



Chemical defense of a troglobiont millipede, *Tetracion jonesi* Hoffman (Diplopoda, Callipodida, Abacionidae)

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Abstract

Tetracion jonesi Hoffman, 1956 is a troglomorphic cave-limited abacionid millipede found in caves in the southern Cumberland Plateau of Alabama and Tennessee, USA. Specimens collected in a cave in Franklin Co., Tennessee, were live-extracted with methanol. Gas chromatography/mass spectroscopy of the extract revealed *p*-cresol as the sole component of the millipede's chemical defense. Three other callipodidan species have been studied and also produce *p*-cresol.

Key words

p-cresol, troglobiosis, Polydesmida, Alabama, Tennessee, caves

Introduction

Tetracion jonesi Hoffman, 1956 (Fig. 1) has been the subject of ongoing genetic research by SFL and KSZ. This abacionid millipede, found exclusively in caves in the Cumberland Plateau of Alabama and Tennessee, USA, is one of the three troglobiotic and troglomorphic species of *Tetracion*, a genus endemic to the Cumberland Plateau (Hoffman 1956). As with all callipodidan millipedes so far studied, *T. jonesi* carries obvious paired lateral repugnatorial glands on all diplosegments from the



Figure 1. Male *Tetracion jonesi* Hoffman, showing ozopores (**p**) starting on the sixth trunk segment.

sixth to the penultimate. The glands open through pores (*p*, Fig. 1) located on a dorsolateral metazonital crest, which is significantly broader than the others to accommodate the pore. The chemical identity of the product of these glands was suspected, on the basis of previous work on other species belonging to the families Abacionidae and Schizopetalidae, to contain *p*-cresol. Both Peck (1989) and Shear et al. (2007) have cited unpublished data by M. Blum identifying *p*-cresol as a component of the secretion. Below we confirm by our own analysis that *p*-cresol is the sole volatile compound identifiable in whole-body methanol extracts of *Tetracion jonesi* individuals.

Methods and results

Specimens of *T. jonesi* were collected in Buckets of Blood Cave (Tennessee Cave Survey #FR61), Franklin Co., Tennessee, and dropped alive into vials containing 1 ml or less of methanol. To avoid contamination, the glass vials had caps lined with Teflon. The specimens, now preserved in 70% ethanol, have been placed as vouchers in the collection of the Virginia Museum of Natural History, Martinsville, Virginia, USA. The methanol extract of the specimens was analyzed by ISM and THJ using a Shimadzu QP-5000 GC/MS equipped with an RTX-5, 30 m, 0.25-mm i.d. column. Analysis of the methanol extract of *T. jonesi* specimens revealed the presence of a single volatile component, EIMS m/z 108 [Mp] (80), 107 (100), 90 (13), 79 (25), and 77 (38), matching those reported for the isomeric cresols. Direct comparison with authentic samples of *o*-cresol, *m*-cresol, and *p*-cresol showed that the natural material had a retention time and mass spectrum identical to those of *p*-cresol, and a retention time different from those of *o*-cresol and *m*-cresol.

Discussion

Previous reports of the presence of *p*-cresol in the defensive secretions of abacionid millipeds include that on *Abacion magnum* (Loomis, 1943), by Eisner et al. (1963) and on *Delophon georgianum* Chamberlin 1943, by Shear et al. (2007). Ćurčić et al. (2009) found *p*-cresol in the secretion of the European schizopetalid *Apfelbeckia insculpta* (L. Koch, 1867). *Abacion magnum*, *D. georgianum* and *T. jonesi* are native to the Appalachian Mountains of the United States, and the family Abacionidae is endemic to North America. While callipodidan millipeds belonging to other families occur in western North America and Mexico, the Mediterranean region of Europe, mountainous Central Asia, eastern China and Southeast Asia (Shear et al. 2003), our knowledge of the defensive secretions of members of the order is limited to the three above-named Appalachian abacionid species, and the European schizopetalid *A. insculpta*, all of which produce *p*-cresol.

