# *Trypanosoma rangeli* interactions within the vector *Rhodnius prolixus* - A mini review

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*This article is an integrative mini review of the research on the interactions between* Trypanosoma rangeli *and the insect vector,* Rhodnius prolixus. *Special attention is given to the interactions of these parasites with the gut environment, gut walls, with hemolymph invasion, hemocytes, hemocyte microaggregations, prophenoloxidase-activating system, superoxide, and nitric acid generation and eicosanoid pathways. We described factors affecting vectorial capacity and suggested that* T. rangeli *may modulate the hemocoelic invasion and the survival of the parasites by overcoming the cellular and humoral defense reactions of the insect vector at different physiological events. The mechanisms of these interactions and their significance for parasite transmission are discussed.*

Key words: *Rhodnius prolixus* - *Trypanosoma rangeli* - lectins - prophenoloxidase - hemocyte agglutination - superoxide - nitric acid - eicosanoids

*Rhodnius prolixus* is a strictly hematophagous triatomine of the order Hemiptera, known for its remarkable ability to ingest enormous meals in a short period of time. Blood feeding is essential for all five instar nymphs and adult insects as a protein source to complete their development and egg production (Buxton 1930). *R. prolixus,* like other insects, are constantly challenged in a world full of microbial pathogens. The environment and their hematophagous behavior make these insects potential transmitters of many pathogens, including *Trypanosoma cruzi*, the causative agent of Chagas disease, (Garcia & Azambuja 1991, Kollien & Schaub 2000), and *T. rangeli*, which is apparently harmless to humans but can be pathogenic to the insect vector (Watkins 1971, Hecker et al. 1990). Unlike *T. cruzi*, which develops in the gut of its invertebrate hosts, *T. rangeli* also develops in the gut, but commonly invades the hemolymph of the insect vector (Watkins 1971, Hecker et al. 1990). Interestingly, it has been observed that triatomines have different susceptibility to different strains; for example strains originated in Colombia and Costa Rica have distinct kinetics of development with hemocelic and salivary glands invasions, depending on species of the genus *Rhodnius* (D' Alessandro 1976).

In a recent review, Vallejo et al. (2002) and Guhl and Vallejo (2003) revealed some biochemical and molecular aspects of *T. rangeli* and discussed about two major phylogenetic lineages having different characteristics, which can lead to a better understanding of the epidemiology and interactions of this parasite and the triatomine vector.

A potential new approach for controlling parasite transmission is to interrupt its biological cycle in the insect vector by targeting mechanisms fundamental for the establishment and development of parasite infection. Such strategy requires detailed investigation of the interactions between parasites and their insect vectors, especially identifying and characterizing key molecules and/or pathways that are crucial for successful development of the parasites in the invertebrate hosts and their transmission to mammals.

The main point of this mini review is to focus on the interactions of the insect's immune reactions and other systems, that may influence the outcome of *T. rangeli* infection in the vector and therefore be potential targets for new approaches and technologies to block parasite transmission.

## *Trypanosoma rangeli* and gut interactions

In the *T. rangeli* and *R. prolixus* interactions, the point of contact between parasite and vector begins with the ingestion of bloodstream trypomastigotes by the insect vector during the feeding process. After ingestion, *T. rangeli* transforms into epimastogote form, multiplies in the gut of its insect vector, and invades the hemolymph to continue the establishment of its infection and development in the salivary glands, where metacyclogenesis takes place (Watkins 1971, Hecker et al. 1990). To complete its biological cycle in nature, *T. rangeli* is transmitted to the vertebrate host through salivary secretion during feeding (Garcia et al. 1994). The basis of *T. rangeli* infectivity in the gut of *R. prolixus* is still poorly understood. The parasite engages in a series of interactions involving differentiation, multiplication, and parasite penetration through epithelial cells of the digestive tract for entering into the hemocoel. Since the gut of *R. prolixus* is the first environment for the establishment of *T. rangeli* infection, the influence of digestive enzymes and stomach lytic factor (Azambuja et al. 1983, 1989, 2004) on parasite development remains as possibility. But it is still not clear whether the proteolytic enzymes and hemolytic factor are implicated in *T. rangeli* development in the inver-

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tebrate host. It was demonstrated that feeding *R. prolixus* with the SH-proteinase inhibitor, pepstatin, had no effect on the rates of *T. cruzi* infection (Garcia & Gilliam 1980), and *T. rangeli* growth, even in an aggressive environment against *T. cruzi* (Azambuja & Garcia, unpublished results). Mello et al. (1996) demonstrated differences in the development of three strains of *T. cruzi* in the gut of *R. prolixus*, and related the infectivity of these to the ability of the gut lectins to agglutinate the parasites in vitro. However, the effects of gut lectins on *T. rangeli* interactions were not yet investigated.

