



Differences in mating behavior between two allopatric populations of a Neotropical scorpion



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ARTICLE INFO

Article history:

Received 29 December 2016
Received in revised form 20 June 2017
Accepted 21 June 2017
Available online 12 July 2017

Keywords:

Scorpions
Bothriuridae
Courtship behavior
Intraspecific variation

ABSTRACT

Courtship and mating behavior generally evolve rapidly in diverging populations and species. The adaptation to different environments may cause behavioral divergence in characteristics involved in mate choice. Our objective in this study was to compare the sexual behavior of two distant populations of the scorpion *Bothriurus bonariensis*. This species has a broad distribution in South America, inhabiting Central Argentina, Uruguay and south-eastern Brazil. It is known that in this species there is a divergence in morphological patterns (body size, coloration, allometry and fluctuating asymmetry indexes) among distant populations. Considering the differences in environmental conditions between localities, we compare the sexual behavior in intra-population and inter-population matings from Central Argentina and southern Uruguay populations. We found significant differences in mating patterns, including differences in the frequency and duration of important stimulatory courtship behaviors. In addition, most inter-population matings were unsuccessful. In this framework, the differences in reproductive behavior could indicate reproductive isolation between these populations, which coincides with their already known morphological differences. This is the first study comparing the sexual behavior of allopatric populations of scorpions; it provides new data about the degree of intraspecific geographical divergence in the sexual behavior of *B. bonariensis*.

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1. Introduction

In the more conventional view, speciation in animals is due to accumulation of differences by genetic drift and selection in allopatry (Mayr, 1942; Sobel et al., 2010). Allopatric populations in the first phases of divergence can help in the study of the role of environmental adaptation, sexual selection and genetic divergence in generating reproductive barriers between populations (Jennings et al., 2011). Differential intensity of sexual selection among populations can lead to divergent morphological and behavioral evolution and speciation (Andersson, 1994; Kraaijeveld and Pomiankowski, 2004; Mobley and Jones, 2007). It is known that sexually selected traits are affected by different environmental factors and, therefore, these factors could influence the corresponding preferences both within and among populations (Kokko and Rankin, 2006; Bro-Jørgensen, 2010; Maan and Seehausen, 2011; Wilkins, 2012). The adaptation to different environments may cause behavioral divergence in characteristics involved in mate

choice (Schluter, 2001; Eraly et al., 2009). Courtship and mating behavior generally can evolve rapidly in diverging populations and species; therefore, these features are easily detected in speciation events (Barlow, 2002; González et al., 2013). Particularly species with a wide distribution may present variations in sexual behavior among populations across a distribution, initiating a speciation process (Zann, 1976; Hoikkala and Welbergen, 1995; Miller et al., 1998; Lachlan and Servedio, 2004; González et al., 2013).

Geographical variations in courtship behavior have been observed in many taxa, including vertebrates (Ryan et al., 1990; Ishikawa et al., 2006; Prohl et al., 2006; Kuppennagel and Baur, 2011), insects (Saarikettu et al., 2005; Briceño et al., 2007) and spiders (Miller et al., 1998; Elias et al., 2006; González et al., 2013). Much is known about the mating behavior and reproductive isolation of spiders (Persons et al., 2001; Masta and Maddison, 2002; Hebets and Maddison, 2005; González et al., 2013, 2014, 2015; Bollatti et al., 2017). Miller et al. (1998) reported differences in the sexual behavior between different populations of the spider *Schizocosa crassipes* (Walckenaer 1837) (Lycosidae). In addition, González et al. (2013) observed large differences in the patterns and frequency of occurrence of courtship behaviors in different populations in the spider *Aglaoctenus lagotis* (Holmberg 1876) (Lycosidae).

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However, there is less information about the behavior involved in mate selection and reproductive isolation among populations of scorpions.

Courtship and mating of scorpions involve a complex series of ritualized behaviors, many of which are conserved among taxa (Polis and Sissom, 1990). Classically, the male grasps the female with his pedipalps and starts the typical mating dance. During courtship, the male performs different behavioral patterns to decrease female aggressiveness. Then the male locates a suitable place to deposit the spermatophore and induces the female to collect the sperm from it. After the sperm transfer, the partners finally separate (Polis and Sissom, 1990; Peretti, 1993; Peretti and Acosta, 1999; Benton, 2001; Toscano-Gadea, 2010). Sexual recognition occurs because of small changes in some courtship behaviors (e.g., frequency of kissing and leg rubbing, duration of sexual sting, etc.) (Peretti, 1993). In the present study, our objective was to describe and compare the sequence of sexual behaviors (courtship and sperm transfer) of two distant populations, from southern Uruguay and Central Argentina, of the Neotropical scorpion *Bothriurus bonariensis* (Koch 1842).

Bothriurus bonariensis is the type species of the scorpion family Bothriuridae. This burrowing species represents an important model for many research topics, including sexual biology and sexual selection (Peretti, 1995, 1996, 1997, 2000, 2003; Peretti and Acosta, 1999; Peretti et al., 2001; Peretti and Battán-Horenstein, 2003; Carrera et al., 2009; Olivero et al., 2012, 2014, 2015; Vrech et al., 2014). It has a broad continuous distribution in South America (Mattoni and Acosta, 2005; Ojanguren-Affilastro, 2005), inhabiting Central Argentina, Uruguay, and south-eastern Brazil (Mattoni and Acosta, 2005; Ojanguren-Affilastro, 2005). The populations from Argentina and Uruguay are separated by conspicuous biogeographical barriers, represented by the Uruguay River between Argentina and Uruguay, and the Negro River within Uruguay. In Central Argentina, the climate is drier, with a lower level of rains and higher temperatures than in southern Uruguay (Cabrera and Willink, 1973; see Argentina's national meteorological service at www.smn.gov.ar and Uruguay's National direction of meteorology

at www.meteorologia.com.uy). Grassland vegetation is dominant in southern Uruguay while hills with scrubby vegetation dominate in Central Argentina (Cabrera, 1971; Grela, 2004). There is a divergence in morphological patterns (body size, coloration, allometry and fluctuating asymmetry indexes) among distant populations in *B. bonariensis* (Peretti et al., 2001; Ojanguren-Affilastro, 2005; Olivero et al., 2012, 2014). In addition, the courtship and mating behavior of the species has been widely studied (Peretti 1993, 1995, 1996, 1997, 2000; Olivero, 2014; Olivero et al., 2015). Males in distant populations of *B. bonariensis* present different chemical compositions of an external sexual gland exudate and perform different patterns of stimulatory behaviors (e.g., 'rubbing with telson' and 'pull and approximation') during courtship (Olivero et al., 2015). Also, they can modulate these sexual behaviors with females of different populations to achieve sperm transfer (Olivero et al., 2015). Before this background and considering the differences in environmental conditions between the selected localities, we expect to find differences in the mating behavior patterns of the two populations studied as well as some degree of inconsistency in the inter-population mating. In this context, this is the first study comparing the sexual behavior among allopatric populations of scorpions, with the aim to provide new data about the degree of intraspecific geographical divergence in the sexual behavior of *B. bonariensis*.

