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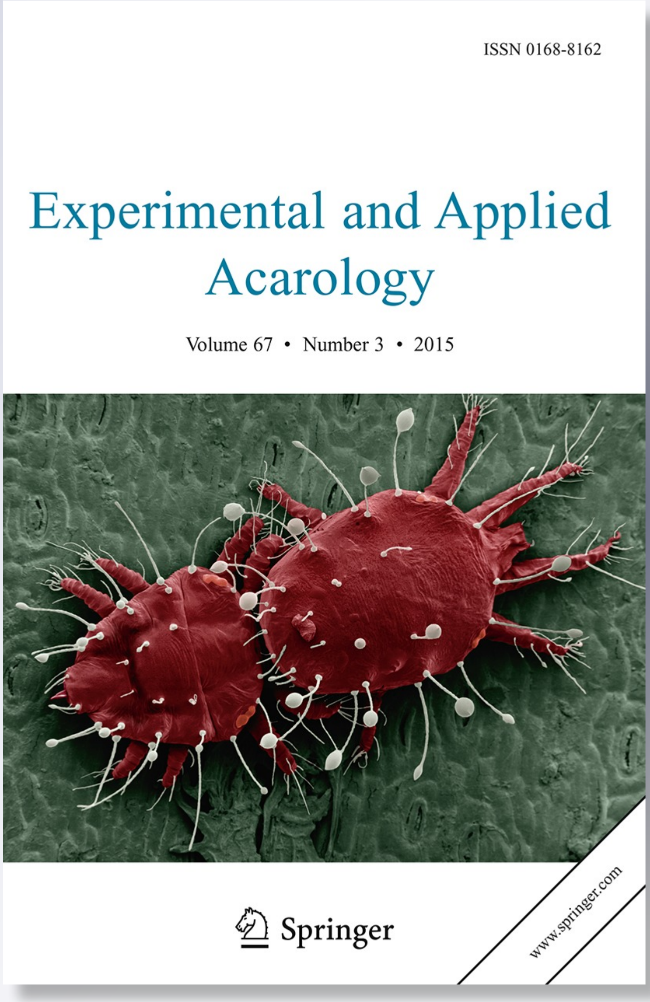

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Taxonomic distribution of defensive alkaloids in Nearctic oribatid mites (Acari, Oribatida)

Ralph A. Saporito[1] · **Roy A. Norton**[2] · **Martin H. Garraffo**[3] · **Thomas F. Spande**[4]

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Abstract The opisthonotal (oil) glands of oribatid mites are the source of a wide diversity of taxon-specific defensive chemicals, and are likely the location for the more than 90 alkaloids recently identified in oribatids. Although originally recognized in temperate oribatid species, alkaloids have also been detected in related lineages of tropical oribatids. Many of these alkaloids are also present in a worldwide radiation of poison frogs, which are known to sequester these defensive chemicals from dietary arthropods, including oribatid mites. To date, most alkaloid records involve members of the superfamily Oripodoidea (Brachypylina), although few species have been examined and sampling of other taxonomic groups has been highly limited. Herein, we examined adults of more than 60 species of Nearctic oribatid mites, representing 46 genera and 33 families, for the presence of alkaloids. GC–MS analyses of whole body extracts led to the detection of 15 alkaloids, but collectively they occur only in members of the genera *Scheloribates* (Scheloribatidae) and *Protokalumma* (Parakalummidae). Most of these alkaloids have also been detected previously in the skin of poison frogs. All examined members of the oripodoid families Haplozetidae and Oribatulidae were alkaloid-free, and no mites outside the Oripodoidea contained alkaloids. Including previous studies, all sampled species of the cosmopolitan oripodoid families Scheloribatidae and Parakalummidae, and the related, mostly tropical families Mochlozetidae and Drymobatidae contain alkaloids. Our findings are consistent with a generalization that alkaloid presence is widespread, but not universal in

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✉ Ralph A. Saporito
rsaporito@jcu.edu

[1] Department of Biology, John Carroll University, University Heights, OH 44118, USA

[2] College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, USA

[3] Clinical Mass Spectrometry Core, NIDDK, NIH, DHHS, Bethesda, MD 20892, USA

[4] Laboratory of Medicinal Chemistry, NIDA, NIH, DHHS, Bethesda, MD 20892, USA

Oripodoidea. Alkaloid presence in tropical, but not temperate members of some non-oripodoid taxa (in particular Galumnidae) deserves further study.

Keywords Chemical defense · Dendrobatids · Opisthonotal (oil) gland · Poison frogs · Soil mites · *Scheloribates* · *Protokalumma*

Introduction

Oribatid mites (in their traditional sense, excluding Astigmata) are among the most abundant and diverse arthropods in soil, leaf-litter, and arboreal microhabitats worldwide (Maraun and Scheu 2000; Franklin et al. 2004; Lindo and Winchester 2006), and the approximately 10,000 described species represent more than 1200 genera and 170 families (Subías 2004; Schatz et al. 2011). Many are opportunistic feeders on nematodes, small dead arthropods and algae, but most are fungivores or ingest dead plant remains (Schneider et al. 2004; Norton and Behan-Pelletier 2009 and references therein). Among their hallmarks is an exceptional diversity of defensive mechanisms, particularly in the adult, the evolution of which has been linked to low secondary production in predator-rich environments (Norton 2007; Pachl et al. 2012).

