Chemical defenses in the opilionid infraorder Insidiatores: divergence in chemical defenses between Triaenonychidae and Travunioidea and within travunioid harvestmen (Opiliones) from eastern and western North America

W. A. Shear[1], **T. H. Jones**[2], **H. M. Guidry**[2], **S. Derkarabetian**[3,4], **C. H. Richart**[3,4], **M. Minor**[5] and **J. J. Lewis**[6]:   [1]Department of Biology, Hampden-Sydney College, Hampden-Sydney, VA 23943, USA. E-mail: wshear@hsc.edu; [2]Department of Chemistry, Virginia Military Institute, Lexington, VA 24450, USA; [3]Department of Biology, San Diego State University, San Diego, CA 92182, USA; [4]Department of Biology, University of California, Riverside, Riverside, CA 92521, USA; [5]Ecology Group, Institute of Agriculture & Environment, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand; [6]J. Lewis & Associates Biological Consulting, 217 W. Carter Avenue, Clarksville, IN 47129, USA

Abstract. Live whole specimens of two species of the harvestman Superfamily Travunioidea Absolon & Kratchovíl 1932 from the eastern United States, eight species from the western United States, six morphospecies of the family Triaenonychidae Sørensen 1886 from New Zealand, and specimens of the phylogenetically early-diverging North American triaenonychid *Fumontana deprehendor* Shear 1977 were extracted in methanol, and the solvent analyzed for components from their defensive secretions. The components were then mapped on a recent phylogeny of the taxa. In both eastern cladonychiid species, *Erebomaster flavescens* Cope 1872 and *Theromaster brunneus* (Banks 1902), the major component found was anabaseine, an alkaloid related to nicotine. In the western species, *Paranonychus brunneus* (Banks 1893), *Cryptomaster leviathan* Briggs 1969, *Speleomaster lexi* Briggs 1974, *S. pecki* Briggs 1974, *Speleonychia sengeri* Briggs 1974, *Metanonychus idahoensis* Briggs 1971, *Briggsus flavescens* (Briggs 1971) and *Sclerobunus nondimorphicus* Briggs 1971, the major component was N,N-dimethylphenylethylamine, implying that the travunioids from the two regions represent different phyletic lines. The secretions of the triaenonychid species, members of the genera *Soerensenella* Pocock 1903 and *Nuncia* Loman 1902, were dominated by 4-methyl-3-hexanone, and that of *F. deprehendor* by phenol. The completely different chemistry of the two taxa, Travunioidea and Triaenonychidae, implies significant phylogenetic differences, and the presence of phenol in *F. deprehendor* may suggest a long period of separate evolution for this species.

Keywords: Nicotine, benzothiazole, 2-3′ dipyridyl, salicyl alcohol, mellein, N,N-dimethylphenylethylamine, 4-methyl-3-hexanone

Harvestmen, arachnids of the order Opiliones (also known in North America as daddy-long-legs) defend themselves chemically with secretions from paired glands in the prosoma, which open through pores on either side of the body. Information on the chemical composition of these secretions has accumulated since the initial studies of Estable et al. (1955) that identified gonyleptidine, the first defensive substance from a harvestman to be chemically determined. Developments in the field have been ably summarized in a chapter by Gnaspini & Hara (2007), which revealed that research on defensive chemistry in Opiliones has focused disproportionally on South American gonyleptids and their relatives (see also Föttinger et al. 2010). Since the 2007 review, information has been added regarding more disparate taxa for which the chemistry of the secretions was previously unknown. Raspotnig et al. (2005) published the first report on the chemistry of sironids (Cyphophthalmi Simon 1879), and Jones et al. (2009) added data for a stylocellid. Raspotnig et al. (2010) provided the first report of secretion chemistry among Dyspnoi, from *Paranemastoma quadripunctatum* (Perty 1833), and Shear et al. (2010a, b) studied two North American phalangodids, *Bishopella laciniosa* (Crosby & Bishop 1924) and *Texella bifurcata* (Briggs 1968), and a stygnopsid, *Chinquepellobunus madlae* (Goodnight & Goodnight 1967). These more recent developments have been summarized by Raspotnig (2012 [2013]), who also mentioned preliminary results for many additional harvestman species. Thus while progress has been made filling taxonomic gaps in our knowledge of harvestman defensive secretions, much remains to be done.

While these studies focused primarily on reporting the composition of secretions from individual species, some recent work has been more analytical. Rocha et al. (2013) discussed possible chemical pathways for the synthesis of secretion components. Attempts at a phylogenetic analysis of the distribution of defensive secretions include those of Caetano & Machado (2013) and Raspotnig et al. (2014). The hope has frequently been expressed that data on defensive secretions may be of value in the phylogenetics and taxonomy of Opiliones (Hara et al. 2005; Jones et al. 2009; Shear et al. 2010a, b, Föttinger et al. 2010, Raspotnig 2012 [2013]), but we see an emerging picture that may be blurred by a great deal of homoplasy. Indeed, the results of the analyses of the same data by Caetano & Machado (2013) and Raspotnig et al. (2014) came to opposite conclusions concerning the polarity of chemical transformations in Grassatores.

