

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOLOGIA

SISTEMA DE ACASALAMENTO E EVOLUÇÃO DO CUIDADO
PATERNAL EM DUAS ESPÉCIES DE OPILIÕES DA SUBFAMÍLIA
HETEROPACHYLINAE (OPILIONES: GONYLEPTIDAE)

TAÍS MARIA DE NAZARETH GONÇALVES

2008

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Orientador

Prof. Dr. Glauco Machado

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RESUMO

Fêmeas dos opiliões *Chavesincola inexpectabilis* e *Pseudopucrolia* sp. utilizam cavidades naturais em barrancos como sítios de oviposição. Essas cavidades são monopolizadas por alguns machos através de brigas com outros machos, permitindo o acesso apenas de fêmeas ovígeras. O sucesso reprodutivo dos machos está diretamente associado à posse de um ninho e o sistema de acasalamento das espécies se encaixa na definição de poliginia por defesa de recursos. Observações de laboratório com *Pseudopucrolia* indicam que o tamanho dos machos não é uma característica selecionada pelas fêmeas. Características comportamentais, entretanto, parecem estar relacionadas à probabilidade de obtenção de cópulas pelos machos: quanto maior a fidelidade e a permanência de um macho dentro de um dado ninho, maior a chance desse macho obter uma cópula. Adicionalmente, resultados obtidos para *Pseudopucrolia* fornecem apoio para algumas das principais previsões da teoria de evolução do cuidado paternal em artrópodes via seleção sexual: (1) as fêmeas são iteropáricas; (2) há muitas oportunidades de cópula para os machos; (3) o cuidado paternal libera a fêmea para forragear; (4) os ovos aumentam a atratividade dos machos; e (5) os machos estão dispostos a guardar ovos geneticamente não relacionados a eles. O mapeamento das formas de investimento parental na filogenia de Gonyleptidae permite inferir que o cuidado paternal evoluiu pelo menos três vezes dentro da família: uma vez em Heteropachylinae, pelo menos uma vez em Gonyleptinae e uma vez na base do clado Progonyleptoidellinae + Caelopyginae.

Termos de Indexação: Comportamento, Gonyleptidae, poliginia por defesa de recurso, seleção sexual, sucesso reprodutivo.

ABSTRACT

Females of the harvestmen *Chavesincola inexpectabilis* and *Pseudopucroliia* sp. lay eggs inside natural cavities in ravines. These cavities are monopolized by some males, which fight against conspecific males, allowing the access only of ovigerous females. The reproductive success of males is directly associated to the ownership of a nest and the mating system of both species may be characterized as “resource defense polygyny”. Laboratory observations with *Pseudopucroliia* indicate that male size is not a sexually selected trait. On the other hand, male behavioral features seem to be related to the probability of achieving copulations: the greater the nest tenure and the permanence of a given male inside a nest, the higher his chances of mating. Additionally, results obtained with *Pseudopucroliia* sp. provide support to some of the main predictions of the theory of evolution of paternal care in arthropods via sexual selection: (1) females are iteroparous; (2) guarding males have many mating opportunities; (3) paternal care creates more opportunities for females to forage; (4) females prefer to copulate with guarding males; and (5) males are willing to guard unrelated eggs. Mapping the forms of parental investment in the phylogeny of the Gonyleptidae, it is possible to infer that paternal care has evolved at least three times within the family: once in the Heteropachylinae, at least once in the Gonyleptinae, and once at the base of the clade Progonyleptoidellinae + Caelopyginae.

Index terms: Behavior, Gonyleptidae, reproductive success, resource defense polygyny, sexual selection.

INTRODUÇÃO GERAL

A ordem Opiliones

A ordem Opiliones é o terceiro maior grupo da classe Arachnida, com quase 6.000 espécies descritas (Machado *et al.*, 2007). As espécies da ordem estão presentes em diversos ambientes terrestres, sendo encontradas em todos os continentes, com exceção da Antártica. Elas ocorrem no solo, serrapilheira, debaixo de pedras e entulhos, em troncos de árvore, entre tufo de gramíneas e sobre a vegetação. Embora muitas espécies sejam amplamente distribuídas, podendo ser encontradas em diversos habitats, outras são restritas a determinados ambientes, tais como cavernas e ninhos de formigas (revisão em Curtis & Machado, 2007).

