

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

A New Species and Karyotype Variation in the Bordering Distribution of *Mepraia spinolai* (Porter) and *Mepraia gajardoi* Frías *et al* (Hemiptera: Reduviidae: Triatominae) in Chile and its Parapatric Model of Speciation

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ABSTRACT - In the present study, the morphology, color pattern, chromosomal complement and aspects of meiosis in natural populations at the borders of the distributions of *Mepraia gajardoi* Frías *et al* and *Mepraia spinolai* (Porter) are described. The males of these bordering populations are brachypterous or macropterous, while females are always micropterous. Morphological and cytogenetic data indicated that the populations that border the distributions of *M. gajardoi* and *M. spinolai*, belong to a different species of parapatric origin.

KEY WORDS: Wing polymorphism, heterochromatin variation, vector of Chagas' disease

Mepraia spinolai (Porter) and *Mepraia gajardoi* Frías *et al* are two endemic Chilean Reduviidae. *Mepraia gajardoi*, originally considered as a population of *M. spinolai*, later came to be regarded as a distinct species (Frías *et al* 1998, Jurberg *et al* 2002). It is distributed along the northern coast of Chile, approximately between 18° and 26° S, while *M. spinolai* is distributed approximately between 26° S and 33° S (Frías & Atria 1998). Both species transmit the causal agent of Chagas' disease, *Trypanosoma cruzi*. *Mepraia spinolai* is adapted to sylvatic/peridomestic habitats, and *M. gajardoi* only to sylvatic habitats (Sagua *et al* 2000, Canals *et al* 2001, Carvajal *et al* 2007, Botto-Mahan *et al* 2008). This disease is important in the Neotropical region with nearly 16 million people infected, and another 90 million at risk of contracting the disease, especially in rural areas (Schofield *et al* 1982, Schofield 1994).

Mepraia gajardoi differs from *M. spinolai* in body color, morphology, genitalia and karyotype. Females of both species are invariably wingless (micropterous). Males of *M. gajardoi* are always winged (brachypterous), whereas males of *M. spinolai* may be winged (macropterous, brachypterous) or wingless (micropterous) (Mazza *et al* 1940, Neiva & Lent 1940, Lent & Jurberg 1967, Frías *et al* 1998). Reciprocal crossbreeding experiments showed that the two species are reproductively isolated (Frías *et al* 1998).

The karyotypes of both species are formed by 20 autosomes and an X1 X2 Y sex system. However, males of *M. gajardoi* have a large Y chromosome, whereas those of *M. spinolai* are polymorphic for a small neo-Y chromosome, which may have originated by a fracture of the large holocentric Y chromosome of *M. gajardoi* (Frías & Atria 1998). Also, a particular heteropycnotic chromocenter, typical of *M. spinolai*, differentiates this species from *M.*

gajardoi. It is formed by sex chromosomes surrounded by several autosomal heteropycnotic dots. Other heteropycnotic regions outside this chromocenter can also be observed (Frías & Atria 1998, Perez *et al* 2004)

In the present study, I report the morphological traits of adults and the chromosomal complement of populations of *Mepraia* species bordering the distribution of *M. gajardoi* and *M. spinolai*, with a discussion of their mode of speciation.

Material and Methods

Populations studied. The specimens examined were collected during the summer of 1996 by D Frías and A Alviña, and during spring, in November 2002, by D Frías, from region III of Atacama and region II of Antofagasta (Fig 1). The former region is located in the Parque Nacional Pan de Azúcar: Pan de Azúcar Island, 3 km SW of Porto Pan de Azúcar (26° 9' 05" S, 70° 40' 53" W, 50 m a.s.l.): six males and two females; associated with *Spheniscus humboldti* (Humboldt penguin); in the coastal area of the mainland in the Caleta Pan de Azúcar (26° 9' 01" S; 70° 40' 01" W, 0 m a.s.l.): two females, two males associated with lizards; Cerro El Soldado, 3 km S of Porto Pan de Azúcar (26° 10' 10" S, 70° 40' 03" W, 231 m a.s.l.): one female and two males, associated with seagulls *Larus dominicanus*. Inca de Oro, Mina San Vicente, 16 km NE of Estación Chimbero (26° 45' 01" S, 69° 54' 06" W, 1600 m a.s.l.): one female, three macropterous males, two micropterous males. The second was in the Caleta Bandurrias, 21 km S of Papos (25°12' 20" S, 70° 26' 7" W, 0 m a.s.l.): one female, six males, associated with goats *Capra hircus*; Cachinales, 22 km S of Papos (25°12' 58" S, 70° 26' 12" W, 0 m a.s.l.): two males associated with *L. dominicanus*.

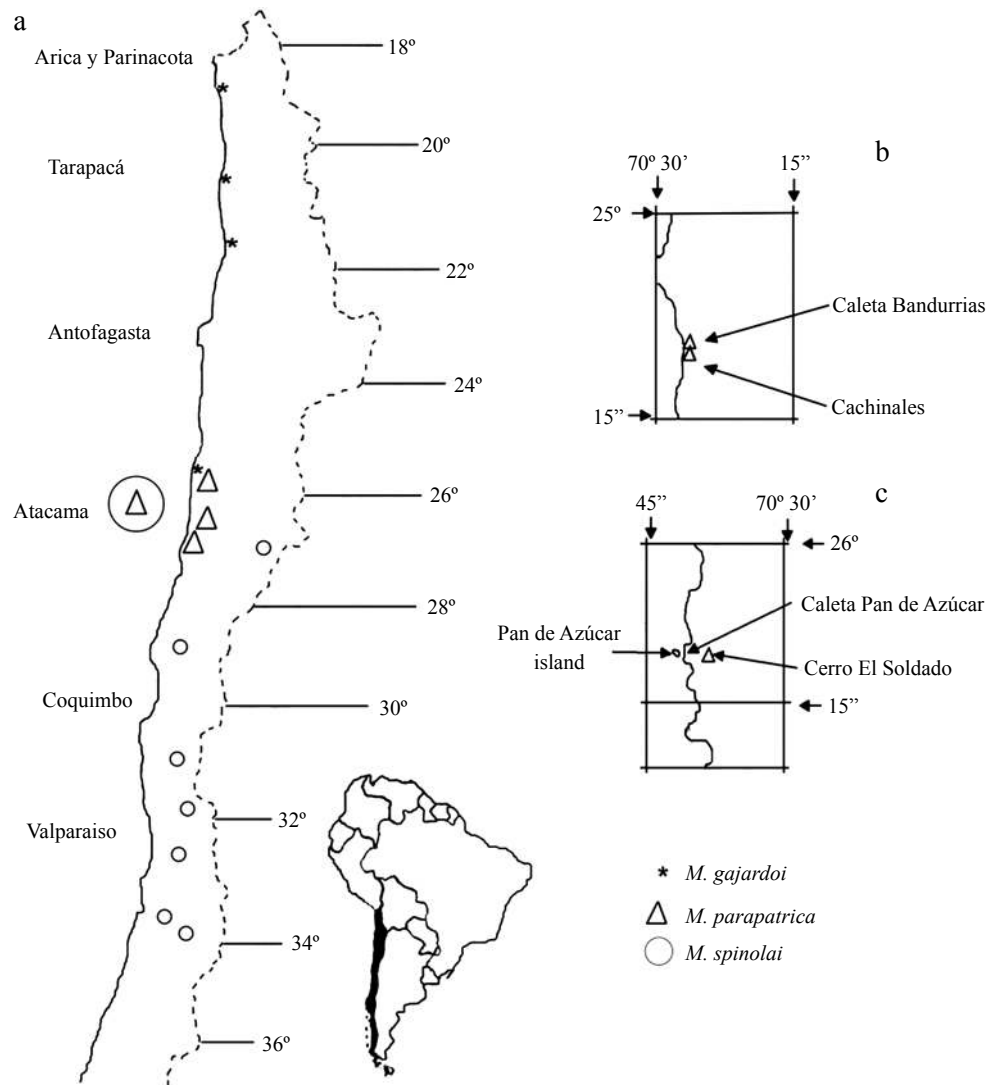


Fig 1 Geographical distribution of *Mepraia parapatrica*, *M. spinolai* and *M. gajardoi*. General view (a). Details of the distribution of *M. parapatrica* in region II of Antofagasta (b) and in region III of Atacama (c).