2. Materials and methods

2.1. Collection and rearing

We collected adult males and females of *B. bonariensis* at the beginning of the reproductive season to ensure sexual receptivity of individuals. From November to December in 2009–2012 (i.e. before the beginning of the summer season in South America), we collected individuals of two distant populations: in Central Argentina (Mendiolaza, Córdoba) and in southern Uruguay (Piedras de Afilas, Canelones; Fig. 1). There were no differences in temperature and precipitation levels between the different mating

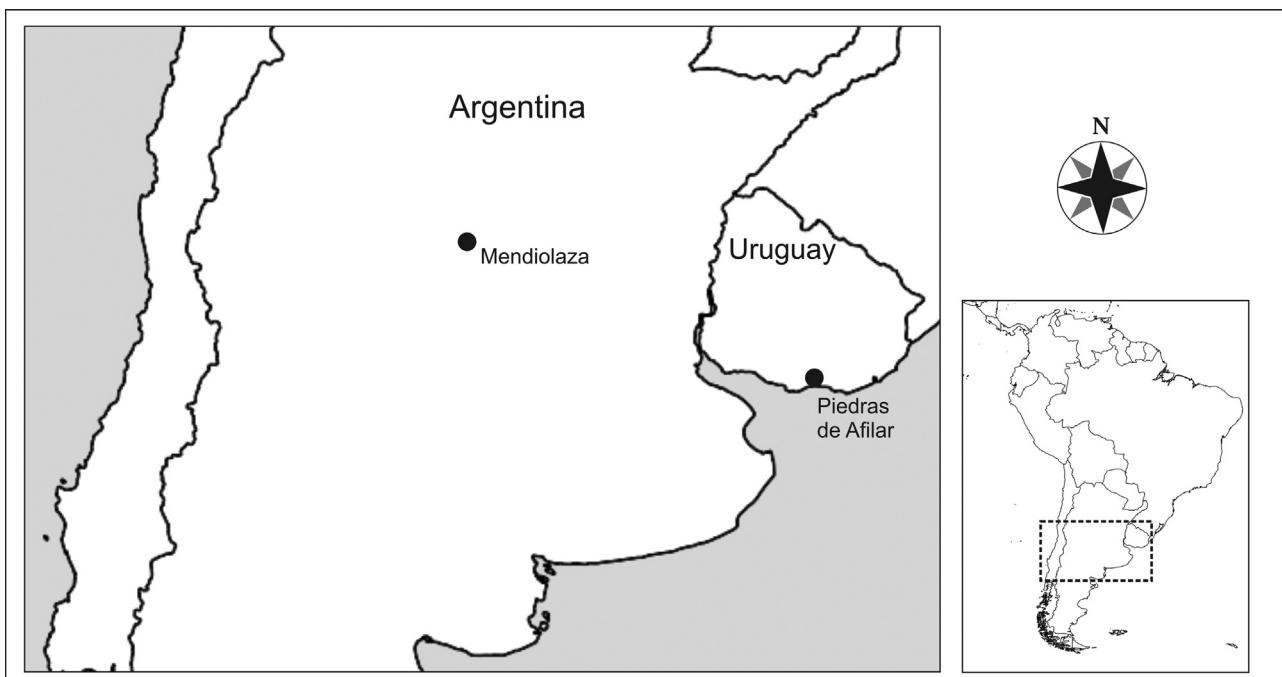


Fig. 1. Location of the studied populations of *Bothriurus bonariensis*: Central Argentina population from Mendiolaza, Córdoba, Argentina. Southern Uruguay population from Piedras de Afilas, Canelones, Uruguay.

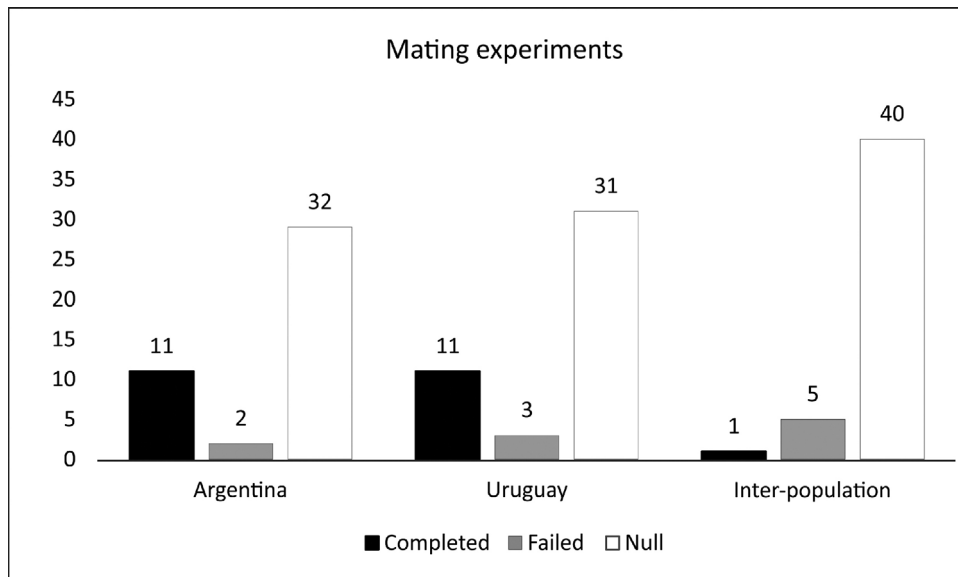


Fig. 2. Mating trials observed in intra-population and inter-population crosses with inter-population combinations of Uruguayan males and Argentinian females. Null: couples that did not begin courtship.

seasons (see Argentina's national meteorological service at www.smn.gov.ar and Uruguay's National direction of meteorology at www.meteorologia.com.uy). We kept specimens individually in plastic cages and they were fed with mealworm larvae (*Tenebrio* sp.) once a week. Each cage included one moist cotton ball to provide humidity. The temperature varied from 28 to 34 °C during the

period of breeding. We kept all the animals under a reversed 12:12 h light:dark cycle.

