UNIVERSIDAD DE CHILE -FACULTAD DE CIENCIAS -ESCUELA DE PREGRADO

“Descripción morfológica del surco estridulatorio en las tres especies del género *Mepraia* (Hemiptera: Reduviidae: Triatominae)”

Seminario de Título entregado a la Universidad de Chile en cumplimiento parcial de los requisitos para optar al Título de Bióloga con Mención en Medio Ambiente

Nicol Andrea Quiroga Hidalgo

Director del Seminario de Título: Carezza Botto Mahan

Co-Director del Seminario de Título: Mario Penna Varela

Co-Director del Seminario de Título: Matías Muñoz Sandoval

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INFORME DE APROBACIÓN SEMINARIO DE TITULO

Se informa a la Escuela de Pregrado de la Facultad de Ciencias, de la Universidad de Chile, que el Seminario de Título presentado por la **Srta. Nicol Quiroga Hidalgo**

“DESCRIPCIÓN MORFOLÓGICA DEL SURCO ESTRIDULATORIO EN LAS TRES ESPECIES DEL GÉNERO *MEPRAIA* (HEMIPTERA: REDUVIIDAE:TRIATOMINAE)”

Ha sido aprobado por la Comisión de Evaluación, en cumplimiento parcial de los requisitos para optar al Título de Bióloga con mención en Medio Ambiente.

Director Seminario de Título ___________________________________
Carezza Botto Mahan

Co-Director del Seminario de Título ___________________________________
Mario Penna Varela

Co-Director del Seminario de Título ___________________________________
Matías Muñoz Sandoval

Comisión Revisora y Evaluadora

Presidente Comisión ___________________________________
Claudio Veloso Iriarte

Evaluador ___________________________________
Luis Flores Prado

Santiago de Chile, octubre 2017

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RESUMEN

La estridulación es un mecanismo de comunicación vibroacústica generada por la fricción de dos estructuras rígidas. Se ha descrito este tipo de comunicación en varios grupos de insectos, entre ellos Reduviidae. Los hemípteros hematófagos, más conocidos como vinchucas (Reduviidae: Triatominae), participan como vectores del protozoo *Trypanosoma cruzi,* agente etiológico de la enfermedad de Chagas en humanos. En este grupo la estridulación resulta de la fricción del estilete sobre el surco estridulatorio, estructura ubicada en la parte ventral del protórax. Se ha descrito que la estridulación es emitida en respuesta a la manipulación, como mecanismo de defensa contra depredadores y como forma de rechazo de la hembra hacia el macho en su intento de copulación. En Chile, los triatominos endémicos del género *Mepraia* habitan zonas áridas, semiáridas y mediterráneas, y se caracterizan por la existencia de polimorfismo alar en machos adultos. En *Mepraia* existen individuos alados (braquípteros y macrópteros) y no alados (micrópteros), siendo las hembras de las tres especies invariablemente micrópteras. En *Mepraia gajardoi* los machos son exclusivamente braquípteros, en *Mepraia parapatrica* braquípteros y macrópteros*,* y en *Mepraia spinolai* braquípteros, macrópteros y micrópteros. El objetivo de este estudio es describir la morfología del surco estridulatorio de las tres especies del género *Mepraia*: *M. gajardoi, M. parapatrica* y *M. spinolai*, de manera de comparar entre sexos, especies y morfotipo alar de los machos. Para la descripción morfológica se utilizaron tres individuos de cada especie, sexo y morfotipo alar. Se observó la ultraestructura del surco estridulatorio mediante microscopía electrónica de barrido y se obtuvieron imágenes del protórax mediante microscopía confocal. Las imágenes obtenidas mediante ambos métodos se utilizaron para medir largo, ancho y profundidad del surco. Adicionalmente, se contó el

número de crestas y la distancia entre éstas. Se realizó un análisis de similitud (ANOSIM) para evaluar diferencias entre grupos y, análisis de similitud de porcentajes (SIMPER) para evaluar el aporte de las variables a la disimilitud entre sexos, especies y tipo alar. Se realizó una descripción general de surco y se encontró que es similar al surco de otros Triatominos. Se detectó que la morfología del surco estridulatorio presenta diferencias significativas sólo entre morfotipos alares, pero no se encontraron diferencias significativas entre especies y sexos. Aun cuando la señal producida por la estridulación es desconocida en *Mepraia,* este trabajo aporta información relevante para el estudio de la comunicación vibroacústica en triatominos chilenos y además descarta a esta estructura como carácter morfológico para diferenciar entre las tres especies del género.

