Chemical and evolutionary analysis of the scent gland secretions of two species of Gonyleptes Kirby, 1819 (Arachnida: Opiliones: Laniatores)

Matheus Lima Silva Vieira (matheuslimasv@usp.br)
Universidade de São Paulo: Universidade de Sao Paulo
Marcos Ryotaro Hara
Universidade de Sao Paulo
Rodrigo Hirata Willemart
Universidade de Sao Paulo
Deborah Yara Alves Cursino dos Santos
Universidade de Sao Paulo
Miriam Sannomiya
Universidade de São Paulo: Universidade de Sao Paulo https://orcid.org/0000-0003-3306-9170

Research Article

Keywords: Chemotaxonomy, Chemical secretion, Benzoquinones, Gonyleptinae, Vinyl ketones

Posted Date: September 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2049113/v1

Version of Record: A version of this preprint was published at Chemoecology on February 14th, 2023. See the published version at https://doi.org/10.1007/s00049-023-00380-4.

Abstract

The subfamily Gonyleptinae is the second largest in Gonyleptidae, harboring over 100 species. Gonyleptinae is polyphyletic, nestled in the clade K92, and despite its richness, several species of that subfamily have not had their chemicals of the defensive secretions analyzed. Among these are *Gonyleptes curticornis* (Mello-Leitão, 1940) and *G. horridus* Kirby, 1819, the latter being particularly important because it is the type species of the genus, which in turn names the subfamily. *Gonyleptes horridus* is also used in many phylogenetic analyses, be it using morphological or molecular data. The chemical study of the secretions of these two species by GC-MS and NMR ^{1}H showed the presence of 1-(6-isopropyl-3,4-dihydro-2H-pyran-2-yl)-methylbutanone, 1-(6-isopropyl-3,4-dihydro-2H-pyran-2-yl) isobutanone and 4- methyl-1-hepten-3-one in both species. On the other hand, 4-methyl-1 hexen-3-one, benzaldehyde and 3-octanone were observed only in *G. curticornis*. Both species are Gonyleptinae and chemical mapping of the group corroborates that vinyl ketones are synapomorphy of K92 and that the compound 1-(6-isopropyl-3,4-dihydro-2H-pyran-2-yl) isobutanone is synapomorphic to *G. curticornis* and *G. horridus*, but homoplastic to the genus *Sodreana* Mello-Leitão, 1922. 1-(6-(1-methyl-propyl)3,4-dihydro-2H-pyran-2yl)2-methylbutanone and 4-methyl-1-hepten3-one is also synapomorphic to *G. curticornis* and *G. horridus*, but homoplastic in *Moreiranula saprophila*.

Introduction

With approximately 6900 described species (Kury et al. 2022), harvestmen (Opiliones) are the third largest group of Arachnida, after Acari (ticks and mites) and Araneae (spiders) (Selden 2007). Opiliones is taxonomically arranged in four monophyletic subfamilies: Cyphophthalmi, Eupnoi, Dyspnoi and Laniatores (Giribet and Kury 2007). Scent glands have been the focus of behavioral (Hara and Gnaspini 2003; Caetano and Machado 2013; Segovia et al. 2015) and chemical studies (Hara et al. 2005; Caetano and Machado 2013; Raspotnig et al. 2014, 2017).

The scent glands produce secretions primarily used for defense (Gnaspini and Hara 2007). It has been shown to repel some predators, but not others (Machado et al. 2005; Souza and Willemart 2011; Silva et al 2018). It can also be used as an alarm pheromone (Machado et al. 2002). Whereas scent gland secretions in harvestmen have traditionally been considered to be products of de novo synthesis, there is evidence for the unusual case of sequestration-derived gland constituents (Raspotnig et al. 2022). Defensive secretions can be emitted in different ways, forming a chemical shield around the body: (i) simply exhaling from the ozopore (with little or no emission of enteric liquid); (ii) by increasing the evaporating surface; and (iii) by directly rubbing the secretion-soaked leg on the aggressor, or emitting the secretion as a jet (Gnaspini and Hara 2007). These forms of emission of the defensive secretion are potential sources of characters for phylogenetic analyses. Caetano and Machado (2013), for example, proposed the non-emission of secretions in droplets or non-accumulation of them between the bases of legs I and II as a synapomorphy of the clade K92.