The midgut epithelium is the main barrier against invasion by the parasite. In the process of interaction of *T. rangeli* with gut epithelial cells of *R. prolixus*, the parasites attach to the surface of some epithelial cells either through the flagellum, mainly in the areas of contact between two epithelial cells, or through the posterior end of the protozoan, especially to the perimicrovilar membranes and penetrate into cells that show less dense cytoplasm organelles (Oliveira & Souza 2001). The ultrastructural disorganization of the midgut epithelial cells, mainly the perimicrovillar membranes and microvilli, facilitates *T. rangeli* invasion of the hemolymph (Gomes et al. 2002). The flagellates pass through the gut epithelium by intracellular route (Hecker et al. 1990). After damaging the surface, the parasite moves within the cytoplasm of the epithelial cell, reaches the basal region, crosses the basal lamina, and enters into the hemocoel (Oliveira & Souza 2001). However, the penetration rate of the gut is low, only less than 10% of parasites pass through the gut cell wall (Hecker et al. 1990).

### *Trypanosoma rangeli* and hemolymph interactions

Several laboratories have investigated hemolymph cellular and humoral responses involved in parasite-vector interactions. Details of the molecules and signaling pathways of the defense reactions are still being elucidated. In many insect species, cellular immune responses to pathogens have been related to two kinds of reactions: (i) nodulation and encapsulation, and (ii) phagocytosis by hemocytes (Ratcliffe & Rowley 1979).

Once in the hemocoel, *T. rangeli* may uptake lipophorin, the main lipid-transporting particle, and localize close to the flagellar pocket and in vesicles at the posterior region of the parasite body. Interestingly, the parasites do not uptake any other hemolymphatic protein (Folly et al. 2003). Furthermore, the parasite may penetrate into the hemocyte or freely survive and multiply in the hemolymph. Under these conditions the parasite can be recognized and activates the immune reactions of its insect vector. The hemocytes, such as plasmatocytes, are able to ingest epimastigote forms of the parasite, which are then found within parasitophorus vacuoles. Apparently, fusion of the host cell lysosomes with the vacuole takes place. Intravacuolar parasites in process of digestion but no dividing parasites are seen within the vacuole (Oliveira & Souza 2003) in contrast to what is observed outside the host cells in the hemolymph, where parasites can intensively multiply (Tobie 1970, Mello et al. 1995, Garcia et al. 2004a,b). In addition, during the course of infection, some plasmatocytes appeared disrupted, showing released parasites (Oliveira & Souza 2003).

Humoral immune reactions more frequently investigated in these interactions are: lysozymes, lectins and trypanolytic activity (Gregorio & Ratcliffe 1991a, Mello et al. 1995), prophenoloxidase (proPO) activation (Gregorio & Ratcliffe 1991b, Mello et al. 1995, Gomes et al. 2003, Garcia et al. 2004a,b), hemolymph agglutination (Gregorio & Ratcliffe 1991a, Mello et al. 1996), superoxide and nitric oxide generation (Whitten et al. 2001) and eicosanoid pathways (Garcia et al. 2004a,b).

Gregorio and Ratcliffe (1991a) investigated hemolymph and extracts of different tissue from *R. prolixus* and *Triatoma infestans* and tested for agglutination and lytic activities against erythrocytes and culture epimastigote forms of *T. rangeli*. Agglutination activity against rabbit erythrocytes was found in the crop, midgut, and hindgut extracts of *T. infestans* but only in the hemolymph of *R. prolixus*. In addition, the extracts of *T. infestans* salivary glands, but not those of *R. prolixus*, showed a lytic activity against *T. rangeli* (Gregorio & Ratcliffe 1991a). They concluded that *T. infestans*, which is refractory to infection by *T. rangeli*, appears to contain a much wider distribution of agglutinins and trypanolytic factors in its tissue than the susceptible species, *R. prolixus*. Amino et al. (2002) characterized a pore-forming lytic protein from the saliva of *T. infestans*. This protein, named trialysin, lysed protozoan parasites and bacteria indicating that it plays a role in the control of microorganism growth in the salivary glands.

