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Post-zygotic reproductive barriers confirm absence of continuous interspecific gene flow between *Mepraia* spp. (Hemiptera, Triatominae)

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Mepraia **is a genre composed of three species endemic to Chile. Climate projections demonstrate that these triatomines could modify their potential geographical range and does not rule out that hybridization events could be occurring. Furthermore, recent phylogenomic studies suggested possible ancestral events of hybridization and gene flow and, based on the most recent speciation model that divides** ***Mepraia*** **in two geographically distinct groups (the North, composed of** ***M. garjadoi*** **and** ***M. parapatrica*****, and the South, composed of** ***M. spinolai*****), was also suggested an alternative model that may be compatible with ancient hybridization events or continued gene flow. Thus, we conducted a comprehensive study of the capacity to produce hybrids and to reproductive barriers present between** ***Mepraia*** **spp., with the aim of evaluating the possibility of hybridization and interspecific gene flow be occurring in natural conditions and confirming the specific status of the three** ***Mepraia*** **species. Even though hybrids have been obtained in all directions, the combination of different evolutionary events demonstrated here, such as low hatching rate and high mortality rate of hybrids, as well as the presence of post-zygotic reproductive barriers and possible action of Haldane's rule indicate that there is no continuous interspecific gene flow between** ***Mepraia*** **spp. and confirms the specific status of species based on the biological species concept.**

The subfamily Triatominae (Hemiptera, Reduviidae) is a monophyletic group composed of 158 species distributed in 19 genera[1,2]. The *Mepraia*[3] genus is composed of three species endemic to Chile (distributed between 18° and 33° S of the coastal region and valleys in the interior of Chile)[4]: *M. gajardoi*[5] distributed along the northern coast of Chile, approximately between 18° and 26° S; *M. spinolai*[6], distributed approximately between 26° and 33° S; and *M. parapatrica* Frías-Lasserre, 2010, distributed between 24° and 27° S, northern coast of Chile, region of Antofagasta and Atacama[4–9]. These three species have already been found naturally infected by *T. cruzi*[10], with human blood being one of the food sources for *M. spinolai* e *M. parapatrica*[11,12]. Although these vectors are responsible for the transmission of the protozoan in the wild, Rosa et al.[9] have already reported specimens in peridomiciliary regions.

Garrido et al.[13] evaluated the potential impact of climate change on the geographical distribution of *M. spinolai* and *M. gajardoi*. The authors made a climate change projections until 2070 and concluded that: under future climate conditions, these species could modify their potential geographical range; the suitable areas for both species may be greater than currently known, generating new challenges in terms of vector control and

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prevention; and preventive measures to avoid accidental human vectorial transmission by wild vectors of *T. cruzi* become critical, considering the uncertainty of the future suitable areas projected in this study.

In addition to the above assumptions, the authors observed an overlap area encompassing around 24° 40′ to 26° 35′ S by the coast, distribution area of *M. parapatrica*, and highlight that the climate of this area would be suitable for both *M. gajardoi* and *M. spinolai*[13]. Thus, based on the experimental crosses carried out by Campos-Soto et al.[14], the authors do not rule out that hybridization events could be occurring between the three species in natural conditions[13].

Experimental crosses between *M. gajardoi* and *M. spinolai* allowed us to observe that these species are capable of producing hybrids (demonstrating absence of interspecific prezygotic reproductive barrier)[14,12]. However, low hatching rates of hybrid offspring were observed (ranging from 5.9 to 10.4%)[14–5]. In addition, only the cross between *M. gajardoi* ♀ with *M. spinolai* ♂ resulted in adult hybrids, the other direction produced hybrids that died before reaching adulthood (hybrid inviability)[14].

As the hybrids presented normal gametogenesis (without chromosomal errors), the adult male were backcrossed with the parents and resulted in second generation hybrids (F2) with inviable gametes (resulting from chromosome pairing errors)[14]. On the other hand, some hybrid females resulted in fertile F2 when backcrossed, being the hybrid breakdown of the third generation hybrids (F3) resulting from the hybrid collapse—first generation hybrids (F1) viable and fertile, being the inviability or sterility present in the F2 or later generations of interspecific crosses[14].

Initially, the specific status of *Mepraia* spp. was based on phenotypic and chromosomal data[14–19]. Later, phylogenetic data supported the status of *M. spinolai* and *M. gajardoi*[20,21] and raised doubts about the status of *M. parapatrica*, since introgression/hybridization events in some populations of this putative species were suggested[5,20,22]. More recently, more complex phylogenetic[21] and phylogenomic[22] studies confirmed that the three species are valid (in addition to a possible fourth cryptic taxon with a small distribution range enclosed between Santa María Island and Morro Moreno)[22].

Belintani et al.[22], through phylogenomic studies with transcriptomes of the head and salivary glands, suggested possible ancestral events of hybridization and gene flow among *Mepraia* spp. (which would explain the inconsistencies in the genetic trees), once they observed significant signs of introgression between these species using different models (ABBA-BABA test, HyDe analysis, maximum pseudo-likelihood Inference model implemented in PhyloNet and JML test). Furthermore, the authors suggested that the diversification of *Mepraia* occurred recently and was significantly influenced by interspecific gene flow during its early stages of divergence.

On the other hand, based on the most recent speciation model proposal by Campos-Soto et al.[21]—which suggests two geographically distinct groups (the North, composed of *M. garjadoi* and *M. parapatrica*, and the South, composed of *M. spinolai*)—, Belintani et al.[22] highlight that the authors suggest an alternative model that may be compatible with ancient hybridization events or continued gene flow.

Thus, since knowledge of hybridization events in *Mepraia* is limited to experimental crosses between *M. gajardoi* and *M. spinolai*, we conducted a comprehensive study of the capacity to produce hybrids and to reproductive barriers present between *Mepraia* spp., with the aim of evaluating the possibility of hybridization and interspecific gene flow be occurring in natural conditions and confirming the specific status of the three *Mepraia* species, based on the biological species concept[23,24].

