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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.

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).

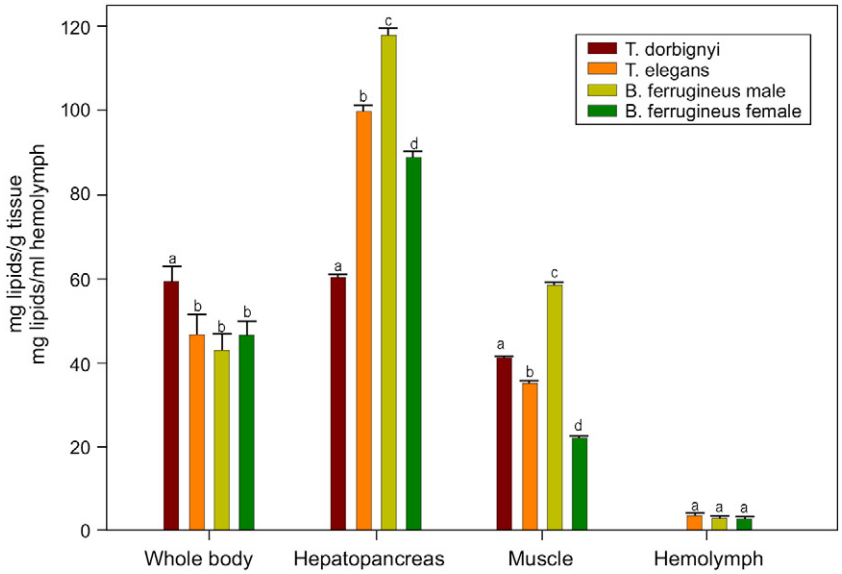

Fig. 1. Quantity of lipids present in the whole body, hepatopancreas, muscle and hemolymph of males of *Timogenes elegans*, *T. dorbignyi*, and *Brachistosternus ferrugineus* males and females. Values are expressed as total lipid mg/wet weight. Values express the means ± SD of three pools of tissue analyzed separately. Different letters (a, b, c, d) above the bar indicate statistically significant differences in quantity of lipids between the four different models of study analyzing whole body, hepatopancreas, muscle and hemolymph determined by Tukey's post hoc test, same letter above are not significantly different; level of significance $p < 0.05$.

3. Results

After quantifying the lipids of the different samples, it could be determined that the quantity of lipid remained constant in the whole body of the four models analyzed, and ranged between 42.9 mg/g (wet weight) for males of *B. ferrugineus* and 59 mg/g in males of *T. dorbignyi*. In the hepatopancreas the quantity of lipids was higher, with values ranging from 62 to 118 mg/g. In hepatopancreas of females *B. ferrugineus* the value was significantly lower than in hepatopancreas of males (88.9 to 118 mg/g). In muscles the quantity varied between 22 mg/g in *B. ferrugineus* females to 58.4 mg/g in *B. ferrugineus* males. Finally, the quantity of hemolymphatic lipids was about 3 mg/ml (Fig. 1).

Principal lipids in whole body and hepatopancreas are the triacylglycerides (TAG), around 42%, both contain phosphatidylcholine (PtdCho) with 20% and 38% respectively. Also, 18% of hydrocarbons (HC) were found in whole body. Hemolymphatic lipids are represented by 57% of PtdCho. A great percentage of phosphatidylserine (PtdSer) was found (20%) (Fig. 2).

In both whole body and muscle of *T. dorbignyi* the predominant lipid was PtdCho (around 60%), whereas it TAG was in the hepatopancreas (75%). (Fig. 3).

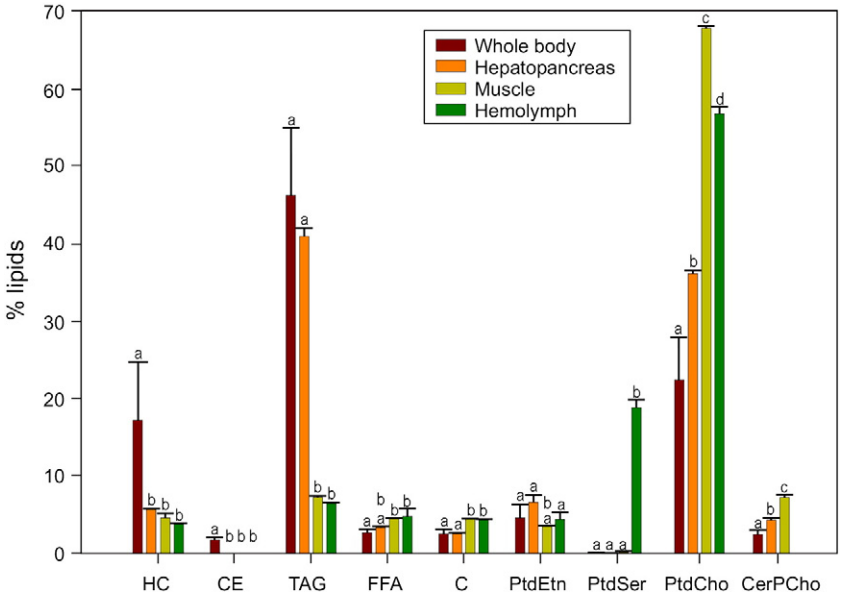

Fig. 2. Percentage of different lipid classes in whole body, hepatopancreas, muscle and hemolymph of male of *Timogenes elegans*. Percentages are analyzed for each lipid in particular. Values are the means ± SD of three pools, analyzed separately through chromatography coupled to a flame ionization detector. Different letters (a, b, c, d) above the bar indicate statistically significant differences between whole body, hepatopancreas, muscle and hemolymph for each class of lipid determined by Tukey's post hoc test, same letter above are not significantly different level of significance $p < 0.05$. HC (hydrocarbons), CE (cholesteryl esters), TAG (triacylglycerides), FFA (free fatty acids), C (cholesterol), PtdEtn (phosphatidylethanolamine), PtdSer (phosphatidylserine), PtdCho (phosphatidylcholine), and CerPCho (sphingomyelin).