Currently, approximately 140 species from all suborders have had their chemical defenses studied, mainly from the suborders Laniatores and Eupnoi (approximately 50 species from each one). Since scent glands are synapomorphic for the order, this makes Opiliones suitable for phylogenetic chemosystematics, i.e. making evolutionary inferences (Hara et al. 2005; Caetano and Machado 2013; Wouters et al. 2013; Raspotnig 2012; Raspotnig et al. 2014, 2017) based on secretion from homologous glands (Raspotnig et al. 2017). In Laniatores, the infraorder Grassatores features phenols and benzoquinones, as well as acyclic compounds that superficially resemble some compounds found in Eupnoi (Hara et al. 2005; Raspotnig 2012). As more compounds have been identified in the defensive secretion, gradually more precise mapping, and inferences of the evolution of such trait became possible for the whole order (Raspotnig 2012; Caetano and Machado 2013; Wouters et al. 2013; Raspotnig et al. 2010, 2017). Despite recent efforts to identify the compounds in the defensive secretions in an increasing number of species, a few, taxonomic-wise key species have been overlooked so far. Gonyleptidae is one of the largest families of Grassatores, and it has been the focus of many phylogenetic analyses (Pinto-da-Rocha et al. 2014; Benavides et al. 2021). One of the most used species in analyses like these is *Gonyleptes horridus* Kirby, 1819 (for instance, Pinto-da-Rocha et al. 2014; Benedetti and Pinto-da-Rocha 2019; Ázara et al. 2020), because it names both the polyphyletic subfamily Gonyleptinae (second largest one; Pinto-da-Rocha et al. 2014) and the family. However, despite its pivotal taxonomic importance, the defensive secretion of *G. horridus* is still unknown. In this study, we identify the compounds in the defensive secretion of this key species. *Gonyleptes curticornis* (Mello-Leitão, 1940), a species phylogenetically related to *G. horridus*, and abundant in the Atlantic Rain Forest in northern coast of São Paulo State, is also studied here for comparison. Both species are included in the clade K92, a repeatedly retrieved clade in phylogenetic analyses, composed of the subfamilies Gonyleptinae, Sodreaninae, Caelopyginae, Hernandariinae and Progonyleptoidellinae (Kury 1992). Thus, we hope to provide further data that combined with those already available, hopefully will shed some light to solve the polyphyly of Gonyleptinae regarding chemical data, or at the very least, contribute to the understanding of scent gland secretion´s evolution in clade K92.

Material And Methods

Collection and maintenance

We collected four females and nine males (totalizing 13 adult specimens) of *Gonyleptes horridus* at Tijuca National Park, Rio de Janeiro city, Rio de Janeiro State, Brazil, from 2 to 4 of December of 2019. We collected 10 males and 10 females (totalizing 20 adult specimens) of *Gonyleptes curticornis* at the Cachoeira da Fazenda in the city of Ubatuba (State of São Paulo, Brazil) on February 20, 2018. We collected all the animals manually at night. Once in the laboratory, we maintained specimens in plastic containers with paper towel on the bottom and a wet cotton ball to provide humidity.

Collection of secretions

We collected the defensive secretion only from adult specimens in one–two days following the field collection. We used each specimen only once, and we deposited voucher specimens in the Museum of Zoology of the University of São Paulo, in Brazil (MZUSP). To collect secretions, we held the specimen between our fingers and pressed the abdomen dorso-ventrally We placed a microscopy glass slide on the dorsal scutum between legs III and IV and used it as a screen to retain the defensive secretion. We used micropipetttes (capillary glass tube of known volume) to collect the released defensive secretion. Its content was dissolved either in vials with 3.5 µL of methanol (HPLC grade - TEDIA) for CG-MS analysis or 1 µl of DMSO-d_6 for the ^{1}H NMR analysis, always separating the sexes. We kept vials for both type of analyses in the freezer at -10ºC.

Chemical analysis

We performed the CG-MS analysis at the Department of Botany, Institute of Biosciences of University of São Paulo. We injected the samples dissolved in 3.5 µL of methanol into a gas chromatograph coupled to a mass spectrometer (GC-MS), Agilent 6850 System, with a fused DB-5 silica capillary column (30 m x 0.25 mm x 0.25 µm).

We calculated the retention index for each compound detected in the output identified with 90% or more of similarity to spectra available in the NIST library. To perform this calculation, we injected a standard C_8 - C_{20} hydrocarbon column (Sigma-Aldrich), and used the following formula: IR = 100 *((tc - tn / tn + 1 - tn) + n) (Viegas and Bassoli 2007). Afterwards, we compared the calculated values with the retention index described in the literature, thus allowing the identification of the compounds.

We used spectral data such as NMR ^{1}H and mass spectra to elucidate the structure of the compounds. We obtained the NMR spectra on a Bruker Ascend™ III 600 14.1 T instrument operating at 600 MHz. We used $CDCl_3$ as solvent and tetramethyl silane (TMS) as internal reference. We recorded chemical shifts δ in ppm and coupling constants J in Hz.