The specimens were transported to the laboratory in plastic boxes and maintained on live chicken.

Specimens collected in these studies were deposited in the Collection of the Institute of Entomology, Universidad Metropolitana de Ciencias de la Educación (IEUMCE), Santiago, Chile.

For comparative purposes, I utilized 168 specimens, 107 females and males of *M. spinolai* and 61 of *M. gajardoi* from different localities, collected between 18°S and 33°S, and those deposited at IEUMCE, Santiago, Chile. Specimens of *M. gajardoi* from region II and *M. spinolai* from region III were especially studied because they overlap parapatrically with the new species at some of these localities. Herein, I also redescribe *M. spinolai* and compare it to the new species and to *M. gajardoi* (Frias *et al* 1998, Jurberg *et al* 2002).

Characteristics of each locality. Pan de Azúcar Island and other coastal localities of regions II and III lie in a coastal-desert climate regime, with less than 200 mm of rain annually

(Di Castri & Hajek 1976). Pan de Azúcar Island has been preserved as a national park because the Humboldt penguin *Spheniscus humboldti* and the sea otter *Lutra felina* reproduce on this island. The fauna and flora are similar to those of the coast, in the Atacama Desert. Cacti, marine birds, carrion birds, lizards and other marine mammals such as the sea lion *Otaria flavescens* are numerous on the island (Sagua *et al* 2000). Inca de Oro is a continental city located 1,600 m a.s.l., near the Domeyko Cordillera. This locality is in the most arid part of northern Chile. It has a desert climate with cold mountains and scarce and irregular rainfall during winter, rarely reaching 100 mm per year. The fauna that serves as a host to sylvatic *M. spinolai* is mainly composed by small rodents, such as *Chinchilla lanigera*, *Lagidium viscacia*, *Oligoryzomys longicaudatus*, *Phyllotis darwini*, *Phyllotis xanthopygus*, *Abrocoma benetti* and *Octodon degus*.

Morphology. I counted the number of wingless (micropterous) and winged (brachypterous and macropterous) males collected

in each locality. Micropterous males, like females, have their hemelytra reduced to small suboval sclerites, not extending beyond the posterior margin of the mesonotum. Males with hemelytra shorter than the abdomen are considered brachypterous. Males having the hemelytra longer than or as long as the abdomen are considered macropterous.

The following measurements were taken for females and males: total length of the body (except the rostrum), head length (except the rostrum), maximum width of the head at the eye level, pronotum length and width (Fig 2a), hemelytra length (Fig 2b), abdomen length and width (Fig 2c). Body coloration and the color pattern of the connexivum of the abdomen were also analyzed. Terminology follows that of Lent & Wygodzinsky (1979).

The genitalia were dissected and treated overnight in 10% KOH. In males, the median process of the pygophore, parameres and phallus were studied following Lent & Jurberg (1967) and Jurberg *et al* (1998, 2002). In female genitalia, the gonocoxite 8 (Gc8), gonocoxite 9 (Gc9), gonapophysis 8 (G8) and gonapophysis 9 (G9) were particularly analyzed, following Lent & Jurberg (1967).

Cytological preparation. Chromosomes were obtained from the gonads of adults that were fixed in 3:1 ethanol-acetic acid. The gonads were crushed in 50% acetic acid, and frozen in liquid nitrogen. Coverslips were removed with a razor blade. Karyotypes were obtained by the classic lacto-acetic orcein method (Solferini & Morgante 1987). The barium hydroxide method of Sumner (1972) was used for C-Banding. Chromosome preparations were examined using an optical

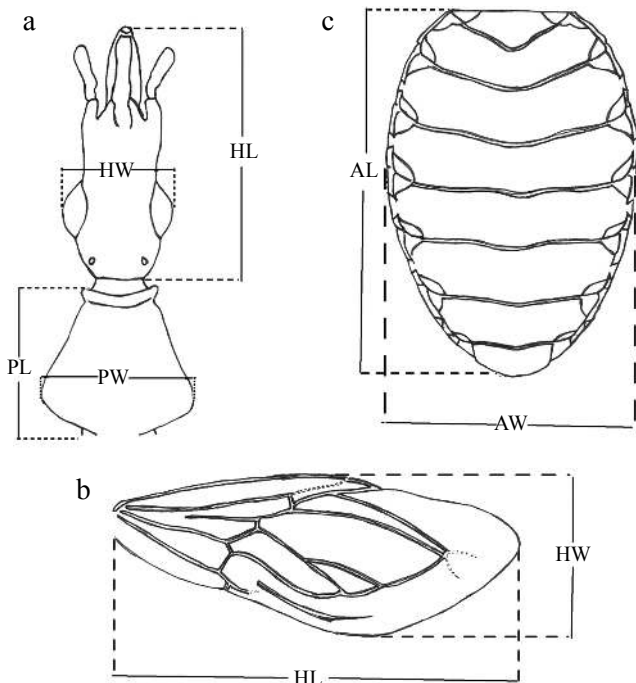


Fig 2 Body measurements of *Mepraia parapatric n. sp.* Head and thorax (a), HW = head width, HL = head length, PL = pronotum length, PW = pronotum width. Hemelytra (b) HL = hemelytra length, HW = hemelytra width. Abdomen (c), AL = abdomen length, AW = abdomen width.

Leitz Laborlux K Microphoto microscope in addition to a laser scanning microscope 633 (Helium-Neon, Carl Zeiss, Inc. Model 210).

Mepraia parapatric n. sp.

Holotype: Brachypterous male, region II of Antofagasta, Caleta Bandurrias, three November 2002, associated with goats, deposited at IEUMCE. **Paratypes:** one female and six males (one macropterous and five brachypterous), same data and locality as holotype. **Cachinales,** one female and two males (one brachypterous and one macropterous), associated with lizards, same data and locality as holotype. Collector D Frías.

Region III of Atacama, Parque Nacional Pan de Azúcar: Pan de Azúcar Island: two females and six males (one macropterous and five brachypterous), 5 November 2002, Caleta Pan de Azúcar, in coastal area of mainland: two females and two males (one macropterous and one brachypterous) associated with lizards, 6 November 2002, D Frías coll. **Cerro el Soldado:** one female and two brachypterous males, associated with sea birds, D Frías and A Alviña colls., 15 December 1996. The paratypes were deposited at IEUMCE.

Type locality. Caleta Bandurrias, region II of Antofagasta, 25° 12' 20"S, 70° 26' 07" W, 0 m a.s.l., 21 km S of Papos city.

Distribution. *Mepraia parapatric n. sp.* is distributed along the northern coast of Chile, region II of Antofagasta and region III of Atacama, in "desierto litoral" approximately between 25° 12' 20"S, 70° 26' 07"W and 26° 10' 10"S, 70° 40' 03" W, 231 a.s.l. This species also occurs on Pan de Azúcar Island 26° 9' 05"S, 70° 40' 53" W, 50 m a.s.l. (Fig 1).