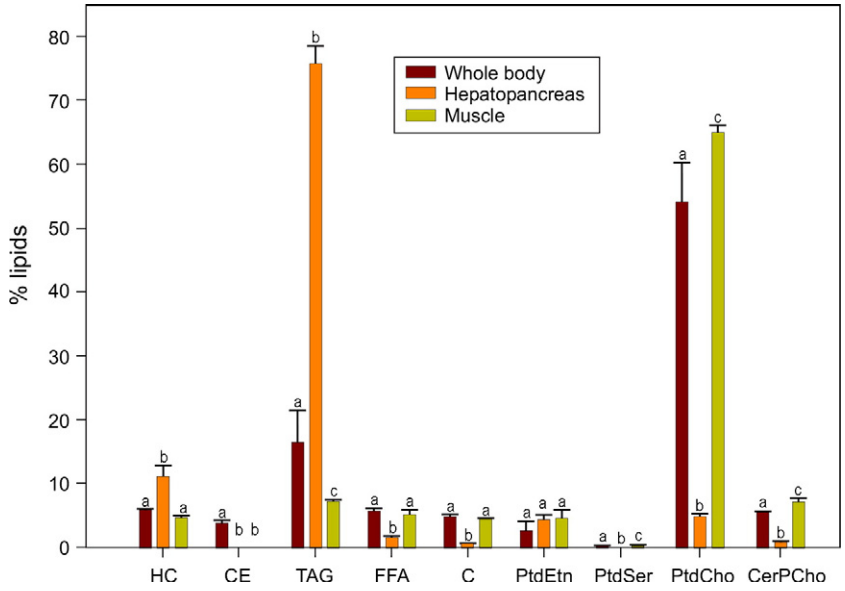

Fig. 3. Percentage of different lipid classes in whole body, hepatopancreas and muscle of male of *Timogenes dorbignyi*. Percentages are analyzed for each lipid in particular. Values are the means ± SD of three pools, analyzed separately mediante chromatography coupled to a flame ionization detector. Statistical analyses and abbreviations of lipids are as in Fig. 2.

Most abundant lipids in males of *B. ferrrugineus* were as follows: in whole body TAG and PtdCho (40%), in hepatopancreas TAG (82%), in muscle PtdCho (68%) and in the hemolymph PtdCho (52%) and PtdSer and HC (around 15%) (Fig. 4).

In Fig. 5 the lipid composition of females of *B. ferrugineus* is shown. In whole body and hepatopancreas, TAG turned out to be the main component with around 60%, followed by PtdCho with 30% and 14% respectively. In muscle and hemolymph PtdCho was found to make up around 70%, TAG 10%. Again, a high percentage of PtdSer (15%) was found in hemolymph.

With respect to *T. elegans* males the most abundant fatty acids were 18:1 in hepatopancreas, gonads, whole body and hemolymph (50%, 46%, 40%, and 28% respectively); in muscle they were 18:2, 18:0, 18:1 with around 26% and in telson they were 18:0 with 36% (Table 1).

With relation to *T. dorbignyi* males, the most abundant fatty acids of whole body, hepatopancreas, gonad and hemolymph were 18:1 with 35% for the first two and 43% for the last two ones), in muscle 18:2 (31%) and in telson it is 48% of 18:0 (Table 2).

Table 3 represents the percentages of fatty acids of *B. ferrugineus* males. In whole body, muscle and telson the predominant tones were 18:1 and 18:2 (between 32% and 40%), in hepatopancreas 18:0 with 36%, in gonads 18:1 with 45% and in the hemolymph 16:0 and 18:1 (around 30%). Finally, the most abundant fatty acids found in females of *B. ferrugineus* were 43% and 37% of 18:0 in the whole body and hepatopancreas, respectively. In muscle the values found were around 32% 18:2 and 18:0. In both telson and gonads the most abundant FA were 18:1 with 31% and 42%, respectively, .Meanwhile in hemolymph was found to have 42% of 16:0 (Table 4). From the comparative analysis of

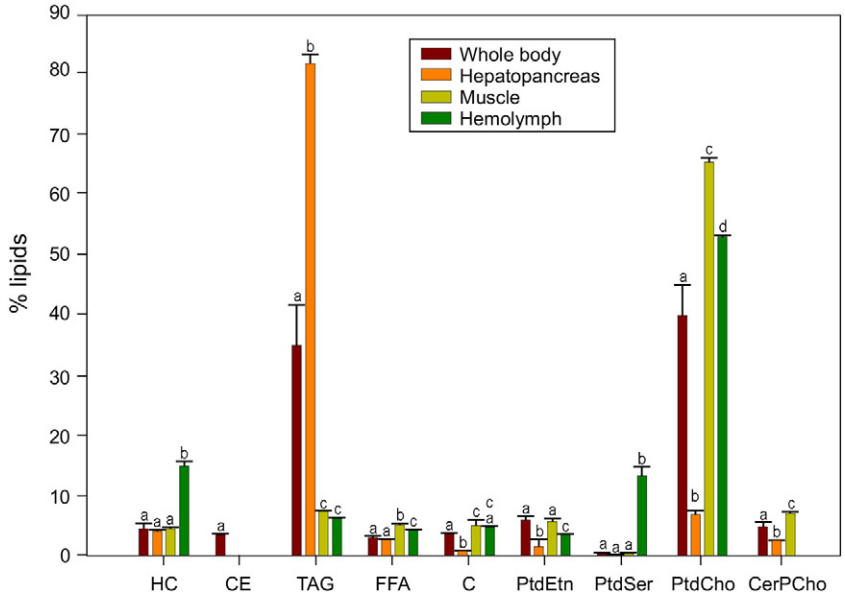

Fig. 4. Percentage of different lipid classes in whole body, hepatopancreas, muscle and hemolymph of males of *Brachistosternus ferrugineus*. Percentages are analyzed for each lipid in particular. Values are the means ± SD of three pools, analyzed separately through chromatography coupled to a flame ionization detector. Statistical analyses and abbreviations of lipids are as in Fig. 2.

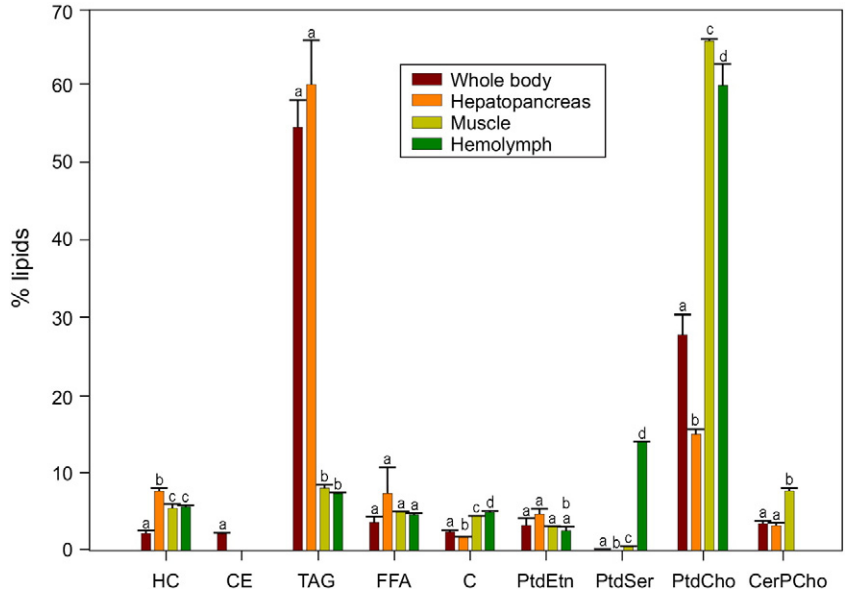

Fig. 5. Percentage of different lipid classes in whole body, hepatopancreas, muscle and hemolymph of *Brachistosternus ferrugineus* females. Percentages are analyzed for each lipid in particular. Values are the means ± SD of three pools, analyzed separately through chromatography coupled to a flame ionization detector. Statistical analyses and abbreviations of lipids are as in Fig. 2.

