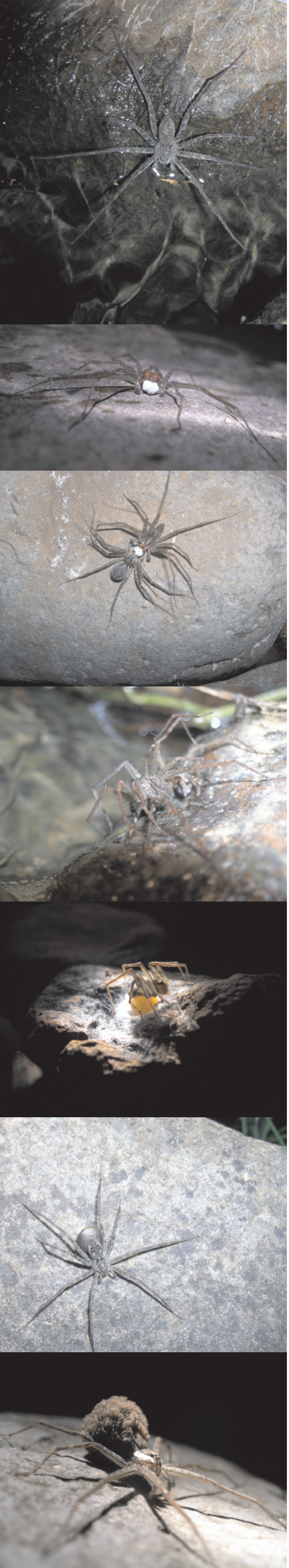

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# Comportamento sexual e morfometria geométrica em caracteres somáticos e sexuais em *Paratrechalea* (Araneae; Trechaleidae): evidências para a proposta de um novo táxon.

Tese de Doutorado

**Luiz Ernesto Costa Schmidt**

Porto Alegre

Maio de 2008

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Tese submetida ao Programa de Pós-Graduação em Genética
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**Orientador: Prof. Dr. Aldo Mellender de Araújo**

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# Sumário

# Resumo

O estudo de comportamentos inusitados observados em populações naturais de qualquer organismo exige que suas bases descritivas sejam primeiramente estabelecidas antes de permitir a aplicação de abordagens experimentais. A Ordem Araneae é reconhecida pela enorme diversidade de formas e hábitos, onde destaco as aranhas do gênero *Paratrechalea* Carico, 2005 tanto pela presença de um comportamento atípico quanto por uma particularidade ecológica entre algumas de suas espécies. Estudos preliminares relativos à distribuição das espécies do gênero indicaram uma região de distribuição sintópica entre duas delas: *P. azul* Carico, 2005 e *P. ornata* (Mello-Leitão, 1943). Além disso, durante o comportamento sexual destas espécies, os machos oferecem presentes nupciais na forma de uma presa envolta em seda, sendo o primeiro registro deste comportamento para a fauna de aranhas Neotropicais. Para as descrições apresentadas na tese, foram utilizadas combinações de amostras que compõem o conjunto de dados total: uma amostra de *P. azul* e outra de *P. ornata* em condição de sintopia provenientes do Brasil, uma amostra Brasileira de *P. galianoae* Carico, 2005, e duas amostras Uruguaias de *P. ornata*. A análise descritiva do comportamento sexual mostrou que presentes nupciais não apenas fazem parte do cortejo sexual destas espécies, mas determinam o sucesso reprodutivo dos machos. A forma das quelíceras de machos e de fêmeas é afetada pelo cortejo sexual, onde machos as utilizam para sinalização intersexual e fêmeas apresentam padrões alométricos indicando cooperação durante a cópula. Análises morfométricas, envolvendo técnicas bidimensionais e de morfometria geométrica, foram aplicadas com o intuito de descrever aspectos básicos sobre as populações estudadas, como o dimorfismo sexual existente e a variabilidade morfológica das genitálias. O dimorfismo sexual foi mais evidente para as populações sintópicas, e os caracteres somáticos avaliados separam *P. ornata* em dois grupos: população do Brasil e populações do Uruguai. Para as genitálias, a divisão de *P. ornata* em dois grupos foi mantida, e a partir disso assume-se que estes grupos constituem duas espécies. Padrões observados para alometria de genitália foram melhores explicados por hipótese de seleção sexual do que por hipótese de conflito sexual. Como conclusão geral é apresentada uma proposta para a evolução da divisão de *P. ornata* em duas espécies, tendo como elemento-chave o mecanismo de deslocamento de caracteres atuando sobre as populações das espécies sintópicas.

# Abstract

The study of unusual behaviors found in natural populations of any organism demands a strong descriptive ground before an experimental applications approach can be applied. The Order Araneae is one of the most diverse animal groups, both in terms of morphology and behavior. Here I highlight the genus *Paratrechalea* Carico, 2005 for two reasons: the occurrence of an unusual behavior and some ecological particularities shared by some of its species. Preliminary studies related to the geographical distribution of some species of the genus revealed a syntopic geographical region between two of them: *P. azul* Carico, 2005 and *P. ornata* (Mello-Leitão, 1943). Moreover, during courtship, males of these species offer a prey wrapped in silk as a nuptial gift, being the first record of such behavior for the Neotropical spider fauna. For the description presented along the thesis, it was used a combination of samples that compose the data set: a syntopic sample of *P. azul* and *P. ornata* from Brazil, a Brazilian sample of *P. galianoae* Carico, 2005, and two Uruguayan samples of *P. ornata*. Descriptive analysis of the sexual behavior of these species showed that nuptial gifts not only makes part of the courtship process, but also determine male's reproductive success. Male and female chelicerae morphology is affected by the courtship process, where males use them as a signaling trait, and females showed allometric patterns for this structure linked to cooperation during copulation. Traditional and geometric morphometrics analyses were applied in order to investigate basic population aspects of these species, like sexual dimorphism and genital variability. Sexual dimorphism was stronger within the syntopic populations, and the analyzed somatic structures indicate that *P. ornata* can be split in two geographically related groups: a Brazilian group and an Uruguayan group. The analysis of genitalia also held the same *P. ornata* split, and given the use of such structures for species recognition among arthropods, we assume that these are two different species. Patterns of genitalic allometry observed were better explained using sexual selection hypothesis than using sexual conflict hypothesis. It is presented a hypothesis for the *P. ornata* evolutionary split, taking character displacement as the main mechanism acting over the syntopic species.

# Capítulo 1

# Introdução Geral

A Ordem Araneae é um dos grupos animais classificados como megadiverso no planeta, apresentando hábitos peculiares, moldados a partir de um padrão comportamental e fisiológico predatório terrestre (Wise 1993; Foelix 1996; Gonzaga *et al.* 2007). Atualmente é representada por 39.882 espécies distribuídas em 108 famílias ao redor do mundo (Platnick 2007). Dentre esta enorme diversidade, é de se esperar que ocorram exemplos com hábitos diferenciados. Para a fauna de aranhas do Rio Grande do Sul destaco as aranhas de hábito semi-aqüático da família Trechaleidae.

A escolha deste grupo, como será apresentado mais adiante, não se deu apenas por esta característica semi-aqüática, mas também por uma situação peculiar de duas espécies desta família, com uma relação filogenética bastante próxima e de distribuição sintópica na região nordeste do Rio Grande do Sul. Não obstante, estas espécies possuem uma outra característica que as destaca das demais espécies de aranhas, que é o oferecimento de um presente nupcial durante o cortejo sexual (Costa-Schmidt *et al.* 2005a).

As dificuldades em trabalhar com tal grupo são inúmeras, mas principalmente pelo total desconhecimento de aspectos elementares da história natural destas espécies. Elucidar alguns destes aspectos elementares é o principal tema desta tese, avaliando questões comportamentais e morfológicas que mostraram serem pertinentes ao conjunto amostral disponível.

Segue agora a apresentação das protagonistas desta história, bem como os temas escolhidos para dar início a esta descrição.

## 1.1. As aranhas da família Trechaleidae Simon 1890

A família Trechaleidae apresenta restrições biogeográficas marcantes: composta por 78 espécies distribuídas em 17 gêneros, apenas uma única espécie é descrita para o Japão (*Shinobius orientalis*, gênero monoespecífico) (Platnick 2007). Todas as demais espécies têm distribuição restrita ao Neotrópico, onde o Brasil abriga 35 espécies, sendo 25 delas registradas com exclusividade para este país (Platnick 2007). Este panorama de distribuição das espécies tende a ser modificado, à medida que novas amostragens vão sendo realizadas em pontos ainda não explorados do território Brasileiro, e ao mesmo tempo em que novas técnicas de análise vão sendo implementadas auxiliando na identificação de novas espécies (Capítulo 5 desta tese).

Atualmente o Rio Grande do Sul abriga oito espécies de Trechaleidae: *Trechalea bucculenta, Trechaleoides biocellata*, *T. keyserlingi*, *Paradossenus longipes, Paratrechalea azul, P. galianoae, P. longigaster* e *P. ornata*, de acordo com o material disponível na coleção do Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul (MCN – FZB/RS), determinado a partir dos trabalhos de descrição destas espécies (Carico 1993, 2005; Silva *et al.* 2006).

### 1.1.1. As aranhas do gênero *Paratrechalea* Carico, 2005

Este gênero é atualmente composto por sete espécies (Carico 2005; Silva *et al.* 2006; Platnick 2007), todas elas com distribuição restrita à porção austral da região Neotropical (Fig. 1). Quatro delas são encontradas no Rio Grande do Sul: *Paratrechalea azul*, *P. galianoae*, *P. longigaster* e *P. ornata* (Carico 2005; Silva *et al.* 2006). Muito do que se conhece sobre a história natural destas espécies provém de observações em campo ainda não publicadas, resumos de congressos (Costa-Schmidt *et al.* 2005a, 2005b), além do material contido no corpo desta tese.

Estas aranhas apresentam hábito semi-aqüático e noturno, restritas a corpos d’água lóticos, e se distribuem principalmente na Bacia Hidrográfica do Rio La Plata (Fig. 1). Vivem associadas à vegetação ripária destes sistemas hídricos quando jovens, e durante a fase adulta são encontradas principalmente entre os seixos rochosos que afloram na superfície da água (Fig. 2). Uma característica peculiar neste gênero é a presença de um cortejo sexual elaborado, onde o macho oferece à fêmea uma presa envolta em seda como forma de presente nupcial (Fig. 2). Se este é um fato relativamente comum em insetos (Vahed 1998, 2007), em aranhas, no entanto, ele é muito raro, sendo o primeiro caso descrito para espécies da fauna de aranhas Neotropical (Costa-Schmidt *et al.* 2005a; Capítulo 2 desta tese).

Fig. 1. Área de distribuição predominante do gênero *Paratrechalea*, coincidente com a área de distribuição da Bacia Hidrográfica do Rio La Plata (em verde no mapa). A cobertura total da bacia atinge os países: Brasil, Argentina, Paraguai e Uruguai. Mapa modificado de < http://www.icsu-scope.org >.

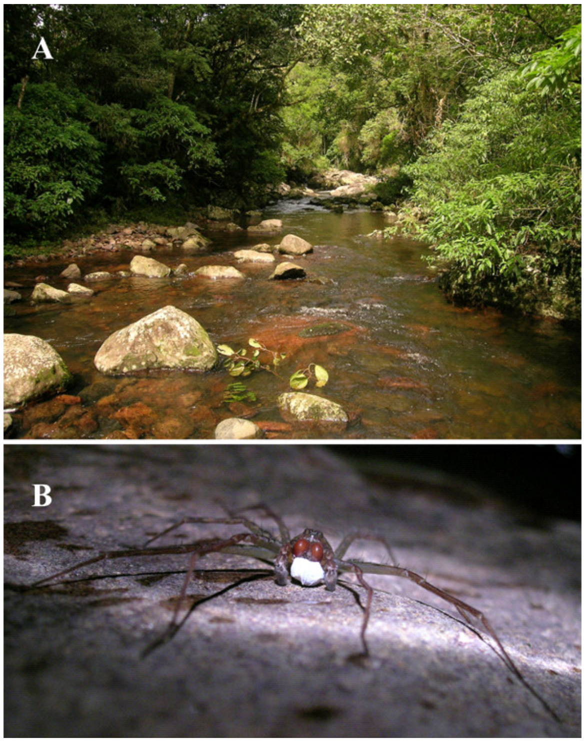

Fig. 2. A) Hábitat típico de ocorrência das espécies do gênero *Paratrechalea* na região nordeste do Estado do Rio Grande do Sul (encosta da Serra Geral, Maquiné, RS). B) Macho de *Paratrechalea azul* carregando em suas quelíceras um presente nupcial.

### 1.1.2. *Paratrechalea azul* Carico, 2005 e *Paratrechalea ornata* (Mello-Leitão, 1943)

Entre as espécies de *Paratrechalea* que ocorrem no Rio Grande do Sul, *P. azul* e *P. ornata* merecem destaque, tanto pelo elevado grau de cripticidade morfológica, onde apenas a análise detalhada de suas genitálias permite uma identificação precisa destas espécies (Fig. 3), como sua ocorrência sintópica em parte de sua distribuição geográfica conhecida (Fig. 4), mais especificamente na encosta da Serra Geral do Rio Grande do Sul (Costa-Schmidt & Silveira, em preparação; Carico 2005; Silva *et al.* 2006). A faixa de simpatria no entanto é mais ampla, ocorrendo entre a região nordeste do Rio Grande do Sul e o Paraná (Fig. 4). Ao sul da região simpátrica são encontradas populações alopátricas de *P. ornata*, sendo que *P. azul* é encontrada em alopatria somente ao norte desta faixa.

Estudos relacionados ao comportamento reprodutivo mostraram uma segregação completa entre indivíduos provenientes de populações sintópicas destas espécies durante o cortejo sexual (Costa-Schmidt *et al.* 2005b), ou seja, existem mecanismos etológicos de isolamento reprodutivo muito eficientes.

## 1.2. Temas escolhidos para serem descritos nesta tese

### 1.2.1. Cortejo sexual em aranhas e presentes nupciais

Cortejo sexual em aranhas há muito vem sendo estudado, principalmente pelo seu caráter de reconhecimento interespecífico (Krafft 1982; Robinson 1982; Costa & Capocasale 1984; Foelix 1996; Costa *et al.* 1997; Jackson & Pollard 1997; Miller *et al.* 1998; Fernandez-Montraveta *et al.* 2001; Simó *et al.* 2002; Peretti *et al.* 2006). A evolução do cortejo sexual está relacionada com a ação da seleção sexual, e pode inclusive servir como um mecanismo efetivo de isolamento reprodutivo (Templeton 1989; Jocqué 2002).

De acordo com Foelix (1996: 176), “as aranhas desenvolveram um comportamento de cortejo sexual especial, que geralmente precede o acasalamento (…) este cortejo é espécie específico, e garante que a hibridação seja evitada”. Este mesmo autor ainda sustenta que mesmo havendo mecanismos mecânicos de isolamento reprodutivo entre espécies de aranhas, “(…) diferenças comportamentais são aparentemente mais importantes para o isolamento”.

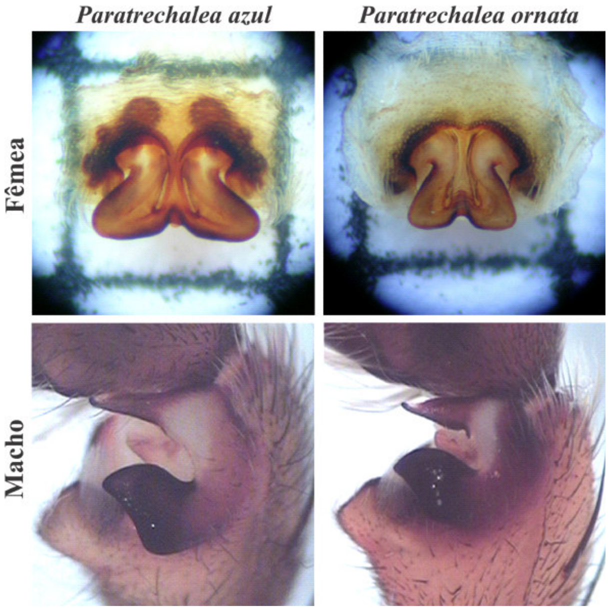

Fig. 3. Genitálias externas (epígenos) de fêmeas e apófises tibiais retrolaterais de machos de *Paratrechalea azul* e *P. ornata*.

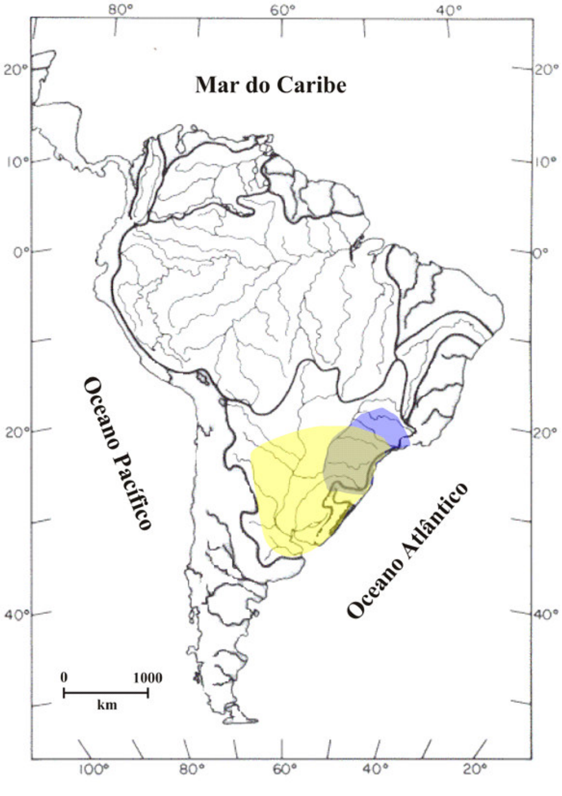

Fig. 4. Distribuição geográfica aproximada conhecida para as espécies *Paratrechalea azul* (área em azul) e *P. ornata* (área em amarelo). Dados das distribuições das espécies compilados de: Carico (2005), Costa-Schmidt & Silveira (em preparação), Silva *et al.* (2006). Mapa modificado de <http://www.icsu-scope.org>.

O oferecimento de um presente nupcial é um mecanismo taxonomicamente difundido (Stålhandske 2001), sendo encontrado tanto em aves quanto em várias ordens de artrópodes, principalmente insetos. Presentes nupciais compreendem qualquer forma de transferência de nutrientes do macho para a fêmea, durante ou imediatamente após o cortejo sexual e/ou cópula (Vahed 1998). Eles podem ser oferecidos sob várias maneiras: presas capturadas pelo macho, espermatóforos, secreções específicas produzidas por glândulas especializadas, regurgitos, bem como o seu próprio corpo (Vahed 1998; Sakaluk 2000).

Uma forma de presente nupcial peculiar entre as aranhas ocorre de maneira isolada dentro das propostas filogenéticas atuais para a Ordem Araneae (Coddington & Levi 1991; Griswold 1993; Silva 2003; Santos 2007), mais especificamente no Clado Lycosoidea (Fig. 5). Este tipo especial de presente nupcial tem descrição na literatura apenas para poucas espécies da família Pisauridae (*Pisaura mirabilis* (Clerck 1757): Van Hasselt 1884, Bristowe 1958; *Pisaura lama* Bösenberg & Strand 1906: Itakura 1993; *Perenethis fascigera* (Bösenberg & Strand, 1906): Itakura 1998), e consiste no oferecimento de uma presa capturada especificamente para esta finalidade. Também pertencente ao Clado Lycosoidea está a família Trechaleidae, à qual *Paratrechalea azul* e *P. ornata* fazem parte, e para estas espécies foi confirmado o registro de oferecimento do mesmo tipo de presente nupcial (Fig. 2).

Justamente devido ao ineditismo deste comportamento para as espécies de aranhas Neotropicais, a descrição das etapas do cortejo sexual de *P. azul* e *P. ornata* foi escolhida como o primeiro aspecto a ser abordado nesta tese (Capítulo 2).

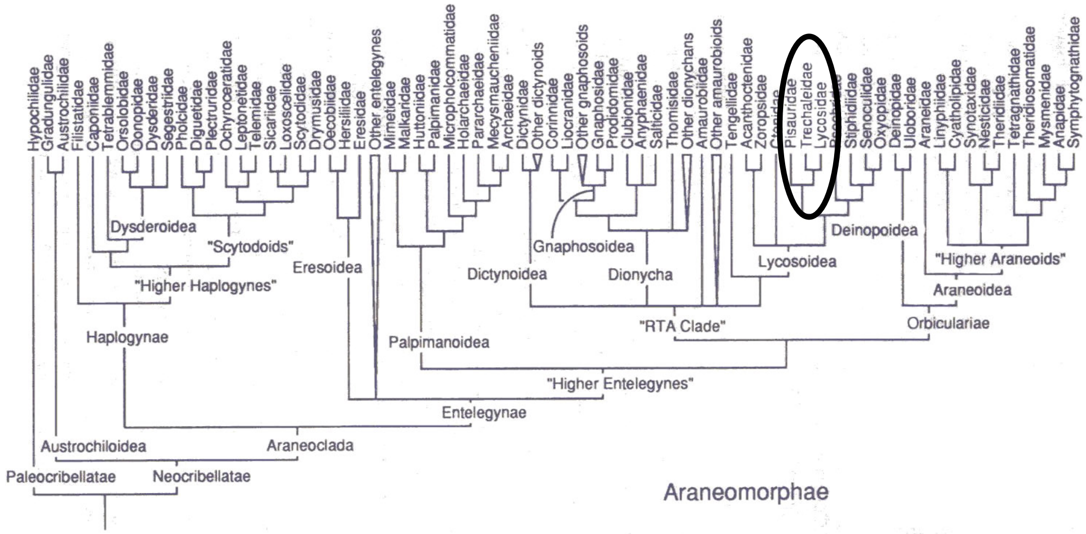

Fig. 5. Cladograma representativo da filogenia das famílias da Sub-Ordem Araneomorphae, com especial destaque ao Clado Lycosoidea, à qual pertence a família Trechaleidae. Modificado de Coddington & Levi (1991).

### 1.2.2. Deslocamento de caracteres

Uma propriedade muito importante da biodiversidade é a complexidade de interações entre os elementos que a constituem (Thompson 1994). Dentre todas as interações possíveis, a competição sempre teve um importante papel em modelos teóricos e no delineamento de experimentos do ponto de vista ecológico, genético e evolutivo (Darwin 1859; Brown & Wilson 1956; Schluter 2000; Begon *et al.* 2006). Modelos recentes têm demonstrado que a competição pode levar ao aumento da diversidade, através de mecanismos que tem como conseqüência o desencadeamento de um processo de especiação (Rice & Pfennig 2007), com casos extremos de radiação adaptativa (Schluter 2000). Para tanto, espécies concorrentes devem ser capazes de criar estratégias que atenuem a pressão competitiva exercida pela interação. Esta relação pode ser interpretada então como um processo co-evolutivo, onde duas entidades filogenéticas distintas têm suas rotas evolutivas restringidas pela presença de uma em relação à outra.

A partir do conhecimento da condição sintópica das populações de *P. azul* e *P. ornata* na região nordeste do Rio Grande do Sul, é possível que o *deslocamento de caracteres* seja este mecanismo de atenuação competitiva para estas populações, tendo como referência os trabalhos de Brown & Wilson (1956), Rueffler *et al.* (2006) e Rice & Pfennig (2007). O termo deslocamento de caracteres foi originalmente descrito por Brown & Wilson (1956) como sendo *"(...) a seldom-recognized and poorly known speciation phenomenon (...)"*, ou seja, desde sua proposta já apresentava uma propriedade de aumento de diversidade. A definição original deste mecanismo é muito extensa para ser transcrita, mas é bem sintetizada em Kawano (2003) e de Adams & Rohlf (2000), respectivamente:

> *"(...) character displacement (...) is recognized when the morphological difference between two interacting species in sympatry is greater than that between the species in allopatry (...)."*
>
> *"Ecological character displacement describes a pattern where morphological differences between sympatric species are enhanced through interspecific competition."*

A maneira pela qual este mecanismo atua nas populações envolvidas é apresentada a seguir. Caso a competição sobre um recurso seja mais intensa entre os indivíduos com fenótipos médios dos grupos concorrentes, indivíduos extremos poderão apresentar uma aptidão maior, principalmente se a intensidade competitiva for dependente da freqüência de recursos disponíveis (Schluter 2000; Rueffler *et al.* 2006). Isso pode acarretar em uma força de seleção disruptiva, que, se mantida no sistema por um certo período, irá gerar divergência entre os grupos competidores (Rueffler *et al.* 2006). Esta divergência poderá ocorrer em qualquer caractere relacionado à dimensão de nicho compartilhada pelos competidores. Se a divergência ocorre em caracteres taxonomicamente importantes, uma nova entidade pode ser proposta em relação ao grupo de origem (Brown & Wilson 1956; Rice & Pfennig 2007), muito embora a divergência em si não defina integralmente uma nova espécie (Mayr 1942). Fica claro, assim, que além do ponto de vista teórico existem questões filosóficas importantes acerca do que é considerado como uma espécie (Bock 2004).

A ocorrência de deslocamento de caracteres nas populações sintópicas de *Paratrechalea* foi avaliada nos seguintes aspectos da expressão fenotípica de caracteres somáticos e genitais: dimorfismo sexual de tamanho e forma (Capítulo 4) e variação interespecífica e intraespecífica de genitálias (Capítulo 5). Para tanto foram utilizadas técnicas de morfometria geométrica para marcos anatômicos já estabelecidas na literatura (Rohlf & Slice 1990; Marcus *et al.* 1996; Adams & Funk 1997; Alibert *et al.* 2001; Bond *et al.* 2001; Bond & Sierwald 2002; Ohno *et al.* 2003; Zelditch *et al.* 2004; Mutanen 2005; entre outros).

### *Dimorfismo sexual*

Aranhas são amplamente reconhecidas pelos casos extremos de dimorfismo sexual de tamanho observados (Vollrath & Parker 1992; Fairbairn 1997; Hormiga *et al.* 2000), principalmente para aranhas orbiculares. Não há apenas uma única força evolutiva determinante deste padrão, e sim um conjunto de fatores que abrangem desde a seleção para uma maior fecundidade de fêmeas (Head 1995; Prenter *et al.* 1999; Hormiga *et al.* 2000), bem como seleção sexual e relações de custo-benefício presentes na história de vida das espécies (Elgar 1991; Vollrath & Parker 1992).

Aranhas cursoriais, i.e., que não utilizam estruturas como as teias para a captura de suas presas, apresentam níveis de dimorfismo sexual pouco evidentes (Vollrath & Parker 1992; Head 1995; Gasnier *et al.* 2002), principalmente quando comparados aos padrões observados ao já citado grupo das aranhas orbiculares. Mesmo assim, a análise dos padrões de dimorfismo neste grupo é fundamental para o entendimento de suas relações intersexuais.

O gênero *Paratrechalea* apresenta um padrão esperado de baixo dimorfismo sexual de tamanho, muito embora análises morfométricas tradicionais tenham apontado diferenças robustas (Costa-Schmidt *et al.* 2005b). A inclusão da análise do dimorfismo sexual de forma nestas populações pretende preencher uma lacuna sobre as informações existentes não apenas para estas populações, mas para apresentar a metodologia morfométrica empregada em estudos aracnológicos.

### *Variação interespecífica e intraespecífica de genitálias*

Como para a maioria dos artrópodes, grande parte da identificação de espécies de aranhas é baseada na morfologia de genitália (Eberhard *et al.* 1998; Huber 2003, 2004). Isto ocorre pelo simples fato de que genitálias apresentam um padrão de evolução morfológica marcada por uma taxa evolutiva mais rápida e divergente do que caracteres somáticos (Eberhard 1985, 1996, 2004; entre outros).

Os caracteres diagnósticos propostos por Carico (2005) e Silva *et al.* (2006) para a identificação das espécies de *Paratrechalea* mostraram uma variabilidade intrapopulacional não esperada para algumas populações amostradas. Assim, foi avaliada quantitativamente esta variação, utilizando não apenas as populações sintópicas de *P. azul* e *P. ornata*, mas também amostras de outras duas populações alopátricas de *P. ornata* (provenientes do Uruguai), e mais uma outra população de *P. galianoae* proveniente da região nordeste do Rio Grande do Sul.

### 1.2.3. Alometria

Alometria é uma propriedade intrínseca do todo o qualquer organismo, e pode ser definida pela relação de desenvolvimento de diferentes estruturas anatômicas em relação ao tamanho corporal geral. Alometria pode ser dividida em pelo menos três níveis (Klingenberg 1996): alometria ontogenética, que diz respeito a covariação observada nas dimensões de uma estrutura ao longo do desenvolvimento do organismo; alometria evolutiva, trata da covariação das relações diferenciais de tamanho entre estruturas homólogas de diferentes espécies; alometria estática, relacionada à variação populacional de uma estrutura em uma determinada etapa do ciclo de vida.

A análise do padrão alométrico foi amplamente utilizada em ecologia evolutiva para explicar e descrever a presença de forças seletivas atuantes sobre caracteres sexuais secundários (Maynard-Smith & Harper 2003) e genitálias (Eberhard *et al.* 1998). Recentemente, dados empíricos e modelos teóricos mais completos, que incluem parâmetros relacionados à história de vida das espécies estudadas, demonstraram que estas interpretações não são tão intuitivas como anteriormente sustentadas (Bonduriansky & Day 2003; Bonduriansky 2007; Bertin & Fairnbairn 2007). Mesmo assim, as técnicas tradicionalmente utilizadas em estudos sobre alometria continuam válidas, apenas a interpretação de seus resultados demanda uma avaliação mais integrada.

Tendo em vista estas possibilidades de inferência, o último conjunto de dados tratados nesta tese diz respeito aos padrões alométricos observados para duas estruturas funcionalmente distintas, mas potencialmente selecionadas sexualmente.

Uma delas são as quelíceras de machos e fêmeas de *P. azul*, *P. ornata* e *P. galianoae* (Capítulo 3). A fundamentação de tal análise diz respeito ao uso desta estrutura por machos e fêmeas durante a cópula desta aranhas (descrita no Capítulo 2). Assume-se uma condição sinalizadora para esta estrutura, e que teoricamente deve apresentar padrões de alometria positiva caso a seleção sexual seja o principal mecanismo de evolução da forma desta estrutura.

Finalizando o conjunto de dados da tese, é apresentada uma abordagem preliminar para a análise dos padrões alométricos das genitálias de machos e de fêmeas de *P. azul* e *P. ornata* (Capítulo 6). Sobre alometria de genitálias existem pelo menos três teorias concorrentes, e nem sempre mutuamente excludentes: a teoria de chave-fechadura, a teoria de conflito sexual, e a teoria de seleção críptica da fêmea (Eberhard 1985, 1996, 2004). Todas elas tentam explicar os padrões divergentes de genitálias e sua taxa evolutiva acelerada. Estes dados visam complementar àqueles apresentados no Capítulo 5.

## Objetivo Geral

Definir os critérios biológicos necessários para o reconhecimento das duas espécies sintópicas do gênero *Paratrechalea*, avaliando aspectos comportamentais e morfológicos, bem a como a integração dos mesmos.

## Objetivos específicos

1. Descrever o comportamento sexual de duas espécies sintópicas do gênero *Paratrechalea*, na busca de padrões qualitativos de segregação interespecífica (Capítulo 2);
2. Apresentar uma hipótese para a evolução do dimorfismo sexual nas quelíceras destas aranhas e sua relação com o oferecimento do presente nupcial (Capítulo 3);
3. Descrever a magnitude do dimorfismo sexual nestas espécies, através da avaliação do grau de variabilidade morfológica de tamanho e forma de estruturas somáticas (Capítulo 4);
4. Verificar o grau de variabilidade de caracteres genitais de machos e fêmeas em amostras interespecíficas e intraespecíficas (Capítulo 5);
5. Descrever as relações alométricas das genitálias de machos e de fêmeas destas espécies, com uma discussão preliminar para os padrões observados (Capítulo 6).