Traditional Opiliones taxonomic groups have now been robustly supported with genomic data sets (Hedin et al. 2012), and include the mite-like suborder Cyphophthalmi as sister to remaining harvestmen, the Phalangida Latrielle 1796. Within Phalangida, the raptorially-pedipalped Laniatores Thorell 1876 are sister to the Palpatores Thorell 1876, comprised of the often long-legged suborder Eupnoi Hansen & Sørensen 1904 and the suborder Dyspnoi Hansen & Sørensen 1904. The division of the suborder Laniatores into two infraorders, Insidiatores Loman 1900 and Grassatores Kury 2003, was proposed by Kury (2003) to taxonomically recognize two divergent phyletic lines of harvestmen. Insidiatores includes

those taxa presently grouped as Triaenonychidae Sørensen 1886, Synthetonychiidae Forster 1954, and a group of species of unsettled family-level taxonomy presently referred to as Travunioidea Absalon & Kratchovíl 1932. It is not clear that Insidiatores as composed is monophyletic (but Grassatores almost certainly is). Representative Insidiatores examined here can be seen in Fig. 1.

Synthetonychiidae is a poorly studied but probably monophyletic taxon including minute harvestmen limited to New Zealand (Forster 1954, Kury 2007). In some recent phylogenies, synthetonychiids have been resolved as an outgroup to the remaining Laniatores (Giribet et al. 2010). Triaenonychidae is composed of numerous genera and species that are important, if not dominant, elements of the harvestman fauna of the southern hemisphere (Australia, New Zealand, Madagascar, South Africa, and southern South America [Kury 2007]), but one species, *Fumontana deprehendor* Shear 1977, is known from the southern Appalachian Mountains in North America (Shear 1977, Thomas & Hedin 2008). Triaenonychid taxonomy is somewhat problematical (Mendes & Kury 2008). No triaenonychids had been examined for the chemistry of their defensive secretions prior to this study, and synthetonychiids remain unstudied.

Genera and species of the ''superfamily'' Travunioidea have been recorded from Europe (Kury & Mendes 2007) and Japan, but North America appears to host the most diverse and probably the best understood fauna (Fig. 1; Shear & Derkarabetian 2008, Derkarabetian et al. 2010, 2011). Only a single North American species from this phylogenetically important taxon has been examined from the viewpoint of chemical defense. Specimens from New Mexico were studied by Epka et al. (1984); at the time they referred their material to *Sclerobunus robustus* (Packard 1877), but recent work (Derkarabetian et al. 2010, 2011; Derkarabetian & Hedin 2014) has shown that at least three additional species occur in New Mexico, so the exact identity of their specimens is now unclear. Epka et al. (1984) found an extraordinary array of molecules in the secretion of *S. robustus:* N,N-dimethylphenylethylamine, nicotine, bornyl acetate, bornyl propionate, camphene and limonene.

Raspotnig et al. (2011) examined four species in the European travunioid genus *Holoscotolemon* Roewer 1915: *H. jaqueti* (Corti 1905), *H. oreophilum* Martens 1978, *H. lessiniense* Martens 1978 and *H. unicolor* Roewer 1915. They found that the secretions of *H. jaqueti* and *H. oreophilum* were dominated by nicotine, while that of *H. lessiniense* primarily consisted of the similar alkaloid anabaseine. No results were obtained from adults of *H. unicolor*.

For this study, we analyzed extracts from 15 species of Insidiatores from North America and New Zealand. While our findings for the North American species might have been predicted from the earlier examinations of *Sclerobunus ?robustus* and the European species of *Holoscotolemon,* the chemistry of the New Zealand forms was quite unexpected.

METHODS

Specimens studied were collected alive and dropped in the field into vials containing less than 1 ml of USP methanol; the vials had Teflon-lined caps. Collection localities for the specimens studied are given in Table 1. All specimens will be placed as vouchers in the collection of the Virginia Museum of Natural History, Martinsville, Virginia.

Although when it was possible to extract more than one specimen of a species separately, the results were consistent, in most cases we were restricted to a single specimen by the rarity of the species involved and the difficulties in collecting them, or analyzed extracts from several specimens collected into the same vial. For this reason, some of our results must be regarded as preliminary, and we are working to follow up with additional specimens. However, at the level we are studying, simply characterizing components without detailed quantitative analysis, previous studies have shown little variation within species in the composition of their secretions, though relative amounts of components may differ.

The analysis of the extracts was performed by HMG and THJ. Gas chromatography-mass spectrometry was carried out in the EI mode using a Shimadzu QP-5000 or QP-2010 GC/MS equipped with an RTX-5, 30 m × 0.25-mm i.d. column. The instruments were programmed from 60 ° C to 250 ° C at 10 °/min. Identification of components was accomplished using NIST/EPA/NIH mass spectral library on CD-rom, version 1.7 (1999) and the NIST/EPA/NIH mass spectral library version 2.0d (2005).