Results

Hybrids were obtained in all experimental crosses (Fig. 1A–F) (confirming absence of pre-zygotic barriers between *Mepraia* spp.), with hatching rates ranging from 05 to 49% (Table 1). However, the offspring from crosses between ♀ *M. parapatrica* x *M. spinolai* ♂ (Fig. 1C) and ♀ *M. gajardoi* x *M. parapatrica* ♂ (Fig. 1B) was composed only of females (Table 2) (which made it impossible to carry out intercrossing, but drew our attention to the possible application of Haldane's rule). All other directions resulted in adult hybrids of both sexes (Table 2, Fig. 2A–P) and thus intercrosses (F1 × F1) were performed (Table 1). F2 hybrids were obtained in all crosses—demonstrating absence of the post-zygotic barrier of hybrid sterility. Morphological and cytogenetic analysis of the testes in the F1 hybrids that reached adulthood confirmed the absence of hybrid sterility by gonadal dysgenesis (since the gonads were not atrophied) (Fig. 3E,F) or chromosomal errors (since the metaphases showed 100% pairing between the chromosomes from the different species) (Fig. 3A–D). However, all F2 offspring died before reaching adulthood (possibly by action of the post-zygotic barrier of hybrid collapse) (Table 1).

Discussion

For the first time, hybrids were obtained for all possible crosses of *Mepraia* spp. (Table 1). Different from what was observed by Campos-Soto et al.[14] that only obtained adult hybrids for the cross between *M. gajardoi* ♀ with *M. spinolai* ♂, we obtained adult offspring for all directions and were able to characterize all possible interspecific reproductive barriers present between *M. parapatrica*, *M. gajardoi* and *M. spinolai*. While the authors suggest that the phenomenon of hybrid inviability acts in the hybrid breakdown resulting from the cross between *M. gajardoi* ♂ with *M. spinolai* ♀[14], we demonstrated that these hybrids are viable, fertile and the phenomenon responsible for the hybrid breakdown is the hybrid collapse which resulted in the total mortality of the F2 hybrids (Table 1). Likewise, we demonstrate that the same phenomenon is also responsible for hybrid breakdown when *M. gajardoi* ♀ and *M. spinolai* ♂ are crossed (Table 1).

From the hybrids resulting from crosses between *M. gajardoi* ♀ with *M. spinolai* ♂, Campos-Soto et al. [11] performed backcrosses with the parentals, as the authors aimed to evaluate whether the segregation of the wing size pattern was sex-linked or autosomal. In addition to characterizing that the inheritance was not sex-linked, they reported some reproductive barriers that resulted in the hybrid breakdown (among them, the hybrid collaps): when they evaluated the male progeny of one of the first backcrosses analyzed (hybrid F1 ♂ with *M.*

