, mainly smaller methyl or dimethyl-branched ethyl ketones (Meinwald et al., 1971; Blum and Edgar, 1971; Jones et al., 1976, 1977) are widespread among phalangid Palpatores, and 1,4-naphthoquinone and 6-methyl-1,4-naphthoquinone also have been found in the scent gland secretion of one phalangid species (Wiemer et al., 1978). On the other hand, chlorinated naphthoquinones, to our knowledge, have never been reported previously from exocrine secretions of any arthropod species. Chlorinated exocrine components of arthropods seem to be rare, with the only well-known source for such compounds being the foveal glands of ticks, which produce chlorophenols for sexual communication (Berger, 1972; Yoder et al., 2002; Benoit et al., 2004).

With regard to current concepts of opilionid phylogeny (Martens, 1976; Martens et al., 1981; Shultz and Regier, 2001; Giribet et al., 2002) and the suggested near-basic position of Cyphophthalmi, the *Siro* secretions may represent examples for the ancestral composition of opilionid scent gland chemistry. Interestingly, as mentioned above, phalangid Palpatores, but not Laniatores display characters of the *Siro* secretions. Thus, the ketone-rich secretion profiles of *Leiobunum* and *Hadrobunus* may be derived from ketones analogous to those found in *Siro*. Also, the naphthoquinones detected in *Phalangium opilio* may originate from a cyphophthalmid-like chemistry. In contrast, the lack of ketones and naphthoquinones in scent gland secretions of Laniatores, at least with respect to hitherto known data, would either indicate the complete loss of these compounds and replacement by other compounds in the course of evolution or, alternatively, it might even suggest a distinct evolutionary root for Laniatores, not sharing a common ancestor with Cyphophthalmi. The former case may be supported by preliminary results suggesting the presence of ketones in the scent gland secretion of the laniatorid species, *Parampheres ronae* (Gonzalez et al., 2004), whereas the latter case would confirm the Cyphopalpatores concept of Martens (1976). In this concept, Cyphophthalmi are placed within the classic Palpatores (*sensu* Shear, 1982), and Laniatores represent a separate lineage. However, to begin a comprehensive chemosystematic analysis of Opiliones, chemical data from scent glands of groups hitherto not investigated such as Caddoidea, Ischyropsalidoidea, Troguloidea, and Oncopodoidea must be obtained.

Biology and Roles of Scent Glands of Sironidae. As generally assumed for scent glands of opilionids, also the small, short-legged and mitelike repre-

TABLE 2. RELATIVE ABUNDANCES OF SCENT GLAND SECRETION COMPONENTS OF *S. duricorius* AND *S. exilis*. (CONCLUSIVELY IDENTIFIED COMPOUNDS ARE MARKED WITH AN ASTERISK)

		S. duricorius[a]		*S. exilis*[b]	
Peak no.	Component	Adults	Juveniles	Adults	Juveniles
A	Acetophenone*	0.45 ± 0.25	0.54 ± 0.36	0	0
B	Undecan-2-one*	**9.71 ± 1.59**	**8.84 ± 1.70**	0.57 ± 0.19	0.82 ± 0.30
C	Dodecan-2-one (branched isomer)	1.66 ± 0.50	1.97 ± 0.40	0.34 ± 0.10	0.49 ± 0.13
D	Dodecan-2-one*	2.01 ± 0.61	2.04 ± 0.75	0.89 ± 0.22	0.88 ± 0.17
E	1,4-Naphthoquinone*	**17.61 ± 2.82**	**18.63 ± 7.40**	**14.01 ± 1.90**	**14.58 ± 1.99**
F	Tridecan-2-one (isomer 1, branched)	1.00 ± 0.37	1.09 ± 0.45	0.59 ± 0.20	0.61 ± 0.12
G	6-Tridecen-2-one*	**4.02 ± 1.03**	3.72 ± 1.96	**4.13 ± 0.92**	3.19 ± 1.06
H	7-Tridecen-2-one*	**18.98 ± 2.38**	**19.67 ± 3.56**	**15.47 ± 1.35**	**13.40 ± 2.15**
I	Tridecadienone	3.27 ± 1.56	2.99 ± 0.73	0.65 ± 0.40	0.47 ± 0.26
J	Tridecan-2-one*	**20.21 ± 3.56**	**22.73 ± 2.84**	**20.28 ± 3.79**	**25.98 ± 4.25**
K	Tetradecanone (isomer 1)	0.06 ± 0.08	0.03 ± 0.07	0.17 ± 0.08	0.12 ± 0.04
L	6-Methyl-1,4-naphthoquinone	**12.15 ± 2.03**	9.47 ± **1.63**	**13.08 ± 1.43**	**11.50 ± 2.07**
M	Tetradecanone (isomer 2)	0.52 ± 0.24	0.70 ± 0.30	1.15 ± 0.28	1.29 ± 0.24