Diagnosis. Females: connexivum of second urotergite with two separate red spots, urotergites III to VII with small red spots at intersection with inter-segmental suture, urotergites VIII to X black; in ventral view, gonapophysis (G9) 0.60-0.70 mm long, slightly concave posteriorly, curved anteriorly, sharp and bifurcated at apex. Males: medial region of parameres with spiniform sclerotized area, basal plate of articular apparatus of phallus slightly sclerotized, 0.71-0.81 mm long, phallosome anteriorly, very small at apex, 0.11-0.14 mm wide, phallosome plate slightly sclerotized, rhomboid posteriorly, aedagus 1.29-1.31 mm long.

System of sex determination X1X2Y. The Y chromosome is C positive and longer than the X chromosomes. Both X1 and X2 chromosomes are euchromatic with a small block of heterochromatin in one of their telomeres.

Description

Region III populations. Pan de Azúcar Island, Caleta Pan de Azúcar, and Cerro el Soldado.

Females. Black overall, micropterous, 18.40-22.40 mm long (Fig 3a). Head: length 4.22-4.78 mm, width 2.00-2.55 mm, black, strongly granulate, first and second segments of antennae black, first segment with short curved hairs, second

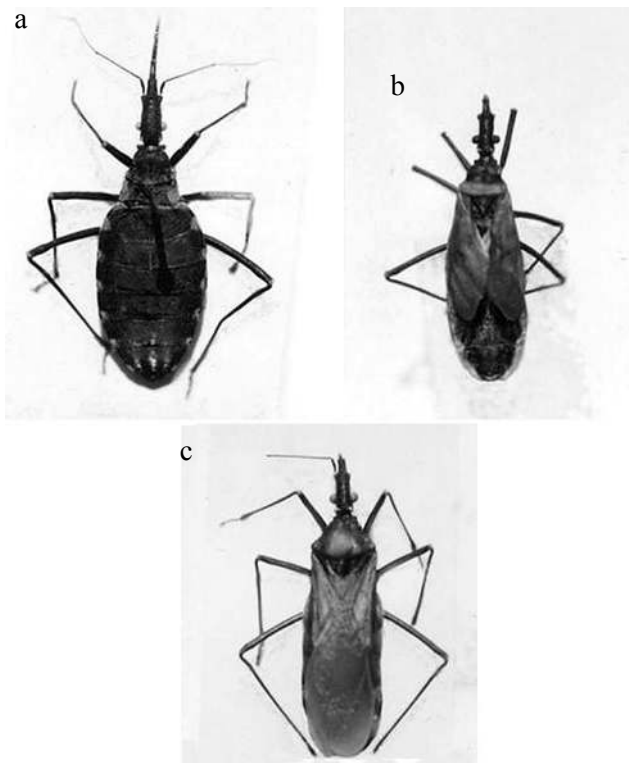


Fig 3 Female and males of *Mepraia parapatrica* from Pan de Azúcar island. Female (a), brachypterous male (b), macropterous male (c).

segment with long hairs, third and fourth segments brown with long and short hairs. Rostrum, black with slender rostral segments. Eyes metallic gray, never reaching inferior level of head. Ocellus small, 0.16-0.21 mm in diameter, reddish or whitish. Neck shiny black. Thorax: pronotum subtrapezoidal, uniformly black, 2.00-3.00 mm long, 2.23-3.53 mm wide, anterior lobe conspicuously convex, posterior lobe rugose, shorter than anterior lobe, humerus and posterior margin of pronotum rounded. Anterolateral projections of pronotum conical, collar curved. Scutellum triangular, wider than long, heavily rugose, with median concavity. Hemelytra length 1.00-1.51 mm, reduced to small suboval sclerites. Legs uniformly black, except for dark-brown tarsi, femur with short curved hairs, tibia with short curved erect hairs, tarsus with long erect hairs. Abdomen: ovoid, black, 11.00-14.00 mm long, 7.00-10.00 mm wide; dorsally, first urotergite black, connexivum of second urotergite with two separate red spots, urotergites III to VII with small red spots on intersection of intersegmental suture, urotergites VIII, IX and X black (Fig 3a), in ventral view, urostermites I, II and III black or with tiny red spots, connexival membrane dark brown. Genitalia black overall (Fig 4a), and overall size smaller than *M. spinolai* and *M. gajardoi* (Fig 4d, g). Gonocoxite 8 (Gc8) rounded with numerous black bristles, gonapophysis 9 (G 9) 0.60-0.70 mm long, black, slightly concave posteriorly, curved anteriorly, with long bristles, sharp and bifurcated at apex (Fig 4b); gonapophysis 8 (G 8) triangular, with long bristles (Fig 4c), gonocoxite 9 (Gc 9) 0.90-1.00 mm long, concave, with external margin darker than central area, rounded at apex, with numerous long

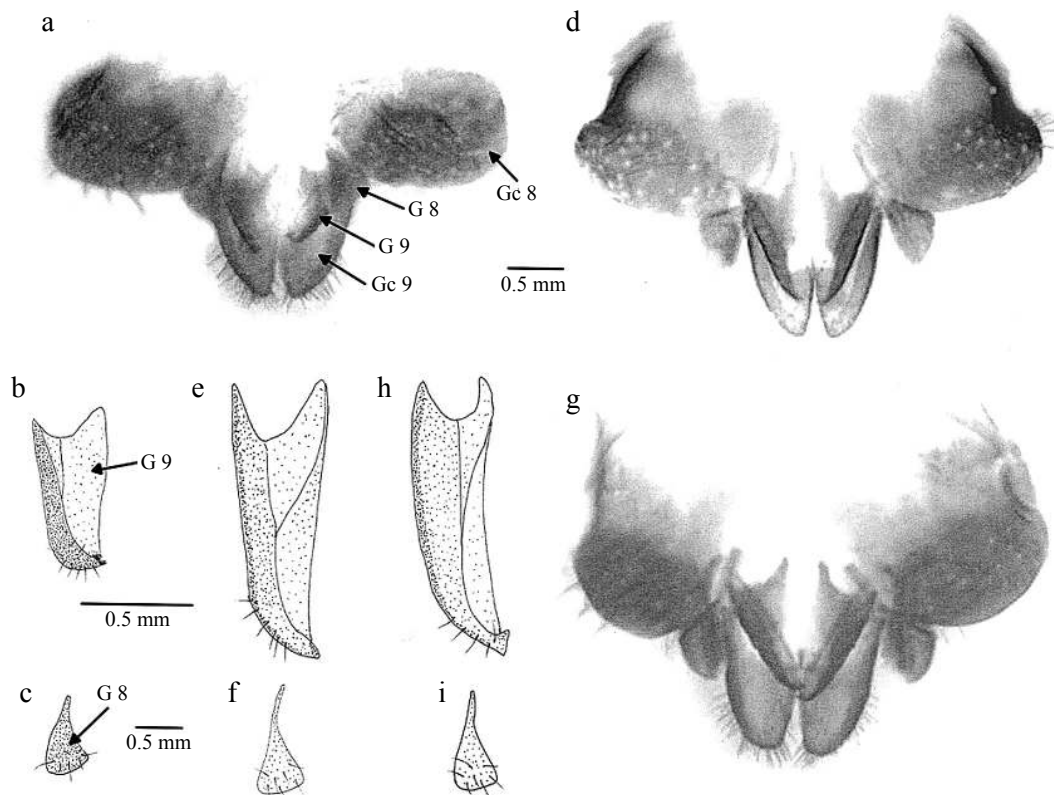


Fig 4 Female genitalia in ventral view. *Mepraia parapatrica* (a,b,c), *M. spinolai* (d,e,f), *M. gajardoi* (g,h,i). Gonocoxite 8 (Gc8), gonocoxite 9 (Gc9), gonapophysis 8 (G8), gonapophysis 9 (G9).

bristles anteriorly (Fig 4a). In *M. spinolai* and *M. gajardoi* these structures are very different, in particular gonapophysis 8 (Fig 4f-i) and gonapophysis 9 (Fig 4e-h).