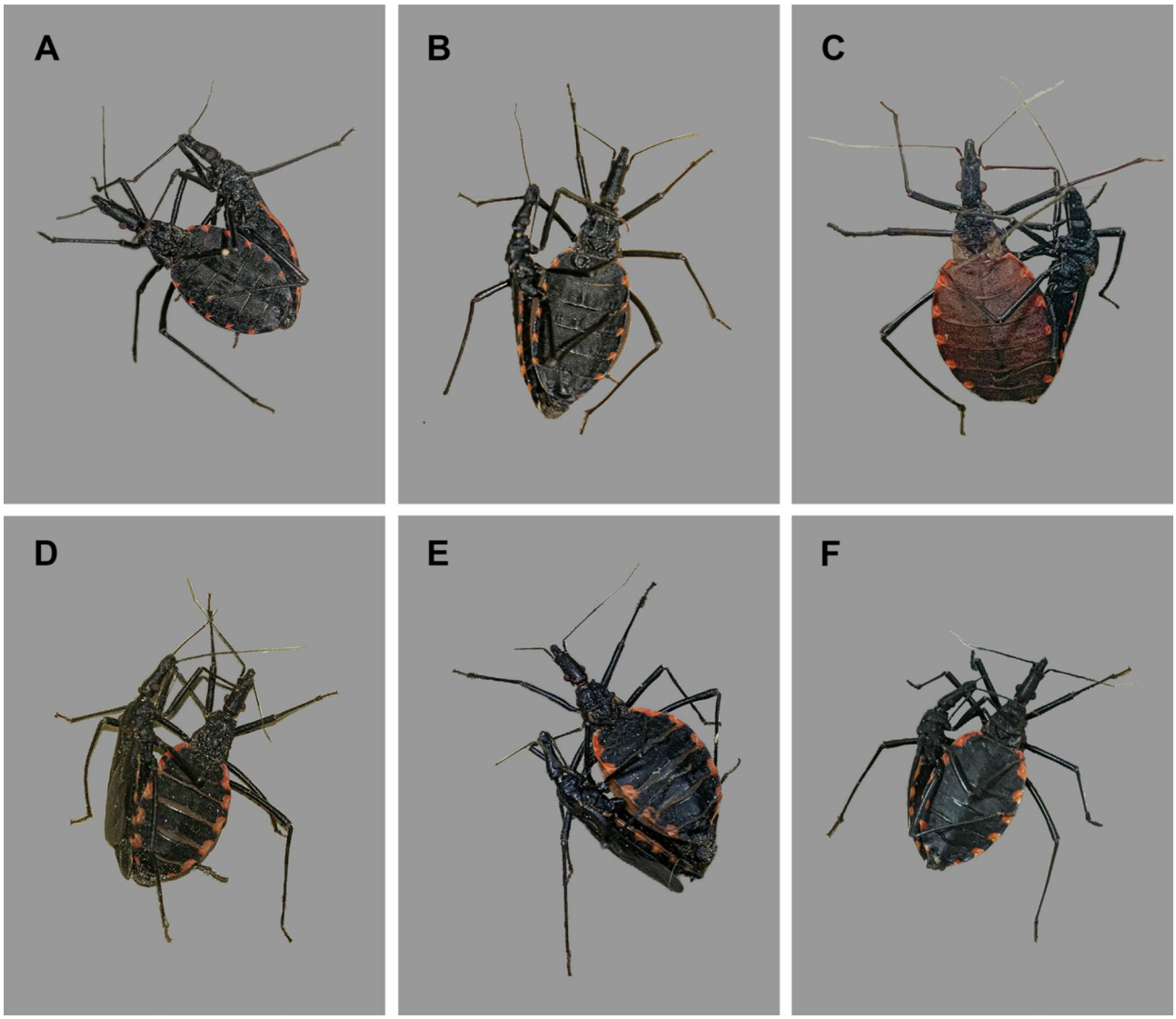

Fig. 1. Interspecific mating of *Mepraia* spp. (**A**) *M. parapatrica* ♀ × *M. gajardoi* ♂; (**B**) *M. gajardoi* ♀ × *M. parapatrica* ♂; (**C**) *M. parapatrica* ♀ × *M. spinolai* ♂; (**D**) *M. spinolai* ♀ × *M. gajardoi* ♂; (**E**) *M. gajardoi* ♀ × *M. spinolai* ♂; (**F**) *M. spinolai* ♀ × *M. parapatrica* ♂.

spinolai ♀), they observed unbalanced chromosome numbers that resulted in non-viable gametes[14]. Our results, once again, differ from what was observed, since our F2 offspring did not reach adulthood. This phenomenon may have occurred because, unlike the authors who performed backcrosses, we performed intercrosses and, possibly, the genetic deregulation resulting from recombination during meiosis of the F1 hybrids[25] was greater because we crossed a male hybrid with a female hybrid (in which both suffered deregulation[25], unlike the backcross in which only one of the specimens used suffered deregulation[14]).

Hybrid collapse was initially characterized for *Triatoma* Laporte, 1832 species of the *T. brasiliensis* complex[25–27]. The authors observed that F1 hybrids were viable and fertile (with 100% pairing between chromosomes), that F2 offspring reached adulthood and that they practically did not produce third-generation hybrids (F3) (since the gametes of F2 hybrids were rendered unviable by chromosomal errors)[25]. Both phenomena, namely, inviability (as observed in *Mepraia*) or sterility (as observed in *Triatoma*) of the hybrid from F2 characterize the hybrid collapse. It is believed that the species of the *T. brasiliensis* complex have been diversified recently[29]. Curiously, the same phenomenon was recently suggested by Belintani et al.[22] for *Mepraia* spp. Given these factors, we can suggest that, possibly, hybrid collapse is the post-zygotic barrier responsible for preventing natural hybridization and gene flow between species that derived more recently.

Likewise, the same post-zygotic barrier was responsible for the hybrid breakdown in one direction of the crosses between *M. parapatrica* and the other species of the genus *Mepraia*, that is, ♀ *M. parapatrica* x *M. gajardoi* ♂ and ♀ *M. spinolai* x ♂ *M. parapatrica* (Table 1). The other direction did not allow intercrossings to be carried out, as all hybrids that reached adulthood were female (Table 2). This phenomenon is very interesting, because although the number of adult hybrids was relatively low (Table 2), the total absence of males suggests the

Experimental crosses	Number of eggs	Hatching rate	Number of nymphs	Mortality rate
Interspecific crosses				
♀ *M. spinolai* x *M. parapatrica* ♂	80	20%	16	83%
♀ *M. parapatrica* x *M. spinolai* ♂	65	10%	06	100%
♀ *M. gajardoi* x *M. parapatrica* ♂	36	05%	05	100%
♀ *M. parapatrica* x *M. gajardoi* ♂	174	26%	46	43%
♀ *M. spinolai* x *M. gajardoi* ♂	70	34%	24	68%
♀ *M. gajardoi* x *M. spinolai* ♂	108	49%	53	89%
Intercrosses				
♀ Hybrid x Hybrid ♂[1]	119	15%	18	100%
♀ Hybrid x Hybrid ♂[2]	82	33%	29	100%
♀ Hybrid x Hybrid ♂[3]	60	12%	07	100%
♀ Hybrid x Hybrid ♂[4]	127	14%	18	100%
Intraspecific Crosses (Control)				
♀ *M. spinolai* x *M. spinolai* ♂	217	71%	154	–
♀ *M. gajardoi* x *M. gajardoi* ♂	440	80%	350	–
♀ *M. parapatrica* x *M. parapatrica* ♂	93	76%	71	–

Table 1. Experimental crosses conducted among *Mepraia* spp. [1]Hybrids from the cross between ♀ *M. parapatrica* x ♂ *M. gajardoi*; [2]Hybrids from the cross between ♀ *M. spinolai* x ♂ *M. gajardoi*; [3]Hybrids from the cross between ♀ *M. gajardoi* x ♂ *M. spinolai*; [4]Hybrids from the cross between ♀ *M. spinolai* x ♂ *M. parapatrica*.

Experimental crosses	Adult hybrids		
	Males (M)	Females (F)	M%
♀ *M. spinolai* x *M. parapatrica* ♂	08	08	50%
♀ *M. parapatrica* x *M. spinolai* ♂	00	06	00%
♀ *M. gajardoi* x *M. parapatrica* ♂	00	05	00%
♀ *M. parapatrica* x *M. gajardoi* ♂	20	16	67%
♀ *M. spinolai* x *M. gajardoi* ♂	09	14	39%
♀ *M. gajardoi* x *M. spinolai* ♂	08	10	44%

Table 2. Analysis of Haldane's rule application in *Mepraia* spp. M%: male offspring rate (number of males/total number of hybrids × 100).

possible application of Haldane's rule in these crosses. Selivon et al.[28] highlighted that the sterility or unfeasibility of heterogametic sex—in the case of triatomines, the male (XY, X_1X_2Y or $X_1X_2X_3Y$)[29]—is the first step in the evolution of processes associated with unfeasibility or complete sterility, being the study of the occurrence of Haldane's rule in incipient or evolutionarily related species of particular interest for the knowledge of the processes associated with speciation. Thus, Haldane's rule, previously reported as absent in Triatominae[1,14,30,31]—including for *Mepraia*[14]—, possibly played an important role in the diversification of *Mepraia*.