All chemicals were mapped onto a modified phylogeny based on the molecular phylogenetic analysis of Derkarabetian et al. (2010), trimmed to include only those genera with chemical data presented here. An ultrametric tree was used for character mapping, which was conducted in Mesquite 2.75 using the ancestral state reconstruction module using parsimony. Additionally, we mapped chemicals onto a phylogeny including triaenonychids analyzed here and the genus *Holoscotolemon*. The taxa were added according to their placement in the maximum likelihood phylogeny of Giribet et al. (2010).

RESULTS

Results of the analysis are presented in Tables 2–4, and structural formulae of detected components are shown in Fig. 2. As seen in Table 2, the major component of the secretion in both eastern North American travunioid species (*Erebomaster flavescens* Cope 1872 and *Theromaster brunneus* (Banks 1902)) was the alkaloid anabaseine. Minor or trace components were anabasine (a related alkaloid), phenol, benzothiazole, salicyl alcohol, 2,3′-dipyridyl and mellein. Four individuals of *T. brunneus* were analyzed; no significant differences were found between individuals, except that salicyl alcohol was not found in two of the specimens. A specimen of *E. flavescens* from Indiana was analyzed separately, and six specimens of the species from Ohio were extracted and analyzed as a group. The results for *E. flavescens* differed from those for *T. brunneus* in that trace amounts of 4-hydroxybenzine-ethanol were found in the *E. flavescens* extract, and that phenol, anabasine and mellein were minor components (1–10%) rather than traces (< 1%).

Table 3 summarizes the results from the analyses of extracts from eight species of travunioids from western North America. Components in common with the eastern species were phenol and benzothiazole, and as with the eastern species, these compounds were present only in trace amounts. The major component in all western species was N,N-
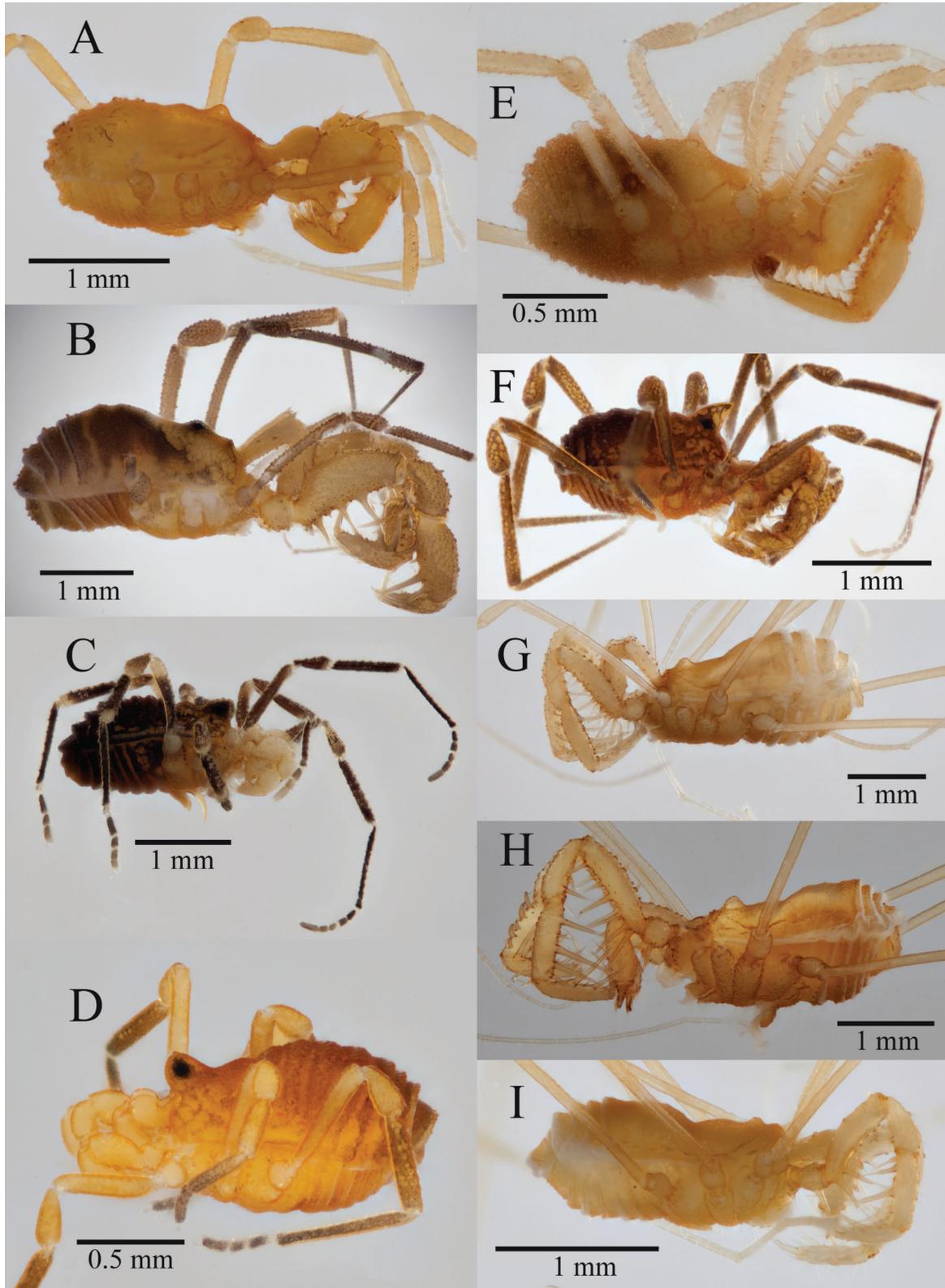
Figure 1.—Representatives of North American Insidiatores. High resolution images for all specimens figured here are available on Morphbank under publication ID 835667 (http://www.morphbank.net/835667). A. *Briggsus flavescens*, B. *Cryptomaster leviathan*, C. *Paranonychus brunneus*, D. *Metanonychus idahoensis*, E. *Fumontana deprehendor*, F. *Erebomaster* sp., G. *Speleomaster lexi*, H. *Speleomaster pecki*, I. *Speleonychia sengeri*.