O corpo dos opiliões é compacto e tem duas partes principais, um prossoma anterior (também chamado cefalotórax) e um opistossoma posterior (também chamado abdômen). No prossoma estão presentes as quelíceras, os pedipalpos e os quatro pares de pernas, sendo o segundo par mais alongado e usado como um apêndice sensorial, uma característica única entre os aracnídeos (Shultz & Pinto-da-Rocha, 2007). A parte dorsal do prossoma, conhecida como carapaça, geralmente tem um par de ocelos que é certamente a principal estrutura sensorial envolvida com a fotorrecepção, mas provavelmente incapaz de formar imagens (veja Acosta & Machado, 2007). Lateralmente à carapaça, encontram-se as aberturas das glândulas defensivas, outra característica exclusiva da ordem Opiliones. Essas glândulas produzem uma variedade de secreções voláteis que são liberadas quando o opilião é ameaçado por algum predador (Gnaspini & Hara, 2007).

A ordem Opiliones é dividida em quatro subordens, contendo ao todo 45 famílias e aproximadamente 1.500 gêneros (Giribet & Kury, 2007). Representantes da subordem Cyphophthalmi são geralmente pequenos (1 a 3 mm de comprimento de corpo) e possuem

pernas curtas (Giribet, 2007). A subordem Eupnoi possui seis famílias e 1.780 espécies de corpo geralmente arredondado, pernas longas e pedipalpos não armados (Cokendolpher *et al.*, 2007). A subordem Dyspnoi, distribuída exclusivamente no Hemisfério Norte, é dividida em sete famílias e 290 espécies, que possuem uma grande diversidade de tamanho e de morfologia (Gruber, 2007). A subordem mais diversa é a Laniatores, composta por 26 famílias e 3.748 espécies distribuídas em regiões tropicais e temperadas, principalmente no Hemisfério Sul. As espécies que fazem parte desse grupo possuem corpo mais robusto, pernas de tamanho variáveis e pedipalpos armados com espinhos (Kury, 2007).

Assim como as aranhas, provavelmente o grupo mais bem estudado dentre os aracnídeos, os opiliões têm se mostrado organismos especialmente adequados como modelos para trabalhos comportamentais. A família Gonyleptidae (subordem Laniatores), a segunda maior entre os opiliões, concentra a maioria dos estudos ecológicos e comportamentais realizados com espécies da ordem até o momento em regiões neotropicais (veja referências em Pinto-da-Rocha *et al.*, 2007). Muitas espécies dessa família foram usadas em manipulações experimentais diretamente no campo, permitindo testar hipóteses ecológicas de maneira refinada (e.g., Machado & Oliveira, 1998; 2002; Machado *et al.*, 2002, 2005; Buzatto *et al.*, 2007). Outras espécies foram facilmente mantidas em cativeiro, onde executaram comportamentos similares aos observados no campo (e.g., Capocasale & Bruno-Trezza, 1964; Elpino-Campos *et al.*, 2001; Willemart, 2001; Pereira *et al.*, 2004; Willemart & Chelini, 2007; Osses *et al.*, no prelo). No entanto, o estudo dos opiliões está em seu princípio e informações básicas sobre a história natural das espécies ainda são cruciais para a formulação de hipóteses testáveis sobre o significado adaptativo de diferentes comportamentos. Da mesma forma que aranhas e escorpiões, o aumento no conhecimento biológico sobre os opiliões poderá trazer importantes contribuições teóricas para a ecologia comportamental como um todo.