ABSTRACT

Stridulation is a mechanism of vibroacoustic communication generated by the friction of two rigid structures. Stridulation is emitted in several groups of insects, including the hematophagous hemipterans better known as kissing bugs (Reduviidae: Triatominae). These insects are vectors of *Trypanosoma cruzi,* responsible of Chagas disease in humans. In this group, stridulation results from the friction of the proboscis against the stridulatory sulcus. The sulcus is placed at the center of the prostern, and is composed of numerous horizontal ridges. Stridulation in Triatominae is emitted in response to predators, handling and by females in order to reject males during copulation attempts. In Chile, the endemic genus *Mepraia* inhabits arid, semiarid and Mediterranean zones and is characterized by conspicuous alary polymorphism. Females are always micropterous (wingless) and males can be brachypterous, macropterous or micropterous. The goal of this study is to describe the morphology of the stridulatory sulcus in the three species of *Mepraia*: *M. gajardoi* (with only brachypterous males), *M. parapatrica* (with brachypterous and macropterous males) and *M. spinolai* (with micropterous, brachypterous and macropterous males), and to compare between sexes, species and alary types. For the three species, three individuals of each sex and alary type were analyzed. Scanning electron microscopy (SEM) and confocal microscopy photomicrographs were used to measure length, width and depth of the sulcus. Additionally, the number of ridges and the inter-ridge distance were quantified. An Analysis of Similarities (ANOSIM) was performed to evaluate differences among groups. The contribution of the variables to differences among groups was evaluated by a Similarity of Percentages (SIMPER). Significant differences were detected in alary types but no differences were detected between sexes and among species. The results

obtained in the present study provide important information for future research aiming at studying vibroacoustic communication in Chilean triatomines and preclude the use of this structure as a morphological character to differentiate among the three species of the *Mepraia* genus.

INTRODUCTION

Stridulation is a mechanism of vibroacoustic communication in which a sound or vibratory signal is produced by the friction of two rigid structures (Cocroft et al. 2014). The sound or vibration can be transported by the substrate, air or body. This type of communication is widespread in Arthropoda, involving a rich diversity of structures and signals (Drosopoulos & Claridge 2005).

The hematophagous hemipterans known as kissing bugs (Reduviidae: Triatominae) are vectors of the protozoan parasite *Trypanosoma cruzi*, responsible of Chagas disease in humans. Stridulation has been described in the Reduviidae (Schofield 1977, Lent & Wigodsinsky 1979, Manrique & Schilman 2000, Reyes-Lugo et al. 2006, Silva et al. 2010), in which the friction of the proboscis against the stridulatory sulcus produces the sound or vibration. The stridulatory sulcus consists of a series of ridges along the central part of the prostern, and the shape, width, length and number of ridges have been used to describe the morphology of this structure. The sulcus has been reported to vary among species and nymphal stages, being a useful structure to identify species and their nymphal stages (Lent & Wygodzinsky 1979, Di Luciano 1981, Gonçalves et al. 1985, Silva et al. 2003).

In the triatomine species so far studied, stridulation is emitted in response to handling, in the presence of predators and in a sexual context, when females reject copulation attempts by males (Manrique & Lazzari 1994, Manrique & Schilman 2000, Lazzari et al. 2013). The stridulatory signal consists of the fast repetition of a two-noted syllable composed of a short and a long chirp, and a dominant frequency of approximately 2000 Hz (Lazzari et al. 2013). In *Triatoma infestans, T. guasayana, T. sordida, Rhodnius prolixus* and *Dipetalogaster maxima* no differences between sex in the disturbance stridulation signal were found, however, there are within-species differences in the duration of chirps and inter-chirp intervals (Schilman et al. 2001). Notwithstanding, the relationship between the structure of the signal and the morphology of the stridulatory sulcus remains unclear (Roces & Manrique 1996, Manrique & Schilman 2000).

In Chile two genera of triatomines have been described: *Triatoma* with only one species, *Triatoma infestans,* and *Mepraia* with three species. The genus *Mepraia* is endemic to Chile, and composed by *Mepraia gajardoi* (Frías et al. 1998), *Mepraia parapatrica* (Frías-Laserre 2010) and *Mepraia spinolai* (Porter 1933). These species inhabit arid, semi-arid and Mediterranean regions of Chile. *M. gajardoi* (18º - 25°S) and *M. parapatrica* (25º - 26°S) are distributed in the northern coasts, with a diet including mainly birds, marine mammals and lizards (Sagua et al. 2000). *M. spinolai* (26º - 33°S) has the most extensive distribution and its diet include mainly small mammals and occasionally livestock (Frías-Laserre 2010). *Mepraia* is characterized by the alary polymorphism present in adult males. In all *Mepraia* species, females are invariantly micropterous (wingless), while in *M. gajardoi* all males exhibit brachypterous wings (Frías et al. 1998), in *M. parapatrica* males present macropterous or brachypterous wings (Frías-Laserre 2010), and in *M. spinolai* males can be micropterous or winged (brachypterous and macropterous) (Lent et al. 1994).

Even though *Mepraia* species present stridulatory sulcus, the morphology of this structure has not been described, and thus potential differences among species remain unexplored. In addition, the stridulatory signal of *Mepraia* species has not been recorded and there is no information available about the context in which these signals are emitted.