# Capítulo 2

# Nuptial gifts and sexual behaviour in two species of spider (Araneae, Trechaleidae, *Paratrechalea*)

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## Abstract

Male delivering of a prey packed in silk as a nuptial gift is rare in spiders and restricted until now to Pisauridae. Here we describe this behavioural pattern found in two Trechaleidae species, *Paratrechalea azul* Carico, 2005, and *Paratrechalea ornata* (Mello-Leitão, 1943), mainly based on field observations. We observed the following steps of sexual behaviour: sperm induction, nuptial gift construction, mate searching, pre-copulatory courtship, copulation, and copulatory ending. In this group, a nuptial gift consists of a prey wrapped in silk, which appears as a white rounded shape. The male carries his nuptial gift in his chelicerae while searching for a female. When he finds a female, he shows a stereotyped courting behaviour consisting of a hyperflexed posture that is also assumed by the receptive female while they face each other. The copulatory position and pattern is similar to that found in other Lycosoidea: the male mounts the female and makes a total of four palpal insertions while alternating sides. However, after each palpal insertion the male dismounts and returns to a frontal position while biting the gift. Copulatory courtship is evidenced by palpal and leg movements. The copulation ends by female initiative and she almost always retains the nuptial gift. No case of pre-copulatory or post-copulatory cannibalism has been recorded. The occurrence of male behavioural adaptations to nuptial gift construction and copulation, as well as hypotheses about the origin of nuptial gift construction among spiders are presented. These descriptions are the first records of such nuptial gift offering for Neotropical spiders and to non-Pisauridae species as well.

**Key words:** Nuptial gift, sexual behaviour, *Paratrechalea ornata*, *Paratrechalea azul*, Trechaleidae

**Short title:** Nuptial gifts in *Paratrechalea* spiders.

## Introduction

Spider sexual behaviour has been a subject of study for a long time (Robinson 1982), although studies focusing on sexual selection among spiders are not as common as in other animal groups (Huber 2005). Courtship is traditionally viewed as an intersexual communication mechanism (Krafft 1982; Robinson 1982; Fernandez-Montraveta *et al.* 2001; Peretti *et al.* 2006) acting during the entire mating process, providing the stage for species recognition (Costa and Capocasale 1984; Costa *et al.* 1997; Jackson and Pollard 1997; Simó *et al.* 2002), mate choice (Fernandez-Montraveta and Schmitt 1994) and cryptic female choice (Eberhard 1985; Eberhard 1996; Peretti *et al.* 2006). Some authors assume that sexual behaviour differences among spiders, mainly during courtship, are the first and most efficient mechanism of reproductive isolation, with clear influence on evolutionary histories and speciation patterns (Costa and Capocasale 1984; Foelix 1996; Miller *et al.* 1998).

In a broad sense, sexual behaviour in spiders follows some discrete steps including sperm induction (charging the palpal organ with sperm), mate searching, courtship, copulation and copulatory ending. Behavioural modulation processes coordinate these steps, where aggressive attenuation and mutual arousal (or stimulation) are the best-known functional examples (Robinson 1982; Schneider and Lubin 1998). It is probable that some of these properties act together during courtship, or at least have some influence on the evolution of courtship and speciation processes (Miller *et al.* 1998).

Nuptial gift offering during courtship and mating is well known in arthropods, particularly in insects (Vahed 1998). Three hypotheses for the evolutionary origin of nuptial gifts are usually considered: male investment in his brood, male mating effort, and cannibalism defense (Vahed 1998; Sakaluk 2000; Stålhandske 2001; Bilde *et al.* 2007). Each of these hypotheses seem to have more influence in some systems than in others, even though they do not exclude one another (Stålhandske 2001) and, as said by Bilde *et al.* (2007), all of them assume that females receive direct benefits from the nuptial gift. An alternative hypothesis has been proposed by Sakaluk (2000) postulating that nuptial gifts could be a means to exploit female feeding motivation (sensory exploitation), thus reducing sexual conflict by attenuating female control during the insemination process, and not necessarily involving direct benefits to the female.

In spiders, nuptial gift offerings have been reported exclusively in pisaurids. The best known example of this unique behavior is found in *Pisaura mirabilis* (Clerck, 1757) (Pisauridae) (Bristowe and Locket 1926; Austad and Thornhill 1986; Stålhandske 2001; Stålhandske 2002; Bilde *et al.* 2006; Bilde *et al.* 2007), although a similar pattern has also been mentioned in two other pisaurids: *Pisaura lama* Bösenberg and Strand, 1906 and *Perenethis fascigera* (Bösenberg and Strand, 1906) (Itakura 1993; Itakura 1998).

Until now these data suggest an evolutionary origin of nuptial gifts restricted to Pisauridae and maintained as a sexually selected male trait (Bilde *et al.* 2007). However, to date the lack of more examples has limited a wider analysis about how nuptial gifts have evolved in spiders.

During a field trip along the east coast of Rio Grande do Sul State (Brazil), one of the authors (JEC) found males of a Trechaleidae species, each carrying a white structure in its chelicerae. After those first observations, new expeditions confirmed the occurrence of nuptial gifts in *Paratrechalea azul* Carico, 2005, and *Paratrechalea ornata* (Mello-Leitao, 1943) (Trechaleidae). The aims of this work are: 1) to report for the first time the nuptial gift offering behaviour for Neotropical species, 2) to report for the first time the occurrence of such behaviour in a non-Pisauridae spider family; 3) to compare the present descriptions with those available to Pisauridae species, mainly *Pisaura mirabilis*.

## Methods

### Study organisms

Trechaleids from Brazil are poorly known, although Carico (2005) recently revised the genus *Trechalea* Thorell 1869, and described the genera *Paratrechalea* and *Trechaleoides*. He also transferred *Trechalea ornata* Mello-Leitão, 1943 to the genus *Paratrechalea*, and described two new species (*Paratrechalea azul* and *P. galianoae*) from some museum specimens previously assigned to *T. ornata*. According to Carico (2005), these spiders were limited to the southern part of South America (Northern Argentina, Uruguay, and Southern Brazil).

The genus *Paratrechalea* contains semi-aquatic spiders which present differences in the use of the habitat according to their developmental stages: juveniles occupy the riparian zone of streams and rivers, while adults can be found over the rocks along these same water courses (Silva *et al.* 2006). They are all active at night, emerging from their refuges during the crepuscular hours. *Paratrechalea azul* and *P. ornata* adults present a slight sexual dimorphism (males show a more robust and reddish chelicerae) and can be found all year, but copulations are more frequent from November to February. Besides their partial geographical overlap, other important characteristics of these two species include their cryptic morphology and ecological habits. There is only a slight difference in size between these two species: individuals of *P. azul* are larger than those of *P. ornata*; thus the species can only be determined confidently by morphological analysis of their reproductive characters (Carico 2005; Silva *et al.* 2006).

### Area of study

Field observations took place at Pedra de Amolar Stream (Tramandaí Basin, Maquiné County, Rio Grande do Sul State, Brazil – 29º32’20” S, 50º14’47” W) during the spring and summer seasons from 2004 to 2006 (approximately 180 hours of observation). Studies in the field were conducted during the first hours of night (ca. 1h after sunset) with the use of a headlamp beam. Males and females were located by light reflection from the eyes as they emerged from their refuges and wandered over the rocks in the stream. Observations of behavioural activities were made using the light from the headlamp.

Numbers of observations were as follows: six of sperm induction, thirty of nuptial gift construction, fifty of mate searching, fifty of pre-copulatory courtship, fifty of copulation and copulatory courtship, and fifty of copulatory ending. Observations ended around five minutes after sperm induction/nuptial gift construction, or as soon as the pair separated. Some copulation encounters were recorded with a JVC S-VHS camcorder (model GR-SZ9) for later analyses. Samples of males, females and the nuptial gifts were collected in 80% alcohol immediately after copulation, allowing later species determination, whereas other individuals were collected and transported alive to the laboratory. Voucher specimens were deposited at the Museu de Ciências Naturais – Fundação Zoobotânica do Rio Grande do Sul (MCN-FZB/RS).

### Laboratory observations

Under laboratory conditions, field-collected juvenile spiders were kept until maturity in individual vials (200ml plastic cups covered by a soft textile net) under natural light and room temperature conditions (18ºC to 25ºC). Spiders were offered termites or houseflies (*Musca domestica*) on a daily basis. All the behavioural observations were conducted inside a circular plastic arena (20 cm diameter x 10 cm height) with rocks as substrate and a Petri dish containing water. One female was released inside the arena 24h before the male for acclimation. Males were then released into the arena with a prey already captured but not yet wrapped in silk as a nuptial gift. Observations were terminated in the following cases: 1) after more than 30 min transpired without contact between the spiders (*n* = 5); 2) when the female made frequent attacks towards the male (*n* = 14); 3) when the male used an escape behaviour (suggesting that the female was from another species) (*n* = 8); 4) immediately after copulatory ending (*n* = 15).

## Results

Sixteen units of behaviour were identified during courtship and mating of the two *Paratrechalea* species (boldface terms along with their corresponding descriptions appear in Appendix 1). No differences were detected between the two species in either quality or sequence of units. However, preliminary data suggests the existence of a strong interspecific recognition mechanism between these species, which acts long before the male starts to court the female. All courtships executed by males without nuptial gifts resulted in no copula (*n* = 15), not because of physical limitation but probably for female choice. So, the following descriptions of units of behaviour are related to the tests involving males that were necessarily carrying a nuptial gift.

**Sperm induction.** This behaviour was only recorded in the first hours after sunset in the natural habitat. Each male built a small triangular sperm web (Fig 1 a-c), usually placed on the side of the rocks, protected from the watercourse and hidden from direct visual observation. From the six observations of sperm induction, we could observe only three without having to move the rock in order to get a better view of the process. The male stands above the sperm web during ejaculate deposition and sperm induction. The palps were charged once, alternately, with sperm, which lasted only a few seconds (~15sec).

**Nuptial gift construction.** As soon as they left their refuges under the coastal rocks, males assumed a hunting posture, i.e., resting the first two pairs of legs on the water surface (Fig 2 a). They captured a large variety of aquatic insects, basically Ephemeroptera adults, aquatic stages of Odonata, and some moths that can be found along the river margin. Once captured, the prey was placed over a rock (or over any other solid substrate – Fig 2 b) and was covered with silk threads (**silk covering**). The male performed concentric abdominal movements over the prey, starting from the periphery to the centre, while he rotated his body and continuously drummed palps on the silk sheet (**drumming**), which is wider than the prey (Fig 2 c). Leg and **palpal grooming** behaviour usually occurred during this phase. After the silk covering step was complete, the male bit the sheet in the centre and, in a single movement, raised his body by extending his first, second and fourth pair of legs, thus removing the covered prey from the substrate (Fig 2 d). Then, the male's palps and third pair of legs executed prey **wrapping**. These appendages surrounded the uncovered part of the prey (which was facing the substrate) and were the only structures that touched the nuptial gift while it was carried with the chelicerae. No silk line connected the carried nuptial gift with the male spinnerets. After wrapping, the male assumed a resting position with the nuptial gift held by the chelicerae (Fig 2 e), and started **tarsal rubbing**.

**Mate searching.** In the field, males seemed to search for mates as they walked randomly over the rocks. Under laboratory conditions some males recognized the silk lines deposited by females but without discrimination between homospecific/heterospecific silk. When a female was perceived, the male immediately changed his pattern to a **stroboscopic movement**, interpreted as a sign of sexual excitement.

**Pre-copulatory courtship.** When the male approached the female and she did not perceive him, the male started a series of **lateral tapping** movements, followed by a **hyperflexed posture**, thus displaying the nuptial gift (Fig 3 a). This step could be abbreviated if the female perceived his presence, allowing the male to assume the hyperflexed posture immediately. The receptive female turned to face the male, assumed a similar hyperflexed posture, and grasped the nuptial gift with the chelicerae (**frontal position**; Fig 3 b). While in this face-to-face position, the female started to pull the nuptial gift away as the male pulled back.

**Copulation and communication signals.** The copulatory position varied from the typical lycosid mating position, where the male **mounts** the female, with the two facing in opposite directions (Fig 4 a-c). The main differences were that, in *Paratrechalea*, the male mounted between the female's first and second leg femora (Fig 4 c) instead of directly on top of her carapace, and the female was not grasped by the male's second and third legs, as occurs with other lycosoids. The male maintained contact with the nuptial gift by holding it with the third pair of legs while he mounted (**gift holding** – Fig 4 a-b), whereas the female kept the nuptial gift within her chelicerae while she ate it. There was a strong suggestion of female and male communication signals within these species. Before mounting, while in the frontal position, the female executed the **slow waving** of the first pair of legs, and both sexes made **palpal contact** while they maintained this position. The male performed a series of movements with his first and second pair of legs upon the dorsal side of the female abdomen (**quick touching**), followed by the female twisting her abdomen towards the same touched side (**rotation**), thus exposing the epigynum. These movements were executed on alternate sides: when the male touched the female's left side, he mounted towards this side and performed the right palpal insertion, and then vice-versa. For each mount, there was only one **insertion** consisting of two or three hematodochal expansions (Fig 4 a, c). The male returned to the frontal position after each mount over the female and bit the gift, releasing the third pair of legs from it. In the field, males performed an average of two alternate insertions per palp ($n$ = 15); in the laboratory, there was a large variation in this pattern, but fewer exceeded two insertions per palp ($n$ = 4).

**Copulatory ending.** For all observations made, copulatory ending seems to be initiated by the female, which assumed an aggressive behaviour towards the male. For most of the cases, the females extracted the gift from the males, and walked away with it, except for a few cases where the male retained the nuptial gift after copulation ($n$ = 4). Despite this behaviour, no case of pre-copulatory or post-copulatory cannibalism was recorded for these species. There was some change of male behaviour right before copulatory end. The male started to behave as if trying to maintain the current palpal insertion while omitting specific steps of the copulatory courtship sequence, i.e., gift holding.

## Discussion

The kind of nuptial gift offering described here for two species of Trechaleidae is the first reported in any family outside the family Pisauridae. However, other trechaleids including *Paratrechalea galianoae* Carico 2005 (LECS, field observation), *Trechalea bucculenta* (Simon 1898) (Álvaro Laborda and Miguel Simó, pers. com.) and other unidentified species (Paulo Motta, pers. com.) were observed transporting probable nuptial gifts.

An important outcome of our data is that we did not find qualitative differences in the courtship of the two species that could be used for taxonomic discrimination.

Moreover, our results highlight the crypticity between the species, previously related only to morphological and ecological aspects of their biology and now extended to reproductive behavioral aspects. In spite of this similarity, preliminary heterospecific reproductive tests resulted in no copulations (authors' unpublished data) because males did not even court the female.

Possible explanations for the adaptive significance of nuptial gifts within *Paratrechalea*

Four hypotheses explaining the adaptive significance of nuptial gifts in arthropods was introduced previously (Stålhandske 2001, 2002; Sakaluk 2000; Bilde *et al.* 2007; Vahed 1998, 2007). The first hypothesis is related to paternal investment resulting from natural selection (Vahed 1998; Stålhandske 2001). Our observations cannot provide evidence to support or reject this question, which is related to female direct benefits. We are currently conducting experiments to test the paternal investment hypothesis. The second hypothesis considers the use of a nuptial gift as a male mating effort that results from sexual selection (Vahed 1998; Stålhandske 2001; Huber 2005). Males in our study were only able to copulate when holding a nuptial gift, i.e., females only copulate when presented with a gift, and this can be seen as evidence that the trait could be a male mating effort. The third hypothesis emphasizes the protection that a nuptial gift may confer against sexual cannibalism (Vahed 1998; Stålhandske 2001). Because we observed not a single case of sexual cannibalism (neither pre- nor post-copulatory cannibalism), we cannot exclude the importance of nuptial gift in order to protect the male against an eventual attack. The fourth hypothesis is more related to the evolution of nuptial gifts as a sensory exploitation of female foraging instincts (Sakaluk 2000; Vahed 2007), but the same argument was also applied to the maternal care instincts in the case of *Pisaura mirabilis* (Stålhadske 2002). However, our observations give a little support of a possible maternal care exploitation for the species studied here, based on important egg-sac structural and female egg-sac carrying behavioral differences observed between *Paratrechalea* (Fig 5) and *Pisaura*.

These hypotheses may not have a direct relation to the adaptive significance of the trait at this evolutionary moment, but it is possible that they could have had a decisive role in the evolutionary past. Based only on the present descriptions, all raised hypotheses can be applied to *Paratrechalea*. They are not mutually exclusive, and further studies based on experimental evidence will help us to understand not only which hypothesis fits best in this case, but also if the trait is a convergence or homology when compared with the Pisauridae cases.

### Sexual behaviour of *Paratrechalea azul*, *P. ornata* and *Pisaura mirabilis*

There is a strong overlap of the sexual behaviour of the two *Paratrechalea* species presented here with the description of *Pisaura mirabilis* (Bristowe and Locket 1926; Stålhandske 2001; Bilde *et al.* 2006). All the courtship processes seem to be the same, but differ in the mating position: the male of *Pisaura mirabilis* reaches the female epigynum ventrally (Bristowe and Locket 1926), i.e., he does not mount on the top of the female's carapace as is the case with *Paratrechalea*.

There are two important similarities among the three species that deserve emphasis. First, the male uses the third pair of legs to hold the nuptial gift during palpal insertion (this report for *Paratrechalea azul* and *P. ornata*; Bristowe and Locket 1926, Bilde *et al.* 2006 for *Pisaura mirabilis*), and this may be related to intersexual communication (further comments below). Second, after an insertion, the male returns to the frontal position (this report for *Paratrechalea azul* and *P. ornata*; Stålhandske (2001) for *Pisaura mirabilis*), which also may serve as a key point during intersexual communication in order to prepare the female for another mount and also to guide the male in adopting the correct position before mounting.

One important difference with these comparisons is the thanatosis behaviour for *P. mirabilis* (Bilde *et al.* 2006). Males of *Paratrechalea azul* and *P. ornata* do not assume this death feigning posture, but instead will briefly fight to retain nuptial gifts and eventually abandon them to the females.

### Third-leg use by the male

Another important feature in the gift-wrapping procedure of *Paratrechalea* is that the third leg pair is used in wrapping the nuptial gift in addition to holding the completed gift after construction. The use of this leg pair for prey wrapping is unusual for spiders (William Eberhard, pers. comm.) and no previous reports of this behaviour were found in the literature. Perhaps this is the same process used by *Pisaura mirabilis*, but no descriptions of it have been found.

The sudden change of male behaviour just before copulatory ending may suggest an important aspect of male/female copulatory communication that could be achieved by the male's third leg during the gift holding posture. The *Pisaura mirabilis* male makes the same special use of its third leg, i.e., the gift holding posture during copulation (Bristowe and Locket 1926). Further, Bilde *et al.* (2006) explicitly raised the possibility that it could be related to some communication channel between the couple. Our data do not allow us to go further in explaining what stimulus triggers the copulatory ending, but special attention to gift holding could shed some light upon the subject.

### Final remarks

Obviously, much work remains to be done in order to draw an evolutionary scenario that can be applied to the known cases of nuptial gifts among spiders. The present report is the first effort to raise the nuptial gift offering to another level of analysis, inviting us to search for the evolutionary forces that probably created the observed behavioural patterns within Trechaleidae and Pisauridae.

Comparing our present observations with the descriptions available for *Pisaura mirabilis* (Bristowe and Locket 1926; Stålhandske 2001; Bilde *et al.* 2006), we believe that all the similarities found among these courtship descriptions allow us to say that nuptial gift offering is probably a homologous character between the spider families Trechaleidae and Pisauridae. Moreover, it could be a plesiomorphic character for these two families, since they belong to the same clade (Lycosoidea) according to known phylogenetic relationships (Griswold 1993; Silva 2003; Coddington 2005). However, analysis of such relationships within these spider families demands information derived from morphological and molecular data from sources not mentioned here.

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**Appendix 1.** Descriptions of the different behavioral steps observed during *Paratrechalea* spp. sexual behaviour (as presented in order of appearance in the text), the sex involved in each step, and references that mention the same steps for other spider species.

| **Step** | **Sex** | **Description** | **References** |
|---|---|---|---|
| Silk covering | M | Consists of a continuous addition of silk over the prey that is maintained directly on the substrate. | - |
| Drumming | M | Alternating palp movements, rapidly tapping against the substratum. | a,b |
| Grooming | M, F | Cleaning the palps and legs with the mouth. | b,c |
| Wrapping | M | A complex pattern of movements that involves the chelicerae, palps and the pair leg III, at the final stage of nuptial gift construction. | - |
| Tarsal rubbing | M | Two homolateral and contiguous legs, semiflexed, rub against each other with opposing and alternate fast movements. | a,b |
| Stroboscopic movement | M | Searching movement towards the female where the male moves in a discontinuous way. Can be followed by a lateral stimulation and/or hyperflexion. | - |
| Lateral tapping | M | Tapping made with the first two pair of legs on the lateral side of the female, mainly on the abdomen. | - |
| Hyperflexion | M, F | The two first leg pairs are raised and folded above the cephalotorax. | a |
| Frontal position | M, F | Male and female stand facing each other in a hyperflexed position, both holding the nuptial gift with their chelicerae. | - |
| Mounting | M | Climbing movement on the female cephalotorax. The male sternum stays in | - |

| | | | |
|---|---|---|---|
| | | contact with the dorsal side of female cephalotorax. Male body passes between the female legs I and II. | |
| Gift holding | M | The nuptial gift is held by the male with its III leg pair while he is copulating, i.e., during the mount. There is a slow movement of this leg pair, while holding the nuptial gift. | - |
| Slow waving | F | Leg I vertically raised, almost hyperflexed, followed by a slow back and forth movement with a small angle. | a;b;c;d |
| Palp contacting | M, F | Male and female palpal interaction, simply touching each other's palps, while maintaining a frontal position. | - |
| Quick touching | M | Similar to tarsal rub, except that there is contact of the male legs with the female abdomen. | - |
| Rotation | F | Abdominal orientation towards the side of male palpal insertion. | b |
| Insertion | M | "The physical act of the coupling of the male palp with the female epigynum." [e] | e |

References: a. Fernandez-Montraveta *et al.* (2001); b. Costa *et al.* (1997); c. Roberts and Uetz (2004); d. Roland and Rovner (1983); e. Stratton *et al.* (1996).

**Fig 1 A-C** Male of *Paratrechalea azul* during the sperm induction process. **A** A side-view of the male above the sperm web (arrow). **B** A side-view of the sperm web (arrow). **C** Top-view of the sperm web, showing its triangular shape and the exact position of the anchor points to the substrate.

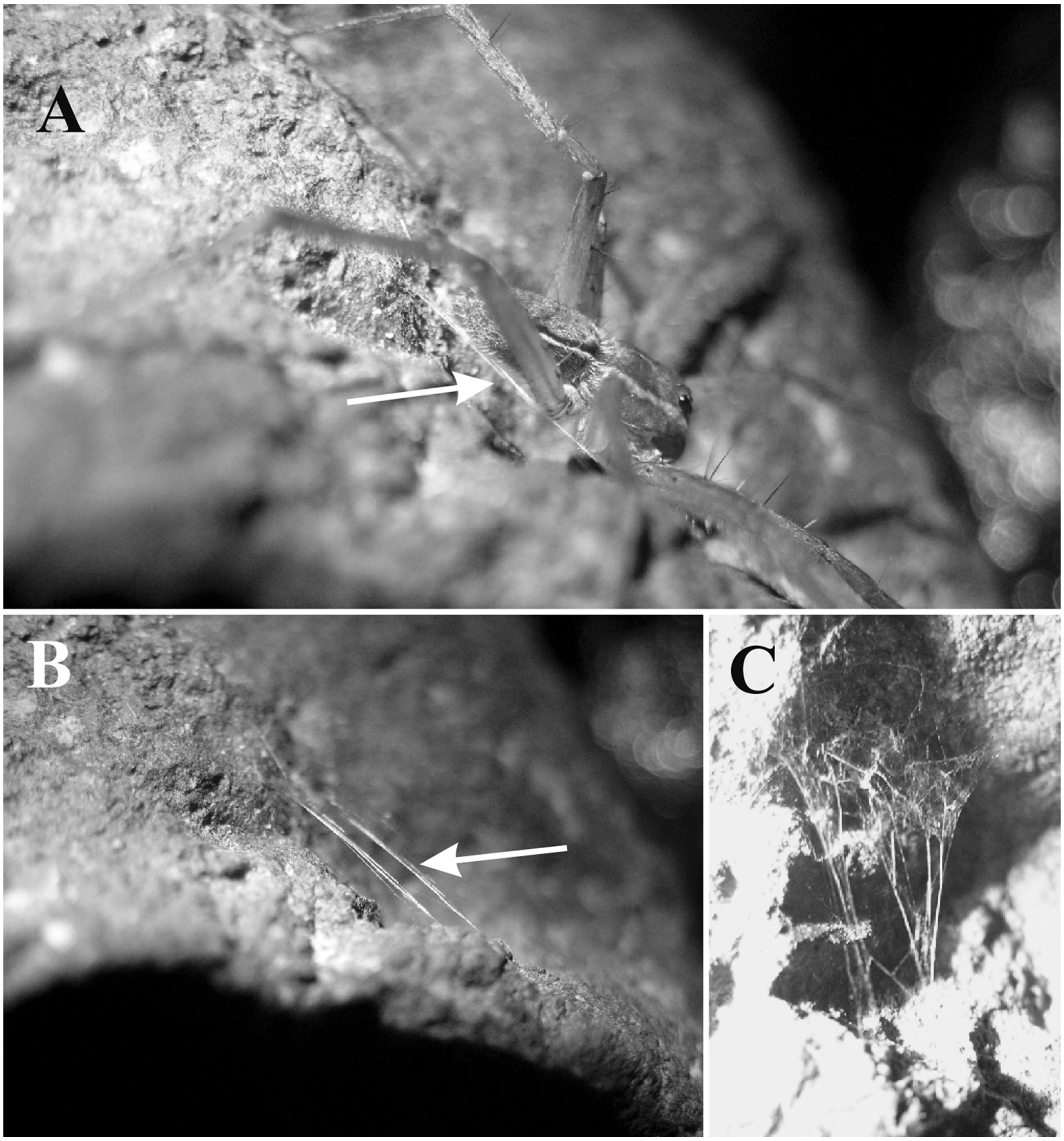


**Fig 2 A-E. A** Hunting position adopted by *Paratrechalea azul* and *P. ornata* males and females in the field at the stream margin. **B** Initial step of nuptial gift construction when the male fixes the gift onto the substrate. In this particularly case, the male is re-constructing a previous nuptial gift. **C** Intermediate step of nuptial gift construction showing when the male has already added silk threats over the prey. The silk sheet is wider than the wrapped prey (arrow). **D** Final step of nuptial gift construction (wrapping) showing the male raised on its I, II, and IV leg pairs and the use of the III leg pair and palps in order to complete the prey wrapping. **E** Male carrying the already constructed nuptial gift within its chelicerae.

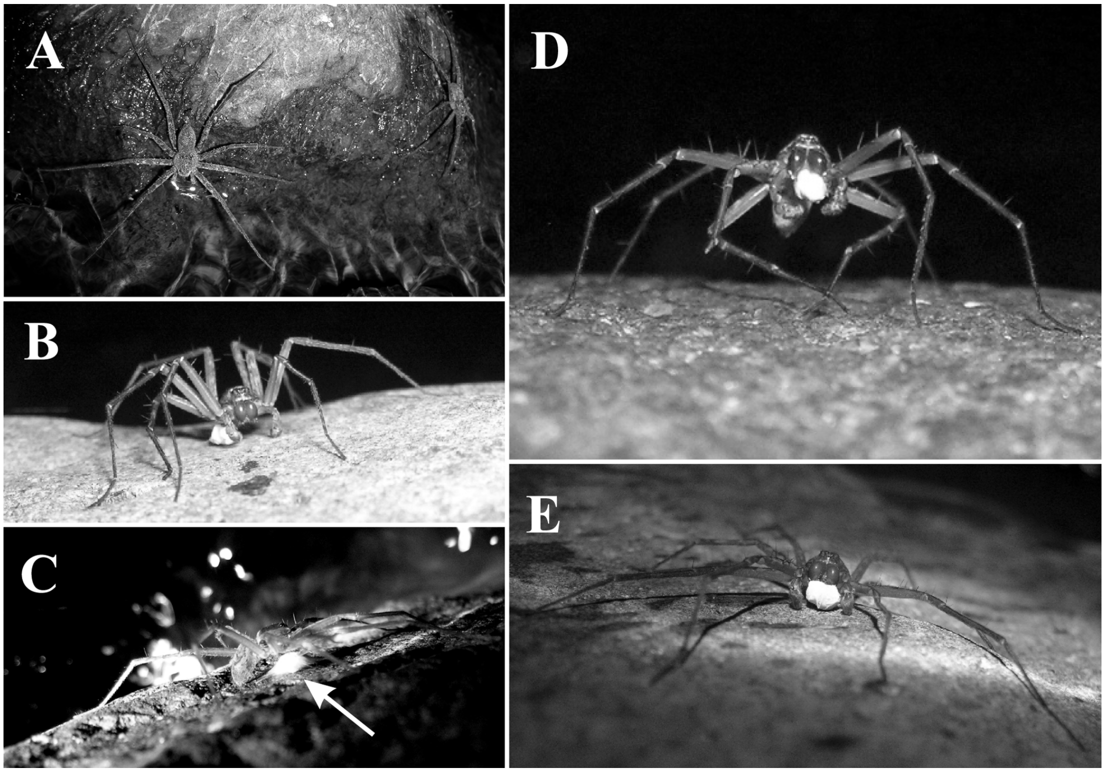


**Fig 3 A-B. A** Male in hyperflexed position next to a female showing the nuptial gift within its chelicerae. **B** Male and female in frontal position.

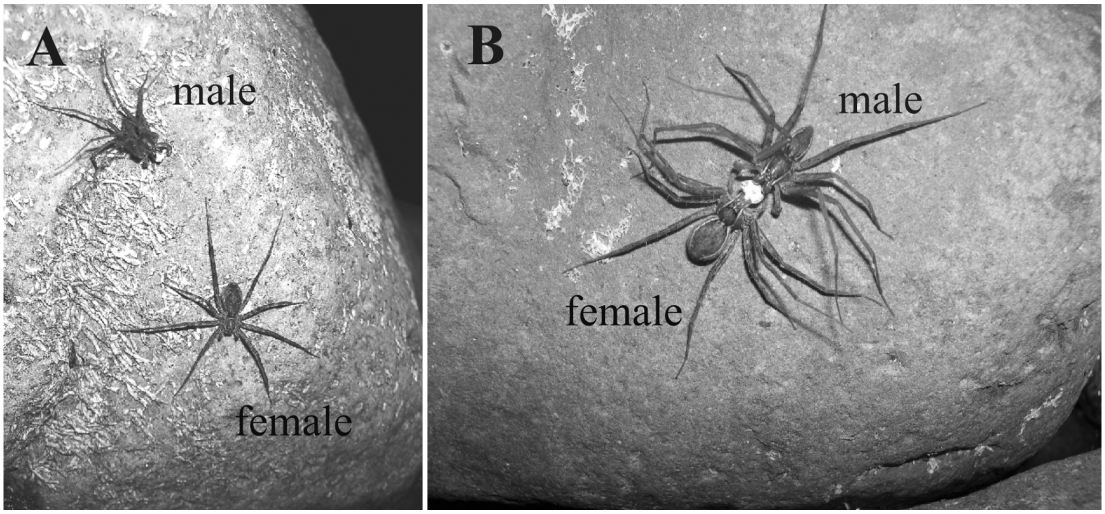


**Fig 4 A-C. A** A side-view of a copulation, showing the male palpal insertion evidenced by the expanded hematodocha (white arrow) while the nuptial gift is held by its III pair of legs within the female chelicerae (gray arrows). **B** Female frontal-view during copulation, showing the male left III leg holding the nuptial gift (arrow) that is being bitten by the female. **C** Male frontal-view during copulation, showing that its body passes between the female legs I (gray arrow) and II. Sperm transfer can be evidenced by the expanded hematodocha during the palpal insertion (white arrow).

