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Chapter 8

HEMOLYMPH AND HEMOCYTES OF TARANTULA SPIDERS: PHYSIOLOGICAL ROLES AND POTENTIAL AS SOURCES OF BIOACTIVE MOLECULES

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ABSTRACT

Arachnids compose the most important and numerous group of chelicerates and include spiders, scorpions, mites and ticks. Some arachnids have a worldwide distribution and can live for more than two decades. This is in part due to their efficient defense system, with an innate immunity that acts as a first line of protection against bacterial, fungal and viral pathogens. The adaptive success of the spiders stimulates the study of their defense mechanisms at cellular and molecular levels with both biological and biotechnological purposes. The hemocytes (plasmatocytes, cyanocytes, granulocytes, prohemocytes, and leberidocytes) of spiders are responsible for phagocytosis, nodulation, and encapsulation of pathogens as well as produce substances that mediate humoral mechanisms such as antimicrobial peptides and factors involved in the coagulation of hemolymph and melanization of microorganisms. This chapter discusses on the morphophysiology of tarantula spider hemocytes and bioactive molecules isolated from hemocytes and hemolymph. In addition, there is a special focus on the Brazilian tarantula *Lasiodora* sp., which is currently under systematic review. Although there are many gaps to be filled, significant progress has been achieved on the identification of *Lasiodora* sp.hemocytes and study of bioactive molecules present in hemocytes of these spiders.

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1. INTRODUCTION

The arthropods have developed an innate immunity that is the first line of protection against bacteria, fungi and viral pathogens. The high diversity of invertebrates evidences the efficiency of their defense system and indicates that the absence of acquired immunity did not hinder their successes (Coates and Nairn, 2014).

The Chelicerata is a sub-phylum of arthropods and includes the classes Arachnida, Xiphosura and Picnogonida. The arachnids compose the most important and numerous groups of chelicerates and are represented by the forms most known by humans, such as spiders, scorpions, mites and ticks. Spiders are the most diverse and successful group of terrestrial invertebrates (Rash and Hodgson, 2002) and the tarantula spiders are among the largest living spiders and the richest spider groups (Bertani et al. 2012; Platnick, 2015). The adaptive success of spiders encourages studies based on their immunity.

Cellular and humoral pathways accomplish the innate immunity of arthropods (Hoebe et al. 2004; Fukuzawa et al. 2008). The hemocytes of spiders, which can be identified based on their morphology, ultrastructure, and physiological roles, are responsible for phagocytosis, nodulation and encapsulation. The humoral mechanisms involve the action of antimicrobial peptides (AMPs), coagulation of the hemolymph and melanization of microorganisms (Jiravanichpaisal et al. 2006, 2010). In spiders, in addition to AMPs, the defensive mechanisms involve other molecules such as phenoloxidase, coagulation factors, complement factors, lectins, proteases, and protease inhibitors, which can be found in the hemolymph and hemocytes of these animals.

Detection, isolation and evaluation of mode of action of these molecules can contribute to a broader understanding of the processes involved in the immune system of arachnids. Moreover, the isolation and characterization of new antimicrobial molecules may also contribute with strategies for control of human and phytopathogens.

2. SPIDERS

Spiders belong to the order Araneae of the class Arachnida. The Araneae can still be divided into two groups: Mesothelae and Opisthothelae (Ruppert, 1994). The suborder Mesothelae comprises a single family (Liphistiidae) of primitive spiders that are characterized by the segmented abdomen, while the Opisthothelae spiders do not show external segmentation of the abdomen (Dutra, 2006). The infraorder Mygalomorphae, which is the most basal of the Opisthothelae group, include spiders that have a pair of chelicera parallel to the direction of the body and a pair of leaf lungs. The Mygalomorphae comprises the families Theraphosidae, Dipluridae and Hexatelidae (Vizzotto, 2009).