Table 1.—Collecting localities.

Species	Voucher number	Collection localities
Cryptomaster leviathan	07-177	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp, 28 May 2007, A.Richart, C.Richart (CHR 1354)
	08-188	OR: Coos Co., Golden and Silver Falls St. Pk., 4 April 2008, S.Derkarabetian, C.Richart (CHR 2029)
	07-176	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp, 28 May 2007, A.Richart, C.Richart (CHR 1335)
Erebomaster flavescens	07-179	IN: Crawford Co., Sibert's Well Cave (near Wyandotte Cave), 3 mi NE Leavenworth, 19 Nov 2007, J. Lewis
	07-180	IN: Harrison Co., Devils Graveyard Cave, 7 mi SW Corydon, 19 Nov 2007, J. Lewis
	07-181	IN: Harrison Co., Devils Graveyard Cave, 7 mi SW Corydon, 19 Nov 2007, J. Lewis
	12-336	OH: Adams Co., Edge of Appalachia Preserve, 8 June 2011, W. A. Shear
Theromaster brunneus	08-211	NC: Haywood Co., Cullowhee Mtn. Road at Wolf Creek, 22 October 2008, W. A. Shear
Speleomaster lexi	08-172	ID: Lincoln Co., Tee Cave, 30 June 2007, A.Richart, C.Richart (CHR 1577)
	08-178	ID: Lincoln Co., Gwinn Cave, 29 June 2007, A.Richart, C.Richart (CHR 1568)
Speleomaster pecki	08-174	ID: Butte Co., Beauty Cave, 30 June 2007, A.Richart, C.Richart (CHR 1581)
Speleonychia sengeri	08-175	WA: Klickitat Co., Cheese Cave, 9 June 2007, N.Richart, C.Richart (CHR 1621)
	08-176	WA: Skamania Co., Cave #27, 9 June 2007, N.Richart, C.Richart (CHR 1622)
	08-177	WA: Skamania Co., Big Cave, 8 June 2007, N.Richart, C.Richart (CHR 1588)
	08-179	WA: Skamania Co., Slime Cave (Cave #39) 8 June 2007, N.Richart, C.Richart (CHR 1607)
Paranonychus brunneus	07-174	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp, 28 May 2007, A.Richart, C.Richart (CHR 1356)
	07-175	OR: Lane Co., Willamette Nat. For., Clark Creek Organization Camp, 28 May 2007, A.Richart, C.Richart (CHR 1357)
Metanonychus idahoensis	09-248	ID: Shoshone Co., Hobo Cedar Grove, 25 July 2008, C.Richart (CHR 2361)
Briggsus flavescens	08-190	OR: Clatsop Co., Saddle Mt. Rd. near U.S. 26, 3 April 2008, S.Derkarabetian, C.Richart (CHR 2016)
Nuncia sp.	10-275	NZ: South Island, Westland, Dancing Creek, Haast Pass, 11 February 2010, M. Minor
Nuncia sp.	10-278	NZ: South Island, Buller, Aratika, 9 February 2010, M. Minor
Nuncia sp.	10-279	NZ: South Island, Buller, Springs Junction, 5 February 2010, M. Minor
Soerensenella sp.	10-271	NZ: North Island, Wanganui, Totara Reserve, 28 March 2010, M. Minor
Soerensenella prehensor	10-272	NZ: North Island, Taupo, Whakapapa Bush, 4 April 2010, M. Minor

dimethylphenylethylamine, with nicotine and N,N-dimethyli-soamylamine as minor or trace components. An exception was *Briggsus flavescens* (Briggs 1971), in which the major component was phenol, with N,N-dimethylphenylethylamine as a minor component and a trace amount of benzothiazole. This unexpected result came from one small specimen and requires confirmation.