From a taxonomic point of view, the specific status of the three species is confirmed by the presence of reproductive barriers, based on the biological species concept[23,24]. The characterization of reproductive isolation, even if post-zygotic, is an important step for integrative taxonomy of *Mepraia*, once it is in accordance with the phenotypic[14–16] (phenetic species concept)[32] and molecular[21,22] (phylogenetic species concept)[33] characteristics. The combination of different study approaches to characterize the specific status of a triatomine was initiated, in 1998, with the description of *M. gajardoi*[5,34]. In addition to assisting in the characterization of new taxa with greater robustness[35], integrative taxonomy (combining the application of experimental crosses with other approaches) has contributed to revalidation[36] and synonymization[1,37] events of triatomines.

Even though hybrids have been obtained in all directions (Table 1), the combination of different evolutionary events demonstrated here, such as low hatching rate and high mortality rate of hybrids, as well as the presence of post-zygotic reproductive barriers and possible action of Haldane's rule indicate that there is no continuous interspecific gene flow between *Mepraia* spp. Therefore, if these species start to live in sympatry—due to climate change, as indicated by Garrido et al.[13]—and, in the best-case scenario, backcrossing occurs under natural conditions, the probability of introgression is non-existent given the barriers observed by Campos-Soto et al.[14] when backcrossing hybrids with parents.

Methods

Experimental crosses

Interspecific crosses were performed between the three species of *Mepraia* (Table 1). The species used were provided of colonies maintained in the by the Triatominae Insectarium of the School of Pharmaceutical Sciences

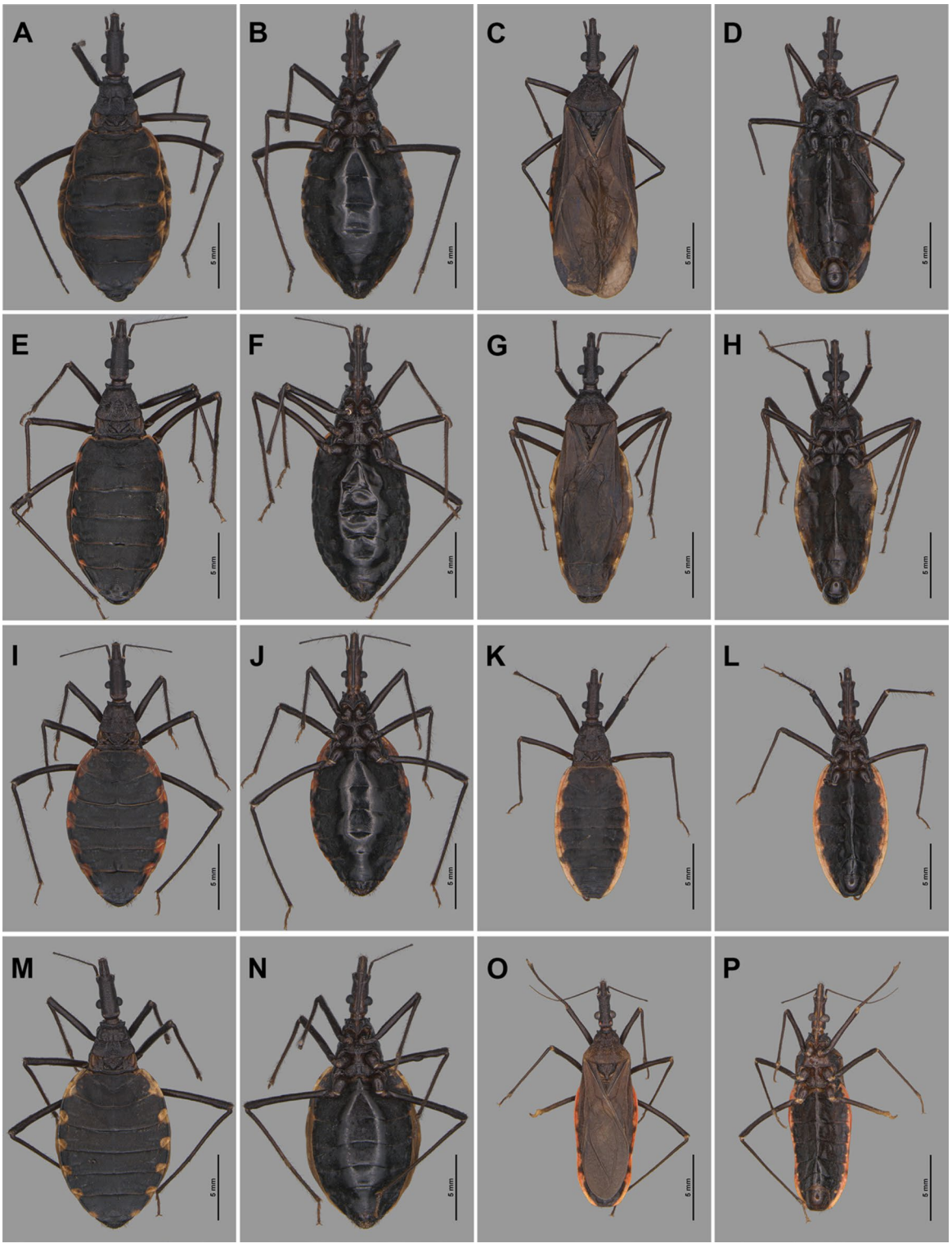

Fig. 2. Adult hybrids of *Mepaia* spp. (**A**–**D**): *M. gajardoi* ♀ × *M. spinolai* ♂ in dorsal (A,C) and ventral view (B,D); (**E**–**H**): hybrids from the cross between *M. parapatrica* ♀ × *M. gajardoi* ♂ in dorsal (E,G) and ventral view (F,H); (**I**–L): hybrids from the cross between *M. spinolai* ♀ × *M. gajardoi* ♂ in dorsal (I,K) and ventral view (J,L); (**M**–**P**): hybrids from the cross between *M. spinolai* ♀ × *M. parapatrica* ♂ in dorsal (M,O) and ventral view (N,P).