Sistemas de acasalamento em opiliões

Existe uma grande variedade de sistemas de acasalamento entre os animais e a evolução desses sistemas se deve principalmente às condições ecológicas (Emlen & Oring, 1977). Na maioria das espécies animais, o investimento da fêmea na prole é maior do que o do macho, pois estas produzem uma quantidade limitada de gametas custosos energeticamente (Trivers, 1972). Nesse sentido, a maximização do sucesso reprodutivo das fêmeas é obtida selecionando um bom parceiro e garantindo a sobrevivência do limitado número de filhotes que elas conseguem produzir. Já para os machos, o modo ideal de maximizar seu sucesso reprodutivo é obtendo cópulas com o maior número possível de fêmeas e, geralmente, não provendo cuidado à prole (Bateman, 1948). Portanto, a distribuição espacial e temporal de recursos utilizados pelas fêmeas para a produção ou incubação de ovos exerce uma grande influência sobre o modo como os machos se comportam e otimizam o seu sucesso de acasalamento (Emlen & Oring, 1977).

A poliginia talvez seja o padrão de acasalamento mais amplamente difundido na natureza (Emlen & Oring, 1977). Nesse sistema de acasalamento, a minoria dos machos controla ou têm acesso a múltiplas fêmeas, enquanto a maioria dos machos tem pouco ou nenhum acesso a parceiras sexuais (Shuster & Wade, 2003). A poliginia é geralmente favorecida quando alguns machos são capazes de monopolizar um conjunto de fêmeas receptivas (Emlen & Oring, 1977). De acordo com a forma que os machos usam para monopolizar as fêmeas, a poliginia pode ser dividida em poliginia por defesa de recursos e poliginia por defesa de fêmeas. A poliginia por defesa de recursos ocorre quando alguns machos controlam as fêmeas indiretamente, defendendo territórios ou recursos contra machos co-específicos. Já a poliginia por defesa de fêmeas ocorre quando alguns machos controlam as fêmeas diretamente, defendendo-as contra o acesso de machos co-específicos.

As estratégias de acasalamento na ordem Opiliones foram estudadas em poucas espécies, apesar da grande diversidade e abundância destes organismos na natureza. Em particular, as estratégias de acasalamento das espécies das subordens Cyphophthalmi e Dyspnoi são praticamente desconhecidas. Entretanto, a escassa informação que existe para as subordens Eupnoi e Laniatores sugere que os sistemas de acasalamento entre os opiliões são muito diversos. Um dos poucos estudos detalhados sobre o assunto foi conduzido com o opilião neotropical *Zygopachylus albomarginis* (Manaosbiidae), cujos machos constroem ninhos de barro sobre troncos caídos que são utilizados por fêmeas como sítios de oviposição. Após a oviposição, são os machos, e não as fêmeas, que permanecem guardando os ovos (Mora, 1990).

Outra espécie que foi bem estudada é o opilião *Leiobunum vittatum* (Sclerosomatidae), uma espécie abundante no leste dos Estados Unidos (Macías-Ordóñez, 1997, 2000). Os machos dessa espécie defendem rochas no solo que são visitadas por fêmeas em busca de substrato para oviposição. Os machos brigam entre si pelo controle dessas pedras e, conseqüentemente, pelo acesso às fêmeas. Ao contrário de *Z. albomarginis*, os machos de *L. vittatum* não exercem nenhum cuidado parental e, após depositarem seus ovos em fissuras nas rochas, as fêmeas abandonam o sítio reprodutivo. Nesse caso, o sucesso reprodutivo dos machos de *Leiobunum* está diretamente associado ao número de fêmeas com que copulam no seu território e o sistema de acasalamento se encaixa na definição de “poliginia por defesa de recursos” (Macías-Ordóñez, 1997).

Cuidado paternal em opiliões

Muitos fatores e condições ecológicas foram propostos para explicar qual dos sexos tem maior propensão a exercer cuidado parental (Queller, 1997). A diferença sexual no tamanho dos gametas e a grande abundância dos gametas dos machos fazem com que estes tenham que competir pelo acesso aos raros gametas femininos (Bulmer & Parker, 2002). A

