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Study of the Salivary Glands in Triatominae (Hemiptera, Reduviidae, Triatominae): Their Color and Application to the Chagas Disease Vector Evolution

Jader de Oliveira,[1]* Amanda Ravazi,[2] Eder dos Santos Souza,[1] Felipe Ferraz Figueiredo Moreira,[3] Cleber Galvão,[4] João Aristeu da Rosa,[1] and Kaio Cesar Chaboli Alevi[2]

[1]*Laboratório de Parasitologia, Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (FCFAR/UNESP), Araraquara, Brazil;* [2]*Laboratório de Biologia Celular, Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (IBILCE/UNESP), São José do Rio Preto, Brazil;* [3]*Laboratório de Biodiversidade Entomológica, Instituto Oswaldo Cruz (LABE/IOC/FIOCRUZ), Rio de Janeiro, Brazil;* [4]*Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz (LNIRTT/IOC/FIOCRUZ), Rio de Janeiro, Brazil*

Abstract. Chagas disease is caused by *Trypanosoma cruzi* and transmitted by feces of a triatomine that has the habit of defecating during blood feeding. The salivary glands of triatomines are important to hematophagy because their saliva is rich in anticoagulant and hemolytic proteins. The salivary glands of some *Rhodnius* species analyzed are reddish due to the presence of nitrophorins (antihemostatic activity). The present study aimed to analyze the color pattern of the salivary glands of 67 triatomine species to evaluate whether the presence of nitrophorins is a synapomorphy of *Rhodnius* or the tribe Rhodniini, or if it is shared with triatomines of the tribes Triatomini and Cavernicolini. Since only the species of the tribe Rhoniini present red glands, it is admitted that the presence of nitrophorin proteins is a synapomorphy of the tribe Rhodniini and that this tribe has derived more recently when compared with Triatomini and Cavernicolini.

Chagas disease is a vector-borne and potentially life-threatening illness caused by the protozoan *Trypanosoma cruzi* (Chagas, 1909). It occurs mainly in endemic areas in 21 Latin American countries, where it is transmitted to humans mostly by contact with feces of triatomines, known as "kissing bugs." It is estimated that about 6–7 million people are infected worldwide, mostly in Latin America.[1]

Currently, there are 151 species of triatomines distributed in 18 genera and five tribes, all species being considered potential vectors of Chagas disease.[2,3] As Chagas disease has no cure and treatment with benznidazole and nifurtimox is effective only in the acute phase of the disease (which is often asymptomatic), vector control is the most effective method of preventing this neglected disease.[1] Thus, all knowledge about these hematophagous insects is important and can help and improve vector control programs.

Although the transmission of *T. cruzi* to the host occurs mostly through the feces of triatomines, hematophagous behavior is fundamental to the contamination with the protozoan, as such insects have the habit of defecating during blood feeding.[2] It is believed that hematophagy was derived from ancestral generalist predators that initiated this behavior as opportunistic hematophagy, then it became facultative and finally evolved to mandatory hematophagy.[4]

The salivary glands of triatomines perform a fundamental role during hematophagy because their saliva is rich in proteins and anticoagulant and hemolytic enzymes.[5,6] These structures have been studied anatomically,[7] histologically,[7] biochemically,[5] molecularly,[8] and cytogenetically[9] based on a few triatomines of the genera *Triatoma*, *Rhodnius*, and *Panstrongylus*.

Based on the analysis of *Rhodnius prolixus*, it has been suggested that the salivary glands (principal glands) of the species of the genus *Rhodnius* are reddish.[10] This characteristic results from the presence of nitrophorins,[10] which are proteins with antihemostatic activity[11] whose molecules contain a heme group responsible for that color. The relationship between red salivary glands and the presence of nitrophorins has recently been confirmed by Pacheco[12] which inoculated nitrophorin inhibitors into *Rhodnius* eggs and the adult salivary glands were transparent.

Considering that the presence of nitrophorins is suggested for all species of the genus *Rhodnius*,[13] and only *R. prolixus*,[13] *Rhodnius robustus*,[13] and *Rhodnius domesticus*[14] have been studied, the present study aimed to analyze the color pattern of the salivary glands of 67 triatomine species, distributed in 10 different genera and grouped into three tribes, to evaluate whether the presence of nitrophorins is a synapomorphy of *Rhodnius* or the tribe Rhodniini, or if it is shared with triatomines of the tribes Triatomini and Cavernicolini.

At least two adult specimens of each species (males and/or females) were analyzed (tribe Cavernicolini: *Cavernicola pilosa*; tribe Rhodniini: *Psammolestes tertius*, *Psammolestes coreodes*, *Psammolestes arthuri*, *Rhodnius brethesi*, *Rhodnius colombiensis*, *R. domesticus*, *Rhodnius ecuadoriensis*, *Rhodnius marabaensis*, *Rhodnius milesi*, *Rhodnius montenegrensis*, *Rhodnius nasutus*, *Rhodnius neglectus*, *Rhodnius neivai*, *Rhodnius pallescens*, *Rhodnius pictipes*, *R. prolixus*, *R. robustus, Rhodnius stali*, tribe Triatomini: *Dipetalogaster maxima*, *Eratyrus cuspidatus*, *Meccus pallidipennis*, *Meccus longipennis*, *Meccus picturata, Meccus phylossoma*, *Mepraia spinolai*, *Nesotriatoma bruneri* sn *Nesotriatoma flavida*, *Panstrongylus herreri* sn *Panstrongylus lignarius*, *Panstrongylus lignarius*, *Panstrongylus megistus*, *Panstrongylus lutzi*, *Triatoma arthurneivai*, *Triatoma bahiensis*, *Triatoma baratai*, *Triatoma brasiliensis*, *Triatoma brasiliensis macromelasoma*, *Triatoma carcavalloi*, *Triatoma circummaculata*, *Triatoma costalimai*, *Triatoma delpontei*, *Triatoma dimidiata*, *Triatoma garciabesi*, *Triatoma guasayana*, *Triatoma guazu*, *Triatoma infestans*, *Triatoma juazeirensis*, *Triatoma jurbergi*, *Triatoma klugi*, *Triatoma lectularia*, *Triatoma lenti*, *Triatoma maculata*, *Triatoma matogrossensis*, *Triatoma melanica*, *Triatoma melanocephala*, *Triatoma petrocchiae*, *Triatoma platensis*, *Triatoma protracta,*