fatty acids of the main saponifiable lipids, differences between TAG of hepatopancreas and phospholipids (PL) of the same tissue were observed in *T. elegans* (Table 5). Here, an increase of 16:0, 18:1, and a decrease in 18:2 was observed. This pattern is repeated in *T. dorbignyi* and *B. ferrugineus* (Table 6, 7, and 8). For the case of the muscle of *T. elegans*, *T. dorbignyi*, and males of *B. ferrugineus*, there is a great difference at the level of the sum of saturated where in TAG is between 78% and 87% and in PL only from 35% to 48%. This difference is given by an increase in 16:0 and 18:0, and a decrease in mono unsaturated and poly unsaturated (Table 5, 6 and 7). In females of *B. ferrugineus* an increase in TAG of muscle is also observed, but with a pattern of fatty acids different from the aforementioned (Table 8).

4. Discussion

As expected, in the three species studied here, it was observed that the organ with greater lipid content was the hepatopancreas (60 to 118 mg/g). It is possibly the most important storage organ of scorpions, not only for the quantity of lipids stored in it, but also for the presence of TAG as majority lipid (see further). This organ is described in other scorpions because of its great importance for nutrient storage and metabolism (Ramamurthi et al., 1975; Warburg et al., 2002) and has likewise high lipid concentrations (Raghavaiah and Ramamurthi, 1977). It is analogous to the M-diverticula of spiders (Laino et al., 2009), the insect's fat body (Seifert and Rosenberg, 1977; Warburg et al., 2002) and the hepatopancreas of crustaceans (O'Connor, J.M., Gilbert, L.I., 1968; Al-Mohanna and Nott, 1986; García et al., 2002b).

In the three species of scorpions studied, a majority content of TAG was observed in the hepatopancreas ranging from 42% to 82%, coinciding with that found in the hepatopancreas of the scorpion *Leiurus quinquestriatus* (El-Salhy et al., 1981). In the M-diverticula of the spider *Polybetes pythagoricus* TAG constitutes the main lipid storage form, representing about 74% of the total lipid (Laino et al., 2009). In insects they represent around 70%–90% in the fat body (Bailey et al., 1975; Sobotnik et al., 2006) and in crustaceans' hepatopancreas around 68% (González Baró and Pollero, 1988).

Table 1
Fatty acid composition of whole body, hepatopancreas, muscle, telson, gonad and hemolymph of males of *Timogenes elegans*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Different letters (a, b, c, d and e) indicate statistically significant differences between whole body, hepatopancreas, muscle, telson, gonad and hemolymph for each percentage of fatty acid determined by Tukey's post hoc test; same letters indicate non-significantly different levels of significance, $p < 0.05$. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

T. elegans male	Whole body	Hepatopancreas	Muscle	Telson	Gonad	Hemolymph
14:0	1.11 ± 1^{a}	1.59 ± 1^{a}	0.50 ± 0.4^{a}	1.11 ± 0.5^{a}	1.06 ± 1^{a}	1.80 ± 0.7^{a}
15:0	0.40 ± 0.3^{a}	0.47 ± 0.4^{a}	0.20 ± 0.2^{a}	0.41 ± 0.3^{a}	0.24 ± 0.3^{a}	2.60 ± 0.1^{b}
16:0	15.90 ± 6^{a}	20.11 ± 3^{a}	11.20 ± 4^{ab}	14.23 ± 3^{ab}	15.33 ± 4^{a}	25.90 ± 0.3^{ac}
16:1	0.90 ± 0.6^{a}	1.33 ± 1.1^{a}	0.90 ± 0.7^{a}	2.14 ± 0.3^{a}	0.54 ± 0.4^{a}	4.40 ± 0.1^{b}
17:0	0.90 ± 0.7^{a}	0.80 ± 0.9^{a}	0.60 ± 0.5^{a}	1.03 ± 0.7^{a}	0.21 ± 0.3^{a}	ND
17:1	1.70 ± 1.2^{a}	0.55 ± 0.5^{a}	0.90 ± 0.8^{a}	ND	0.19 ± 0.2^{a}	ND
18:0	16.50 ± 3^{a}	8.38 ± 2^{a}	26.50 ± 5^{b}	36.42 ± 5^{c}	14.52 ± 3^{a}	14.80 ± 1.4^{a}
18:1	40.80 ± 7^{a}	50.74 ± 7.5^{a}	24.20 ± 4^{b}	18.92 ± 2^{b}	46.6 ± 6.5^{a}	28.40 ± 1^{ab}
18:2	19.40 ± 3^{a}	13.83 ± 1^{a}	30.80 ± 4.1^{b}	20.42 ± 2.1^{ac}	16.5 ± 1.5^{a}	11.00 ± 0.8^{ad}
18:3	0.50 ± 0.4^{a}	1.85 ± 1^{a}	1.60 ± 1.2^{a}	0.46 ± 0.3^{a}	0.85 ± 0.7^{a}	0.20 ± 1.5^{a}
20:0	0.90 ± 0.9^{a}	ND	0.30 ± 0.4^{a}	0.14 ± 0.3^{a}	ND	4.20 ± 0.5^{b}
20:3	ND	ND	1.50 ± 1.3^{a}	2.80 ± 0.7^{a}	3.19 ± 0.5^{a}	ND
22:0	0.60 ± 0.5^{a}	0.26 ± 0.2^{a}	0.30 ± 0.2^{a}	1.70 ± 1.1^{a}	0.26 ± 0.4^{a}	6.30 ± 0.8^{b}
∑ SFAs	36.31 ± 12.4^{a}	31.62 ± 7.9^{a}	39.60 ± 10.7^{a}	55.0 ± 10.9^{a}	31.6 ± 8.7^{a}	55.60 ± 3.8^{a}
∑ MUFAs	43.40 ± 8.8^{a}	52.62 ± 9.1^{a}	26.00 ± 5.6^{ab}	21.06 ± 2.3^{b}	47.4 ± 7.1^{a}	32.80 ± 1.1^{ab}
∑ PUFAs	19.90 ± 3.4^{a}	15.68 ± 2^{ab}	33.90 ± 6.6^{ac}	23.68 ± 9.4^{a}	20.5 ± 2.7^{a}	11.20 ± 2.3^{ab}
∑ UFAs	63.30 ± 12.2^{a}	68.30 ± 11.1^{a}	59.90 ± 12.2^{a}	44.7 ± 11.7^{a}	67.9 ± 9.8^{a}	44.00 ± 3.4^{a}