2.2. Observation and analysis of sexual behaviors

All observations were made in the Laboratorio de Biología Reproductiva y Evolución, Instituto de Diversidad y Ecología Ani-

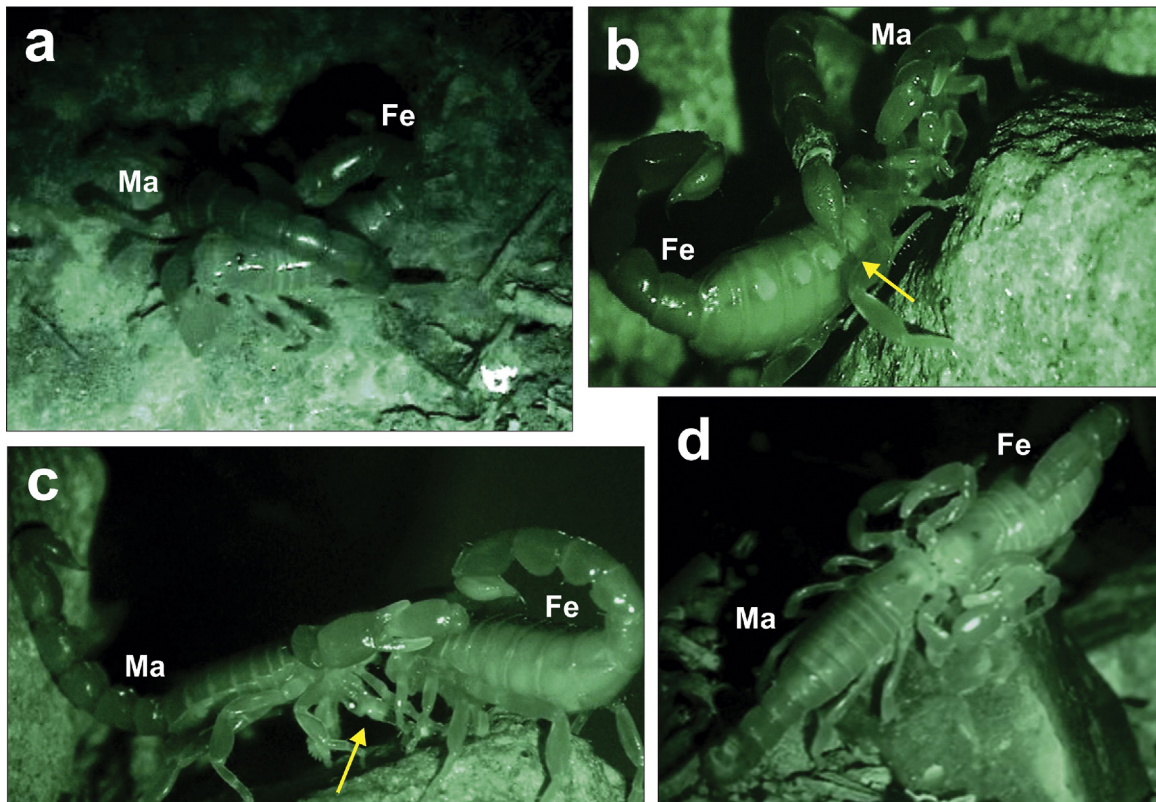


Fig. 3. Mating pair of *Bothriurus bonariensis* during courtship. (a) 'Rubbing with telson' in a mating pair of the Uruguayan population. (b) 'Sexual sting' in a mating pair of the Argentinian population. The arrow indicates the sting of the male puncturing the female's body in the pleural membrane. (c) 'Spermatophore deposition' in a mating pair of the Argentinian population. The arrow indicates the spermatophore. (d) 'Pull and approximation' in a mating pair of the Argentinian population. Fe, female, Ma, male.

Table 1
Behaviors performed by males and females of *Bothriurus bonariensis* during courtship and sperm transfer. The terms and definitions are based on scorpion literature for the Bothriuridae family (See Peretti, 1993, 1995; Peretti et al., 2000).

Behavior pattern	Description	Sex
Exploration	Individuals wander around exploring the environment.	Male and female
Alert position	Individuals are motionless, the metasome is curved over the mesosome and the pedipalp chelae are open.	Male and female
Grasping the female	The male grasps any part of the female's body with his pedipalps.	Male
Reorientation	With the female grasped, the male rotates his body until they both stand facing the chelicera, in a position ready to begin mating.	Male and female
Pedipalp grip	The male grasps the female in a pedipalp to pedipalp grip.	Male and female
Mating dance	The motile stage of courtship, during which the male grasps the female's pedipalps and then leads her as the pair moves together.	Male and female
Pull and approximation	The male pulls slightly at the female and approaches her, reaching her mouth region. Then he moves away and repeats the sequence.	Male and female
Rubbing with telson	The male spreads the product of the telson gland, rubbing the dorsal side of the telson on the female body.	Male
Sexual sting	The male uses its sting to puncture the female's body, usually at the mesosome pleura.	Male
Pull	The male pulls the female and forces her to walk when she is not willing to move.	Male
Male push	The male extends his metasome rearward and pushes his body down, leaving his genital opening in contact with the substrate to continue with the spermatophore deposition.	Male
Spermatophore deposition	The male presses his genital opening against the substrate and sticks the base of the spermatophore to the substrate.	Male
Female goes over spermatophore	The male assists the female in positioning her genital aperture over the spermatophore, allowing sperm uptake.	Male and female
Cheliceral grip	The male grasps the female's chelicerae with his chelicerae.	Male and female
Grasping of the legs	The male grasps the third pair of legs of the female with his pedipalps.	Male
Sperm transfer	The male supports its metasoma on the substrate and pushes the female back. The spermatophore bends slightly under the female's weight, allowing sperm to enter the female's reproductive tract.	Male
Slow backward movement	The male pushes the female backwards, allowing the removal of the spermatophore from the female genital opening.	Male and female

mal (IDEA), CONICET-UNC, in Argentina. We kept the individuals in the plastic cage during a week for acclimation before the observations. We randomly selected male–female pairs and placed them in a mating arena (18 cm W x 30 cm L x 30 cm H) with soil as substrate, and stones and pieces of tree bark from the capture sites. We performed the observations during four mating seasons, from 2009 to 2012. We recorded mating sequences with a digital video camera (Sony DCR-TRV 351) equipped with +4 close up lenses and “night shot” function. We tested 45 mating trials of pairs from the Argentinian population, 45 mating trials of pairs from the Uruguayan population and 46 inter-population mating trials with males from Uruguay and females from Argentina. Males from Argentina and females from Uruguay did not mate so we excluded this pairing from the analysis.

2.3. Statistical analysis

We analyzed the observations with JWatcher 1.0 (www.jwatcher.ucla.edu). We performed all the statistical analyses with the program NCSS 2007 (NCSS, LLC, Kaysville, UT, USA). We determined the normality of the data and the homogeneity of variances using kurtosis and the modified Levene test for equal variances, respectively. We analyzed absolute and relative frequency and duration of the behavioral patterns with analysis of variance tests (ANOVA or Kruskal–Wallis one-way ANOVA, depending on the normality of the data) and post hoc tests (Tukey or Dunn).