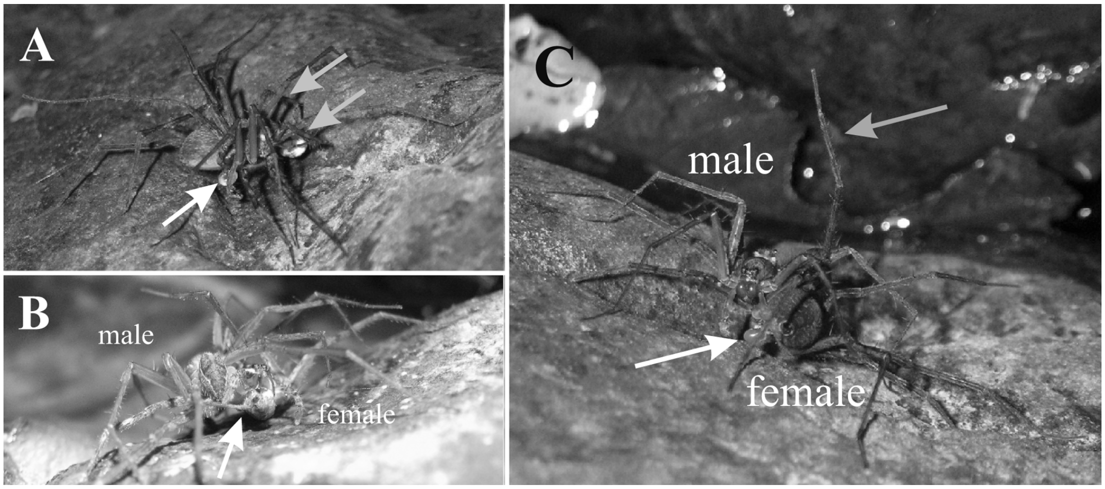
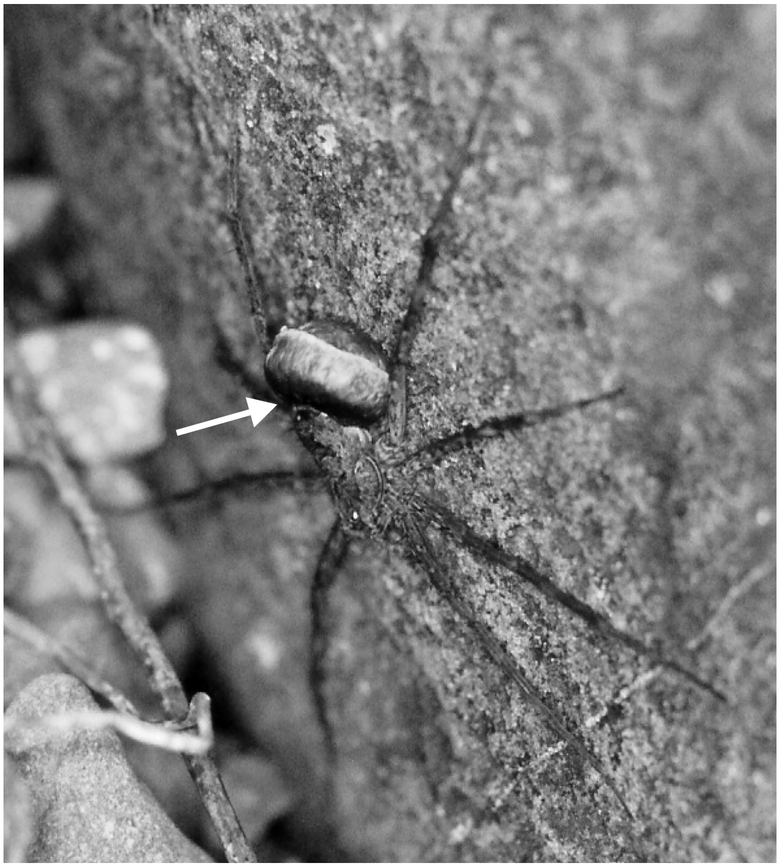


**Fig 5.** *Paratrechalea azul* female carrying the eggsac by the spinnerets in field situation.



# Capítulo 3

## Sexual dimorphism in chelicerae size in three species of nuptial-gift spiders: a discussion of possible functions and driving selective forces

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## Abstract

Positive allometric patterns observed for intersexual signaling characters are related to directional sexual selection, and supported by theoretical and empirical data. Recent models have showed that positive allometry may not hold as a rule, if the influence of natural selection is added to the model. Here we tested these models applying traditional morphometrical techniques for the analysis of chelicerae sexual dimorphism and allometric patterns within the *Paratrechalea* genus: *P. azul*, *P. galianoae,* and *P. ornata*. Spider chelicerae are basically used for prey capture, but males of *Paratrechalea* also use the chelicerae to offer a nuptial gift during courtship, also presenting a clear size and color sexual dimorphism supporting its possible role as a signal. Chelicerae size was male biased for all the variables studied and showed an isometric pattern, while females showed a higher variation. Our findings are in accordance to models of viability-related function for prey capture, questioning some statements proposed by the positive allometry model.

**Keywords:** chelicerae; sexual selection; sexual dimorphism; allometry; spider; *Paratrechalea*.

## Introduction

The premise that characters associated to intersexual signaling show a positive allometric pattern under directional sexual selection is supported by empirical data and theoretical models (Eberhard *et al.* 1998; Maynard-Smith & Harper 2003; Funke & Huber 2005). However, some authors have demonstrated that such arguments may not hold as a rule, and the patterns previously stated could be a consequence of sampling biases of the cases studied (Bonduriansky 2007). Furthermore, signaling structures imply resource allocation trade-offs between body size and trait size which maximize fitness. While sexual selection could favour these signaling traits, natural selection may counteract this trend (Arnqvist 1997; Bonduriansky & Day 2003).

Recently, Costa-Schmidt, Carico & Araújo (submitted manuscript) described the sexual behavior of *Paratrechalea azul* Carico 2005 and *P. ornata* (Mello-Leitão, 1943), where the male offers a nuptial-gift during courtship. The same behaviour was also registered at the field for a third species of the same genus, *P. galianoae* Carico 2005 (Costa-Schmidt *et al.* submitted). These are the first records of such nuptial-gift offering for Neotropical spider species, previously seen amongst a few Pisauridae species (Huber 2005).

The nuptial-gift consists of a prey wrapped in silk that can be adult stages of aquatic insects (mainly Ephemeroptera). Males perform stereotyped behaviours during courtship, starting with the adoption of a hyperflexed posture in front of the female while holding the nuptial gift with the chelicerae (Fig. 1). In case of acceptance, the female adopts the same posture in front of the male and bites the nuptial gift. While biting the nuptial gift, male courtship is followed by a series of stimulatory movements over the female body, with alternate mountings over the female prosoma in order to proceed with the palpal insertion and sperm transfer. A detailed description of the courtship process is presented in Costa-Schmidt *et al.* (submitted manuscript).

According to preliminary observations, male and female chelicerae within these species are sexually dimorphic in both size and color aspects, where males present more robust and reddish chelicerae (Costa-Schmidt *et al.* submitted – Fig. 2). Based on that, there seems to be behavioral and morphological evidences suggesting that male chelicerae could function as intersexual signals during courtship.

This article aims to describe the degree of chelicerae sexual dimorphism in *P. azul*, *P. ornata* and *P. galianoae*, and discuss the possible use of this structure as a signal character by analyzing the data with traditional linear morphometric tools.

## Material and methods

One hundred and seven female and 89 male adult *Paratrechalea* spiders were collected from natural populations. One sample of *P. azul* and another sample of *P. ornata* were collected at Pedra de Amolar River (Maquiné Municipality, Rio Grande do Sul State, Brazil – 29°32'20.52"S; 50°14'46.83"W), from May to August 2005. *Paratrechalea galianoae* was collected from a single population located at Pedra Branca Fall (Pedras Brancas River, Itati Municipality, Rio Grande do Sul State, Brazil – 29°23'45.59"S; 50°02'42.44"W) in January 2006.

For the measurement of chelicerae dimensions, we used a Nikon SMZ600 stereomicroscope with a scaling eyepiece for recording the size of the following chelicerae dimensions: basal segment frontal length (BL), basal segment frontal width (BW), basal segment lateral width (BLW), and fang length (FL) (Fig. 2). All measurements are presented in millimeters.

We also considered the cephalothorax centroid size as an indicator of overall body size (CSize), once this structure has a well known correlation with body size in spiders (Eberhard *et al.* 1998; Prenter, Elwood & Montgomery 1999; Walker & Rypstra 2001). The centroid size corresponds to the square root of summed squared distances of landmarks from their centroid (Swiderski 2003). Cephalothorax centroid size is also presented in millimeters.

In order to estimate cephalothorax centroid size, digital images from the dorsal view of cephalothorax were taken using a digital camera (Nikon Coolpix 5400) attached to the ocular of a stereomicroscope (Nikon SMZ600). All pictures were taken in the same magnification, and an ocular scale was used to make posterior pixels/millimeters conversion. Only Type II landmarks (Slice *et al.* 1996) were plotted in each image, except for two Type I landmarks in the cephalothorax (Fig. 3, landmarks #11-12). The identified landmarks for the cephalothorax were: #1 & #3, maximum width of the anterior end, which correspond to the clypeus width; #2 & #7, cephalothorax length; #4 & #10, inflexion points between the cephalic region and the thoracic region; #5 & #9, cephalothorax width; #6 & #8, maximum curvature of the posterior end; #11 & #12: thoracic furrow length.

Variables were tested for normal distribution using the Kolmogorov-Smirnov model test, and most of the variables showed normal distribution (p-values ranging from 0.05361 to 0.9945). Only the chelicerae BW of *P. ornata* males and FL of both *P. ornata* males and *P. azul* females showed deviations from normal distribution (p-values equal to 0.03594, 0.00372, and 0.0104, respectively). Mean differences between sexes for each species were analyzed with the Student t-test in order to detect chelicerae sexual size dimorphism. When any of the two assumptions of normality or equal variances were not met, Mann-Whitney U-tests were performed.

The coefficient of variation (CV), which is the standard deviation divided by the mean, was estimated for each variable within each sex. We applied a test proposed in Zar (1999, p. 141) to compare the CV of each variable between sexes. However, Eberhard *et al.* (1998) calls a special attention to the fact that CV is affected by two factors that should be analyzed separately, mainly for comparative studies involving correlated quantitative traits.

One factor is the slope of the estimated allometric line between the trait of interest and a particular body size trait, which will be presented below. The other factor is the dispersions of points around this allometric line, which we estimated using the alternative coefficient of variation ($CV' = CV(y) \times (1 - r^2)^{1/2}$) proposed by Eberhard *et al.* (1998). CV′ represents the coefficient of variation that $y$ would have if $x$ were held constant (Eberhard *et al.* 1998), which is the portion of the total CV that is more prone to variation in the context of sexual selection.

Allometric indexes (the regression coefficient of the study variable over an overall body size variable) were obtained by the linear regression of the log transformed original data. Two sets of comparisons were made for each structure and for each sex. First, we used the CSize as the overall body size variable. Second, we used the size of the structure to infer the relations among dimensions within the structure. We arbitrarily chose the BL as a measure of overall chelicerae size.

Two models of linear regression were used: the ordinary least-squares model (OLS), which implies the assumption of dependent variable error only; and the reduced major axis model (RMA), a model that assumes some amount of error for both variables (dependent and independent). The RMA model seems to be more realistic, since all the measurements were taken using the same technique, one that does not control for measurement errors within the independent variable, although it has some statistical requirements, like significant levels of correlation coefficients (Eberhard, Huber & Rodriguez 1999; Ohno *et al.* 2003; Bonduriansky 2007). The OLS model still holds as the most used model along the comparative data available, although the possible overestimation of the regression coefficient. The allometric indexes were tested for the null hypothesis of isometry, i.e., assuming a regression coefficient equal to one ($\beta = 1$). For each species, two sets of intersexual comparison of the estimated regression slopes was performed using one-way ANCOVA test for homogeneity of regressions, with CSize (body size allometry) and BL (chelicerae size allometry) as covariates.

Regression models were ran with PAST v. 1.75b (Hammer, Harper & Ryan 2001), using the default bootstrap procedures for the estimation of confidence limits (2000 repetitions). Cephalothorax CSize were estimated using the RMorph library (Baylac 2007) developed for R environment (R Development Core Team 2007).

## Results

### *Chelicerae sexual size dimorphism*

Overall chelicerae dimensions confirmed the already expected male biased sexual size dimorphism (Table 1). The only exceptions were the inversion of this pattern for the FL in *P. azul* and the absence of statistical difference for this same variable in *P. galianoae*.

Females showed higher CV values for all measurements in the three species. CV comparisons between sexes within each species showed different significant levels (Table 2). For *P. azul*, CV values are statistically equal between males and females. An opposite pattern was observed for *P. ornata*, which showed significant differences in CV for all measurements, except for BW. The other species, *P. galianoae*, showed a pattern closer to *P. azul*, with different CV values for FL.

The absolute values of the alternative CV´ did not have a consistent pattern (Table 3). From 12 comparisons, the only observed statistical significance was for BLW in *P. galianoae*, with males showing more variation than females (Table 3). If we consider marginally significant results, *P. ornata* and *P. galianoae* showed a tendency for an increase of male variation in BW in relation to overall body size, while *P. azul* showed a female variation bias for FL in relation to chelicerae size (Table 3).

### *Chelicerae allometric analyses*

Males have a constant isometric pattern for both regression models (OLS and RMA) in relation to CSize (Table 4). We found only two significant coefficients in opposite directions from 24 estimated coefficients, i.e., one hyperallometric coefficient estimated using RMA (BW in *P. ornata*), and a hypoallometric coefficient using OLS (FL in *P. galianoae*). As expected, RMA tended to show higher allometric coefficients than OLS. When the measured variables were regressed against the BL (Table 5), they maintained the isometric pattern, except for the BW of *P. ornata* which showed the same hyperallometric pattern for the RMA estimate.

Females showed different patterns for each species. From the 12 estimated RMA slopes, five were significant for hyperallometry (Table 4). Two measures showed the same relation for both *P. ornata* and *P. galianoae* (BL and FL), and a third was only observed for *P. ornata* (BLW). A single case of hypoallometry using OLS was observed for BL in *P. azul*. However, within chelicerae size regressions showed a distinct pattern (Table 5), where *P. azul* presented a slight tendency for hyperallometry (marginally significant RMA slopes for BW and BLW), and *P. ornata* and *P. galianoae* showed an hypoallometric slope for BW.

Male and female comparisons showed statistically the same allometric slopes for most of the traits in all species considered (Table 6). The only exceptions were related to BW and FL allometric slopes for *P. galianoae* when considering the overall body size. Another trait that showed distinct intersexual allometric slopes was BW in *P. ornata* when BL was the covariate. However, three other comparisons showed marginally significant results: *P. ornata* FL in relation to overall body size, *P. ornata* BW and *P. galianoae* FL in relation to chelicerae size (Table 6).

## Discussion

### *Chelicerae sexual dimorphism*

The present results confirm that chelicerae size dimorphism is male biased in the three species studied, which strongly coincides with the hypothesis of a selective force acting over this structure. Chelicerae sexual size dimorphism could be explained in terms of sexual selection acting on that trait. A possible and intuitive interpretation, supported by behavioral (Costa-Schmidt *et al.* submitted) and now by morphometric data, could be that the males developed bigger chelicerae in order to fulfill the requirements to use this structure during courtship advertisement. If so, theory predicts that male chelicerae not only would be bigger but also should show higher coefficient of variation. However, these statements were only partially met by our data. Even though we found a male biased sexual size dimorphism for most chelicerae dimensions, CV values (numerical and/or significant) were female biased (Table 2), and CV´ values did not show a regular pattern for higher variation level for any sex gender (Table 3).

Such discordance from theoretical predictions leads us to imagine another possible evolutionary scenario where sexual selection is not necessarily the main driving force. We may interpret this pattern as a consequence of a natural selection process over the chelicerae evolution of these *Paratrechalea* species. Differences in chelicerae size could be a product of specialization for prey capture (Walker & Rypstra 2001), where males can be more prone to capture bigger and/or more difficult preys than females. This could lead to an ecological outcome of niche divergence, a mechanism rose by Hedrick & Temeles (1989) for the origin of sexual size dimorphism within a species. In fact, this hypothesis does not have empirical support. Field observations (LECS) suggest that both sexes have the same prey capture ability and a complete diet overlapping. However, the investigation of differences in prey capture abilities between sexes deserves an experimental approach.

Alternatively, we also have another interpretation were both selective forces, sexual and natural selection, may be concomitantly working. Sexual selection could indeed act for intersexual signaling, while natural selection may act as a constraint to male chelicerae development, in order to maintain the functionality of this structure for prey capture (viability-related functional selection). Reduction on chelicerae functionality for prey capture has been observed in cases where males have morphological modifications in their chelicerae for male-male combat (Walker & Rypstra 2001), even though male-male combat was never observed for *P. azul* and *P. ornata* (LECS field observations). The lower CV levels observed for males fits with this model and can be interpreted as the occurrence of such constraint, i.e., most males have reached an optimal level of chelicerae size and now are constrained to this optimal (maximum) limit. However, it is important to emphasize the fact that three of the four CV´ values that were significant or marginally significant were male biased for higher variation, which may be interpreted as the presence of a sexual selection force over these chelicerae traits.

### *Chelicerae allometric coefficients*

The overall findings of an isometric pattern along male allometric coefficients call our attention for the possibility that *Paratrechalea* male chelicerae are not a signaling trait driven by sexual selection. In this case, the hypothesis of a signaling trait was raised under the assumption of visual communication along courtship process - but according to present results the observed dimorphism would be guided by natural selection.

However, we insist that since there is a consistent chelicerae sexual dimorphism (shape and size) and a full behavioral description of the use of this trait use during courtship (Costa-Schmidt *et al.* submitted), the provided data does not exclude that male chelicerae are indeed a sexual signaling trait. As Bonduriansky (2007) states, allometric patterns cannot necessarily support the action of sexual selection over a specific trait, i.e., the fact that we found an isometric relation along the measured chelicerae dimensions - which are not different from those compared to females - does not mean that sexual selection is not acting over them. Furthermore, we should pay a special attention to the species life-history in order to draw our conclusions, since an isometric pattern had already been modeled for sexual selected traits that have also viability-related functions (Bonduriansky and Day 2003).

### *Conclusion*

The fact that females showed higher coefficients of variation agrees with our hypothesis that male chelicerae did not present higher allometric slopes because they are under a viability-related functional selection. This was supported by the CV´ data, which showed little evidence for differences in variation in the context of sexual selection. The *Paratrechalea* chelicerae sexual dimorphism can be an example of the model number 3 presented by Bonduriansky & Day (2003). *Paratrechalea* male chelicerae probably have reached an optimal variation level, and so have a restricted CV for chelicerae traits. Females would be under a natural selection regime for this structure, maintaining the functionality of the structure with higher level of variation than males.

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Table 1. Mean and standard deviation (mm) for each chelicerae variable and overall body size in males and females and the corresponding result of the statistical comparison. BL: basal segment length; BW: basal segment width; BLW: basal segment lateral width; FL: fang length; CSize: cephalothorax centroid size.

| Character | Males | Females | Statistic comparison | *p* |
|---|---|---|---|---|
| *P. azul* | *n* = 35 | *n* = 38 | | |
| BL | 2.57 (±0.1393) | 2.41 (±0.1359) | t = 4.86; df = 71 | < 0.00001 |
| BW | 1.26 (±0.0670) | 1.04 (±0.0694) | t = 13.54; df = 71 | < 0.00001 |
| BLW | 1.68 (±0.0890) | 1.37 (±0.0881) | t = 15.18; df = 71 | < 0.00001 |
| FL | 1.16 (±0.0634) | 1.23 (±0.0715) | U = 299.5 | < 0.0001 |
| C Size | 8.06 (±0.4089) | 8.45 (±0.4943) | t = -3.68; df = 71 | < 0.001 |
| *P. ornata* | *n* = 37 | *n* = 35 | | |
| BL | 2.21 (±0.1055) | 1.87 (±0.1345) | t = 12.00; df = 70 | < 0.00001 |
| BW | 1.07 (±0.0644) | 0.82 (±0.0565) | U = 1290 | < 0.00001 |
| BLW | 1.39 (±0.0740) | 1.08 (±0.0763) | t = 17.34; df = 70 | < 0.00001 |
| FL | 1.01 (±0.0478) | 0.97 (±0.0714) | U = 884 | 0.0056 |
| C Size | 6.96 (±0.3004) | 6.68 (±0.3978) | t = 3.33; df = 70 | 0.0014 |
| *P. galianoae* | *n* = 17 | *n* = 34 | | |
| BL | 2.10 (±0.1350) | 1.93 (±0.1705) | t = 3.83; df = 49 | < 0.001 |
| BW | 1.07 (±0.0620) | 0.85 (±0.0703) | t = 10.81; df = 49 | < 0.00001 |
| BLW | 1.36 (±0.1196) | 1.13 (±0.1068) | t = 6.99; df = 49 | < 0.00001 |
| FL | 0.96 (±0.0538) | 0.98 (±0.0876) | U = 241 | 0.3382 |
| C Size | 6.47 (±0.4137) | 6.57 (±0.4939) | t = -0.71; df = 49 | 0.4823 |

Table 2. Coefficients of variation (× 100) for males and females for each chelicerae variable and overall body size. BL: basal segment length; BW: basal segment width; BLW: basal segment lateral width; FL: fang length; CSize: cephalothorax centroid size.

| | *P. azul* | | | *P. ornata* | | | *P. galianoae* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Male $n = 35$ | Female $n = 38$ | $p$ | Male $n = 37$ | Female $n = 35$ | $p$ | Male $n = 17$ | Female $n = 34$ | $p$ |
| BL | 5.42 | 5.64 | 0.8135 | 4.77 | 7.20 | 0.0161 | 6.41 | 8.87 | 0.1596 |
| BW | 5.32 | 6.64 | 0.1925 | 6.03 | 6.87 | 0.4422 | 5.90 | 8.28 | 0.1431 |
| BLW | 5.34 | 6.44 | 0.2698 | 5.33 | 7.06 | 0.0986 | 8.78 | 9.44 | 0.7419 |
| FL | 5.46 | 5.81 | 0.7128 | 4.75 | 7.39 | 0.0099 | 5.60 | 8.94 | 0.0496 |
| C Size | 5.07 | 5.85 | 0.3979 | 4.32 | 5.95 | 0.0600 | 6.39 | 7.52 | 0.4655 |

Table 3. Alternative coefficient of variation (CV´× 100), for males and females, for each chelicerae variable in relation to overall body size (CSize) and chelicerae size (BL). CSize: cephalothorax centroid size; BL: basal segment length; BW: basal segment width; BLW: basal segment lateral width; FL: fang length.

| | *P. azul* | | | *P. ornata* | | | *P. galianoae* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Male *n* = 35 | Female *n* = 38 | *p* | Male *n* = 37 | Female *n* = 35 | *p* | Male *n* = 17 | Female *n* = 34 | *p* |
| Body Size | | | | | | | | | |
| BL | 2.86 | 2.88 | 0.9669 | 2.66 | 2.99 | 0.4893 | 2.19 | 2.48 | 0.5727 |
| BW | 3.39 | 3.60 | 0.7212 | 4.12 | 2.99 | 0.0616 | 3.29 | 2.32 | 0.0879 |
| BLW | 2.98 | 3.18 | 0.6998 | 3.58 | 2.77 | 0.1332 | 5.27 | 2.95 | 0.0037 |
| FL | 2.88 | 2.86 | 0.9669 | 3.09 | 3.22 | 0.8076 | 3.57 | 3.29 | 0.7010 |
| Chelicerae Size | | | | | | | | | |
| BW | 2.71 | 3.03 | 0.5084 | 3.18 | 3.62 | 0.4440 | 2.69 | 3.04 | 0.5791 |
| BLW | 2.33 | 2.52 | 0.6418 | 3.40 | 3.08 | 0.5603 | 3.83 | 2.95 | 0.2078 |
| FL | 2.14 | 2.96 | 0.0577 | 2.72 | 2.72 | 1.0000 | 3.12 | 3.05 | 0.9159 |

Table 4. Ordinary Least Squares ($b_{OLS}$) allometric coefficients, Reduced Major Axis ($b_{RMA}$) allometric coefficients, and correlation coefficients ($r$) between the analyzed chelicerae dimensions and cephalothorax centroid size (CSize). All correlation coefficients were statistically significant for $p < 0.0001$. BL: basal segment length; BW: basal segment width; BLW: basal segment lateral width; FL: fang length.

| | BL | | | BW | | | BLW | | | FL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $b_{OLS}$ | $b_{RMA}$ | $r$ | $b_{OLS}$ | $b_{RMA}$ | $r$ | $b_{OLS}$ | $b_{RMA}$ | $r$ | $b_{OLS}$ | $b_{RMA}$ | $r$ |
| Males | | | | | | | | | | | | |
| *P. azul* ($n$ =35) | 0.90 | 1.06 | 0.85 | 0.81 | 1.04 | 0.77 | 0.86 | 1.04 | 0.83 | 0.92 | 1.08 | 0.85 |
| *P. ornata* ($n$ = 37) | 0.91 | 1.10 | 0.83 | 1.03 | 1.40* | 0.73 | 0.93 | 1.25 | 0.74 | 0.83 | 1.09 | 0.76 |
| *P. galianoae* ($n$ = 17) | 0.95 | 1.01 | 0.94 | 0.77 | 0.92 | 0.83 | 1.17 | 1.46° | 0.80 | 0.68* | 0.87 | 0.77 |
| Females | | | | | | | | | | | | |
| *P. azul* ($n$ =38) | 0.82* | 0.95 | 0.86 | 0.94 | 1.12 | 0.84 | 0.95 | 1.09 | 0.87 | 0.84° | 0.99 | 0.85 |
| *P. ornata* ($n$ = 35) | 1.11 | 1.21* | 0.91 | 1.03 | 1.15 | 0.90 | 1.09 | 1.18* | 0.92 | 1.12 | 1.24* | 0.90 |
| *P. galianoae* ($n$ = 34) | 1.11 | 1.16* | 0.96 | 1.04 | 1.09 | 0.96 | 1.04 | 1.10 | 0.95 | 1.09 | 1.18* | 0.93 |

° Marginally significant.

* $p < 0.05$; ** $p < 0.01$

Table 5. Ordinary Least Squares ($b_{OLS}$) allometric coefficients, Reduced Major Axis ($b_{RMA}$) allometric coefficients, and correlation coefficients ($r$) between the analyzed chelicerae dimensions and chelicerae basal segment length (BL). All correlation coefficients were statistically significant for $p < 0.0001$. BW: basal segment width; BLW: basal segment lateral width; FL: fang length.

| | BW | | | BLW | | | FL | | |
|---|---|---|---|---|---|---|---|---|---|
| | $b_{OLS}$ | $b_{RMA}$ | $r$ | $b_{OLS}$ | $b_{RMA}$ | $r$ | $b_{OLS}$ | $b_{RMA}$ | $r$ |
| Males | | | | | | | | | |
| *P. azul* (*n* =35) | 0.85 | 0.98 | 0.86 | 0.88 | 0.98 | 0.90 | 0.94 | 1.02 | 0.92 |
| *P. ornata* (*n* = 37) | 1.08 | 1.27* | 0.85 | 0.88 | 1.13 | 0.77 | 0.81° | 0.99 | 0.82 |
| *P. galianoae* (*n* = 17) | 0.81 | 0.91 | 0.89 | 0.94 | 1.05 | 0.90 | 0.71 | 0.86 | 0.83 |
| Females | | | | | | | | | |
| *P. azul* (*n* =38) | 1.05 | 1.18° | 0.89 | 1.05 | 1.14° | 0.92 | 0.89 | 1.04 | 0.86 |
| *P. ornata* (*n* = 35) | 0.81* | 0.95 | 0.85 | 0.88 | 0.98 | 0.90 | 0.96 | 1.03 | 0.93 |
| *P. galianoae* (*n* = 34) | 0.88* | 0.94 | 0.93 | 0.90° | 0.95 | 0.95 | 0.96 | 1.02 | 0.94 |

° Marginally significant.

* $p < 0.05$

Table 6. Comparison between allometric coefficients (b values) of males and females in relation to cephalothorax centroid size (CSize: Body Size) and basal segment length (BL: Chelicerae Size). BL: basal segment length; BW: basal segment width; BLW: basal segment lateral width; FL: fang length.

| | *P. azul* | | *P. ornata* | | *P. galianoae* | |
|---|---|---|---|---|---|---|
| | F | *p* | F | *p* | F | *p* |
| Body Size | | | | | | |
| BL | 0.4171 | 0.5205 | 1.9998 | 0.1619 | 1.8246 | 0.1832 |
| BW | 0.7627 | 0.3855 | 0.0003 | 0.9853 | 4.8409 | 0.0327 |
| BLW | 0.4291 | 0.5146 | 1.1149 | 0.2948 | 0.1216 | 0.7289 |
| FL | 0.3629 | 0.5489 | 3.6321 | 0.0609 | 6.9228 | 0.0115 |
| Chelicerae Size | | | | | | |
| BW | 2.7057 | 0.1045 | 3.3439 | 0.0718 | 0.2774 | 0.6009 |
| BLW | 2.5396 | 0.1156 | 0.0016 | 0.9678 | 1.1905 | 0.2808 |
| FL | 0.1665 | 0.6845 | 1.7714 | 0.1877 | 3.4399 | 0.0699 |

Figure 1. Male of *Paratrechalea azul* holding a nuptial gift with its chelicerae while in the hyperflexed position. This sequence of pictures was taken under laboratory conditions by presenting a mature female to the male, illustrating how the male chelicerae are used during courtship.

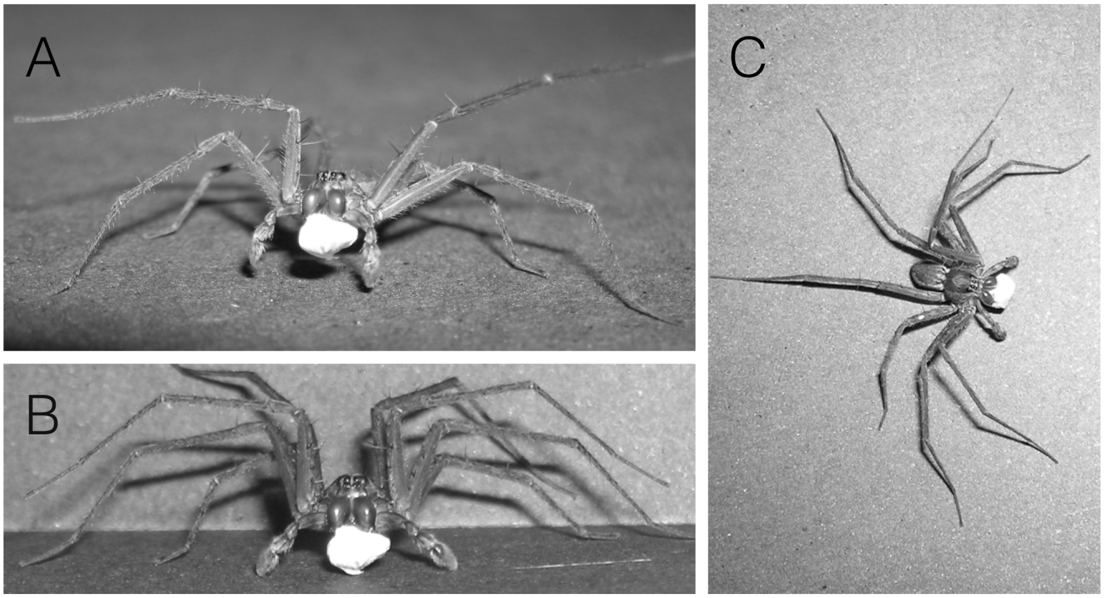


Figure 2. Overall *Paratrechalea* sp. chelicerae morphology. All specimens and dissected structures shown are *Paratrechalea azul*. A) Male frontal view; B) female frontal view; C) male (left) and female (right) lateral view. BL: basal segment frontal length; BW: basal segment frontal width; BLW: basal segment lateral width; FL: fang length. Scale bars = 1 mm.