competição dos machos por fêmeas resulta em uma baixa certeza da paternidade, principalmente em espécies com fertilização interna (Williams, 1975). Adicionalmente, a competição entre machos pelo acesso às fêmeas e a escolha dos melhores machos pelas fêmeas faz com que apenas um grupo restrito de machos da população tenha chance de conseguir cópulas (Bateman, 1948). Portanto, a incerteza da paternidade e a perda de oportunidades de cópulas fazem com que os custos do cuidado paternal para os machos sejam, na grande maioria das espécies, maiores do que os benefícios (Kokko & Jennions, 2003). De fato, o cuidado paternal é extremamente raro na natureza, sendo encontrado em poucas espécies (Clutton-Brock, 1990). Em artrópodes, esse comportamento evoluiu independentemente somente em 15 *taxa* (Tallamy, 2000, 2001; Machado, 2007). De acordo com a hipótese de evolução do cuidado paternal via seleção sexual, a guarda da prole pelo macho minimizaria os custos da reprodução para as fêmeas, pois as liberaria para forragear após a oviposição. Adicionalmente, o cuidado paternal seria um sinal honesto da qualidade do macho para as fêmeas. Sob essas condições, machos que provejam cuidado parental devem obter um maior número de cópulas quando comparados a machos que não o façam (Tallamy, 2000, 2001).

Todos os casos de cuidado paternal descritos até o momento para os aracnídeos estão restritos à ordem Opiliones (Machado & Macías-Ordóñez, 2007). Em *Lepchana spinipalpis* (Assamiidae) e *Karamea* spp. (Triaenonychidae), machos guardam ovos em diferentes estágios de desenvolvimento e até mesmo ninfas (Martens, 1993; Machado, 2007). Já machos de *Leytpodoctis oviger* (Podoctidae) carregam de 1-13 ovos atados ao fêmur do quarto par de pernas (Martens, 1993). Em outras espécies de Gonyleptidae, tais como *Ampheres leucopheus*, *Gonyleptes saprophilus*, *Neosadocus* sp., *Iporangaia pustulosa*, *Iguapeia melanocephala* e *Progonyleptoidellus striatus*, os machos também já foram observados cuidando de desovas contendo ovos em diversos estágios de desenvolvimento embrionário (Hara *et al.*, 2003; Machado *et al.*, 2004). Em todos esses casos, mais de uma fêmea contribui com ovos para a

desova e diferenças na quantidade de ovos entre desovas podem estar relacionadas com a atratividade do macho ou do sítio de oviposição.

OBJETIVOS

A família Gonyleptidae é a mais diversa dentre os opiliões neotropicais, com quase 1.000 espécies divididas em 17 subfamílias (Kury, 2003). O dimorfismo sexual das espécies dessa família é acentuado, com machos maiores e mais fortemente armados que as fêmeas. Dada a enorme variedade de formas de dimorfismo sexual na família, é provável que a seleção sexual tenha exercido um papel importante na evolução do grupo (Machado & Macías-Ordóñez, 2007). As formas de investimento parental entre os Gonyleptidae também são bastante diversificadas, com casos de cuidado maternal, paternal e nenhum cuidado, que provavelmente é o comportamento ancestral na família (Machado & Raimundo, 2001). O cuidado paternal, que é a forma mais rara de investimento parental entre os artrópodes, evoluiu pelo menos duas vezes independentemente entre os Gonyleptidae: uma na subfamília Gonyleptinae e outra na base do clado formado pelas subfamílias Caelopyginae e Progonyleptoidellinae (Machado *et al.*, 2004).

Heteropachylinae é uma subfamília basal de Gonyleptidae (Kury, 1994) composta por oito gêneros e 12 espécies cuja distribuição registrada para a Mata Atlântica vai desde o Ceará até o norte do Espírito Santo (Kury, 2003). Em geral, os representantes da subfamília são opiliões de médio porte, com coloração marrom ou preta, que ocorrem predominantemente no chão de florestas e formações abertas. Não existe nenhuma informação biológica sobre as espécies de Heteropachylinae. Assim, o objetivo geral desta dissertação é descrever a biologia reprodutiva e o sistema de acasalamento com ênfase no cuidado paternal exibido por duas espécies pertencentes a essa subfamília.