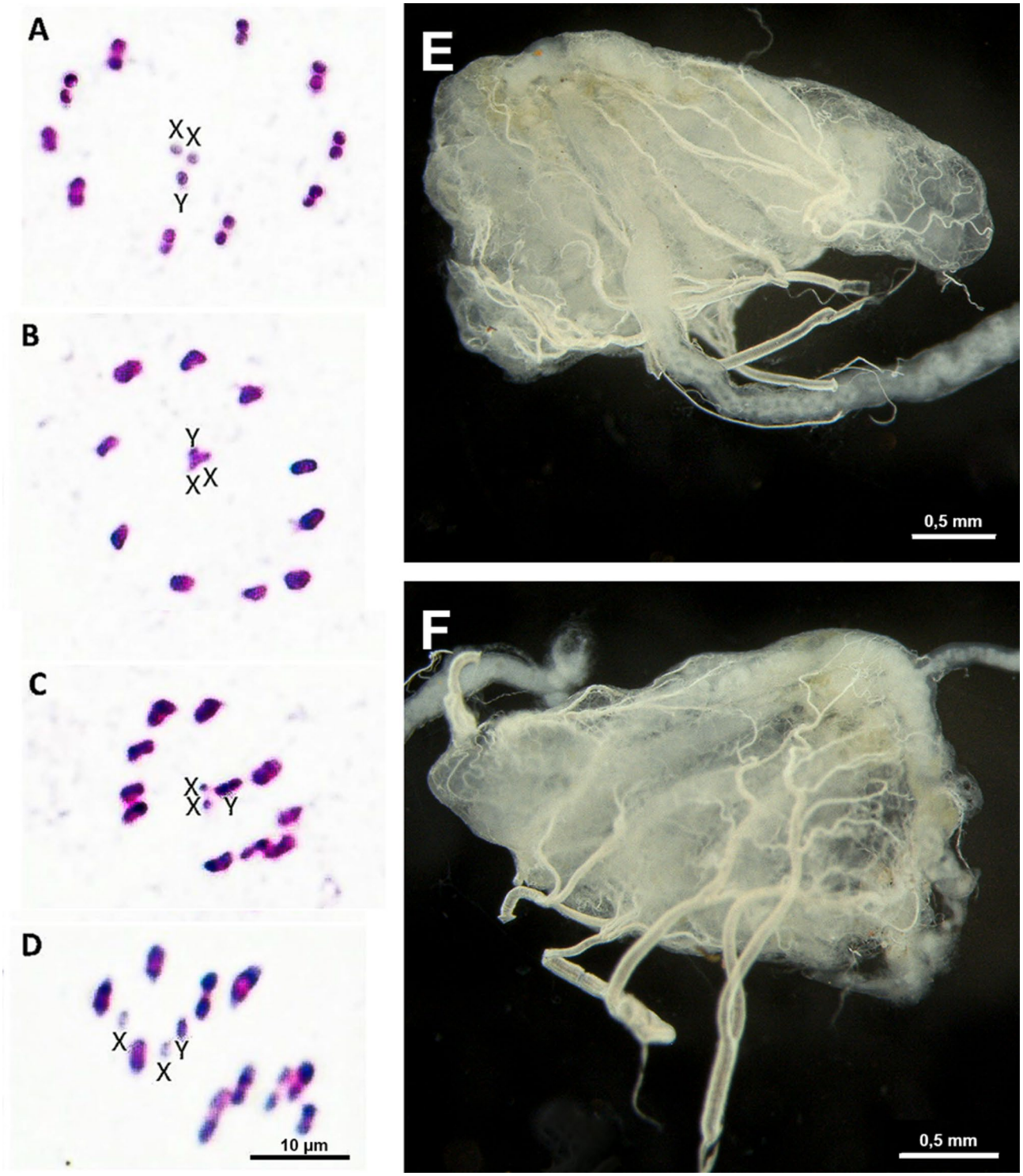

Fig. 3. Cytogenetic (**A**–**D**) and morphological (E,F) characterization of the testes of *Mepraia* hybrids. A,D. Hybrids from the cross between ♀ *M. parapatrica* × ♂ *M. gajardoi*, B. Hybrids from the cross between ♀ *M. spinolai* × ♂ *M. gajardoi*, C. Hybrids from the cross between ♀ *M. gajardoi* × ♂ *M. spinolai*, D,F. Hybrids from the cross between ♀ *M. spinolai* × ♂ *M. parapatrica*. Note that all metaphases show 100% chromosome pairing (**A**–**D**) and that the testicle does not present gonadal dysgenesis (E,F).