Takle (1988) described that the hemocyte aggregation process following injection of *T. rangeli* appears only a few days after *R. prolixus* infection, when free trypanosomes are abundant. Mello et al. (1995) demonstrated that after inoculation of *T. rangeli* into the hemocoel of *R. prolixus*, the parasite engages in high rates of infection through successive division during the extracellular development, and increases the lysozyme activity levels in the hemolymph. They also showed that *T. rangeli* infection neither induced trypanolytic nor peptide antibacterial activities. More recently, a galactose-binding lectin from *R. prolixus* hemolymph, which enhanced the activation of clump formation by *T. rangeli* in *R. prolixus* hemocyte monolayers, with an increase in clump size and hemocyte aggregation, was described (Mello et al. 1999). This purified lectin also affected the motility and survival of *T. rangeli* culture short forms, but not the long forms, which predominated in the hemolymph after two days of inoculation (Mello et al. 1995), when they were incubated in vitro (Mello et al. 1999).

An important biological event in the *T. rangeli* cycle in the invertebrate host is its ability to activate the proPO system of *R. prolixus*. Gregorio and Ratcliffe (1991b) demonstrated that *T. infestans*, but not *R. prolixus* preparations, present a very active proPO system when activated by laminarin and lipopolysaccharides. For both species of insects neither *T. rangeli* from culture nor parasite lysates were able to trigger proPO activation in vitro. However, the presence of the parasite in *R. prolixus* hemolymph/laminarin assays reduced the level of proPO activation. These authors suggest that the susceptibility of

*R. prolixus* to *T. rangeli* hemolymph infection may, at least in part, be explained by the suppression of the inset immune defense system i.e. inhibition of the proPO in the presence of this parasite. Interestingly, Gomes et al. (1999) clearly demonstrated using in vitro experiments that the activation of the proPO pathway occurred when the hemolymph was incubated with fat body homogenates and short epimastigote forms of *T. rangeli*. The same authors showed using in vivo experiments that short, but not long, epimastigotes activated directly the formation of melanin (Gomes et al. 1999). In addition, insects previously feeding on blood containing either short or long epimastigotes were able to suppress the proPO-activating pathway induced by thoracic inoculation of the short forms, indicating that the reduction of the PO activity was a result of parasite ingestion. It was suggested that oral *T. rangeli* infection in *R. prolixus* might facilitate the establishment of the infection in the hemolymph of the insect vector (Gomes et al. 2003).

Our laboratory is investigating the role of complex glycoconjugates in the vector-parasite interactions. Using glycosylphosphatidylinositol (GPI)-anchors, specifically glycoinositolphospholipids (GIPLs) and GPI-mucins purified from *T. rangeli* epimastigotes, we demonstrated that both compounds activated proPO pathway (Gomes et al. 2001). In addition, GIPLs and GPI-mucins when inoculated into *R. prolixus* also induced the formation of hemocyte microaggregates, while membrane phospholipids had no effect upon this physiological event (Gomes & Azambuja, unpublished data).

One of the most exciting outcomes in the investigation of *T. rangeli* in triatomines is its ability to relate to superoxide and nitric acid generation and eicosanoid pathways in the insect vector. Whitten et al. (2001) studied the immune responses of *R. prolixus* in vivo following the injection of two strains and two developmental forms of *T. rangeli*. They observed that after 24 h the H14 strain, which failed to multiply and invade the salivary glands, stimulated higher levels of proPO, superoxide and nitrites than the Choachi strain, which rapidly multiplied in the hemolymph to invade the salivary glands. Usually, short forms of epimastigotes stimulated greater superoxide and proPO responses than long epimastigotes in both parasite strains in the hemolymph of *R. prolixus*. In addition, when the NADPH oxidase inhibitor, N-ethylmaleimide, or the inducible nitric oxide synthase inhibitor, S-methyl isohiourea sulfamide, are inoculated into *R. prolixus*, they caused higher insect mortalitiy after inoculation with parasites of either strains compared with those of uninfected control insects (Whitten et al. 2001). This indicates that both NADPH oxidase and nitric oxide synthase activities may be involved in the immune responses of *R. prolixus* infection by *T. rangeli*. In this insect, nitric oxide synthase gene expression and nitrite/nitrate levels are modulated in the midgut epithelial cells and fat body following in vivo challenge with *T. cruzi* or *T. rangeli* (Ratcliffe, personal information).