Brachypterous male. Black overall, 15.50-19.00 mm long (Fig 3b). Head: 3.10-3.60 mm long, 2.3-2.7 mm wide (Fig 5a), black, strongly granulate, overall size smaller than *M. spinolai* (Fig 5b) and *M. gajardoi* (Fig 5c). First and second segments of antennae black, third and fourth segments brown, first segment with some short curved hairs, second, third and fourth segments with long and short hairs. Rostrum brown with slender rostral segments. Eyes metallic black or grayish, never reaching inferior level of head. Ocellus prominent, reddish or whitish, 0.19-0.27 mm in diameter. Neck shiny dark brown. Thorax: pronotum subtrapezoidal, black, 2.00-2.38 mm long, 3.00-3.53 mm wide, anterior lobe conspicuously convex, posterior lobe rugose, longer than anterior lobe, posterior margins of pronotum and humerus rounded. Anterolateral projections of pronotum conical, collar curved. Scutellum triangular, as wide as long, and heavily rugose with prominent median concavity. Hemelytra shorter than abdomen, 7.00-9.00 mm long, rounded at apex. Legs uniformly black except for dark-brown tarsi, femur of first, second, and third pairs of legs with short hairs, tibia and tarsus with long hairs. Abdomen: slightly ovoid, 8.70-10.00 mm long, 4.20-5.70 mm wide, black; connexivum with small red spots on urotergites II to VII extending onto inter-segmental sutures, black in ventral view, with small red spots. Spiracles black, parameres and pygophore black, median area of parameres spiniform, strongly curved anteriorly, rhombus-like and strongly sclerotized at apex (Fig 6a), parameres of *M. spinolai* and *M. gajardoi* lacking this spine-shaped area (Fig 6b,c); V-shaped median process of pygophere (Fig 6d) larger and broader than in *M. spinolai* and *M. gajardoi* (Fig 6e,f), basal plate of articulatory apparatus of phallus slightly sclerotized, 0.71-0.81 mm long, phallosome

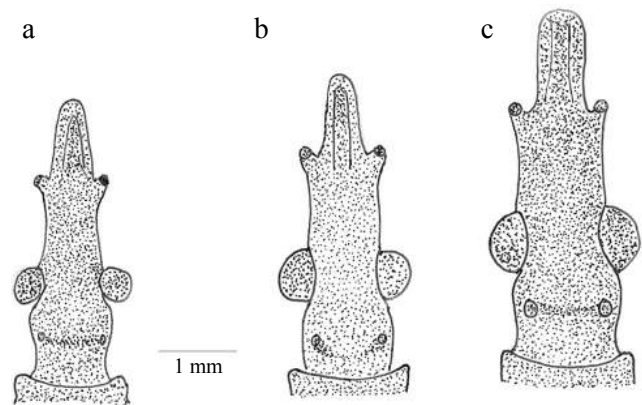


Fig 5 Male heads in dorsal view. Brachypterous male of *Mepraia parapatricae* (a), micropterous male of *M. spinolai* (b), brachypterous male of *M. gajardoi* (c).

smaller than in *M. gajardoi* and larger than in *M. spinolai*, aedagus 1.29-1.30 mm long, longer than in *M. spinolai* and shorter than in *M. gajardoi* (Fig 7a,b,c), phallosome anteriorly, very small at apex, 0.11-0.14 mm wide, smaller than in *M. spinolai* and *M. gajardoi* (0.68-0.72 mm and 0.35-0.42 mm wide in *M. spinolai* and *M. gajardoi*, respectively) (Fig 7a-f). Phallosome plate of *M. parapatricae* sclerotized, posteriorly rounded and rhombus-shaped (Fig 7d), whereas in *M. spinolai* this plate is posteriorly concave and rounded at its apex (Fig 7e), and in *M. gajardoi* the plate is rounded at its apex (Fig 7f). Endosome process in *M. parapatricae* anteriorly conical at apex and median area with small bristles (Fig 7g); in *M. spinolai* it is cylindrical (Fig 7h), and in *M. gajardoi* it is conical and larger than in *M. parapatricae* (Fig 7i).

Macropterous male. Similar to brachypterous males in color, but with larger body, 19.00-20.00 mm long (Fig 3c).

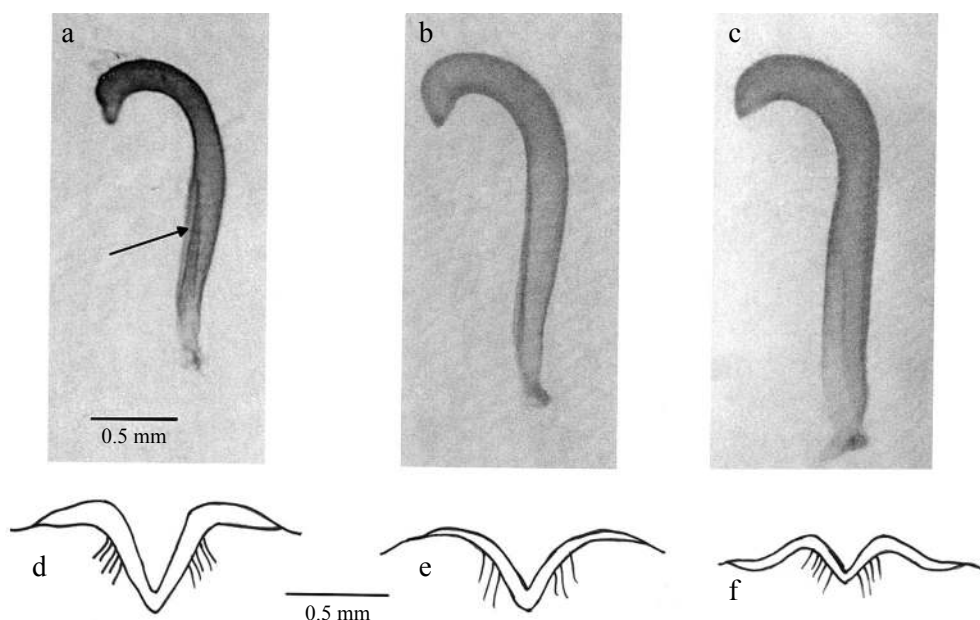


Fig 6 Parameres of males (a,b,c) and median process of pygophere of male genitalia (d,e,f). *Mepraia parapatricae* (a, d), *M. spinolai* (b, e), *M. gajardoi* (c, f). Arrow indicates median spiniform structure.