(FCFAR/UNESP), Araraquara, São Paulo, Brazil [*M. gajardoi* originating from Iquique, Caleta San Marcos, 21° 00.535′ S, 070° 10.104′ W, 23 m (Figure 4A–D), *M. parapatrica* originating from Taltal, Caleta Loreto, 24° 50.062′ S, 070° 32.447′ W, 7 m (Figure 4E–H), and *M. spinolai* originating from Colina, cantera 1, 33° 05.279′ S 070° 40.800′ W, 755 m (Figure 4I–L)]. To ensure the virginity of the crossed insects, fifth instar nymphs were

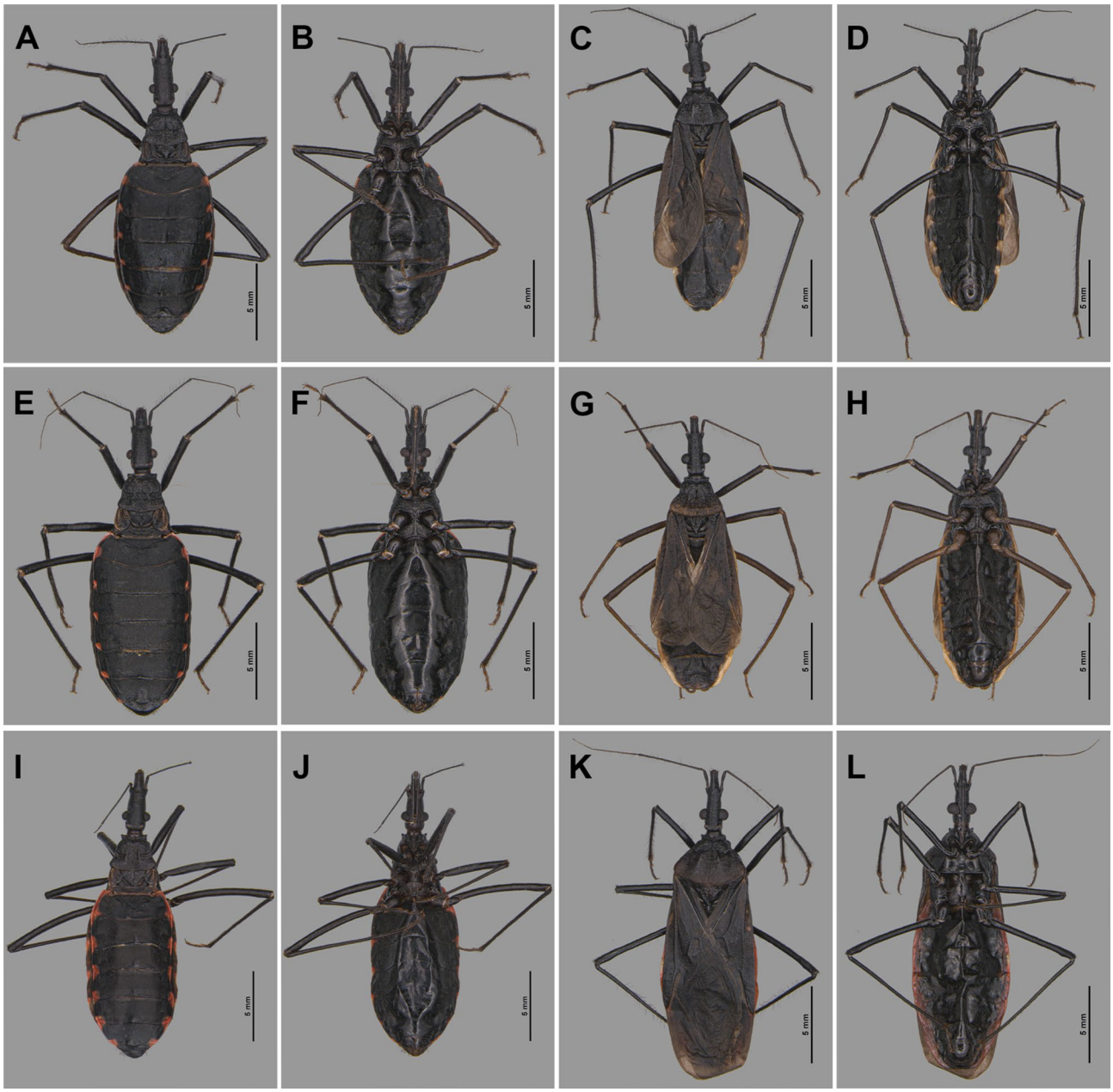

Fig. 4. Parental adults of *Mepaia* spp. (**A**–**D**): *M. gajardoi* in dorsal (A,C) and ventral view (B,D); (**E**–**H**): *M. parapatrica* in dorsal (E,G) and ventral view (F,H); (**I**–**L**): *M. spinolai* in dorsal (I,K) and ventral view (J,L); A,B,E,F,I,J: female hybrids; C,D,G,H,K,L: male hybrids.

sexed and separated in sex-specific pots[25,38]. After reaching the adult stage, five experimental crosses for each direction (Table 1) were initiated (each couple kept separate in a plastic jar) and lasted 4 months[38]. Insect feeding were performed weekly during this period[38]. The insects were kept at room temperature (average of 24 °C) and relative humidity of 63%[39]. The hatching rate of eggs from each cross and mortality of hybrids were monitored weekly (Table 1). With hybrids that have reached the adult stage, intercrosses (♀ hybrid x ♂ hybrid) were performed to evaluate the fertility of the hybrid offspring (Table 1). In addition, intraspecific crosses were performed as a control group (Table 1). Finally, the hybrids that reached adulthood were sexed to assess whether Haldane's rule[40] was acting. This rule predicts that if hybrids hatch, the heterogametic sex is the first affected by the evolutionary events that make this organism unfeasible or lead to sterility[40,41]. We justify that for all quantitative data collected, the relative frequency was calculated.

Morphology and cytogenetic of the gonads

Five adult male hybrids from each cross were dissected and their testes removed and stored in a methanol: acetic acid solution (3:1). The morphology of the hybrid and parental gonads (control group) was analyzed under a stereomicroscope microscope (SM) (model MZ APO; Leica Microsystems GmbH, Wetzlar, Germany) fitted with

the Motic Advanced 3.2 Plus Image Analysis System (Motic, Hong Kong) to evaluate the presence of GD (which may be uni- or bilateral)[42]. Later, slides were prepared by the cell-crushing technique (as described by Alevi et al.[43]), and cytogenetic analyses were performed to characterize spermatogenesis of the hybrids, with emphasis on the degree of pairing between the homologous chromosomes[25], using the lacto-acetic orcein technique[43,44]. The slides were examined under a light microscope (Jenamed; Carl Zeiss, Jena, Germany) that was coupled with a digital camera with a 1000-fold magnification; AxioVision LE version 4.8 imaging software (Carl Zeiss) was used for analysis.

