

Karyotype Evolution of Chagas Disease Vectors (Hemiptera, Triatominae)

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Abstract. The Triatominae subfamily is composed of 153 hematophagous species that are potential vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease. Karyotypic studies in triatomines were initiated in 1909. There are 92 karyotypes described, all grouped into the tribes Rhodniini and Triatomini. Recently, a phylogenetic study of the triatomines that combines molecular data with geological changes was performed. We now discuss how the karyotype evolved with the diversification of the triatomines.

The subfamily Triatominae consists of 153 hematophagous species (151 living species and two fossil ones) considered as potential vectors of *Trypanosoma cruzi* (Chagas, 1909), the etiological agent of Chagas disease.^{1–3} Chagas disease is a neglected disease distributed in 21 countries in Latin America, and it is estimated that approximately 6 to 7 million people are infected worldwide.⁴

Currently, the subfamily Triatominae is divided into 18 genera and five tribes (Alberproseniini, Bolboderini, Cavernicolini, Rhodniini, and Triatomini),¹ which are distributed from Southern United States of America to Patagonia, with a few species of Triatomini known from India and Australia. According to Justi et al.,⁵ the first three tribes (Alberproseniini, Bolboderini, and Cavernicolini) comprise only 15 of the 153 known species. Rhodniini and Triatomini are the most diverse and epidemiologically relevant tribes and therefore, have been the object of more research.¹

Karyotypic studies in Triatominae were initiated in 1909 with the description of the karyotype of *Triatoma sanguisuga* (Leconte, 1855).⁶ In 1950, karyology was resumed, and new karyotypes were described.⁷ In 1966, Ueshima⁸ described 20 new karyotypes and proposed cytogenetic studies as a tool in the taxonomy of these vectors (cytotaxonomy). So far, 92 karyotypes have been described,^{3,9–11} all species being grouped into the tribes Rhodniini and Triatomini.

Recently, there was a proposal of a phylogenetic study of triatomines that combines molecular data with geological changes.⁵ The authors observed that most of the diversification events that occurred in the tribes Rhodniini and Triatomini are linked mainly to the climatic and geological changes caused by the Andean uplift in South America. Based on phylogenetic relationships presented by Justi et al.,⁵ we will discuss for the first time how the karyotype of triatomines evolved according to their diversification.

The subfamily Triatominae was recovered as monophyletic by Justi et al.⁵ For a long time, it was discussed if Triatominae was monophyletic, paraphyletic, or polyphyletic.¹² Nokkala and Nokkala¹³ suggest that the primitive karyotype of the order Hemiptera is XY. Thus, based on the latest theory

that triatomines originated from a common ancestor (monophyletic group)⁵ and that the main events of variation in their number of chromosomes are related to fission (agmatoploidy) and fusion (simploidy) of the X sex chromosome,⁹ we can confirm that all triatomines evolved from an ancestral karyotype $2n = 22$ (20A + XY) (as initially suggested by Ueshima⁸).

The species of the tribes Alberproseniini, Bolboderini, and Cavernicolini do not have the described karyotype. However, as Cavernicolini was presented as a sister tribe to Rhodniini and all *Rhodnius* (15 of the 20 species) and *Psammolestes* (two of the three species) species analyzed have $2n = 22$ (20A + XY),^{3,9} we suggest that all species of the tribes Rhodniini and Cavernicolini have 22 chromosomes. A recent cytogenetic study of *Cavernicola pilosa* (Barber, 1937) suggests that this species has an XY sex-determination system,¹⁴ supporting our hypothesis.

The most diverse genera within the tribes Rhodniini (*Rhodnius*) and Triatomini (*Triatoma*) have classically been divided into subgroups (groups, complexes, and subcomplexes) primarily based on morphology and geographical distribution.¹⁵ Among the different groups of *Rhodnius* (*pallescens*, *pictipes*, and *prolixus*), there was no observation of events that resulted in changes in the number of chromosomes of these vectors when compared with the ancestral karyotype $2n = 22$.^{3,9} On the other hand, there are various events associated with the chromosomal evolution of Triatomini that are discussed next.