* Address correspondence to Jader de Oliveira, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista (FCFAR/UNESP), Rodovia Araraquara-Jaú km 1, 14801-902, Araraquara, Sao Paulo, Brazil. E-mail: jdr.oliveira@hotmail.com

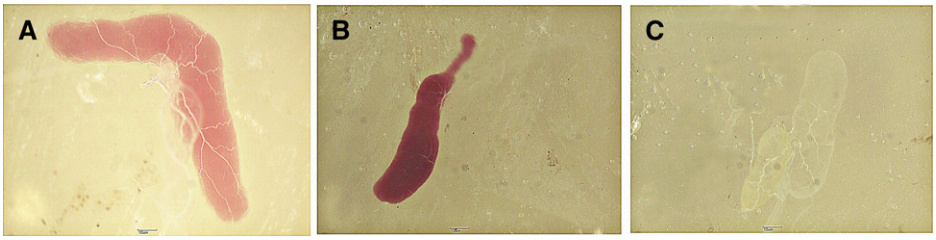

FIGURE 1. Salivary glands of (**A**) *Psammolestes tertius*, (**B**) *Rhodnius montenegrensis*, and (**C**) *Triatoma infestans*. Note the red glands in (**A**) *P. tertius* and (**B**) *R. montenegrensis* (tribe Rhodniini). Bar = 10 μm. This figure appears in color at www.ajtmh.org.

Triatoma pseudomaculata, *Triatoma pintodiasi*, *Triatoma rubrovaria*, *Triatoma sherlocki*, *Triatoma sordida*, *Triatoma tibiamaculata*, *Triatoma vandae*, *Triatoma vitticeps*, *Triatoma williami*, *Triatoma wygodzinskyi*). The specimens were provided by the Triatominae Insectarium of Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Araraquara, São Paulo, Brazil, and the Insectarium of the National and International Triatominae Taxonomy Reference Laboratory at Fiocruz, Rio de Janeiro, Brazil. The bugs were dissected and the salivary glands were then removed and examined by stereoscope microscope.

Through the analysis of the salivary glands, it was observed that the species of the tribe Rhodniini present red glands (represented by *P. tertius* [Figure 1A] and *R. montenegrensis* [Figure 1B]). On the other hand, all the other species analyzed exhibited transparent glands (represented by *T. infestans* [Figure 1C]).

It is estimated that the tribes Triatomini and Rhodniini diverged at 48.9–64.4 mya, when South America was already separated from Africa.[15] Recently, it was suggested that the uplift of the Andes in South America and the variations in sea levels in North America are the events involved in the diversification of these tribes.[16] Nitrophorin heme proteins could have appeared (and later they were positively selected) after the divergence of the tribes, more specifically in the common ancestor of the tribe Rhodniini.

Although there is no dating of the divergence of the tribe Cavernicolini, recently this tribe was presented as a brother group of Rhodniini.[16] However, the absence of nitrophorins in the salivary glands of *C. pilosa* suggest that this tribe derived before Rhodniini. A possible conclusion is that the tribe Triatomini derived first, followed by Cavernicolini, and finally came the tribe Rhodniini. This highlights the need for studies using molecular clocks in Triatominae with representatives of all the tribes.

One of the antihemostatic activities of the nitrophorins is the storage and transport of nitric acid ligated into the center of ferric heme,[6] which promotes vasodilation and inhibition of platelet aggregation when it is released in the microcirculation.[17] This study demonstrates that the species of the tribes Triatomini and Cavernicolini do not have this heme protein in the composition of their salivary glands. In the few studies that characterize the salivary glands of other genera of the tribe Triatomini, the substances isolated were triabin and pallidipin in *M. pallidipennis*[18]; triafestins, triplatin, and trialysin in *T. infestans*[19,20]; procalin in *T. protracta*[20]; dipetalodipin in *D. maxima*[20]; and lipocalin in *T. lectularia*[8] and *P. herreri*.[8]

Therefore, this study highlights the presence of nitrophorin proteins as a synapomorphy of the tribe Rhodniini and suggests that this tribe has derived more recently when compared with the tribes Triatomini and Cavernicolini, which contributes to understanding the evolutionary history of this important vector group.

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Authors' addresses: Jader de Oliveira, Eder dos Santos Souza, and João Aristeu da Rosa, Laboratório de Parasitologia, Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (FCFAR/UNESP), Araraquara, Brazil, E-mails: jdr.oliveira@hotmail.com, ederss1@hotmail.com, and rosaja@fcfar.unesp.br. Amanda Ravazi and Kaio Cesar Chaboli Alevi, Laboratório de Biologia Celular, Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (IBILCE/UNESP), São José do Rio Preto, Brazil, E-mails: amandaravazi95@gmail.com and kaiochaboli@hotmail.com. Felipe Ferraz Figueiredo Moreira, Laboratório de Biodiversidade Entomológica, Instituto Oswaldo Cruz Fundacao Oswaldo Cruz, (LBE/IOC/FIOCRUZ), Rio de Janeiro, Brazil, E-mail: felipento@hotmail.com. Cleber Galvão, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, E-mail: clebergalvao@gmail.com.

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