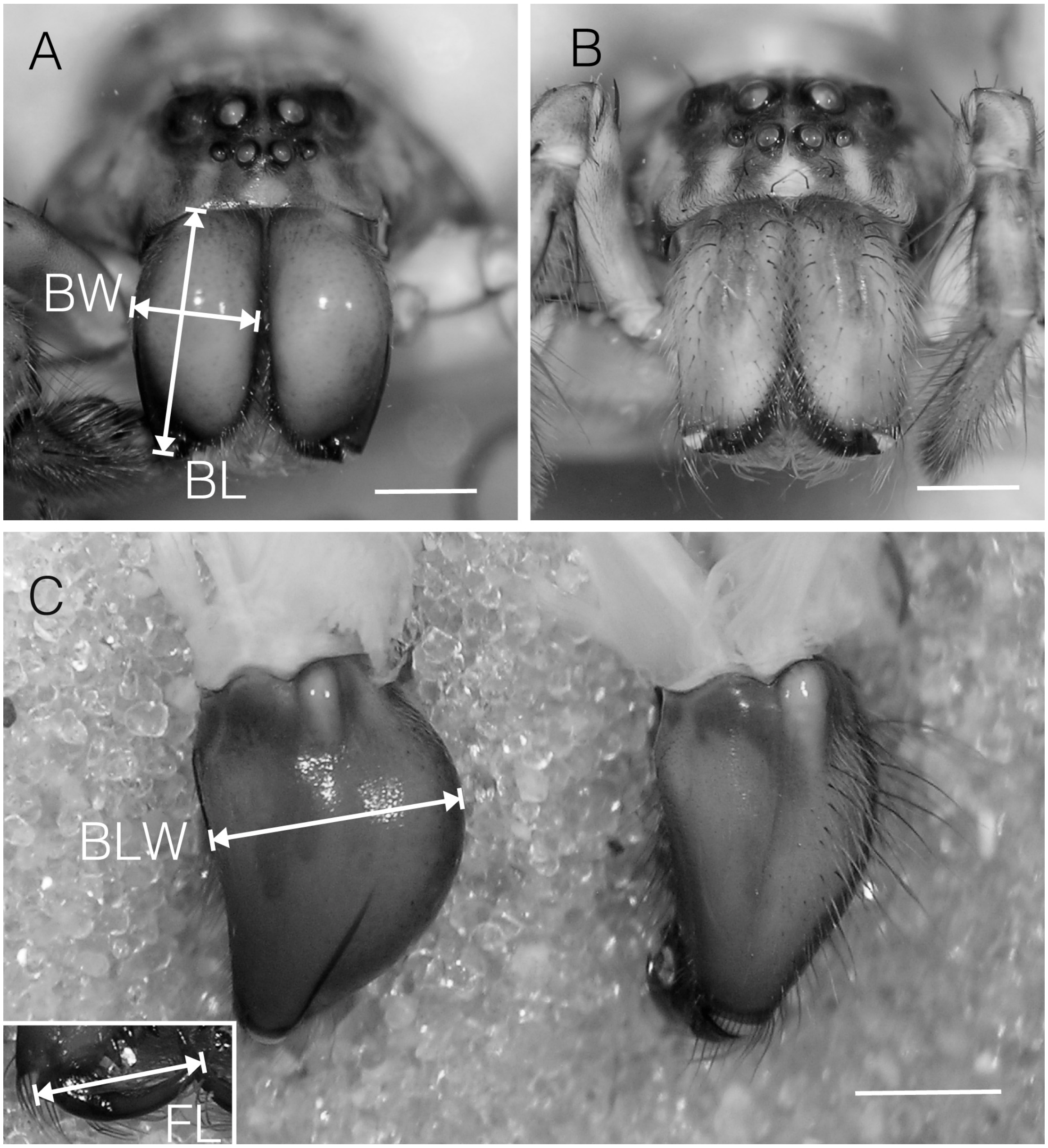
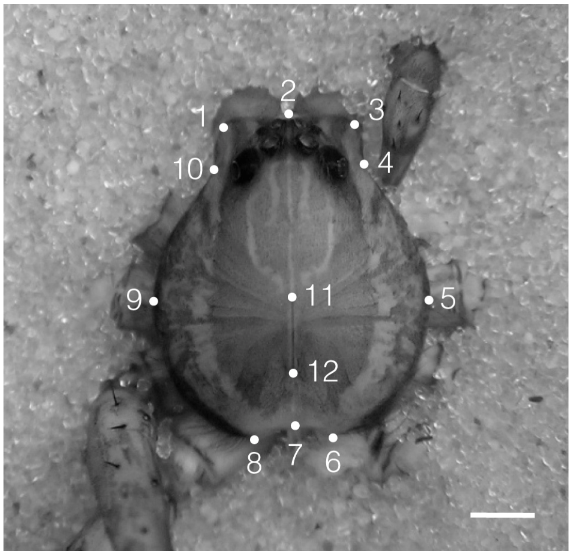


Figure 3. Locations of landmarks on cephalothorax in dorsal view. The specimen corresponds to a *Paratrechalea ornata* male. Scale bar = 1 mm.



# Capítulo 4

## Morphometric relations and sexual dimorphism in *Paratrechalea* (Araneae, Trechaleidae): a geometric morphometrics approach.

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## Abstract

Sexual dimorphism is almost ubiquitous among animals, and is originated by several causes. In spiders, much of the efforts in studying sexual dimorphism were related to highly dimorphic groups, with a strong bias to web-building spiders. Few cases are related to wandering spiders, which tend to be less dimorphic. Also, most of the sexual dimorphism studies focus on size relationship between sexes (sexual size dimorphism). Here we present the description of sexual dimorphism within a genus of cryptic wandering spiders, the *Paratrechalea* Carico 2005. Using geometric morphometrics techniques, we present a quantitative description of both shape and size sexual dimorphism for three species of the genus (interspecific analysis) and also the variation within three populations of one of these species (intraspecific analysis), using two somatic structures (cephalothorax and sternum) as the objects of analysis. Sexual shape dimorphism proved to be very consistent among species and populations for both structures, while sexual size dimorphism was absent but for a single population. Besides their cryptic aspect, the applied techniques were capable to show consistent discriminant results for species and populations. Moreover, the data suggests the split of the intraspecific group in two different taxonomic entities. Hypothesis concerning the observed patterns are presented.

**Keywords:** sexual dimorphism, Araneae, *Paratrechalea*, geometric morphometrics.

## Introduction

Sexual dimorphism is almost ubiquitous among animals, and can be described by morphological, physiological and behavioral traits (Walker & Rypstra 2001; Bertin *et al.* 2002). Extreme cases of sexual dimorphism received most of the attention, and spiders are one of the groups with these extreme cases (Vollrath & Parker 1992; Fairbairn 1997; Hormiga *et al.* 2000). It is widely accepted that sexual dimorphism is the result of different selective pressures (Hedrick & Temeles 1989; Prenter *et al.* 1999; Gasnier *et al.* 2002). For spiders, the main hypothesis for the evolution of sexual dimorphism is fecundity selection (Head 1995; Prenter *et al.* 1999; Hormiga *et al.* 2000), although sexual selection (Elgar 1991) and life-history trade-offs (Vollrath & Parker 1992) have also empirical evidence of their action on the determination of sexual dimorphism.

Besides spider sexual size dimorphism is no more conspicuous than shape dimorphism, most studies are based on body size correlated traits (Vollrath & Parker 1992; Head 1995; Prenter *et al.* 1999; Gasnier *et al.* 2002; Hormiga *et al.* 2000). This certainly underestimates the influence of sexual interaction over sexual shape dimorphism evolution, particularly for groups like wandering spiders where size dimorphism is almost absent (Vollrath & Parker 1992; Head 1995).

Based on this possible bias, we present here a geometric morphometrics approach for describing sexual dimorphism in a wandering spider group. During the last decades the establishment of geometric morphometrics has made huge contribution for the study of shape variation (Rohlf & Marcus 1993). In summary, geometric morphometrics allow us to analyze separately shape and size properties from a given structure, but even more interesting is the possibility to analyze the shape of a structure in an integrative way, and not only few of its dimensions. For example, using geometric morphometrics, sexually dimorphic species can be quantitatively described by its morphological properties, which are used to be tested for differences between shape consensus of each sex.

The aim of this work is to describe morphometric properties of shape and size from three *Paratrechalea* species (Araneae; Trechaleidae) based on quantitative analyses. The specific aim is to describe the degree of sexual shape and size dimorphism found for each species, and the degree of variation of sexual shape and size dimorphism among three populations of one of the studied species.

## Material and Methods

### Study species

We studied three semi-aquatic spider species from the Trechaleidae family: *Paratrechalea azul* Carico 2005, *P. ornata* (Mello-Leitão, 1943) and *P.galianoae* Carico 2005. Morphometric aspects among them have been suggested based on few specimens (Carico 2005), and such descriptions demands further investigations with bigger samples. The three species have nocturnal semi-aquatic habits, and are easily found living next to streams and rivers. More information about the natural history of these species can be found elesewhere (Carico 2005; Silva *et al.* 2006; Costa-Schmidt *et al.* submitted).

### Sampling design

The study has two levels of comparative analyses. The first, the interspecific analysis, deals with the comparison among the three species on a local scale. The second level is the intraspecific one, and deals with three samples taken along part of *P. ornata* geographic range.

Adult male and female specimens were sampled from natural populations in Brazil and Uruguay. Population locations are summarized in Table 1. Except for *P. ornata* (Que) population, all populations were sampled during a single season. *Paratrechalea azul* and *P. ornata* (Maq) were the only sample populations collected at the same location (Table 1), representing the syntopic condition of these species for the northeastern region of Rio Grande do Sul (Brazil). All sample locations share the same habitat structure features: they are located at the edge of a river or stream with exposed rocks where the spiders rest and/or hide, and are associated with riparian vegetation.

All specimens were conserved in 80% alcohol. Voucher specimens were deposited at the Museu de Ciências Naturais – Fundação Zoobotânica do Rio Grande do Sul (MCN-FZB/RS, curator: Erika Buckup; Porto Alegre/Brazil), and at the Facultad de Ciências – Seccíon Entomologia (FCE, curator: Miguel Simó; Montevideo/Uruguay).

### Geometric morphometrics data collection

Digital images were taken using a digital camera (Nikon Coolpix 5400) attached to the ocular of a stereomicroscope (Nikon SMZ600). All pictures were taken in the same magnification for each structure. An ocular scale was used to make posterior pixels/millimeters conversion. For each specimen, we digitalized two non-genital (somatic) body parts (dorsal view of cephalothorax and sternum) (Fig. 1).

Only Type II landmarks (Slice *et al.* 1996) were plotted in each image, except for the cephalothoraxes images that have two Type I landmarks (Fig. 1a, landmarks #11-12). For the cephalothorax, the identified landmarks were: #1 & #3, maximum width of the anterior end, which correspond to the clypeus width; #2 & #7, cephalothorax length; #4 & #10, inflexion points between the cephalic region and the thoracic region; #5 & #9, cephalothorax width; #6 & #8, maximum curvature of the posterior end; #11 & #12: thoracic furrow length. For the sternum, the identified landmarks were basically the acute points found among the legs projections (at the right side: landmarks #1 to #4, at the left side: landmarks #6 to #9), and also the acute point at the posterior region (landmark #5).

General Procrustes Superimposition analysis (GPS) was applied in order to separate shape from size properties. This process is fully described in Rohlf & Slice (1990). From the GPS results is possible to calculate the scores of a Principal Component Analysis made over the projection of the shapes found in the pre-form space in the tangent space (shape variables) (see also Alibert *et al.* 2001).

For sexual shape dimorphism analysis, two-way MANOVA were used to test differences in the estimation of the statistical parameters of shape variables (represented by the scores of the PC's) among the considered levels for both interspecifc and intraspecific models. The first factor was different for each model, corresponding to the **species** (levels: *P. azul*, *P. galianoae* and *P. ornata*), and **population** (levels: *P. ornata* (Maq), *P. ornata* (Pas), and *P. ornata* (Que)), for interspecific and intraspecific models respectively. **Sex** was the second factor for both models. Sexual shape dimorphism was also estimated by a one-way MANOVA using sex as comparison factor within species.

We calculated the Mahalanobis distance as a disimilarity measure of shape distance among the mean shape of the levels under analysis, which ranges from 0 to infinite. Comparisions were made between sexes within species, and also among sexes and populations for intraspecific analysis.

We investigate size differences between sexes (sexual size dimorphism) and among species and populations, using the centroid size as a variable. The centroid size is defined as "the square root of summed squared distances of landmarks from their centroid" (Swiderski 2003). This variable showed normal distribution for our samples (Kolmogorov-

Smirnov test: $p \geq 0.2603$). The comparison of sexes within species was done using t-tests. Also, we ran a two-way ANOVA in order to test differences in size for the same factors used in the MANOVA analysis. If significant, a post hoc Tukey test was performed to compare all levels.

### Software

TpsUtil v. 1.33 (Rohlf 2004) was used to create a file containing the sequence of pictures to be analyzed. This file was loaded in the tpsDig 2.04 software (Rohlf 2005) for landmarks plotting. Both programs are available at <http://life.bio.sunysb.edu/morph/>. Posterior morphometrical statistic analyses and graphic outcomes were made using the RMorph library (Baylac 2007) developed for R environment (R Development Core Team 2007).

## Results

We found strong morphological differentiation for shape and size components of the structures in both interspecific differentiation and intraspecific variation. The sexual dimorphism was even higher than interspecific differences in some analysis. Cephalothorax and sternum showed a strong sexual dimorphism for shape component, and a not so regular sexual size dimorphism. We will present the results in topics related to the level of analysis (inter or intraspecific), followed by the specific morphometric component (shape or size).

### 1. Interspecific analysis

#### 1.1. Shape analysis

Principal component analysis (PCA) clearly indicated that most of the variation found was related to the sexual dimorphism (Fig 2). The factor species showed higher overlapping among the 95% confidence ellipses that represent each species, with *P. ornata* as being the species which shares most of its shape proprieties with the two other species. Both structures showed the same patterns of percent variation for the first two principal components (PCs) (cephalothorax = 50.9%, sternum = 52.3%).

All tested MANOVA models have a strong significance for each factor separately, for both cephalothorax and sternum (Table 2). Only the cephalothorax has significant interaction between factors, i.e., sexual shape dimorphism is not the same among the species. Comparisons among species showed strong significant levels of shape differentiation for both structures (Table 3), with *P. azul* and *P. ornata* (Maq) showing a significant but closer shape.

All comparisons within species were significant (Table 4), and sexual shape dimorphism within each species seems to be the same, where females have wider thoracic region and males have wider cephalic region (Fig 3).

The sternum showed the same significant level of sexual shape dimorphism as for the cephalothorax, but the factor species had little more influence in the tested model (Table 2). Even significant, within species comparisons showed lower significant levels than those found for cephalothorax (Table 3). The main shape difference between sexes within species is found along the anteroposteiror axis, being more elongated for males at its posterior end, with a special elongation within *P. azul* (Fig 3).

The discriminant analysis corroborates with the PCA findings that these structures have a strong sexual dimorphism, especially for cephalothorax that showed high levels of correct sex determinations (Table 5). The factor species is not a good descriptor of the observed variation, with cephalothorax presenting a smaller percentage of determination when compared to the sternum (Table 5). For this data, sternum shape is more informative than cephalothorax for a correct classification of an individual within one of these three species.

The Mahalanobis distance estimates not only corroborate the observation that cephalothorax is more sexually dimorphic than sternum, but also indicates that *P. azul* and *P. ornata* (Maq) are closer within the shape space (Table 6). Mahalanobis estimates for sternum presented smaller distances between sexes within species, and a more tenuous tendency of proximity between *P. azul* and *P. ornata* (Maq) (Table 6).

### 1.2. Size analysis

The ANOVA models showed different results for each structure. The centroid size data revealed to be highly related to the factor species and to the interaction of factors for both models, but the factor sex was only significant for the sternum (Table 7).

Pairwise comparison among levels of each significant factor for each structure showed strong differences. Cephalothorax upper probability levels for the factor species was $p = 7.85\times10^{-4}$. Sternum pairwise comparison for the factor species showed an upper probability level of $p = 10^{-7}$, while sternum pairwise comparison and factor sex the upper probability level was $p = 5.31\times10^{-5}$. The significant interaction between factors means that there are different relations of sexual size dimorphism among the species (see below).

Cephalothorax sexual size dimorphism was present in both *P. azul* and *P. ornata* (Maq), but in opposite directions (Table 8), while sternum sexual size dimorphism was female biased, and only observed for *P. azul*. *Paratrechalea galianoae* did not show sexual size dimorphism in either structure.

## 2. Intraspecific analysis

### 2.1. Shape analysis

Principal component analysis (Fig 4) showed that the first two PCs explains near 50% of data variation (cephalothorax = 49.2%, sternum = 44.7%), which is similar to what was found for the interspecific analysis. Sexual dimorphism holds as the main factor explaining the observed variation (Fig 4), which can be viewed by plotting the 95% confidence ellipses of each factor over the data. From Fig 4 we can easily see that the factor population showed an almost complete overlapping of the confidence ellipses among levels.

Discriminant analysis indicates that the cephalothorax is a better descriptor of population and sex than sternum (Table 5).

The factors population and sex tested in the MANOVA models were highly significant for both structures, except for the interaction of factors in the sternum (Table 2). Sex is indeed the main factor of variation, and holds highly significant among all pairwise comparisons (Table 3). Therefore, the factor population has different significance patterns for each somatic structure. Cephalothorax pairwise comparisons showed an interesting relation of Maquine as compared to all Uruguayan populations (Table 3), suggesting two divergent groups, with the Uruguayan populations having the same shape accordingly to these analyses. The sternum presented another shape pattern among populations, where each population and sex seems to have its own shape. Sexual shape dimorphism was significant for both structures in all populations (Table 4).

The Mahalanobis distance among populations shows that the Uruguayan populations have more similar cephalothorax shapes and the similar distance from the Maquine population (Table 6). The sternum did not show the same pattern, but an approximately constant relation of shape divergence among all populations. Within species Mahalanobis distances showed the same pattern found for the interspecific analysis, with cephalothorax having higher shape divergence than sternum (Table 6).

### 2.2. Size analysis

Intraspecific size analysis showed a strong effect of population along the two-way ANOVA models for cephalothorax and sternum, where factor sex was only significant for cephalothorax (Table 7). Both structures showed no interaction among the factors, and most of the data variation observed was not explained by the tested factors (evaluated by the residuals sum squares – Table 7). Pairwise comparison showed that each population has its own cephalothorax and sternum size (cephalothorax: $p \leq 6.49 \times 10^{-3}$; sternum: $p \leq 0.02136$).

Sexual size dimorphism was only present for cephalothorax size in the Maquine population (mentioned in the interspecific analyses results – Table 8), although Quebrada de los Cuervos and Paso del Molino did show marginal significant p values for cephalothorax and sternum, respectively (Table 8).

## Discussion

In spiders, the use of somatic characters for taxonomic recognition is usually related to taxonomic levels above species (Huber 2003, 2004; Bond & Beamer 2006). Here we presented quantitative data demonstrating the use of such structures for the recognition of closely and cryptic species. Moreover, the finding of the distinction between *P. ornata* (Maq) and the *P. ornata* Uruguayan populations may be one of two hypotheses: an influence of geographic variation or that we are dealing with two different taxonomic entities.

There is a strong sexual shape dimorphism for cephalothorax and sternum in both inter and intraspecific levels. Sexual size dimorphism pattern was similar to those described for wandering spiders, which do not show such size biases as those found for orb-weavers (Head 1995; Hormiga *et al.* 2000; Gasnier *et al.* 2002). Besides a tendency of having a female bias, we have found a case of male size bias within our data.

Table 9 presents the main results of our analyses, and may be used as a guide along the remaining discussion.

### *Sexual dimorphism*

We have found that the main source of sexual dimorphism for these *Paratrechalea* species is related to shape variation. Sexual size dimorphism was not regular among the studied species or even populations of *P. ornata*, suggesting a possible relation with ecological interactions and/or available resources. The observed sexual shape and size dimorphisms follow current theoretical statements for wandering spiders, i.e., with moderate levels of dimorphism when compared to orb-building species (Walker & Rypstra 2001).

Sexual shape dimorphism is stronger within cephalothorax along the studied species, suggesting the action of different developmental programs leading to such divergence. Based in our personal experience working with these spiders, we interpreted the cephalothorax shape variation may be an indirect response for a selective pressure over males' chelicerae (Costa-Schmidt *et al.* submitted; Costa-Schmidt & Araújo, unpublished data). *Paratrechalea* males present more robust chelicerae than females, which are used during intersexual signaling (Costa-Schmidt *et al.* submitted), and may also increase males' abilities to cope with bigger preys (Costa-Schmidt & Araújo, unpublished data). Our analyses show that males have wider cephalic region, which could be explained by its connection with chelicerae: in order to preserve their mechanical functionality, wider chelicerae demands a wider clypeus border. This pattern was found for both interspecific and intraspecific comparisons, which may be explained by the phylogenetic dependence.

The pattern of sexual size dimorphism in each somatic structure analyzed was not as clear as the shape component, and this probably have an influence of other factors not controlled in our sampling design. The very fact that the residuals of our two-way ANOVA models have higher sum of squares values indicate this.

Besides the fact that size is the most studied aspect of sexual dimorphism within the Order Araneae (Head 1995; Vollrath 1998; Hormiga *et al.* 2000; Foellmer & Fairbairn 2005; among others), particular reproductive and ecological aspects of these *Paratrechalea* species may confound further direct comparisons to other cases.

The observed small level of sexual size dimorphism and its variable pattern within the studied populations make us suggest a hypothesis that those species were originally monomorphic for size, and that the observed cases of sexual size dimorphism are basically responses to environmental conditions (e.g. resources availability and/or social interactions). This response to environmental conditions can be compared to the dimorphic niche hypothesis (Hedrick & Temeles 1989), were the sexes may occupy different niches to attenuate some competitive pressures. Therefore, it is obvious that these hypothesis and statement demands experimental data in order to be confirmed.

Geographic variation may play an important role on the patterns observed. From the *P. ornata* populations, the only one that presented a significant sexual size dimorphism was *P. ornata* (Maq), and only for cephalothorax size. This population must be analyzed with attention because of its syntopic relation with *P. azul*, and this syntopy may be influencing the pattern at the interspecific level. Curiously, *P. azul* and *P. ornata* (Maq) showed inversed sexual size as mentioned above.

### *Paratrechalea azul* ***and*** *P. ornata* ***taxonomic status***

We brought here strong statistical support that *P. azul* and *P. ornata* are indeed two morphometrically separate species as previously described by Carico (2005), and confirms that *P. azul* is bigger than *P. ornata*. Moreover, they have different shapes of cephalothorax and sternum, besides they cryptic appearance.

This morphometric isolation is corroborated by reproductive behavioral aspects (Costa-Schmidt *et al.* submitted). Even with this sharp definition, it is important to note that both species are still cryptic for most of their niche dimensions (authors' personal observation), and we are positive that the morphological differences found among species were only detected thanks to the strength of the morphometric techniques applied. Species determination in field situations still poses some limitation to us, since the only visual informative aspect of their morphology is size. This is something very problematic when we try to correctly determine overlapping size specimens of these species.

Some aspects of our results make us to suggest that *P. azul* and *P ornata* are closely related species, which was previously assumed by the male and female genital resemblance (Carico 2005; Silva *et al.* 2006). The observed divergent level among them supports this sibling relationship, mainly by the significant levels found for both structures (Table 3) and also by the estimated Mahalanobis distances (Table 5). Therefore, this suggestion demands further cladistic treatment, and while the phylogeny of this genus is not solved, we can not go any further in our statements about phylogenetic relationships among those species.

### *Other taxonomic issues related to shape variation*

Some aspects about the geographic variation found within *P. ornata* populations are helpful in order to create an evolutionary scenario for these two sister species. Most of the data showed an intraspecific divergence pattern between Maquine and Uruguayan populations that could be interpreted as different phenetic taxonomic entities.

Even if the observed variation among *P. ornata* populations is a consequence of geographical variation, this hypothesis do not exclude another (but more complex) mechanism called character displacement (Goldberg & Lande 2006; Adams & Collyer 2007). This mechanism has a strong relation with the syntopic distribution of *P. ornata* and *P. azul* along the northern region of Rio Grande do Sul State, and depends on the strength of interspecific competition between them at this overlapping area. The outcome of this interaction can be divergence in morphology and/or behavior (Brown & Wilson 1956; Schluter 2000; Kawano 2002, 2003), depending on the resource which the species are competing. The opposite effect it would be competitive exclusion (Rice & Pfennig 2007). Therefore, we can not go any further over this hypothesis, once our data do not fulfill the requirements for its description (Schluter 2000; Kawano 2002, 2003). Properly sampling design must be done in order to have a stronger evidence of the presence of such evolutionary mechanism.

### *Conclusions*

In a broader sense, the observed patterns were expected in relation to empirical and theoretical data. The amount of shape difference among species was lower between *P. azul* and *P. ornata*, which may induce us to believe that they are closely related species. *P. galianoae* was the more divergent species for all comparisons. Finally, this description of morphological variation among few species of the same genus only responds for a small part of their recent evolutionary history. In other words, we have presented just a glimpse of the phenotypic answers of these populations for such recent selective forces.

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Table 1. Sampling location and sample size of each studied population.

| Species | Location | $n_{\text{male}}$ | $n_{\text{female}}$ |
|---|---|---|---|
| *P. azul* | Maquiné / Brazil | 35 | 38 |
| *P. galianoae* | Itati / Brazil | 17 | 34 |
| *P. ornata* | | | |
| *P. ornata* (Maq) | Maquiné / Brazil | 37 | 35 |
| *P. ornata* (Pas) | Paso del Molino / Uruguay | 14 | 21 |
| *P. ornata* (Que) | Quebrada de los Cuervos / Uruguay | 19 | 20 |

Table 2. MANOVA of shape principal components with factors species/population (interspecific / intraspecific) and sex (the first 20 and 14 PCs were selected for cephalothorax and sternum respectively).

| INTERSPECIFIC | D.f. | Wilks | approx. F | num. D.f. | den. D.f. | p |
|---|---|---|---|---|---|---|
| Cephalothorax | | | | | | |
| Species | 2 | 0.287 | 7.405 | 40 | 342 | $< 2.2\ e^{-16}$ |
| Sex | 1 | 0.122 | 61.621 | 20 | 171 | $< 2.2\ e^{-16}$ |
| Interaction | 2 | 0.685 | 1.779 | 40 | 342 | 0.0036 |
| Residuals | 190 | | | | | |
| Sternum | | | | | | |
| Species | 2 | 0.236 | 13.399 | 28 | 354 | $< 2\ e^{-16}$ |
| Sex | 1 | 0.551 | 10.285 | 14 | 177 | $< 2\ e^{-16}$ |
| Interaction | 2 | 0.835 | 1.195 | 28 | 354 | 0.2311 |
| Residuals | 190 | | | | | |
| INTRASPECIFIC | D.f. | Wilks | approx. F | num. D.f. | den. D.f. | p |
| Cephalothorax | | | | | | |
| Population | 2 | 0.261 | 5.795 | 40 | 242 | $< 2.2\ e^{-16}$ |
| Sex | 1 | 0.128 | 41.335 | 20 | 121 | $< 2.2\ e^{-16}$ |
| Interaction | 2 | 0.503 | 2.477 | 40 | 242 | $1.259\ e^{-5}$ |
| Residuals | 140 | | | | | |
| Sternum | | | | | | |
| Population | 2 | 0.452 | 4.424 | 28 | 254 | $5.613\ e^{-11}$ |
| Sex | 1 | 0.420 | 12.540 | 14 | 127 | $< 2.2\ e^{-16}$ |
| Interaction | 2 | 0.808 | 1.018 | 28 | 254 | 0.4452 |
| Residuals | 140 | | | | | |

Table 3. Pairwise comparisons among factor levels. Each column represents the F value and its respective p value for the considered factors, and their interaction. Species and population names are represented by the acronym of their specific and populational names.

| INTERSPECIFIC | Species | | Sex | | Interaction | |
|---|---|---|---|---|---|---|
| | F | p | F | p | F | p |
| Cephalothorax | | | | | | |
| azul - orn | 4.185 | $1.09\ e^{-6}$ | 47.912 | $6.6\ e^{-16}$ | 1.906 | 0.0524 |
| azul - gal | 9.666 | $3.57\ e^{-15}$ | 36.401 | $6.6\ e^{-16}$ | 1.856 | 0.0726 |
| orn - gal | 9.363 | $1.08\ e^{-14}$ | 39.746 | $6.6\ e^{-16}$ | 2.006 | 0.0390 |
| Sternum | | | | | | |
| azul - orn | 5.479 | $1.14\ e^{-7}$ | 68.419 | $6.6\ e^{-16}$ | - | - |
| azul - gal | 14.038 | $6.0\ e^{-16}$ | 50.438 | $6.0\ e^{-16}$ | - | - |
| orn - gal | 13.613 | $6.0\ e^{-16}$ | 58.304 | $6.0\ e^{-16}$ | - | - |
| INTRASPECIFIC | Population | | Sex | | Interaction | |
| | F | p | F | p | F | p |
| Cephalothorax | | | | | | |
| Maq - Pas | 6.431 | $2.63\ e^{-9}$ | 33.731 | $1.32\ e^{-15}$ | 0.963 | 1.0000 |
| Maq - Que | 8.890 | $5.95\ e^{-13}$ | 34.180 | $1.32\ e^{-15}$ | 6.061 | $6.42\ e^{-9}$ |
| Pas - Que | 1.877 | 0.2174 | 17.319 | $2.05\ e^{-15}$ | 1.851 | 0.2370 |
| Sternum | | | | | | |
| Maq - Pas | 4.347 | $4.60\ e^{-5}$ | 8.008 | $5.43\ e^{-10}$ | - | - |
| Maq - Que | 4.937 | $5.35\ e^{-6}$ | 8.922 | $2.91\ e^{-11}$ | - | - |
| Pas - Que | 4.579 | $1.11\ e^{-4}$ | 9.196 | $2.58\ e^{-9}$ | - | - |

Table 4. MANOVA results for the within species/population analysis for sexual shape dimorphism (the first 20 and 14 PCs were selected for cephalothorax and sternum respectively; for each comparison: *d.f.* = 1).

| | D.f. res. | Wilks | approx. F | num. D.f. | den. D.f. | p |
|---|---|---|---|---|---|---|
| Cephalothorax | | | | | | |
| *P. azul* | 71 | 0.1176 | 19.5101 | 20 | 52 | $< 2.2\ e^{-16}$ |
| *P. galianoae* | 49 | 0.0968 | 13.9949 | 20 | 30 | $3.4\ e^{-10}$ |
| *P. ornata* (Maq) | 70 | 0.0852 | 27.3903 | 20 | 51 | $< 2.2\ e^{-16}$ |
| *P. ornata* (Pas) | 33 | 0.0786 | 8.2055 | 20 | 14 | $1.1\ e^{-4}$ |
| *P. ornata* (Que) | 37 | 0.0390 | 22.2030 | 20 | 18 | $7.3\ e^{-9}$ |
| Sternum | | | | | | |
| *P. azul* | 71 | 0.4799 | 4.4904 | 14 | 58 | $2.2\ e^{-5}$ |
| *P. galianoae* | 49 | 0.4965 | 2.6082 | 14 | 36 | 0.0103 |
| *P. ornata* (Maq) | 70 | 0.4599 | 4.7823 | 14 | 57 | $1.1\ e^{-5}$ |
| *P. ornata* (Pas) | 33 | 0.2758 | 3.7506 | 14 | 20 | 0.0037 |
| *P. ornata* (Que) | 37 | 0.1935 | 7.1439 | 14 | 24 | $1.6\ e^{-5}$ |

Table 5. Correct classification percentages achieved by the discriminant analyses for interspecific and intraspecific comparisons.

| INTERSPECIFIC | Species | | | Sex | |
|---|---|---|---|---|---|
| | azul | orn | gal | female | male |
| Cephalothorax | 69.9% | 65.3% | 76.5% | 99.0% | 98.9% |
| Sternum | 83.6% | 77.8% | 86.3% | 82.2% | 79.8% |
| INTRASPECIFIC | Population | | | Sex | |
| | Maq | Pas | Que | female | male |
| Cephalothorax | 90.3% | 65.7% | 69.2% | 100% | 100% |
| Sternum | 83.3% | 48.6% | 71.8% | 89.5% | 82.8% |

Table 6. Estimated Mahalanobis distances between the average shape of the compared groups for interspecific and intraspecific comparisons. Above diagonal: species/population comparisons for cephalothorax. Below diagonal: species/population comparisons for sternum. Main diagonal (boldface numbers): sex comparisons within species for sternum\cephalothorax.

| **INTERSPECIFIC** | *P. azul* | *P. galianoae* | *P. ornata* (Maq) |
|---|---|---|---|
| *P. azul* | **3.10 \ 28.92** | 7.07 | 2.25 |
| *P. galianoae* | 9.63 | **5.01 \ 29.32** | 4.66 |
| *P. ornata* (Maq) | 4.88 | 5.26 | **4.30 \ 32.77** |
| **INTRASPECIFIC** | *P. ornata* (Maq) | *P. ornata* (Pas) | *P. ornata* (Que) |
| *P. ornata* (Maq) | **4.14 \ 28.67** | 6.85 | 7.58 |
| *P. ornata* (Pas) | 2.11 | **9.96 \ 26.51** | 2.37 |
| *P. ornata* (Que) | 3.02 | 2.93 | **6.93 \ 35.46** |

Table 7. Centroid size two-way ANOVA of each structure for the interespecific and intraspecific analysis.

| INTERSPECIFIC | Df | Sum Sq | Mean Sq | F | p |
|---|---|---|---|---|---|
| Cephalotorax | | | | | |
| Species | 2 | 114.173 | 57.086 | 317.6823 | $< 2.2\ e^{-16}$ |
| Sex | 1 | 0.229 | 0.229 | 1.2766 | 0.2600 |
| Interaction | 2 | 4.053 | 2.027 | 11.2775 | $2.3\ e^{-5}$ |
| Residuals | 190 | 34.142 | 0.180 | | |
| Sternum | | | | | |
| Species | 2 | 24.498 | 12.2489 | 290.55 | $< 2.2\ e^{-16}$ |
| Sex | 1 | 0.715 | 0.7147 | 17.479 | $4.4\ e^{-5}$ |
| Interaction | 2 | 0.863 | 0.4315 | 10.553 | $4.5\ e^{-5}$ |
| Residuals | 190 | 7.769 | 0.0409 | | |

| INTRASPECIFIC | Df | Sum Sq | Mean Sq | F | p |
|---|---|---|---|---|---|
| Cephalotorax | | | | | |
| Population | 2 | 10.236 | 5.118 | 26.144 | $2.2\ e^{-10}$ |
| Sex | 1 | 1.488 | 1.488 | 7.600 | $6.6\ e^{-3}$ |
| Interaction | 2 | 0.932 | 0.466 | 2.381 | 0.0962 |
| Residuals | 140 | 27.407 | 0.196 | | |
| Sternum | | | | | |
| Population | 2 | 2.497 | 1.249 | 27.649 | $7.6\ e^{-11}$ |
| Sex | 1 | 0.011 | 0.011 | 0.251 | 0.6175 |
| Interaction | 2 | 0.250 | 0.125 | 2.772 | 0.0660 |
| Residuals | 140 | 6.322 | 0.045 | | |

Table 8. Mean and standard deviation for cephalothorax and sternum (centroid size) in millimeters within species/population, and t-test results for sexual size dimorphism.

| | Females | | Males | | | | |
|---|---|---|---|---|---|---|---|
| | n | MEAN(SD) | n | MEAN(SD) | *t* | *d.f.* | *P* |
| Cephalothorax | | | | | | | |
| *P. azul* | 38 | 8.454 (.4943) | 35 | 8.062 (.4089) | 3.6763 | 71 | 0.00046 |
| *P. galianoae* | 34 | 6.569 (.4939) | 17 | 6.470 (.4137) | 0.7080 | 49 | 0.4823 |
| *P. ornata* (Maq) | 35 | 6.683 (.3978) | 37 | 6.958 (.3004) | -3.3324 | 70 | 0.00138 |
| *P. ornata* (Pas)[1] | 21 | 7.142 (.6392) | 14 | 7.055 (.3527) | 0.5175 | 32.13 | 0.6084 |
| *P. ornata* (Que) | 20 | 7.302 (.5687) | 19 | 7.621 (.3974) | -2.0158 | 37 | 0.05113 |
| Sternum | | | | | | | |
| *P. azul* | 38 | 4.024 (.2136) | 35 | 3.744 (.1885) | 5.9088 | 71 | $1.09\ e^{-7}$ |
| *P. galianoae* | 34 | 3.091 (.2462) | 17 | 2.980 (.1945) | 1.6161 | 49 | 0.1125 |
| *P. ornata* (Maq)[2] | 35 | 3.253 (.2158) | 37 | 3.282 (.1379) | -0.0672 | 57.26 | 0.5044 |
| *P. ornata* (Pas) | 21 | 3.509 (.2791) | 14 | 3.340 (.1681) | 2.0269 | 33 | 0.0508 |
| *P. ornata* (Que) | 20 | 3.561 (.2830) | 19 | 3.588 (.1835) | -0.3484 | 37 | 0.7295 |

[1] Variance difference: F = 3.2842; num d.f. = 20; den. d.f. = 13; p = .03207

[2] Variance difference: F = 2.4501; num d.f. = 34; den. d.f. = 36; p = .009246

Table 9. Summary of the main patterns observed along the applied analyses.

| | **Shape** | **Size** |
|---|---|---|
| **Interspecific** | 1. Sex is the main factor of variation (sexual shape dimorphism); | 1. Cephalothorax sexual size dimorphism observed only for the syntopic populations, and in inversed way; |
| | 2. Higly significant differences among species besides the cripticity; | 2. Sternum sexual size dimorphism only for *P. azul;* |
| | 3. Sternum is a better discriptor of species than cephalothorax; | |
| | 4. *P. azul* and *P. ornata* have closer shapes (more similar). | |
| **Intraspecific** | 1. Strong sexual shape dimorphism; | 1. Population is the main factor for both structures; |
| | 2. Populations can be split in two groups (Brazil and Uruguay); | 2. Each population has its own cephalothorax and sternum size; |
| | 3. Cephalothorax is a better descriptor of population and sex. | 3. Only *P. ornata* (Maq) showed sexual size dimorphism (see also interspecific comments above). |

Figure 1. Landmarks positions in each analyzed structure. A) Cephalothorax; B) Sternum. Scale bars = 1 mm.