3. Results

3.1. Intra-population matings

3.1.1. Argentinian population

In Argentinian pairs, 81% of matings (11/13) were completed (i.e., sperm transfer occurred; Fig. 2). The total duration of mating

was 51.21 ± 6.54 min (mean \pm SE). The duration of the first part of courtship (before spermatophore deposition) was 35.89 ± 5.58 min and the duration of the second part of courtship (after spermatophore deposition; Fig. 3c) was 15.31 ± 3.03 min. All behaviors described in Table 1 were observed. The most frequent behaviors performed during courtship were ‘pull and approximation’ (Table 2 and Video S1 in the supplementary online Appendix) and ‘rubbing with telson’ (Fig. 3a and Table 2). Another typical behavior performed was the ‘sexual sting’. All males performed this behavior at least once during courtship (Fig. 3b). We also observed that 2 of the 13 males of this population performed a ‘metasoma vibration’ when they detected a female. During this behavior, the male moved his metasoma rapidly and repeatedly with a short up-and-down movement. Males performed the behavior ‘male push’ (Tables 2 and 3; Video S2) to attach the spermatophore to the soil and transfer the sperm. The duration of sperm transfer was 4.86 ± 1.65 min. In a single case the female cannibalized the male immediately after sperm transfer, i.e. before the couple separated.

3.1.2. Uruguayan population

In the Uruguayan pairs, 80% of the matings (11/14) were completed (Fig. 2). Total duration of mating was 56.92 ± 6.77 min. The duration of the first part of courtship was 41.51 ± 5.78 min, and the duration of the second part was 15.42 ± 3.14 min. All the behaviors described in Table 1 were observed. The most frequent behavior performed during courtship was ‘rubbing with telson’ (Fig. 3a and Table 2; Video S3). ‘Pull and approximation’ was observed with very low frequency (Fig. 3d and Table 2). Uruguayan males also performed the ‘sexual sting’ at least once during courtship (Fig. 3b). The frequency of ‘male push’ was high in Uruguayan males (Table 2). The duration of sperm transfer was 6.92 ± 1.59 min.

Table 2

Comparison of frequencies of courtship behaviors (means \pm standard error) in the studied populations of *Bothriurus bonariensis*: Argentina (Mendiolaza, Córdoba), Uruguay (Piedras de Afilar, Canelones), inter-population matings (males from Uruguay, females from Argentina). A and B indicate the grouping and separation of populations by the Tukey and Dunn tests.

Behavior frequency	Argentina	Inter-population	Uruguay	Statistics
Pull and approximation	A 11.77 \pm 2.36	B 0.67 \pm 3.48	B 1.64 \pm 2.28	$H = 8.17; p = 0.017$
Rubbing with telson	A 10 \pm 2.72	AB 15.17 \pm 4.01	B 24.07 \pm 2.62	$F = 7.03; p = 0.003$
Male push	A 58.31 \pm 10.22	AB 57.67 \pm 15.04	B 92.86 \pm 9.85	$F = 3.59; p = 0.0401$
Grasping the female	A 1.31 \pm 0.23	B 2.83 \pm 0.34	A 1.07 \pm 0.22	$H = 7.81; p = 0.0201$
Reorientation	1.23 \pm 0.23	1.83 \pm 0.34	0.78 \pm 0.23	$F = 3.31; p = 0.0502$

3.2. Inter-population comparison

The total duration of mating was similar in both populations ($F = 0.32, p = 0.731$). The durations of the first and the second part of courtship were also similar between the two populations ($F = 0.45, p = 0.644$ and $F < 0.001, p = 0.977$, respectively). The main behavioral differences between the populations appeared during courtship. The behavior 'rubbing with telson' was observed with a higher frequency and duration in Uruguayan pairs. Conversely, the frequency and duration of 'pull and approximation' was higher in Argentinian pairs (Tables 2 and 3). We observed significant differences in the behavior 'male push' between the two different populations. Argentinian specimens showed a less frequent occurrence of 'male push' (Table 2) than the Uruguayan population. In addition, the Argentinian population showed the behavior 'vibration of the metasoma', a behavior not observed in the Uruguayan population.

3.3. Inter-population matings

13% percent of the inter-population couples (Uruguayan males and Argentinian females) started courtship but only one mating was completed (sperm transfer occurred; Fig. 2). In all inter-population trials, females offered resistance to males, resulting in a higher frequency of the behavior 'grasping the female' and a higher duration of the behavior 'reorientation' (Tables 2 and 3). Males had difficulties with the 'pedipalp grip' on females of the other population, resulting in a weak grasping and subsequent separation of the couple. In the inter-population matings, males from Uruguay performed the behavior 'rubbing with telson' with intermediate and significantly different frequency and duration in comparison to intra-population matings (Tables 2 and 3).

4. Discussion

In accordance with previous studies (Peretti, 1993, 1995, 1996, 1997, 2000; Olivero, 2014; Olivero et al., 2015), we could observe

the principal behavioral units described for *B. bonariensis* (Fig. 4). However, the populations studied herein showed significant differences in mating patterns, including differences in the frequency and duration of important stimulatory courtship behaviors. Individuals from the Uruguayan population are larger and present more robust traits (Mattoni, 2003; Olivero et al., 2012). Some of the differences observed in the patterns of behavior might partly be due to these morphological differences. Males from Uruguay show a longer duration of the 'male push' behavior. During this behavior, the male pushes against the ground, leaving the genital opening in contact with the substrate to proceed with spermatophore deposition. Spermatophores of males from the Uruguayan population are bigger than spermatophores of Argentinian males, with longer laminae and longer dorsal folds (Olivero et al., 2012). The morphology of these structures is very important since the lamina and the dorsal fold of the spermatophore fits into the intercoxal space of the female's legs to ensure sperm transfer (Peretti, 2003). These differences in spermatophore morphology between males from the two populations could result in a higher effort in spermatophore deposition for Uruguayan males, and as result a longer duration of the behavior for them.

Differences in the stimulatory behaviors 'rubbing with telson' and 'pull and approximation' were also observed. The Uruguayan males performed 'rubbing with telson' with higher frequency and duration than Argentinian males. Conversely, males from the Argentinian population performed 'pull and approximation' behavior with a higher frequency and duration than Uruguayan males. The differences observed in these behaviors may occur because of a correlation between performance and the individual's quality. During the 'rubbing with telson' behavior, the male spreads the product of a gland located in the dorsal side of its telson on the female's body. The secretion of this gland has the function to increase the sexual receptivity of the female (Peretti, 1997; Olivero et al., 2015). To spread the secretion during courtship, the male performs a series of movements, including rubbing the dorsal side of the telson on both sides of the female's metasoma and mesosoma (Video S3). On the other hand, the behavior 'pull and approxima-

Table 3

Comparison of the duration (min; means \pm standard error) of courtship behaviors in the studied populations of *Bothriurus bonariensis*: Argentina (Mendiolaza, Córdoba), Uruguay (Piedras de Afilar, Canelones), inter-population matings (males from Uruguay, females from Argentina). A and B indicate the grouping and separation of populations by the Tukey and Dunn tests.