Morphologically, the body of a spider consists of two main parts (Figure 1): an anterior portion or carapace called prosome and a posterior portion (the abdomen) called opisthosome. The structure that connects the prosome and the opisthosome is called pedicel. The prosome supports four pairs of legs, a pair of chelicerae and a pair of pedipalps (modified in males on copulatory organs) (Platnick, 1971; Foelix, 1996). In addition, there are the eyes (Figure 1) in the prosome, which can be found in number of two, six or eight, and are extremely important

for taxonomy. The opisthosome contains the respiratory, circulatory, digestive and reproductive systems (Foelix, 1996).

The Mygalomorphae spiders are commonly called as tarantulas. Thousands of mygalomorph species are described, distributed in the families Actinopodidae, Antrodiaetidae, Atypidae, Barychelidae, Ctenizidae, Cyrtaucheniidae, Dipluridae, Hexathelidae, Idiopidae, Mecicobothriidae, Migidae, Microstigmatidae, Nemesiidae, Paratropididae, and Theraphosidae. The Theraphosidae family is represented by around 976 species divided in 128 genera, distributed across the globe and occurring in various habitats (Platnick, 2015). This family possesses a genus (*Lasiodora*) that is under systematic review and includes a group of spiders found in Brazilian northeast (*Lasiodora* sp.), which will be discussed forward.

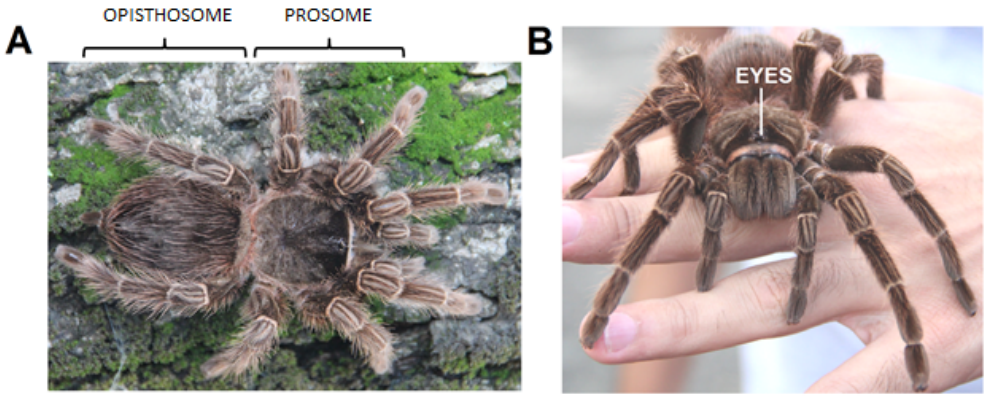

Figure 1.A tarantula spider from *Lasiodora* genus, evidencing the major anatomical divisions in mygalomorphs. (A) A top view of the body, emphasizing the division in opisthosome and prosome. (B) A frontal view of the body, highlighting the position of the eyes.

Similar to all arthropods, the spiders have an exoskeleton that surrounds the body of the animal and is constituted by chitin secreted by the monolayered epidermis. Numerous trunk and appendicular muscles are attached to this structure. The external layer of the exoskeleton is sclerotized (Bitsch and Bitsch, 2002).

During the growth process, the arthropods pass by the molt and, in general, most of the spiders cease growth and the exchange of exoskeleton when achieve the reproductive stage (Silva Jr., 2000).

However, in large tarantulas, the adult females continue to carry out the molt once a year or at irregular intervals (Silva Jr., 2000). Accidents caused by spiders are classified in distinct groups according to the effects of venom in humans: those that produce necrotic ulceration and those that not produce necrosis (Schwatrz et al. 2012). Although many people consider tarantula spiders as the most dangerous, their bites do not induce local necrosis or systemic effects, being only painful. Nevertheless, tarantulas have been a relatively popular pet for several years. Indeed, these arachnids are popular as household pets and several websites in social networks can be found containing informations about the maintenance of spiders as pets, making it a fascinating hobby.

