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Trends in Taxonomy of the Rhodniini Tribe (Hemiptera, Triatominae): Reproductive Incompatibility between *Rhodnius neglectus* Lent, 1954 and *Psammolestes* spp. Confirms the Generic Status of *Psammolestes* Bergroth, 1911

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Citation: Ravazi, A.; Oliveira, J.d.; Madeira, F.F.; Reis, Y.V.d.; Oliveira, A.B.B.d.; Galvão, C.; Azeredo-Oliveira, M.T.V.d.; Rosa, J.A.d.; Alevi, K.C.C. Trends in Taxonomy of the Rhodniini Tribe (Hemiptera, Triatominae): Reproductive Incompatibility between *Rhodnius neglectus* Lent, 1954 and *Psammolestes* spp. Confirms the Generic Status of *Psammolestes* Bergroth, 1911. *Diversity* **2022**, *14*, 761. https://doi.org/10.3390/d14090761

Academic Editor: Luc Legal

Received: 1 August 2022
Accepted: 14 September 2022
Published: 15 September 2022

Abstract: The tribe Rhodniini is a monophyletic group composed of 24 species grouped in two genera: *Rhodnius* and *Psammolestes*. *Rhodnius* is a paraphyletic genus formed by 21 species. The event of paraphilia is supported by the greater evolutionary proximity of the species of the *prolixus* groups with the genus *Psammolestes* than with the other groups of *Rhodnius*. Based on this phenomenon, it was proposed the alteration of the genus of the species of *Psammolestes* to *Rhodnius*. Thus, we performed experimental crosses between *Psammolestes* spp. and *R. neglectus* to assess the degree of reproductive compatibility between *Psammolestes* and *Rhodnius*. Hybrids were not obtained for intergeneric crosses. Based on this, we have demonstrated that *Rhodnius* and *Psammolestes* have reproductive incompatibility. These data are important to confirm the validity of the specific status of *Psammolestes*, based on the biological concept of the species.

Keywords: Chagas disease vectors; hybridization; experimental crosses; evolution

1. Introduction

Chagas disease is a neglected disease caused by the protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) which affects about 8 million people worldwide [1,2]. The main form of transmission of the protozoan is through hematophagous insects known as triatomines (Hemiptera, Triatominae) [1,2]. Currently 157 species of triatomines are known, divided in 18 genera and five tribes [3–5], being all considered as potential Chagas disease vectors.

The tribe Rhodniini Pinto, 1926 is a monophyletic group composed of 24 species grouped in two genera: *Rhodnius* Stål, 1859 with long thin legs and a long head, living mainly in palm trees, and *Psammolestes* Bergroth, 1911 having a short head, strong legs, wide femora, a very wide rostrum (the widest in all the subfamily) and living in nests of birds [6]. However, there are several reports of these genera living in sympatry in natural conditions, as *P. tertius* Lent & Jurberg, 1965 living in sympathy with *R. neglectus* Lent, 1954 in nests of *Phacellodomus ruber* (Vieillot, 1817) (Passeriformes, Furnariidae) in the federal district, Brazil [7] and *P. arthuri* (Pinto, 1926) found in sympatry with *R. prolixus* Stål, 1859 on bird nests in Anzoátegui state, Venezuela [*P. rufifrons* (Wied-Neuwied, 1821) and *Icterus nigrogularis* (Hahn, 1819) (Passeriformes, Icteridae)] [8].

Rhodnius is a paraphyletic genus formed by 21 species divided into three groups: *pallescens*, *pictipes* and *prolixus* groups [3,9]. The event of paraphilia is supported by the greater evolutionary proximity of the species of the *prolixus* groups with the genus *Psammolestes* than with the other groups of *Rhodnius* [10–12]. Based on this phenomenon, Hypsa et al. [13] proposed the alteration of the genus of the species of *Psammolestes* to *Rhodnius*.