Justi et al.⁵ observed that the uplift of the western Cordillera acted as a vicariant event separating the clade *venosa* from the remaining Triatomini. The clade *venosa*, represented by the species of the complex *dispar* [*Triatoma boliviana* (Martinez et al., 2007), *Triatoma carrioni* (Larrousse, 1926), *Triatoma dispar* (Lent, 1950), *Triatoma nigromaculata* (Stål, 1872), and *Triatoma venosa* (Stål, 1872)],¹⁵ was the first to diverge from the tribe Triatomini. Recently, the karyotype $2n = 22$ (20A + XY) was described for *T. boliviana* and *T. carrioni*,¹⁰ which may indicate that all species of this complex have $2n = 22$ chromosomes and have not undergone agmatoploidy or simploidy during the divergence of the clade from the karyotype ancestor.

Subsequently, Justi et al.⁵ observed that the Northern Andean uplift separated *Triatoma maculata* (restricted to the Amazon) from the other members of the group *infestans* (an evolutionary event that has not caused changes in the number

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of chromosomes). Furthermore, the authors report that *Triatoma melanocephala* (Neiva and Pinto, 1923) and *Triatoma vitticeps* (Stål, 1859) (subcomplex *vitticeps*),¹⁷ although present in South America, are considered exceptions because they appear to have reached the Atlantic coast by dispersal and diversified before that event. Different from the species of the group *infestans*, which present $2n = 22$ chromosomes,^{9,11} *T. melanocephala* and *T. vitticeps* have $2n = 24$ ($20A + X_1X_2X_3Y$).^{6,16} In the case of the subcomplex *vitticeps*, agmatoploidy resulted in a karyotype that makes it possible to distinguish these species from all the others in South America.¹⁷

The group *infestans* includes the subcomplexes *brasilensis*, *infestans*, *maculata*, *matogrossensis*, *rubrovaria*, and *sordida*.^{15,18} Taking into account that many karyotypes of these subcomplexes have been described, all species analyzed have $2n = 22$ ($20A + XY$),^{9,11} and different subcomplexes emerged from different selective pressures (*brasilensis* in the Caatinga Province, *rubrovaria* in the Pampean Province, *infestans* in the Chacoan Province, and *sordida*, *matogrossensis*, and some species of the subcomplex *maculata* in the Cerrado Province),^{5,18} we suggest that all group *infestans* species have $2n = 22$ chromosomes. Although the complex *spinolai* was long considered as a sister group to South American triatomines (being part of the group *infestans*),¹⁵ the recent study presented by Justi et al.⁵ demonstrated that the species of this complex are more closely related to the clades *geniculatus* and *rubrofasciata*.

The complex *spinolai* consists of the species *Triatoma breyeri* (Del Ponte, 1929), *Triatoma eratyrisiformis* (Del Ponte, 1929), *Mepraia spinolai* (Porter, 1934), *Mepraia gajardoi* (Frias, Henry & Gonzalez, 1998), and *Mepraia parapatrica* (Frias-Lasserre, 2010).¹⁵ *Triatoma eratyrisiformis* (and possibly *T. breyeri* by phylogenetic proximity)⁵ presents $2n = 24$ ($20A + X_1X_2X_3Y$)⁸ and *Mepraia* spp. present $2n = 23$ ($20A + X_1X_2Y$).¹⁹ From the ancestral karyotype ($2n = 22$), the species of this complex that diverged in *Mepraia* have suffered one agmatoploidy event for the X sex chromosome (X_1X_2), and the species that diverged in *Triatoma* have suffered two events ($X_1X_2X_3$). These results corroborate the phylogenetic relationship presented by Justi et al.⁵ because most species of the clades *geniculatus* and *rubrofasciata* have 23 (sex-determination system X_1X_2Y), 24 (sex-determination system $X_1X_2X_3Y$), or exceptionally 25 chromosomes (sex-determination system X_1X_2Y).^{8,17-19}

Gardim et al.²⁰ pointed out the need for a general revision in the tribe Triatomini because *Panstrongylus* cannot be clustered separately from *Triatoma*. For example, the clade *geniculatus* consists of *Panstrongylus* spp., *flavida* complex, and *Triatoma tibiamaculata*,⁵ and all studied species of this clade [except *Panstrongylus megistus* (Burmeister, 1835) and *Panstrongylus lutzi* (Neiva & Pinto, 1923)]^{21,22} presented $2n = 23$ ($20A + X_1X_2Y$),^{9,21} which confirms the evolutionary relationship proposed. Based on the ancestral karyotype ($2n = 22$), we suggest that during the divergence of the common ancestor of the clade *geniculatus* an agmatoploidy in the X sex chromosome has happened, which resulted in karyotype $2n = 23$ (karyotype shared by *Panstrongylus* spp., *Nesotriatoma* spp., and *T. tibiamaculata*). However, during the karyotypic evolution of *Panstrongylus*, two events occurred: simploidy in a pair of autosomes in *P. megistus* $2n = 21$ ($18A + X_1X_2Y$)²¹ [a less common event, possibly related to a vicariant divergence

between *P. megistus* and *T. tibiamaculata* (Pinto, 1926) from the separation of the common ancestor when the connection between the Amazon Rainforest and the Atlantic Forest was lost as a result of climatic changes caused by the Andean uplift]⁴ and agmatoploidy in the X sex chromosome in *P. lutzi* $2n = 24$ ($20A + X_1X_2X_3Y$).²²