Data availability

All data generated or analysed during this study are included in this published article.

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References

1. Campos, F. F. et al. One genome, multiple phenotypes: Would *Rhodnius milesi* Carcavallo, Rocha, Galv.o & Jurberg, 2001 (Hemiptera, Triatominae) Be a valid species or a phenotypic polymorphism of *R. neglectus* Lent, 1954?. *Diversity* **16**, 472 (2024).
2. Paiva, V. F. et al. *Hospesneotomae* n. gen. of the Triatomini tribe presents a turnaround in the taxonomy of the Triatoma protracta species complex. *Sci. Rep.* **15**, 8143. https://doi.org/10.1038/s41598-025-91399-w (2025).
3. Mazza, S., Tobar, R.G. & Jörg, M.E.. Mepraia novum genus de Triatomidae. Mepraia spinolai (Porter) 1933, nov. comb., redescipción de ♂ y descripción de ♀. Investigaciones sobre Triatomidae. Universidad de Buenos Aires, Misión de Estudios de Patología Regional Argentina (Jujuy), **44**, 1–30 (1940).
4. Campos, R. et al. High phylogeographic structure in sylvatic vectors of Chagas disease of the genus *Mepraia* (Hemiptera: Reduviidae). *Infect. Genet. Evol.* **19**, 280–286 (2013).
5. Frías, D. & Atria, J. Chromosomal variation, macroevolution and possible parapatric speciation in *Mepraia spinolai* (Porter) (Hemiptera: Reduviidae). *Genet. Mol. Biol.* **21**, 179–184 (1998).
6. Porter, C. E. Una Triatoma nueva chilena. *Revista Chilena de Historia Natural* **37**, 192–193 (1933).
7. Galvão, C., Carcavallo, R., Rocha, D. S. & Jurberg, J. A checklist of the current valid species of the subfamily Triatominae Jeannel, 1919 (Hemiptera, Reduviidae) and their geographical distribution, with nomenclatural and taxonomic notes. *Zootaxa* **202**, 1–36 (2003).
8. Frías-Lasserre, D. A new species and karyotype variation in the bordering distribution of *Mepraia spinolai* (Porter) and *Mepraia gajar* Frías et al. (Hemiptera, Reduviidae, Triatominae) in Chile and its parapatric model of speciation. *Neotrop. Entomol.* **39**, 572–583 (2010).
9. Rosa, J. A., de Oliveira, J. & Laserre, D. A. F. New records of *Mepraia* (Hemiptera, Reduviidae, Triatominae) in Chile. *J. Vector Ecol.* **44**, 195–198 (2019).
10. Campos-Soto, R. et al. Interactions between *Trypanosoma cruzi* the chagas disease parasite and naturally infected wild *Mepraia* vectors of Chile. *Vector Borne Zoonotic Dis.* **16**, 165–171 (2016).
11. Quiroga, N. et al. Blood-meal sources and *Trypanosoma cruzi* infection in coastal and insular Triatomine bugs from the Atacama Desert of Chile. *Microorganisms* **10**, 785 (2022).
12. San Juan, E. et al. Humans as blood-feeding sources in sylvatic triatomines of Chile unveiled by next-generation sequencing. *Parasit. Vectors* **16**, 225 (2023).
13. Garrido, R. et al. Potential impact of climate change on the geographical distribution of two wild vectors of Chagas disease in Chile: *Mepraia spinolai* and *Mepraia gajardoi*. *Parasit. Vectors* **12**, 478 (2019).
14. Campos-Soto, R. et al. Experimental crosses between *Mepraia gajar* and *M. spinolai* and hybrid chromosome analyses reveal the occurrence of several isolation mechanisms. *Inf. Gen. Evol.* **45**, 205–212 (2016).
15. Frías, D.; Atria, J. Chromosomal variation, macroevolution and possible parapatric speciation in Mepraia spinolai (Porter) (Hemiptera: Reduviidae). *Genet. Mol. Biol.* **21**, 179–184 (1998).
16. Frías, D., Henry, A. & González, C. *Mepraia gajardoi*: A new species of triatominae (Hemiptera, Reduviidae) from Chile and its comparison with Mepraia spinolai. *Rev. Chil. Hist. Nat.* **71**, 177–188 (1998).
17. Lasserre, D. F., de Oliveira, J., Pinotti, H. & da Rosa, J. A. Morphological description of *Mepraia* spp. females (Hemiptera: Reduviidae, Triatominae). *Acta Trop.* **190**, 389–394 (2019).
18. Calleros, L. et al. Systematics of *Mepraia* (Hemiptera-Reduviidae): Cytogenetic and molecular variation. *Infect. Genet. Evol.* **10**, 221–228 (2010).
19. Campos, R. et al. High phylogeographic structure in sylvatic vectors of Chagas disease of the genus *Mepraia* (Hemiptera, Reduviidae). *Infect. Genet. Evol.* **19**, 280–286 (2013).
20. Campos, R. et al. Wing shape differentiation of *Mepraia* species (Hemiptera, Reduviidae). *Infect. Genet. Evol.* **11**, 329–333 (2011).
21. Campos-Soto, R. et al. Testing phylogeographic hypotheses in *Mepraia* (Hemiptera: Reduviidae) suggests a complex spatio-temporal colonization in the coastal Atacama Desert. *Insects.* **13**, 419 (2022).
22. Belintani, T. et al. Transcriptome-based phylogenomic analysis reveals evidence of ancient hybridization events in the *Mepraia* genus (Hemiptera: Reduviidae: Triatominae). *Front. Ecol. Evol.* **11**, 1215319 (2023).
23. Mayr, E. *Animal Species and Evolution* Vol. 797 (Harvard University Press, 1963).
24. Dobzhansky, T. *Genetics of the Evolutionary Process* Vol. 505 (Columbia University Press, 1970).
25. Mendonça, V. J. et al. Cytogenetic and morphologic approaches of hybrids from experimental crosses between *Triatoma lenti* Sherlock & Serafim, 1967 and *T. sherlocki* Papa et al., 2002 (Hemiptera: Reduviidae). *Inf. Gen. Evol.* **26**, 123–131 (2014).
26. Alevi, K. C. C. et al. Hybrid collapse confirms the specific status of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera, Triatominae), an endemic species in Brazil. *Am. J. Trop. Med. Hyg.* **98**, 475–477 (2018).
27. Pinotti, H. et al. Revisiting the hybridization processes in the *Triatoma brasiliensis* complex (Hemiptera, Triatominae): interspecific genomic compatibility point to a possible recent diversification of the species grouped in this monophyletic complex. *PLoS ONE* **16**, e0257992 (2021).
28. Selivon, D., Perondini, A. L. P. & Morgante, J. S. Haldane's rule and other aspects of reproductive isolation observed in the *Anastrepha fraterculus* complex (Diptera: Tephritidae). *Gen. Mol. Biol.* **22**, 507–510 (1999).
29. Alevi, K. C. C., Oliveira, J., Rosa, J. A. & Azeredo-Oliveira, M. T. V. Karyotype evolution of Chagas disease vectors (Hemiptera, Triatominae). *Am. J. Trop. Med. Hyg.* **99**, 87–89 (2019).
30. Villacís, A. G. et al. Chagas vectors *Panstrongylus chinai* (Del Ponte, 1929) and *Panstrongylus howardi* (Neiva, 1911): Chromatic forms or true species?. *Parasit. Vectors* **13**, 226 (2020).
31. Panzera, F., Pita, S. & Lorite, P. Chromosome structure and evolution of triatominae: A review. In *Triatominae - The Biology of Chagas Disease Vectors* (eds Guarneri, A. & Lorenzo, M.) 65–99 (Springer, 2021).
32. Ridley, M. Evolution. *J. Evol. Biol.* **6**, 615–617 (1993).