Although the change in the generic status of *Psammolestes* was suggested by these molecular studies [13] (based on the phylogenetic species concept [14]), from the morphological and ecological divergences [6] the genus *Psammolestes* was kept as valid (considering the phenetic and ecological species concepts [15]). However, recently Fileé et al. [12], using more complex phylogenetic analyzes with mitochondrial and nuclear data (phylogenetic species concept [14]), again recommended the reclassification of species from *Psammolestes* to *Rhodnius*.

Thus, considering that studies of hybridization can help to understand the taxonomy and systematics of species (based on the biological species concept [16,17]), can be used to analyze the isolating mechanisms that limit gene flow between species, as well as experimental hybridization can be employed to establish the role of natural hybridization in generating new genetic variants (that may lead to adaptive evolution and/or in founding new evolutionary lineages) [18,19], we performed experimental crosses between *Psammolestes* spp. and *R. neglectus* to assess the degree of intergeneric reproductive compatibility between *Psammolestes* and *Rhodnius*.

2. Materials and Methods

In order to evaluate the genetic and reproductive compatibility [16] between the genus of the Rhodniini tribe, reciprocal crossing experiments were conducted between *R. neglectus* (origin: Brazil, São Paulo, Jaboticabal, second generation) and *P. coreodes* (Bergroth, 1911) (origin: Brazil, Mato Grosso do Sul, Corumbá, second generation) and between *R. neglectus* and *P. tertius* (origin: Brazil, Bahia, Castro Alves, third generation). The choice of *R. neglectus* among the species of the *prolixus* groups was based on the phylogenetic proximity of the species with *Psammolestes* spp. [10]. The crossing experiments were conducted in the Triatominae insectary of the School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil, in according to the experiments of Neves et al. [20] and Ravazi et al. [21]: the insects were sexed as 5th instar nymphs [22], and males and females were kept separately until they reached the adult stage in order to cross adult virgins [23]. For the crosses, 3 couples from each set were placed in plastic jars (5 cm in diameter ×10 cm in height) and kept at room temperature. Furthermore, intragenerics crosses were also performed for control. Weekly, the triatomines were fed on duck blood, the eggs were collected throughout the female's oviposition periods (as this period may vary between species, for standardization, we set the "female's oviposition periods" as "four months after the first oviposition") and the egg fertility rate was calculated.

3. Results and Discussion

Hybrids were not obtained for intergeneric crosses between *Rhodnius* and *Psammolestes* (Table 1, Figure 1B [24]). On the other hand, intrageneric crosses between species of *Rhodnius* (*R. prolixus* x *R. neglectus* (Figure 1A [24]), *R. prolixus* x *R. robustus* Larrousse, 1927 and *R. pictipes* Stål, 1872 x *R. prolixus* [25]) and between species of *Psammolestes* (*P. coreodes* x *P. tertius* (Figure 1C [24]) [21]) resulted in the formation of hybrids (which were made unfeasible by post-zygotic barriers) [21,25].

Table 1. Results of the experimental crosses performed between *Rhodnius* and *Psammolestes*.

Experimental Crosses	Number of Eggs				
	C1	C2	C3	Total	Egg Fertility
P. coreodes ♀x *R. neglectus* ♂	55	74	52	181	0%
R. neglectus ♀x *P. coreodes* ♂	60	121	156	337	0%
P. tertius ♀x *R. neglectus* ♂	1	26	28	55	0%
R. neglectus ♀x *P. tertius* ♂	114	130	97	341	0%
Control experiments					
P. coreodes ♀x *P. coreodes* ♂	61	10	90	161	67.7%
P. tertius ♀x *P. tertius* ♂	52	47	75	174	72.3%
R. neglectus ♀x *R. neglectus* ♂	337	409	175	901	89.8%