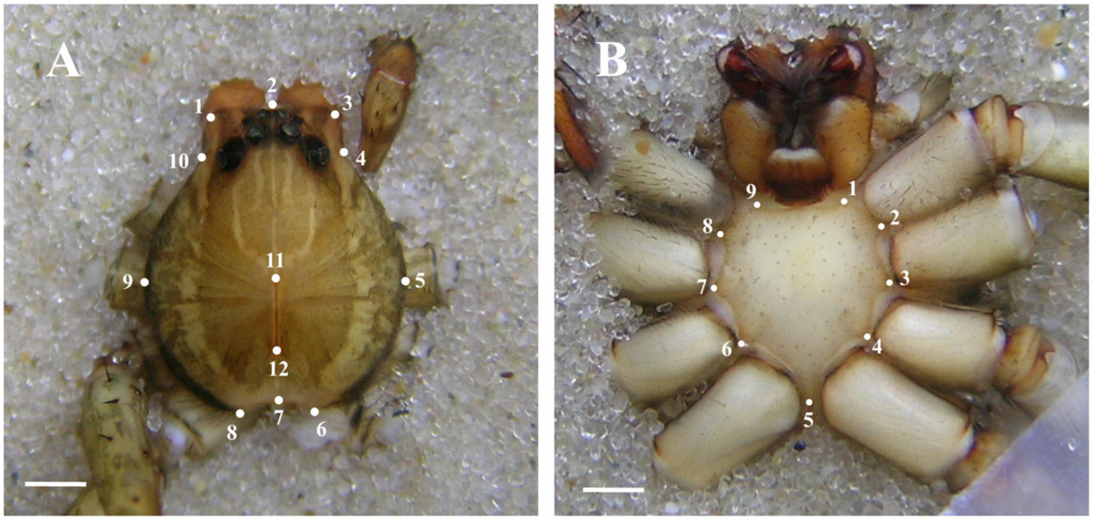


Figure 2. Principal Component Analysis for cephalothorax and sternum of interspecific data. Simbols represents the average shape of each factor level, with delimiting 95% confidence ellipses.

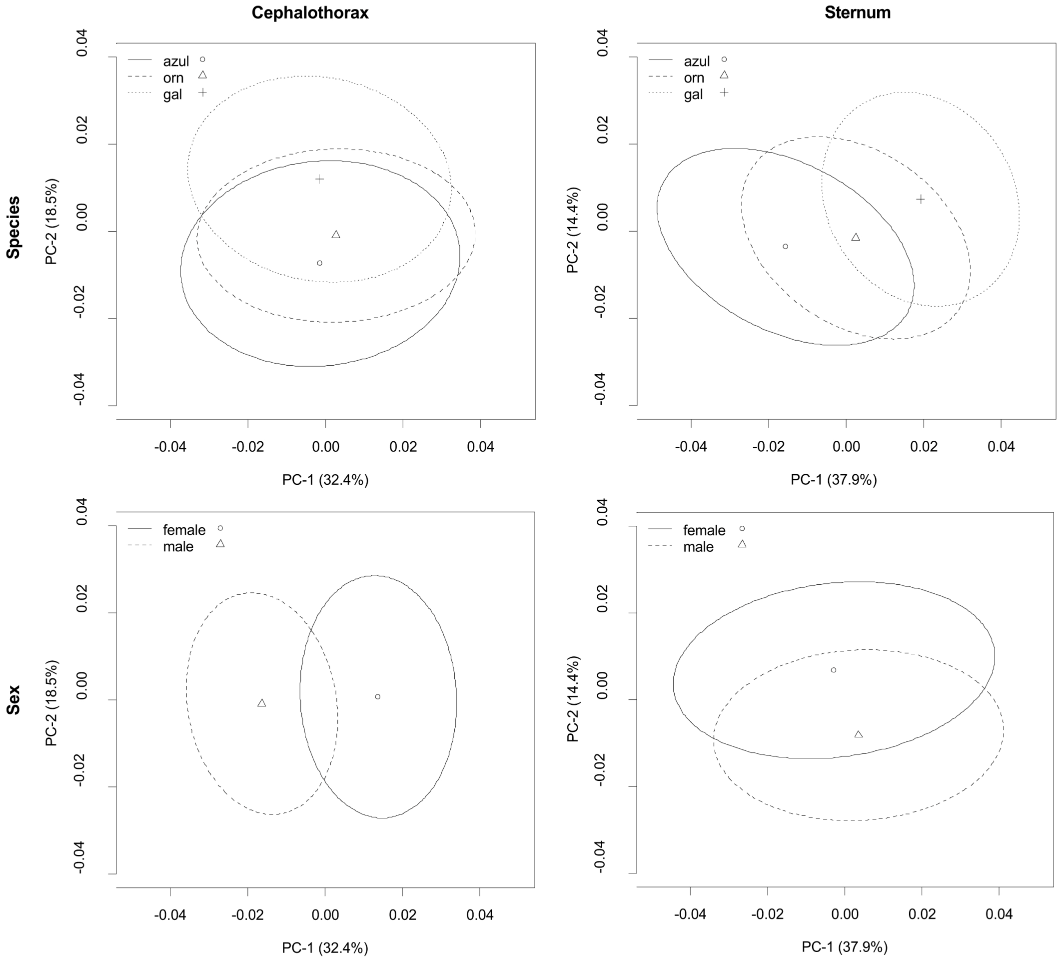


Figure 3. Extreme shapes found along the shape space spectrum described by the first principal component for cephalothorax and sternum in the interspecific and intraspecific data. Grey lines: males; black lines: females. Intermediate shapes probably do not have such evident dimorphism.

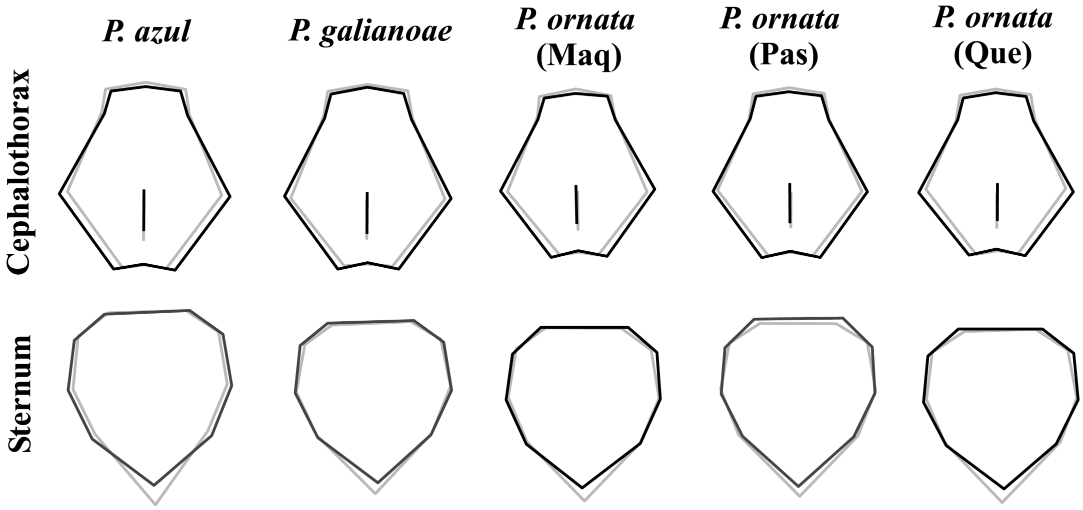
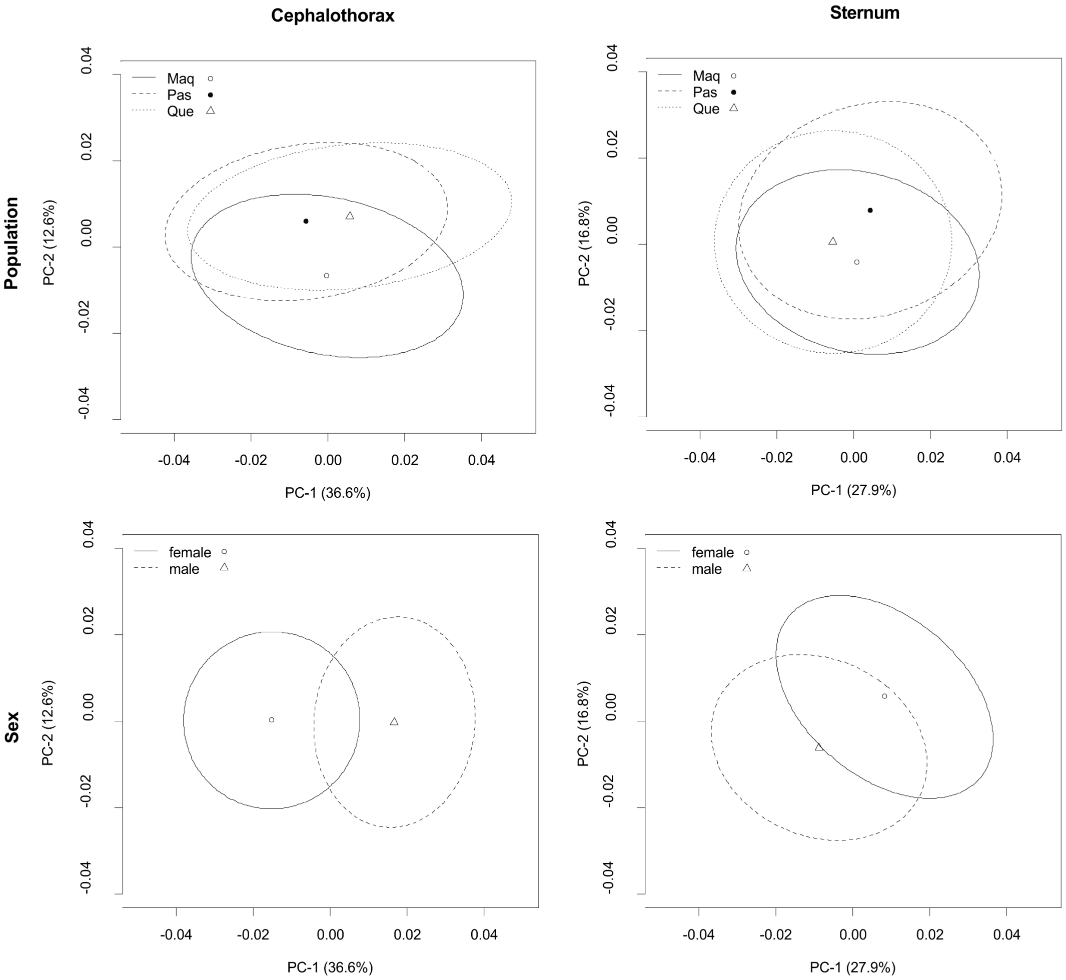


Figure 4. Principal Component Analysis for cephalothorax and sternum of intraspecific data. Simbols represents the average shape of each factor level, with delimiting 95% confidence ellipses.



# Capítulo 5

# Genitalic variation and taxonomic discrimination in *Paratrechalea* (Araneae; Trechaleidae)

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## Abstract

For spiders, morphological differentiation within genitalic traits is the main diagnostic criteria of a species. Besides some well described exceptions of genitalic polymorphism and crypticity, spider genitalic variation is seldom quantitatively analyzed. Using geometric morphometrics landmark analysis, we report clear evidence of quantitative interspecific divergence and intraspecific variation in the genital shape of three species of the genus *Paratrechalea* (*P. azul*, *P. ornata* and *P. galianoae*). The genitalic species recognition was very consistent with our quantitative data for both sexes. Interspecific variation suggested a character displacement pattern between two syntopic populations of *P. azul and P ornata*, and also a possible case of species crypticity in *P. ornata* that will involve the splitting of the Uruguayan populations from the Brazilian ones.

**Key words:** genitalic evolution; geometric morphometrics; taxonomy discrimination.

## Introduction

The line between what distinguishes a true species from a simple case of variation is not only subtle but also dependent on the species definition that is assumed (Bond *et al.* 2001; Huber 2004; Mutanen 2005). Within this fuzzy zone lies a large number of separate biological entities which remain undetected because of their morphological similarities (Adams & Funk 1997). Therefore, morphological species delimitation may pose a problem for some specific groups with a high degree of resemblance.

Systematic practice is based strongly upon the correlation of morphology and the recognition of the limits of true species, and there is no exception for spiders (Huber 2003, 2004; Huber *et al.* 2005). Spiders are one of the most diverse metazoan groups, with 40000 described species (Platnick 2008). The structure with the most diagnostic traits for spider species is the genitalia, which in general have a high degree of specificity associated with a faster evolutionary pace guided by mechanisms of sexual selection (Eberhard 1985; Arnqvist 1997; Hosken & Stockley 2004; Mutanen 2005; but see also Costa & Capocasale 1984; Pérez-Miles 1989; Jocqué 2002; Huber 2003; Huber *et al.* 2005).

Leaving processes aside, two opposing cases of genitalic phenotypic expression may pose a problem for the usual systematic species recognition: cryptic species and polymorphism. Both cases have been described for spiders (crypticity: Ramirez & Chi 2004; Johannesen *et al.* 2005; Huber *et al.* 2005; polymorphism: Pérez-Miles 1989; Huber & González 2001; Jocqué 2002), suggesting that neither may be an exception. Cryptic species complexes can be found in several organisms (Bond & Sierwald 2002; Muster *et al.* 2004) and they are often revealed by integration of molecular and/or behavioral tools, and not by the definition of morphological apomorphies (Adams & Funk 1997). On the other hand, genitalic polymorphism can be discovered by several methods, the best evidence being the hatching of different morphs from a single egg-sac.

During recent decades the establishment of geometric morphometrics as a new morphometric paradigm has made a significant contribution to the study of shape variation (Rohlf & Marcus 1993). For example, using geometric morphometrics, a cryptic complexes can be not only quantitatively described by its morphological properties, but also can allow the testing of differences in shape consensus for the observed groups. This approach has already been applied in some spider studies (Bond & Beamer 2006; Costa-Schmidt & Araújo, in review), and despite the limitations of using morphometrical data as phylogenetic characters (Zelditch *et al.* 2004; Bond & Beamer 2006), geometric morphometric tools can improve the capacity of taxonomic discrimination.

Here we present some quantitative analyses dealing with genital morphological variation within three species of the semi-aquatic spider genus *Paratrechalea* Carico 2005 (Araneae, Trechaleidae). The taxonomic status of two species in this genus, *P. azul* Carico 2005 and *P. ornata* (Mello-Leitão 1943), can be questioned, since they share strong morphological and ecological crypticity and an considerable visual intraspecific genitalic variation (Carico 2005; Silva *et al.* 2006). This last feature means that descriptions are difficult to apply to some populations, even though further analyses confirmed that these two species are indeed two different entities (Silva *et al.* 2006), which was corroborated by behavioral and non-genital morphometrical data (Costa-Schmidt *et al.* submitted; Costa-Schmidt & Araújo, in review).

The general aim of this work is to improve our knowledge of the morphometric properties of shape and size for genital traits of these species based on quantitative analyses. The specific aims are: 1) to quantify the visual genitalic variation observed within each species, 2) to identify what are the main differences among species, and 3) to present some systematic hypothesis regarding the patterns that this quantitative approach raises, such as taxonomic discrimination.

## Material and Methods

### *Study species and sampling design*

Three *Paratrechalea* species were analyzed in this work: *P. azul* Carico 2005, *P. ornata* (Mello-Leitão, 1943), and *P. galianoae* Carico 2005. These were the same species analyzed by Costa-Schmidt & Araújo (in review) for non-genital morphometrical relations. Besides the cryptic aspect among these species, *P. azul* and *P. ornata* have a marked genital resemblance in both males and females. *P. galianoae* is easily recognized based on genital morphology, thus the insertion of this species within the data set will serve as a control group for the levels of divergence found between the two other species.

The three species have nocturnal semi-aquatic habits, and are easily found living next to streams and rivers. *P. azul* and *P. ornata* have a syntopic distribution on the northeast region of Rio Grande do Sul (Brazil). The zone of overlap is not yet established. Sampling efforts did not find any isolated population of *P. azul* in the Rio Grande do Sul

State. We assume that they are sibling species, based on ecological requirements (authors' personal observations), on genitalic resemblance (Carico 2005; Silva *et al.* 2006), and by quantitative morphological analyses of non-genitalic characters (Costa-Schmidt & Araújo, in review).

Within their known distributions, *P. azul* and *P. ornata* can be found in second to higher order streams with altitudes ranging from sea level to 200m. *P. galianoae* seems to be strongly associated with a dense riparian vegetation coverage, typically found along first and second order streams in an altitudinal level starting from ca 300m. to above. There is a clear checker-board distribution between *P. ornata* and *P. galianoae* (LECS, unpublished data), and we assume that such distribution is a direct response of the habitat structure and altitudinal restrictions.

Comparisons were made of field-collected adult males and females from four different locations (Table 1). This is the same data set presented elsewhere (Costa-Schmidt & Araújo, in review) for non-genital characters, but analyzed here using a single model, i.e., we considered each sampled population as a single level of a classification factor. All specimens were preserved in 80% alcohol. Voucher specimens are deposited in the Museu de Ciências Naturais – Fundação Zoobotânica do Rio Grande do Sul (MCN-FZB/RS), and in the Facultad de Ciências (Montevideo – Uruguay).

### *Geometric morphometrics procedures*

Digital images were made by attaching a camera (Nikon Coolpix 5400) to the ocular of a stereomicroscope (Nikon SMZ600), made at the same magnification for each structure. An ocular scale was subsequently used to make pixel/millimeter conversions.

We have chosen from the literature as our object for analysis the genital structures used in species diagnoses. The genus diagnosis was presented by Carico (2005), emphasizing the ventral division of the median apophysis of the male pedipalpus and the external posterior-median scape of the female epigynum. Additionally, the same author and Silva *et al.* (2006) used the ectal division of the retrolateral tibial apophysis (Fig. 1A, RTA) as a component of the male diagnosis. So, based on these evidences and also on Huber (1995), we assumed that the analyses of the male ectal division of the RTA and the female epigynum scape (Fig. 1B) present most diagnostic information needed to infer the degree of divergence among species. Since males have two symmetrical palps (assumption made visually), we chose to analyze only the left palp RTA. Only Type II landmarks (Slice *et al.* 1996), i.e., those defined by local properties such as maximum curvatures, were plotted in each image. For the RTA, the following landmarks (Fig. 1A) were analyzed: #1, insertion point of the distal lobe into the palpal tibia; #2, maximum curvature of the distal lobe anterior margin; #3, mid-point of the distal lobe anterior margin; #4, tip of the distal lobe; #5, mid-point of the distal lobe posterior margin; #6, maximum curvature between the distal and basal lobes; #7, point of maximum inflection of the basal lobe anterior margin; #8, tip of the basal lobe; #9 & #10, equidistant inflexion points along the basal lobe posterior margin; #11, insertion point of the basal lobe into the palpal tibia. For the epigynum scape, the following landmarks (Fig. 1B) were analyzed: #1, #2, #8 & #9, connection points of the scape with the posterior margin of the anterior field; #3, #4, #6, #7, maximum inflexion points on the margin of the inner fold; #10, #11, #13, #14, points of maximum inflexion on the rim of the scape; #12, midline point on the posterior rim of the scape; #5, midline point on the margin of the inner fold.

Using a simple morphological formula, “form = shape + size”, we first isolated these two morphological components (also addressed here as morphological properties). To isolate all landmark configurations, we applied a General Procrustes Superimposition analysis (GPS). Each landmark configuration was translated, rotated and scaled to the unit centroid size, using a least squares procedure. After this treatment, each configuration was described by a single point within a multidimensional space, called the pre-form space. Because of its multidimensionality and non-Euclidian properties, the pre-form residuals were projected into a plane that is tangent to the mean pre-form shape, where Euclidian properties are fulfilled and conventional statistical analyses were applied. After this projection, a Principal Component Analysis was made over the scores of this projection, composing the shape variables. More information about these methods can be found elsewhere (Rohlf & Slice 1990; Zelditch *et al.* 2004).

Size component was estimated by centroid size, which is defined as “the square root of summed squared distances of landmarks from their centroid” (Swiderski 2003). The advantage of this method is that it takes an overall size measurement of the form, while linear estimates of size underestimate other two-dimensional variables of the same structure.

### *Shape analysis*

Genital structures were tested by a one-way MANOVA using species and populations as a descriptor factor composed by the following levels: *P. azul*, *P. galianoae*, *P. ornata* (Maq) (*Maquiné* population), *P. ornata* (Pas) (*Paso del Molino* population), and *P. ornata* (Que) (*Quebrada de los Cuervos* population). Pairwise comparisons were performed between all possible combination levels using the same model in the case of significant analysis. The probability results of these pairwise tests were corrected with a Bonferroni correction for multiple comparisons. Discriminant analysis based on shape variables was applied in order to verify the percent of correct classifications achieved by the tested factor, which is a way to confirm if the informed classification within the factor levels do represent variation among groups.

Because we could describe an average shape for each considered level using shape variables only, we were able to estimate the Mahalanobis distance as a disimilarity measure of shape distance among the average shapes under analysis. This estimate is directly related to morphological divergence, i.e., the higher this estimated value the bigger the morphological divergence. Permutation tests among individuals of each pairwise group comparison were done in order to verify if the estimated distances between those groups could not be achieved by random sampling.

### *Size analysis*

We investigated size differences among species and populations using the centroid size as a variable. Centroid size showed a normal distribution for our samples (Kolmogorov-Smirnov test: epyginum, $p \geq 0.3853$; RTA, $p \geq 0.7304$). We performed a one-way ANOVA in order to test differences in size among the levels (same factor used in the MANOVA analysis). If significant, a post-hoc Tukey test was performed to compare them.

### *Software*

TpsUtil v. 1.33 (Rohlf 2004) was used to create a file containing the sequence of pictures to be analyzed. This file was loaded in the tpsDig 2.04 software (Rohlf 2005) for plotting of landmarks. Both programs are available at <http://life.bio.sunysb.edu/morph/>.

Most morphometric analyses and graphic outcomes were made using the RMorph library (Baylac 2007) developed for R environment (R Development Core Team 2007).

## Results

The analyses suggest that shape is the most informative morphological property in assigning groups. Size showed different patterns within each genital structure, demanding different interpretations of their size variation. The overall findings can be summarized by four statements. First, both structures presented the same behavior, i.e., they gave similar results about species or populations grouping. Second, *P. galianoae* showed the most divergent shape pattern in all analyses, thus corroborating the assumption of its use as an outgroup for the analysis of *P. azul* and *P. ornata* relationship. Third, there is an expected shape difference among species for both genital structures, with *P. azul* and *P. ornata* populations being closer within the shape space. Fourth, all shape analyses indicate that two populations, initially presumed to be *P. ornata,* can be interpreted as being two different taxonomic entities.

### *Shape analysis*

Genital structures showed strong differentiation among groups for all performed analyses. Principal Component Analysis (PCA) indicated the presence of at least three groups based on the first two components (PC1 and PC2 – Fig. 2 and Fig. 3), corresponding to the three studied species. The addition of the third PC allows us to distinguish another group, composed of the Uruguayan populations of *P. ornata* (supplement material).

Discriminant analysis confirms that the assumed classification explains the observed shape variation for both RTA and epigynum shape variables (Table 2), i.e., genital structures are good predictors of species classification. This analysis also suggests that the group formed by the Uruguayan populations is consistent, with a few misclassifications including a single female specimen from Paso del Molino that was initially placed as being from Maquine.

MANOVA models were highly significant for both genital structures (Table 3). All pairwise comparisons showed the same highly significant levels, i.e., the samples have different shapes of RTA and epigynum (Table 3). Again, we observed a splitting behavior between Uruguayan *P. ornata* populations from the Brazilian population. The comparison between Paso del Molino and Quebrada de los Cuervos samples were also significant for both structures, but with contrasting lower significant levels in relation to other comparisons (Table 3, last row).

Mahalanobis distance between the samples mean shapes also confirms the same diverging pattern of *P. ornata* (Table 4). It is important to emphasize that the observed Mahalanobis distance for RTA among Maquiné and the Uruguayan populations were even higher than that between *P. ornata* and *P. azul*. This was not observed for the epigynum, however, which showed lower Mahalanobis distances when comparing the Maquine population with the Uruguayan ones (Table 4). Permutation tests indicate that the estimated distances were significant for all comparisons and for both structures ($p \leq 0.0003$ for *P. ornata* (Pas) and *P. ornata* (Que) comparisons; $p \approx$ zero for all other comparisons).

### *Size analysis*

The statistical summary for the genital structures is presented in Table 5. From this table we show that the *P. ornata* (Maq) population not only presented the smaller mean epigynum size, but also the smaller coefficient of variation (Table 5). All other samples have similar range values, which indeed have little overlap with the *P. ornata* (Maq) range.

The size of genital structures was highly significant different among the samples (Table 6), but without concordance along the pairwise comparisons (Table 7), demanding separate descriptions for each structure. The RTA showed a pattern that can be described by species differences, since the only non-significant comparisons were among the three *P. ornata* populations (Table 7). Among the three species, *P. azul* has the largest RTA, followed by *P. ornata*, while *P. galianoae* has the smallest (Table 5).

The epigynum showed a pattern of differentiation that is not straightforward. Most of the observed differences were in relation to *P. ornata* (Maq) (Table 7). The only exception was between *P. galianoae* against *P. ornata* (Pas) that presented significant different epigynum mean sizes, but with a lower significant level compared with those involving *P. ornata* (Maq) (Table 7).

## Discussion

Here we report the application of a robust morphometric method to discriminate among a small group of samples representing three putatively related spider species. The outcome of such analyses ranged from the obvious determination of spider species using their genital shape, to the recognition of a division within what was once considered a single species. This latter conclusion has important and deep roots in the standard methodology used in spider systematics. Much of spider systematics has been based upon the assumption that genital specificity (Huber 2003, 2004) is directly linked to the widespread pattern of a faster rate of genital evolution (Eberhard 1985; Hosken & Stockley 2004). So, for genital traits, we are facing the challenging task of distinguishing between interspecific shape divergence or intraspecific shape variation.

The application of geometric morphometrics proved to be highly important for the discrimination of cryptic spider species within two different taxonomic situations. The first one deals with two already described taxonomic entities that have a strong niche overlap (name species here), but with known behavioral reproductive isolation and non-genital morphometric differences (Costa-Schmidt *et al.* submitted; Costa-Schmidt & Araújo in prep.). The second case deals with our findings that within *P. ornata*, there are two cryptic entities which exibit clear spatial niche segregation (geographical allopatry).

Evolutionary explanations for the observed pattern are suggested, based on the available information about the involved species and populations, which opens the subject for further studies and/or interpretations. On the other hand, the observed split of *P. ornata* demands a taxonomic description based on consistent characters identified in each group.

### ***Genital variation between*** *P. azul* ***and*** *P. ornata*

This work fulfills an important aspect related to the morphological variation of genital structures and the recognition of distinct species within the genus *Paratrechalea*. Since the beginning of our efforts in studying *P. azul* and *P. ornata*, we were able to gather fundamental information supporting their taxonomic status as two different species (non-genital morphometry: Costa-Schmidt & Araújo, in review; reproductive behavior: Costa-Schmidt *et al.* submitted), and this was also corroborated here by information gained from genitalic analysis.

The previously described genital variation within these two species shows quantitatively that the variation does not overlap along their shape space, mainly for the syntopic interspecific samples from Maquine. We believe that such divergence may be due to a reinforcement mechanism, such as character displacement (Brown & Wilson 1956), avoiding possible hybrid formation between the syntopic populations. The hypothesis of character displacement was also raised in the analysis of non-genital structures, especially for those populations sampled along the region of syntopy (Costa-Schmidt & Araújo, in review; further comments below).

### *Possible evolutionary explanations for the* P. ornata *variation*

The degree of divergence found among *P. ornata* populations raises important and complementary evolutionary hypotheses to explain the observed pattern. Each hypothesis demands an appropriate sample design, and the following comments place us upon the unstable ground of speculative explanation. Even so, we will present our interpretation for the patterns observed within our data.

#### *Intraspecific geographic variation*

The intraspecific differences observed in our analyses can be considered a simple case of geographical variation resulting from ecological restriction of species that live near the margins of bodies of water. Creation of new colonies is more feasible **within** the same river basin, while a gene flow barrier may impede such colonization **between** river basins. Thus, in the latter case, morphological variation may result from genetic drift in founder populations which develop from individuals that cross the barrier.