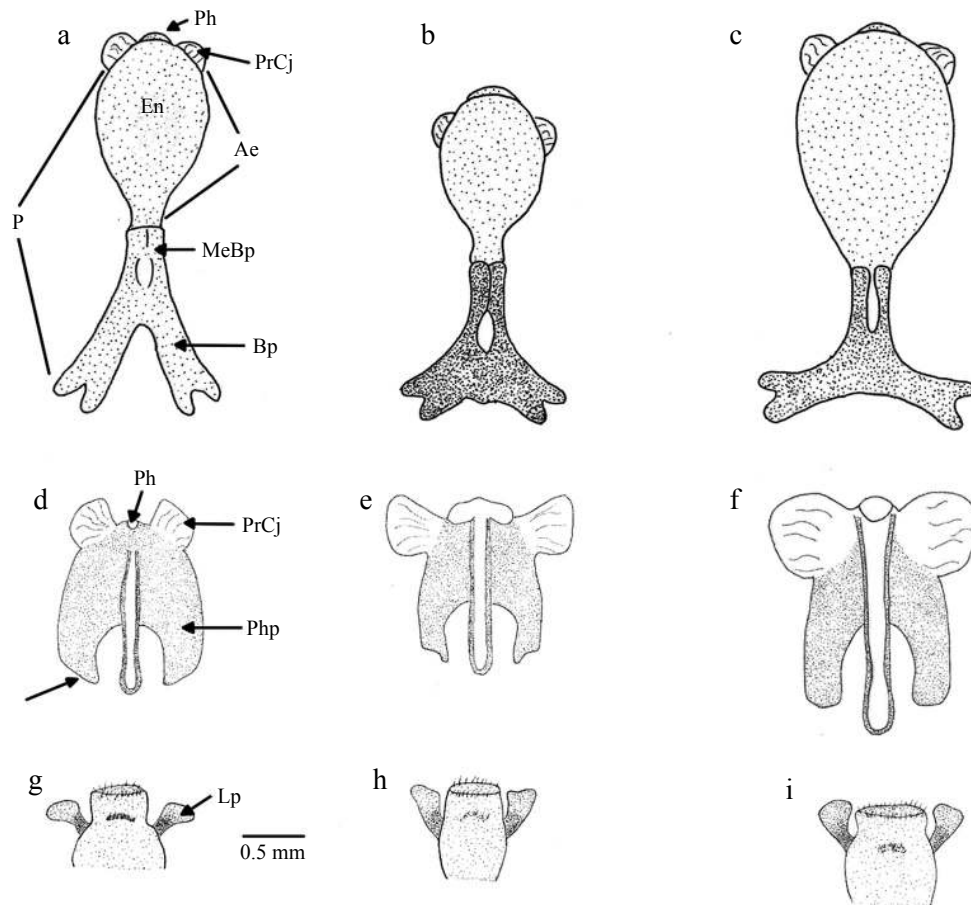


Fig 7 Phallus of male genitalia in dorsal view (a,b,c). Phallosoma plate in ventral view (d,e,f), endosoma process in ventral view (g,h,i) *Mepraia parapatrica* (a,d,g), *M. spinolai* (b,e,h), *M. gajardoi* (c,f,i). Phallus (P) phallosoma (Ph), process of conjunctive (PrCj), aedagus (Ae), endosome (En), basal plate (Bp), median extension of basal plate (MeBp), lateral plate (Lp). Arrow indicates posterior apex of phallosoma plate.

Pronotum width 4.19-4.61 mm; hemelytra longer than those of brachypterous males, between 12.00 mm and 13.00 mm, reaching to or past end of abdomen. Collar straight or slightly curved, in ventral view, spiracles reddish. Genitalia similar to those of brachypterous male.

Populations of region II. Caleta Bandurrias and Cachinales

Female. Similar to female of region III from Pan de Azúcar Island, Caleta Pan de Azúcar and Cerro el Soldado.

Brachypterous and macropterous males. These are morphologically similar to those described from region III.

Cytogenetics. The karyotype of the Bandurrias and Cachinales populations is formed by 20 autosomes and an X1, X2, Y system of sex determination. The Y chromosome is C positive and longer than the X1 and X2 chromosomes. All autosomes have constitutive heterochromatin in both telomeres. The X1 and X2 chromosomes are euchromatic, but have a small block of constitutive heterochromatin in one of their telomeres (Fig 8a,b). Specimens from Pan de Azúcar Island show a karyotype similar to specimens from Bandurrias (Fig 8c).

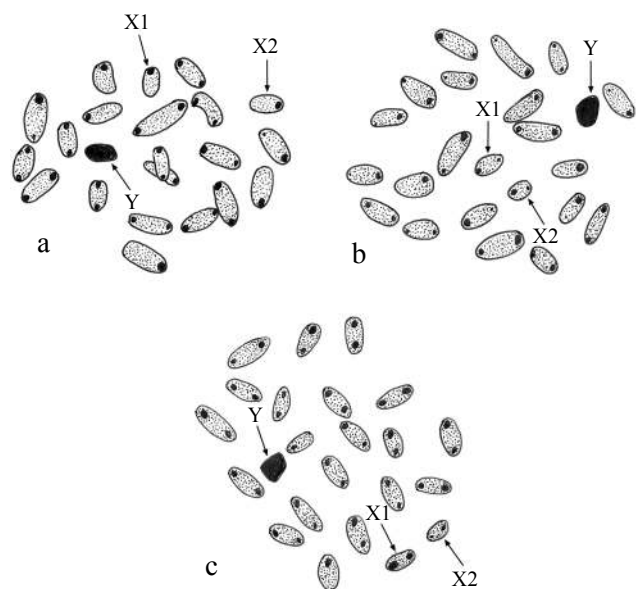


Fig 8 Gonial metaphase in a brachypterous male from Bandurrias (a,b), gonial metaphase in a brachypterous male from Pan de Azúcar Island (c).

Etymology. The name of this species is related to its probable mode of speciation. The specific name refers to the Spanish word *parapatrica*, based on Articles 31.2.2 and 31.2.3 of the International Code of Zoological Nomenclature.

Redescription of *Mepraia spinolai*

Population of region III. Inca de Oro

Female. Overall dark brown, micropterous, 19.60-21.00 mm long. Head: 4.61-5.50 mm long, 1.97-2.00 mm wide, dark brown, strongly granulate, antennae brown, first segment with some short and curved hairs, second segment with long hairs, third and fourth segments with long and short hairs, rostrum dark brown with slender rostral segments. Eyes metallic black or grayish, never reaching inferior level of head. Ocellus reddish or whitish, 0.09-0.20 mm in diameter. Neck shiny brown. Thorax: pronotum subtrapezoidal, uniformly dark brown, 3.56-4.33 mm long, 3.05-4.07 mm wide, anterior lobe conspicuously convex, posterior lobe rugose and about as long as anterior, posterior margin of pronotum and humerus rounded. Scutellum triangular, wider than long, heavily rugose. Hemelytra length 1.25-1.50 mm, reduced to small suboval sclerites; legs uniformly brown, femur with short curved hairs, tibia and tarsus with long and short erect hairs. Abdomen: ovoid, black, 12.00-13.00 mm long, 8.00-9.00 mm wide, connexivum with dorsal red spots on each urotergite II to VIII. Second urotergite with dorsal continuous orange or red band on the edges; connexival sutures of urosternites I-VII with ventral red spots on intersection with inter-segmental suture, reddish connexival membrane. Genitalia black overall (Fig 4d), gonocoxite 8 with numerous long black bristles, gonapophysis 9 (G9) 0.90-1.00 mm long, curved and rounded at apex, with long bristles anteriorly, concave posteriorly (Fig 4e), gonapophysis 8 (G8) triangular, with long bristles (Fig 4f); gonadocoxite 9 (Gc 9) 0.90-1.10 mm long, concave, with external margin darker than central area, numerous long bristles anteriorly, rounded at apex (Fig 4d).