Justi et al.⁵ presented the group *phyllosoma* containing *Triatoma mexicana* (Herrich-Schaeffer, 1848), *Triatoma dimidiata* (Latreille, 1811), *Triatoma recurva* (Stål, 1868), *Triatoma gerstaeckeri* (Stål, 1858), *Meccus pallidipennis* (Stål, 1872), *Meccus longipennis* (Usinger, 1939), *Meccus mazzottii* (Usinger, 1941), and *Meccus picturatus* (Usinger, 1939) and suggested that *Triatoma sanguisuga* was separated from the other members by a vicariant event (high sea level that inundated Florida and the Gulf Coast). This group is basically a combination of species of the complexes *lecticularia* [*Triatoma gerstaeckeri*, *Triatoma indictiva* (Neiva, 1912), *Triatoma lecticularia* (Stål, 1859), *Triatoma recurva*, *Triatoma rubida* (Uhler, 1894), and *Triatoma sanguisuga*] and *phyllosoma* [*Meccus bassolsae* (Aguillar et al., 1999), *Triatoma bolivari* (Carcavallo, Martinez & Pelaez, 1987), *Meccus longipennis*, *Meccus mazzottii*, *Triatoma mexicana*, *Meccus pallidipennis*, *Meccus phyllosoma*, *Meccus picturata*, and *Triatoma ryckmani* (Zeledón & Ponce, 1972)].¹⁵ With the exception of *T. lecticularia* (which was recovered as a sister species to *Paratriatoma hirsuta* (Barber, 1939), both having karyotype $2n = 22$), all the species of the complexes *lecticularia* and *phyllosoma* that were cytogenetically studied have karyotype $2n = 23$ ($20A + X_1X_2Y$).⁹ We suggest that this is the karyotype of all species of the group *phyllosoma*.

Considering that *T. ryckmani* and *T. rubida* present $2n = 23$ and *T. lecticularia* and *P. hirsuta* $2n = 22$, we can raise two hypotheses: i) the ancestor of these triatomines had 22 chromosomes, and during the divergence of *T. ryckmani* and *T. rubida*, an agmatoploidy of the X sex chromosome took place or ii) the common ancestor had $2n = 23$, and during the divergence of *T. lecticularia* and *P. hirsuta*, a simploidy of the X sex chromosome occurred. However, as agmatoploidy is much more common in the subfamily Triatominae²¹ and simploidy is known only to autosomes,⁹ we understand that the first hypothesis is the most likely.

Finally, Justi et al.⁵ showed that the separation of the Old World clade [consisting of *Triatoma rubrofasciata* (De Geer, 1773) and *Linshcosteus* spp.], dates to a time as late as the Mid-Oligocene. Furthermore, the authors note that *T. rubrofasciata* and the species of the genus *Linshcosteus* [*Linshcosteus carnifex* (Distant, 1904), *Linshcosteus chota* (Lent & Wygodzyski, 1979), *Linshcosteus confumus* (Ghauri, 1976), *Linshcosteus costalis* (Ghauri, 1976), *Linshcosteus kali* (Lent & Wygodzyski, 1979), and *Linshcosteus karupus* (Galvão et al., 2002)] form a monophyletic group. Based on the peculiar karyotype $2n = 25$ ($22A + X_1X_2Y$) of *T. rubrofasciata* (which presents an agmatoploidy in the autosomes, making it possible to differentiate it from all triatomine species)²³ and the phylogenetic relationship presented between *T. rubrofasciata* and the species of genus *Linshcosteus*,⁵ we suggest that agmatoploidy has occurred in the common ancestor of the Old World clade, that is, we believe that all species of the genus *Linshcosteus* also have 25 chromosomes.

Therefore, based primarily on the evolutionary data presented by Justi et al.,⁵ we highlight new and important

information (also in the form of hypotheses) on the evolution of the karyotype of triatomines that will guide new studies on these vectors of Chagas disease.

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