3. HEMOLYMPH AND HEMOCYTES

The fresh hemolymph of a spider shows a blue color due to the presence of copper contained in the respiratory pigment hemocyanin. The hemolymph is analogue to the blood of vertebrates and shows a variety of circulating cells called hemocytes. This organic fluid include substances such as hemocyanin (accounting for about 80%), amino acids (glutamine is the most abundant), carbohydrates (mainly glucose), and fatty acids, including palmitic, linoleic and stearic acids (Foelix, 1996; Tillinghast and Townley, 2008; Coates and Nairn, 2013). One of the main ways of collecting the hemolymph is by cardiac puncture in the presence of anticoagulant (Soares et al. 2011). The heart of tarantula spiders can be clearly viewed after scraping of the dorsal hairs (Figure 2).

Figure 2.The heart (indicated by the arrow) of a *Lasiodora* spider viewed by transparency after scraping of the dorsal spider hairs.

The hemocyanins are multimeric copper-containing proteins that transport oxygen in the hemolymph, but also have multiple other functions (Coates and Nairn, 2013, 2014; Starrett et al. 2013). In chelicerates, hemocyanin appears to play an important role in innate immunity system, homeostasis and molt (Nagai et al. 2001; Adachi et al. 2003; Cerenius and Söderhäll, 2004; Kuballa and Elizur, 2008; Kuballa et al. 2011; Glazer et al. 2013). *In vitro* coagulation cascade components and antimicrobial factors can induce hemocyanin to exert phenoloxidase activity (Nagai et al. 2001; Adachi et al. 2003; Baird et al. 2007; Kuballa et al. 2011; Jeanicke and Decker, 2008) as well as antimicrobial and antiviral activities (Zhang et al. 2004; Pan et al. 2005; Riciluca et al. 2012; Coates and Nairn, 2014; Qiu et al. 2014).

Mechanical injuries and the presence of invader objects or microorganisms in hemolymph may also result in the deposition of melanin around the damaged tissue or the foreign body. The melanin physically holds an attacker, preventing or slowing its growth. Also, reactive and toxic intermediates, such as quinines, are produced during the formation of melanin and act causing the microorganism death (Cerenius and Söderhäll, 2004). The melanization mechanism is part of the innate immune system and is present in the hemolymph as a protein complex mixture of several molecules including proteases. The activation of these proteases is carefully regulated by phenoloxidase system, which consists

of a cascade of proteins capable of binding to polysaccharides and other compounds typically associated with microorganisms such as peptidoglycans and lipopolysaccharides (Silva, 2002).

The goal of the immune response is to remove or perceive the invader, through non antigen-specific mechanisms that involve specific cells and molecules such as enzyme inhibitors, antimicrobial peptides and lectins. The hemocytes are the cells that have the ability to defend invertebrates against pathogens, parasites and other foreign bodies that had penetrated the hemocele. Defense reactions directly exerted by hemocytes are phagocytosis, encapsulation and damage repair (Lavine and Strand, 2002). The circulating hemocytes are also involved in the release of antimicrobial peptides, the coagulation of the hemolymph, the melanin formation and the complement activation. The hemocytes are extremely sensitive to bacterial lipopolysaccharide (LPS), responding through the release of granular components, including antimicrobial peptides. The production of these peptides is also mediated by Toll-like receptors (Iwanaga and Lee, 2005). Upon reaching the site of infection, the hemocytes can secrete the components of the coagulation cascade in the hemocele (Fukuzawa et al. 2008). The hemolymph coagulation phenomenon was firstly identified as a defense system in the horseshoe crab *Limulus polyphemus* (Bang, 1956).

In the horseshoe crab, the hemocytes are known as granulocytes because they have granules of various sizes. When a hemocyte detects bacterial endotoxins (such as LPS groups), it releases their granules through a rapid exocytosis. Among the released granular content, there are two serineprotease zymogens (called factors C and G), which are catalytically activated in response to LPS and β-1,3-D-glucan, the main components of cell wall of Gram-negative bacteria and fungi, respectively (Muta and Iwanaga, 1996). This activation results in the conversion of the protein coagulogen to coagulin, which forms a gel that hinders the spread of the microorganism. The invaders detained by the gel (clot) are phagocytosed by hemocytes and then killed by action of lectins, antimicrobial compounds and protease inhibitors found in these cells.