The goal of this study is to describe and compare the morphology of the stridulatory sulcus in the three species of the *Mepraia* genus. To this end, individuals of both sexes and of all the alary morphotypes were studied. Additionally, I examine if the morphology of the stridulatory sulcus is a useful character to identify species of the

Mepraia genus. The information obtained in this study will be relevant for future studies investigating the role of vibroacoustic communication in Triatominae.

MATERIAL AND METHODS

A total of 24 adult individuals of *Mepraia gajardoi* (Figure 1), *Mepraia parapatrica* (Figure 2) and *Mepraia spinolai* (Figure 3) were manually collected in the field. For the three species, three males and three females of each alary morphotype were collected. However, no *M. parapatrica* macropterous males were analyzed because of their low abundance in the population studied.

Figure 1. *Mepraia gajardoi* adults: female (left) and brachypterous male (right).

Figure 2. *Mepraia parapatrica* adults: female (left) and brachypterous male (right).

Figure 3. *Mepraia spinolai* adults from left to right: female, micropterous male, brachypterous male, macropterous male

Individuals were captured in the coast and valleys between 18°and 32°S, location of capture sites is shown in Figure 4. After triatomines were euthanized with a cold-shock, the thorax of specimens was extracted using a scalpel, and the six legs were removed with forceps. Then, a ventral photograph of the mesothorax was obtained on a graph-paper using a digital camera (Cannon, Power Shot D30, 12.1 Mega pixels). Two microscopy techniques were used to describe the morphology of the sulcus. First, the specimens were gold coated and the sulcus observed using a scanning electron microscope (Hitachi TM-3000, Tabletop SEM at 15 kV) following Silva et al. (2003). Various sulcus photomicrographs were obtained with this technique, including a low-magnification (100-120×) image of the entire sulcus, a high-magnification (1000-4000×) photomicrograph of the ridges located at the middle of the sulcus and between three to five consecutives photomicrographs (300-600×) covering of the entire sulcus length. The number of ridges were counted on these images. In addition, a confocal microscope (Zeiss 710, Plan-Apochromat 10×, 0.3 M27) was used to obtain a 3D projection of the stridulatory sulcus.

The morphometry of the stridulatory sulcus was characterized with the following measures: maximum sulcus width (MSW), number of sulcus ridges (NSR) and inter-ridge distance (IRD). The IRD corresponded to the average distance among 11 to 20 consecutive ridges located at the center of the sulcus. In addition, the 3D projection obtained by means of confocal microscopy was used to measure the sulcus length (SL) and sulcus depth (SD).