The Brazilian population is several hundred kilometers distant from the Uruguayan ones (~ 550km from Quebrada de Los Cuervos; ~ 700km from Paso del Molino). Therefore, we did not sample geographically intermediate populations to confirm or reject our conclusion that the observed pattern can be a result of the colonizing stages of marginal populations (e.g., Maquine).

#### *Character displacement between P. azul and P. ornata*

The hypothesis that the observed variation among *P. ornata* populations is a consequence of geographical variation does not exclude the action of other mechanisms of shape variation, such as character displacement (Goldberg & Lande 2006; Adams & Collyer 2007). This hypothesis was previously proposed by Costa-Schmidt & Araújo (in review), and we believe that the morphological relations between the syntopic samples from Maquine further indicates the occurrence of such mechanism.

Two predictions for the occurrence of character displacement were taken from Ware & Opell (1989 – p. 150). First, genitalic features should show greater differences in regions of sympatry than in regions of allopatry. Our data fit with this prediction, at least for the epigynum, based on the estimated Mahalanobis distances in shape consensus of each group (Table 4). Second, the genitalia should be less variable within areas of sympatry. Our data showed that the range of genital size and respective CV values were more pronounced within the isolated *P. ornata* populations than in the Maquine population (Table 5). Based on these two predictions we believe that the present data serve as evidence that such mechanism took place within this system.

If we assume that the observed morphological patterns represent a case of character displacement, we must show which major factor caused it rather than competitive exclusion. Field observations indicate that the morphological crypticity between *P. azul* and *P. ornata* includes strong niche overlapping. The only divergent aspect found until now is sexual communication with a strong recognition mechanism which involves a supposed chemical component (unpublished data). So, if character displacement is truly a mechanism acting along those syntopic populations, it is probably related to reproductive isolation, i.e., to a reinforcement in reproductive signaling, which affect the morphological properties of these spiders.

The possible action of character displacement is more related to non-sexual selection explanations of genitalic evolution, such as lock-and-key mechanisms, which are at odds with current explanations for genitalic evolution (Eberhard 1985; Ware & Opell 1989; Hosken & Stockley 2004; Huber 2004). However, even stating that lock-and-key may have its importance for genitalic evolution, we do not close the possible action of other sexual selective mechanisms due to the fact that we only analyzed one specific genitalic structure from each sex. As posed by Costa-Schmidt & Araújo (in review), a proper sampling design must be done in order to have stronger evidence for the presence of such an evolutionary mechanism.

### *Genital morphology species-specificity*

Another important aspect is the almost impossible discovery of genital polytypism within a species (Huber 2003), mainly if those morphs are dispersed along a geographical continuum where they can be easily misinterpreted as different species. In other words, genitalic differences found along the spatial distribution of a single species may lead to taxonomic inflation because of systematic methodological bias. Moreover, we cannot deny the huge influence that Mayr's non-dimensional definition has on taxonomic practice (Mayr 1963; Huber 2003), which is the basis of species-specific genitalic morphology.

As we did find sharp limits for shape component of male and female genitalia, we must try to translate those differences into something that can be used by a taxonomist when analyzing a particular sample, i.e., we must apply some effort in order to describe which shape trait is shared in each group (Zelditch *et al.* 2004). Such information was necessarily proposed elsewhere (Carico 2005, Silva *et al.* 2006), consisting of formal descriptions of diagnostic characters of each species.

This effort will be presented elsewhere, when additional diagnostic characters that better describe the studied species will be followed with the description of a new taxonomic entity. This is not done in this paper for the following reasons: First, we are not sure if we have gathered enough information that justifies the splitting of *P. ornata* in two species, or if we maintain a more conservative strategy to place them at the sub-species level. Second, even if a new species is proven to true, we must compare the identified groups to the type material of *P. ornata* in order to conclude which group (Maquine or the Uruguayan populations) is considered the new species, and construct the proper description.

### *Conclusions*

In a broader sense, the observed interspecific divergence patterns were expected in relation to empirical and theoretical data of genital traits used in taxonomy. The number of shape differences among species was lower between *P. azul* and *P. ornata*, which may induce us to believe that they are sister species, even though such assumption will be only solved after a robust cladistic analysis. Intraspecific analysis showed an interesting divergent pattern suggesting that we have two separate taxonomic entities within the *P. ornata* dataset.

It is still early to state an evolutionary explanation in this system since fundamental reproductive aspects (like the presence of polyandry) remain to be answered. Subsequent approaches must be applied in order to better evaluate the hypothesis raised. For example, careful sampling design associated with the analytical procedures presented here would allow us to understand if character displacement did have influence over the evolutionary history of these spider species.

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Table 1. Sampling location, city, country, geographic coordenates, and sample size of each studied population.

| | | | **Sample size** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | ***P. azul*** | | ***P. ornata*** | | ***P. galianoae*** | |
| **Sampling location** | **City; Country** | **Coordinates** | **Females** | **Males** | **Females** | **Males** | **Females** | **Males** |
| Pedra de Amolar River - Barra do Ouro | Maquiné; Brazil | 29°32'20.52"S; 50°14'46.83"W | 38 | 35 | 35 | 37 | - | - |
| Santa Lucía River - Paso del Molino | Minas; Uruguay | 34°16'40.10"S; 55°14'00.80"W | - | - | 21 | 14 | - | - |
| Yerbal Chico River - Quebrada de los Cuervos | Treinta y Tres; Uruguay | 32°55'30.50"S; 54°27'33.10"W | - | - | 20 | 19 | - | - |
| Pedras Brancas River - Pedra Branca Fall | Itati; Brazil | 29°23'45.59"S; 50°02'42.44"W | - | - | - | - | 34 | 17 |

Table 2. Correct classification percentages achieved by discriminant analyses.

| | RTA | Epigynum |
|---|---|---|
| *P. azul* | 100% | 100% |
| *P. galianoae* | 100% | 100% |
| *P. ornata* (Maq) | 100% | 100% |
| *P. ornata* (Pas) | 85.7% | 71.4% |
| *P. ornata* (Que) | 100% | 90% |

Table 3. MANOVA of shape principal components for species/population (the first 18 and 24 PCS were selected for RTA and epigynum scape respectively), and pairwise comparisons for all levels combinations.

| | RTA[a] | | | EPIGYNUM[b] | | |
|---|---|---|---|---|---|---|
| | Wilks | F | p | Wilks | F | p |
| Samples (df = 4) | 0.0003 | 38.804 | $< 2.2\ e^{-16}$ | 0.0007 | 26.501 | $< 2.2\ e^{-16}$ |
| Residuals (df = 117) | | | | | | |
| **Pairwise comparisons:** | | | | | | |
| *P. azul* vs. *P. galianoae* | 0.0484 | 109.1597 | $2.3\ e^{-57}$ | 0.0463 | 103.0024 | $1.3\ e^{-68}$ |
| *P. azul* vs. *P. ornata* (Maq) | 0.0828 | 61.5561 | $7.7\ e^{-46}$ | 0.1267 | 34.4585 | $8.7\ e^{-43}$ |
| *P. azul* vs. *P. ornata* (Pas) | 0.0598 | 87.3079 | $8.2\ e^{-53}$ | 0.2015 | 19.8172 | $4.0\ e^{-31}$ |
| *P. azul* vs. *P. ornata* (Que) | 0.0564 | 92.9630 | $4.4\ e^{-54}$ | 0.2411 | 15.7367 | $1.1\ e^{-26}$ |
| *P. galianoae* vs. *P. ornata* (Maq) | 0.0590 | 88.5747 | $4.2\ e^{-53}$ | 0.0217 | 225.5691 | $3.0\ e^{-88}$ |
| *P. galianoae* vs. *P. ornata* (Pas) | 0.1092 | 45.3268 | $6.2\ e^{-40}$ | 0.0299 | 162.4213 | $5.9\ e^{-80}$ |
| *P. galianoae* vs. *P. ornata* (Que) | 0.0851 | 59.7632 | $2.9\ e^{-45}$ | 0.0300 | 161.9082 | $7.2\ e^{-80}$ |
| *P. ornata* (Maq) vs. *P. ornata* (Pas) | 0.1438 | 33.0732 | $4.4\ e^{-34}$ | 0.4001 | 7.4973 | $1.4\ e^{-14}$ |
| *P. ornata* (Maq) vs. *P. ornata* (Que) | 0.1176 | 41.6779 | $2.4\ e^{-38}$ | 0.3196 | 10.6436 | $7.7\ e^{-20}$ |
| *P. ornata* (Pas) vs. *P. ornata* (Que) | 0.5919 | 3.8304 | $7.7\ e^{-6}$ | 0.7302 | 1.8473 | 0.016485 |

[a] Samples: num df = 72, den df = 395.6; Pairwise comparisons: df1 = 18, df2 = 100

[b] Samples: num df = 96, den df = 477.9; Pairwise comparisons: df1 = 24, df2 = 120

Table 4. Estimated Mahalanobis distances between the average shape of the compared groups. Above diagonal: species/population comparisons for epigynum scape. Below diagonal: species/population comparisons for RTA.

| | *azul* | *gal* | *orn* (Maq) | *orn* (Pas) | *orn* (Que) |
|---|---|---|---|---|---|
| *azul* | - | 273.80 | 65.98 | 37.32 | 30.05 |
| *gal* | 207.19 | - | 374.06 | 357.83 | 367.72 |
| *orn* (Maq) | 56.37 | 160.14 | - | 16.34 | 23.92 |
| *orn* (Pas) | 147.95 | 124.33 | 68.58 | - | 5.16 |
| *orn* (Que) | 140.68 | 140.28 | 69.92 | 10.01 | - |

Table 5. Sample size (n), mean, standard deviation (s.d.), range, and coefficient of variation (CV) of centroid size for RTA and epigynum scape.

| **Species/Population** | **n** | **Mean** | **s.d.** | **Range** | **C.V.** |
|---|---|---|---|---|---|
| **RTA** | | | | | |
| *P. azul* | 35 | 1.055 | 0.03744 | 0.956 - 1.135 | 0.03549 |
| *P. galianoae* | 17 | 0.866 | 0.05849 | 0.776 - 0.971 | 0.06754 |
| *P. ornata* (Maq) | 37 | 0.914 | 0.03190 | 0.838 - 1.005 | 0.03490 |
| *P. ornata* (Pas) | 14 | 0.941 | 0.03889 | 0.870 - 0.994 | 0.04135 |
| *P. ornata* (Que) | 19 | 0.922 | 0.04328 | 0.856 - 1.014 | 0.04695 |
| **Epigynum** | | | | | |
| *P. azul* | 38 | 1.001 | 0.05806 | 0.892 - 1.124 | 0.05800 |
| *P. galianoae* | 34 | 0.964 | 0.09717 | 0.769 - 1.164 | 0.10080 |
| *P. ornata* (Maq) | 35 | 0.847 | 0.04220 | 0.760 - 0.940 | 0.04982 |
| *P. ornata* (Pas) | 21 | 1.025 | 0.06506 | 0.903 - 1.143 | 0.06347 |
| *P. ornata* (Que) | 20 | 1.004 | 0.06980 | 0.780 - 1.123 | 0.06952 |

Table 6. Centroid size one-way ANOVA of RTA and epigynum scape.

| | Df | Sum Sq | Mean Sq | F | p |
|---|---|---|---|---|---|
| RTA | | | | | |
| Samples | 4 | 0.569 | 0.1422 | 86.438 | $< 2.2 e^{-16}$ |
| Residuals | 117 | 0.192 | 0.0016 | | |
| Epigynum | | | | | |
| Samples | 4 | 0.640 | 0.1599 | 33.922 | $< 2.2 e^{-16}$ |
| Residuals | 143 | 0.674 | 0.0047 | | |

Table 7. Significant levels from Tukey test for centroid size of RTA and epigynum scape.

| | RTA (p)[a] | Epigynum (p)[b] |
|---|---|---|
| *P. azul* vs. *P. galianoae* | < 2.2 $e^{-16}$ | NS |
| *P. azul* vs. *P. ornata* (Maq) | < 2.2 $e^{-16}$ | < 2.2 $e^{-16}$ |
| *P. azul* vs. *P. ornata* (Pas) | < 2.2 $e^{-16}$ | NS |
| *P. azul* vs. *P. ornata* (Que) | < 2.2 $e^{-16}$ | NS |
| *P. galianoae* vs. *P. ornata* (Maq) | 8.4 $e^{-4}$ | < 2.2 $e^{-16}$ |
| *P. galianoae* vs. *P. ornata* (Pas) | 1.3 $e^{-5}$ | 0.0141 |
| *P. galianoae* vs. *P. ornata* (Que) | 6.7 $e^{-4}$ | NS |
| *P. ornata* (Maq) vs. *P. ornata* (Pas) | NS | < 2.2 $e^{-16}$ |
| *P. ornata* (Maq) vs. *P. ornata* (Que) | NS | < 2.2 $e^{-16}$ |
| *P. ornata* (Pas) vs. *P. ornata* (Que) | NS | NS |

[a] NS: p > 0.2362

[b] NS: p > 0.1636

Fig 1. Landmarks positions within each analyzed structure: A) ectal division of the retrolateral tibial apophysis (11 landamarks); B) epigynum scape (14 landmarks). Pictures take from *Paratrechalea ornata* specimens. Bars = 1mm.

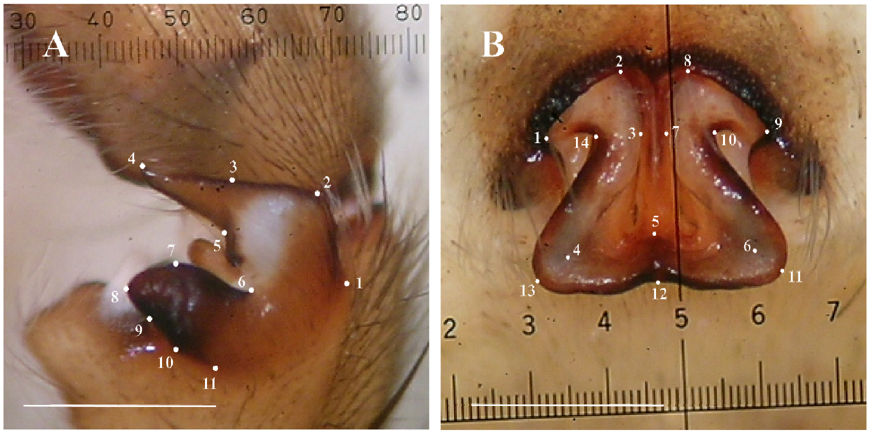


Fig 2. Projections of the first and second scores of the Principal component analysis for RTA and epigynum scape. Symbols represents the consensus shape of each species/population, with delimiting 95% confidence ellipses.

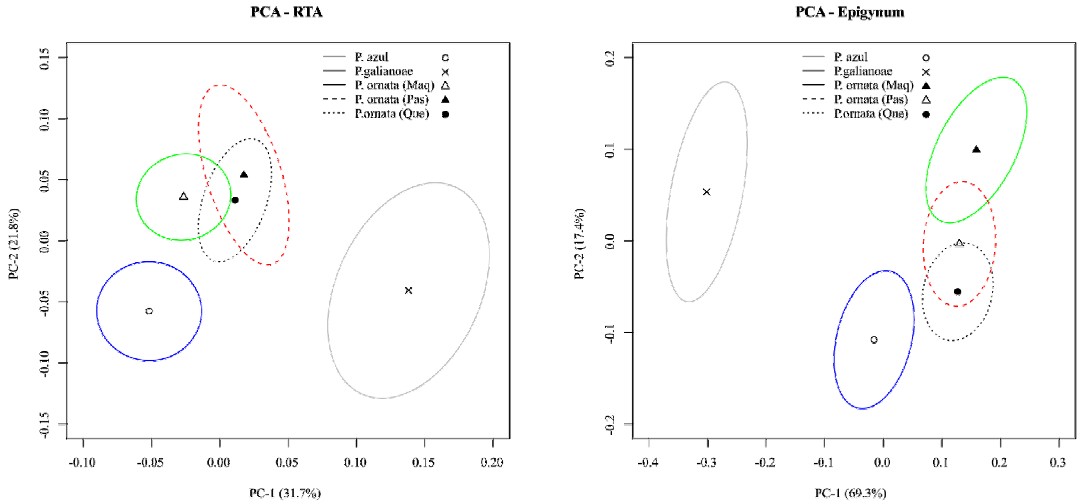
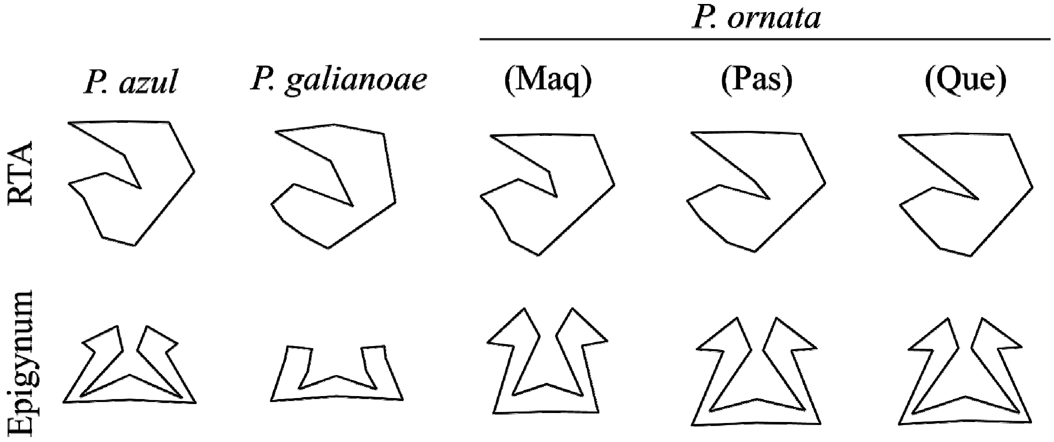
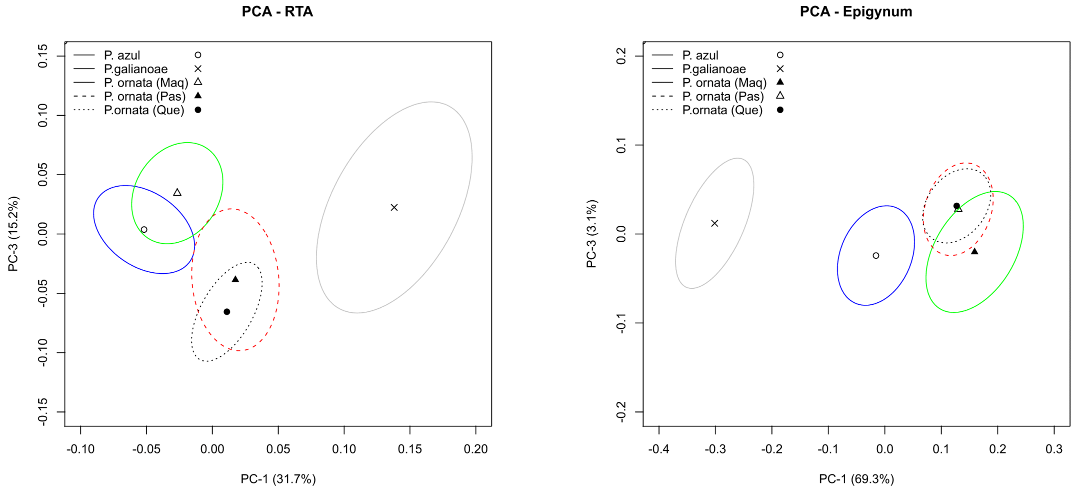


Fig 3. Shape consensus observed for each species/populations within the first component of a Principal Component Analysis.



**Supplement material.** Projections of the first and third scores of the Principal component analysis for RTA and epigynum scape. Symbols represents the consensus shape of each species/population, with delimiting 95% confidence ellipses.



# Capítulo 6

# Alometria estática de genitália em *Paratrechalea*

Grande parte da teoria sobre evolução da forma da genitália tem como referência o trabalho de Eberhard *et al.* (1998), onde foi proposta a chamada hipótese *one-size-fits-all*, onde seus pressupostos teóricos foram estabelecidos e discutidos a partir de dados empíricos de 20 espécies de insetos e aranhas. Toda a argumentação deste trabalho segue uma linha de raciocínio elegante e obviamente vinculada à teoria do próprio Eberhard de evolução da genitália através da escolha críptica da fêmea (Eberhard 1985, 1996, 2004).

Os pressupostos da *one-size-fits-all* podem ser resumidos da seguinte maneira: a) caracteres genitais de machos apresentam um padrão de alometria negativa quando escalonados por alguma medida de tamanho corporal total; b) os coeficientes de variação (CV) de caracteres genitais de machos são menores do que os CV de caracteres somáticos; c) alometria negativa e valores reduzidos de CV indicariam a presença de uma força seletiva (sexual) estabilizadora.

Recentemente alguns autores demonstraram, através de modelos teóricos e dados empíricos, que a interpretação dos coeficientes de alometria como assinaturas dos processos seletivos atuantes sobre as estruturas analisadas não é tão intuitiva quanto imaginada, contrapondo o terceiro pressuposto apresentado acima (Bonduriansky & Day 2003; Bonduriansky 2007; Bertin & Fairbairn 2007). Sendo assim, podemos dizer que alometria negativa e valores reduzidos de CV para caracteres genitais de machos é um fato disseminado em artrópodes, porém o que origina tal relação permanece um mistério.

Um fato curioso é a escassez de exemplos onde a genitália das fêmeas também é contemplada pelas análises (Polihronakis 2006). Alguns trabalhos envolvendo fêmeas de aranhas estão disponíveis (Pérez-Miles 1989; Uhl & Vollrath 2000; Ramos *et al.* 2005), onde são apresentados alguns resultados contraditórios, mas que representam a complexidade em buscar um padrão possivelmente aplicável a todo o grupo. Destes, destaco o trabalho de Ramos *et al.* (2005), que apresenta uma hipótese de independência na taxa evolutiva das genitálias de machos e de fêmeas (*positive genitalic divergence hypothesis*).

## Materiais e métodos

### *Espécies de estudo*

Para a análise das relações alométricas das genitálias de machos e de fêmeas, optamos por concentrar nossas análises nas populações de espécies sintópicas de Maquiné (*P. azul* e *P. ornata*) e as duas populações oriundas do Uruguai.

### *Medidas morfológicas avaliadas*

As dimensões das genitálias de machos e de fêmeas foram tomadas a partir do conjunto de marcos anatômicos apresentados no Capítulo 5. A estrutura genital avaliada em machos foi a apófise tibial retrolateral (ATR). Dada a simetria desta estrutura para cada palpo, estabelecemos que apenas as ATR dos palpos esquerdos seriam analisadas. Para as fêmeas, foi avaliado o escapo do epígeno, estrutura genital externa usualmente utilizada para a determinação específica. Maiores detalhes sobre os procedimentos de aquisição e tratamento das imagens, posicionamento dos marcos anatômicos e critérios de escolha das estruturas genitais podem ser encontrados no Capítulo 5.

A partir das matrizes de coordenadas dos marcos anatômicos provenientes de cada peça, medimos as distâncias lineares entre marcos anatômicos de interesse, e que representam as principais dimensões de cada estrutura. Para a ATR foram analisadas (Fig 1a): a) margem anterior do lóbulo distal (DAM); b) comprimento do lóbulo distal (DL); c) margem posterior do lóbulo distal (DPM); d) margem anterior do lóbulo basal (BAM); e) largura da ATR (RW); f) comprimento da ATR (RL). Para o epígeno foram analisadas as seguintes dimensões (Fig 1b): a) largura da margem posterior do campo médio (PMW); b) largura anterior do escapo (SAW); c) largura posterior do escapo (SPW); d) comprimento lateral do escapo (SLL); e) comprimento central do escapo (SCL).

Como uma medida de tamanho corporal, utilizamos o tamanho do centróide do cefalotórax (CSize), estimado através dos mesmos marcos anatômicos apresentados no Capítulo 4. A utilização desta estrutura como um estimador do tamanho corporal total do indivíduo é tradicional em aracnologia, muito embora esta seja a segunda em vez que uma medida mais integrada desta estrutura é utilizada (a outra ocasião é referente ao Capítulo 3 desta tese). Os tamanhos do centróide para as duas estruturas genitais em análise também foram calculados (RSize e ESize para ATR e epígeno, respectivamente), visando assim ter uma variável mais integrada.

*Análises estatísticas*

Todas as variáveis foram testadas para normalidade através do modelo de Kolmogorov-Smirnov (valores de $p$ variando de 0.272 a 0.999).

Índices de alometria (a declividade da reta de regressão da variável de estudo pela variável de tamanho corporal) foram obtidos pela regressão linear dos dados originais *log* transformados. Dois conjuntos de comparação foram realizados para cada estrutura genital, sendo o primeiro referente a regressão das variáveis de interesse pelo CSize, e a segunda relacionada a regressão destas variáveis pelo tamanho global da estrutura (RSize e ESize).

Dois modelos de regressão linear foram utilizados: o modelo dos mínimos quadrados (*ordinary least squares* – OLS) e o modelo do eixo principal reduzido (*reduced major axis* – RMA). Para maiores comentários sobre cada destes modelos, consultar o Capítulo 3 desta tese. Cada índice de alometria foi testado para a hipótese nula de isometria, isto é, onde $\beta = 1$.

Todos os procedimentos envolvendo o cálculo das distâncias lineares foram realizados utilizando R (R Development Core Team 2007), bem como o cálculo dos tamanhos dos centróides. Os modelos de regressão foram rodados através do programa PAST v. 1.75b (Hammer *et al.* 2001), utilizando os procedimentos de auto-reamostragem padrões do programa (2000 repetições) para o cálculo dos intervalos de confiança dos índices de alometria.

## Resultados

O tamanho das genitálias dos machos e das fêmeas apresentaram um nítido padrão de alometria negativa quando regredidos em relação ao tamanho do cefalotórax, onde as fêmeas apresentaram menores valores de alometria (Tabela 1). A única exceção foi observada em *Paratrechalea* sp.n., que é a mesma espécie que possui dimorfismo sexual de tamanho invertido ($\text{Cefalotórax}_{\text{machos}} > \text{Cefalotórax}_{\text{fêmeas}}$; Capítulo 4).

O padrão de alometria negativa é mantido quando as distâncias lineares internas das estruturas genitais são regredidas pelo tamanho do cefalotórax (imagens nas linhas superiores das Fig. 2 e 3; para dados completos ver Apêndices I e II para machos e fêmeas, respectivamente). Entretanto, algumas amostras não foram tão consistentes ao padrão de alometria negativa, principalmente para a ATR em *P. ornata* que apresentou medidas isométricas (linhas superiores das Fig. 2 e 3). Mesmo assim, os valores absolutos dos coeficientes de regressão obtidos pelo modelo de *OLS* apresentaram uma tendência a alometria negativa. Um único caso de alometria positiva foi observado para a *SCL* das fêmeas de *P. azul* pelo modelo de *RMA*, que por sua vez contrasta com o valor absoluto obtido para a mesma medida pelo modelo *OLS*.

Quando as medidas lineares são escalonadas em relação ao tamanho da genitália, surgem algumas diferenças nos padrões de alometria entre as amostras (linhas inferiores das Fig. 2 e 3; Apêndices III e IV para machos e fêmeas, respectivamente). Para os machos (Fig. 2), *P. azul* e *Paratrechalea* sp.n. compartilham um padrão de alometria negativa para as mesmas medidas se incluirmos o coeficiente alométrico de *DL* de *Paratrechalea* sp.n. que foi marginalmente não-significativo ($p$ = 0.0642). Estas duas espécies apresentam um padrão inverso para as medidas de largura e comprimento da ATR, onde *P. azul* apresentou alometria positiva para as larguras e isometria para comprimento, enquanto que *Paratrechalea* sp.n. apresentou o padrão inverso (Fig. 2). Já as populações Uruguaias compartilharam os mesmos padrões alométricos para a maioria das dimensões analisadas, onde a principal diferença foi em relação ao *DL*.

Já para as fêmeas foi observada uma tendência para alometria positiva, principalmente para as dimensões relacionadas á largura da genitália (*SAW* e *SPW*; Fig. 3). A única medida que apresentou coeficientes alométricos negativos significantes foi *SLL*, e apenas para *Paratrechalea* sp.n. e *P. ornata* (Pas).

### *Coeficientes de variação*

Os coeficientes de variação (CV) observados para o tamanho do cefalotórax e os tamanhos das genitálias apresentaram valores relativamente similares, ou pelo menos não discrepantes, independentemente do tipo de CV utilizado (Apêndices I e II). Entretanto, quando consideradas as dimensões lineares internas das estruturas genitais, alguns valores de CV foram relativamente maiores do que aqueles observados para o caractere somático de comparação.

## Discussão

Apresentamos neste capítulo a descrição dos padrões de alometria para genitálias de machos e fêmeas de três espécies de *Paratrechalea*. Basicamente, nossos dados concordam com a interpretação de outros trabalhos relacionados à análise alométrica de genitálias, onde tanto o modelo *one-size-fits-all* (Eberhard 1985; Eberhard *et al.* 1998; Hosken & Stockley 2004) quanto o modelo *lock-and-key* (Mutanen *et al.* 2006) podem ser utilizados para a interpretação dos dados. Devido ao caráter descritivo dos dados por ora disponíveis, e das críticas metodológicas recentemente apresentadas por alguns autores questionando a validade das interpretações sobre as forças seletivas responsáveis pelos padrões alométricos observados (Bonduriansky & Day 2003; Bonduriansky 2007; Bertin & Fairbairn 2007), nos limitaremos a comentar aspectos relativos ao conhecimento da biologia reprodutiva de *Paratrechalea*, sem maiores pretensões de generalizar estes comentários além deste grupo de aranhas estudadas.

### *ATR e funcionalidade das genitálias*

O comportamento copulatório de *Paratrechalea* foi recentemente descrito por Costa-Schmidt *et al.* (submetido; Capítulo 2). Sabemos que a ATR tem um papel essencial no sucesso do acoplamento da genitália do macho na genitália da fêmea (LECS dados não publicados), seguindo o padrão funcional de ATR apresentado por Huber (1995). Este mesmo autor afirma que a evolução da ATR é determinada pela seleção sexual.

Uma vez que a porção distal das ATR's dos machos de *Paratrechalea* foi a que apresentou maior estabilidade ao padrão de alometria negativa, possivelmente esta região é a que tem maior interação durante o contato desta estrutura com a genitália da fêmea. Neste caso, para esta estrutura da genitália dos machos, podemos sustentar a hipótese de chave-e-fechadura (*lock-and-key*) para a manutenção do funcionamento da ATR e do sucesso de cópula destas espécies, e possivelmente mantida pela seleção sexual (Huber 1995).

### ***Alometria da genitália e o dimorfismo sexual de tamanho em*** *Paratrechalea*

Os dados apresentados na Tabela 1 indicam que mesmo sendo em geral negativamente alométricos, os valores observados para os coeficientes de alometria podem estar sendo influenciados por outras propriedades populacionais. Nosso argumento é sustentado pela comparação com os dados apresentados em no Capítulo 4 para dimorfismo sexual em *Paratrechalea*.

A partir da Tabela 1, podemos ver que os valores dos coeficientes alométricos dos tamanhos das genitálias dos machos e das fêmeas, quando escalonados pelo tamanho do cefalotórax, parecem ter uma associação inversa com a direção do dimorfismo sexual de tamanho observado, principalmente para a genitália das fêmeas. A interpretação deste padrão pode ser ampliada e explicada a partir da seguinte hipótese: a condição sintópica das populações estudadas de *P. azul* e *Paratrechalea* sp.n. levou estas espécies a um processo de deslocamento de caracteres observado na *forma* dos caracteres somáticos (cefalotórax e esterno; Capítulo 4) e genitais (ATR e escapo do epígeno; Capítulo 5). Caracteres somáticos, como o cefalotórax, também apresentaram diferenças de *tamanho*, e podem ser interpretados como reflexo deste mesmo processo. As genitálias, entretanto, não seguem o mesmo padrão de deslocamento para o tamanho, ocorrendo a manutenção (canalização?) dos tamanhos de genitália, constatado através da diferença dos coeficientes alométricos.

Esta hipótese será devidamente testada com o aumento do número de populações amostradas em situações de alopatria e simpatria. Caso confirmada a influência deste processo sobre as populações de *P. azul* e *Paratrechalea* sp.n., poderemos afirmar então que diferenças alométricas podem ter uma origem na seleção natural atuante devido à competição interespecífica.

### *Alometria negativa para a genitália das fêmeas*

Este padrão observado pode ser visto como um importante mecanismo de manutenção da espécie. Mantendo um tamanho “ideal”, machos e fêmeas garantem de certa maneira que indivíduos colonizadores (tanto imigrantes como emigrantes) com tamanhos corporais distintos possam ainda ter sucesso de cópula uma vez que as genitálias não estão envolvidas em tal segregação por tamanho.

