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Analysis of lipid and fatty acid composition of three species of scorpions with relation to different organs

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ABSTRACT

Within arthropods most of the information related to the type of mobilization and storage of lipids is found in insects and crustaceans. Literature is scarce with relation to scorpions. This order is a remarkably important model of the biochemistry, since it is characterized as an animal with very primitive traits which have varied minimally through time. In the present study we characterize and compare lipids and fatty acids present in three species of scorpion: *Timogenes elegans*, *Timogenes dorbignyi*, and *Brachistosternus ferrugineus*, focusing the study on the main organs/tissues involved in the dynamics of lipids. As found in the fat body of insects, hepatopancreas of crustaceans and midgut diverticula of spiders, the hepatopancreas of the three species studied here turned out to be the organ of lipid storage (great quantity of triacylglycerides). With relation to the hemolymph and muscles, a great quantity of phospholipids was observed, which is possibly involved in membrane formation. It is important to highlight that unlike what happens in insects, in scorpions the main circulating energetic lipid is the triacylglyceride. This lipid is found in greater proportion in the hepatopancreas of females, surely for reproduction. The fatty acid of the different organs/tissues analyzed remained constant in the three species studied with certain characteristic patterns, thus observing saturated and unsaturated most abundant fatty acids of C16 and C18. Finally, it could be observed that in *T. elegans*, *T. dorbignyi and B. ferrugineus* scorpions, there is a lack of 20:4 that generates a special condition within fatty acids of arthropods.

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1. Introduction

Lipid molecules are important in different organisms because they represent an effective energy storage, are main components of biological membranes and also have signaling function of remarkable importance. Within a given lipid class, the incorporation of a variety of fatty acids differing in chain length and unsaturation gives rise to a great variability of different lipid species. How this immense lipid variability is deployed *in vivo*, and to what extent it can be altered in response to exogenous factors (such as nutrition) without jeopardizing the lipid homeostasis at the organism level remains unclear (Shevchenko and Simons, 2010). Nowadays much effort is being made to improve the traditional methodology employed in the analysis of the lipids of insects, as for example the work recently performed by Tzompa-Sosa et al. (2014) in which the influence of the different methods of lipid extraction (aqueous versus organic solvent) in the lipid composition of 5 species of insects is analyzed, or that performed by Carvalho et al. (2012) where mass spectrometry is employed for building lipidomes of insects related to the different stages of development.

Although it is known that lipids are stored in an organ named fat body in insects (Gilbert and Chino, 1974; Arrese and Soulages, 2010), and hepatopancreas in crustaceans (O'Connor, J.M., Gilbert, L.I., 1968; García et al., 2004), little is known about Class Arachnida with regard to the organ of lipid storage and the nature of stored lipids and their distribution to their respective places of utilization (Laino et al., 2009, 2011a).

Class Arachnida presents a great diversity of species distributed in 11 orders, in which the information related to the lipids is in some cases disperse, in others scarce, and in a great majority nonexistent.

It is unquestionable that Araneae is the Arachnid order containing the most information related to this subject. The first study performed where the uptake, storage, and mobilization of lipids were described, was recently reported in the spider *Polybetes pythagoricus* (Holmberg, 1875) (Laino et al., 2009); subsequently, in the same species, lipid transference was observed between midgut-diverticula and lipoproteins (Laino et al., 2011a). Finally, an advance on the knowledge of the role of different lipids in the embryo development of *Schizocosa malitiosa*

Abbreviations: C, Cholesterol; CE, Cholesteryl esters; CerPCho, Sphyngomielin; FFA, Free fatty acids; HC, Hydrocarbons; MUFA, Monounsaturated fatty acid; PL, Phospholipids; PtdCho, Phosphatidylcholine; PtdEtn, Phosphatidylethanolamine; PtdSer, Phosphatidylserine; PUFA, Polyunsaturated fatty acid; SFA, Saturated fatty acid; TAG, Triacylglycerols; UFA, Unsaturated fatty acid.

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(Tullgren, 1905) and *P. pythagoricus* was also recently achieved (Laino et al., 2011b, 2013).

Scorpions (Class Arachnida, Order Scorpiones) are chelicerates with an ancient history that have changed little since the Silurian (450 million years) (Millot and Vachon, 1968) and are considered as one of the oldest known terrestrial lineages (Zouari et al., 2006). Therefore, they have been chosen as models to perform biochemical studies as those carried out by Louati et al. (2011) and Zouari et al. (2005) where digestive enzymes were studied, thus allowing for the interpretation of the digestive process in animals with primitive traits.

Many studies have described scorpion morphology, digestive system (Goyffon and Martoja, 1983; Warburg et al., 2002; Gefen, 2008; Warburg, 2012) and their venoms (Almaaytah and Albalas, 2014; Harrison et al., 2014), but very few have studied the lipids and fatty acids of these organisms. The only study related to lipids and fatty acids of TAG and PL in scorpions has been performed in the hemolymph and the hepatopancreas of the buthid *Leiurus quinquestriatus* (Ehrenberg, 1828) through the use of thin layer and gas–liquid chromatography (El-Salhy et al., 1981). Fatty acids of the body of other buthid: *Centruroides vittatus* (Say, 1821) were analyzed comparatively with other terrestrial arthropods (Uscian and Stanley-Samuelson, 1994). Lipoproteins of the scorpionid *Pandinus imperator* (Koch, 1842) were studied by Schenk et al. (2009), in which the authors described an unusual quantity of phosphatidylserine (PtdSer) in the lipoprotein which is possibly related to the production or the efficiency of the venom.