According to Xylander and Nevermann (2006) and Xylander (2009), the arthropod hemocytes can be classified as prohemocytes, plasmatocytes, granulocytes, spherulocytes, coagulocytes, discoid hemocytes, adipohemocytes, cystocytes, oenocytoids and cyanocytoids. In contrast to insects and crustaceans, the immune system of the most groups of chelicerates is not well-investigated. Studies addressing arthropod hemocytes have mainly focused on vectors of diseases that affect humans directly (Araújo et al. 2008, Castilho et al. 2006, Hillyer and Strand, 2014) in detriment to ecologically important and species-rich taxa, such as scorpions and spiders, which also contain many species of medical importance (Kuhn-Nentwig et al. 2014).

Sherman (1981) classified the hemocytes of the tarantula spider *Eurypelma marxi* according to insect nomenclature in plasmatocytoids, oenocytoids and granular hemocytes. Foelix (1996) classified the spider cells in four types: granulocytes, leberidocytes, cyanocytes and prohemocytes. The more actual classification for hemocytes of spiders comprises five cell types, named based on the nomenclature used for insects: plasmatocytes or hyaline cells (immune response), cyanocytes or oenocytes (involved in respiration and immune response), granulocytes (mainly acting in the immune response), prohemocytes (stem cells) and leberidocytes (present in moulting individuals) (Kuhn-Nentwig et al. 2014).

The cells differ in shape and cytochemical/electron microscopic staining of their cytoplasma and granules. Frequently, the researchers use more than one type of microscopy

to insure the different types of cells. Other aspects that may influence the results are the method of punction and spider health state. These are one of the reasons for that the authors differ so much on the spider hemocytes classification.

Lectins, protease inhibitors, and antimicrobial peptides have been isolated from both hemolymph plasma and hemocytes of arthropods. As some examples, it has been reported the protease inhibitor from plasma of silkworm *Antheraea mylitta* (Shrivastava and Ghosh, 2003), the serine proteinase inhibitor from plasma of *Manduca sexta* (Wang & Jiang, 2004), the trypsin and subtilisin inhibitor from hemocytes of shrimp *Litopenaeus vannamei* (Vega and Albores, 2005), and an AMP from hemocytes of the tick *Boophilus microplus* (Fogaça et al. 2006). Foradori et al. (2006) investigated the digestive fluid of spider *Argiope aurantia* and isolated two peptidases of lower molecular mass, called p16 and p18. The authors also showed evidences of the presence of serine peptidase inhibitor. Wan et al. (2013) reported that the chymotrypsin inhibitor of the spider *Araneus ventricosus* also acts as elastase inhibitor and microbial serine protease inhibitor.

In arthropods, protease inhibitors play important roles controlling endogenous activity of proteases involved in digestion and activation of phenoloxidase cascade as well as of microbial proteases that act as virulence factors (Kanost, 1999). Protease inhibitors are classified into five groups (serine, threonine, cysteine, aspartyl and metalloprotease inhibitors) according to the mechanism employed at the active site of proteases that they inhibit (Fear et al. 2007).

Protease inhibitors are essential for organisms for controlling protein damage caused by self and non-self proteases (Simonet et al. 2002). Most of the invertebrate protease inhibitors were isolated initially from insect hemolymph and could be classified in two groups: Kunitz-type family, corresponding to low molecular mass proteins; and serpin superfamily, corresponding to proteins of approximately 45 kDa (Polanowski and Wilusz, 1996). Another two main families of arthropods proteases inhibitors are pacifastin and cystatin. The pacifastin family constitutes a family of peptidic inhibitors of serine proteases that are considered to be important regulators of several physiological processes in arthropods (Breugelmans et al. 2008). Cystatins are involved in various physiological and cellular processes, including immune responses, protein homeostasis, signaling pathways, and apoptosis (Wan et al. 2013).