Padrões alométricos negativos para a genitália de fêmeas já foram descritos para outras espécies de aranhas (Uhl & Vollrath 2000), e tais padrões podem ser vistos com uma extensão da aplicação da hipótese *one-size-fits-all* proposta por Eberhard *et al.* (1998).

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Tabela 1. Coeficientes de alometria para o modelo dos mínimos quadrados (*Ordinary Least Squares* - $b_{OLS}$) entre o tamanho do centróide das genitálias e o tamanho corporal (CSize), tipo de dimorfismo sexual de tamanho para o cefalotórax e distribuição geográfica das amostras analisadas. RSize: tamanho do centróide da apófise tibial retrolateral; ESize: tamanho do centróide do escapo do epígeno.

| | Genitálias[a] | | Dimorfismo[b] | |
|---|---|---|---|---|
| | $b_{OLS}$ (RSize) | $b_{OLS}$ (ESize) | Cefalotórax | Distribuição |
| *P. azul* | $0.46^{\S}$ | $0.14^{\S}$ | viés para fêmeas | sintopia |
| *Paratrechalea* sp.n. | $0.38^{\S}$ | $0.41^{\S}$ | viés para machos | sintopia |
| *P. ornata* (Pas) | 0.67* | $0.22^{\S}$ | ns | alopatria |
| *P. ornata* (Que) | 0.59* | $0.29^{\S}$ | ns | alopatria |

$^{*}p < 0.05$; $^{\S}p < 0.0001$

[a] Informação compilada dos Apêndices I e II.

[b] Informação compilada do Capítulo 4.

Figura 1. Medidas lineares analisadas para: A) apófise tibial retrolateral (ATR) dos machos; B) escapo do epígeno das fêmeas. DAM: margem anterior do lóbulo distal; DL comprimento do lóbulo distal; DPM: margem posterior do lóbulo distal; BAM: margem anterior do lóbulo basal; RW: largura da ATR; RL: comprimento da ATR; PMW: largura da margem posterior do campo médio; SAW: largura anterior do escapo; SPW: largura posterior do escapo; SLL: comprimento lateral do escapo; SCL: comprimento central do escapo. Barras de escala = 1mm.

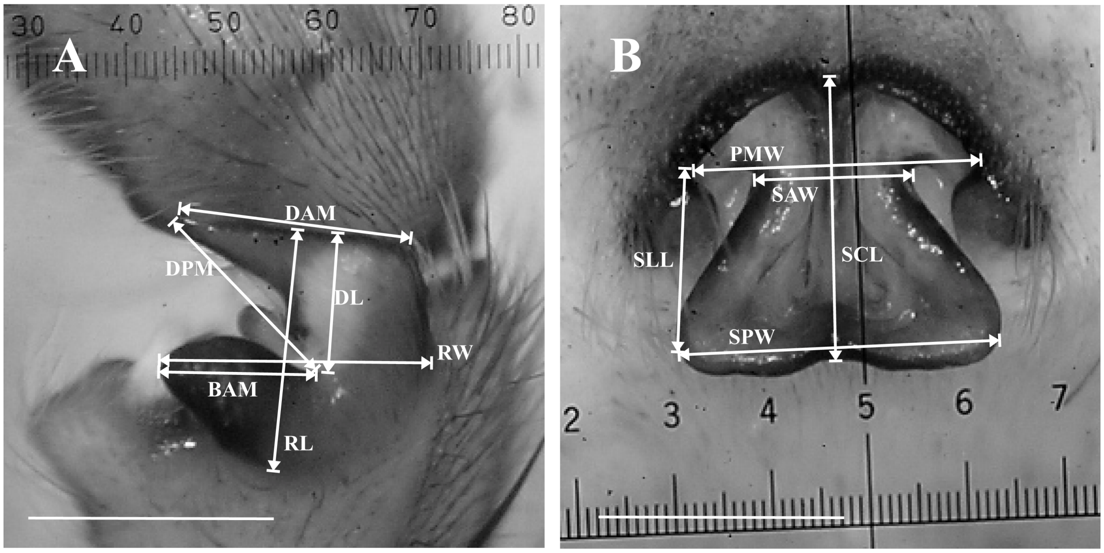


Figura 2. Coeficientes alométricos estimados das dimensões lineares da apófise tibial retrolateral (ATR) dos machos de *Paratrechalea azul*, *Paratrechalea* sp.n., *P. ornata* (Pas) e *P. ornata* (Que). Na linha superior são apresentados os coeficientes relativos ao tamanho corporal geral (CSize: tamanho do centróide do cefalotórax); na linha inferior são apresentados os coeficientes relativos ao tamanho total da ATR (RSize: tamanho do centróide da ATR). Linhas tracejadas: alometria negativa; linhas contínuas simples: isometria; linhas contínuas grossas: alometria positiva. Para cada par de coeficientes: método do quadrado-mínimo ($b_{\mathrm{OLS}}$): acima e em fonte normal; método do eixo principal reduzido ($b_{\mathrm{RMA}}$): abaixo e em itálico. Significâncias: ° marginalmente não-significante; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $^{\S}$ $p < 0.0001$. Pas: Paso Del Molino; Que: Quebrada de los Cuervos.

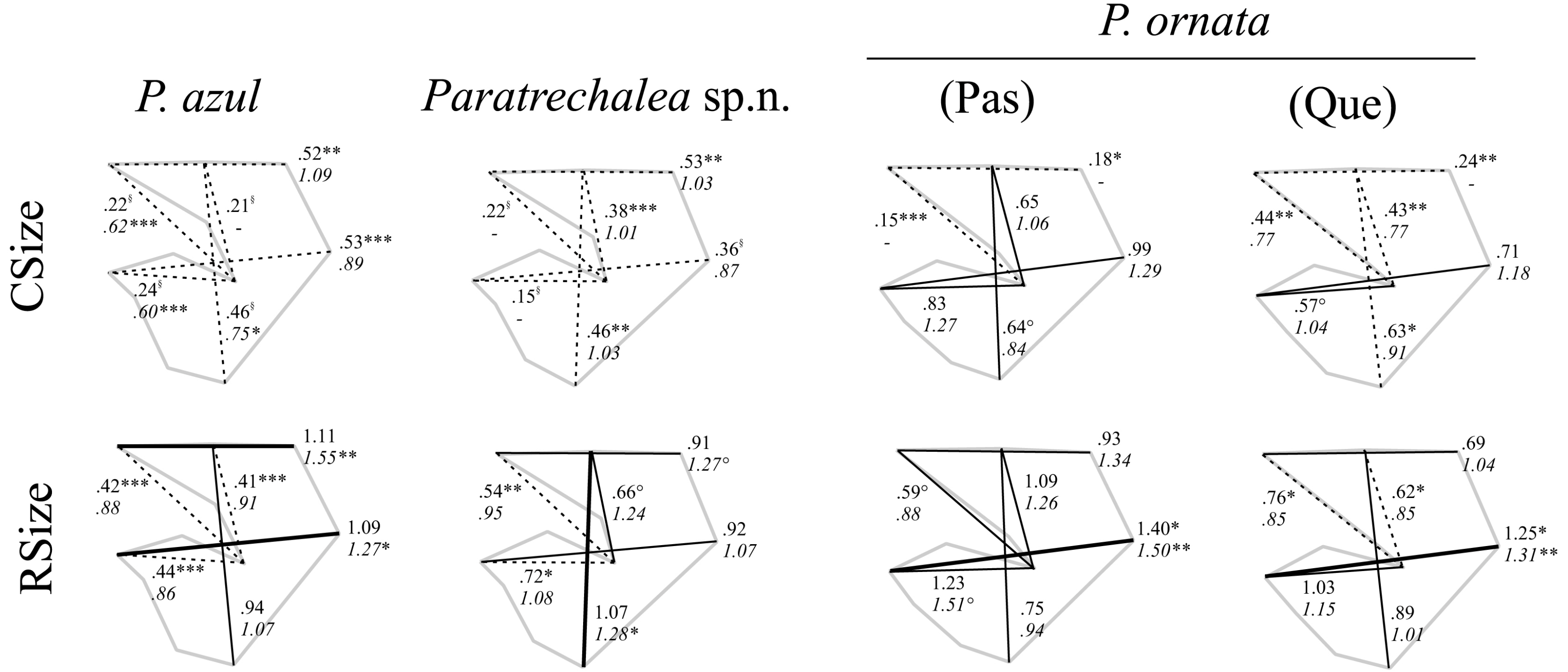
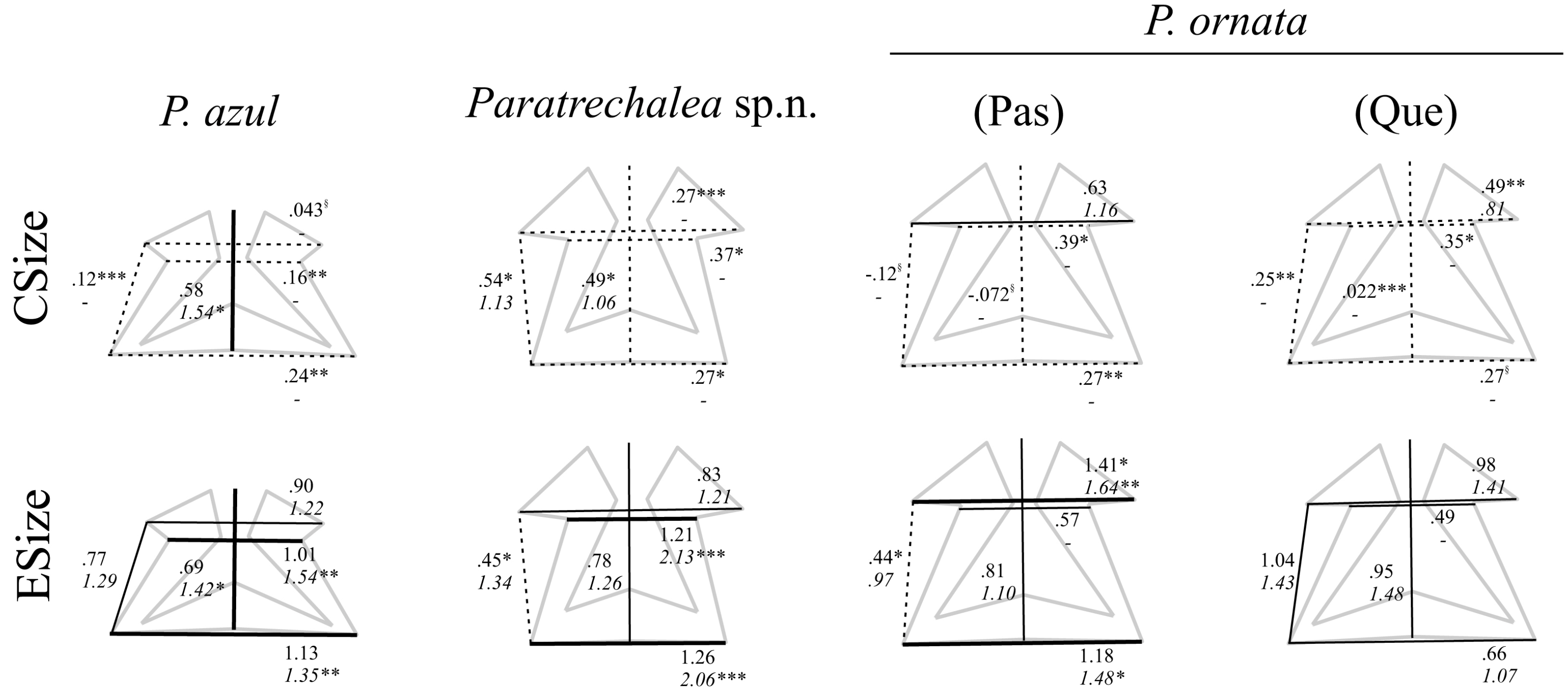


Figura 3. Coeficientes alométricos estimados das dimensões lineares do escapo do epígeno das fêmeas de *Paratrechalea azul*, *Paratrechalea* sp.n., *P. ornata* (Pas) e *P. ornata* (Que). Na linha superior são apresentados os coeficientes relativos ao tamanho corporal geral (CSize: tamanho do centróide do cefalotórax); na linha inferior são apresentados os coeficientes relativos ao tamanho total da ATR (RSize: tamanho do centróide da ATR). Linhas tracejadas: alometria negativa; linhas contínuas simples: isometria; linhas contínuas grossas: alometria positiva. Para cada par de coeficientes: método do quadrado-mínimo ($b_{OLS}$): acima e em fonte normal; método do eixo principal reduzido ($b_{RMA}$): abaixo e em itálico. Significâncias: ° marginalmente não-significante; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $^{\S}$ $p < 0.0001$. Pas: Paso Del Molino; Que: Quebrada de los Cuervos.



**Apêndice I.** Média, desvio padrão (DP), coeficiente de variação (CV), coeficiente de correlação (*r*); coeficiente de alometria para o método dos mínimos-quadrados ($b_1$); intervalo de confiança para a estimativa do coeficiente de alometria (IC – 95%); coeficiente de alometria para o método do eixo principal reduzido ($b_2$); coeficiente de variação modificado (CV´); para as variáveis de análise da apófise tibial retrolateral (ATR) dos machos das espécies de *Paratrechalea* estudadas em relação ao tamanho corporal (CSize: tamanho do centróide do cefalotórax). RSize: tamanho do centróide da ATR; DAM: margem anterior do lóbulo distal; DL comprimento do lóbulo distal; DPM: margem posterior do lóbulo distal; BAM: margem anterior do lóbulo basal; RW: largura da ATR; RL: comprimento da ATR.

| Espécie | Variável | Média | DP | CV | r | $b_1$ | IC - 95% | | EP | $b_2$ | IC - 95% | | CV' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Paratrechalea azul* | | | | | | | | | | | | | |
| | C Size | 8.0622 | 0.4089 | 0.0507 | - | - | - | - | - | - | - | - | - |
| | R Size | 1.055 | 0.03744 | 0.0355 | 0.6540$^{\S}$ | 0.4602$^{\S}$ | 0.2654 | 0.6849 | 0.09267 | 0.7037** | 0.5107 | 0.9421 | 0.0268 |
| | DAM | 0.5721 | 0.03162 | 0.0553 | 0.4763** | 0.5195** | 0.2663 | 0.7719 | 0.1669 | 1.0906 | 0.7996 | 1.528 | 0.0486 |
| | DL | 0.3989 | 0.013 | 0.0326 | 0.3306 | 0.2124$^{\S}$ | 0.02415 | 0.373 | 0.1055 | - | - | - | - |
| | DPM | 0.5578 | 0.01763 | 0.0316 | 0.3505* | 0.2178$^{\S}$ | 0.03455 | 0.3776 | 0.1013 | 0.6215*** | 0.4323 | 0.8959 | 0.0296 |
| | BAM | 0.4097 | 0.01247 | 0.0304 | 0.4034* | 0.2432$^{\S}$ | 0.06477 | 0.4604 | 0.09605 | 0.6030*** | 0.4501 | 0.8332 | 0.0279 |
| | RW | 0.7185 | 0.03234 | 0.0450 | 0.5927*** | 0.5307*** | 0.2906 | 0.8082 | 0.1255 | 0.8954 | 0.6527 | 1.232 | 0.0363 |
| | RL | 0.7124 | 0.02734 | 0.0384 | 0.6152$^{\S}$ | 0.4644$^{\S}$ | 0.2629 | 0.6829 | 0.1036 | 0.7549* | 0.5813 | 1.034 | 0.0303 |
| *Paratrechalea* sp.n. | | | | | | | | | | | | | |
| | C Size | 6.9584 | 0.3004 | 0.0432 | - | - | - | - | - | - | - | - | - |
| | R Size | 0.9142 | 0.0319 | 0.0349 | 0.4698** | 0.3802$^{\S}$ | 0.1091 | 0.6311 | 0.1208 | 0.8094 | 0.5866 | 1.062 | 0.0308 |
| | DAM | 0.5072 | 0.02253 | 0.0444 | 0.5151** | 0.5308** | 0.2073 | 0.7996 | 0.1493 | 1.0303 | 0.7922 | 1.311 | 0.0381 |
| | DL | 0.3121 | 0.01345 | 0.0431 | 0.3787* | 0.3813*** | 0.09465 | 0.6565 | 0.1575 | 1.0068 | 0.7085 | 1.365 | 0.0399 |
| | DPM | 0.4408 | 0.01457 | 0.0331 | 0.2911 | 0.2234$^{\S}$ | -0.0595 | 0.4762 | 0.1241 | - | - | - | - |
| | BAM | 0.372 | 0.01408 | 0.0378 | 0.1691 | 0.1477$^{\S}$ | -0.1876 | 0.5075 | 0.1455 | - | - | - | - |
| | RW | 0.6578 | 0.02467 | 0.0375 | 0.4115* | 0.3583$^{\S}$ | 0.04685 | 0.6313 | 0.1341 | 0.8706 | 0.6277 | 1.138 | 0.0342 |
| | RL | 0.5955 | 0.02635 | 0.0442 | 0.4424** | 0.4576** | 0.1302 | 0.7328 | 0.1568 | 1.0345 | 0.7885 | 1.349 | 0.0397 |
| *Paratrechalea ornata* (Pas) | | | | | | | | | | | | | |
| | C Size | 7.0551 | 0.3527 | 0.0500 | - | - | - | - | - | - | - | - | - |
| | R Size | 0.9407 | 0.03889 | 0.0413 | 0.8032*** | 0.6655* | 0.4329 | 0.9835 | 0.1488 | 0.8286 | 0.5622 | 1.181 | 0.0246 |
| | DAM | 0.5358 | 0.03002 | 0.0560 | 0.1782 | 0.1846* | -0.4291 | 0.9031 | 0.3073 | - | - | - | - |
| | DL | 0.3419 | 0.01771 | 0.0518 | 0.6144* | 0.6531 | 0.2384 | 1.157 | 0.2529 | 1.0631 | 0.5972 | 1.54 | 0.0409 |
| | DPM | 0.5002 | 0.01846 | 0.0369 | 0.2338 | 0.1512*** | -0.1305 | 0.4835 | 0.1896 | - | - | - | - |
| | BAM | 0.4002 | 0.0251 | 0.0627 | 0.6561* | 0.8328 | 0.1867 | 1.359 | 0.2888 | 1.2693 | 0.8757 | 1.75 | 0.0473 |
| | RW | 0.6791 | 0.04225 | 0.0622 | 0.7692** | 0.9892 | 0.5653 | 1.469 | 0.2478 | 1.286 | 0.9732 | 1.792 | 0.0398 |
| | RL | 0.5862 | 0.02263 | 0.0386 | 0.7566** | 0.6361° | 0.3911 | 0.9962 | 0.1657 | 0.8407 | 0.4629 | 1.286 | 0.0252 |
| *Paratrechalea ornata* (Que) | | | | | | | | | | | | | |
| | C Size | 7.6206 | 0.3974 | 0.0521 | - | - | - | - | - | - | - | - | - |
| | R Size | 0.9218 | 0.04328 | 0.0470 | 0.6479** | 0.5858* | 0.229 | 0.8684 | 0.167 | 0.9041 | 0.6443 | 1.298 | 0.0358 |
| | DAM | 0.5313 | 0.0257 | 0.0484 | 0.2541 | 0.24** | -0.2024 | 0.5479 | 0.2215 | - | - | - | - |
| | DL | 0.3344 | 0.01316 | 0.0394 | 0.5645* | 0.4333** | 0.1486 | 0.7265 | 0.1536 | 0.7675 | 0.5268 | 1.131 | 0.0325 |
| | DPM | 0.4913 | 0.01934 | 0.0394 | 0.5686* | 0.4374** | 0.09674 | 0.743 | 0.1535 | 0.7694 | 0.4758 | 1.107 | 0.0324 |
| | BAM | 0.3768 | 0.01989 | 0.0528 | 0.55* | 0.5706° | 0.1725 | 0.9243 | 0.2101 | 1.0374 | 0.6809 | 1.597 | 0.0441 |
| | RW | 0.6413 | 0.03936 | 0.0614 | 0.6004** | 0.7119 | 0.2298 | 1.043 | 0.23 | 1.1857 | 0.8599 | 1.733 | 0.0491 |
| | RL | 0.5941 | 0.02839 | 0.0478 | 0.6944*** | 0.6358* | 0.3022 | 0.9564 | 0.1598 | 0.9157 | 0.6288 | 1.33 | 0.0344 |

° marginalmente significnate; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $^{\S}$ $p < 0.0001$

**Apêndice II.** Média, desvio padrão (DP), coeficiente de variação (CV), coeficiente de correlação (*r*); coeficiente de alometria para o método dos mínimos-quadrados ($b_1$); intervalo de confiança para a estimativa do coeficiente de alometria (IC – 95%); coeficiente de alometria para o método do eixo principal reduzido ($b_2$); coeficiente de variação modificado (CV´); para as variáveis de análise da apófise tibial retrolateral (ATR) dos machos das espécies de *Paratrechalea* estudadas em relação ao tamanho corporal (CSize: tamanho do centróide do cefalotórax). ESize: tamanho do centróide do escapo do epígeno; PMW: largura da margem posterior do campo médio; SAW: largura anterior do escapo; SPW: largura posterior do escapo; SLL: comprimento lateral do escapo; SCL: comprimento central do escapo.

| Espécie | Variável | Média | DP | CV | r | $b_1$ | IC - 95% | | EP | $b_2$ | IC - 95% | | CV' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Paratrechalea azul* | | | | | | | | | | | | | |
| | C Size | 8.4544 | 0.4943 | 0.0585 | - | - | - | - | - | - | - | - | - |
| | E Size | 1.001 | 0.05806 | 0.0580 | 0.1402 | 0.136$^{\S}$ | -0.1583 | 0.6259 | 0.1602 | - | - | - | - |
| | PMW | 0.5237 | 0.0369 | 0.0705 | 0.03632 | 0.04307$^{\S}$ | -0.3464 | 0.4683 | 0.1975 | - | - | - | - |
| | SAW | 0.3956 | 0.03533 | 0.0893 | 0.1061 | 0.159** | -0.2464 | 0.6408 | 0.2485 | - | - | - | - |
| | SPW | 0.729 | 0.0563 | 0.0772 | 0.187 | 0.2454** | -0.0871 | 0.8054 | 0.2148 | - | - | - | - |
| | SLL | 0.3457 | 0.02576 | 0.0745 | 0.09338 | 0.1172*** | -0.2505 | 0.6185 | 0.2082 | - | - | - | - |
| | SCL | 0.4078 | 0.03384 | 0.0830 | 0.3783* | 0.5824 | 0.1866 | 1.021 | 0.2443 | 1.5392* | 1.109 | 2.28 | 0.0768 |
| *Paratrechalea* sp.n. | | | | | | | | | | | | | |
| | C Size | 6.6825 | 0.3978 | 0.0595 | - | - | - | - | - | - | - | - | - |
| | E Size | 0.847 | 0.0422 | 0.0498 | 0.4873** | 0.4087$^{\S}$ | 0.1903 | 0.6439 | 0.1275 | 0.8387 | 0.6371 | 1.11 | 0.0435 |
| | PMW | 0.5207 | 0.03183 | 0.0611 | 0.2685 | 0.2738*** | -0.0741 | 0.5844 | 0.171 | - | - | - | - |
| | SAW | 0.2984 | 0.03282 | 0.1100 | 0.2063 | 0.3681* | -0.2557 | 0.9156 | 0.3038 | - | - | - | - |
| | SPW | 0.4555 | 0.04572 | 0.1004 | 0.1549 | 0.2677* | -0.2784 | 0.7799 | 0.2972 | - | - | - | - |
| | SLL | 0.3075 | 0.02084 | 0.0678 | 0.4781** | 0.5391* | 0.1845 | 0.8887 | 0.1724 | 1.1276 | 0.8224 | 1.476 | 0.0595 |
| | SCL | 0.4489 | 0.02877 | 0.0641 | 0.4604** | 0.4873* | 0.2365 | 0.8205 | 0.1636 | 1.0585 | 0.732 | 1.467 | 0.0569 |
| *Paratrechalea ornata* (Pas) | | | | | | | | | | | | | |
| | C Size | 7.1422 | 0.6392 | 0.0895 | - | - | - | - | - | - | - | - | - |
| | E Size | 1.0254 | 0.06506 | 0.0634 | 0.3022 | 0.2153$^{\S}$ | -0.0994 | 0.5669 | 0.1558 | - | - | - | - |
| | PMW | 0.5836 | 0.05972 | 0.1023 | 0.5464* | 0.6318 | 0.1925 | 1.046 | 0.2282 | 1.1561 | 0.8089 | 1.578 | 0.0857 |
| | SAW | 0.3318 | 0.03153 | 0.0950 | 0.3718 | 0.3937* | -0.0884 | 0.8293 | 0.2256 | - | - | - | - |
| | SPW | 0.6278 | 0.0576 | 0.0917 | 0.2562 | 0.2706** | -0.1906 | 0.7934 | 0.2343 | - | - | - | - |
| | SLL | 0.3725 | 0.02337 | 0.0627 | -0.1701 | -0.1176 § | -0.4275 | 0.1651 | 0.1562 | - | - | - | - |
| | SCL | 0.5125 | 0.03606 | 0.0704 | -0.09229 | -0.07257 § | -0.4045 | 0.2815 | 0.1796 | - | - | - | - |
| *Paratrechalea ornata* (Que) | | | | | | | | | | | | | |
| | C Size | 7.3023 | 0.5687 | 0.0779 | - | - | - | - | - | - | - | - | - |
| | E Size | 1.0037 | 0.06979 | 0.0695 | 0.5016* | 0.289$^{\S}$ | 0.1185 | 0.5814 | 0.1209 | 0.5761** | 0.3158 | 0.8846 | 0.0602 |
| | PMW | 0.5368 | 0.04809 | 0.0896 | 0.6031*** | 0.4919** | 0.1791 | 0.806 | 0.1578 | 0.8156 | 0.5941 | 1.115 | 0.0715 |
| | SAW | 0.3098 | 0.03497 | 0.1129 | 0.3203 | 0.3538* | -0.2329 | 0.8823 | 0.2538 | - | - | - | - |
| | SPW | 0.6266 | 0.04016 | 0.0641 | 0.4376° | 0.2695$^{\S}$ | 0.07169 | 0.5496 | 0.1343 | - | - | - | - |
| | SLL | 0.357 | 0.03177 | 0.0890 | 0.2986 | 0.2461** | -0.008 | 0.5364 | 0.1908 | - | - | - | - |
| | SCL | 0.4864 | 0.04137 | 0.0851 | 0.02577 | 0.02199*** | -0.2832 | 0.4527 | 0.2069 | - | - | - | - |

° marginalmente significnate; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $^{\S}$ $p < 0.0001$

**Apêndice III.** Média, desvio padrão (DP), coeficiente de variação (CV), coeficiente de correlação (*r*); coeficiente de alometria para o método dos mínimos-quadrados ($b_1$); intervalo de confiança para a estimativa do coeficiente de alometria (IC – 95%); coeficiente de alometria para o método do eixo principal reduzido ($b_2$); coeficiente de variação modificado (CV´); para as variáveis de análise da apófise tibial retrolateral (ATR) dos machos das espécies de *Paratrechalea* estudadas em relação ao tamanho da genitália (RSize: tamanho do centróide da ATR). DAM: margem anterior do lóbulo distal; DL comprimento do lóbulo distal; DPM: margem posterior do lóbulo distal; BAM: margem anterior do lóbulo basal; RW: largura da ATR; RL: comprimento da ATR.

| Espécie | Variável | Média | DP | CV | r | $b_1$ | IC - 95% | | EP | $b_2$ | IC - 95% | | CV' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Paratrechalea azul* | | | | | | | | | | | | | |
| | R Size | 1.055 | 0.03744 | 0.0355 | - | - | - | - | - | - | - | - | - |
| | DAM | 0.5721 | 0.03162 | 0.0553 | 0.714$^{\S}$ | 1.1066 | 0.7969 | 1.558 | 0.1889 | 1.5498** | 1.168 | 2.051 | 0.0387 |
| | DL | 0.3989 | 0.013 | 0.0326 | 0.4485** | 0.4094*** | 0.155 | 0.6408 | 0.142 | 0.9127 | 0.6906 | 1.288 | 0.0291 |
| | DPM | 0.5578 | 0.01763 | 0.0316 | 0.4806** | 0.4244*** | 0.1918 | 0.715 | 0.1348 | 0.8831 | 0.6612 | 1.262 | 0.0277 |
| | BAM | 0.4097 | 0.01247 | 0.0304 | 0.5197** | 0.4453*** | 0.2401 | 0.6759 | 0.1274 | 0.8568 | 0.6571 | 1.213 | 0.0260 |
| | RW | 0.7185 | 0.03234 | 0.0450 | 0.8604$^{\S}$ | 1.0947 | 0.8887 | 1.256 | 0.1129 | 1.2723* | 1.129 | 1.459 | 0.0229 |
| | RL | 0.7124 | 0.02734 | 0.0384 | 0.8762$^{\S}$ | 0.94 | 0.7862 | 1.202 | 0.0900 | 1.0728 | 0.8937 | 1.363 | 0.0185 |
| *Paratrechalea* sp.n. | | | | | | | | | | | | | |
| | R Size | 0.9142 | 0.0319 | 0.0349 | - | - | - | - | - | - | - | - | - |
| | DAM | 0.5072 | 0.02253 | 0.0444 | 0.7162$^{\S}$ | 0.9117 | 0.6099 | 1.09 | 0.1502 | 1.2729° | 1.098 | 1.548 | 0.0310 |
| | DL | 0.3121 | 0.01345 | 0.0431 | 0.53*** | 0.6593° | 0.3053 | 0.9929 | 0.1783 | 1.2439 | 0.9356 | 1.682 | 0.0365 |
| | DPM | 0.4408 | 0.01457 | 0.0331 | 0.5731*** | 0.5433** | 0.2445 | 0.7651 | 0.1313 | 0.9481 | 0.7662 | 1.242 | 0.0271 |
| | BAM | 0.372 | 0.01408 | 0.0378 | 0.6658$^{\S}$ | 0.7187* | 0.3502 | 0.9218 | 0.1361 | 1.0794 | 0.9145 | 1.313 | 0.0282 |
| | RW | 0.6578 | 0.02467 | 0.0375 | 0.8533$^{\S}$ | 0.9178 | 0.7562 | 1.082 | 0.0948 | 1.0756 | 0.9222 | 1.269 | 0.0196 |
| | RL | 0.5955 | 0.02635 | 0.0442 | 0.8413$^{\S}$ | 1.0752 | 0.8741 | 1.328 | 0.1168 | 1.278* | 1.068 | 1.609 | 0.0239 |
| *Paratrechalea ornata* (Pas) | | | | | | | | | | | | | |
| | R Size | 0.9407 | 0.03889 | 0.0413 | - | - | - | - | - | - | - | - | - |
| | DAM | 0.5358 | 0.03002 | 0.0560 | 0.6966** | 0.9329 | 0.4154 | 1.37 | 0.2774 | 1.3392 | 0.9881 | 1.871 | 0.0402 |
| | DL | 0.3419 | 0.01771 | 0.0518 | 0.8589$^{\S}$ | 1.0868 | 0.6487 | 1.473 | 0.1871 | 1.2654 | 0.891 | 1.607 | 0.0265 |
| | DPM | 0.5002 | 0.01846 | 0.0369 | 0.6726** | 0.5947° | 0.1971 | 0.9442 | 0.1889 | 0.8842 | 0.5899 | 1.196 | 0.0273 |
| | BAM | 0.4002 | 0.0251 | 0.0627 | 0.8156*** | 1.2352 | 0.829 | 1.72 | 0.253 | 1.5146° | 1.119 | 2.044 | 0.0363 |
| | RW | 0.6791 | 0.04225 | 0.0622 | 0.9311$^{\S}$ | 1.3981* | 1.171 | 1.781 | 0.1581 | 1.5016** | 1.237 | 1.859 | 0.0227 |
| | RL | 0.5862 | 0.02263 | 0.0386 | 0.7918*** | 0.7474 | 0.3479 | 1.086 | 0.1664 | 0.944 | 0.5436 | 1.231 | 0.0236 |
| *Paratrechalea ornata* (Que) | | | | | | | | | | | | | |
| | R Size | 0.9218 | 0.04328 | 0.0470 | - | - | - | - | - | - | - | - | - |
| | DAM | 0.5313 | 0.0257 | 0.0484 | 0.6595** | 0.6888 | 0.4193 | 1.058 | 0.1904 | 1.0445 | 0.7285 | 1.596 | 0.0364 |
| | DL | 0.3344 | 0.01316 | 0.0394 | 0.7348*** | 0.6238* | 0.3994 | 0.7783 | 0.1396 | 0.8489 | 0.6743 | 1.209 | 0.0267 |
| | DPM | 0.4913 | 0.01934 | 0.0394 | 0.8961$^{\S}$ | 0.7626* | 0.5785 | 0.9059 | 0.09159 | 0.8509 | 0.6889 | 1.015 | 0.0175 |
| | BAM | 0.3768 | 0.01989 | 0.0528 | 0.9012$^{\S}$ | 1.0341 | 0.772 | 1.283 | 0.1206 | 1.1474 | 0.9361 | 1.436 | 0.0229 |
| | RW | 0.6413 | 0.03936 | 0.0614 | 0.951$^{\S}$ | 1.2471* | 1.04 | 1.463 | 0.09836 | 1.3114** | 1.143 | 1.57 | 0.0190 |
| | RL | 0.5941 | 0.02839 | 0.0478 | 0.8748$^{\S}$ | 0.886 | 0.5851 | 1.053 | 0.119 | 1.0128 | 0.8489 | 1.226 | 0.0232 |