Behavior duration	Argentina	Inter-population	Uruguay	Statistics
Pull and approximation	A 0.74 \pm 0.16	B 0.06 \pm 0.23	B 0.11 \pm 0.15	$H = 7.88; p = 0.02$
Rubbing with telson	A 2.02 \pm 0.77	A 2.83 \pm 1.13	B 7.12 \pm 0.74	$H = 14.63; p < 0.001$
Male push	4.86 \pm 0.99	4.92 \pm 1.47	7.97 \pm 0.96	$F = 2.98; p = 0.07$
Grasping the female	0.82 \pm 0.17	0.74 \pm 0.25	0.19 \pm 0.16	$H = 5.28; p = 0.07$
Reorientation	A 1.64 \pm 0.54	A 1.69 \pm 0.79	B 0.28 \pm 0.52	$H = 6.17; p = 0.04$
Sperm transfer	4.86 \pm 1.65	2.30 \pm 2.43	6.92 \pm 1.59	$H = 2.99; p = 0.22$

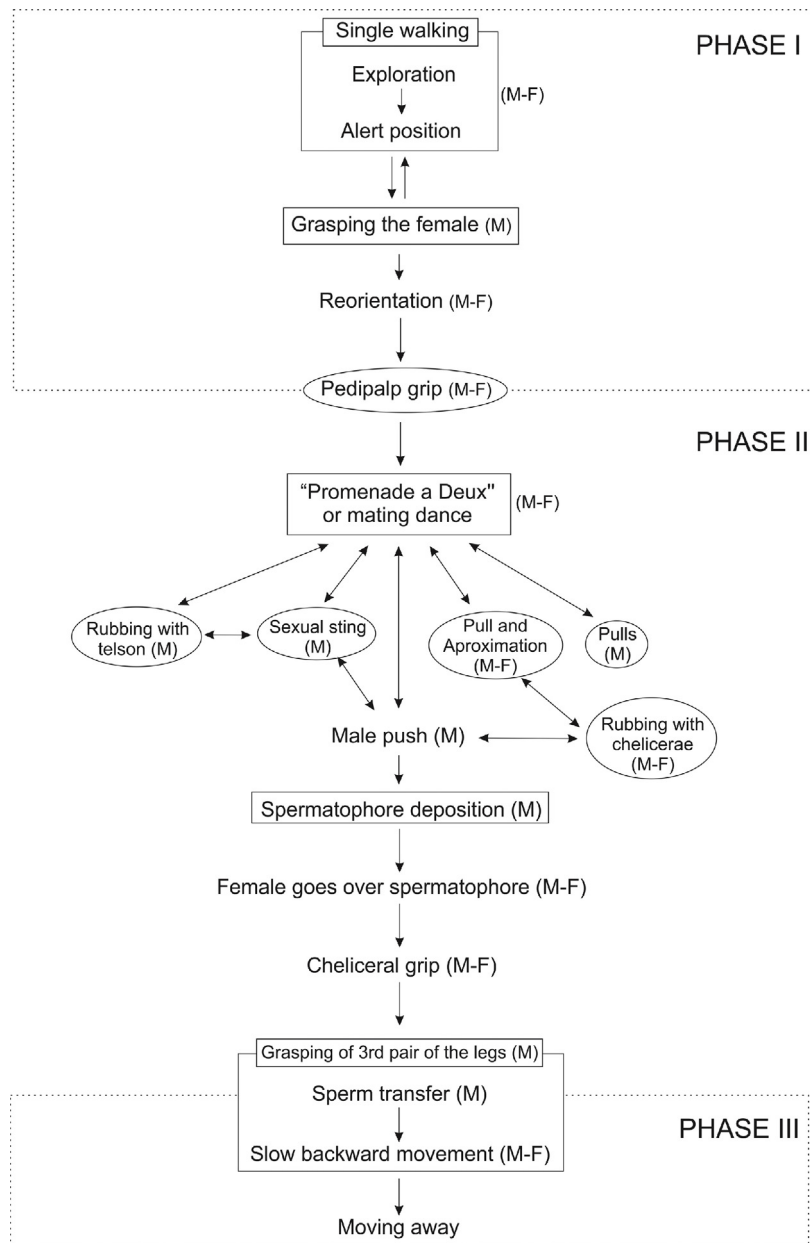


Fig. 4. Schematic diagram showing the main behaviors in a mating sequence of *Bothriurus bonariensis*, based on Peretti (1993). M, male, F, female.

tion' involves simpler movements for the male, pulling the female slightly to reach her mouth region. Then he moves away to repeat the sequence (Video S1). For males of lower quality it would be more efficient to perform less costly actions than for higher quality males (Számádó, 2011). Because of the differences in the environments of the two populations (Cabrera and Willink, 1973; Olivero et al., 2012, 2014), the individuals tolerate different stress conditions (Møller, 1995; Auffray et al., 1999; Olivero et al., 2012; 2014). Several studies support the idea that environmental stressors of sufficient intensity generate a reduction of developmental stability, which makes individuals less efficient in terms of growth, survival and reproduction (Graham et al., 2000; Santos et al., 2006; Vishalakshi and Singh, 2008a,b), even generating different levels of fluctuating asymmetry (Parsons, 1990; Møller, 1993). Individuals from the Argentinian population are smaller, with smaller structures, and present different types of asymmetry (directional asymmetry, fluctuating asymmetry or antisymmetry) compared to individuals from the Uruguayan population (Olivero et al., 2012,

2014). In addition, the caudal glands of the Uruguayan males are larger than the caudal glands of the Argentinian males. Therefore, they could be able to allocate more energy during courtship, including the production of more substances exuded by the caudal gland (Blaul and Ruther, 2012). Consequently, males from the Argentinian population could replace the 'rubbing with telson' behavior with a less costly behavior, as 'pull and approximation', which implies expending less energy to the same stimulatory intention (Blaul and Ruther, 2012; Olivero et al., 2015).

While in the present study we could observe matings between individuals of different populations, most inter-population matings were unsuccessful. In general, males from Uruguay tried to court females from Argentina, but the females' selectivity was very high and the matings were interrupted in different stages of courtship, before reaching sperm transfer. The high frequency of the behaviors 'grasping the female' and 'reorientation' observed in the inter-population mating trials could have occurred due to differences in the size of the pedipalp between individuals from the two pop-

ulations. The pedipalps are significantly larger and more robust in Uruguayan individuals. Consequently, Uruguayan males could not correctly hold on to Argentinian females, finally resulting in the separation of the couple.