Serine proteases involved in hemolymph coagulation and activation of prophenoloxidase processes, which are restricted to arthropods, are controlled by serine protease inhibitors of Kunitz, serpin and pacifastin families present in the hemolymph of arthropods. These inhibitors can act on specific proteases or on more than one type of proteases (Theopold et al. 2004; Fear et al. 2007). Inhibitors of serine proteases could also mediate the production of antimicrobial peptides (Fogaça et al. 2006).

Present in all living organisms, the AMPs consist in small molecules with about one hundred amino acids in length (Pasupuleti et al. 2012) and are evolutionarily conserved (Hancock and Sahl, 2006). The AMPs are used by the host for protection against different types of pathogens and are a heterogeneous group with respect to their primary and secondary structures, antimicrobial action and effects on host cells. The AMPs usually have a positive charge provided by arginine and lysine residues and an amphipathic structure that allows them interact with the bacterial membrane (Auvynet and Rosenstein, 2009).

The AMPs have been classified into families and sub-families based on their primary sequences and structures (Yeaman and Yount, 2003). The AMP families described differ

among each other in physicochemical and chemical structure (Khamis et al. 2015). The AMPs isolated until now have been organized in public databases like APD (Wang and Wang, 2004), ANTIMIC (Brahmachary et al. 2004), APD2 (Wang et al. 2009), DAMPD (Sundararajan et al. 2012) and CAMP (Thomas et al. 2010; Waghu et al. 2014). The database APD contains 2529 antimicrobial peptides described with the following activities: antibacterial, antiviral, antifungal, antiparasitic, anticancer, antiprotozoal, insecticidal, spermicidal, chemotactic, and antioxidant (Wang and Wang, 2009).

Several AMPs have been isolated from the venom and hemolymph of scorpions and spiders (Kuhn-Nentwig, 2003). Silva Jr. (2000) purified and characterized four molecules present in the hemolymph of the tarantula spider *Acanthoscurria gomesiana*. The first was the theraphosin, purified from plasma, and the other three peptides, called mygalin, gomesin and acanthoscurrin, were isolated from hemocytes (Silva Jr., 2000; Lorenzini et al. 2003; Pereira et al. 2007). The theraphosin is a 4052.5 Da peptide with activity against *Micrococcus luteus*. The mygalin is a peptide of 415.9 Da with activity against *Escherichia coli* and able to induce the production of hydrogen peroxide. Gomesin is a 2270.4 Da peptide, with high similarity to tachyplesins and protegrins (peptides from the horseshoe crab), with broad activity against bacteria, fungi, yeasts and *Leishmania*. The gomesin structure includes a pyroglutamic acid as the N-terminal, one α-C-terminal amide arginine and four cysteine residues that form two disulfide bonds. The acanthoscurrin is a glycine-rich peptide presenting two isoforms with 10132.4 and 10249.1 Da, which differ by the presence of two additional glycine residues; the acanthoscurrin showed activity against *E. coli* and *Candida albicans* and similarity to insect antifungal proteins and proteins related to defense in plants (Silva Jr., 2000; Lorenzini et al. 2003; Pereira et al. 2007).

A study demonstrated that mygalin is not cytotoxic to murine cells *in vitro* and does not affect cell proliferation or IL-2 production. This peptide activates the innate immune response through induction of Th1 cytokines and proinflammatory mediators, such as TNF-α and nitric oxide, that are essential for defense against infectious pathogens (Mafra et al. 2012). Moreover, as a proinflammatory factor that regulates the immune response, mygalin can be exploited with therapeutic purposes alone or in combination with other molecules such as gomesin, which has anti-cancer activity (Soletti et al. 2010; Mafra et al. 2012).