Table 4 shows results from the analyses of extracts of triaenonychids. Three small specimens of *F. deprehendor* were extracted and analyzed together. *Fumontana deprehendor* had phenol as a major component, with traces of salicyl alcohol. Each record of a New Zealand triaenonychoid represents either one or two specimens. The major components of the New Zealand triaenonychoids were quite different from both *F. deprehendor* and the travunioids. While the travunioids and *F. deprehendor* were dominated by cyclic compounds frequently containing nitrogen, the New Zealand triaenonychoids showed linear aldehydes, alcohols and ketones. The secretions were also much less complex, with only one or two minor or trace components in *Nuncia* sp.

Results of the character mapping analyses including the triaenonychids and *Holoscotolemon* are shown in Fig. 3. This analysis indicates that if Insidiatores is monophyletic, the ancestral state for all species is phenol, with changes to 4-

Table 2.—Compounds present in eastern North American travunioids and species of *Holoscotolemon* (data on *Holoscotolemon* from Raspotnig et al. 2011). Plus sign indicates major component, "o" a minor component (<10%) and "t" a trace component (<1%). The "Unknown" is an undetermined component at m/z = 174.

Fig. 2	Component	*Erebomaster flavescens*	*Theromaster brunneus*	*Holoscotolemon jaqueti*[1]	*Holoscotolemon lessiniense*[1]	*Holoscotolemon oreophilum*[1]
1	Phenol	o	t			
2	Benzothiazole	t	t			
3	Salycyl alcohol	t	t			
4	4-Hydroxybenzenethanol	t				
5	Anabasine	o	t			
6	2,3′-Dipyridyl	t	t		t	
7	Anabaseine	+	+		+	
8	Mellein	o	t			
10	Nicotine			+		+
	Unknown*		t			

Table 3.—Compounds present in western North American travunioids. Plus sign indicates major component (<10%), "o" a minor component (<10%) and "t" a trace component (<1%).

Fig. 2	Component	Paranonychus brunneus	Cryptomaster leviathan	Speleomaster lexi	Speleomaster pecki	Speleonychia sengeri	Metanonychus idahoensis	Sclerobunus nondimorphicus	Briggsus flavescens
1	Phenol	t	t	o	t	t			+
2	Benzothiazole	t				t			t
9	N,N-dimethylphenylethylamine	+	+	+	+	+	+	+	o
10	Nicotine						o	o	
11	N,N-dimethylisoamylamine						o	t	

Table 4.—Compounds present in *Fumontana deprehendor* and six morphospecies of New Zealand triaenonychids. Plus sign indicates major component (<10%), "o" a minor component (<10%) and "t" a trace component (<1%).

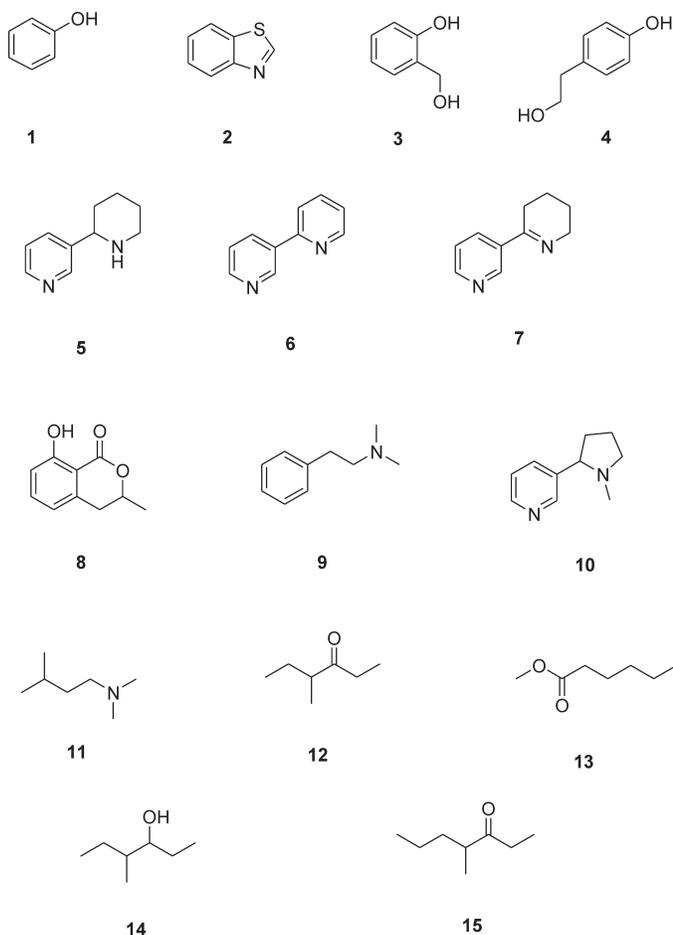| Fig. 2	Component	Soerensenella sp.	Soerensenella prehensor	Nuncia sp. Dancing Creek	Nuncia sp. Aratika	Nuncia sp. Springs Junction 1	Nuncia sp. Springs Junction 2	Fumontana deprehendor
1	Phenol							+
3	Salicylic alcohol							o
12	4-methyl-3-hexanone	+	+	+	+	+	+	
13	Methylhexanoate					t		
14	4-methyl-3-hexanol						+	
15	4-methyl-3-heptanone					t		