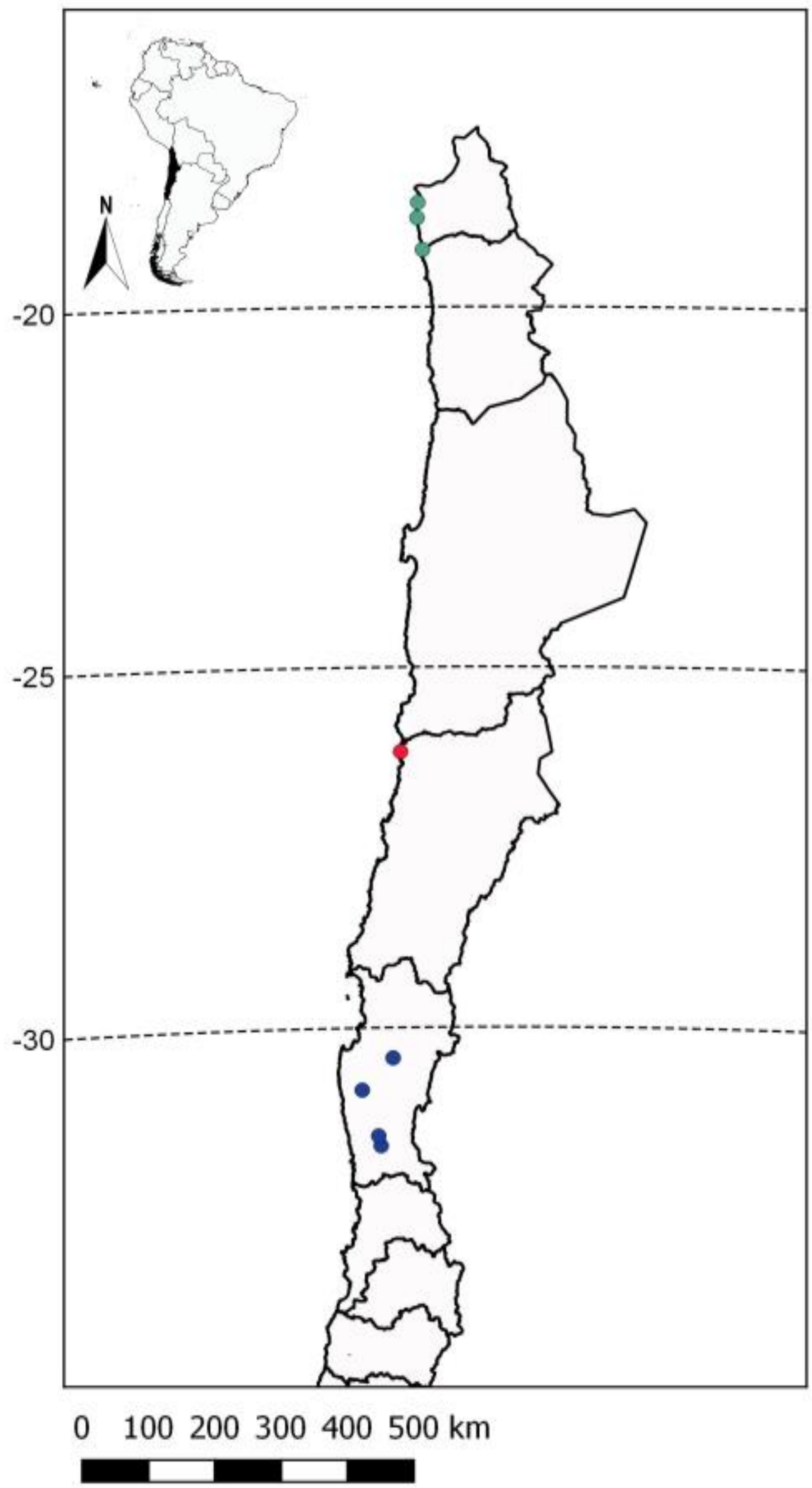

Figure 4. Map of the northern region of Chile. Localities where *Mepraia gajardoi* (green circles), *M. parapatrica* (red circle) and *M. spinolai* (blue circles) individuals used in this study were captured.

Sulcus measurements (i.e., SL, NSR, MSW, IRD and SD) were standardized by body size, by dividing these measurements by the maximum width of the mesothorax of each individual. All the morphometric measures were obtained with the free software Image J (Rasband 2016). Because of the small sample size, non-parametric analyses were performed. First, to analyze the similarity between groups (species, sex and alary type) an Analysis of Similarity (ANOSIM) was used. Subsequently, a Similarity of Percentages (SIMPER) was performed on the groups for which the ANOSIM showed significant dissimilarity (see results). SIMPER was conducted to identify the sulcus variables that contributed to discriminate among the levels of the groups. ANOSIM and SIMPER were performed in PAST (Hammer et al. 2001, version 3.14).

RESULTS

Description of the sulcus

In the three species, the sulcus is in the central portion of the prostern, from under the collar to the end of this segment. The sulcus is subtriangular, longer than wider (Figures 5 and 6), reaching its maximum width at about its mid-length. The sulcus is surrounded by two anterolateral processes that are well defined in both sexes but larger in males. Trichobothria are usually present on these processes and near the sulcus in males and females, and at the posterior edge of the segment trichobothria become numerous. The sulcus is composed by numerous ridges and at its anterior and posterior end these ridges are inconspicuous, but in the mid portion these structures are well defined. Ridges are more numerous and IRD is larger in females than males (Table 1). As shown by confocal microscopy, sulcus depth is highly variable (Figure 7), and winged males present a deeper sulcus than females. The length of the sulcus is larger in females than in males and the sulcus is wider in females of *M. gajardoi* and *M. parapatrica*, but in *M. spinolai* males have a wider sulcus. No cuticular trait was found to differentiate among species, alary morphotype or between sexes.