Esta dissertação está dividida em dois capítulos e os objetivos específicos de cada um deles são:

- (A) CAPÍTULO 1 - Descrever aspectos básicos da biologia reprodutiva de *Chavesincola inexpectabilis* Soares & Soares, 1946, tais como o comportamento de corte, cópula e oviposição. Adicionalmente, são apresentadas informações inéditas sobre o cuidado paternal na espécie.
- (B) CAPÍTULO 2 - Descrever mais um novo caso de cuidado paternal na subfamília Heteropachylinae, referente à espécie *Pseudopucrolia* sp. e explorar de forma detalhada seu sistema de acasalamento. Além disso, o capítulo testa as principais predições da hipótese de evolução do cuidado paternal via seleção sexual proposta por Tallamy (2000; 2001).

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CAPÍTULO 1

Mating behavior of *Chavesincola inexpectabilis* (Opiliones: Gonyleptidae), with the description of a new and independently evolved case of paternal care in harvestman*

Taís M. Nazareth & Glauco Machado



ABSTRACT

In this paper, we investigate the mating behavior of the gonyleptid *Chavesincola inexpectabilis* (Heteropachylinae) and provide basic descriptive information on courtship, copulation, oviposition, and paternal care. Like most gonyleptids, males of *C. inexpectabilis* present a strong armature on the fourth pair of legs and use its spines and apophyses to fight other males and to repel them from their nesting sites. The mating pair presents a short interaction before copulation and touches on the female by the male occurs both during and after penetration, while she oviposits. The oviposition behavior presents a marked difference when compared to other Laniatores: females hold the eggs on the chelicerae before depositing them on the substrate. After oviposition, the eggs are left under the guard of the male, which defends the eggs against the attack of cannibalistic conspecifics. Mapping the available data on reproductive biology of the Gonyleptidae, it is possible to infer that paternal care has evolved at least three times independently within the family: once in the clade Progonyleptoidellinae + Caelopyginae, once in the Gonyleptinae, and once in the Heteropachylinae, which occupies a basal position within the group.

Keywords: Copulation, courtship, evolution, Heteropachylinae, oviposition, sexual dimorphism.

INTRODUCTION

Although Opiliones may have been the first group of arthropods to evolve an intromittent organ (Dunlop *et al.*, 2003), several aspects of their reproductive biology were investigated in the last 10 years (review in Machado & Macías-Ordóñez, 2007). Harvestman fertilization is internal, and the transfer of spermatozoa may occur indirectly through spermatophores in representatives of the suborder Cyphophthalmi, or directly by means of a long and fully intromittent male genitalia in the suborders Eupnoi, Dyspnoi, and Laniatores (Machado & Macías-Ordóñez, 2007). Courtship before intromission is generally quick and tactile, but there are some cases in which males offer a glandular secretion produced in their chelicerae before copulation as a nuptial gift for their mates. Courtship during intromission, on the other hand, may be intense and involve leg tapping and rubbing. Copulation is often followed by a period of mate guarding in which the female is held or constantly touched by the male (see Table 12.1 in Machado & Macías-Ordóñez, 2007).

Females may lay their eggs immediately or months after copulation, and the oviposition strategies seem to be related to the length of the ovipositor. Most species of the suborders Cyphophthalmi and Eupnoi have a long ovipositor and hide their eggs inside small holes in the soil, trunk crevices, or under stones. Representatives of suborders Dyspnoi and Laniatores, constrained by their short ovipositor, lay their eggs on exposed substrates such as leaves, wood, and rocks (Machado & Macías-Ordóñez, 2007). The forms of parental care range from microhabitat selection for oviposition to active egg guarding by a parental individual. In most species, eggs are laid singly in shallow natural cavities or are covered by debris by the female. In some species, however, females lay eggs in a single large batch and brood eggs throughout the embryonic development, remaining with the newly hatched nymphs for some days until they disperse (Machado & Raimundo, 2001). Maternal care has

been reported for many families of the suborder Laniatores, especially among the Neotropical representatives of the superfamily Gonyleptoidea (see Machado & Warfel, 2006).