N	Tetradecanone (isomer 3)	0.65 ± 0.25	0.67 ± 0.27	0.45 ± 0.23	0.31 ± 0.06
O	Tetradecenone (isomer 1)	0.01 ± 0.01	Trace	0.24 ± 0.14	0.19 ± 0.07
P	Tetradecenone (isomer 2)	0.06 ± 0.09	0.03 ± 0.08	0.19 ± 0.11	0.20 ± 0.05
Q	Tetradecanone* (isomer 4)	0.05 ± 0.08	0.02 ± 0.05	1.10 ± 0.33	1.14 ± 0.17
R	4-Chloro-1,2-* naphthoquinone	**7.09 ± 2.44**	**6.64 ± 2.81**	**11.83 ± 1.68**	**8.62 ± 1.10**
S	Pentadecadienone	0.04 ± 0.06	0.02 ± 0.05	3.00 ± 0.86	2.72 ± 0.75
T	Pentadecatrienone	0.05 ± 0.08	0.02 ± 0.05	0.92 ± 0.35	0.49 ± 0.38
U	Pentadecenone	0.03 ± 0.05	0.01 ± 0.03	**4.57 ± 1.14**	**4.94 ± 0.89**
V	Unknown	0.01 ± 0.01	Trace	0.37 ± 0.19	0.36 ± 0.13
W	Pentadecan-2-one*	0.01 ± 0.02	0.01 ± 0.02	1.68 ± 0.50	2.12 ± 0.35
X	6-Methyl-4-chloro-1, 2-naphthoquinone	0.36 ± 0.51	0.17 ± 0.12	**4.30 ± 1.24**	**5.59 ± 1.68**

[a]Mean values based on 95 adult and 19 juvenile profiles; [b]mean values based on 26 adult and 9 juvenile profiles.
Main components (comprising more than 3% relative peak area) are printed in bold.

sentatives of Sironidae use their secretions for defense. When disturbed, *S. duricorius*, for example, expels a small yellowish to brownish droplet from ozophores and transfers this droplet to the aggressor by leg dabbing. This behavior is also known from other species of Sironidae (Juberthie, 1961). The defensive or toxic potential of the secretions to predatory microarthropods seems to be high: as reported for *S. rubens*, spiders and isopods died immediately when contaminated with the secretion, and when exposed to vapors of secretions, narcosis occurred (Juberthie, 1976). For *S. rubens*, no chemical data are available yet, but a ketone–naphthoquinone-dominated chemistry of scent gland secretions, comparable to our congeneric study species, is to be expected. Thus, similar defensive properties against predatory arthropods may be proposed for secretions of *S. duricorius* and *S. exilis* as well. However, bioassays with single secretion components have not yet been performed. Also, a possible alarm pheromonal role, as recently demonstrated for an opilionid scent gland secretion (Machado et al., 2002), should be investigated. In addition, compounds such as naphthoquinones are known to possess antimicrobial properties. For example, 6-methyl-1,4-naphthoquinone inhibits growth of *Staphylococcus aureus* (Bendz, 1951). Thus, the *Siro* secretion might also have a role in protecting against microorganisms, which would be of particular importance to inhabitants of a humid, fungi- and bacteria-rich environment in soil.

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