Figure 2.—Compounds identified in this study. 1. Phenol, 2. Benzothiazole, 3. Salycyl alcohol, 4. 4-Hydroxybenzenethanol, 5. Anabasine, 6. 2,3′-dipyridryl, 7. Anabaseine, 8. Mellein, 9. N,N-dimethylphenylethylamine, 10. Nicotine, 11. N,N-dimethylisoamylamine. 12. 4-methyl-3-hexanone, 13. Methylhexanoate, 14. 4-methyl-3-hexanol, 15. 4-methyl-3-heptanone.

methyl-3-hexanone in New Zealand triaenonychids and to N,N-dimethylphenylethylamine in travunioids.

DISCUSSION

The qualitative near-identity of the extracts from *E. flavescens* and *T. brunneus* supports the close phylogenetic relationship hypothesized on the bases of morphology and genetics by Derkarabetian et al. (2010). The strong differences between the secretions of this "eastern clade" and that of the hypothetical "western clade" of travunioids supports that distinction.

Raspotnig et al. (2011) found anabaseine as the major component in the secretion of *Holoscotolemon lessiniense,* but nicotine dominated that of *H. jaqueti* and *H. oreophilum* (Table 2). These three species appear to be closely related from morphological evidence and numerous characters, especially genitalic, place them close to *Erebomaster* Cope 1872 and *Theromaster* Briggs 1969 (Martens 1978). Trace components in these three species were pyridines with the same core structure as anabaseine and nicotine. Both chemical and morphological evidence, therefore, argue for a closer relation-

ship of the eastern North American genera with European *Holoscotolemon* than with the travunioid genera from western North America.

For the western travunioids, N,N-dimethylphenylethylamine was the major component in all species except *Briggsus flavescens. Metanonychus idahoensis* Briggs 1971 and *Sclerobunus nondimorphicus* Briggs 1971 had nicotine and N,N-diethylisoamylamine as minor components, as well as two unidentified compounds not shown. For the other species, phenol was present as either a minor component or a trace, and benzothiazole was found as a trace in *Paranonychus brunneus* (Banks 1893) and *Speleonychia sengeri* Briggs 1974. The complex mixtures found in the eastern cladonychiids and in *S. ?robustus* (Epka et al. 1984) were not recovered from the western species we studied. The complexity of the secretion extracted from the two eastern cladonychiid species is similar to that found by Epka et al. (1984) for *Sclerobunus ?robustus*, but quite different chemically. New Mexico *Sclerobunus* Banks 1893 require re-examination.

Both the complexity and the diversity of chemical composition within Insidiatores is unusual among opilionids, because in previous studies, similar classes of compounds (though different molecules) have been found in large taxonomic groupings. For example, sclerosomatids utilize a variety of ketones and alcohols, and many Grassatores produce alkylphenols and hydroquinones (Hara et al. 2007, Raspotnig 2012 [2013], Caetano & Machado 2013, Raspotnig et al. 2014). In some cases the secretion consists of a single compound (Shear et al. 2010a, b). However, in the case of the cyphophthalmids, the two species so far studied show as diverse an array of molecules as do the travunioids or even more so (Raspotnig et al. 2005, Jones et al. 2009, Raspotnig 2012 [2013]). Because cyphophthalmids are sister to all remaining Opiliones, the scanty data collected so far could be construed to suggest that early-evolving defensive secretions were complex mixtures, later winnowed down to only a few, or to single, components. Evidence against this view is that gonyleptoids, a derived group, also have complex mixtures, though the compounds are nearly all methylated and/or ethylated benzoquinones or alkylphenols (Föttinger et al. 2010, Raspotnig 2012 [2013]). However, the question that remains unexamined so far is the extent to which the method of collecting the secretions and the processing for analysis may have influenced the results; it is possible that chemical changes in some of the components could be induced during study, and this could account for the mixtures obtained.

