



## Article/Artigo

# Comparative biology of the two sister species of Triatominae (Hemiptera: Reduviidae)

Biologia comparativa de duas espécies irmãs de Triatominae (Hemiptera: Reduviidae)

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### ABSTRACT

**Introduction:** *Triatoma pseudomaculata* and *T. wygodzinskyi* (Hemiptera: Reduviidae: Triatominae) are two Brazilian vectors of Chagas disease. The first is an arboricolous species in sylvatic environment and considered a vector of *T. cruzi* in peridomestic structures; the second, a rupicolous species in the wild environment of no epidemiological importance. In order to test the assumption that sister species share biological traits, comparative studies of their development cycle and blood ingestion were conducted. **Methods:** Eggs laid by virgin females of each species were randomly selected. The nymphs were observed daily and fed on mice weekly. The time required to pass through the different stages to adulthood was recorded in days. The triatomines were weighed individually before and after feeding. The mortality rate according to each nymphal stage was calculated. **Results and conclusions:** Analysis of the results shows that they display only minor biological differences even though they exhibit a distinct ecology. This suggests that the biological traits are important criteria to determine the relationship between species.

**Key-words:** *Triatoma wygodzinskyi*. *Triatoma pseudomaculata*. life cycle. feeding behavior.

### RESUMO

**Introdução:** *Triatoma pseudomaculata* e *T. wygodzinskyi* (Hemiptera: Reduviidae: Triatominae) são dois vetores Brasileiros da doença de Chagas. A primeira é uma espécie arbóricola em ambiente silvestre e considerada vetor do *T. cruzi* em estruturas peridomesticas. A segunda é rupícola em ambientes silvestres e sem importância epidemiológica. Com o objetivo de testar a hipótese que espécies irmãs compartilham características semelhantes, realizamos um estudo comparativo do ciclo biológico e ingestão alimentar. **Métodos:** Ovos pertencentes a cinco fêmeas de cada espécie provenientes do campo foram selecionados aleatoriamente. As ninfas foram observadas diariamente e alimentadas com camundongos semanalmente. O tempo requerido para passar até o estágio adulto foi registrado em dias. Os triatomíneos foram pesados individualmente antes e depois da alimentação. A mortalidade de cada estágio foi calculada. **Resultados e conclusões:** Análise dos resultados mostram que eles exibem pequenas diferenças biológicas apesar de apresentarem diferenças marcantes quanto a sua ecologia. Isso sugere que as características biológicas são critérios importantes para determinar a relação entre as espécies.

**Palavras-chaves:** *Triatoma wygodzinskyi*. *Triatoma pseudomaculata*. Ciclo de Vida. Hábito alimentar.

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### INTRODUCTION

Autochthonous species of Triatominae originally restricted to the wild environment are increasingly reported invading houses and peridomestic structures where they may act as vectors of *Trypanosoma cruzi* (Chagas, 1909), the agent of Chagas disease. Among these, *Triatoma pseudomaculata* (Corrêa & Espínola, 1964), an arboricolous species in sylvatic environment, is considered a vector of *T. cruzi* in peridomestic structures. Nevertheless, it does not display significant ability for colonizing human dwelling, as verified by the scarcity of domestic colonies notified indoors<sup>1,2,3</sup>. Recently, one case of *T. cruzi* transmission by *T. pseudomaculata* to a woman was reported in Ceará State<sup>4</sup>. Therefore, the role of this species as an effective vector needs to be accurately investigated. A second autochthonous species is *Triatoma wygodzinskyi* Lent, 1951 (morphologically very similar to *T. pseudomaculata*). This rupicolous species was described among a small number of specimens collected in southern Minas Gerais State<sup>5,6</sup>. Recently, genetic and morphometric comparisons suggest that both species belong to the same evolutionary lineage; consequently, they should be classified together in the same group<sup>7,8</sup>.

The development cycle of triatomines is related to the species and environmental conditions and is deeply influenced by the accessibility of adequate blood sources<sup>9</sup>. The hypothesis of this work is that sister species, such as *T. pseudomaculata* and *T. wygodzinskyi*, share biological traits. In order to test this assumption, comparative studies of the development cycle and blood ingestion of both species were conducted under the same laboratory conditions.

## METHODS

### Sample origin

*T. pseudomaculata* specimens were collected from peridomestic structures in the rural area of Curaçá (9°28' S, 39°44' W), Bahia State, while *T. wygodzinskyi* was captured in cracks between rocks near Vargem Grande do Sul, São Paulo State (21°79' S, 46°84' W). Once captured, the specimens were kept in the laboratory under a natural light/dark regime, at a temperature of  $28 \pm 2^\circ\text{C}$ , and  $70 \pm 5\%$  relative humidity.