In the present study we characterize and compare lipids and fatty acids present in three neotropical species of the scorpion Family Bothriuridae, *Timogenes elegans* (Mello-Leitão, 1931), *Timogenes dorbignyi* (Guérin Méneville, 1843), and *Brachistosternus ferrugineus* (Thorell, 1876). We focused the study on the main organs/tissues involved in the dynamics of lipids (hepatopancreas, hemolymph, muscle and gonad). In addition, the possible differences between males and females of *Brachistoternus ferrugineus* were analyzed.

This is the first time this kind of study is performed in Bothriuridae. This family presents a Gondwanic distribution, but most of its genera and species occur in the neotropics (Kovařík and Ojanguren-Affilastro, 2013). The three studied species in this contribution where chosen because they are well known species from the Chaco phytogeographic province as defined by Cabrera (1976), an area that possess the highest scorpion diversity of Argentina, in which we are performing several ethological and ecological studies (Nime et al., 2013, 2014). These three species are ground dwellers that construct their burrows in open soil, are active during spring and summer, spending the rest of the year in hibernation inside of their burrows. *Brachistosternus ferrugineus* is the smallest and most abundant of them; with an average size of 4 cm, is also the most abundant scorpion in meridional Chacoan environments (Nime et al., 2013, 2014). *Timogenes* species are less abundant and bigger; *T. dorbignyi* is a medium sized species (CA. 6 cm), and *T. elegans* is by far the biggest of them (and also of the family), reaching 12 cm (Kovarik and Ojanguren-Affilastro, 2013). All these scorpion species are active and generalist predators of the epigean arthropod fauna, their preys being limited mostly by their size and capability of manipulation.

The main objectives of this work were to characterize and compare lipids and fatty acids present in three species of scorpion: *Timogenes elegans*, *Timogenes dorbignyi*, and *Brachistosternus ferrugineus*, focusing the study on the main organs/tissues involved in the dynamics of lipids. In one species, *Brachistosternus ferrugineus*, the abundance and availability of materials allowed us to search for differences between sexes.

2. Materials and methods

2.1. Animal and organ/tissue isolation

A total of 90 adult scorpions of *Brachistosternus ferrugineus* (45 females and 45 males), 20 adults of *Timogenes elegans* (males) and 10 adults of *Timogenes dorbignyi* (males) were analyzed. The individuals were collected using UV lamps at night in March 2014 in Reserva Natural Formosa and neighboring areas, in Formosa Province, Argentina [24°17′00″S61°48′00″W], after which they were moved to the laboratory and kept in glass cages at 25 °C with 16/8 h light/dark cycle for two days unfed, before being sacrificed.

Animals were anesthetized with ethyl ether previous to treatment, and finally sacrificed. Hemolymph was obtained by severing their legs, and the scorpions were centrifuged in a tube at low speed, using the same technique as Cunningham et al., 1994 for spiders. A ventral incision was made in the mesosomal tegument hepatopancreas and gonads were carefully dissected out, similar to that performed in spiders with modifications (Laino et al., 2009). Legs, chelas, and telson were also dissected. Legs and chelas together were considered as muscle.

2.2. Lipid characterization

Lipids from hepatopancreas, hemolymph, muscle, and whole body were extracted following the procedure by Folch et al. (1957). Quantitative determination of lipid classes was performed by thin layer chromatography coupled to a flame ionization detector in a latroscan apparatus model TH-10 (Iatron Laboratories, Tokyo, Japan), after separation on Chromarods type S-III (Ackman et al., 1990; García et al., 2002a). Lipid classes were quantified with monoacylglycerol as an internal standard. The total lipids were determined by gravimetry (Cunningham and Pollero, 1996). Due to the scarcity of *T. dorbignyi* hemolymph, we failed to perform lipid characterization, however we were able to characterize fatty acids (see below).

Lipids were separated by a sequence of three different solvent systems. Firstly, by the use of hexane/benzene (70:30 v/v) chromarods were dried and partially scanned to determine apolar lipids. In order to determine neutral lipids, the development in benzene/chloroform/formic acid (70:25:1 v/v) was performed. Finally, the determination of polar lipids was achieved by the development in chloroform/acetone/methanol/acetic acid/ water (30:40:10:10:5 v/v). Quantification was performed with calibration curves of standards run under the same conditions. Reference lipids used as standards were hydrocarbons of C24, cholesteryl oleate, tripalmitin, oleic acid, cholesterol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and sphingomyelin. Three determinations of three independent pools were performed.

2.3. Fatty acid characterization

Fatty acid methyl esters from total lipid samples were prepared with BF_3–MeHO according to the method by Morrison and Smith (1992). The analysis was performed by gas–liquid chromatography in a HP-6890 capillary chromatograph (Hewlett Packard, Palo Alto, CA) on an Omegawax 250 30 m × 0.25 mm fused silica column with a 0.25 μm phase (Supelco, Bellefonte, CA). The column temperature was programmed for a linear increase of 3 °C per min from 175 to 230 °C. Peaks were identified by comparison with retention times of Supelco 37 component fatty acid methyl esters mix (Supelco). For the case of the different tissues, three pools independent from one another were analyzed.

2.4. Statistical analyses

Statistical comparison of quantity of lipids and percentage of different lipid classes of whole body, hepatopancreas, muscle and hemolymph was done using a one-way ANOVA after checking for normality and homogeneity of variances. Lipids and fatty acid composition are shown as mean ± standard deviation (SD). Significant differences ($p < 0.05$ or $p < 0.001$) were compared using the Tukey's post hoc test and Student's *t*-test. Data were analyzed using GraphPad InStat 3.01 (GraphPad Software, San Diego California USA).

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