While maternal egg guarding is widespread among arachnids, exclusive paternal care is present only in the order Opiliones (Machado *et al.*, 2005). Male assistance has evolved in at least five families belonging to three non-closely related superfamilies of the suborder Laniatores: Travunioidea, Epedanoidea, and Gonyleptoidea (Machado, 2007). Within the Gonyleptidae, which comprises nearly 1,000 species and corresponds to the largest family of the suborder Laniatores, there are eight cases of paternal care recorded so far (Machado & Macías-Ordóñez, 2007). In this paper, we investigate the mating behavior of the gonyleptid *Chavesincola inexpectabilis* Soares & Soares, 1946 (Heteropachylinae). We provide basic descriptive information on the courtship, copulation, oviposition, and paternal care of this small (ca. 5 mm width of the dorsal scute) harvestman that presents a marked sexual dimorphism, with males larger and more armed than females (Soares & Soares, 1946). This study is the first description of the reproductive biology of a representative of the subfamily Heteropachylinae.

MATERIAL AND METHODS

Nine females and 14 males of *C. inexpectabilis* were collected in the borders of a small (ca. 8 ha) urban forest fragment in Santa Teresa city (19° 58' S; 40° 32' W; 675 m alt.), Espírito Santo state, southeastern Brazil. The individuals were found under rotting logs and piles of tree fern trunks discarded from a green house nearby. They were brought to our laboratory in the Natural History Museum of the Universidade Estadual de Campinas (São Paulo state, Brazil) and were maintained in a communal terrarium (40 x 90 cm base, 20 cm height) containing soil, small pieces of tree fern trunks collected in the study site, and 10 artificial

nests built in clay blocks (with 6 x 2 cm base, 3 cm height). Each artificial nest had a central hole (1 cm in diameter and 2 cm depth) crossing the clay block from side to side. These blocks were placed against the glass wall of the terrarium so that it was possible to observe the harvestmen behavior inside the nests through the glass (Figures 1 - 2). These nests simulated natural cavities in ravines in which males of another Heteropachylinae species (*Pseudopucrolia* sp.) were found taking care of eggs in the field (Nazareth & Machado, unpubl. data). During the study period, the abiotic conditions in the laboratory were (mean $\pm$ SD): temperature of 25.5 ± 1.2 °C, humidity of $82.0 \pm 5.4\%$, and photoperiod of 13L:11D.

Individuals were measured (dorsal scute width) and individually marked on their dorsal scute with colored dots of enamel paint. They were fed pieces of dead cockroaches and an artificial diet for ants (Bhatkar & Whitcomb, 1970) three times a week. The nests were individually numbered and, at each observation, the identity of the individuals inside each nest was recorded. Behavioral data are based on 45 days of laboratory work comprising nearly 50 h of *ad libitum* observations (sensu Altman, 1974), of which 43 h were conducted at night (from 18:00 to 00:00 h), when individuals are more active. Nocturnal observations were made with a red lamp to avoid disturbing the animals (cf. Elpino-Campos *et al.*, 2001; Pereira *et al.*, 2004). Continuous recording (sensu Martin & Bateson, 1994) was made of all relevant behavioral events, such as fights between males, copulations, and ovipositions. Voucher specimens of males and females were deposited in the arachnological collection of the Museu de Zoologia da Universidade de São Paulo (MZSP), São Paulo state, Brazil.