We observed an intermediate duration of the behavior ‘rubbing with telson’ in inter-population matings. It is possible that Uruguayan males (they perform the behavior more frequently and with greater duration with Uruguayan females) decrease the duration of the behavior ‘rubbing with telson’ because it is unnecessary for acceptance by the Argentinian females. According to [Patricelli et al. \(2002\)](#) and [Peretti and Carrera \(2005\)](#), males can modify their sexual display, changing the frequency or duration of stimulatory behaviors in accordance with the response of the female. In this case, it is possible that males from the Uruguayan population modulate this behavior with females from Argentina, which would expect a different pattern of courtship. However, the results of the present study show that 93% of the mating trials between the two populations failed. Even though males from Uruguay tried to court females from Argentina, they did not reach the stage of sperm transfer with them. This could be caused by the differences in the preferences of females from different populations during courtship. Females are more selective than males in their choice of mates. They make a great reproductive effort and therefore should avoid unsuccessful matings ([Trivers, 1972](#); [Andersson, 1994](#)). Before this background, the differences in reproductive behavior and the low frequency of inter-population matings could indicate reproductive isolation between these populations. Also, these differences coincide with morphological differences in body size, pedipalp size and differences in the morphology and types of asymmetry of the spermatophore ([Olivero et al., 2012, 2014](#)).

Founder effects and divergent sexual selection in different populations are important reasons for geographic differences in courtship behavior ([Briceño et al., 2007](#)). Differences in morphology, behavior and genetic characters between allopatric populations have been observed in many taxa, including insects and arachnids ([Yamashita and Polis, 1995](#); [Holwell, 2008](#); [Jenning et al., 2011](#); [Olivero et al., 2012](#); [González et al., 2013](#); [Postiglioni, 2015](#); [Bollatti et al., 2017](#)). Particularly, it is known that the geographical barriers between Argentina and Uruguay cause divergence among other arachnid populations. In the wolf spider *Aglaoctenus lagotis*, populations from Central Argentina and southern Uruguay present a different sexual behavior, with different duration and frequencies of sexual units involved in courtship. In addition, the individuals from southern Uruguay are larger than the individuals from Central Argentina and have a different coloration ([González et al., 2013](#)). In the same way, differences in patterns of copulatory behaviors have been observed between Uruguayan and Argentinian individuals in the spider *Allocosa senex* ([Mello-Leitão 1945](#)). Individuals from Uruguay are bigger and heavier than individuals from Argentina ([Bollatti et al., 2017](#)).

In *B. bonariensis* the geographical barriers, together with the morphological evidence and the differences in mating behavior, could suggest the presence of particular characteristics in each population. Also, the limitations in moving large distances and the adaptation to particular environments is a common characteristic among scorpions ([Polis, 1990](#); [Prendini, 2001](#)). This may result in a divergent evolution of the groups and the generation of two different taxa, separating Central Argentinian and southern Uruguayan individuals. Also, the intermediate populations of the distribution have intermediate morphological characteristics, showing a gradient of size and coloration from east to west of the distribution range ([San Martín, 1962](#); [Olivero et al., 2012](#)). Recent studies indicate that different populations of *B. bonariensis* from Argentina (Tandil, Buenos Aires, Entre Ríos) share morphological characters and that these are notably different from the characters of northern and southern Uruguayan populations ([Olivero et al., 2012](#)). Individuals

from Brazil are larger and have a darker coloration than Uruguayan and Argentinian individuals ([Mattoni, 2003](#); [Ojanguren-Affilastro, 2005](#); [Olivero et al., 2012](#)). In previous studies, the occurrence of successful inter-population matings between individuals from Buenos Aires (Argentina) and Córdoba ([Peretti, 1993](#)) has been observed. However, there are no additional data about matings between other allopatric populations of the species (e.g., northern Uruguay or Brazil and Argentina populations).

Data of matings between *B. bonariensis* and a sister species, *B. chacoensis*, which have an allopatric distribution, showed that the courtship behavior between these sister species is very similar, even proceeding to sperm transfer ([Peretti, 1993](#)). However, we should consider that these mating trials, in the same way as the mating trials observed in the present study, were performed under laboratory conditions and would never occur in nature due to the allopatric distribution.

In conclusion, although there are differences in morphology and sexual behavior between the studied populations, we consider that it is not yet possible to confirm the identity of two groups. In general, there is no clear barrier between the populations to mark a specific limit in the distribution. Among the more distant populations, almost complete differentiation can be observed; however, with the available data intermediate populations cannot be classified with 100% certainty within one of these two groups. New studies of population genetics and more data of the mating behavior of the intermediate location populations could provide new data to test the existence of a cryptic species complex in a multidisciplinary and evolutionary framework.

Acknowledgments

We are grateful to Carlos Toscano-Gadea, David Vrech and Germán Robert for their help in collecting the scorpions in the field in Uruguay and Argentina. We thank also two anonymous reviewers and the editor for their constructive comments on the manuscript. Financial support was provided by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Fondo para la Investigación Científica y Tecnológica (FONCYT) and Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SECYT).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.zool.2017.06.009>.

References

- Andersson, M., 1994. *Sexual Selection*. Princeton University Press, Princeton.
- Auffray, J.-C., Renaud, S., Alibert, P., Nevo, E., 1999. Developmental stability and adaptive radiation in the *Spalax ehrenbergi* superspecies in the Near-East. *J. Evol. Biol.* 12, 207–221.
- Barlow, G.W., 2002. How behavioral studies contribute to the species problem: a piscine perspective. *Fish Fish.* 3, 197–212.
- Benton, T.G., 2001. Reproductive biology. In: Brownell, P., Polis, G.A. (Eds.), *Scorpion Biology and Research*. Oxford University Press, Oxford, pp. 278–301.
- Blaul, B., Ruther, J., 2012. Body size influences male pheromone signals but not the outcome of mating contests in *Nasonia vitripennis*. *Anim. Behav.* 84, 1557–1563.
- Bollatti, F., García Díaz, V., Peretti, A.V., Aisenberg, A., 2017. Geographical variation in sexual behavior and body traits in a sex role reversed wolf spider. *Naturwissenschaften* 104, 40.
- Briceño, D., Eberhard, W., Vilardi, J., Cayol, J.P., Shelly, T., 2007. Courtship behavior of different wild strains of *Ceratitis capitata* (Diptera: Tephritidae). *Fla. Entomol.* 90, 15–18.
- Bro-Jørgensen, J., 2010. Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends Ecol. Evol.* 25, 292–300.
- Cabrera, A.L., 1971. Fitogeografía de la República Argentina. *Bol. Soc. Arg. Bot.* XIV (1–2), 1–42.
- Cabrera, A.L., Willink, A.W., 1973. *Biogeografía de América Latina. Serie Biología, Monografía No. 13*. OEA, Washington.
- Carrera, P., Mattoni, C.I., Peretti, A.V., 2009. Chelicerae as male grasping organs in scorpions: sexual dimorphism and associated behaviour. *Zoology* 112, 332–350.