Macropterous male. Brown overall, 19.00-20.00 mm long. Head: 4.5-4.62 mm long, 2.0-2.13 mm wide (Fig 5b), dark brown, strongly granulate, brown antennae, first segment with some short curved hairs, second, third and fourth segments with long and short hairs. Rostrum brown, with slender rostral segments. Eyes metallic brown or grayish, never reaching inferior level of head. Ocellus prominent, black, reddish or whitish, 0.20-0.27 mm diameter. Neck shiny brown. Thorax: pronotum subtrapezoidal and uniformly brown, 2.85-3.27 mm long, 4.00-4.70 mm wide, anterior lobe conspicuously convex, posterior lobe rugose and longer than anterior lobe, posterior margins of pronotum and humerus rounded. Anterolateral projections conical, collar curved. Scutellum triangular, wider than long, heavily rugose, with prominent median concavity. Hemelytra longer than abdomen or not exceeding its length, 14.00-15.00 mm long, rounded at apex. Legs uniformly brown, femur of first pair of legs with long erect and short curved hairs; femur of second and third pairs of legs with short and curved hairs, tibia and tarsus with long and short erect hairs. Abdomen slightly ovoid, 10.05-11.00 mm long, 6.00-8.00 mm wide, black, connexivum with dorsal red spots on urotergites

II to VII in ventral view, black with red spots on urosternites II to VII, spiracles yellow, parameres strongly curved anteriorly, rhomboid and moderately sclerotized at apex, medial area not spiniform (Fig 6b), pygophore dark brown or black, median process of pygophore V-shaped (Fig 6e), basal plate of phallus articular apparatus heavily sclerotized 0.33-0.36 mm long, phallosome anteriorly, very broad at apex, 0.68-0.72 mm wide, aedagus 1.14-1.19 mm long (Fig 7b,e), phallosoma plate sclerotized, concave posteriorly and rounded at apex (Fig 7e), endosoma process anteriorly cylindrical, at apex and median area with small bristles (Fig 7h).

Micropterous male. Black overall, 18.50-19.00 mm long. Head: 3.90-4.33 mm long, 1.87-2.10 mm wide, black, strongly granulate, antennae brown, first segment with some short and curved hairs, second, third, and fourth segments with long and short erect hairs. Rostrum brown, with slender rostral segments. Eyes metallic black or grayish, never reaching inferior level of head. Ocellus prominent, reddish or whitish, 0.16-0.21 mm in diameter. Neck shiny brown. Thorax: Pronotum subtrapezoidal, uniformly brown, 2.21-2.97 mm long, 2.78-3.10 mm wide, anterior lobe conspicuously convex, posterior lobe rugose and about as long as anterior lobe, posterior margins of pronotum and humerus rounded. Anterolateral projections conical. Scutellum triangular, wider than long, heavily rugose, with pronounced median concavity. Hemelytra reduced to small suboval sclerites, 1.00-1.44 mm long. Legs uniformly brown, femur of first pair of legs with long erect hairs, femur of second and third pairs of legs with short curved hairs; tibia and tarsus with long and short erect hairs. Abdomen slightly ovoid, 9.45-10.00 mm long, 5.00-6.00 mm wide; black, connexivum with two dorsal red spots on urotergites II to VII on intersection of intersegmental suture, in ventral view black with red spots, spiracles yellow, parameres similar to Fig 6b, pygophore dark brown or black, median process of pygophore V-shaped, similar to Fig 6e; basal plate of phallus articular apparatus heavily sclerotized and shorter than 0.5 mm, phallosoma anteriorly very broad at apex, wider than 0.15 mm (similar to Fig 7b,e), aedagus length similar to macropterous male, phallosoma plate sclerotized, posteriorly concave and rounded at apex, similar to Fig 7e, endosoma process anteriorly cylindrical, with small bristles at apex and median area, similar to Fig 7h.

Cytogenetics. The karyotype of the Inca de Oro population is formed by 20 autosomes plus X1 X2 Y in males and X1 X1 X2 X2 in females (Fig 9a,b). All autosomes have C positive blocks on both telomeres; the X1 sex chromosome has one dot of heterochromatin in one of its telomeres, whereas the X2 chromosomes are totally euchromatic (Fig 9b). During metaphase II of meiosis (males), the tenth half bivalents form a ring-shaped configuration with three of the sex chromosomes in the center (Fig 9a). In anaphase II, the X1 and X2 chromatids segregate to the same pole, while the Y chromosome migrates to the opposite pole (Fig 9c). Therefore, the second division is reductional for sex chromosomes, as in other Hemiptera. The pachytene of prophase I has a particular heteropycnotic chromocenter formed by sex chromosomes surrounded by several autosomal heteropycnotic dots. Also, other heteropycnotic regions outside this chromocenter can be observed (Fig 9d). In one winged male (macropterous),

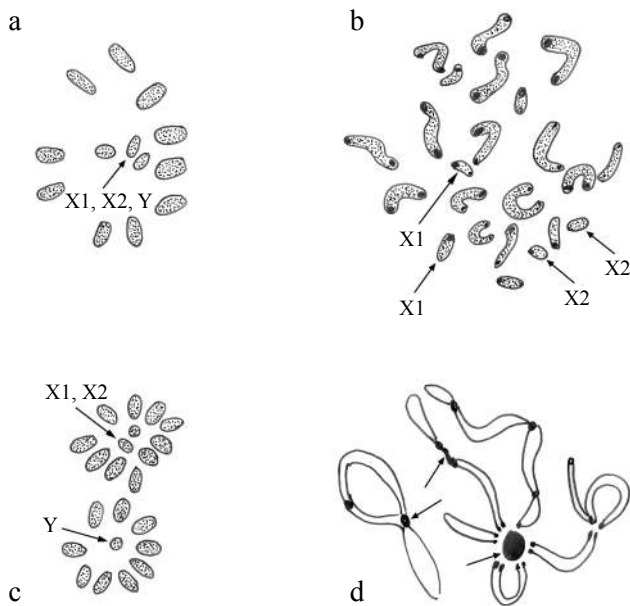


Fig 9 Karyotype of *Mepraia spinolai* from Inca de Oro. Metaphase II in a macropterous male, classic squash lacto-acetic orcein method (a). Gonial metaphase in a female, C banding method (b). Anaphase II in polar view in a macropterous male, classic squash lacto-acetic orcein method (c). Pachytene of prophase I in a macropterous male, classic squash lacto-acetic orcein method. Arrow indicates chromocenters (d).

different types of diakinesis plates were observed. In two of these plates the three sex chromosomes formed a chromocenter that seems to be attached to one bivalent autosome (Fig 10a,b). In another plate, one of the X chromosomes seems to be free, and in the other the X is attached to the Y chromosome and to one bivalent autosome (Fig 10c). The X chromosome also appears free, near a bivalent autosome (Fig 10d). This

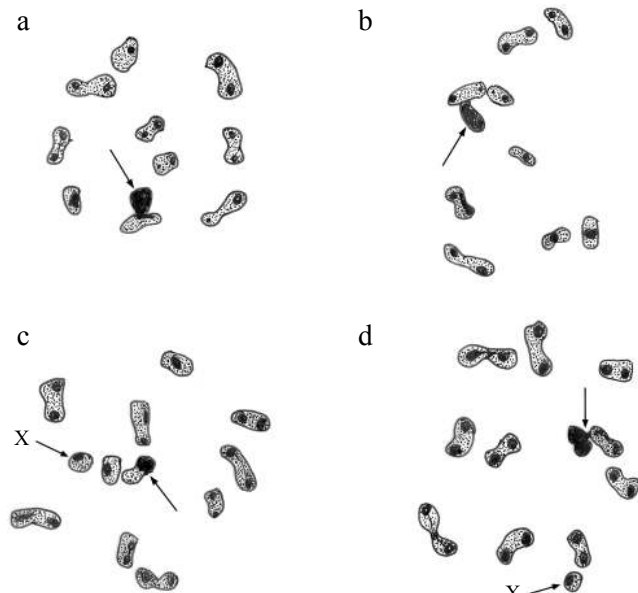


Fig 10 Diakinesis plates in a macropterous male from Inca de Oro, C banding method (a,b,c,d).

population has a chromosome complement similar to that of *M. spinolai* as described by Panzera *et al* (1998) and Frías and Atria (1998).