With so many variables involved, we investigated the importance of the eicosanoid pathways in the *T. rangeli* interactions within its vector, *R. prolixus*. Eicosanoids are known to mediate cellular reaction in insects inoculated with bacteria, fungi (Stanley 2000), and parasitoids (Carton et al. 2002). We have shown that eicosanoid pathway is also involved in the microaggregates formation in *R. prolixus* inoculated with *T. rangeli* (Garcia et al. 2004a,b). In one experimental protocol, designed to test the effects of eicosanoid biosynthesis inhibitors, we demonstrated that hemocoelic injection of *T. rangeli* epimastigotes into insects that were previously fed on blood containing an inhibitor of phospholipase A2, dexamethasone, a specific inhibitor of the cyclooxygenase pathway, indomethacin, and a non-selective lipoxygenase inhibitor, NDGA, besides reducing hemocyte microaggregation, attenuated the proPO system in the hemolymph and consequently, enhanced parasitemia and mortality induced by the parasite challenge (Garcia et al. 2004a). We also demonstrated that all the effects obtained by dexamethasone administered orally were counteracted by injection with arachidonic acid, and suggested that *Rhodnius*' immune responses to the parasite infection might be modulated by a physiological system that includes eicosanoid biosynthesis pathways (Garcia et al. 2004a).

In another approach, Garcia et al. (2004b) demonstrated a reduced number of hemocyte microaggregations, enhanced number of parasites in the hemolymph as well as increased mortality in insects fed on blood containing *T. rangeli* epimastigotes and challenged with the same parasite. All these effects were reversed by the combined inoculation of *R. prolixus* with *T. rangeli* together with arachidonic acid. Similar results could be observed in in vitro assays using hemolymph taken from insects previously fed on blood containing parasites i.e. hemocyte microaggregation reactions were attenuated when *T. rangeli* was used as inducer of the reaction (Garcia et al. 2004b).

### *Trypanosoma rangeli* and salivary glands interactions

The salivary glands of *R. prolixus* are infected by *T. rangeli* after successful penetration of the gut epithelium into the hemocoel. Bassery et al. (2002) investigated the interaction between *T. rangeli* and *R. prolixus* salivary glands. They demonstrated that in in vitro adhesion inhibition assays using long epimastigote forms, some sugars were able to block the receptors on both the surfaces of the salivary glands and on *T. rangeli*. Among the sugars tested, N-acetylglicosamine, N-acetylgalactosamine, and galactose showed the highest inhibitory effect on the adhesion tests. They suggested that the carbohydrates and lectins are important to parasite-vector interactions, mainly for facilitating parasite invasion of the salivary glands of the invertebrate host. After attachment to the surface, the flagellates penetrate the outer membranes of the salivary glands, disrupting the inner layer to pass between the muscle cells and reach the gland cell basement membrane. This latter is also penetrated by the parasite flagellum and invaginates the gland cell to create a vacuole in which the trypanosome crosses the gland cells to reach the central lumen, often only losing its involving vacuole just before leaving the cell (Ellis et al. 1980).

Interestingly, infected salivary glands reduced both the apirase activity and the nitric oxide content, two im-

portant components of the saliva, which help the insect finding the blood vessel during the probing phase to initiate feeding (Garcia et al. 1994). If this is true, insects with salivary gland infection should have some difficulty to feed. This was exactly what we observed: insects with salivary glands infected with *T. rangeli* pierce the host skin more and frequently draw less blood and at a slower rate than uninfected controls when feeding on rabbits (Garcia et al. 1994). Undoubtedly, the repeated piercing for locating blood vessels and attempts of sucking more blood facilitate the transmission of *T. rangeli* to the vertebrate host. However, the difficult blood ingestion is not the reason for the increase in the mortality of insects infected with *T. rangeli*. Probably, the pathogenic effects of *T. rangeli* on *R. prolixus* are due to mechanical lesions leading to a loss of cytoplasm in gut epithelial cells and in muscle from salivary glands during the penetration of high number of parasites (Hecker et al. 1990, Garcia et al. 1994). With these data at hands, Garcia et al. (1994) hypothesized that *T. rangeli* infection modulates the insect vector ability to locate blood vessels by affecting the salivary antihemostatic properties, consequently enhancing the probability of intradermal inoculation of parasites into mammalian hosts.