Table 2
Fatty acid composition of whole body, hepatopancreas, muscle, telson, gonad and hemolymph of males of *Timogenes dorbignyi*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Different letters (a, b, c, d and e) indicate statistically significant differences between whole body, hepatopancreas, muscle, telson, gonad and hemolymph for each percentage of fatty acid determined by Tukey's post hoc test; same letters indicate non-significantly different levels of significance, $p < 0.05$. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

T. dorbygnyi male	Whole body	Hepatopancreas	Muscle	Telson	Gonad	Hemolymph
14:0	0.90 ± 0.7^{a}	0.90 ± 0.1^{a}	0.43 ± 0.2^{a}	0.66 ± 0.4^{a}	1.49 ± 0.2^{a}	4.10 ± 1.8^{b}
15:0	0.40 ± 0.5^{a}	0.50 ± 0.3^{a}	0.22 ± 0.1^{a}	0.23 ± 0.1^{a}	0.60 ± 0.3^{a}	1.50 ± 1^{a}
16:0	17.30 ± 2.1^{a}	21.97 ± 1^{ac}	11.13 ± 1.5^{ad}	22.60 ± 2.5^{ac}	20.48 ± 2.1^{ac}	25.00 ± 1.5^{bc}
16:1	2.10 ± 0.9^{a}	1.40 ± 0.5^{a}	0.43 ± 0.2^{a}	0.80 ± 0.4^{a}	0.88 ± 0.6^{a}	4.30 ± 1^{b}
17:0	0.20 ± 0.1^{a}	1.30 ± 0.2^{b}	0.60 ± 0.3^{ac}	0.50 ± 0.2^{a}	1.06 ± 0.2^{bc}	ND
17:1	0.76 ± 0.6^{a}	ND	1.24 ± 0.6^{a}	ND	ND	ND
18:0	23.40 ± 3.1^{a}	12.04 ± 1.5^{b}	26.61 ± 4.4^{a}	48.00 ± 6.1^{c}	13.08 ± 2.7^{b}	10.40 ± 1^{b}
18:1	32.16 ± 4.7^{a}	38.00 ± 5.1^{ab}	24.17 ± 2.4^{ac}	14.43 ± 1.5^{c}	45 ± 1^{b}	42.00 ± 1.5^{ab}
18:2	20.18 ± 2.9^{a}	17.45 ± 3^{ab}	31.56 ± 5.7^{d}	11.63 ± 2^{bc}	13.82 ± 2^{ac}	9.40 ± 0.5^{b}
18:3	0.60 ± 0.3^{a}	5.23 ± 0.6^{b}	0.77 ± 0.3^{a}	0.35 ± 0.2^{a}	1.02 ± 0.3^{a}	ND
20:0	0.70 ± 0.1^{a}	0.30 ± 0.2^{b}	0.29 ± 0.1^{b}	0.15 ± 0.1^{b}	ND	ND
20:3	0.70 ± 0.2^{a}	0.60 ± 0.1^{a}	1.40 ± 2.1^{a}	0.50 ± 0.3^{a}	2.15 ± 0.3^{a}	1.60 ± 1
22:0	0.32 ± 0.3^{a}	0.29 ± 0.1^{a}	0.83 ± 0.4^{a}	0.17 ± 0.1^{a}	0.52 ± 0.4^{ab}	1.10 ± 0.5^{ac}
∑ SFAs	43.22 ± 6.9^{a}	37.29 ± 3.5^{a}	40.11 ± 7^{a}	72.31 ± 9.5^{b}	37.24 ± 5.7^{a}	42.1 ± 5.8^{a}
∑ MUFAs	35.02 ± 6.2^{a}	39.40 ± 5.6^{a}	25.84 ± 3.2^{ac}	15.23 ± 1.9^{bc}	45.88 ± 1.6^{a}	46.3 ± 2.5^{ad}
∑ PUFAs	21.48 ± 3.2^{a}	23.27 ± 3.6^{ab}	33.73 ± 8.1^{b}	12.48 ± 2.5^{a}	16.99 ± 2.6^{a}	11 ± 1.5^{a}
∑ UFAs	56.50 ± 9.4^{a}	62.67 ± 9.2^{a}	59.57 ± 11.3^{a}	27.71 ± 4.4^{b}	62.87 ± 4.2^{a}	57.3 ± 4^{a}

With regard to the composition of fatty acids of the hepatopancreas of the three species studied, the chromatographic patterns match those described for fatty acids of PL and TAG of the hepatopancreas of the scorpions *L. quinquestriatus* and *Centruroides vittatus*, where the main fatty acids were 18:1, 18:2, 18:0 and 16:0 (El-Salhy et al., 1981; Uscian and Stanley-Samuelson, 1994). This same pattern was also observed in whole body preparations and M- diverticula of four labidognath spider species (Uscian and Stanley-Samuelson, 1994; Laino et al., 2009), as well as in one millipede species (Order Polydesmida) (Uscian and Stanley-Samuelson, 1994). It is known that in insects, lipids have a relatively high quantity of unsaturated fatty acids C18 (Uscian and Stanley-Samuelson, 1994). Also, in general, the profile found in the present work matches with that recently reported for five species of insects (Tzompa-Sosa et al., 2014). In our case it is important to highlight that although in the three species studied here, 18 carbons were the most abundant fatty acids found, there is a difference in the percentage of 18:0 between the two species of *T. elegans* and males and females of *B. ferrugineus*, the difference being more than double in this last species. This difference is possibly due to the alimentary habit of the different species as determined by Schartau and Leidescher (1983) when they compared fatty acids of tarantula *Aphonopelma hentzi* (Girard 1852) with those of the scorpion *Leiurus quinquestriatus*.