° marginalmente significnate; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $^{\S}$ $p < 0.0001$

**Apêndice IV.** Média, desvio padrão (DP), coeficiente de variação (CV), coeficiente de correlação (*r*); coeficiente de alometria para o método dos mínimos-quadrados ($b_1$); intervalo de confiança para a estimativa do coeficiente de alometria (IC – 95%); coeficiente de alometria para o método do eixo principal reduzido ($b_2$); coeficiente de variação modificado (CV´); para as variáveis de análise da apófise tibial retrolateral (ATR) dos machos das espécies de *Paratrechalea* estudadas em relação ao tamanho da genitália (ESize: tamanho do centróide do escapo do epígeno). PMW: largura da margem posterior do campo médio; SAW: largura anterior do escapo; SPW: largura posterior do escapo; SLL: comprimento lateral do escapo; SCL: comprimento central do escapo.

| Espécie | Variável | Média | DP | CV | r | $b_1$ | IC - 95% | | EP | $b_2$ | IC - 95% | | CV' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Paratrechalea azul* | | | | | | | | | | | | | |
| | E Size | 1.001 | 0.05806 | 0.0580 | - | - | - | - | - | - | - | - | - |
| | PMW | 0.5237 | 0.0369 | 0.0705 | 0.7344$^{\S}$ | 0.8972 | 0.586 | 1.224 | 0.1382 | 1.2216 | 0.9637 | 1.512 | 0.0478 |
| | SAW | 0.3956 | 0.03533 | 0.0893 | 0.6568$^{\S}$ | 1.0144 | 0.5319 | 1.425 | 0.1941 | 1.5444** | 1.188 | 1.929 | 0.0673 |
| | SPW | 0.729 | 0.0563 | 0.0772 | 0.8357$^{\S}$ | 1.1297 | 0.868 | 1.373 | 0.1237 | 1.3517** | 1.114 | 1.589 | 0.0424 |
| | SLL | 0.3457 | 0.02576 | 0.0745 | 0.5945$^{\S}$ | 0.7686 | 0.5063 | 1.121 | 0.1732 | 1.2928 | 0.9564 | 1.72 | 0.0599 |
| | SCL | 0.4078 | 0.03384 | 0.0830 | 0.4833** | 0.6885 | 0.3776 | 1.074 | 0.2078 | 1.4244* | 1.043 | 1.889 | 0.0726 |
| *Paratrechalea* sp.n. | | | | | | | | | | | | | |
| | E Size | 0.847 | 0.0422 | 0.0498 | - | - | - | - | - | - | - | - | - |
| | PMW | 0.5207 | 0.03183 | 0.0611 | 0.6851$^{\S}$ | 0.8329 | 0.474 | 1.148 | 0.1542 | 1.2159 | 0.9625 | 1.505 | 0.0445 |
| | SAW | 0.2984 | 0.03282 | 0.1100 | 0.567*** | 1.206 | 0.5079 | 1.887 | 0.305 | 2.1268*** | 1.597 | 2.805 | 0.0906 |
| | SPW | 0.4555 | 0.04572 | 0.1004 | 0.6121$^{\S}$ | 1.2613 | 0.7384 | 1.929 | 0.2836 | 2.0606*** | 1.518 | 2.726 | 0.0794 |
| | SLL | 0.3075 | 0.02084 | 0.0678 | 0.3379* | 0.4543* | 0.1361 | 0.8455 | 0.2203 | 1.3444 | 0.9443 | 1.872 | 0.0638 |
| | SCL | 0.4489 | 0.02877 | 0.0641 | 0.6183$^{\S}$ | 0.7804 | 0.3065 | 1.085 | 0.1727 | 1.2621 | 1.019 | 1.482 | 0.0504 |
| *Paratrechalea ornata* (Pas) | | | | | | | | | | | | | |
| | E Size | 1.0254 | 0.06506 | 0.0634 | - | - | - | - | - | - | - | - | - |
| | PMW | 0.5836 | 0.05972 | 0.1023 | 0.8624$^{\S}$ | 1.4145* | 1.011 | 1.764 | 0.1905 | 1.6402** | 1.35 | 1.986 | 0.0518 |
| | SAW | 0.3318 | 0.03153 | 0.0950 | 0.3819 | 0.5678 | -0.1638 | 1.276 | 0.3152 | - | - | - | - |
| | SPW | 0.6278 | 0.0576 | 0.0917 | 0.7993$^{\S}$ | 1.1852 | 0.7731 | 1.518 | 0.2044 | 1.4828* | 1.119 | 1.847 | 0.0551 |
| | SLL | 0.3725 | 0.02337 | 0.0627 | 0.4587* | 0.4449* | 0.01731 | 0.8293 | 0.1977 | 0.9698 | 0.5197 | 1.364 | 0.0557 |
| | SCL | 0.5125 | 0.03606 | 0.0704 | 0.7387*** | 0.8152 | 0.5027 | 1.121 | 0.1707 | 1.1036 | 0.813 | 1.407 | 0.0474 |
| *Paratrechalea ornata* (Que) | | | | | | | | | | | | | |
| | E Size | 1.0037 | 0.06979 | 0.0695 | - | - | - | - | - | - | - | - | - |
| | PMW | 0.5368 | 0.04809 | 0.0896 | 0.6941*** | 0.9826 | 0.6610 | 1.482 | 0.2471 | 1.4156 | 1.077 | 2.311 | 0.0645 |
| | SAW | 0.3098 | 0.03497 | 0.1129 | 0.254 | 0.487 | -0.3741 | 1.573 | 0.4498 | - | - | - | - |
| | SPW | 0.6266 | 0.04016 | 0.0641 | 0.6185** | 0.6611 | 0.3558 | 0.9591 | 0.2037 | 1.0689 | 0.7974 | 1.675 | 0.0504 |
| | SLL | 0.357 | 0.03177 | 0.0890 | 0.73*** | 1.0446 | 0.6571 | 1.541 | 0.2372 | 1.4309 | 0.9632 | 2.201 | 0.0608 |
| | SCL | 0.4864 | 0.04137 | 0.0851 | 0.6409** | 0.9493 | 0.4345 | 1.43 | 0.2757 | 1.4812 | 1.021 | 2.221 | 0.0653 |

° marginalmente significnate; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $^{\S}$ $p < 0.0001$

## Capítulo 7

## Discussão Geral

Foram apresentados aqui os argumentos a favor de uma idéia, aqueles relacionados à descrição dos limites de uma espécie enquanto táxon (*sensu* Bock 2004). Não apenas uma, mas inicialmente três destas entidades taxonômicas foram envolvidas ao longo da tese, que a partir dos seus resultados indicou uma possível quarta entidade, identificada apenas graças à complementaridade dos dados aqui apresentados.

O método que permeia e envolve a produção acadêmica também muitas vezes a limita. Por isso, passo neste momento a fazer uma segunda leitura dos mesmos fatos, uma leitura mais livre de restrições de formato, mas uma leitura ainda fiel aos conhecimentos teóricos, e fundamentalmente sustentada por estes. Essa leitura é arriscada, pois trilha caminhos mais sinuosos, mas é como acredito que algo original poderá surgir, muito além do método.

Minha primeira conclusão geral é mais do que elementar: a presença de uma *força* seletiva atuando sobre o sistema estudado (uso a expressão *força* movido pela tradição, já que, como muito bem mostrou Endler (1986), a noção de força para a seleção natural é completamente equivocada). De todas as leituras realizadas, sejam elas de cunho morfológico ou comportamental, todas apontam para a mesma direção, de que a seleção sexual teve um papel determinante na evolução das populações de *Paratrechalea azul*, *P. ornata*, *P. galianoae* e *Paratrechalea* sp.n.. Vimos que um comportamento elaborado de cortejo sexual (Capítulo 2) pode deixar marcas nítidas sobre o fenótipo de uma espécie (Capítulo 3), e que suas similaridades são de fato limitadas, se me permitem fazer uma menção à Teoria da Similaridade Limitante (Abrams 1983).

Minha segunda conclusão é mais audaciosa, e será esta que irá exigir mais de meus esforços em demonstrar sua ocorrência: a presença de uma segunda força seletiva atuando neste mesmo sistema. A influência da seleção natural sobre a evolução fenotípica destas populações foi, durante cada capítulo relativo aos aspectos morfométricos avaliados, identificada como um elemento chave na identificação e proposta de uma nova espécie, através do processo de deslocamento de caracteres. Devido à limitação do método, mais especificamente de um desenho amostral adequado, não foi possível ir além em conclusões e extrapolações.

Parte da tese teve um componente marcado por um elevado grau de ineditismo para fauna de aranhas Neotropicais: o oferecimento de uma presa como presente nupcial (Capítulo 2). Por que não pensar que os machos podem ser tão exigentes quanto às fêmeas na busca de uma parceira? Esta pergunta surge da seguinte premissa: há um investimento energético do macho durante a construção do presente nupcial ainda não mensurado, que nos faz formular uma hipótese intuitiva de que existe uma relação envolvendo benefícios diretos para a fêmea.

Os machos sinalizam suas intenções de cópula nestas espécies, e os fazem com suas quelíceras, contrastantes em relação um presente nupcial mais do que branco, mas quimicamente sinalizado. Sim, presença de substâncias indutivas de um comportamento cooperativo durante a cópula parecem estar atuando neste sistema.

Uma situação interessante e tão inusitada quanto o oferecimento de presentes nupciais é a situação de sintopia das populações de *Paratrechalea azul* e *Paratrechalea* sp.n. em Maquiné. Descrever parte das relações morfométricas destas populações se torna elementar, e ponto de partida para demais estudos envolvendo a origem e manutenção destas unidades biológicas.

Por uma questão de organização apenas, o restante desta discussão está estruturado em dois blocos. O primeiro relacionado às interpretações derivadas da descrição do oferecimento de presentes núpcias em *Paratrechalea*, onde grande parte do que será apresentado constituía uma versão preliminar da discussão do Capítulo 2. O segundo bloco apresenta uma análise integrada dos aspectos morfológicos observados ao longo dos demais capítulos, com algumas indicações dos rumos investigativos que serão tomados a partir desta tese.

## 7.1. Aspectos comportamentais

Como apresentado no Capítulo 2, o oferecimento de uma presa revestida de seda como uma forma de presente nupcial era apenas conhecido para algumas espécies da Família Pisauridae. A descrição deste comportamento para a Família Trechaleidae nos permite desenvolver algumas hipóteses sobre a evolução deste comportamento e as relações filogenéticas dos grupos, muito embora estas suposições ainda sejam evidentemente pouco embasadas em dados empíricos.

É inevitável comparar nossas observações com os dados já disponíveis para *Pisaura mirabilis* (Bristowe & Locket 1926; Stålhandske 2001; Bilde *et al.* 2006; entre outros), e acredito que haja uma grande quantidade de informações indicando que o oferecimento deste tipo de presente nupcial seja um caractere homólogo para estas duas famílias de aranhas. Arrisco inclusive a dizer que este seria um caractere plesiomórfico para o grupo em questão, por apresentarem algumas semelhanças marcantes em seus comportamentos sexuais, bem como por estas duas famílias estarem agrupadas em mesmo clado (Lycosoidea) de acordo com as relações filogenéticas atualmente descritas (Griswold 1993; Silva 2003; Coddington 2005; Santos 2007).

Foram destacadas algumas similaridades importantes que me levam a acreditar nesta hipótese plesiomórfica (Capítulo 2 para *Paratrechalea* sp.n. e *P. azul*; Bristowe & Locket 1926, Stålhandske 2001; Bilde *et al.* 2006 para *Pisaura mirabilis*). O uso da perna III realmente é entre estes o fator de similaridade que merece especial destaque, principalmente por não haver outra descrição de uso deste par de pernas que seja tão elaborado quanto ao seu papel durante o processo de cópula.

A diferença observada no acesso ao epígeno da fêmea pelo macho entre trecaleídeos e pisaurídeos pode ser explicada por outros fatores que intrinsecamente sugerem rotas evolutivas distintas. Justamente por este motivo é que a alta similaridade de algumas etapas do processo de oferecimento de presentes nupciais indica que este seja um comportamento plesiomórfico, caso contrário estas etapas não seriam tão similares, cuja similaridade é mantida inclusive na ordem seqüencial das etapas de cortejo.

Entretanto, fica uma questão pendente (entre tantas outras) do por que os presentes nupciais são tão valiosos a ponto do macho lutar por ele (Capítulo 2), mesmo em um sistema rico em presas de grande qualidade. Seria esperado que o presente nupcial fosse mais valorizado em um sistema onde as presas são escassas e/ou difíceis de capturar, ou onde as fêmeas tenham limitações severas na captura de presas (hipótese de capturabilidade diferencial de presas). Nestes casos, o oferecimento do presente nupcial seria mais facilmente recompensado com a cópula, uma vez que há benefícios diretos para a fêmea. No entanto, a partir da minha experiência de campo na região de Maquiné (Rio Grande do Sul, Brasil) posso fazer as seguintes afirmações: que os habitats destas duas espécies de *Paratrechalea* possuem recursos abundantes, que as fêmeas têm as mesmas habilidades de captura que os machos, e que estas espécies não sofrem nenhuma pressão de predação evidente durante seus períodos de atividade.

### *Origem e evolução dos presentes nupciais em aranhas*

Barrantes & Eberhard (2007) recentemente levantaram a hipótese de que o empacotamento de presas em aranhas (o comportamento geral, não apenas aquele específico para presentes nupciais), seria uma condição derivada da construção da ooteca. Esta hipótese é facilmente aplicada para o caso de *Paratrechalea*, a partir da comparação do processo de construção de presentes nupciais (Capítulo 2) e ootecas (dados não publicados). Fêmeas de *Paratrechalea* usam o terceiro par de pernas para a finalização do processo de construção da ooteca da mesma maneira que os machos utilizam este mesmo par de pernas para a finalização da construção do presente nupcial. O par de pernas III é a única parte do corpo destas aranhas, além das fiandeiras e palpos para ambos os sexos e das quelíceras para os machos, que entram em contato com estas estruturas (ootecas e presentes nupciais). Além disto, os mesmos movimentos corporais são utilizados por machos e fêmeas para a construção de presentes nupciais e ootecas, respectivamente (observações pessoais).

Estes detalhes no uso das pernas III e dos movimentos corporais sugerem que a construção dos presentes nupciais feita pelos machos esteja sendo determinada pelo mesmo programa comportamental utilizado pelas fêmeas para construir suas ootecas. Entretanto, este processo de "exaptação" (entre aspas pois o conceito de exaptação proposto por Gould & Vrba [1982] não pode ser diretamente aplicado), não foi direto, mas passou sim por pelo menos uma etapa intermediária. O empacotamento de presas para facilitação no transporte teria sido o primeiro estágio no uso deste programa de empacotamento (Fernando G. Costa [Instituto de Investigaciones Biológicas Clemente Estable, Montevideo/Uruguai], comunicação pessoal; Barrantes & Eberhard 2007). Desta etapa para uma outra seguinte, onde a presa capturada passa a ser utilizada como um sinal no cortejo sexual, o processo é mais direto. Machos que além de capturar e empacotar suas presas, mas que também oferecem estas às fêmeas, podem ter tido um aumento nas suas aptidões, onde o presente passa a servir como um sinal honesto das qualidades reprodutivas do macho (Kokko 1997).

Determinar a origem evolutiva deste tipo de oferecimento de presente nupcial em aranhas é difícil frente ao conhecimento disponível, uma constatação também feita por Bilde *et al.* (2006). Algumas tentativas em explicar a origem deste comportamento foram apresentadas por Vahed (1998) para insetos, e recentemente este mesmo autor ampliou sua revisão abrangendo também alguns exemplos com aranhas (Vahed 2007), incluindo o caso de *Pisaura mirabilis*. Irei agora ampliar esta discussão incluindo os casos descritos em *Paratrechalea*, principalmente visando sugerir o ponto de origem deste comportamento na filogenia das aranhas.

Existe uma forte concordância na literatura que a evolução dos presentes nupciais em artrópodes pode ter sua origem em mecanismos de exploração sensorial (*sensory exploitation hypothesis* – Vahed 1998; Sakaluk 2000; Kokko *et al.* 2003; Arnqvist 2006; Vahed 2007), e provavelmente isto tenha ocorrido da mesma maneira em aranhas. A hipótese da exploração sensorial não assume (ou exclui – Kokko *et al.* 2003; Vahed 2007) a participação de benefício diretos na origem ou durante a manutenção dos caracteres de interesse, mas possui necessariamente uma importante influência dos benefícios indiretos. Além disto, esta hipótese pode ser interpretada como caso específico de esforço de acasalamento (*mating effort*), além de concordar com a associação da origem evolutiva do presente com o empacotamento de presas para transporte.

Stålhandske (2002) levanta a hipótese de que a origem dos presentes nupciais em *Pisaura mirabilis* está relacionada a um exemplo peculiar de exploração sensorial, onde os machos que oferecem para as fêmeas uma estrutura similar a uma ooteca têm a agressividade das fêmeas reduzida. Esta hipótese só pode ser aplicada para o caso de *Paratrechalea* após a modificação dos mecanismos perceptivos envolvidos no modelo. Além de apresentarem formas distintas para ootecas e presentes nupciais, sendo pouco provável que visualmente sejam confundidos (Fig. 1), a maneira pela qual os machos e as fêmeas de *Paratrechalea* carregam estas estruturas é funcionalmente diferente. Machos carregam os presentes nupciais pelas quelíceras, enquanto que as fêmeas transportam as ootecas aderidas às fiandeiras.

Mesmo se ampliarmos o modelo para um canal de sinalização químico e/ou tátil, onde as fêmeas poderiam interpretar a superfície dos presentes nupciais como sendo um ooteca, algumas inconsistências se manteriam em *Paratrechalea*. Fêmeas invariavelmente se alimentam do presente nupcial, um comportamento nada adaptativo se os machos estivessem, por assim dizer, mimetizando a superfície de uma ooteca.

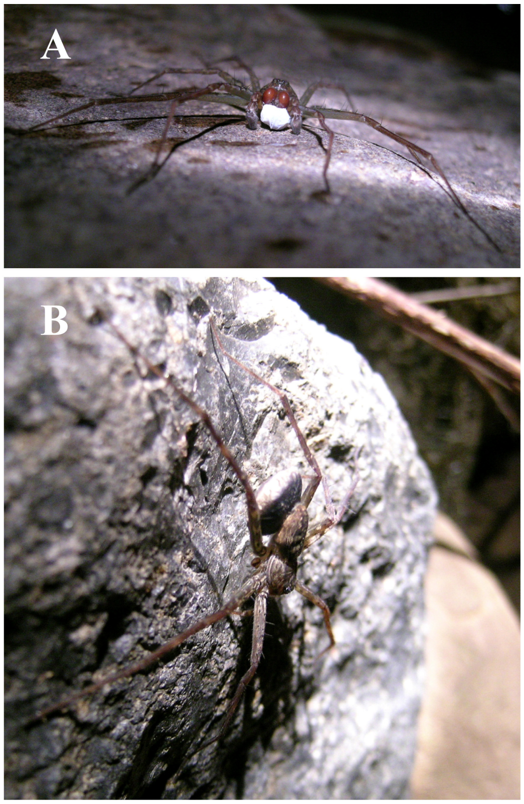


Figura 1. Presente nupcial e de uma ooteca de *Paratrechalea*, ressaltando a maneira pelo qual são transportados e as diferenças de forma que possuem. A) macho de *Paratrechalea azul* carregando um presente nupcial em suas quelíceras. B) fêmea de uma espécie de *Paratrechalea* não identificada com sua ooteca, em posição usual em campo.

### *Cortejo como isolamento heteroespecífico*

Um importante resultado obtido através dos dados apresentados é a ausência de diferenças qualitativas no cortejo sexual de *Paratrechalea* sp.n. e *P. azul* que pudessem ser utilizadas na determinação taxonômica destas espécies. Estes dados apenas reforçam o caráter críptico relatado para aspectos morfológicos e ecológicos, e agora ampliado para caracteres relacionados ao comportamento reprodutivo das espécies. Apesar desta similaridade na descrição do padrão, dados experimentais (não apresentados) indicam que cópulas heteroespecíficas não ocorrem em condições naturais ou experimentais, uma vez que os machos nem ao menos cortejam fêmeas da outra espécie. Em outras palavras, há um isolamento comportamental muito eficaz atuando sobre este sistema.

Cortejo e oferecimento do presente nupcial possuem, muito provavelmente, origens evolutivas independentes (como argumentado na seção anterior), mas quando combinados podem impor uma condição determinística sobre a escolha da fêmea. Mesmo tendo observado por várias vezes machos cortejando fêmeas sem oferecer o presente nupcial, nenhum destes obteve êxito em copular.

É possível que esta determinação do sucesso reprodutivo possa estar associado à condição particular de sintopia das populações observadas, e que não necessariamente deva ser mantida em situações de alopatria. Se assim for, o oferecimento do presente não apenas é um esforço ao acasalamento por parte do macho, mas também comporta sinalizações importantes para o reforço do mecanismo de reconhecimento interespecífico (reforço de isolamento).

Para a situação sintópica observada em Maquiné, há indícios de que o oferecimento heteroespecífico do presente nupcial possa ter sido alvo de seleção (uma constatação que seria até mesmo óbvia), uma vez que seria um comportamento pouco adaptativo por ser um investimento sem recompensa (sem cópula). Algumas observações de laboratório demonstraram um comportamento não-discriminatório entre *P. azul* e *Paratrechalea* sp.n. em diferentes momentos do comportamento reprodutivo.

Durante a busca de uma fêmea pelo macho, os machos de *P. azul* apresentaram o mesmo comportamento de excitação (movimentos estroboscópicos) quando confrontados com a seda proveniente de uma fêmea de *Paratrechalea* sp.n.. Este padrão de estimulação heteroespecífica já é conhecido e descrito para outros grupos de aranhas (licosídeos: Roberts & Uetz 2004), e é até mesmo esperado uma vez que estamos lidando com espécies com alta proximidade filogenética. Logo que a fêmea heteroespecífica foi encontrada, estas adotaram a postura de hiperflexão, enquanto que os machos interromperam o processo de cortejo. Esta simples narração indica que os machos de *Paratrechalea azul* desta população não possuem a capacidade de identificar fêmeas heteroespecíficas através de sinalizações químicas nas sedas deixadas por elas, mas possuem uma capacidade de discriminá-las através de contato direto.

Outras interpretações sobre estas observações serão devidamente testadas em condições controladas. Por exemplo, o fato de uma fêmea ter aceitado um cortejo heteroespecífico pode parecer contraditório, mas pode ter uma interpretação sobre o valor do investimento deste recurso. É possível que nestes casos as fêmeas consigam discriminar a espécies do macho que corteja, mas aceita este cortejo com intenção de ficar com o presente nupcial.

## 7.2. Aspectos morfológicos: assinaturas de um mecanismo co-evolutivo em *Paratrechalea*

Mesmo tendo algumas limitações quanto ao desenho amostral, irei agora apresentar brevemente o que considerei, ao longo de boa parte desta tese, como sendo a assinatura de um mecanismo co-evolutivo entre as populações sintópicas de *P. azul* e *P. ornata*. Este mecanismo, mesmo tendo sido formalmente apresentado na Introdução Geral da tese, surgiu enquanto hipótese ao longo da apresentação dos dados e suas respectivas discussões, tendo sido concebido como um argumento construído ao longo do texto, ao invés de um objetivo específico previamente estabelecido.

### *Aplicação do conceito de deslocamento de caracteres em* Paratrechalea

A interpretação dos padrões fenotípicos observados para as populações de *Paratrechalea* analisadas podem ser explicadas utilizando a hipótese do deslocamento de caracteres. Assim, há uma íntima associação entre os padrões observados com uma pressão seletiva de fonte ainda desconhecida, mas com influência sobre caracteres somáticos e genitais. A Tabela 1 apresenta um resumo do que considerei serem os principais indícios de que a condição sintópica das populações de *P. azul* e *Paratrechalea* sp.n. só é mantida devido a uma atenuação dos níveis de competição interespecífica através deste mecanismo, e servirá de base para cada referência feita aos capítulos anteriores desta tese ao longo desta parte da Discussão Geral.

Tabela 1. Resumo das principais informações apresentadas na tese que foram interpretadas como assinaturas da ocorrência de deslocamento de caracteres entre as populações de *Paratrechalea*. Maiores detalhes sobre os procedimentos analíticos utilizados podem ser consultados nos respectivos capítulos da tese. Abreviações: DST: dimorfismo sexual de tamanho; $b_{\mathrm{OLS}}$: coeficiente de regressão para o quadrado mínimo; $b_{\mathrm{RMA}}$: coeficiente de regressão para o eixo principal reduzido; RSize: tamanho do centróide da apófise tibial retrolateral; ESize: tamanho do centróide do escapo do epígeno; hipo: hipoalométrico; hiper: hiperalométrico.

| | | Capítulo 4 | Capítulo 3 | | | | Capítulo 6 | |
|---|---|---|---|---|---|---|---|---|
| | | DST | Comprimento da quelícera (BL) | | Comprimento da garra (FL) | | Genitália | |
| | Distribuição | Cefalotórax | $b_{\mathrm{OLS}}$ (macho) | $b_{\mathrm{OLS}}$ (fêmea) | $b_{\mathrm{OLS}}$ (macho) | $b_{\mathrm{OLS}}$ (fêmea) | $b_{\mathrm{OLS}}$ (RSize) | $b_{\mathrm{OLS}}$ (ESize) |
| *P. azul* | sintopia | fêmeas maiores | isométrico | hipo* [a] | isométrico | hipo° | 0.46$^{\S}$ | 0.14$^{\S}$ |
| *Paratrechalea* sp.n. | sintopia | machos maiores | isométrico | hiper* [a] | isométrico | hiper* [a] | 0.38$^{\S}$ | 0.41$^{\S}$ |
| *P. ornata* (Pas) | alopatria | ns | - | - | - | - | 0.67* | 0.22$^{\S}$ |
| *P. ornata* (Que) | alopatria | ns | - | - | - | - | 0.59* | 0.29$^{\S}$ |
| *P. galianoae* | simpatria[b] | ns | isométrico | hiper* [a] | hipo* | hiper* [a] | - | - |

* $p < 0.05$; $^{\S}$ $p < 0.0001$

° Marginalmente significante.

[a] Significante para $b_{\mathrm{RMA}}$.

[b] Simpatria regional.

Acredito que a pergunta sobre qual pressão seletiva estaria atuando nesta condição de sintopia seja fundamental para um melhor entendimento do modelo de evolução fenotípica em *Paratrechalea.* Há sem dúvida uma influência da seleção sexual, considerando que boa parte dos meus indícios estão vinculados à ocorrência de um comportamento de cortejo pouco usual em aranhas (Capítulo 2) associado aos caracteres utilizados durante o cortejo copulatório (quelíceras – Capítulo 3). Por outro lado, acredito que a seleção natural tem também uma participação direta nos padrões descritos nos capítulos anteriores, principalmente em relação aos padrões alométricos observados para as quelíceras (Capítulo 3).

O ponto frágil do meu argumento é ter suas raízes em uma suposição: de que a ação do mecanismo de atenuação competitiva seja diferencial entre os sexos. Alguns dados apresentados nos Capítulos 4 e 5 sugerem que as fêmeas estejam mais propensas à ação do deslocamento de caracteres do que os machos. Por este motivo é que são observados padrões invertidos tanto para o dimorfismo sexual de tamanho para caracteres somáticos (Capítulo 4) quanto para os coeficientes de alometria de estruturas utilizadas diretamente durante o processo de cortejo copulatório (Capítulo 3). Os machos, por sua vez, apresentam padrões relativamente mais regulares, e as eventuais diferenças observadas seriam sobre os caracteres relacionados à pressão de seleção sexual.

### *Inversão dos padrões alométricos em fêmeas*

A interpretação dos diferentes padrões alométricos observados para as fêmeas das espécies sintópicas de *Paratrechalea* é interpretada como uma resposta à seleção sexual. Casualmente apenas neste sexo e nestas populações é que as fêmeas parecem estar respondendo de uma maneira cooperativa para o sucesso reprodutivo dos machos, contrapondo as questões envolvendo conflitos sexuais como precursores da evolução fenotípica.

As quelíceras destas espécies são um elemento fundamental durante o cortejo copulatório (Capítulo 2 e 3). Como discutido no Capítulo 3, os padrões isométricos observados para os machos podem ser explicados pela presença de uma pressão de viabilidade funcional da estrutura. A observação de padrões alométricos invertidos para as fêmeas é o primeiro ponto de corroboração para a hipótese de cooperação sexual, onde estes padrões seriam resultantes do processo de cooperação sexual para o cortejo copulatório. Fêmeas de *P. azul*, que são somaticamente maiores do que os machos, apresentaram padrões de alometria negativa para alguns caracteres de quelícera (destaco aqui o comprimento da mesma), enquanto que *Paratrechalea* sp.n. apresentou padrão de dimorfismo sexual de tamanho e de coeficientes de alometria inversos. Interpreto estes resultados como uma sugestão de que há um tamanho ideal de comprimento de quelíceras para fêmeas, e que este tamanho esteja sendo controlado pela seleção sexual, contrário ao padrão isométrico esperado.

Já para as genitálias, a resposta cooperativa das fêmeas é mais contundente, onde proponho que seja ampliada a hipótese do *one-size-fits-all* (Eberhard *et al.* 1998) para a evolução do tamanho de genitália de fêmeas. Mais uma vez os padrões alométricos foram invertidos entre *P. azul* e *Paratrechalea* sp.n., e mais uma vez a espécie com tamanho maior apresentou os menores coeficientes de alometria (Tabela 1). O dado que mais chama a atenção é o elevado coeficiente de alometria observado para a genitália das fêmeas de *Paratrechalea* sp.n., claramente destoando dos valores observados para as demais espécies.

Para as genitálias, o campo de investigação aberto pelas abordagens apresentadas se mostra muito promissor. Por exemplo, as diferenças dos coeficientes alométricos para estas estruturas nas fêmeas podem estar relacionadas a um programa de desenvolvimento independente da parte somática, ou seja, não relacionado à alocação diferencial de recursos já descrito para estruturas sexuais secundárias.

### 7.3. Considerações finais

A identificação de uma nova entidade taxonômica entre as populações amostradas foi apenas alcançada graças ao amadurecimento de uma idéia. Abordei, utilizando diferentes métodos e diferentes estruturas anatômicas, a questão da relação da diversidade morfológica e comportamental neste grupo de aranhas semi-aqüáticas. A conclusão consensual de que uma nova espécie pode ser reconhecida em meio ao conjunto amostral disponível pode ser facilmente explicada de acordo com linhas teóricas há muito estabelecidas, e que podem explicar de uma maneira intuitiva (mas ainda não empírica) a história evolutiva de uma das espécies avaliadas: *Paratrechalea* sp.n..

Minha proposta tem como pressuposto a ocorrência histórica de um processo de contato secundário por parte de *P. ornata* oriunda de sua distribuição norte (ao sul do Rio

Grande do Sul) em direção à região outrora apenas ocupada por *P. azul.* Este contato deu início a um processo de especiação sustentado por um mecanismo co-evolutivo entre as populações das espécies envolvidas. O que observamos hoje como sendo *Paratrechalea* sp.n. é o resultado de um processo de especiação alopátrica entre as populações de *P. ornata*, governado pela atenuação competitiva desta espécie com *P. azul.*

Como insistentemente dito, este é o meu argumento, baseado em dados de populações naturais, cuja elaboração reflete parte de minha bagagem acadêmica, e que está aberta à discussão e a novas interpretações sobre os mesmo dados.

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