Recently our understanding of the interactions between *T. rangeli* and the insect vector, *R. prolixus*, has increased tremendously. Even so, the demand for this kind of study is increasing daily with the need to find new methods of vector control. Factors affecting vectorial capacity have been characterized in this insect and research on this respect is ongoing. The molecules that are involved positively and negatively in these interactions are complex. These complexities are reflected by the interactions between agglutinins, enzymes, proPO system, superoxide and nitric generation and eicosanoid pathways and the flagellate *T. rangeli*. On the basis of the identification and characterization of molecules and pathways that influence the establishment of a *T. rangeli* infection, we begin to understand the network and the role these components play in the parasite-insect vector interactions.

## REFERENCES


Amino R, Martins RM, Procópio J, Hirata IY, Juliano MA, Schenkman S 2002. Trialysin, a novel pore-forming protein from saliva of hematophagous insects activated by limited proteolysis. *J Biol Chem 277*: 6207-6213.

Azambuja P, Feder D, Garcia ES 2004. Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: impact on the establishment of the parasite *Trypanosoma cruzi* in the vector. *Exp Parasitol 107*: 89-96.

Azambuja P, Guimarães JA, Garcia ES 1983. Haemolytic factor from the crop of *Rhodnius prolixus*: evidence and partial characterization. *J Insect Physiol 11*: 833-837.

Azambuja P, Mello CB, D´Escoffier LN, Garcia ES 1989. *In vitro* cytotoxicity of *Rhodnius prolixus* hemolytic factor and mellitin towards different trypanosomatids. *Braz J Med Biol Res 22*: 597-599.

Basseri HR, Tew IF, Ratcliffe NA 2002. Identification and distribution of carbohydrate moieties on the salivary glands of *Rhodnius prolixus* and their possible involvement in attachment/invasion by *Trypanosoma rangeli*. *Exp Parasitol 100*: 226-234.

Buxton PA 1930. Biology of the blood-sucking bug, *Rhodnius prolixus*. *Trans Ent Soc London 78*: 227-236.

Carton Y, Frey S, Stanley DW, Vass E, Nappi AJ 2002. Dexamethasone inhibition of the cellular immune response of *Drosophila melanogaster* against a parasitoid. *J Parasitol 88*: 405-407.

D'Alessandro A 1976. Biology of *Trypanosoma (Herpetosoma) rangeli* Tejera. 1920. In WHR Lumsden, DA Evans (eds), *Biology of Kinetoplastida*, Vol.1, Academic Press, London, p. 327-493.

Ellis DS, Evans DA, Stamford S 1980. The penetration of the salivary glands of *Rhodnius prolixus* by *Trypanosoma rangeli*. *Z Parasitenkd 62*: 63-74.

Folly E, Cunha e Silva NL, Lopes AH, Silva-Neto MA, Atella GC 2003. *Trypanosoma rangeli* uptakes the main lipoprotein from the hemolymph of its invertebrate host. *Biochem Biophys Res Commun 10*: 555-561.

Garcia ES, Azambuja P 1991. Development and interactions of *Trypanosoma cruzi* within the insect vector. *Parasitol Today* 7: 240-244.

Garcia ES, Gilliam FC 1980. *Trypanosoma cruzi* development is independent of the protein digestion in the gut of *Rhodnius prolixus*. *J Parasitol 66*: 1052-1053.

Garcia ES, Machado EMM, Azambuja P 2004a. Effects of eicosanoid biosynthesis inhibitors on the prophenoloxidase-activating system and microaggregation reactions in the hemolymph of *Rhodnius prolixus* infected with *Trypanosoma rangeli*. *J Insect Physiol 50*: 157-165.

Garcia ES, Machado EMM, Azambuja P 2004b. Inhibition of hemocyte microaggregation reactions in *Rhodnius prolixus* larvae orally infected with *Trypanosoma rangeli*. *Exp Parasitol 107*: 31-38.

Garcia ES, Mello CB, Azambuja P, Ribeiro JMC 1994. *Rhodnius prolixus*: salivary anti-hemostatic components decrease with *Trypanosoma rangeli* infection. *Exp Parasitol 78*: 287-293.

Gomes SAO, Almeida IC, Oliveira MF, Garcia ES, Azambuja P 2001. Prophenoloxidase activation by *Trypanosoma rangeli* in *Rhodnius prolixus*: effects of membrane components isolated from short and long epimastigotes. *Soc Bras Protozool 1*: 47-48.

Gomes SAO, Feder D, Thomas NE, Garcia ES, Azambuja P 1999. *Rhodnius prolixus* infected with *Trypanosoma rangeli*: *in vivo* and *in vitro* experiments. *J Invertebr Pathol 73*: 289-293.