The information related to lipids present in muscle in Class Arachnida is very scarce. The best representation perhaps stems from studies performed on spiders, where the transference of radiolabelled lipids from hemolymphatic proteins to muscle was observed (Laino et al., 2009). With relation to the quantity of lipids present in the muscle of the three species studied, values ranging from 25 to 58 mg/g were observed, which correspond to 50% of lipids found in the hepatopancreas; this seems to be reasonable because it is not a storage tissue. PL are the majority, surely due to their importance in membrane formation. It is worth mentioning that the so-called "muscle" of the present work is represented by legs and chelas. The analysis of those appendages was performed separately since they possess different muscle/cuticle relationship. A similar composition of lipids and fatty acids was observed for both appendages (results not shown).

Majority fatty acids found in the muscle of *T. elegans*, *T. dorbignyi*, and *B. ferrugineus* are 18:2, 18:1, and 18:0. This coincides with the only information reported by scorpions, where Uscian and collaborators analyzed fatty acids of TAG and PL of the scorpion *Centruroides vittatus*, though unfortunately legs, head and thorax were analyzed as a whole (Uscian and Stanley-Samuelson, 1994). From these experimental results we can conclude that the lipid composition of fatty acids seems to be quite stable among the different species of scorpions (even

Table 3
Fatty acid composition of whole body, hepatopancreas, muscle, telson, gonad and hemolymph of males of *Brachistosternus ferrugineus*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Different letters (a, b, c, d and e) indicate statistically significant differences between whole body, hepatopancreas, muscle, telson, gonad and hemolymph for each percentage of fatty acid determined by Tukey's post hoc test; same letters indicate non-significantly different levels of significance, $p < 0.05$. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

B. ferrugineus Male	Whole body	Hepatopancreas	Muscle	Telson	Gonad	Hemolymph
14:0	0.61 ± 0.5^{a}	0.90 ± 0.7^{a}	0.90 ± 0.7^{a}	1.48 ± 0.7^{a}	1.00 ± 0.6^{a}	4.46 ± 1.4^{b}
15:0	0.39 ± 0.3^{a}	0.30 ± 0.1^{a}	0.40 ± 0.3^{a}	ND	ND	3.96 ± 1.3^{b}
16:0	12.81 ± 3.1^{a}	20.80 ± 4.2^{ac}	11.60 ± 3.7^{ad}	19.85 ± 2.7^{a}	18.40 ± 2.2^{a}	32.38 ± 2.8^{b}
16:1	0.59 ± 0.4^{a}	1.80 ± 0.9^{a}	0.90 ± 0.7^{a}	ND	1.20 ± 0.7^{a}	7.00 ± 2^{b}
17:0	0.92 ± 0.7^{a}	0.60 ± 0.5^{a}	ND	ND	0.70 ± 0.3^{a}	ND
17:1	1.07 ± 0.5^{a}	ND	0.80 ± 0.5^{a}	ND	ND	1.15 ± 0.2^{a}
18:0	14.79 ± 4.1^{a}	36.40 ± 7.2^{b}	12.40 ± 3.2^{a}	13.12 ± 2.9^{a}	11.50 ± 2.1^{a}	11.47 ± 0.9^{a}
18:1	34.43 ± 4.5^{a}	25.05 ± 3.5^{ab}	34.90 ± 4.7^{a}	40.99 ± 5.2^{ac}	45.50 ± 5.1^{ac}	27.68 ± 2.8^{a}
18:2	31.16 ± 6.8^{a}	11.38 ± 2.3^{b}	33.99 ± 4.8^{a}	22.40 ± 3.7^{ac}	15.28 ± 2.1^{bc}	7.88 ± 0.2^{b}
18:3	1.40 ± 0.8^{a}	0.87 ± 0.5^{a}	1.00 ± 0.2^{a}	ND	1.70 ± 0.3^{a}	0.72 ± 0.1^{a}
20:0	0.72 ± 0.7^{a}	0.57 ± 0.4^{a}	0.56 ± 0.9^{a}	ND	1.90 ± 0.2^{a}	1.77 ± 0.1^{a}
20:3	0.42 ± 0.2^{a}	0.53 ± 0.3^{ab}	1.90 ± 0.3^{bd}	1.36 ± 0.5^{be}	0.50 ± 0.4^{ab}	ND
22:0	0.64 ± 0.3^{a}	0.36 ± 0.4^{a}	0.27 ± 0.1^{a}	0.80 ± 0.5^{a}	1.96 ± 0.7^{b}	1.00 ± 0.4^{a}
∑ SFAs	30.88 ± 9.7^{a}	59.93 ± 13.2^{b}	26.13 ± 8.9^{ac}	35.25 ± 6.8^{a}	35.46 ± 6.1^{a}	55.03 ± 6.8^{ad}
∑ MUFAs	36.10 ± 5.4^{a}	26.85 ± 4.4^{ab}	36.60 ± 5.9^{a}	40.99 ± 5.2^{a}	46.70 ± 5.8^{ac}	35.82 ± 4.8^{a}
∑ PUFAs	32.98 ± 7.6^{a}	12.78 ± 3.1^{b}	36.89 ± 5.3^{ac}	23.76 ± 4.2^{ad}	17.48 ± 2.8^{b}	8.60 ± 0.3^{b}
∑ UFAs	69.08 ± 13^{a}	39.63 ± 7.5^{bc}	73.5 ± 11.2^{a}	64.75 ± 9.4^{ac}	64.18 ± 8.6^{ac}	44.42 ± 5.1^{ac}

Table 4
Fatty acid composition of whole body, hepatopancreas, muscle, telson, gonad and hemolymph of females of *Brachistosternus ferrugineus*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Different letters (a, b, c, d and e) indicate statistically significant differences between whole body, hepatopancreas, muscle, telson, gonad and hemolymph for each percentage of fatty acid determined by Tukey's post hoc test; same letters indicate non-significantly different levels of significance, $p < 0.05$. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