Besides the identification of AMPs isolated from the hemocytes of *A. gomesiana* (Silva Jr., 2000; Lorenzini et al. 2003), only limited information on the innate immune system of spiders is available. Antimicrobial activity was detected in the hemolymph of spider *Acanthoscurria rondoniae* due to the presence of an antifungal peptide called rondonin (Riciluca et al. 2012). Rondonin has the amino acid sequence IIIQYEGHKH and a molecular mass of 1236.776 Da, corresponding to the first report of a fragment of hemocyanin with antifungal activity (Riciluca et al. 2012). Peptides called ctenidins showing activity against Gram-negative bacteria were purified from the hemocytes of the spider *Cupiennius salei* (Baumann et al. 2010).These peptides, with over 70% glycine residues resembling acanthoscurrin, are constitutively expressed in hemocyte and nerve tissues, and their expressions are independent of immune challenges (Baumann et al. 2010).

The appearance of new diseases and increased resistance of bacteria to antibiotics in recent decades have become an increasing threat to human health. This has driven a constant search for new agents that have antibacterial activity, especially against resistant strains, leading to an increasing interest in AMPs (Kamysz et al. 2003; Godoy et al. 2013).

4. HEMOCYTES FROM *LASIODORA* SP.: A BRAZILIAN TARANTULA SPECIES UNDER SYSTEMATIC REVIEW

The tarantulas from *Lasiodora* genus belongs to the Theraphosidae family, can reach the average age of 25 years, and are 15–25 cm long with the legs extended (Bertani, 2001). Members of the genus *Lasiodora* are widely distributed in Brazil, where they are called "caranguejeiras" (Horta et al. 2013). Until now there are 39 species described for the *Lasiodora* genus, according Platnick (2015).

Lasiodora spiders show a black or brown color and has stinging hairs on the abdomen of the types I, III and/or IV, which can be launched when the animal feels threatened (Foelix, 1996). Many American theraphosid spiders possess these hairs covering their opisthosome, which are brushed with the hind legs into the direction of the perceived attack, so defensive bites are rarely necessary (Fuchs et al. 2014).

The different species of *Lasiodora* spiders are difficult to distinguish. Reliable morphological identification of tarantulas is most difficult due to the very similar features of many species. Classical methods are based on the examination of male genitalia, shape of body appendages or hair counts. In addition, the difficulty of accessing habitats often means that putative classification is based on few preserved specimens (Escoubas and Rash, 2004).

Lasiodora spiders can be found at the Northeast, Southeast and Midwest regions of Brazil, especially in the Atlantic Forest (Bertani, 2001). In Brazil, this genus is currently under a process of systematic review, coordinated by Butantan Institute at São Paulo. Despite some significant progress has been achieved, there are still many gaps to be filled and this completion of this systematic review remains a long journey.

Tarantulas can be found in diverse types of environment and this diversity of ecological niches, associated with the diversity of prey capture behavior, contributes to the diversity of their poisons (Vieira et al. 2004). There are many studies on the purification of toxins from the venom of these spiders, including *Lasiodora* species.

Kusmerick et al. (2001) screened the *Lasiodora* sp. venom for activity against ion channels and suggested that venom of this spider evokes vesicular release of acetylcholine from parasympathetic nerve terminals in the heart by activating TTX-resistant Na^+ channels. Kalapothakis et al. (2003) reported that the *Lasiodora parahybana* and *Lasiodora* sp. venom interfere in the heart rate. Vieira et al. (2004) reported the identification of toxins LTx1, LTx2 and LTx3, which are expressed in the venom gland of *Lasiodora* sp. These toxins showed significant similarity at the amino acid level with spider toxins from *Lasiodora parahybana*, *Eurypelma californicum*, *Brachypelma smithii*, *Selenocosmia huwena*. Dutra et al. (2008) showed that the recombinant LTx2 toxin acts on calcium channels of BC3H1 cells, blocking L-type calcium channels.

However, there are few studies on the morphophysiology of hemocytes and bioactive molecules from hemolymph of tarantulas spiders, which could contribute to the systematic classification of *Lasiodora* at species level. In order to contribute in this sense, studies have been conducted *Lasiodora* spiders found in the rain forests at Pernambuco, a state at Northeastern Brazil, which are referred as *Lasiodora* sp.