Mapping the compounds

We mapped the compounds identified in the defensive secretions of *Gonyleptes* spp. based on modified characters based on Caetano and Machado (2013) (Table 3). We used the taxa names based on Kury et al. (2022), but we mapped the characters on a modified phylogeny of Pinto-da-Rocha et al. (2014) because the latter is more comprehensive than the former. Besides, the phylogeny proposed by Pinto-da-Rocha et al. (2014) used more taxa which defensive secretion compounds are known compared to that of Kury et al. (2022). We deleted taxa which defensive secretion´s were not studied except for *Ampheres leucopheus* (Gonyleptidae, Caelopyginae), to avoid an oversimplification of the phylogeny. Keeping those taxa which defensive secretion´s are unknown would hamper the analysis because of the large amount of missing data, thus causing more noise than desirable in the mapping. This paper is a first approach to infer the evolution of the identified compounds in the defensive secretion of *Gonyleptes* spp. in Gonyleptidae phylogeny rather than performing a total evidence type of analysis.

Results And Discussion

Specimens of *G. horridus* and *G. curticornis* emitted the defensive secretion in the form of a jet posteriorly (Gnaspini and Hara 2007). Eventually the emitted secretion spread over the carapace, where it evaporated.

According to the mass spectrometry data and retention index calculations, we identified five compounds in total in the defensive secretion of *G. curticornis* and *G. horridus*. We identified compounds 1, 2, 4 and 5 in the chromatogram of the defensive secretion of *G. curticornis* (Table 1, Fig. 1). On the other hand, we identified compounds 2, 3, 4 and 5 in that of *G. horridus* (Table 1, Fig. 2).

The compound with retention time of 8.78 min (compound 1) in the analysis of the defensive secretion of *G. curticornis* showed the following fragments of *m/z* 112 M+ (15); 97(12); 84(35); 83(12);69(12);58(28); 56(23); 55(100); 41(29) in the mass spectrum. Comparing these data with those in the literature (Wouters et al. 2013) and the calculation of the retention index, resulted in the identification of this compound as 4-methyl-1-hexen-3-one. In the ^{1}H NMR spectra, it was observed the presence of olefinic hydrogen signals of a terminal doublet by the signals of three doublets at δ 6.27 (J = 17.5, 1.4 Hz), δ 5.77 (J = 10.5, 1.4 Hz) and δ 6.44 (J = 17.5, 10.5 Hz). The doublet at δ 1.10 (3H) of J = 7.5 Hz and the multiplet at δ 2.89 (1H) indicate the presence of a methyl vicinal to a methine group. The triplet at δ 0.79 (3H) along with the signal at δ 1.59 (m, 2H) indicate the presence of a methyl coupling with the methyl hydrogens. These data set allowed to identify the compound 1 as 4-methyl-1-hexen-3-one (1) (Table 2, Fig. 1).

The compound with the retention peak at 12.47 min (compound 2) in the defensive secretion of *G. horridus* (Fig. 1) and *G. curticornis* (Fig. 2) showed the molecular ion with *m/z* 126 and fragments of *m/z* 111, 84 and 55 (Table 1). The fragment of *m/z* 55 corresponds to an α-carbonyl cleavage, while the fragment of *m/z* 84 results from McLafferty rearrangement (Silverstein et al. 2005). According to the ^{1}H NMR analysis, the presence of a terminal double bond is evidenced by the presence of three double doublets at δ 6.25 (J = 18.0 and 1.2 Hz), δ 5.85 (J = 10.5 and 1.4 Hz) and δ 6.47 (J = 18 and 10.4 Hz) close to a ketone. The methyl vicinal to the methine carbon can be evidenced by the signals of a multiplet at δ 2.83 (1H) and the doublet at δ 0.98 (J = 7.5 Hz, 3H). The triplet at δ 0.79 (J = 7.8 Hz, 3H) indicates a terminal methyl coupling the methyl hydrogens. The multiplet at δ 1.59 (2H) refers to the methylene at position 5 of the molecule, which is unprotected as a function of the carbonyl, while the signal at δ 1.34 (m, 2H) refers to the methylene hydrogens of carbon 6. All these data set confirmed this compound as 4-methyl-1-heptan-3-one 2 (Table 2).