B. ferrugineus Female	Whole body	Hepatopancreas	Muscle	Telson	Gonad	Hemolymph
14:0	0.90 ± 0.7^{a}	0.90 ± 0.4^{a}	0.52 ± 0.1^{a}	1.19 ± 0.4^{a}	2.00 ± 0.9^{a}	2.40 ± 2^{a}
15:0	0.39 ± 0.3^{a}	0.8 ± 0.4^{a}	0.19 ± 0.1^{a}	0.30 ± 0.2^{a}	0.90 ± 0.7^{ac}	2.90 ± 2^{bc}
16:0	18.73 ± 3.2^{a}	20.51 ± 3.1^{a}	13.1 ± 1.9^{a}	20.38 ± 2.7^{a}	21.8 ± 3.1^{a}	41.70 ± 10^{a}
16:1	1.01 ± 0.1^{a}	1.80 ± 0.4^{a}	0.99 ± 0.7^{a}	2.15 ± 1.3^{a}	1.50 ± 0.7^{a}	ND
17:0	0.83 ± 0.7^{a}	0.92 ± 0.1^{a}	0.77 ± 0.5^{a}	0.82 ± 0.4^{a}	ND	1.20 ± 0.4^{a}
17:1	0.10 ± 0.1^{a}	0.14 ± 0.1^{a}	ND	ND	1.30 ± 0.2^{b}	ND
18:0	43.85 ± 5.2^{a}	37.70 ± 4.7^{a}	34.1 ± 3.9^{a}	19.14 ± 3.7^{b}	10.9 ± 1.9^{b}	17.90 ± 9^{b}
18:1	10.74 ± 2.1^{a}	17.60 ± 2.1^{a}	9.57 ± 2.2^{a}	31.14 ± 4.9^{b}	42.8 ± 5.1^{c}	9.10 ± 2.1^{a}
18:2	18.89 ± 2.1^{a}	14.39 ± 2.1^{a}	35.6 ± 4.1^{b}	19.36 ± 2.1^{a}	13.9 ± 1.5^{a}	17.60 ± 0.2^{a}
18:3	3.00 ± 0.7^{a}	4.76 ± 0.7^{b}	2.04 ± 0.7^{ab}	0.73 ± 0.4^{bc}	2.15 ± 0.7^{ab}	0.70 ± 0.4^{a}
20:0	0.82 ± 0.4^{a}	0.58 ± 0.3^{a}	0.11 ± 0.1^{ab}	0.91 ± 0.7^{a}	1.5 ± 0.3^{ac}	1.40 ± 0.7^{a}
20:3	ND	0.36 ± 0.2^{a}	2.20 ± 0.7^{a}	2.32 ± 2.1^{a}	0.69 ± 0.2^{a}	2.70 ± 1^{a}
22:0	0.38 ± 0.2^{a}	0.26 ± 0.2^{a}	0.71 ± 0.5^{ab}	1.53 ± 0.7^{bc}	0.44 ± 0.4^{ab}	2.43 ± 0.1^{c}
∑ SFAs	65.91 ± 10.7^{a}	$62,47 \pm 9.6^{a}$	49.5 ± 7.1^{a}	44.29 ± 8.8^{a}	37.5 ± 7.3^{a}	69.93 ± 24.2^{a}
∑ MUFAs	11.85 ± 2.3^{a}	19.54 ± 2.6^{a}	10.5 ± 2.9^{a}	33.30 ± 6.2^{b}	45.6 ± 6^{c}	9.10 ± 2.1^{a}
∑ PUFAs	21.89 ± 2.8^{a}	19.50 ± 3^{a}	39.8 ± 5.5^{b}	22.41 ± 4.6^{a}	16.7 ± 2.4^{a}	21.00 ± 1.6^{a}
∑ UFAs	33.74 ± 5.1^{a}	39.05 ± 5.6^{ab}	50.4 ± 8.4^{ac}	55.71 ± 10.8^{bc}	62.3 ± 8.4^{c}	30.10 ± 3.7^{ac}

comparing different families), since in the muscle a higher percentage of 18:2 was found with relation to all the tissues/organs studied. There are several works in insects determining that membranes can be affected by the type of diet of the different species (Carvalho et al., 2012), similar to what can occur in the hepatopancreas, but at muscle level. The results based on analyzing muscle in the present work do not show a significant difference among species. Based on the results of the chromatography analysis of hemolymphatic lipids, PL were found to be the majority lipids in the circulation of *T. elegans*, as well as in males and females of *B. ferrugineus*, possibly transporting these PL to the different organs to cover the structural requirements of plasmatic membranes. This finding is in agreement with the one described in the scorpion *Leiurus quinquestriatus* (El-Salhy et al., 1981), in spiders (Cunningham et al., 2007) and crustaceans (Lee and Puppione, 1978; García et al., 2004). On the other hand, it does not coincide with the results described by Schenk and collaborators in the scorpion *Pandinus imperator*, where only 30% of PL were found in hemolymphatic lipoproteins (Schenk et al., 2009).

As mentioned previously, PL are structural lipids per excellence, containing a PL in particular which is characterized by a highly negative charge and pI 1.2 (Abramson et al., 1964): phosphatidylserine. This PL is found at an unusually high concentration in the scorpion *Pandinus imperator*. It seems that the negative charge of the PL may be related to the efficiency or quantity of venom generated (Schenk et al., 2009). This fact could be certain since in an early study of venom of the scorpion *Leiurus quinquestriatus* a great quantity of PtdSer was observed by thin-layer chromatography (Marie and Ibrahim, 1976). In *T. elegans*, as well as in males of *B. ferrugineus* and females of *B. ferrugineus* the quantity of PtdSer was very high, similar to that found in *P. imperator*. The pattern of fatty acids of telson – a structure with venom gland as majority component – did not show great differences with those of the other tissues, leading to the conclusion that at least in telson there are no lipids with qualitatively and/or

Table 5
Fatty acid composition of TAG and PL of hepatopancreas and muscle of males of *Timogenes elegans*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Student's *t*-test was used to compare the significance of the differences fatty acid composition of PL with respect to TAG in hepatopancreas and PL with respect to TAG in muscle. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

T. elegans Male	PL		TAG	
	Hepatopancrease	Muscle	Hepatopancrease	Muscle
14:0	$3.47 \pm 0.9^{*}$	$1.84 \pm 0.7^{**}$	1.93 ± 0.2	9.20 ± 1.1
15:0	1.42 ± 0.5	$1.34 \pm 0.3^{**}$	0.69 ± 0.5	3.50 ± 0.4
16:0	$16.91 \pm 2.7^{*}$	$16.5 \pm 1.2^{**}$	24.36 ± 2.7	39.80 ± 4.2
16:1	ND	ND	1.48 ± 0.7	4.20 ± 0.5
17:1	0.21 ± 0.1	ND	ND	ND
18:0	13.31 ± 2.7	$15.65 \pm 1.7^{*}$	9.62 ± 0.7	30.00 ± 3.5
18:1	$31.99 \pm 4.5^{*}$	$28.97 \pm 2.7^{**}$	54.19 ± 7.1	9.80 ± 1.3
18:2	$25.55 \pm 2.9^{**}$	$28.05 \pm 2.1^{**}$	7.73 ± 0.4	2.80 ± 0.3
18:3	$2.55 \pm 0.9^{*}$	ND	0.10 ± 0.1	0.30 ± 0.4
20:0	0.71 ± 0.5	ND	ND	ND
20:3	2.83 ± 0.7	7.57 ± 0.3	ND	ND
22:0	1.06 ± 0.6	ND	ND	ND
∑ SFAs	36.88 ± 7.9	$35.33 \pm 3.9^{**}$	36.60 ± 4.1	82.50 ± 9.2
∑ MUFAs	$32.2 \pm 4.6^{*}$	$28.97 \pm 2.7^{**}$	55.67 ± 7.8	14.00 ± 1.8
∑ PUFAs	$30.93 \pm 4.5^{**}$	$35.62 \pm 2.4^{**}$	7.83 ± 0.5	3.10 ± 0.7
∑ UFAs	63.13 ± 9.1	$64.59 \pm 5.1^{**}$	63.50 ± 8.3	17.10 ± 2.5