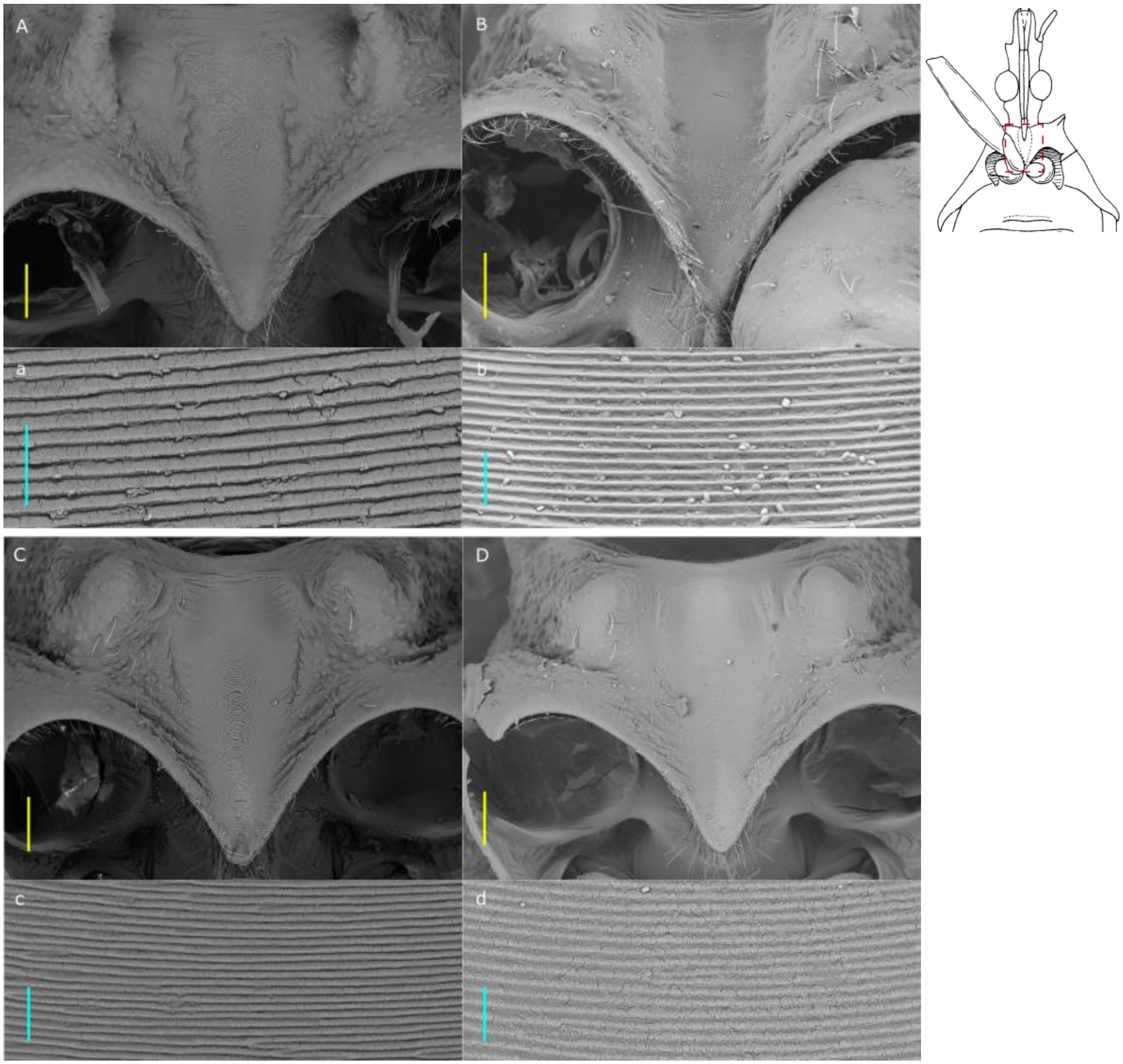

Figure 5. Ultrastructure of the stridulatory groove and details of the ridges. A: female of *Mepraia gajardoi* (100×) and a: magnification of the ridges (3000×); B: brachypterous male of *M. gajardoi* (120×) and b: magnification of the ridges (2000×); C: female of *M. parapatrica* (100×) and c: magnification of the ridges (2000×), D: *M. parapatrica* brachypterous male (100×) and d: magnification of the ridges (2000×). The bottom magnified photomicrograph for each individual show details of the ridges from the middle portion of the sulcus. The yellow and blue scale bars represent 200 and 10 μm, for the low magnification and high magnification pictures, respectively. (Schematic drawing modified from Lent & Wigodzinsky 1979)