Soares et al. (2013) classified the hemocytes of *Lasiodora* sp. in six morphological types: prohemocytes, granulocytes type I, granulocytes type II, spherulocytes, oenocytoids and

plasmatocytes. The most abundant cells were the granulocytes and Table 1 summarizes the ultrastructural characteristics of *Lasiodora* sp. hemocytes.

Fukuzawa et al. (2008) suggested that phagocytosis is not the major defense mechanism activated towards a microbial challenge and probably plays a secondary role, being responsible for clearing cellular debris and remodeling damaged tissues. These authors showed that the injection of particles into the legs of the tarantula *Acanthoscurria gomesiana* clearly activated a coagulation cascade. Soares et al. (2013) reported that the same occurred after cardiac punction of *Lasiodora* sp. and the Figure 3 shows this process observed by optical microscopy. Based on this observation the authors suggested that *Lasiodora* sp. spherulocytes participated in the hemolymph coagulation.

Table 1. Ultrastructural characteristics of hemocyte found in *Lasiodora* sp.

Hemocyte type	Relative population* (%)	Cell characteristics
Prohemocytes	8.1	10–15 µm in diameter Spherical shape Central nucleus Few granules and mitochondria
Granulocytes type I	35.2	25–30 µm in diameter Elongated shape Lobed and central nucleus Numerous dense granules and mitochondria
Granulocytes type II	29.6	35–40 µm in diameter Elliptical shape Central nucleus Elliptical granules and few mitochondria
Spherulocytes	11.1	15–25 µm in diameter Uniform (round) cell and nucleus shape Numerous granules near to the plasma membrane
Oenocytoids	3	15–20 µm in diameter Round cell shape Variable nucleus shape Many mitochondria and some granules
Plasmatocytes	13	15–20 µm in diameter Variable cell and nucleus shape Cytoplasm without granules

*The determination of relative hemocyte populations was performed using slides prepared with fresh hemolymph (10µl) in triplicate analysed by bright-field microscopy (magnification: 100x). Reference: Soares et al. (2013).

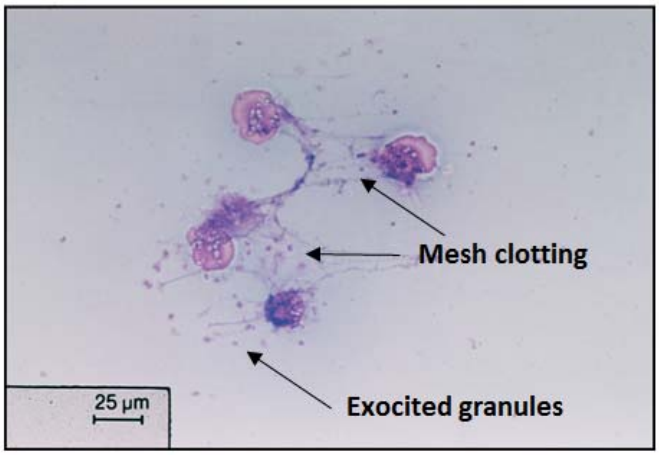

Figure 3. Spherulocyte degranulation and clotting process in *Lasiodora* sp. observed by optical microscopy.The presence of mesh clotting and the exocytosis of contents of granules by spherulocytes evidence that the coagulation cascade was activated.