Discussion

Morphology. The population of *M. parapatrica* from region II (Bandurrias and Cachinales) and those from Parque Nacional Pan de Azúcar from region III differ from *M. spinolai* and *M. gajardoi* in their karyotype and morphology. The main differences are briefly summarized in Tables 1 and 2.

Males of *M. parapatrica* are always winged, brachypterous or macropterous, but never micropterous, whereas males of *M. gajardoi* are always brachypterous. Males of *M. spinolai* are polymorphic in the length of their wings, and can be micropterous, brachypterous or macropterous (Lent *et al* 1994, Frías & Atria 1998, Frías *et al* 1998). These wing differences are important, because it has been reported that the genes responsible for wing development are linked to the Y chromosome. Therefore the phenotypic differences described are inheritable (Frías & Atria 1998).

Chromosomes. The populations of *M. parapatrica* also show differences in their distinctive cytogenetic features in comparison to those occurring in *M. gajardoi* and *M. spinolai* (Table 2). All autosomes have blocks of heterochromatin in both telomeres; the X1 and X2 sex chromosomes have dots of heterochromatin in one of their telomeres (Fig 8a,b,c). This characteristic differentiates it from *M. gajardoi*, in which the autosomes and the X1 and X2 sex chromosomes are completely euchromatic. The Y chromosome in the new species is C-positive and is longer than the X chromosomes. This feature differentiates it from *M. spinolai*, which shows polymorphism in the number of Y sex chromosomes (Y1 and Y2), probably originating from a fracture of an ancestral longer Y chromosome of *M. gajardoi* (Frías & Atria, 1998).

It is probable that the association detected between sex chromosomes and one of the autosomes in the Inca de Oro micropterous males of *M. spinolai* correspond to a translocation, which allowed the fracture of the holocentric Y chromosome. This fracture would have originated the polymorphism in the number of Y chromosomes found in the *M. spinolai* population. This fragmentation of the original longer Y chromosome of *M. gajardoi* and *M. parapatrica* may be associated with the exceptional alary polymorphism detected in *M. spinolai*, especially regarding the origin of wingless males (Frías & Atria 1998, Jurberg *et al* 2002). Panzera *et al* (1998) found a similar association between each X chromosome and autosomes in a wingless male of *M. spinolai*.

Cytogenetic differences, mainly heterochromatinization, translocations and Y chromosome rupture with the formation of chromocenters (as described in *M. spinolai*) may indicate a post-zygotic reproductive isolation between *Mepraia* species in semi-geographical conditions.

Geographical distribution and biology. *Mepraia parapatrica*

Table 1 Morphological differences between *Mepraia parapatrica*, *M. spinolai* and *M. gajardo*.

Character	<i>M. parapatrica</i>	<i>M. spinolai</i>	<i>M. gajardo</i>
Spots of urotergites II in females	Two separate red spots	One continuous long orange-red band	Small or absent, reddish brown
Urotergites II to VIII in females	With small red spots on edges of urotergites	With a continuous red or orange band on edges of urotergites	With small brown spots on edges of urotergites
Color of connexivum in females	Dark-brown	Reddish	Dark-brown
Color of urosternites in females	Black with small red spots at the margins of urosternites II to IV	Dark brown with small red spots on urosternites II to VII	Uniformly dark brown
Gonapophysis 9 in female genitalia	Shorter than 0.70 mm, posteriorly slightly concave, anteriorly curved and bifurcated, sharp at apex	Longer than 0.70 mm, posteriorly strongly concave, anteriorly curved, rounded at apex	Longer than 0.70 mm, posteriorly heavily concave, anteriorly curved and obtuse, and triangular at apex
Length of hemelytra in males	Brachypterous and macropterous	Macropterous, brachypterous and micropterous	Always brachypterous
Parameres	Strongly curved anteriorly, rhomboid and heavily sclerotized at apex, medial region with spiniform sclerotized area	Strongly curved anteriorly, rhomboid and moderately sclerotized at apex, medial area with no spiniform area	Moderately curved anteriorly, rhomboid and slightly sclerotized at apex, medial area with no spiniform area
Aedagus length	1.29-1.31 mm, shorter than <i>M. gajardo</i> and longer than <i>M. spinolai</i>	1.14-1.19 mm	1.27-1.40 mm
Basal plate of articulatory apparatus	Slightly sclerotized, longer than 0.65 mm	Heavily sclerotized, shorter than 0.65 mm	Moderately sclerotized, shorter than 0.65 mm
Phallosoma at apex	Very small, width < 0.15 mm	Broad, width > 0.15 mm	Small, width > 0.15 mm
Phallosoma plate	Slightly sclerotized, posteriorly rounded and rhomboid at apex	Heavily sclerotized, posteriorly with a concavity and rounded at apex	Moderately sclerotized, posteriorly rounded at apex
Endosoma process anteriorly	Conical	Cylindrical	Conical

is apparently confined to Pan de Azúcar Island and also to the coastal border of regions II and III, approximately between 25° 12' 20" S, 70° 26' 07" W and 26° 10' 10" S, 70° 40' 03" W. The presence of *M. gajardo* in region II and *M. spinolai* in region III (Frías & Atria 1998, Frías *et al* 1998) may indicate that they cohabit with *M. parapatrica* in this area. However, the morphology of the female and male genitalia is very different

from that of *M. spinolai* and *M. gajardo*. Furthermore, the cytogenetic characteristics suggest that there is no gene flow between *M. parapatrica* and *M. gajardo* and *M. spinolai*, indicating that *M. parapatrica* is a distinct species that had a parapatric origin. Also, these populations of *M. parapatrica* differ ecologically from the other two species. They feed on wild sea birds and lizards, similar to *M. gajardo*, but they were

Table 2 Cytogenetic differences between *Mepraia parapatrica*, *M. spinolai* and *M. gajardo*.

Character	<i>M. parapatrica</i>	<i>M. spinolai</i>	<i>M. gajardo</i>
Sex determination system	X1X2Y	X1X2Y1Y2	X1X2Y
Y chromosome	Longer than X1 and X2 chromosomes	Similar in size to X1 and X2 chromosomes	Longer than X1 and X2 chromosomes
Heterochromatin	Abundant on both telomeres of autosomes, X1 and X2 have a small block of heterochromatin in one of their telomeres	Abundant on both telomeres of autosomes, X1 has a small block of heterochromatin in one of its telomeres, whereas X2 is totally euchromatic	Absent or scarce in telomeric zone of autosomes, X1 and X2 totally euchromatic

also collected in a goat pen, therefore invading peridomestic environments as does *M. spinolai*. The latter is a wild or peridomestic species that normally feeds on small wild rodents, but also occasionally invades domestic habitats. However, it was never reported to feed on birds (Schofield *et al* 1982, Schofield 1994, Frias & Atria 1998, Frias *et al* 1998).

These feeding differences may be related to the efficiency in the propagation of *T. cruzi*. Birds do not support the life cycle of *T. cruzi* (Schofield *et al* 1982). Sagua *et al* (2000) reported that in *M. parapatrica*, no *T. cruzi* infection was detected on Pan de Azúcar Island where this species is associated with the Humboldt penguin. The recently published information on *T. cruzi* infecting *M. gajardoi* indicates a natural association (11.8% infection in all analyzed insects; Botto-Mahan *et al* 2008). However, *T. cruzi* infection can reach up to 46.2% to 61% in *M. spinolai* in some areas of central Chile (Frias *et al* 1995, Botto-Mahan *et al* 2005).