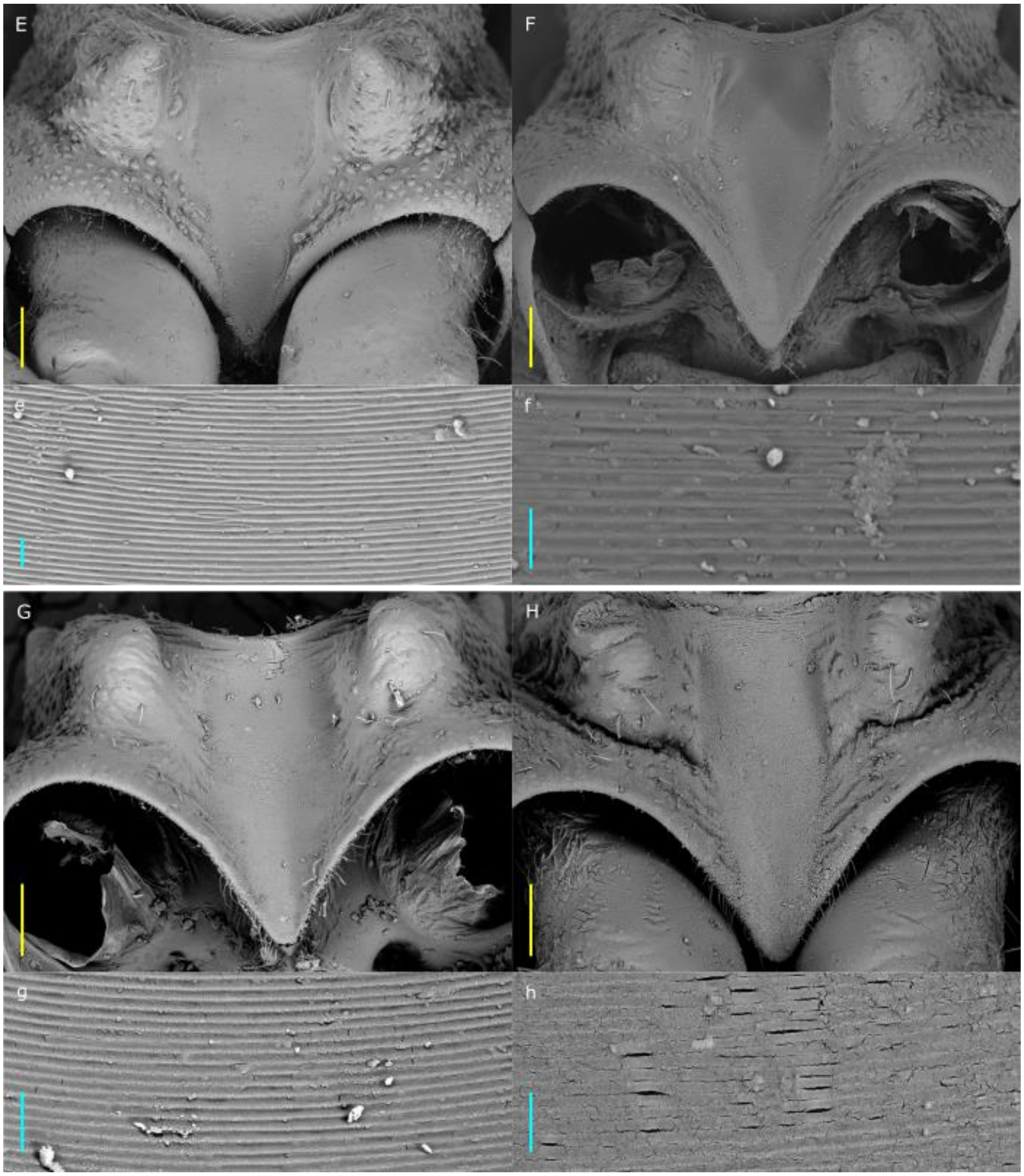

Figure 6. Ultrastructure of stridulatory sulcus and details of the ridges. E: female of *M. spinolai* (100×) and e: magnification of the ridges (5000×); F: micropterous male of *M. spinolai* (100×) and f: magnification of the ridges (2000×); G: brachypterous male *M. spinolai* (120×) and g: magnification of the ridges (2000×), H: macropterous male of *M. spinolai* (120×) and h magnification of the ridges (2000×). The bottom magnified photomicrograph for each individual show details of the ridges from the middle portion of the sulcus. The yellow and blue scale bars represent 200 and 10 μm, for the low magnification and high magnification pictures, respectively.