The compound with the retention time of 14.11 min (compound 3) in the defensive secretion of *G. horridus* showed molecular ion of *m/z* 144, which corresponds to the molecular formula of C_9H_2O. Based on the molecular ion, it is possible to visualize the loss of 15 units, corresponding to one methyl in *m/z* 129. The fragment of *m/z* 45 indicates the presence of an alcohol, whereas the fragments of *m/z* 57 and 87, altogether with the remaining data, and the calculated retention index value, allowed us to identify this

compound as 7-methyl-2-octanol 3. So far, 7-methyl-2-octanol 3 has not been described for other opilionid defensive secretion.

The isomers 4 and 5 in the defensive secretion of both species showed the molecular ions of *m/z* 224 and its characteristic fragmentation pattern allowed the identification led us to compounds 1-(6-(1-methyl-propyl)3,4-dihydro-2H-pyran-2-yl)2-methylbutanone (6) and 1-(6-isopropyl-3,4-dihydro-2H-pyran-2-yl) isobutanone (7) (Wouters et al. 2013) (Table 1).

The defensive secretions of both species of *Gonyleptes* studied here are a mixture of compounds, as usual for Opiliones (Hara et al. 2005; Jones et al. 2009; Raspotnig et al. 2005, 2015; Rocha et al. 2011). The compounds in the defensive secretion of both *Gonyleptes* spp. are similar (Table 1). We mapped those compounds in the modified phylogeny of Pinto-da-Rocha et al. (2014) (Fig. 3). The list of characters and the character matrix are in Tables 3 and 4, respectively.

All the vinyl ketones identified in the defensive secretion of *Gonyleptes* spp. are restricted to the K92 clade, as expected (Kury 1992; Caetano and Machado 2013). This corroborates the current view that vinyl ketones are synapomorphic for K92, derived from alkyl phenols and benzoquinones in the remaining gonyleptids (Caetano and Machado 2013).

The mapping of the characters indicates that all the identified vinyl ketones are homoplastic. However, the combined presence of 4-methyl-1-heptan-3-one; 1-(6-(1-methyl-propyl)3,4-dihydro-2H-pyran-2-yl)2-methyl-butanone (4) and 1-(6-isopropyl-3,4-dihydro-2H-pyran-2-yl) isobutanone (5) are exclusive of *Gonyeleptes* spp., hence allowing to diagnose it. Likewise, Sodreaninae can be diagnosed by the combined presence of 5-methyl-1-hexen-3-one and 1-(6-isopropyl-3,4-dihydro-2H-pyran-2-yl) isobutanone (5). Those results hint that some compounds might be useful for diagnosing monophyletic groups in Gonyleptidae. However, our taxa sampling is reduced, and many other groups close to either *Gonyleptes* or Soderaninae cannot be diagnosable compound wise. Part of those issues might be because of the unsettled phylogenetic relationship among K92 taxa. There are many competing phylogenetic relationships of K92 (Pinto-da-Rocha et al. 2014; Kury et al. 2022) and they can differ quite considerably from one another. Those issues hamper attempts to make inferences of the evolution of the defensive secretion in Gonyleptidae. Soon, when those phylogenetic relationships become more stable, we might have a clearer picture of the usefulness of compounds to diagnose and infer taxonomic groupings in Gonyleptidae.

Because behavioral studies on the effectiveness of defensive chemicals and natural history are scarce in Opiliones, it is unfortunately not possible to comment on the use of such chemicals against predators or in other contexts. Finally, we report 7-methyl-2-octanol (3) that is present in *G. horridus* defensive secretion for the first time in Opiliones. It is autapomorphic for this species and the first alcohol in a gonyleptid secretion to our knowledge.

Table 3
List of modified chemical characters from Caetano and Machado (2013) of the clade K92 (Arachnida, Opiliones) used in the present analysis