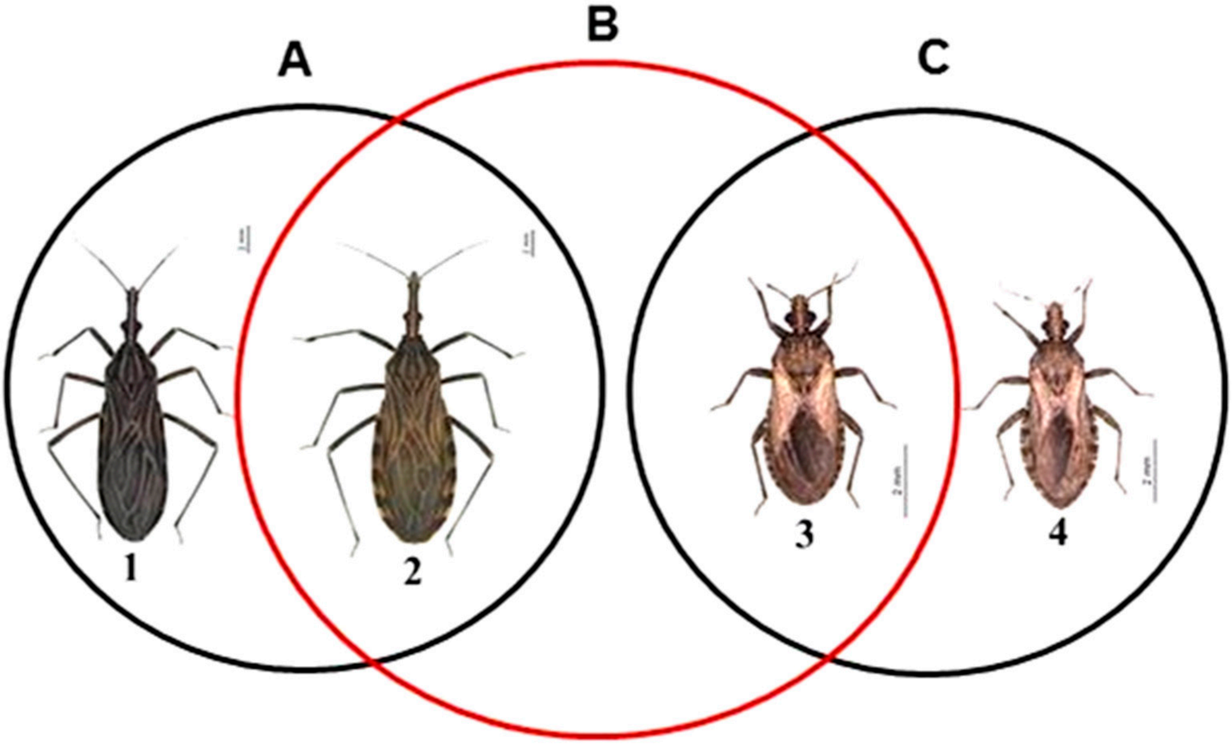

Figure 1. Scheme representing the intrageneric (**A**,**C**) and intergeneric (**B**) crosses of the Rhodniini tribe. Note that the black circles represent reproductive compatibility and hybrid formation between *Rhodnius* spp. (**A**) and between *Psammolestes* spp. (**C**) and the red circle demonstrates reproductive incompatibility and absence of hybrid formation between the *Rhodnius* and *Psammolestes* genera (**B**). 1: *R. prolixus*, 2: *R. neglectus*, 3: *P. tertius* and 4: *P. coreodes*. Bar: 2 mm.

Events that result in pre-zygotic reproductive isolation in Triatominae are more common between species with different chromosome numbers (*Triatoma brasiliensis brasiliensis* Neiva, 1911 x *T. melanocephala* Neiva and Pinto, 1923, and *T. b. brasiliensis* x *T. vitticeps* (Stål, 1859) [20]), between species that are very distant from the phylogenetic point of view ([26]), or between species of different genera (*T. b. brasiliensis* x *Panstrongylus tibiamaculatus* (Pinto, 1926), *T. infestans* (Klug, 1834) x *R. neglectus*, *T. infestans* x *R. prolixus*, *T. sordida* (Stål, 1859) x *R. prolixus* and *T. sordida* x *R. neglectus* [20,26]). Recently, Cesaretto et al. [27] crossed *Meccus* Stål, 1859 and *Triatoma* Laporte, 1832 species and, due to the absence of a pre-zygotic barrier, confirmed the synonymization of *Meccus* with *Triatoma*.