P-cresol and *o*-cresol have also been found in the defensive secretions of millipeds belonging to other orders. Noguchi et al. (1997) reported that *p*-cresol was the major component of the secretion of *Nedyopus patrioticus patrioticus* Attems, 1898 (Polydesmida, Paradoxosomatidae). Taira and Arakaki (2002) found *p*-cresol as a major component in the secretion of the polydesmidan paradoxosomatid *Chamberlinius hualiensis* Wang, 1956, and Taira et al. (2003) discovered it in the secretion of the ubiquitous synanthropic paradoxosomatid *Oxidus gracilis* (C.L. Koch, 1847), although as a minor component. All three of these polydesmidans also possess the typical cyanogenic defensive system of the order Polydesmida. Shear et al. (2007) studied a polydesmidan nearctodesmid, *Leonardemus injucundus* Shelley & Shear, 2007, and found that *p*-cresol was the sole defensive chemical in this species, which lacked the

cyanogenic system. Kluge and Eisner (1971) reported *o*-cresol from the julidan parajulid *Oriulus venustus* (Wood, 1864) (originally reported as *O. delus* Chamberlin, 1940; according to Shelley [2002], *delus* is a synonym of *venustus*). In this species, *o*-cresol occurs along with the benzoquinones typical of julidans; the record should be checked because phenolics have not been found in other julidans, including other parajulids (Eisner et al. 1978).

Thus *p*-cresol occurs only in the two orders Polydesmida and Callipodida, and Shear et al. (2007) suggested that this supported a hypothesis of relationship between the two orders, given that the chemistry of repugnatorial secretions seems to be characteristic of orders, and can provide evidence of relationship. For example, benzoquinones characterize the secretions of the orders Julida, Spirobolida and Spirostreptida, usually united by additional characters in phylogenies as Juliformia (Eisner et al. 1978; Sierwald & Bond 2007; Shear et al. 2007).

The presence of chemical defenses has been reported previously for another troglobiotic arthropod, the harvestman (Opiliones) *Chinquepellobunus madlae* (Goodnight & Goodnight) (Shear et al. 2010). SFL and KSZ have not observed predation on *T. jonesi*, but potential predators with which the millipeds share habitat include cave salamanders (*Eurycea lucifuga*), slimy salamanders (*Plethodon glutinosus*), Alleghany woodrats (*Neotoma magister*) and three species of spiders, *Nesticus barri*, *Meta ovalis*, and *Liocranoides archeri*. Peck and Richardson (1976) found that *E. lucifuga* in captivity would not take *T. jonesi* even if the millipeds were the only food offered the salamanders for as long as eight days, and never found *T. jonesi* in the guts of wild-collected specimens. They attributed this to the defensive secretion of the millipeds. Peck (1974), in a study of the diet of *E. lucifuga* showed that the salamander regularly fed on *Pseudotremia* millipeds, which do not have chemical defenses. In a paper on the cave fauna of Alabama, he reported as a personal communication from M. Blum that the defensive secretion contained *p*-cresol, which we have now verified.

The vertebrate predators could possibly take *T. jonesi* at any size, but the spiders would likely be limited to individuals smaller than themselves, and hence immature. However, *N. barri* and *M. ovalis* weave webs with adhesive silk and could conceivably handle larger prey. In particular, *M. ovalis* has been observed preying on the cave millipid *Causeyella youngsteadtorum* Shear in Arkansas (Slay et al. 2009), but *C. youngsteadtorum*, like all chordeumatidans including species of the above-mentioned *Pseudotremia*, lacks chemical defenses. Duffey et al. (1977) carried out experiments demonstrating the effectiveness of *p*-cresol as a repellent of ants, a conclusion also reported by Eisner et al. (1978), but we can only speculate about the action of *p*-cresol on the animals listed above. Humans find *p*-cresol very irritating, and this may suggest the same reaction for other vertebrates.

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