* $p < 0.05$.
** $p < 0.001$.

Table 6
Fatty acid composition of TAG and PL of hepatopancreas and muscle, of male of *Timogenes dorbignyi*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Student's *t*-test was used to compare the significance of the differences fatty acid composition of PL with respect to TAG in hepatopancreas and PL with respect to TAG in muscle. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

T. dorbygnyi Male	PL		TAG	
	Hepatopancreas	Muscle	Hepatopancreas	Muscle
14:0	$3.19 \pm 0.7^{*}$	$3.43 \pm 0.4^{*}$	1.70 ± 0.4	5.17 ± 0.4
15:0	1.29 ± 0.6	$1.57 \pm 0.2^{**}$	0.79 ± 0.5	6.74 ± 0.7
16:0	$18.82 \pm 2.1^{*}$	$24.35 \pm 2.8^{*}$	27.80 ± 2.9	44.83 ± 4.9
16:1	1.39 ± 0.2	1.57 ± 0.7	1.66 ± 0.9	ND
17:1	1.00 ± 0.7	1.43 ± 0.9	ND	ND
18:0	13.65 ± 3.1	$17.77 \pm 2.1^{*}$	10.04 ± 1.5	30.65 ± 3.1
18:1	$28 \pm 0.9^{*}$	$24.16 \pm 2.9^{**}$	30 ± 1	9.98 ± 0.7
18:2	$29.28 \pm 1.1^{*}$	$17.87 \pm 3.2^{**}$	27.02 ± 1	2.63 ± 0.7
18:3	0.70 ± 0.4	ND	0.80 ± 0.7	ND
20:0	ND	0.65 ± 0.5	ND	ND
20:3	2.29 ± 0.4	6.20 ± 0.9	ND	ND
22:0	ND	1.00 ± 0.8	ND	ND
∑ SFAs	36.95 ± 6.5	$48.70 \pm 6.8^{*}$	40.33 ± 5.3	87.39 ± 9.1
∑ MUFAs	30.39 ± 1.8	$27.10 \pm 4.5^{*}$	31.66 ± 1.9	9.98 ± 0.7
∑ PUFAs	32.27 ± 1.9	$24.00 \pm 4.1^{**}$	27.82 ± 1.7	2.63 ± 0.7
∑ UFAs	62.66 ± 3.7	$51.20 \pm 8.6^{**}$	59.28 ± 3.6	12.61 ± 1.4

* $p < 0.05$.
** $p < 0.001$.

Table 7
Fatty acid composition of TAG and PL of hepatopancreas and muscle of males of *Brachistosternus ferrugineus*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Student's *t*-test was used to compare the significance of the differences fatty acid composition of PL with respect to TAG in hepatopancreas and PL with respect to TAG in muscle. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

B. ferrugineus Male	PL		TAG	
	Hepatopancreas	Muscle	Hepatopancreas	Muscle
14:0	5.26 ± 0.7**	2.6 ± 0.5	0.70 ± 0.6	2.26 ± 0.7
15:0	2.37 ± 0.4*	1.3 ± 0.5**	0.90 ± 0.5	4.50 ± 0.5
16:0	24.20 ± 0.9*	15.9 ± 1.7**	26.39 ± 0.6	41.00 ± 5.4
16:1	1.47 ± 0.7	1.1 ± 0.6**	2.45 ± 0.3	5.40 ± 0.8
17:1	0.61 ± 0.5	1.1 ± 0.2	1.01 ± 0.1	ND
18:0	12.97 ± 2.1*	14 ± 2.3*	7.76 ± 0.3	30.20 ± 5.2
18:1	30.84 ± 2.7*	25.4 ± 4.5*	39.46 ± 3	10.00 ± 3.4
18:2	19.33 ± 2.1*	28.3 ± 3.1*	10.22 ± 1.5	6.33 ± 0.8
18:3	1.47 ± 0.1*	1.51 ± 0.7*	0.62 ± 0.5	0.10 ± 0.1
20:0	ND	ND	3.13 ± 0.3	ND
20:3	1.60 ± 0.8*	5.90 ± 0.7	3.75 ± 0.4	ND
22:0	ND	2.00 ± 0.7	3.03 ± 0.5	ND
∑ SFAs	44.80 ± 4.1	35.8 ± 5.7*	41.91 ± 6.7	77.96 ± 11.8
∑ MUFAs	32.90 ± 3.9	27.6 ± 5.3*	42.90 ± 3.4	15.4 ± 2.8
∑ PUFAs	22.40 ± 3*	35.71 ± 4.5**	14.50 ± 2.4	6.43 ± 0.9
∑ UFAs	55.32 ± 6.9	63.31 ± 9.8*	57.40 ± 5.8	21.83 ± 5.1

* $p < 0.05$.
** $p < 0.001$.

quantitatively different fatty acids. The relation of PtdSer with the venom is not clear enough at present and needs to be studied further.

Although data related to the circulation of energetic lipids in scorpions are restricted only to the species *Leiurus quinquestriatus* (El-Salhy et al., 1981), currently, and including the present work, it seems that in these arthropods the majority energetic lipid is the TAG. This fact coincides with what we described in two araneomorph spiders (Cunningham et al., 1994, 2000, 2007) but differs significantly from what was described for groups with diacylglycerides as the majority energetic lipid, as is the case in two mygalomorph spiders (Haunerland and Bowers, 1987; Laino et al., 2015) and in insects (Thomas and Gilbert, 1968; Peled and Tietx, 1975; Ryan et al., 1988; Rodenburg and Van der Horst, 2005; Weers and Ryan, 2006).