Number	Chemical character present in the odoriferous secretion	State
01	2-ethyl-1,4-benzoquinone	[0] absent; [1] present
02	2-ethyl-3-methyl-1,4-benzoquinone	[0] absent; [1] present
03	2-ethyl-5-methyl-1,4-benzoquinone	[0] absent; [1] present
04	2-ethyl-3,5-dimethyl-1,4-benzoquinone	[0] absent; [1] present
05	5-ethyl-2-methyl-1,4-benzoquinone	[0] absent; [1] present
06	2-methyl-1,4-benzoquinone	[0] absent; [1] present
07	2,3-dimethyl-1,4-benzoquinone	[0] absent; [1] present
08	2,5-dimethyl-1,4-benzoquinone	[0] absent; [1] present
09	2,5-dimethyl-3-ethyl-1,4-benzoquinone	[0] absent; [1] present
10	2,3,5-trimethyl-1,4-benzoquinone	[0] absent; [1] present
11	hept-5-en-3-ona	[0] absent; [1] present
12	1-hexen-3-one	[0] absent; [1] present
13	1-hepten-3-one	[0] absent; [1] present
14	3-methylhexan-2-ona	[0] absent; [1] present
15	4-methylhexan-3-one	[0] absent; [1] present
16	4-methyl-1-hexen-3-one	[0] absent; [1] present
17	4-methyl-1-heptan-3-ona	[0] absent; [1] present
18	5-methyl-1-hexen-3-one	[0] absent; [1] present
19	7-metiloct-6-en-4-ona	[0] absent; [1] present
20	4,5- dimethylheptan-3-one	[0] absent; [1] present
21	1-(6-butyl-3,4-di-hydro-2H-pyran-2-yl) pentanone	[0] absent; [1] present
22	1-(6-(1-methyl-propyl)3,4-dihydro-2H-pyran-2-yl)2-methyl-butanone	[0] absent; [1] present
23	1-(6-(propyl)3,4-dihydro-2H-pyran-2-yl)butanone	[0] absent; [1] present
24	1-(6-propyl-3,4-dihydro-2H-pyran-2-yl)2-methyl-butanone	[0] absent; [1] present
25	1-(6-propyl)3,4-dihydro-2H-pyran-2-yl) isobutanone	[0] absent; [1] present
26	1-(6-isopropyl-3,4-dihydro-2H-pyran-2-yl) isobutanone	[0] absent; [1] present

Number	Chemical character present in the odoriferous secretion	State
27	1-(6-butyl-3,4-di-hydro-2H-pyran-2-yl) pentanone	[0] absent; [1] present
28	2-methyl-5-ethylphenol	[0] absent; [1] present
29	2,3-dimethylphenol	[0] absent; [1] present
30	2,5-dimethylphenol	[0] absent; [1] present
31	2,3,4-trimethylphenol	[0] absent; [1] present
32	2,3,6-trimethylphenol	[0] absent; [1] present
33	7-methyl-2-octanol	[0] absent; [1] present

Declarations

Acknowledgements

We thank to Guilherme Ferreira Pagoti, Norton Felipe Dos Santos Silva and Ludson Neves de Ázara for assisting in collecting harvestmen.

Author contributions

All authors contributed to the conceptualization, formal analyses, investigation, methodology, supervision and writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

The grant awarded to M.L.S.V supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant 2019/13645-6).

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Ázara LN, Hara MR, Ferreira RL (2020) Cladistic analysis of the Brazilian troglobitic harvestmen genus *Iandumoema* Pinto-da-Rocha (Opiliones: Gonyleptidae) with the description of three new species: a brief exercise over the use of troglomorphisms in cladistic analysis. Invertebrate Systematics 34:474–503
2. Benavides LR, Pinto-da-Rocha R, Giribet G (2021) The phylogeny and evolution of the flashiest of the armored harvestmen (Arachnida: Opiliones). Systematic Biology 70:648-659