- Elias, D.O., Hebets, E.A., Hoy, R.R., 2006. Female preference for complex/novel signals in a spider. *Behav. Ecol.* 17, 765–771.
- Eraly, D., Hendrickx, F., Lensa, L., 2009. Condition-dependent mate choice and its implications for population differentiation in the wolf spider *Pirata piraticus*. *Behav. Ecol.* 20, 856–863.
- González, M., Peretti, A.V., Viera, C., Costa, F.G., 2013. Differences in sexual behavior of two distant populations of the funnel-web wolf spider *Aglaoctenus lagotis*. *J. Ethol.* 31, 175–184.
- González, M., Costa, F.G., Peretti, A.V., 2014. Strong phenological differences between two populations of a Neotropical funnel-web wolf spider. *J. Nat. Hist.* 48, 2183–2197.
- González, M., Peretti, A.V., Costa, F.G., 2015. Reproductive isolation between two populations of *Aglaoctenus lagotis*, a funnel-web wolf spider. *Biol. J. Linn. Soc.* 114, 646–658.
- Graham, J.H., Fletcher, D., Tigue, J., McDonald, M., 2000. Growth and developmental stability of *Drosophila melanogaster* in low frequency magnetic fields. *Bioelectromagnetics* 21, 465–472.
- Grela, I.A., 2004. Geografía florística de las especies arbóreas de Uruguay: propuesta para la delimitación de dentrofloras. Universidad de la República, Montevideo, Uruguay, Master Thesis.
- Hebets, E.A., Maddison, W.P., 2005. Xenophilic mating preferences among populations of the jumping spider *Habronattus pugillis* Griswold. *Behav. Ecol.* 16, 981–988.
- Hoikkala, A., Welbergen, P.H., 1995. Signals and responses of females and males in successful and unsuccessful courtships of three Hawaiian lek-mating *Drosophila* species. *Anim. Behav.* 50, 177–190.
- Holwitt, G.L., 2008. Geographic variation in genital morphology of *Ciulfina* praying mantids. *J. Zool.* 276, 108–114.
- Ishikawa, M., Mori, S., Nagata, Y., 2006. Intraspecific differences in patterns of courtship behaviors between the Pacific Ocean and Japan Sea forms of the three-spined stickleback *Gasterosteus aculeatus*. *J. Fish Biol.* 69, 938–944.
- Jennings, J.H., Mazzi, D., Ritchie, M.G., Hoikkala, A., 2011. Sexual and postmating reproductive isolation between allopatric *Drosophila montana* populations suggest speciation potential. *BMC Evol. Biol.* 11, 68.
- Kokko, H., Rankin, D.J., 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Phil. Trans. R. Soc. B* 361, 319–334.
- Kraaijeveld, K., Pomiankowski, A., 2004. Evolution: love thy neighbour. *Curr. Biol.* 14, R419–R421.
- Kuppennagel, S., Baur, B., 2011. Partial precopulatory isolation between two geographically distant populations of land snail *Arianta arbustorum* (L.). *J. Mollusc. Stud.* 77, 87–94.
- Lachlan, R.F., Servodio, M.R., 2004. Song learning accelerates allopatric speciation. *Evolution* 58, 2049–2063.
- Maan, M.E., Seehausen, O., 2011. Ecology, sexual selection and speciation. *Ecol. Lett.* 14, 591–602.
- Masta, S.E., Maddison, W.P., 2002. Sexual selection driving diversification in jumping spiders. *Proc. Natl. Acad. Sci. U. S. A.* 99, 4442–4447.
- Mattoni, C.I., 2003. Patrones evolutivos en el género *Bothriurus* (Scorpiones, Bothriuridae): análisis filogenético. Universidad Nacional de Córdoba, Córdoba, Argentina, Ph.D. Thesis.
- Mattoni, C.I., Acosta, L.E., 2005. A new *Bothriurus* from Brazil (Scorpiones, Bothriuridae). *J. Arachnol.* 33, 735–744.
- Mayr, E., 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.
- Miller, G.L., Stratton, G.E., Miller, P.R., Hebets, E., 1998. Geographical variation in male courtship behavior and sexual isolation in wolf spiders of the genus *Schizocosa*. *Anim. Behav.* 56, 937–951.
- Mobley, K.B., Jones, A.G., 2007. Geographical variation in the mating system of the dusky pipefish (*Syngnathus floridae*). *Mol. Ecol.* 16, 2596–2606.
- Møller, A.P., 1993. Morphology and sexual selection in the barn swallow *Hirundo rustica* in Chernobyl, Ukraine. *Proc. R. Soc. Lond. B* 252, 51–57.
- Møller, A.P., 1995. Patterns of fluctuating asymmetry in sexual ornaments of birds from marginal and central populations. *Am. Nat.* 145, 316–327.
- Ojanguren-Afflastró, A.A., 2005. Estudio monográfico de los escorpiones de la República Argentina. *Rev. Iber. Aracnol.* 11, 74–246.
- Olivero, P.A., Mattoni, C.I., Peretti, A.V., 2012. Morphometry and geographical variation of *Bothriurus bonariensis* (Koch 1842) (Scorpiones, Bothriuridae) along its distribution. *J. Arachnol.* 40, 113–122.
- Olivero, P.A., Vrech, D.E., Peretti, A.V., Mattoni, C.I., 2014. Patterns of asymmetry in body characters and genitalia in two distant populations of a Neotropical scorpion. *J. Nat. Hist.* 49, 853–872.
- Olivero, P.A., González, A., Mattoni, C.I., Peretti, A.V., 2015. Chemical caresses: geographical variation of male sexual signals in a Neotropical scorpion. *Behaviour* 152, 1745–1763.
- Parsons, P.A., 1990. Fluctuating asymmetry and stress intensity. *Trends Ecol. Evol.* 5, 97–98.
- Patricelli, G.L., Uy, J.A.C., Walsh, G., Borgia, G., 2002. Male displays adjusted to female's response. *Nature* 415, 279–280.
- Peretti, A.V., 1993. Estudio de la biología reproductiva en escorpiones argentinos (Arachnida, Scorpiones): un enfoque etológico. Universidad Nacional de Córdoba, Córdoba, Argentina, Ph.D. Thesis.
- Peretti, A.V., 1995. Análisis de la etapa inicial del cortejo de *Bothriurus bonariensis* (Scorpiones, Bothriuridae) y su relación con el reconocimiento sexual. *Rev. Arachnol.* 11, 35–45.