One such mechanism is a pair of defensive exocrine glands called opisthonotal (or oil) glands. These characterize all but the most basal oribatid mite taxa, and secrete a wide range of defensive organic compounds, including monoterpenes, sesquiterpenes, aromatics, aliphatic aldehydes, a ketone, fatty acids, fatty acid esters, alkyl formates, and hydrocarbons (Kuwahara 2004; Raspotnig 2010). The occurrence and profiles of these compounds appear to be taxon-specific and thus would provide unique information on the evolutionary relationships among the oribatid mites (see Sakata and Norton 2001; Raspotnig 2010; Raspotnig et al. 2008, 2011 and references therein).

Opisthonotal glands might also be the source of the more than 90 alkaloids, representing 11 structural classes, that have been characterized from extracts of adult oribatid mites (Takada et al. 2005; Saporito et al. 2007, 2011); however, the specific location of alkaloids has not been established. These alkaloid classes include pumiliotoxins (PTX), homopumiliotoxins (hPTX), 5,8-disubstituted indolizidines (5,8-I), 5,6,8-trisubstituted indolizidines (5,6,8-I), dehydro-5,8-disubstituted indolizidines (d-5,8-I), coccinelline-like tricyclics (Tri), a 1,4-disubstituted quinolizidine (1,4-Q), a 4,6-disubstituted quinolizidine (4,6-Q), a 3,5-disubstituted indolizidine (3,5-I), pyrrolidines (Pyr), a spiropyrrolizidine (Spiro), and numerous alkaloids that could not be assigned to a specific structural class (Unclass; see Saporito et al. 2012 for a review). Like the other defensive compounds, alkaloids appear to be of endogenous origin—they are present in mites irrespective of nutritional source (Takada et al. 2005), are adult-specific (Takada et al. 2005; Saporito et al. 2011)—and have circumscribed taxonomic distributions among oribatid mites. They were first discovered in two members of Scheloribatidae—*Scheloribates azumaensis* Enami et al. and *Scheloribates* sp. from temperate Japan (Takada et al. 2005)—and also occur in a congener, *Scheloribates laevigatus* (C. Koch), based on a population from the USA (Saporito et al. 2011). Oribatid mites from Costa Rica and Panama have provided the richest alkaloid dataset (Saporito et al. 2007), but most mites were not identified to species and most samples were derived from a mixture of several species. Despite these problems, the data are consistent with a generalization that alkaloids are particularly prevalent in the geographically widespread Scheloribatidae and the related, mostly tropical families Mochlozetidae and Drymobatidae—members of the superfamily Oripodoidea in the hyporder Brachypylina (Raspotnig et al. 2011). Some alkaloid-positive collections that contained a mix of different species, however, included no mites from these families and the taxonomic spectrum of alkaloid distribution remains unclear (Vences et al. 2011).

Most of the alkaloids identified in oribatid mites are also present in a worldwide radiation of chemically defended frogs, generally referred to as poison frogs (see Saporito et al. 2012). Composed of five independent evolutionary lineages, poison frogs are recognized by a shared ability to sequester defensive alkaloids from dietary arthropods, including ants, beetles, millipedes and, most recently discovered, oribatid mites (Saporito et al. 2009, 2012; Hantak et al. 2013). These frog lineages include certain dendrobatids from Central and South America, bufonids from South America (*Melanophryniscus*), mantellids from Madagasgcar, myobatrachids from Australia (*Pseudophryne*) and, most recently, miniaturized eleutherodactylid frogs from Cuba (Rodríguez et al. 2011). More than 850 lipophilic alkaloids (arranged into more than 20 structural classes) have been identified from the skin of poison frogs, and the majority of these, particularly those with structural branch points in the carbon backbone, are now presumed to arise from a diet of oribatid mites (for reviews, see Saporito et al. 2009, 2012).

Knowing the distribution of alkaloids in mites is fundamental to understanding both mite chemical defense and the evolution of sequestered defenses in poison frogs. Alkaloid surveys in mites will need to encompass a wide variety of both taxa and geographic locations in order to sort phylogenetic from geographical patterns and to seek possible convergences. As alkaloids were first discovered in temperate-zone mites, far from the distribution of poison frogs, our present objective is to examine a selection of Nearctic oribatid mites for alkaloids. Most of the sampled species are in the derived hyporder Brachypylina, since no earlier-derivative taxon is currently known to have alkaloids. The results of the present study are consistent with previous work, in that Scheloribatidae and some related groups consistently have alkaloids.