Table 8
Fatty acid composition of TAG and PL of hepatopancreas and muscle of females of *Brachistosternus ferrugineus*. Data on fatty acid classes are expressed as weight percentage and quantified by GLC-FID. Values are the means ± SD of three independent analyses of three pools. Student's *t*-test was used to compare the significance of the differences fatty acid composition of PL with respect to TAG in hepatopancreas and PL with respect to TAG in muscle. SFA (saturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid), and UFA (unsaturated fatty acid).

B. ferrugineus Female	PL		TAG	
	Hepatopancreas	Muscle	Hepatopancreas	Muscle
14:0	2.94 ± 0.4	2.0 ± 0.4	3.21 ± 0.4	2.70 ± 0.7
15:0	1.22 ± 0.2	1.4 ± 0.2*	1.29 ± 0.3	2.89 ± 0.4
16:0	23.72 ± 3.1	19.9 ± 2.2*	31.00 ± 4.2	34.40 ± 3.5
16:1	1.35 ± 0.3	1.14 ± 0.2*	1.31 ± 0.2	3.25 ± 0.5
17:1	1.29 ± 0.2	1.15 ± 0.2	0.95 ± 0.7	ND
18:0	11.25 ± 1.3*	16.3 ± 1.7*	8.16 ± 0.5	22.00 ± 2.5
18:1	31.10 ± 4.1	23.1 ± 2.5	38.00 ± 3.9	28.08 ± 3.1
18:2	18.56 ± 2.1*	24.3 ± 3.1**	12.00 ± 1.9	6.30 ± 0.7
18:3	6.02 ± 0.7*	2.50 ± 0.3**	2.96 ± 0.3	0.10 ± 0.1
20:0	ND	ND	0.34 ± 0.1	ND
20:3	1.72 ± 0.4*	6.91 ± 0.9	0.39 ± 0.2	ND
22:0	0.82 ± 0.5	1.06 ± 0.4	ND	ND
∑ SFAs	39.9 ± 5.5	40.8 ± 4.9*	44 ± 5.5	61.99 ± 7.1
∑ MUFAs	33.7 ± 4.6	25.4 ± 2.9	40.26 ± 4.8	31.3 ± 3.6
∑ PUFAs	26.3 ± 3.2*	33.7 ± 4.3**	15.35 ± 2.4	6.4 ± 0.8
∑ UFAs	60.04 ± 7.8	59 ± 7.2*	55.61 ± 7.2	37.73 ± 4.4

* $p < 0.05$.
** $p < 0.001$.

The composition of fatty acids of the hemolymph showed a similar pattern in the three species studied, most of them being fatty acids of 16 and 18 carbons, similar to those described by El-Salhy et al. (1981) for *L. quinquestriatus*, in insects and crustaceans (Gilbert and O'Connor, J.M., 1970; Gilbert et al., 1977; Pattnaik et al., 1979). Another constant characteristic related to the hemolymph of scorpions is that, similarly to what was observed in *L. quinquestriatus*, in *T. elegans*, *T. dorbignyi* and *B. ferrugineus* the C20 fatty acids do not exceed 4%. This differs from the percentages of fatty acids of 20 carbons previously reported for insects and crustaceans, found to be higher than the ones reported here (Gilbert and Chino, 1974). Assays using radiolabelled acetate reinforce the hypothesis of the absence of arachidonic acid (20 carbons) in scorpions (*Centuroides sculpturatus* (Wood, 1863)), and confirm their presence in mygalomorphae spiders (*Aphonepelma sp.*) (Ross and Monroe, 1970). Subsequently, Schartau and Leidescher (1983) while working with another mygalomorph spider, *Aphonopelma hentzi*, confirmed these observations. In a later work (Uscian and Stanley-Samuelson, 1994) they observed almost twice the 20:4 PL of that found for the scorpion *Centruroides vittatus*, when comparing the PL of the tarantula *Grammostola sp*.

It is important to highlight that with regard to the composition of fatty acids of hemolymph and hepatopancreas in the males of the three models, the hepatopancreas is enriched in 18:2 and impoverished in 16:0, coinciding with what was reported in *L. quinquestriatus* (El Salhy et al., 1981). As determined by El Salhy et al. (1981), it is clear that there is more than one pool of fatty acids in the hepatopancreas with different degrees of interchange with the hemolymph. This hypothesis can be extended to the different organs analyzed; for example what was found in gonads, where not only the gonad is enriched in 18:2 but also in 18:1, and impoverished in 16:0. No great differences between patterns of fatty acids or lipids were observed between males and females. Variations are possibly found at the level of quantity of lipids, which are surely accompanied by increasing concentrations of water and glycogen as described for scorpions in general (Warbug, 2012). This fact may be reflected by a lower concentration of lipids found in the hepatopancreas and by a greater percentage of TAG in the whole body of females of *B. ferrugineus*.

In 2011 Lease and Wolf observed in females of terrestrial arthropods (several representatives of Class Arachnida and Class Insecta) the lipid content (dry weight) was higher than that found in males. However, significant differences were not observed for the specific case of scorpions, because their study seems to be incomplete with relation to males $n = 1$. Further comparative studies with other species are necessary in order to clarify this issue.

A differential pattern of fatty acids was observed when the majority saponifiable lipids (PL and TAG) found in the hepatopancreas and muscle of the males of the three species were compared. More specifically, when TAG and PL of the hepatopancreas are compared, it can be noted that TAG have an enrichment of 18:1, 16:0 and an impoverishment of 18:2 with regard to PL, coinciding with the results described for the hepatopancreas of *L. quinquestriatus* (El-Salhy et al., 1981) and complete abdomen of *C. vittatus* (Uscian and Stanley-Samuelson, 1994). For the case of PL and TAG of muscle, a great increase in the percentage of saturated fatty acids of over 75% mainly given by 16:0 and 18:0 was observed in TAG, at the expense of a decrease of over 80% of poly unsaturated and over 35% mono unsaturated. This difference may be in the different studies, disguised by a great quantity of PtdCho in the muscular tissue.

Although it is certain that the physiological requirements and environmental constraints argue against a characteristic fatty acid pattern in the arthropods as discussed for insects earlier (Stanley-Samuelson et al., 1988), it is evident that there are patterns of lipids and fatty acids that are shared by the three studied species (Family Bothriuridae) and that match with what was described for unrelated scorpions, as *L. quinquestriatus*, *C. vittatus* (Family Buthidae) *and P. imperator* (Family Scorpionidae). Further studies are necessary to discover new

information which may lead to new interpretations of the lipidic characteristics of these primitive animals.

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