33. Cracraft, J. Species concepts and speciation analysis. In *Current Ornithology* (ed. Johnston, R. F.) 159–187 (Springer, 1983).
34. Alevi, K. C. C., Oliveira, J., Rocha, D. S. & Galvão, C. Trends in taxonomy of Chagas disease vectors (Hemiptera, Reduviidae, Triatominae): From Linnaean to integrative taxonomy. *Pathogens.* **10**, 1627 (2021).
35. Alevi, K. C. C. et al. *Triatoma rosai* sp. nov (Hemiptera, Triatominae): A new species of Argentinian Chagas disease vector described based on integrative taxonomy. *Insects* **11**, 830 (2020).
36. Mendonça, V. J. et al. Revalidation of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera: Reduviidae) and phylogeny of the *T. brasiliensis* species complex. *Zootaxa* **4107**, 239–254 (2016).
37. Nascimento, J. D. et al. Taxonomical over splitting in the *Rhodnius prolixus* (Insecta: Hemiptera: Reduviidae) clade: Are *R. taquarussuensis* (da Rosa et al., 2017) and *R. neglectus* (Lent, 1954) the same species?. *PLoS ONE* **14**, e0211285 (2019).
38. Reis, Y. V. et al. Trends in evolution of the Triatomini tribe (Hemiptera, Triatominae): Reproductive incompatibility between four species of geniculatus clade. *Parasit. Vectors* **15**, 403 (2022).
39. Olaia, N. et al. Biology of Chagas disease vectors: biological cycle and emergence rates of *Rhodnius marabaensis* Souza et al., 2016 (Hemiptera, Reduviidae, Triatominae) under laboratory conditions. *Parasitol. Res.* **120**, 2939–2945 (2021).
40. Haldane, J. B. S. Sex ratio and unisexual sterility in hybrid animals. *J. Genet.* **12**, 101–109 (1922).
41. Turelli, M. & Orr, H. A. The dominance theory of Haldane's Rule. *Genetics* **140**, 389–402 (1995).
42. Azevedo, L. M. S. et al. First evidence of gonadal hybrid dysgenesis in Chagas disease vectors (Hemiptera, Triatominae): Gonad atrophy prevents events of interspecific gene flow and introgression. *Parasit. Vectors* **16**, 390 (2023).
43. Alevi, K. C. C. et al. Karyotype of *Triatoma melanocephala* Neiva & Pinto (1923). Does this species fit in the Brasiliensis subcomplex?. *Infect. Genet. Evol.* **12**, 1652–1653 (2012).
44. De Vaio, E. S., Grucci, B., Castagnino, A. M., Franca, M. E. & Martinez, M. E. Meiotic differences between three triatomine species (Hemiptera: Reduviidae). *Genetica* **67**, 185–191 (1985).

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Author contributions

J.O. conceived and designed the study, performed the data analysis, contributed to the interpretation of results, wrote the main manuscript text, and prepared figures. K.C.C.A. conceived and designed the study, performed the data analysis, contributed to the interpretation of results, and wrote the main manuscript text. A.R. conducted the experiments, collected the data, and provided critical revisions to the manuscript. J.K.S.S. conducted the experiments, collected the data, provided critical revisions to the manuscript, and prepared figures. D.F.L. conducted the experiments, collected the data, and provided critical revisions to the manuscript. J.A.R. conceived and designed the study and provided critical revisions to the manuscript. C.G. conducted the experiments, collected the data, and provided critical revisions to the manuscript. M.T.M. performed the data analysis, contributed to the interpretation of results, and provided critical revisions to the manuscript. All authors reviewed, edited, and approved the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

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