3. Benedetti AR, Pinto-da-Rocha R (2019) Description of two new species of Progonyleptoidellus (Opiliones: Gonyleptidae), with a cladistic analysis of the genus, an overview of relationships in the K92 group, and taxonomic notes on *Deltaspidium*. Zootaxa 4691:461–490
4. Caetano DS, Machado G (2013) The ecological tale of Gonyleptidae (Arachnida, Opiliones) evolution: phylogeny of a Neotropical lineage of armoured harvestmen using ecological, behavioural and chemical characters. Cladistics 29:589–609
5. Eisner T, Alsop D, Meinwald J (1978) Secretions of Opilionids, Whip Scorpions and Pseudoscorpions. In: Bettini S (eds) Arthropod Venoms. Springer, Berlin, Heidelberg, pp 87-99
6. Giribet G, Kury AB (2007) Phylogeny and Biogeography. In: Pinto-da-Rocha R, Machado G, Giribet G (eds) Harvestmen—the biology of Opiliones. Harvard University Press, Cambridge, pp 62-87
7. Gnaspini P, Hara MR (2007) Defense mechanisms. In: Pinto-da-Rocha R, Machado G, Giribet G (eds) Harvestmen—the biology of Opiliones. Harvard University Press, Cambridge, pp 374–399
8. Hara MR, Cavalheiro AJ, Gnaspini P, Santos DYAC (2005) A comparative analysis of the chemical nature of defensive secretions of Gonyleptidae (Arachnida: Opiliones: Laniatores). Biochem Syst Ecol 33:1210–1225
9. Jones TH, Shear WA, Giribet G (2009) The chemical defenses of a stylocellid (Arachnida,Opiliones, Stylocellidae) from Sulawesi with comparisons to other Cyphophthalmi. J Arachnol 37:147–150
10. Kury AB (1992) The false Cranainae of the Brazilian Atlantic Forest (Opiliones Gonyleptidae). Tropi Zoo 5:279–291
11. Kury AB, Mendes AC, Cardoso L, Kury MS, Granado Ade A, Giribet G, Cruz-López JA, Longhorn SJ, Medrano M, Kury IS & Souza-Kury MA (2022) World Catalogue of Opiliones. WCO-Lite version 2.5.0. Online at: https://wcolite.com/
12. Kury AB, Bernabé TN, de Ázara LN, Araújo D, Benedetti AR (2022) Phylogeny of the clade K92 (Opiliones, Laniatores, Gonyleptidae) with description of a new subfamily and discussion on the evolution of caelopygine facies and sexual dimorphism. Zoologischer Anzeiger 298:70-122
13. Machado G, Bonato V, Oliveira PS (2002) Alarm communication: a new function for the scent-gland secretion in harvestmen (Arachnida: Opiliones). Naturwissenschaften 89:357–360
14. Machado G, Carrera PC, Pomini AM, Marsaioli AJ (2005) Chemical Defense in Harvestmen (Arachnida, Opiliones): Do Benzoquinone Secretions Deter Invertebrate and Vertebrate Predators? J Chem Ecol 31:2519–2539
15. Pinto-da-Rocha R, Bragagnolo C, Marques FPL, Antunes Junior M (2014) Phylogeny of harvestmen family Gonyleptidae inferred from a multilocus approach (Arachnida: Opiliones). Cladistics 30:519–539
16. Raspotnig G (2012) Scent gland chemistry and chemosystematics in harvestmen. Biol Serbica 34:5–18
17. Raspotnig G, Fauler G, Leis M, Leis HJ (2005) Chemical profles of scent gland secretions in the cyphophthalmid opilionid harvestmen, *Siro duricorius* and *S. exilis*. J Chem Ecol 31:1353–1368

18. Raspotnig G, Leutgeb V, Schaider M, Komposch C (2010) Naphthoquinones and Anthraquinones from Scent Glands of a Dyspnoid Harvestman, *Paranemastoma quadripunctatum*. J Chem Ecol 36:158–162
19. Raspotnig G, Schaider M, Stabentheiner E, Leis HJ, Karaman I (2014) On the enigmatic scent glands of dyspnoan harvestmen (Arachnida, Opiliones): first evidence for the production of volatile secretions. Chemoecology 24:43–55
20. Raspotnig G, Bodner M, Schäfer S, Koblmüller S, Schönhofer A, Karaman I (2015) Chemosystematics in the Opiliones (Arachnida): a comment on the evolutionary history of alkylphenols and benzoquinones in the scent gland secretions of Laniatores. Cladistics 31:202–209
21. Raspotnig G, Schaider M, Föttinger P, Schönhofer A (2017) A model for phylogenetic chemosystematics: evolutionary history of quinones in the scent gland secretions of harvestmen. Front Ecol Evol 5:139
22. Raspotnig G, Bodner M, Blesl J, Viquez C (2022) The scent gland chemistry of Gagrellinae (Opiliones, Sclerosomatidae): evidence for sequestration of myrmicacin in a species of *Prionostemma*. Chemoecology 1-8
23. Rocha, DFO, Hamilton K, Gonçalves CCS, Machado G, Marsaioli AJ (2011) 6-Alkyl-3,4-dihydro-2 *H*-pyrans: Chemical Secretion Compounds in Neotropical Harvestmen. J Nat Prod 74:658–663
24. Segovia JM, Hara MR, Pagoti GF, Sannomiya M, Santos DY, Willemart RH (2015) The scent glands of the neotropical harvestman *Discocyrtus pectnifemur*: morphology, behavior and chemistry. J Chem Ecol 41:716-723
25. Selden PA (2017) Arachnids. In Reference Module in Life Sciences, Elsevier. 2017. Published online. doi:10.1016/B978-0-12-809633-8.02243-3
26. Silva MS, Willemart RH, Carbayo F (2018) Sticky flatworms (Platyhelminthes) kill armored harvestmen (Arachnida,Opiliones) but are not immune to the prey´s weapons. J Zool 306(2):88-94
27. Silverstein RM, Webster FX, Kiemle DJ (2005) Spectrometric Identification of Organic Compounds. John Wiley & Sons, New York
28. Souza EDS, Willemart RH (2011) Harvest-ironman: heavy armature, and not its defensive secretions, protects a harvestman against a spider. Anim Behav 81:127-133
29. Viegas MC, Bassoli DG (2007) Utilização do índice de retenção linear para caracterização de compostos voláteis em café solúvel utilizando GC-MS e coluna HP-Innowax. Química Nova 30:2031–2034
30. Wouters FC, Rocha DFO, Goncalves CCS, Machado G, Marsaioli AJ (2013) Additional vinyl ketones and their pyranyl ketones in gonyleptid harvestmen (Arachnida: Opiliones) suggest these metabolites are widespread in this family. J Nat Prod 76:1559–1564

Tables

Table 1, 2 and 4 are available in the Supplementary Files section.