Methods

Mite collection and alkaloid extraction

Adult oribatid mites from more than 60 species, representing 46 genera and 33 families (Table 1), were extracted by Berlese-funnels from soil and leaf-litter samples collected between January and April of 2008 from locations in the states of New York (near Syracuse), West Virginia (Randolph County), and California (Yolo County). Alkaloids were extracted from whole mite bodies by transferring freshly collected living specimens using a small brush into 4-mL glass vials with Teflon-lined caps, each containing ca. 50–500 μl of methanol (depending on the number of individual mites in the sample, see below). As confirmed by detailed study after extraction, each vial contained specimens from a single species, except for two instances in which species were indistinguishable when living. In one instance the mixing was anticipated (several minute, unidentified species of *Suctobelbella*) but the other—a mixture of *Protokalumma depressa* (Banks) and an undescribed species near *Protokalumma neonominatus* (Subias)—was discovered only during confirmation. Our goal was to obtain and extract at least 10 specimens per vial, but this was not always possible, and the numbers ranged from 1 to more than 80. Even single specimens should be sufficient, as *S. laevigatus* individuals contain an average of 17 ng of alkaloid (Saporito et al. 2011), which is well within the detection limits of the instrumentation used in the present study. Methanol contact proceeded for at least 24 h, and alkaloids were detected and identified directly from these mite extracts.

Alkaloid identification

Identification of previously documented alkaloids was based on comparison of mass spectral (MS) properties, Fourier-transformed infrared spectral (FTIR) properties (when possible), and gas chromatograph (GC) retention times with those of previously reported oribatid mite and poison frog alkaloids (Daly et al. 2005; Saporito et al. 2007, 2011). Vapor-phase FTIR spectroscopy was also used in the characterization of a previously unreported tricyclic alkaloid. To date, most of the alkaloids identified in oribatid mites are similar in chemical structure or identical to those previously reported from poison frog skins. Poison frog alkaloids have been assigned code names, consisting of a bold-faced number corresponding to their molecular weight and a bold-faced letter to distinguish alkaloids with the same nominal mass (Daly et al. 2005).

Alkaloid analysis

GC–MS data were obtained on a Thermo-Electron Polaris-Q instrument coupled to a Focus GC with a 30 m × 0.25 mm i.d. Restek-5MS fused silica column, or a Varian Saturn 2100T ion trap MS instrument coupled to a Varian 3900 GC with a 30 m × 0.25 mm i.d. Varian Factor Four VF-5 ms fused silica column. GC separation of alkaloids was achieved using a temperature program from 100 to 280 °C at a rate of 10 °C per minute with He (1 mL/min.) as the carrier gas. Each extract was analyzed with both electron impact-mass spectrometry (EI–MS) and chemical ionization-mass spectrometry (CI–MS) with NH_3 (Polaris-Q instrument) or methanol (Saturn instrument) as the reagent gas. GC–FTIR data were obtained with a Hewlett-Packard model 5890 gas chromatograph fitted with a 30 m × 0.32 mm i.d. Phenomenex Zebron ZB-5 capillary column (same temperature program as above), interfaced with a Hewlett-Packard (HP) model 5971 Mass Selective Detector and a Hewlett-Packard Model 5965B IRD with a narrow range (4000-750 cm^{-1}) infrared detector.

Results

GC–MS analysis of adult oribatid mite extracts showed that most mite species examined in this study did not contain alkaloids (Table 1). Alkaloids were present only in members of the genera *Scheloribates* (Scheloribatidae) and *Protokalumma* (Parakalummidae). Eight alkaloids (including isomers) were identified in members of *Scheloribates*, representing four structural classes (Table 2). Five of these alkaloids have been identified previously in the skin of poison frogs: the tricyclic (Tri) **193C** (precoccinelline; Fig. 1), 5,6,8-trisubstituted indolizidine (5,6,8-I) **193G**, unclassified **207N**, 5,6,8-trisubstituted indolizidine (5,6,8-I) **221Q**, and 5,8-disubstituted indolizidine (5,8-I) **281I**. The remaining three alkaloids have not been observed previously, but are structurally similar to other alkaloids identified in the skin of poison frogs, being two isomers of a tricyclic (Tri) of molecular weight (MW) 275 and a tricylic (Tri) of MW 247. Tricyclics represent a large alkaloid

Table 1 Nearctic oribatid mites examined for the presence of alkaloids

Higher Taxon[a]	Family	Genus/species[b]	Provenance	No. of individuals[c]	Alkaloids detected[d]
Infraorder Enarthronota	Hypochthoniidae	*Hypochthonius rufulus* C. Koch	New York	5	–
Infraorder Mixonomata	Collohmanniidae	*Collohmannia johnstoni* Norton & Sidorchuk	West Virginia	1	–
	Oribotritiidae	*Oribotritia banksi* (Oudemans)	West Virginia	4	–
Infraorder Desmonomata					
Hyporder Nothrina	Nothridae	*Nothrus* cf. *silvestris* Nicolet	New York	ca. 25	–
Hyporder Brachypylina					
Superfamily Hermannielloidea	Hermanniellidae	*Hermanniella* sp.	California	67	–
Superfamily Neoliodoidea	Neoliodidae	*Poroliodes* sp. nr. *farinosus* (C. Koch)	New York	1, 1, 1	–
Superfamily Plateremaeoidea	Gymnodamaeidae	*Adrodamaeus* cf. *musci* Paschoal	New York	7	–
Superfamily Damaeoidea	Damaeidae	*Damaeus angustipes* (Banks)	New York	2	–
		Epidamaeus sp.	New York	6	–
		Parabelbella sp. nr. *inaequipes* (Banks)	New York	15, 17	–
		Parabelbella sp.	California	13	–
Superfamily Eutegaeoidea	Compactozetidae	*Oribatodes mirabilis* Banks	New York	7, 5	–
		Cepheus corae Jacot	New York	6	–
Superfamily Polypterozetoidea	Podopterotegaeidae	*Podopterotegaeus tectus* Aoki	New York	17	–
Superfamily Ameroidea	Ameridae	*Gymnodampia setata* (Berlese)	West Virginia	22	–
	Eremobelbidae	*Eremobelba gracilior* Berlese	New York	25	–
Superfamily Zetorchestoidea	Eremaeidae	*Eremaeus brevitarsus* (Ewing)	New York	5	–
		Eremaeus kevani Behan-Pelletier	New York	18	–
Superfamily Gustavioidea	Liacaridae	*Liacarus* sp.	West Virginia	ca. 20	–
		Liacarus acutidens Aoki	California	5	–
		Adoristes ammoonosuci Jacot	New York	3	–
	Peloppiidae	*Metrioppia atlantica* Jacot	New York	ca. 25	–
		Ceratoppia sp.	West Virginia	1	–
		Ceratoppia bipilis (Hermann)	New York	12	–