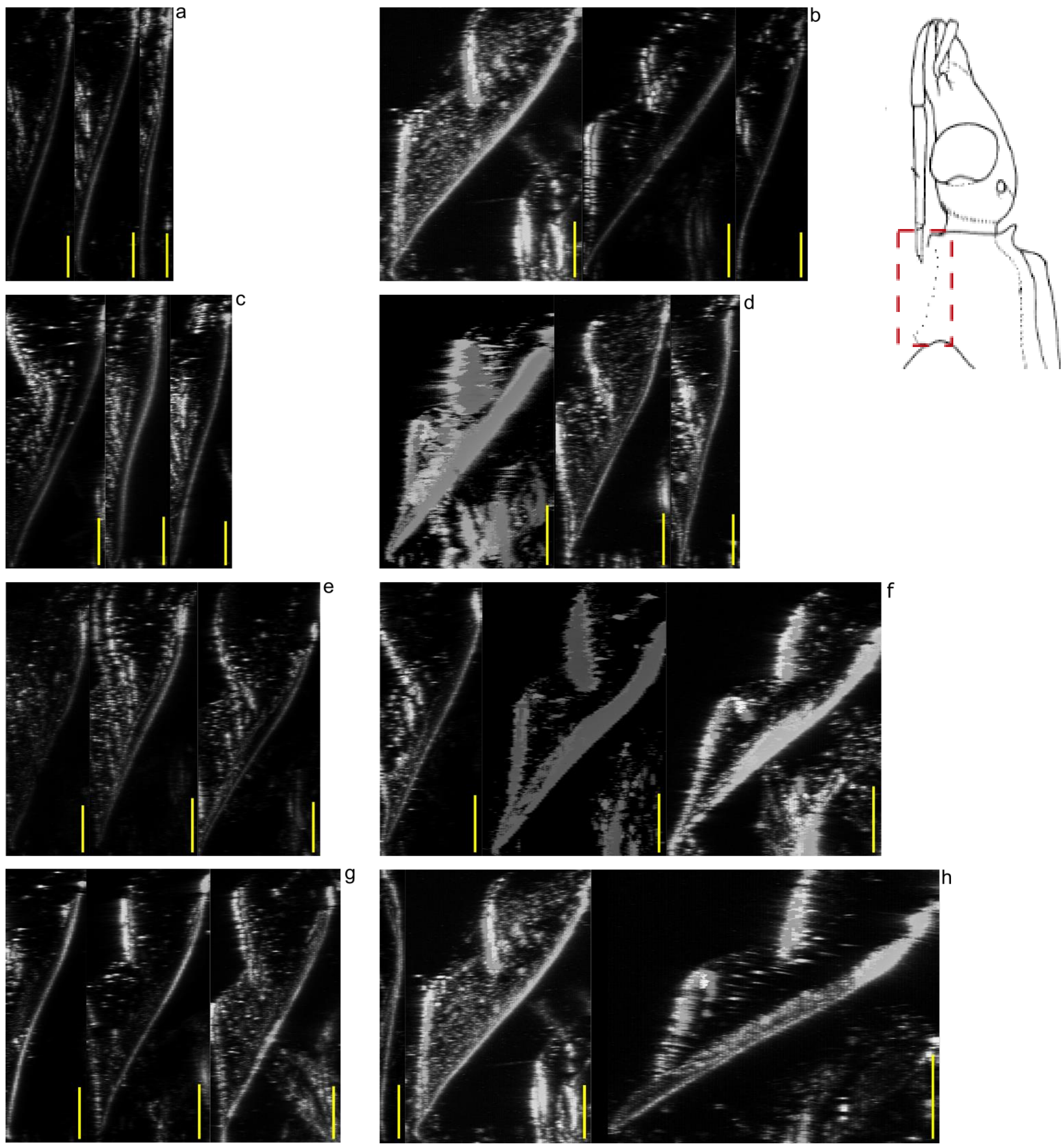

Figure 7. Side view of the prostern (10×) visualized by confocal microscope of all the three individuals of every sex and alary type. a: *Mepraia gajardoi* females, b: *M. gajardoi* brachypterous males, c: *M. parapatrica* females, d: *M. parapatrica* brachypterous males, e: *M. spinolai* females, f: *M. spinolai* brachypterous males, g: *M. spinolai* micropterous males, h: *M. spinolai* macropterous males. The yellow scale bar represents 200 μm. In each picture, the top corresponds to the anterior limit of the sulcus and the bottom corresponds to its posterior region. Left and right correspond to the ventral and dorsal surface of the individual, respectively, as indicated in the schematic drawing on the upper right corner. (Schematic drawing modified from Lent & Wigodzinsky 1979)

Table 1. Measures of the thorax and the stridulatory sulcus of *Mepraia gajardoi, M. parapatrica* and *M spinolai* individuals. The values listed correspond to measures not corrected by body size (mean ± standard error).

Species	Sex	Alar type	Maximum mesosternum width (MW) (cm)	Sulcus length (SL) (µm)	Number of sulcus ridges (NSR)	Sulcus depth (SD) (µm)	Maximum sulcus width (MSW) (µm)	Inter-ridge distance (IRD) (µm)
M. gajardoi	F	Micropterous	3.49 ± 0.15	1104.61 ± 39.44	555.67 ± 35.38	247.23 ± 52.28	406.07 ± 37.03	1.92 ± 0.10
	M	Brachypterous	3.88 ± 0.23	1051.77 ± 21.62	452.00 ± 7.64	500.92 ± 106.36	342.02 ± 13.05	2.46 ± 0.27
M. parapatrica	F	Micropterous	3.35 ± 0.45	1082.45 ± 14.49	555.00 ± 36.59	307.20 ± 47.72	453.32 ± 16.27	1.72 ± 0.13
	M	Brachypterous	3.61 ± 0.17	1012.16 ± 14.80	449.00 ± 37.21	416.06 ± 114.58	395.20 ± 16.83	2.09 ± 0.04
M. spinolai	F	Micropterous	2.73 ± 0.09	935.77 ± 35.53	459.67 ± 27.03	398.54 ± 52.61	327.39 ± 15.45	1.94 ± 0.05
	M	Micropterous	2.77 ± 0.32	996.08 ± 3.29	396.33 ± 12.55	381.91 ± 39.05	365.98 ± 18.39	2.43 ± 0.12
	M	Brachypterous	3.08 ± 0.49	881.08 ± 31.93	376.33 ± 21.31	531.36 ± 85.22	365.84 ± 8.55	2.18 ± 0.16
	M	Macropterous	3.05 ± 0.33	945.27 ± 74.65	352.67 ± 46.83	491.68 ± 197.05	341.45 ± 10.14	2.30 ± 0.13

Morphometry of the sulcus

Results of ANOSIM show that the morphology of the sulcus is similar between sexes and among species. Sex had a marginally significant effect on the structure of the sulcus ($R = 0.137$; $p = 0.060$) and the effect of species was not significant ($R = -0.099$; $p = 0.881$). In contrast, the effect of alary morphotypes was statistically significant ($R = 0.305$; $p = 0.022$). Post-hoc analyses show significant differences between winged and wingless individuals (micropterous-brachypterous, $R = 0.280$; $p = 0.010$; micropterous-macropterous, $R = 0.588$; $p = 0.036$) and no significant differences were detected between brachypterous and macropterous males ($R = 0.018$; $p = 1.00$). Because of the significant differences found among alary types, a SIMPER analysis was performed. Since this analysis compares pairs of individuals, alary types were grouped in winged (brachypterous and macropterous) and wingless (micropterous). Thus, the percentages of every variable that account to dissimilarity are IRD (35.1%), SL (22.5%), SD (18.6%), NSR (14.4%) and, MSW (9.4%). The first three variables contributed to 76.2% to the dissimilarity.