Figures

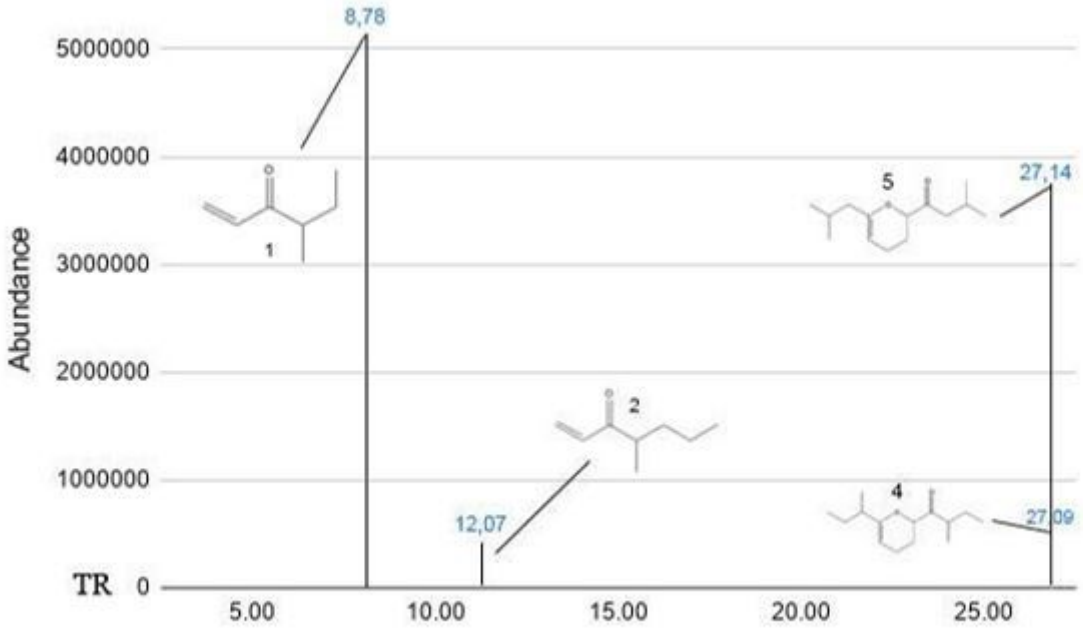

Figure 1

Chemical profile of scent gland secretions of *G. curticornis* (Arachnida, Opiliones)

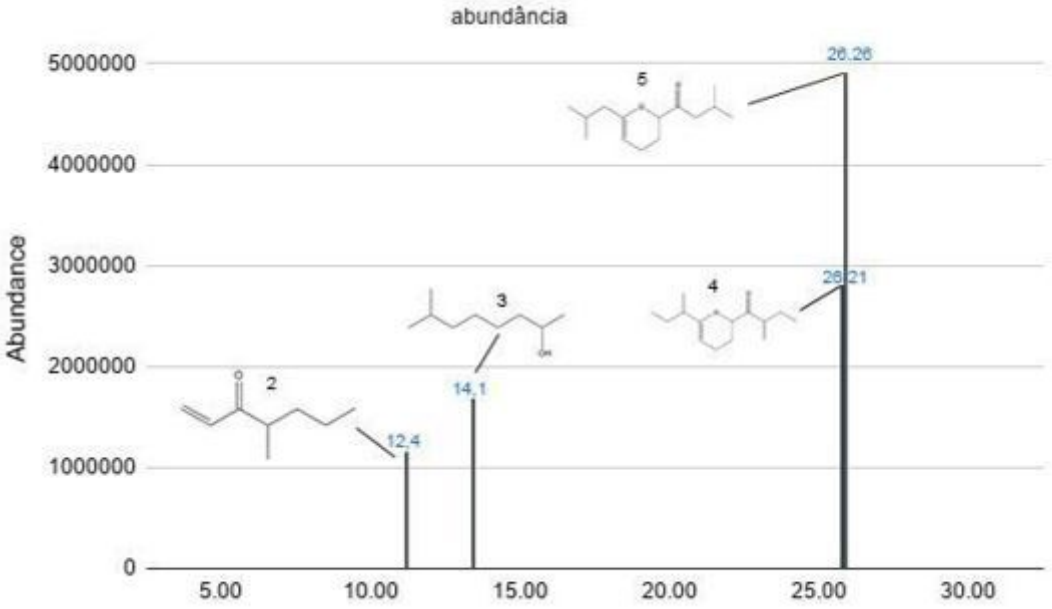

Figure 2

Chemical profile of scent gland secretions of *G. horridus* (Arachnida, Opiliones)