Table 1 continued

Higher Taxon^{a}	Family	Genus/species^{b}	Provenance	No. of individuals^{c}	Alkaloids detected^{d}
Superfamily Carabodoidea	Carabodidae	*Carabodes polyporetes* Reeves	New York	10, 25	–
Superfamily Oppioidea	Oppiidae	*Oppia denticulata* (R. & G. Canestrini)	New York	23	–
		Oppiella nova (Oudemans)	New York	>50	–
	Thyrisomidae	*Gemmazetes* sp. nr. *crosbyi* (Ewing)	New York	36	–
Superfamily Trizetoidea	Suctobelbidae	*Allosuctobelba obtusa* (Jacot)	New York	11	–
		Suctobelbella spp.	New York	ca. 25	–
Superfamily Tectocepheoidea	Tectocepheidae	*Tectocepheus sarekensis* Tragardh	New York	75	–
		Tectocepheus velatus (Michael)	New York	ca. 50	–
Superfamily Limnozetoidea	Hydrozetidae	*Hydrozetes lemnae* (Coggi)	New York	31	–
Superfamily Phenopelopoidea	Phenopelopidae	*Propelops minnesotensis* (Ewing)	New York	12	–
		Propelops sp. nr. *pinicus* Jacot	California	ca. 20	–
Superfamily Achipterioidea	Achipteriidae	*Achipteria catskillensis* Nevin	New York	15, 16	–
		Achipteria armata (Banks)	New York	36	–
		Anachipteria cf. *achipterioides* (Ewing)	New York	14	–
		Anachipteria sp. nr. *magnilamellata* (Ewing)	New York	15	–
Superfamily Oribatelloidea	Oribatellidae	*Oribatella brevicornuta* Jacot	New York	22	–
		Oribatella transtriata Behan-Pelletier	New York	3	–
Superfamily Oripodoidea	Haplozetidae	*Peloribates americanus* Jacot	New York	10	–
		Protoribates capucinus (Berlese)	New York	20	–
		Protoribates oblongus (Ewing)	New York	26	–
		Protoribates robustior (Jacot)	New York	62	–
		Rostrozetes ovulum (Berlese)	New York	17	–
	Oribatulidae	*Oribatula tibialis* (Nicolet)	New York	7, ca. 10	–
	Parakalummidae	*Protokalumma* spp.	New York	8	+
	Scheloribatidae	*Scheloribates latipes* (C. Koch)	New York	21, 15	+
		Scheloribate lanceoliger Berlese	New york	15, ca. 25	+

Table 1 continued

Higher Taxon[a]	Family	Genus/species[b]	Provenance	No. of individuals[c]	Alkaloids detected[d]
Superfamily Ceratozetoidea	Ceratozetidae	*Ceratozetes* sp. nr. *subaquila* (Ewing)	New York	89	–
		Ceratozetes sp. nr. *mediocris* Berlese	New York	15	–
		Fuscozetes bidentatus (Banks)	New York	40	–
		Fuscozetes fuscipes (C. Koch)	New York	41	–
	Chamobatidae	*Chamobates* sp.	New York	20	–
	Mycobatidae	*Punctoribates punctum* (C. Koch)	New York	38	–
	Zetomimidae	*Heterozetes minnesotensis* (Ewing)	New York	67	–
		Zetomimus setosus (Banks)	New York	23	–
Superfamily Galumnoidea	Galumnidae	*Pergalumna emarginata* (Banks)	New York	54, 20	–
		Pergalumna curva (Ewing)	New York	ca. 35	–
		Galumna ithacensis (Jacot)	New York	36	–
		Galumna cf. *octopunctata* (Ewing)	New York	31	–
		Galumna sp.	California	13	–

[a] Classification based on Schatz et al. 2011 (except Compactozetidae = Cepheidae)

[b] Abbreviations are as follows: cf. (may represent named species, but with some doubt), sp. (unidentified species of the named genus), sp. nr. (similar to named species, but probably not conspecific)

[c] Numbers separated by commas represent samples from different populations; samples are listed separately if alkaloids differed