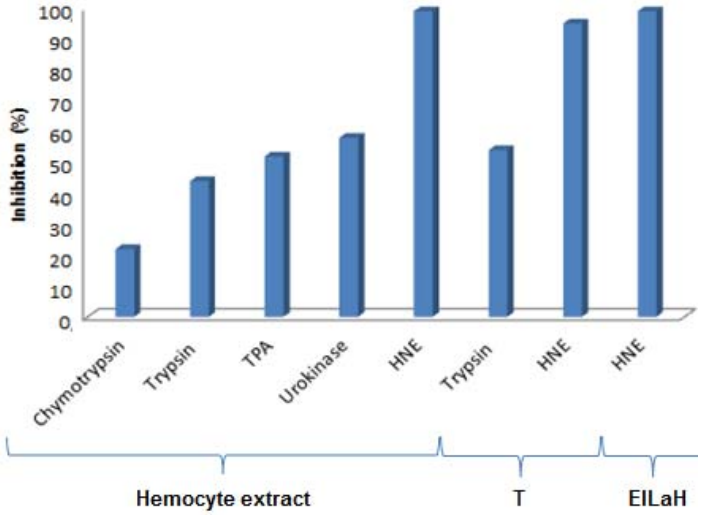

Figure 4. Inhibitory effect on proteases of *Lasiodora* sp. hemocyte extract, a partially purified pool from Trypsin-Sepharose chromatography (T) and isolated elastase inhibitor (EILaH). TPA: tissue plasminogen activator. HNE: human neutrophil elastase.

Hemocytes of *Lasiodora* sp. were also investigated as source of protease inhibitors by Soares et al. (2011).The authors showed that the hemocyte extract was able to inhibit chymotrypsin, trypsin, tissue plasminogen activator, urokinase and mainly human neutrophil elastase (Figure 4). Then, they isolated an antibacterial elastase inhibitor (called EILaH) using affinity chromatography on Trypsin-Sepharose and reversed-phase chromatography. The Figure 5 shows the protein profile of hemocyte extract, Trypsin-Sepharose fraction and isolated inhibitor and Table 2 shows that the inhibitor was active only on *Enterococcus faecalis*.

Table 2. Bacteriostatic activity of preparations from *Lasiodora* sp. hemocytes

Preparation	Bacteria	Minimal Inhibitory Concentration (μg/ml)
Hemocyte extract	*Bacillus subtilis* ATCC-6633	3400
	Enterococcus faecalis ATCC-6057	3400
	Escherichia coli ATCC-25922	NI
	Klebsiella pneumoniae ATCC-29665	NI
	Staphylococcus aureus ATCC-6538	NI
EILaH	*Bacillus subtilis* ATCC-6633	NI
	Enterococcus faecalis ATCC-6057	227.5
	Escherichia coli ATCC-25922	NI
	Klebsiella pneumoniae ATCC-29665	NI
	Staphylococcus aureus ATCC-6538	NI

NI: no inhibition. Reference: Soares et al. (2011).

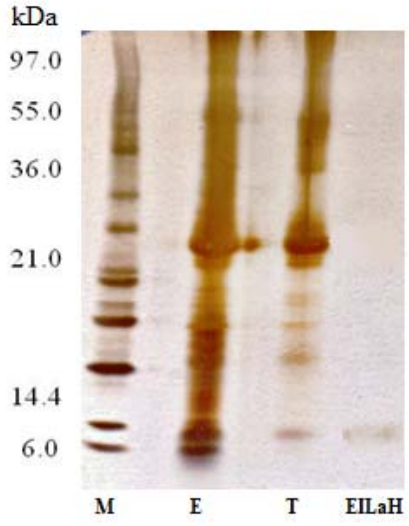

Figure 5. Polyacrylamide gel (12%, w/v) electrophoresis in presence of sodium dodecyl sulfate (SDS-PAGE). Molecular mass standard (M). Extract of *Lasiodora* sp. hemocytes (E), Trypsin-Sepharose column fraction (T) and isolated human elastase inhibitor (EILaH). The isolation of EILaH is described by Soares et al. (2011).

CONCLUSION

Hemocytes and molecules from hemolymph are important components of immunity of spiders, being responsible for phagocytosis, nodulation, encapsulation, coagulation, melanization, and cytotoxicity mechanisms that act against microorganisms. Several types of hemocytes can be found in tarantula spiders, including the *Lasiodora* sp. The identification ofhemocyte types and the isolation of a protease inhibitor from hemocytes of *Lasiodora* sp. were recent contributions for the systematic review of this genus.

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