Results of the character mapping for Travunioidea are shown in Fig. 4. The various compounds are represented by numbers that correspond to those in Fig. 2. Two major findings are seen in the parsimony reconstruction regarding the chemicals that constitute the major components. First, the major component N,N-dimethylphenylethylamine (9) was recovered as the ancestral state for all travunioid genera included in this analysis. Second, there is a transition from N,N-dimethylphenylethylamine (9) to anabaseine (7) as the major component on the branch leading to the eastern Cladonychiidae (*Erebomaster* and *Theromaster*). In addition, these two genera also possess many other minor or trace elements that are unique to this lineage, namely salycyl alcohol (3), anabasine (5), 2,3′-dipyridyl (6) and mellein (8). Also,

1. Phenol

2. Benzothiazole

3. Salycyl alcohol

6,7. 2,3'Dipyridyl and Anabaseine

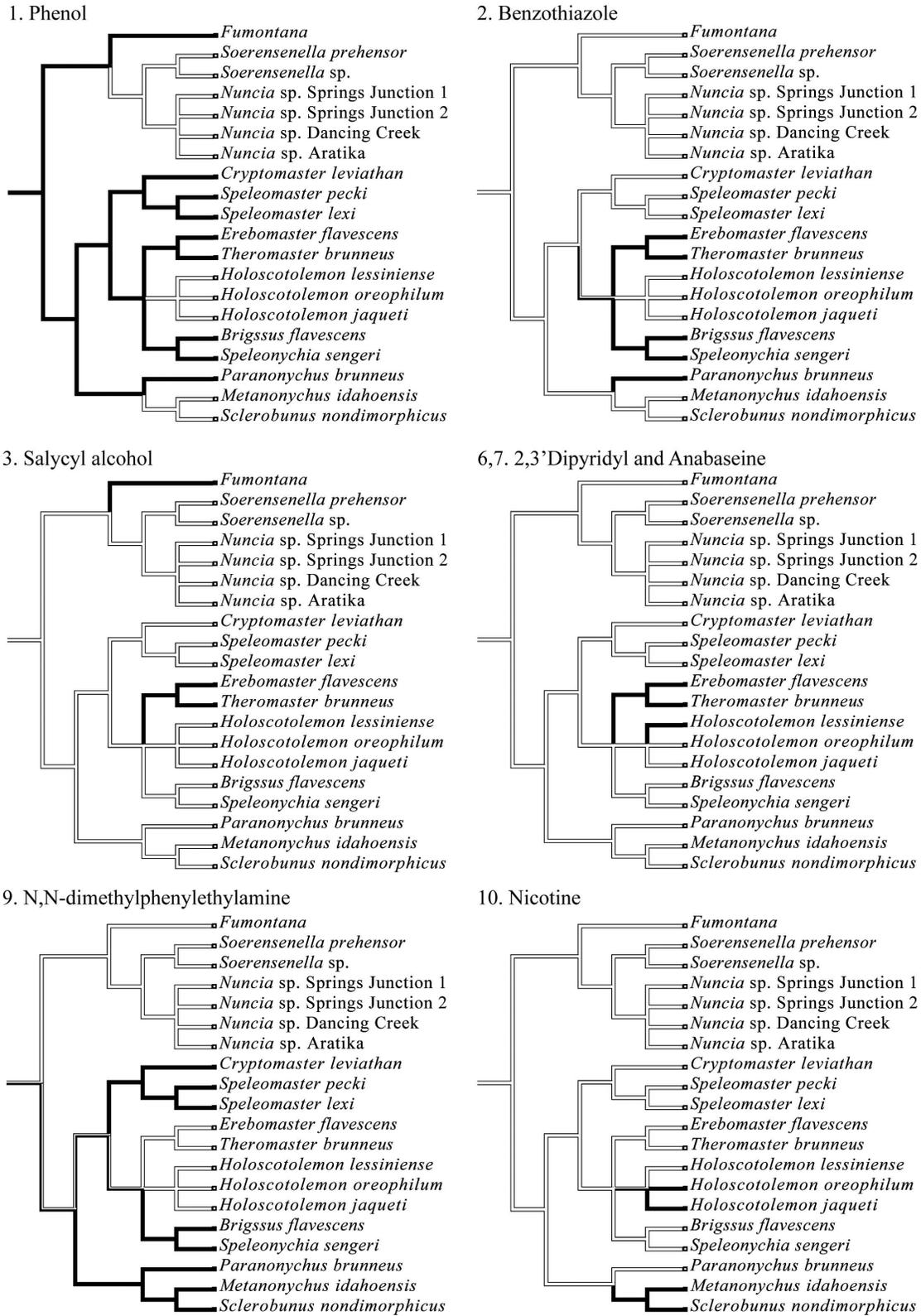9. N,N-dimethylphenylethylamine

10. Nicotine

Figure 3.—Results of chemical character mapping for Insidiatores. Only those chemicals with 2 or more steps are shown. Black = presence, white = absence.
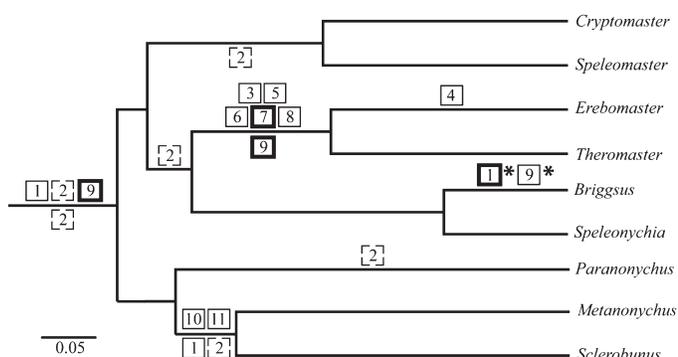
Figure 4.—Results of the chemical character mapping in Travunioidea. Numbers correspond to the chemicals listed in Tables 1 and 2. Boxed numbers above a branch are character gains, those below are losses. Bold boxes are major components and regular boxes are minor/trace components. Dashed boxes represent those chemicals that are equally parsimonious (present/absent) along the branch; but branches with definite gains for these chemicals are also included. Boxes with asterisks indicate a change in component concentration (e.g., change from major to minor).