The chromosome evolution of the Rhodniini tribe was quite homogenous from a numerical and structural point of view and resulted in several identical characteristics for most *Rhodnius* and *Psammolestes* species: karyotype 2n = 22, sex determination system (XY), heterochromatin pattern (C-bands restricted to the Y sex chromosome for most species}, AT and CG base pair composition (X sex chromosome CMA and DAPI and Y DAPI and CMA for most species) and arrangement and location of the 45S rDNA probes (markings restricted to sex chromosomes) [27–30]. These similar chromosomal characteristics, together with the phylogenetic relationship of the species of both genera [9–13], could result in the formation of hybrids. However, our results demonstrate that there is reproductive barrier between *Rhodnius* and *Psammolestes* (Table 1), confirming the generic status of *Psammolestes*.

We suggest that after the divergence of the *Rhodnius* and *Psammolestes* genera from the common ancestor, genotypic modifications (at the genomic level, which did not lead to changes in the numerical and structural composition of the chromosomes [27–30]) and phenotypic [6] resulted in pre-zygotic reproductive isolation. Although the genitalia of *Rhodnius* and *Psammolestes* show differences (*Rhodnius*: i. pygophore with median process pointed, blunt, bifurcate or subrectangular; ii. articulatory apparatus with basal bridge well developed; iii. basal plate struts transformed into two short, basally connected convolute structures and iv. genitalia of female truncate, eighth and ninth tergites subvertical; *Psammolestes*: i. pygophore with short median spinelike process, pointed or rounded apically; ii. articulatory apparatus with basal plate bridge well developed; iii. pedicel about as long as wide; iv. basal plate struts transformed into short curved or convoluted structures and v. genitalia of female truncate, last tergites subvertical [6]), intergeneric copulations were observed (suggesting, therefore, the possible absence of pre-zygotic reproductive isolation due to mechanical incompatibility). These observations are of great taxonomic and evolutionary importance, since it supports the particularities observed between the genera (since there is no intergeneric gene flow) and, above all, it corroborates the generic status of *Psammolestes* questioned by Hypsa et al. [13] and Fileé et al. [12] based on the biological species concept [16,17].

4. Conclusions

Based on this, we have demonstrated that *Rhodnius* and *Psammolestes* have reproductive incompatibility. These data, together with the morphological and ecological data [6] are important to confirm the validity of the specific status of *Psammolestes*, based on the biological, phenotypic and ecological concepts of the species.

Author Contributions: Conceptualization, A.R., J.d.O., and K.C.C.A.; methodology, A.R., J.d.O., F.F.M., Y.V.d.R., A.B.B.d.O., and K.C.C.A.; formal analysis, A.R., J.d.O., F.F.M., Y.V.d.R., A.B.B.d.O., C.G., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; investigation, A.R., J.d.O., F.F.M., Y.V.d.R., A.B.B.d.O., C.G., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; resources, A.R., F.F.M., Y.V.d.R., A.B.B.d.O., C.G., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; writing—original draft preparation, A.R. and K.C.C.A.; writing—review and editing, A.R., J.d.O., F.F.M., Y.V.d.R., A.B.B.d.O., C.G., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; supervision, K.C.C.A., J.d.O. and M.T.V.d.A.-O.; project administration, K.C.C.A., and M.T.V.d.A.-O.; funding acquisition, K.C.C.A., C.G., J.A.d.R., and M.T.V.d.A.-O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Coordination for the Improvement of Higher Education Personnel, Brazil (CAPES)—Finance Code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Carlos Chagas Filho Research Foundation of the State of Rio de Janeiro (FAPERJ).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All relevant data are within the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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