Gomes SAO, Feder D, Garcia ES, Azambuja P 2003. Suppression of the prophenoloxidase system in *Rhodnius prolixus* orally infected with T*rypanosoma rangeli*. *J Insect Physiol 49*: 829-837.

Gomes SAO, Graciano GL, Nogueira NF, Souza W de, Garcia ES, Azambuja P 2002. Effects of gamma irradiation on the development of T*rypanosoma rangeli* in the vector *Rhodnius prolixus*. *J Invertebr Pathol 79*: 86-92.

Gregorio EA, Ratcliffe NA 1991a. The distribution of agglutinins and lytic activity against *Trypanosoma rangeli* and erythrocytes in *Rhodnius prolixus* and *Triatoma infestans*

tissue extracts and haemolymph. *Mem Inst Oswaldo Cruz 86*: 181-186.

Gregorio EA, Ratcliffe NA 1991b. The prophenoloxidase system and *in vitro* interaction of T*rypanosoma rangeli* with *Rhodnius prolixus* and *Triatoma infestans* haemolymph. *Parasite Immunol 13*: 551-564.

Guhl F, Vallejo A 2003. *Trypanosoma* (*Herpetosoma*) *rangeli* Tejera, 1920 – An updated review. *Mem Inst Oswaldo Cruz 98*: 435-442.

Hecker H, Schwarzenbach M, Rudin W 1990. Development and interactions of *Trypanosoma rangeli* in and with the reduviid bug, *Rhodnius prolixus*. *Parasitol Res 76*: 311-318.

Kollien AH, Schaub GA 2000. The development of *Trypanosoma cruzi* in Triatominae. *Parasitol Today 16*: 381-387.

Mello CB, Garcia ES, Ratcliffe NA, Azambuja P 1995. *Trypanosoma cruzi* and *Trypanosoma rangeli*: interplay with hemolymph components of *Rhodnius prolixus*. *J Invertebr Pathol 65*: 261-268.

Mello CB, Azambuja P, Garcia ES, Ratcliffe NA 1996. Differential *in vitro* and *in vivo* behavior of three strains of *Trypanosoma cruzi* in the gut and hemolymph of *Rhodnius prolixus*. *Exp Parasitol 82*: 112-118.

Mello CB, Nigam Y, Garcia ES, Azambuja P, Newtin RP, Ratcliffe NA 1999. Studies of a hemolymph lectin isolated from *Rhodnius prolixus* and its interaction with *Trypanosoma rangeli*. *Exp Parasitol 91*: 289-296.

Oliveira MA de, Souza W de 2001. An electron microscopic study on penetration of *Trypanosoma rangeli* into the midgut cells of *Rhodnius prolixus*. *J Invertebr Pathol 77*: 22-26.

Oliveira MA de, Souza W de 2003. Further morphological studies on the behavior of *Trypanosoma rangeli* in the hemocytes of *Rhodnius prolixus*. *Parasitol Int 52*: 299-307.

Ratcliffe NA, Rowley AF 1979. Role of hemocytes in defence against biological agents. In AP Gupta, *Insect Hemocytes Development, Forms, Functions, and Techniques*, Cambridge University Press, Cambridge, p. 331-414.

Stanley DW 2000. *Eicosanoids in Invertebrate Signal Transduction Systems*, Princeton University Press, Princeton, 125 pp.

Takle GB 1988. Studies on the cellular immune response of insects toward the insect pathogen *Trypanosoma rangeli*. *J Invertebr Pathol 51*: 64-72.

Tobie EJ 1970. Observation on the development of *Trypanosoma rangeli* in the hemocoel of *Rhodnius prolixus*. *J Invertebr Pathol 16*: 118-125.

Vallejo GA, Guhl F, Carranza JC, Lozano LE, Sánchez JL, Jaramillo JC, Gualtero D, Castañeda N, Silva JC, Steindel M 2002. kDNA markers define two major *Trypanosoma rangeli* lineages in LatinAmerica. *Acta Trop 81*: 77-82.

Watkins R 1971. Histology of *Rhodnius prolixus* infected with *Trypanosoma rangeli*. *J Invertebr Pathol 17*: 59-66.

Whitten MM, Mello CB, Gomes SAO, Nigam Y, Azambuja P, Garcia ES, Ratcliffe NA 2001. Role of superoxide and reactive nitrogen intermediates in *Rhodnius prolixus* (Reduviidae)/*Trypanosoma rangeli* interactions. *Exp Parasitol 98*: 44-57.