[d] Details in Table 2

Table 2 Alkaloids present in brachypyline oribatid mites

Superfamily Family Genus	Geographic location	Study	Alkaloids (1) Identified in oribatid mites and poison frogs[a] (2) Identified only in oribatid mites[b]
Superfamily Oripodoidea			
Drymobatidae			
Drymobates sp. A	Bocas del Toro, Panama	Saporito et al. 2007	(1) 5,8-I **207A**; Unclass **227** (2) MW 239, 241
Drymobates sp. B	Southern Limon, Costa Rica	Saporito et al. 2007	(1) None (2) MW 227, 239
Mochlozetidae			
Dynatozetes amplus Grandjean	Bocas del Toro, Panama	Saporito et al. 2007	(1) d-5,8-I **269D** (2) None
Uracrobates (sensu lato) n. sp.	Heredia, Costa Rica	Saporito et al. 2007	(1) 5,6,8-I **237C**; 3,5-I **223AB**; Pyr **183B**; Unclass **209G** (2) None
Parakalummidae			
Protokalumma spp.	New York, USA	Present Study	(1) 5,6,8-I **221Q**; Tri **193C**; Unclass **207N** (2) MW 223 (5,6,8-I), 285 (Tri)[e]
Scheloribatidae			
Scheloribates azumaensis Enami et al.	Fukishima, Japan	Takada et al. 2005	(1) PTX **251D**; Tri **193C** (2) Tri (unknown).
Scheloribates latipes (C. Koch)	New York, USA	Present Study sample 1	(1) 5,6,8-I **221Q**; Tri **193C**; Unclass **207N** (2) MW 247 (Tri)[e]
Scheloribates latipes (C. Koch)	New York, USA	Present Study sample 2	(1) 5,6,8-I **221Q**; Tri **193C**; Unclass **207N** (2) MW 247 (Tri), 275 (Tri)[e]
Scheloribates lanceoliger Berlese	New York, USA	Present Study sample 1	(1) 5,8-I **281I**; 5,6,8-I **221Q**; Tri **193C**; Unclass **207N** (2) MW 247 (Tri), 275 (Tri)[e]

Table 2 continued

Superfamily Family Genus	Geographic location	Study	Alkaloids (1) Identified in oribatid mites and poison frogs[a] (2) Identified only in oribatid mites[b]
Scheloribates lanceoliger Berlese	New York, USA	Present Study sample 2	(1) 5,6,8-I **193G**, **221Q**; Tri **193C**; Unclass **207N** (2) MW 247 (Tri, two isomers), 275 (Tri)[e]
Scheloribates laevigatus (C. Koch)	New York, USA	Saporito et al. 2011	(1) 5,6,8-I **195G**, **209C** (two isomers); PTX **291G** (two isomers) (2) MW 307 (PTX); 247 (Tri); 275 (izidine, two isomers)
Scheloribates sp.	Kyoto, Japan	Takada et al. 2005	(1) 5,6,8-I **223A**; PTX **237A**; dPTX **193H**; 1,4-Q **231A** (2) Additional alkaloids (not identified)
Unknown genus A sp.	Bocas del Toro, Panama	Saporito et al. 2007	(1) 5,8-I **235B″**; PTX **307F″**or **307F‴** (2) None
Unknown genus B[c] sp.	Bocas del Toro, Panama	Saporito et al. 2007	(1) 5,8-I **195I**, **207A**, **209S**, **223D**, **225D**, **235B″**, **237D**; 5,6,8-I **259C′**; hPTX **251R**; Pyr **253I**; Unclass **265K** (2) MW 281, 297 (hPTX), isomer 297 (hPTX)
Unknown genus B sp.	Heredia, Costa Rica	Saporito et al. 2007	(1) 5,8-I **195I**, **209S**, **225D** (2) MW 263, 295 (Tri)
Unknown genus C[d]sp.	Bocas del Toro, Panama	Saporito et al. 2007	(1) 5,8-I **235B″**; 5,8-I **237D**; PTX **251D** (2) None
Unknown genus C sp.	Bocas del Toro, Panama	Saporito et al. 2007	(1) 5,8-I **235B″**; 5,8-I **237D** (2) None
Unknown genus C sp.	Bocas del Toro, Panama	Saporito et al. 2007	(1) 5,8-I **235B″** (2) None
Unknown genus C sp.	Bocas del Toro, Panama	Saporito et al. 2007	(1) 5,8-I **235B″**; PTX **307F″** or **307F‴** (2) None

Table 2 continued

Superfamily Family Genus	Geographic location	Study	Alkaloids (1) Identified in oribatid mites and poison frogs[a] (2) Identified only in oribatid mites[b]
Superfamily Galumnoidea			
Galumnidae			
Galumna sp. 1	Northern Limon, Costa Rica	Saporito et al. 2007	(1) 5,6,8-I **237C**; 4,6-Q **237I**; Unclass **279I**, **323I** (2) MW 207 (dehydroizidine)

See Table 1 for classification notes. Repetitions relate to different samples of the same species

Alkaloid structural classes: 5,8-I (5,8-disubstituted indolizidine); d-5,8-I (dehydro-5,8-disubstituted indolizidine); 5,6,8-I (5,6,8-trisubstituted indolizidine); PTX (pumiliotoxin); hPTX (homopumiliotoxin); dPTX (dehydropumiliotoxin); Tri (tricyclic); 1,4-Q (1,4-disubstituted quinolizidine); 3,5-I (3,5-disubstituted indolizidine); Pyr (pyrrolidine); Unclass (unclassified as to structure)