DISCUSSION

In this study, I found that the overall morphology of the stridulatory sulcus is similar in the three *Mepraia* species. The subtriangular shape of the sulcus of these species is similar to that described for other Triatomines (Silva et al. 2010). The morphology of the stridulatory sulcus shows dissimilarities among alary types, however, no significant differences were found between sexes and among species.

During stridulation, each time a ridge is scraped, a pulse is produced in the vibroacoustic signal, and the consecutive action of scraping the ridges of the sulcus generates the chirp (Golden & Hill 2016). Therefore, an association between the morphology of the sulcus and the structure of the signal is expected to occur. For example, the morphology of the stridulatory organ in Mutillidae wasps has been associated with some spectral variables of the acoustic signal emitted by these insects (Polidori et al. 2013). On the other hand, Schilman et al. (2001) found no association between inter-ridge distance and the signal of five species of triatomines. As the stridulatory signal emitted by *Mepraia* species has not been described, any association between sulcus morphology and signal structure remains unexplored. Previous studies have reported inter-ridge distances ranging from about 4 – 8 μm in triatomines (Schilman et al. 2001), however, in the present study inter-ridge distances of about 2 μm were measured for the three *Mepraia* species. This morphological distinctiveness makes the comparison between the stridulatory signal of *Mepraia* and other triatomines an interesting issue to be addressed in future research.

Hernández et al. (2015) measured the association between morphological traits and the size of wings in *Mepraia spinolai*, finding that the thorax size is larger in winged individuals than in wingless individuals. This relationship suggests that other morphological traits in the thorax may differ among alary morphotypes. Differences in the morphology of the sulcus were only found among different alary morphotypes. In *Mepraia* species, the macropterous and brachypterous individuals had a deeper sulcus than micropterous ones, however, it is unknown if these morphological differences relate to differences in stridulatory signals in species with alary polymorphism. The width and depth of the sulcus had not been previously measured in any species and, therefore, it would be interesting to evaluate whether these morphological variables are associated with the structure of the signals.

Stridulation in triatomines is related to female's rejection of males (Manrique & Lazzari 1994, Manrique & Schilman 2000, Lazzari et al. 2013). In this study, marginal effect between sex in the structure of sulcus were found ($R = 0.137$; $p = 0.060$) and no differences among species, but sex differences may appear if a larger number of individuals are measured. A study of the antennal phenotype, a trait related to sexual recognition reported larger differences between sexes than between species in *M. spinolai* and *M. gajardoi* (Moreno et al. 2006). Furthermore, hybridization between *M. gajardoi* and *M. spinolai* occurs between *M. gajardoi* females and *M. spinolai* males (Campos-Soto et al. 2016). Similarities in secondary sexual characters including stridulating structures and behavior between species could favor the occurrence of hybridization of *Mepraia* in nature.

The morphological analyses conducted in the present study show no evidence of species-specific variation in the morphology of the sulcus in *Mepraia* species. Also, no differences in the morphological cuticular ornamentation of the sulcus were detected; therefore, this structure would not allow to discriminate among the three species of *Mepraia*. Silva et al. (2010) used the morphology of the stridulatory sulcus to differentiate between some nymphal stages of different species of the *Triatoma* genus. Those differences included the number of ridges and the ornamentation around the sulcus; however, the evidence shown in this study suggests that the morphology of the sulcus is not a useful taxonomic character in *Mepraia*.

The behavioral role of vibroacoustic communication in Triatomines remains largely unexplored and this study contributes to the current knowledge by describing the stridulatory organ in kissing bugs from the *Mepraia* genus. This information will be important for future studies investigating the mechanisms employed by triatomines to communicate, and how morphological variability relates to differences in the properties of the signal emitted.

CONCLUSIONS

The stridulatory sulcus in *Mepraia* is subtriangular and similar to the stridulatory sulcus of other species of triatomines. The stridulatory sulcus in *Mepraia species* is highly variable in depth and characterized by inter-ridge distance of ~2 µm. Also, no cuticular traits were found to discriminate between sexes, species and alary types.

Differences in the morphometry of the sulcus were found among winged and wingless individuals. No differences were detected in the morphometry of the stridulatory sulcus between sex and species.

The morphology of the stridulatory sulcus is not a useful character to discriminate among the species of the *Mepraia* genus. It is necessary to record and analyze the stridulatory signal to understand the function of this structure.

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