### Development cycle

Eggs laid by ve old females of each species were randomly selected and individually placed in plastic containers (12 cm high and 3 cm diameter), with lter paper inside to provide the developing insects with access to the food source. Each container was numbered and examined daily until egg hatching. e nymphs were observed daily and fed on mice weekly (Protocol CEUA: P0100-01) and the time required to pass through the di erent stages to adulthood recorded in days (d). e development times (total and by stage) were expressed in median values.

### Blood ingestion

roughout the di erent development stages, the triatomines were weighed individually on an analytical balance (DAHUS®) before and a er feeding and the number of grams of blood ingested (mg) were calculated.

### Mortality

The mortality rate according to each nymphal stage was calculated.

### Statistical methods

For data interpretation concerning the development cycle and blood meal ingestion, normality and homogeneity of variances were assessed using the Levene and Bartle tests, respectively. e median variables were analyzed using the Mann-Whitney test. For statistical tests, we used JMP v.6.0.0 (SAS Institute Inc., 2005). For the Chi square test on mortality range, we used GraphPad InStat v.3.05 (GraphPad Software, San Diego, CA, USA).

## RESULTS

### Development cycle

e total life cycle was slightly longer in *T. pseudomaculata* than *T. wygodzinskyi* (232 and 205 days, respectively,  $p = 0.0014$ ). Nevertheless, interspecies di erences were only observed for egg and N5 duration, all the other stages showed similar durations (Table 1). Moreover, the di erences were exclusively observed among female nymphs (data not shown).

### Blood ingestion

*T. pseudomaculata* and *T. wygodzinskyi* needed a similar number of feeding periods (10 and 11, respectively) to reach the adult stage. No signi cant di erence was observed in the number of blood meals between both species (Table 2); however, the fourth and h nymphal instars of *T. pseudomaculata* ingested more blood than the corresponding stages in *T. wygodzinskyi* (Table 3).

### Mortality

The mortality rates for each nymphal instar are shown in Table 4. Starting with 74 nymphs of *T. wygodzinskyi*, only 31 reached

**TABLE 1** - Time of development (in days) according to the stage in *T. wygodzinskyi* and *T. pseudomaculata*.

Stage	<i>T. wygodzinskyi</i>			<i>T. pseudomaculata</i>			p value
	N	Median	Quantiles (25-75%)	N	Median	Quantiles (25-75%)	
Egg	74	19	18 - 23	48	21	20 - 24	0.0043*
N1	55	29	21 - 46	41	34	26 - 46	N.S.
N2	51	27	19 - 35	31	23	16 - 37	N.S.
N3	46	28	22 - 40	26	29	24 - 34	N.S.
N4	38	41	32 - 49	25	35	27 - 48	N.S.
N5	31	68	55 - 82	21	97	76 - 128	0.0004*
<b>Total</b>	<b>31</b>	<b>205</b>	<b>189 - 230</b>	<b>21</b>	<b>232</b>	<b>215 - 260</b>	<b>0.0014*</b>

N (number of specimens), N1 (1<sup>st</sup> nymphal instar), N2 (2<sup>nd</sup> nymphal instar), N3 (3<sup>rd</sup> nymphal instar), N4 (4<sup>th</sup> nymphal instar), N5 (5<sup>th</sup> nymphal instar). \* Signi cant di erence by the Mann-Whitney test. N.S. = not signi cant.

**TABLE 2** - Number of blood meals according to the nymphal stage in *T. wygodzinskyi* and *T. pseudomaculata*.

Stage	<i>T. wygodzinskyi</i>		<i>T. pseudomaculata</i>		p value
	Median	Quantiles (25-75%)	Median	Quantiles (25-75%)	
N1	2	2 - 3	2	1 - 3	N.S.
N2	2	1 - 2	2	1 - 2.5	N.S.
N3	2	1 - 2	2	1 - 2	N.S.
N4	2	2 - 2.5	2	1 - 2	N.S.
N5	3	2 - 4	4	2 - 5	N.S.
<b>Total</b>	<b>10</b>	<b>9-12</b>	<b>11</b>	<b>9-13</b>	<b>N.S.</b>

N1 (1<sup>st</sup> nymphal instar), N2 (2<sup>nd</sup> nymphal instar), N3 (3<sup>rd</sup> nymphal instar), N4 (4<sup>th</sup> nymphal instar), N5 (5<sup>th</sup> nymphal instar). \* Signi cant di erence by the Mann-Whitney test. N.S. = not signi cant.