Wing polymorphism and heterochrony during development.

All females and males of Triatominae described are winged (Lent & Wygodzinsky 1979). It is probable that the gene for wing development in the ancestors of the genus *Mepraia* was located in an autosome. This would explain the existence of winged males and females in all Triatominae species. Cross-breeding of *M. spinolai* showed that the gene for wing development is linked to the Y chromosome (Frias & Atria 1998). This was probably the first step in the origin of the *Mepraia* complex (Fig 11). In an evolutionary framework, it is possible that *M. gajardoi*, with micropterous females and invariably brachypterous males, was the first ancestral species of the genus *Mepraia* in Chile.

The microptery observed in all species of *Mepraia* and in males of *M. spinolai* is an apomorphic trait (Schofield

et al 1998), probably caused by heterochrony during the development (neoteny). Females are fertile, but their phenotype is similar to fifth instars (Mazza *et al* 1940). The break of the holocentric Y explains the origin of neo-Y chromosomes detected in *M. spinolai*; one form (Y1) bears genes for wings, while the other (Y2) lacks such genes, and this explains the origin of micropterous males (Frias & Atria 1998). The microptery in both males and females in *M. spinolai* shows that, in a parsimony model, *M. spinolai* is the most recent species in the evolution of the genus *Mepraia* in Chile, probably originating semi-geographically from *M. parapatrica* (Fig 11).

Longer wings of macropterous males, with hemelytra longer than the apex of the abdomen, as observed in *M. parapatrica* and *M. spinolai*, correspond to another phenotypic novelty in the *Mepraia* group. This is probably an atavistic trait, because this characteristic has been described in distant ancestors of *Mepraia* species, such as in some species of Triatominae belonging to the genus *Rhodnius*, and also in the genus *Psammolestes* (Lent & Wygodzinsky 1979). However, this character is absent in their closest ancestors such as *Triatoma breyeri* Del Ponte and *Triatoma eratyrisiformis* Del Ponte as described by Lent & Wygodzinsky (1979).

The probable origin of genus *Mepraia*. The species of the *breyeri* complex, formed by *T. breyeri* and *T. eratyrisiformis*, comprise a group related to the species of *Mepraia* (Caracavallo 2000), which occurs in desert and semi-desert areas of Argentina (Curto de Casas *et al* 1999) as do the species of *Mepraia*. Hypsa (2002), based on molecular studies using 16S and 12S rDNA, postulated that *T. eratyrisiformis* belongs to the genus *Mepraia*. This hypothesis does not accord with morphological studies by Caracavallo *et al* (2000). Comparing

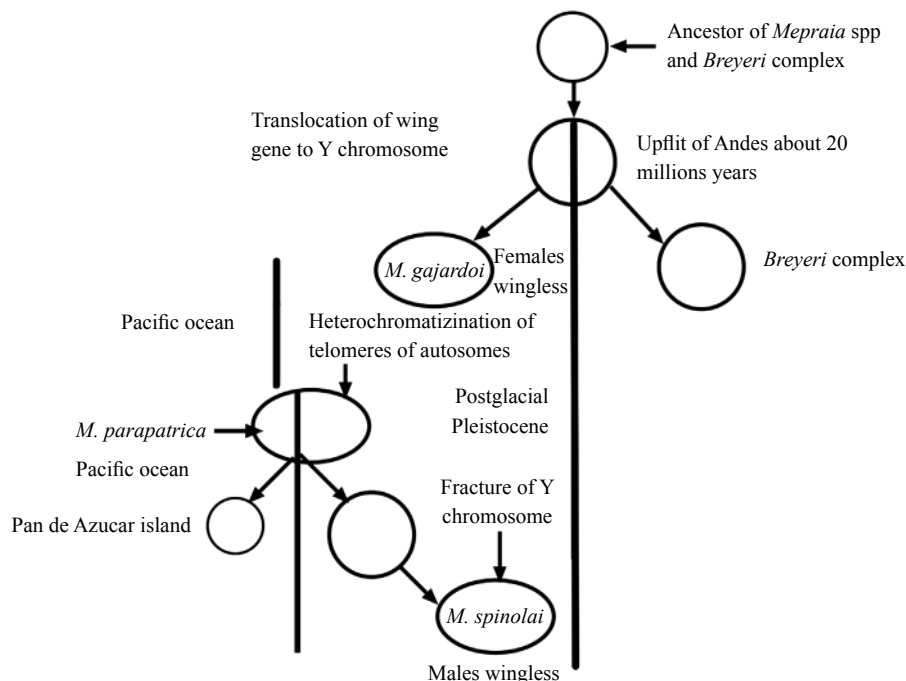


Fig 11 Principal phenotypic and chromosomal and biogeographical steps in the evolution of *Mepraia* species in Chile.

the phenotype of the antennae between *M. gajardoi* and *M. spinolai*, and *T. eratyrisiformis* and *T. breyeri*, Moreno *et al* (2005) found that *Mepraia* species and *T. eratyrisiformis* share similarities in bristles and receptors that are not present in *T. breyeri*. According to Moreno *et al* (2005), *M. gajardoi* and *M. spinolai* have a common ancestor, probably related to the *breyeri* complex.

It is possible that the origin of the genus *Mepraia* is related to the last uplifting of the Andes cordilleras during the Miocene, about 20 million years ago (Fig 11) (Solbrig 1976, Moreno *et al* 2005). This would have caused the separation of the *breyeri* group, which currently occurs in the dry areas of Argentina, along the eastern slope of the Andes (Lent & Wygodzinsky 1979). The speciation process in *Mepraia* would have occurred during the post-glacial Pleistocene about 10,000-8,000 years ago, when floristic, climatic, orogenic, volcanic, and Pacific Ocean-level changes occurred (Cecioni 1970, Vuilleumier 1971, Heusser 1974, Solbrig 1976, Paskoff 1977, Neghme 1982). In this period, Pan de Azúcar Island probably arose, where *M. parapatrica* is currently distributed. Chromosomal changes would have contributed to the reproductive isolation between these species, as described by Frías *et al* (1998). A significant cytogenetic change in the evolution of the *Mepraia* species was the heterochromatinization of telomeres of the autosomes and the X sex chromosomes, described here for *M. parapatrica*. This was a key genetic change that probably gave rise to *M. parapatrica* through meiotic barriers under semi-geographical conditions. Repetitive sequences are located within the constitutive heterochromatin (Gilbert 2003), and within these sequences non-transcriptional genes are found (Frías 2007). They have an important role in genetic regulation (Gatti & Pimpinelli 1992), telomere stabilization (Zakian 1998), DNA replication (Bell *et al* 1993) and chromosomal movement during cell division and chromocenter formation (Gatti & Pimpinelli 1992). Subsequently, in the evolution of *Mepraia* species, the large holocentric Y chromosome detected in *M. gajardoi* and *M. parapatrica* broke and originated the neo-Y chromosomes found in *M. spinolai*. According to the present distribution of these *Mepraia* species, it is likely that all these chromosomal and morphological changes occurred under semi-geographical conditions, similarly to the model described for the micropterous Morabinae grasshoppers in Australia (White 1968, 1978, Key 1974). Therefore, the most parsimonious model that explains the evolution of the *Mepraia* species in Chile is the parapatric model of phyletic speciation by negative heterosis.

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