- Peretti, A.V., 1996. Una probable estrategia para inseminar más hembras en machos de *Bothriurus bonariensis* (Scorpiones, Bothriuridae). *J. Arachnol.* 24, 167–169.
- Peretti, A.V., 1997. Relación de las glándulas caudales de machos de escorpiones (Scorpiones, Bothriuridae) con el comportamiento sexual. *Rev. Arachnol.* 12, 31–41.
- Peretti, A.V., 2000. Existencia de cortejo en campo de machos de *Bothriurus bonariensis* (Scorpiones, Bothriuridae) que carecen de un órgano paraxial. *Rev. Soc. Entomol. Argent.* 59, 96–98.
- Peretti, A.V., 2003. Functional morphology of spermatophores and female genitalia in bothriurid scorpions: genital courtship, coercion and other possible mechanisms. *J. Zool.* 261, 135–153.
- Peretti, A.V., Acosta, L.E., 1999. Comparative analysis of mating in scorpions: the post-transfer stage in selected Argentinian bothriurids (Chelicerata, Scorpiones, Bothriuridae). *Zool. Anz.* 237, 259–265.
- Peretti, A.V., Battán-Horenstein, M., 2003. Comparative analysis of the male reproductive system in Bothriuridae scorpions: structures associated with the paraxial organs and the presence of sperm packages (Chelicerata, Scorpiones). *Zool. Anz.* 242, 21–31.
- Peretti, A.V., Carrera, P.C., 2005. Female control of mating sequences in the mountain scorpion *Zabius fuscus*: males do not use coercion as a response to unreceptive females. *Anim. Behav.* 69, 453–462.
- Peretti, A.V., Acosta, L.E., Martínez, M.A., 2000. Comportamiento de apareamiento en tres especies de *Bothriurus* del grupo *prospicius*: estudio comparado y su relación con *Bothriurus flavidus* (Scorpiones, Bothriuridae). *Revue Arachnol.* 13, 73–91.
- Peretti, A.V., Depiante, L., Battán-Horenstein, M., 2001. Allometry and asymmetry of body characters and spermatophores in *Bothriurus bonariensis* (C. L. Koch) (Scorpiones, Bothriuridae). In: Fet, V., Selden, P.A. (Eds.), *Scorpions 2001*. In Memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks, UK, pp. 345–355.
- Persons, M.H., Walker, S.E., Rypstra, A.L., Marshall, S.D., 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Aranae: Lycosidae). *Anim. Behav.* 61, 43–51.
- Polis, G.A., 1990. Ecology. In: Polis, G.A. (Ed.), *The Biology of Scorpions*. Stanford University Press, Stanford, pp. 247–293.
- Polis, G.A., Sissom, W.D., 1990. Life history. In: Polis, G.A. (Ed.), *The Biology of Scorpions*. Stanford University Press, Stanford, pp. 161–223.
- Postiglioni, R., 2015. Estructuración genética y variación morfológica en ambientes fluviales y oceánico-estuarinos en la araña *Allocausa brasiliensis* (Lycosidae) del sur de Uruguay. Universidad de la República, Montevideo, Uruguay, PhD Thesis.
- Prendini, L., 2001. Substratum specialization and speciation in southern African scorpions: the effect hypothesis revisited. In: Fet, V., Selden, P.A. (Eds.), *Scorpions 2001*. In Memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks, UK, pp. 113–138.
- Prohl, H., Koshy, R.A., Mueller, U., Rand, A.S., Ryan, M.J., 2006. Geographical variation of genetic and behavioral traits in northern and southern tungara frogs. *Evolution* 60, 1669–1679.
- Ryan, M.J., Coccoft, R.B., Wilczynski, W., 1990. The role of environmental selection in intraspecific divergence of mate recognition signals in the cricket frog, *Acris crepitans*. *Evolution* 44, 1869–1872.
- Saarikettu, M., Liimatainen, J.O., Hoikkala, A., 2005. Intraspecific variation in mating behavior does not cause sexual isolation between *Drosophila virilis* strains. *Anim. Behav.* 70, 417–426.
- San Martín, P.R., 1962. Diferencias cromáticas en *Bothriurus bonariensis* Koch, 1842. *Bol. Soc. Taguatú* 1, 97–104.
- Santos, M., Brites, D., Laayouni, H., 2006. Thermal evolution of pre-adult life history traits, geometric size and developmental stability in *Drosophila subobscura*. *J. Evol. Biol.* 19, 2006–2021.
- Schluter, D., 2001. Ecology and the origin of species. *Trends Ecol. Evol.* 16, 372–380.
- Sobel, J.M., Chen, G.F., Watt, L.R., Schemske, D.W., 2010. The biology of speciation. *Evolution* 64, 295–315.
- Számádó, S., 2011. The cost of honesty and the fallacy of the handicap principle. *Anim. Behav.* 81, 3–10.
- Toscano-Gadea, C.A., 2010. Sexual behavior of *Bothriurus buecherli* (Scorpiones: Bothriuridae) and comparison with the *B. prospicius* group. *J. Arachnol.* 38, 360–363.
- Trivers, R.L., 1972. Parental investment and sexual selection. In: Campbell, B. (Ed.), *Sexual Selection and the Descent of Man*. Aldine Publishing Co., Chicago, pp. 52–95.
- Vishalakshi, C., Singh, B.N., 2008a. Effect of environmental stress on fluctuating asymmetry in certain morphological traits in *Drosophila ananassae*: nutrition and larval crowding. *Can. J. Zool.* 86, 427–437.
- Vishalakshi, C., Singh, B.N., 2008b. Effect of developmental temperature stress on fluctuating asymmetry in certain morphological traits in *Drosophila ananassae*. *J. Therm. Biol.* 33, 201–208.
- Vrech, D.E., Olivero, P.A., Mattoni, C.I., Peretti, A.V., 2014. Testes mass, but not sperm length, increases with higher levels of polyandry in an ancient sex model. *PLoS One* 9, e94135.
- Wilkins, M.R., 2012. Evolutionary divergence in acoustic signals: causes and consequences. *Trends Ecol. Evol.* 28, 156–166.
- Yamashita, T., Polis, G.A., 1995. Geographical analysis of scorpion populations on habitat islands. *Heredity* 75, 495–505.
- Zann, R., 1976. Inter- and intraspecific variation in the courtship of three species of grassfinches of the subgenus *Poephila* (Gould) (Estrildidae). *Z. Tierpsychol.* 41, 409–433.