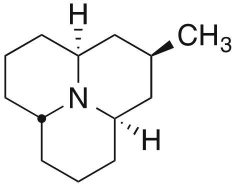
[a] Poison frog alkaloids are assigned code names consisting of a bold-faced number to indicate nominal mass, and a bold-faced letter to distingusih alkaloids with the same mass. Alkaloid classes and code numbers are from Daly et al. 2005

[b] Non-coded alkaloids are indicated by molecular weight (MW) and for some, the probable structural class. Mass spectra and other data are presented in Table 5 of the Supporting Information in Saporito et al. 2007

[c] Genus near *Megascheloribates* in Saporito et al. (2007)

[d] Unknown A (no family classification) in Saporito et al. (2007)

[e] Mass spectral data and GC retention times are in Supplemental Table 1



193C

Fig. 1 Chemical structure of the tricyclic alkaloid precoccinelline, **193C**

group of varied structural types, and are typified by the coccinelline alkaloids (Daly et al. 2005). Tricyclics contain three aliphatic rings and are characterized by mass spectra of many intense peaks that are often separated by 14 atomic mass units, and without any dominant alpha cleavage adjacent to the nitrogen atom.

Seven alkaloids (including isomers) were identified in the mixed sample of *Protokalumma* spp. (two species), representing four structural classes (Table 2). Three of these alkaloids have been detected and/or identified previously in the skin of poison frogs: the tricyclic (Tri) **193C**, unclassified (Unclass) **207N**, and 5,6,8-trisubstituted (5,6,8-I) **221Q**. The remaining four alkaloids have not been observed previously: a 5,6,8-trisubstituted indolizidine (5,6,8-I) of MW 223, a tricyclic (Tri) of MW 285, and two isomers of an unknown structure of MW 357.

In most cases, the previously unreported alkaloids identified in this study were not present in quantities sufficient for full chemical characterization; however, their GC retention times and mass spectral data are given in Supplementary Tables 1 & 2. Vapor-phase FTIR spectral data were obtained for one isomer of the MW 247 tricyclic alkaloid, which is included in Supplementary Figure 1. Additional chemical or physical characterizations (e.g., ^{1}H and ^{13}C NMR) will be necessary in order to assign definitive structures to these alkaloids.

Discussion

Taxonomic distribution of alkaloid presence in oribatid mites

Although limited in scope, the Nearctic oribatid mite diversity examined in the present study is sufficient to show that the presence of alkaloids is highly circumscribed. No mites outside the brachypyline superfamily Oripodoidea contained alkaloids, and all six species from the oripodoid families Haplozetidae and Oribatulidae were alkaloid-free. Consistent with previous work (Takada et al. 2005; Saporito et al. 2007, 2011), all samples of Scheloribatidae contained multiple alkaloids that have branch points in their carbon skeleton, suggesting an isoprenoid origin. A very similar diversity of alkaloids was also detected from the mixed-species sample of Parakalummidae (Table 2).

While alkaloids were not detected in Haplozetidae or Oribatulidae, members of these oripodoid families will require further investigation (Table 1). Takada et al. (2005) suspected the presence of alkaloids in two unidentified Japanese species of *Protoribates* (identified by Takada et al. as '*Xylobates*'; Haplozetidae) based on Dragendorff's reagent staining, yet the three species of *Protoribates* examined in the present study did not contain detectable alkaloids. The haplozetid *Rostrozetes ovulum* examined in the present study also did not contain alkaloids. Two other *Rostrozetes* species were included in alkaloid-positive mixed-species samples reported earlier (Saporito et al. 2007), but each mixed sample also included members of *Scheloribates*, which could have been the ultimate sources of detected alkaloids. *Peloribates americanus* (Haplozetidae) and *Oribatula tibialis* (Oribatulidae) were the first representatives of their respective genera to be examined for alkaloids.

Saporito et al. (2007) found that all mixed samples of oribatid mites from Costa Rica and Panama containing a member of Scheloribatidae yielded alkaloids, and this was also true of three unidentified scheloribatid genera that were represented in single-species samples (see Table 2 of Saporito et al. 2007 and present study). Likewise, single-species samples of the mochlozetids *Dynatozetes amplus* and *Uracrobates* n. sp., the drymobatids *Drymobates* sp. A and sp. B (both undescribed), as well as any mixed sample containing a member of the families Mochlozetidae or Drymobatidae, were all alkaloid-positive (see Table 2 of Saporito et al. 2007 and present study). No member of Mochlozetidae or Drymobatidae was included in the present study. None of the four Galumnidae species sampled in the present study contained alkaloids, but Saporito et al. (2007) found five alkaloids in a single-species sample of *Galumna* sp. (see Table 2 of present study). Further studies of the large superfamily Oripodoidea promise to reveal many new alkaloids and clarify family-level patterns in their distribution, but Galumnidae and the related tropical family Galumnellidae should not be ignored.