Erebomaster is the only taxon known to possess 4-hydroxybenzenethanol (4). The sclerobunines (*Sclerobunus* and *Metanonychus* Briggs 1971) have lost phenol (1) as a component but have gained both nicotine (10) and N,N-dimethylisoamylamine (11). Interestingly, two species of *Holoscotolemon* also produce nicotine.

Raspotnig (2012 [2013]) discussed at length the possible phylogenetic and systematic implications of the diversity of defensive compounds in Opiliones. Overlooking some dissonant results, it appears that the suborder Cyphophythalmi can be characterized by methyl ketones, naphthoquinones and related compounds. Benzoquinones appear in phalangiid Eupnoi, and "sclerosomatid compounds" (noncyclic ketones, alcohols and aldehydes, such as 4-methyl-3-hexanone) are found in sclerosomatid Eupnoi. Few Dyspnoi have been examined, but naphthoquinones and anthraquinones have been found. Grassatores produce predominantly phenols, benzoquinones and hydroquinones. Insidiatores, up to the findings of this study, were characterized by nitrogen-containing alkaloids. Raspotnig (2012 [2013]) is quick to point out that taxonomic sampling within the Opiliones has been erratic and many taxa remain unsampled, or known only from unpublished or preliminary results.

Raspotnig (2012 [2013]) proposed a number of phylogenetic hypotheses that may be summarized as follows: 1) complex mixtures of secretions are plesiomorphic compared to uniform or less diverse mixtures; 2) naphthoquinones and methyl ketones, as found in cyphophthalmids, are basal; 3) naphthoquinones are synapomorphic for a clade Cyphophthalmi + Palpatores; 4) acyclic compounds in Cyphophthalmi and Sclerosomatidae may have a common origin; 5) "sclerosomatid compounds" may represent a synapomorphy for Palpatores; 6) a deep chemical divergence separates Insidiatores and Grassatores; and 7) a link between the chemistry of Cyphophthalmi + Palpatores and Laniatores remains to be found.

But the phylogenetic signal is not so clear as that. The dissonant results mentioned above seem to significantly

disrupt the characterizations given. Among the anomalies Raspotnig (2012 [2013]) mentions which require explanation are the presence of naphthoquinones in some putative sclerosomatids (*Gyas* sp.), ketones in some Gonyleptidae (Grassatores), and now, as a result of our work, methyl ketones ("sclerosomatid substances") in Triaenonychidae and phenol in *Fumontana deprehendor,* a species that consistently is recovered in phylogenies as sister to remaining triaenonychids. At least these latter two make possible a tentative link between Laniatores and some Palpatores.

Caetano & Machado (2013) conducted a phylogenetic analysis of the distribution of scent gland chemistry in Grassatores, and concluded that benzoquinones were ancestral, with alkylphenols evolving independently many times. Using the same data, but a different method of analysis and a different outgroup, Raspotnig et al. (2014) concluded the opposite—that benzoquinones were derived and alkylphenols ancestral. Based on the methods used and the fact that Raspotnig et al. (2014) used a more appropriate outgroup, we agree with the latter conclusion. Our finding that phenol is probably ancestral in Insidiatores (see Fig. 3) reinforces this, although exact phylogenetic relationships between Insidiatores and Grassatores remain unclear.

Raspotnig (2012 [2013]) did not attempt to map the known characters on any established phylogenetic tree of Opiliones. However, study of his Table 2 (pp. 9–10) and our Fig. 3 seems to indicate that at least at the present state of knowledge, there is a great deal of homoplasy present, with various types of compounds being lost and then regained, or evolving independently.

In our results for Insidiatores, the most divergent observation is the presence of 4-methyl-3-hexanone as the major component in all of the New Zealand triaenonychids we studied. If we consider *Fumontana* as a plesiomorphic outgroup, we have the problem of getting from phenol to these noncyclic ketones. The travunioids stand alone with the predominant secretion of either N,N-dimethylphenylethylamine or tobacco alkaloids like nicotine and anabaseine. A major question, which by extension could be applied to the entire phylogenetic scheme of this character, is how one gets from one compound or set of compounds in a supposed plesiomorphic taxon to a chemically completely different compound further up in the tree. In other words, is it reasonable to assume a transition from phenol to 4-methyl-3-hexanone?

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