**TABLE 3** - Blood meal ingested (in mg) according to nymphal stage in *T. wygodzinskyi* and *T. pseudomaculata*.

Stage	<i>T. wygodzinskyi</i>			<i>T. pseudomaculata</i>			p value
	N	Median	Quantiles (25-75%)	N	Median	Quantiles (25-75%)	
N1	55	5.4	3.7 - 6.4	41	4.3	3.2 - 5.9	N.S.
N2	51	12.0	8.9 - 14.1	33	11.4	7.6 - 15.2	N.S.
N3	46	33.0	5.2 - 39.4	26	33.0	25.9 - 40.8	N.S.
N4	38	88.0	75.0 - 106.9	25	100.4	93.5 - 111.0	0.0413*
N5	31	282.6	228.2 - 336.6	21	369.2	288.1 - 450.6	0.0009*
<b>Total</b>	<b>430.9</b>	<b>63.0 - 336.6</b>	<b>511.8</b>	<b>452.3 - 450.6</b>	<b>0.0010*</b>		

N1 (1<sup>st</sup> nymphal instar), N2 (2<sup>nd</sup> nymphal instar), N3 (3<sup>rd</sup> nymphal instar), N4 (4<sup>th</sup> nymphal instar), N5 (5<sup>th</sup> nymphal instar). \* Signi cant di erence by the Mann-Whitney test. N.S. = not signi cant.

**TABLE 4** - Mortality rate according to nymphal stage in *T. wygodzinskyi* and *T. pseudomaculata*

Stage	<i>T. wygodzinskyi</i>				<i>T. pseudomaculata</i>				p value*
	N	n	Mortality %	Cumulative mortality %	N	n	Mortality %	Cumulative mortality %	
N1	74	55	25.7	25.7	48	41	14.6	14.6	N.S.
N2	55	51	7.3	31.1	41	31	24.4	35.4	N.S.
N3	51	46	9.8	37.8	31	26	16.1	45.8	N.S.
N4	46	38	17.4	48.7	26	25	3.9	47.9	N.S.
N5	38	31	18.4	58.1	25	21	16.0	56.3	N.S.

N1 (1<sup>st</sup> nymphal instar), N2 (2<sup>nd</sup> nymphal instar), N3 (3<sup>rd</sup> nymphal instar), N4 (4<sup>th</sup> nymphal instar), N5 (5<sup>th</sup> nymphal instar)

N= Initial number, n= number of nymphs molting for the following stage. N.S. = not signi cant for the Chi square test.

\*Chi-square test.

the adult stage (global mortality rate of 58.1%); a similar rate was determined for *T. pseudomaculata* (56.3%). Mortality for each stage varied from 7.3 to 25.7% for *T. wygodzinskyi* and from 3.9 to 24.4% for *T. pseudomaculata*, the highest mortality rate was registered in N1 (25.67%) for the former species and in N2 (24.4%) for the latter. The interspecies comparison for each stage did not reveal any significant difference between the two species.

## DISCUSSION

Several papers involve studies related to the biology of *T. pseudomaculata*, a triatomine species with an epidemiological role<sup>10,11,12,13,14</sup>. In contrast, no readily available information exists concerning the biology of *T. wygodzinskyi*, which is an exclusively sylvatic vector. In fact, the study conducted by Juarez (1970)<sup>15</sup> on the biological cycle of *T. arthurneivai* actually involves misidentified populations that are currently known as *T. wygodzinskyi*<sup>7,16</sup>.

In most cases, the disparities between the conditions maintained in individual insectaries, the methodologies and statistical analyses complicate the comparison of different studies relating to the same species<sup>11</sup>. For example, the egg to adult life cycle showed an average duration of 255 days in this work, whereas it varied from 212 to 349 days according to other authors<sup>11,14</sup>. The differences observed are partly due to the influence of the blood meal source<sup>13</sup>.

The comparison of biological traits of two species under the same laboratory conditions is more informative. In the present study, the study began with eggs laid by field-collected females in order to limit the endogamy that occurs after a few generations in the laboratory. Concerning the life cycle, the differences observed between the two species are minor and limited to the development time of the egg and N5 stage in female insects. Regarding the blood meal parameters, the number of feeding periods showed no significant differences between the two species. Only the late nymphal instars of *T. pseudomaculata* ingest more blood than *T. wygodzinskyi*. This factor is epidemiologically important and suggests an increased risk of *T. cruzi* transmission in *T. pseudomaculata*. Finally, the interspecies comparison of the mortality rate showed no significant difference.

For a long time, *T. maculata* and *T. pseudomaculata* were considered closely related species<sup>17,18,19</sup>. Recently, this hypothesis was undermined by genetic and morphological studies<sup>7,8,20</sup>. When comparing the life cycles between these two species, it is clear that the life cycle of *T. maculata* is shorter than that of *T. pseudomaculata*. According to different authors, the N1-adult life cycle does not exceed 144 days for *T. maculata*, a much shorter duration than that observed for *T. pseudomaculata* (Table 5). The coherence of our results with the genetic and morphological studies suggests that

**TABLE 5**– Comparison between *T. maculata* and *T. pseudomaculata* life cycles according to the feeding source.

Species	Blood source	N1-adult life cycle in days (average)	References
	bird	144	Espinola <i>et al.</i>

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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