Origin and variation of oribatid mite alkaloids

All of the mites examined for alkaloids in the present study were wild-caught adults. The two alkaloid-containing *Scheloribates* species studied by Takada et al. (2005) were instead cultured in the laboratory and, having been raised on yeast, there seems little doubt that their alkaloids are not sequestered from dietary sources. Furthermore, alkaloids were absent from nymphal and larval stages of these two *Scheloribates* species (Takada et al. 2005) and *S. laevigatus* (Saporito et al. 2011), suggesting that alkaloids are adult-specific.

The ultimate origin of mite alkaloids is not known, and the whole body alkaloid extraction used in the present study does not allow for this determination, but the current evidence suggests that they are probably produced within specialized glands. The large, defensive opisthonotal glands (see Introduction) seem the most likely source, and it may be relevant that non-alkaloid defensive compounds (e.g., terpenes) have not been reported from adults of alkaloid-containing species, even though the glands are present. The opisthonotal glands, however, are not the only ones to consider. All of the species in Table 2 are members of the brachypyline sub-group Poronota, generally considered highly-derived and characterized by an octotaxic system (four pairs) of dermal glands. While the cuticular parts of these glands (porose areas or saccules) may be small, the cluster of secretory cells below them is not (Alberti et al. 1997; Norton and Alberti 1997). Also, in Mochlozetidae there is a tendency toward enlargement or multiplication (up to 50 pairs) of these glands. Alternatively microsymbionts, such as bacteria, could be the ultimate source of alkaloids in mites and ‘poison frogs’. Microsymbionts are responsible for chemical defenses (including the alkaloid tetrodotoxin) in a number of invertebrates and vertebrates (Steinert et al. 2000; Daly 2004; Loudon et al. 2014). However, the factors involved in such symbiotic interactions, in particular the production of defensive chemicals, remain relatively understudied (Mebs 2001).

All five of the mite species that have been repeatedly sampled showed some differences in their alkaloid profiles (Table 2), suggesting that alkaloid production is variable. Alkaloid variation between populations of *Scheloribates latipes* and *S. lanceoliger*, collected a few kilometers apart, was rather minor. More extensive differences were observed between two samples of the scheloribatid 'Unknown genus B sp.', which were collected from two different Central American countries. None of the four samples of the scheloribatid 'Unknown genus C sp.' had an identical alkaloid profile, although one alkaloid (5,8-I **235B″**) was consistently present in all samples. Although the reason for this variation is not known, alkaloids produced by members of the ant genus *Solenopsis* are known to vary among caste members as well as workers of different sizes and ages (e.g., Deslippe and Guo 2000; Torres et al. 2001; Vander Meer 2012).

If alkaloids are gland-produced, their production could differ with maturity, size, or in the progress of regeneration after discharge; however, the occurrence of oil gland products exhibit species-specific profiles, with little intraspecific variation (see Sakata and Norton 2001; Raspotnig 2010; Raspotnig et al. 2008, 2011 and references therein). Furthermore, Heethoff and Raspotnig (2012) found only minor differences in the regeneration of non-alkaloid defensive compounds in the opisthonotal glands of the nothrine species *Archegozetes longisetosus* (Heethoff and Raspotnig 2012). If they are the product of microsymbionts, factors such as symbiont strain (or species), density, and internal and external mite environment are potential variables that could affect alkaloid production.

Alkaloid specificity and taxonomic implications

The alkaloid classes present among the different taxa of oribatids are remarkably similar, and are generally dominated by the likely isoprenoid derived, branched-chain 5,8-disubstituted indolizidines, 5,6,8-trisubstituted indolizidines, pumiliotoxins, and tricyclics (Table 2). On the basis of these alkaloid classes, members of Scheloribatidae and Parakalummidae are most similar to each other, followed by members of Mochlozetidae and Drymobatidae. The alkaloid complement of Parakalummidae therefore supports their inclusion in Oripodoidea (Travé 1970), which has not been unanimously accepted (e.g. Subías 2004).

The diversity of individual alkaloids is surprisingly high, considering the few species that have been sampled; however, the variability of these alkaloids among samples interferes with the search for strong taxonomic patterns. Still, some preliminary observations are notable. Based on morphology, perhaps the two most closely related species in Table 2 are *Scheloribates latipes* and *S. lanceoliger*, and their alkaloid complements are very similar. *Scheloribates lanceoliger* is native to North America, while S. *latipes* appears to be introduced; in New York *S. latipes* is found in anthropogenic habitats often together with *S. laevigatus*, which also seems introduced. The latter shares one alkaloid (a tricyclic of MW 247) with the former two species, which may prove to be a taxonomic character for the genus, as they appeared in no other oribatid mite species (alkaloids from the two Japanese *Scheloribates* were not fully analyzed). Table 2 shows no specific alkaloids to be common to all Oripodoidea, nor to all Scheloribatidae or Mochlozetidae, but both representatives of Drymobatidae potentially share at least one alkaloid (MW 239). Unfortunately, the chemical properties of many potentially taxonomically informative alkaloids have yet to be fully characterized.