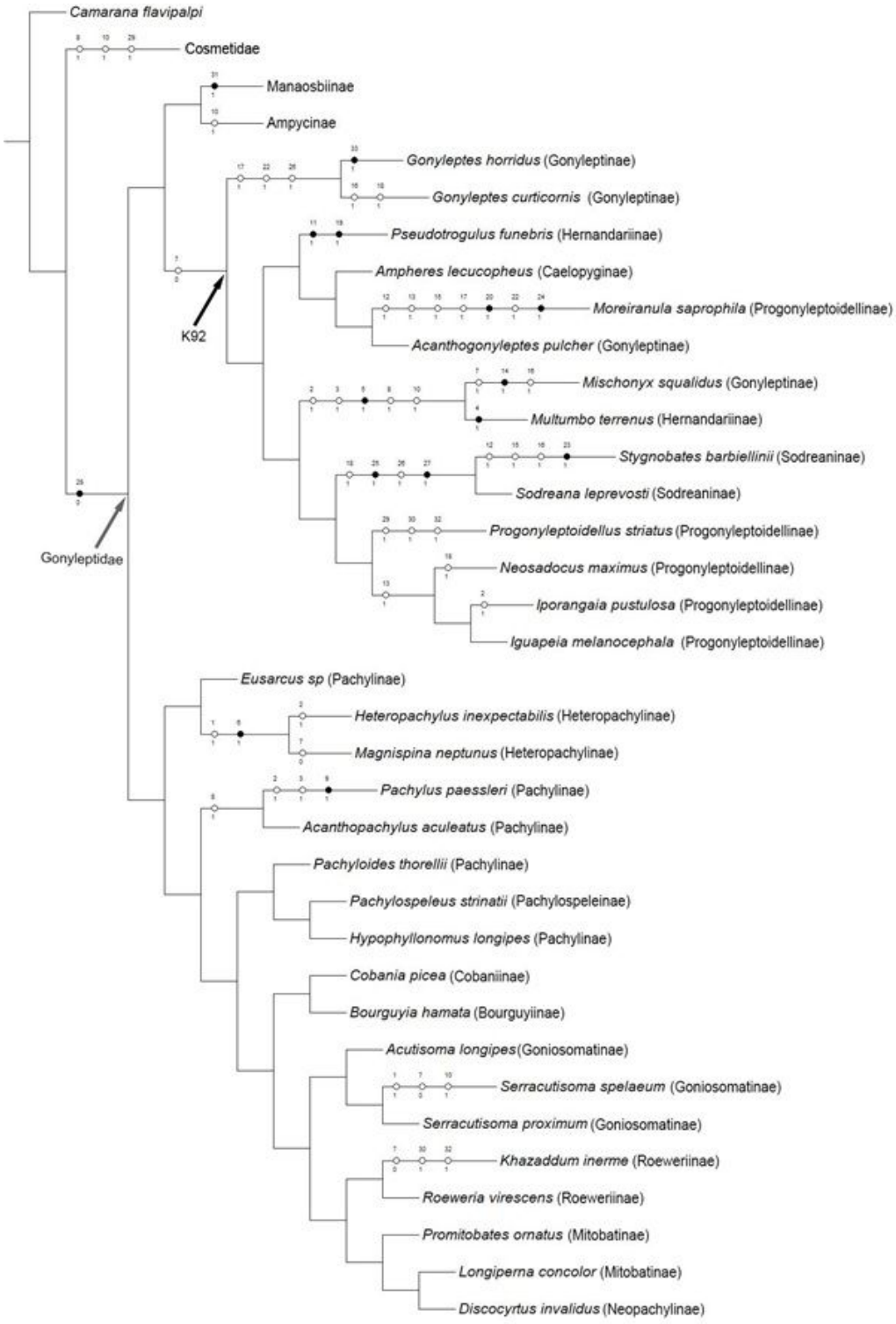

Figure 3

Chemical characters mapped in hypothesized K92 species (Arachnida, Opiliones) relationship, modified from Pinto-da-Rocha et al. (2014). Black circles indicate single transformations, and white circles indicate homoplastic transformations. Numbers above the circle indicate the character number, and the numbers below indicate their state

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table124.docx