Geographic patterns and predator defense

There are taxonomic patterns in alkaloid distribution among oribatid mites, but there also may be broad geographic patterns, in that some data suggest a higher incidence of alkaloid-containing mites in the tropics. Most of the taxonomic diversity in the mite families Scheloribatidae and Mochlozetidae is in tropical latitudes, and Drymobatidae are found only in the tropics (distributions in Subías 2004; Subías et al. 2012). Currently, Galumnidae is the single example of a group that has an alkaloid-containing species in the tropics yet not, to our knowledge, in temperate regions. But there are hints of others. A mixed sample of Trhypochthoniidae and Hypochthoniidae from Isla Bastimentos, Panama, contained alkaloids (Saporito et al. 2007), but different genera in these families lacked alkaloids in the current study. Similarly, a mixed sample of Lohmanniidae, Hypochthoniidae, and Oppiidae from Tortuguero, Costa Rica contained alkaloids, but no member of Hypochthoniidae or Oppiidae in the current study had alkaloids, and we tested no Nearctic member of Lohmanniidae. The latter family lacks opisthonotal glands, but many tropical or subtropical species, including the *Meristacarus* sp. in the mixed sample of Saporito et al. (2007), are known to have numerous dermal glands (Norton et al. 1997), which are absent from the few temperate members of the family. If the single example whose ultrastructure has been studied (*Mixacarus* sp.; Alberti et al. 1997) is representative, these glands are innervated, which is not true of dermal glands in most oribatid mites.

If the geographic pattern proves real, the apparent convergence in alkaloid possession, and the apparent radiation of alkaloid-containing taxa in the tropics will need explanation. It may relate to a particularly strong chemical defense provided by these compounds. Many of the same alkaloids in poison frogs function as a defense against predators, microbes, and parasites (Saporito et al. 2007; Weldon et al. 2006; Mina et al. 2015) and alkaloids may serve a similar role in mites. But we lack the knowledge needed to link alkaloid defense with geography. Are alkaloid-susceptible predators and/or pathogens more of a problem in the tropics? Mochlozetidae and Drymobatidae, in particular, include medium to relatively very large species that inhabit living plants and other surfaces where they would be exposed to predators, including poison frogs (Norton and Behan-Pelletier 2009 and references therein). Do alkaloids and non-alkaloid chemical defenses target different predators and/or pathogens? Non-alkaloid opisthonotal gland secretions are effective deterrents to predatory stenine and scydmaenine staphylinid beetles (Raspotnig 2006; Heethoff et al. 2011; Heethoff and Raspotnig 2012), but they have not been tested against small vertebrate predators. And while alkaloids may serve as a chemical defense against some vertebrate and invertebrate predators, they seem ineffective against the scydmaenine beetle studied by Jałoszyński and Olszanowski (2015), which readily ate adults of three of the four offered species of Scheloribatidae, one of which was *Scheloribates laevigatus*. Additional research will be necessary to elucidate the function of alkaloids in mites and—if they indeed are defensive—to identify the predator(s) that they target.

Alkaloids common to mites and poison frogs

Many of the alkaloids identified in oribatid mites (including those in the present study) are identical to the arthropod-derived alkaloids also present in skin extracts of poison frogs. The absolute stereochemistry, however, remains to be determined for the mite alkaloids and then compared with the frog skin alkaloids. Tricyclics represent a large class of alkaloids that are well known from coccinelline beetles (e.g., precoccinelline; Daloze et al. 1994), and have therefore been the assumed dietary source of these alkaloids in poison frogs. Poison frogs consume beetles (e.g., Biavati et al. 2004), but mites tend to constitute a more significant prey item in most species (e.g., Simon and Toft 1991; Bonansea and Vaira 2007; Valderrama-Vernaza et al. 2009). Given the diversity of tricyclics present in mites (including precoccinelline **193C**), it is more likely that mites are the main dietary source for most (if not all) tricyclic alkaloids in poison frogs. Additional frog dietary studies that include better taxonomic resolution of arthropod prey items will allow for a more detailed understanding of the dietary origin for alkaloids in poison frogs, in particular those alkaloids that are present in different arthropods.

Conclusions

Oribatid mites undoubtedly contain a large diversity of alkaloids. Including the present study, more than 100 alkaloids from 11 previously described structural classes have been reported in them. Many of the alkaloids present in oribatids are also present in poison frogs from both the new and old worlds, suggesting that mites are a significant dietary source of alkaloid defenses in these frogs. Although mites appear to be a rich source, these alkaloids appear relatively restricted in their taxonomic distribution among oribatids—at this point, limited to members of a few families of poronotic Brachypylina, mostly in the superfamily Oripodoidea. Many poison frogs are considered dietary mite specialists (e.g., Simon and Toft 1991; Caldwell 1996; Vences et al. 1998), but it is not known if these frogs are specialists on particular species of mites, maybe targeting brachypylines as an alkaloid-rich food source, or if they are simply ‘mite’ specialists. Whether alkaloids or their profiles are informative taxonomic markers for mites, similar to other defensive chemicals, will require further study to determine. In many cases, further chemical characterization will be necessary to elucidate taxonomic patterns. Future studies should aim to include a wider taxonomic and geographic sampling of the Brachypylina, plus replication within and among populations to assess alkaloid variability. Finally, it will be important to determine the ultimate source (e.g., mite-produced vs. microsymbiont) and the anatomical location of alkaloids in oribatid mites.

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