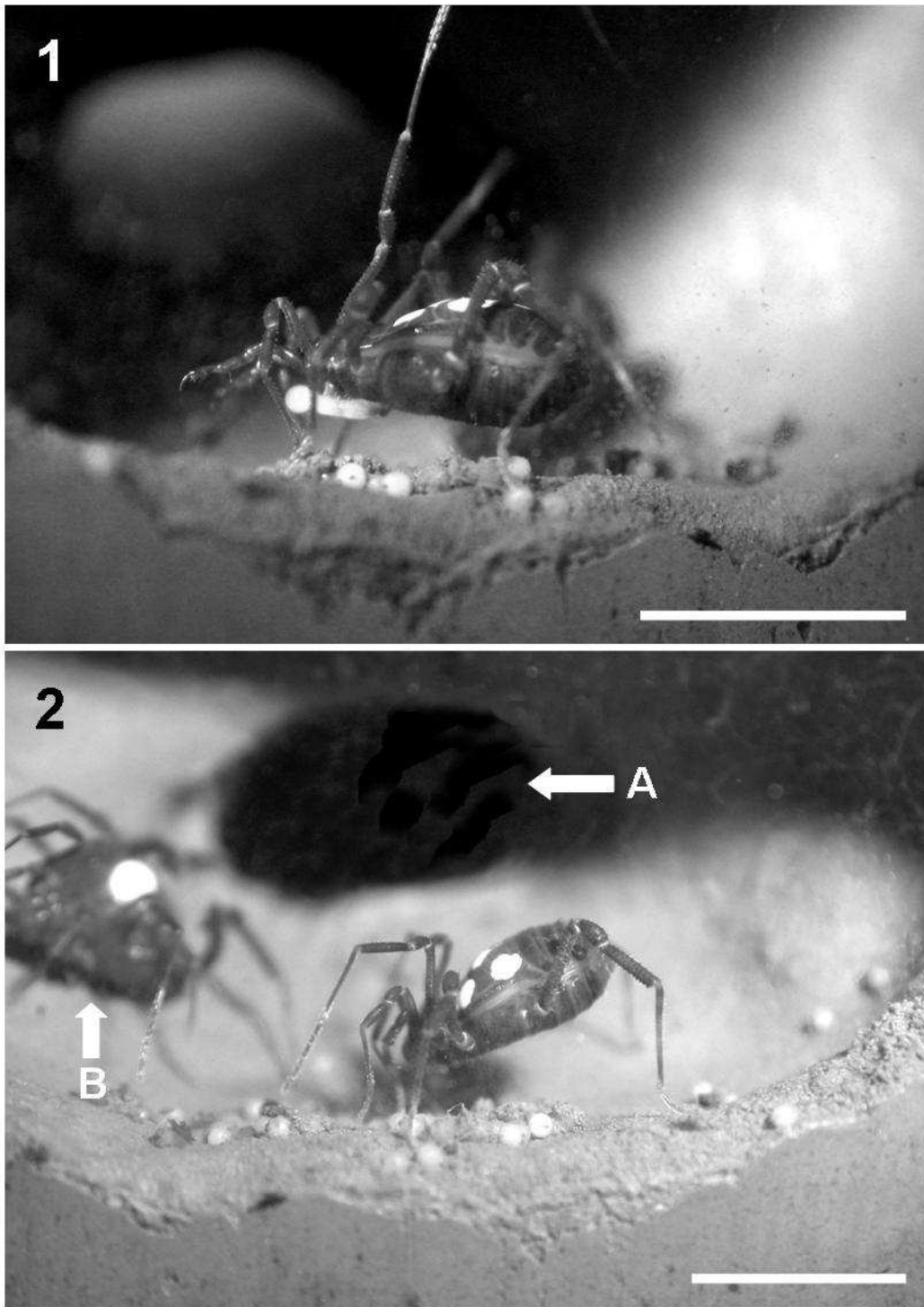
RESULTS

Ten males were observed occupying and occasionally fighting for the ownership of the nests. Only two males were observed mating: the first one (M1) achieved copulation after staying in the same nest for four consecutive days, and the second (M2), after five consecutive days. These males had a dorsal scute width of 4.99 mm (M1) and 4.71 mm (M2), and were, respectively, the first and the third largest males in the terrarium (mean male size $\pm$ SD = 4.48 ± 0.27 mm; $n = 14$). In two occasions, as soon as an intruder male entered a nest occupied by one of these two males, a brief period of intense mutual tapping with the second pair of legs occurred. After that, the individuals turned their backs to each other and intertwined the fourth pair of legs, which is full of spines and tubercles. In this position, the males seemingly tried to capsize each other by means of sudden upward movements in which each male brought its femur IV close to the body, pinching his opponent's fourth pair of legs (this phase lasted nearly 30 s in both fights observed). In both cases, resident males managed to pull the intruders out of the nests.

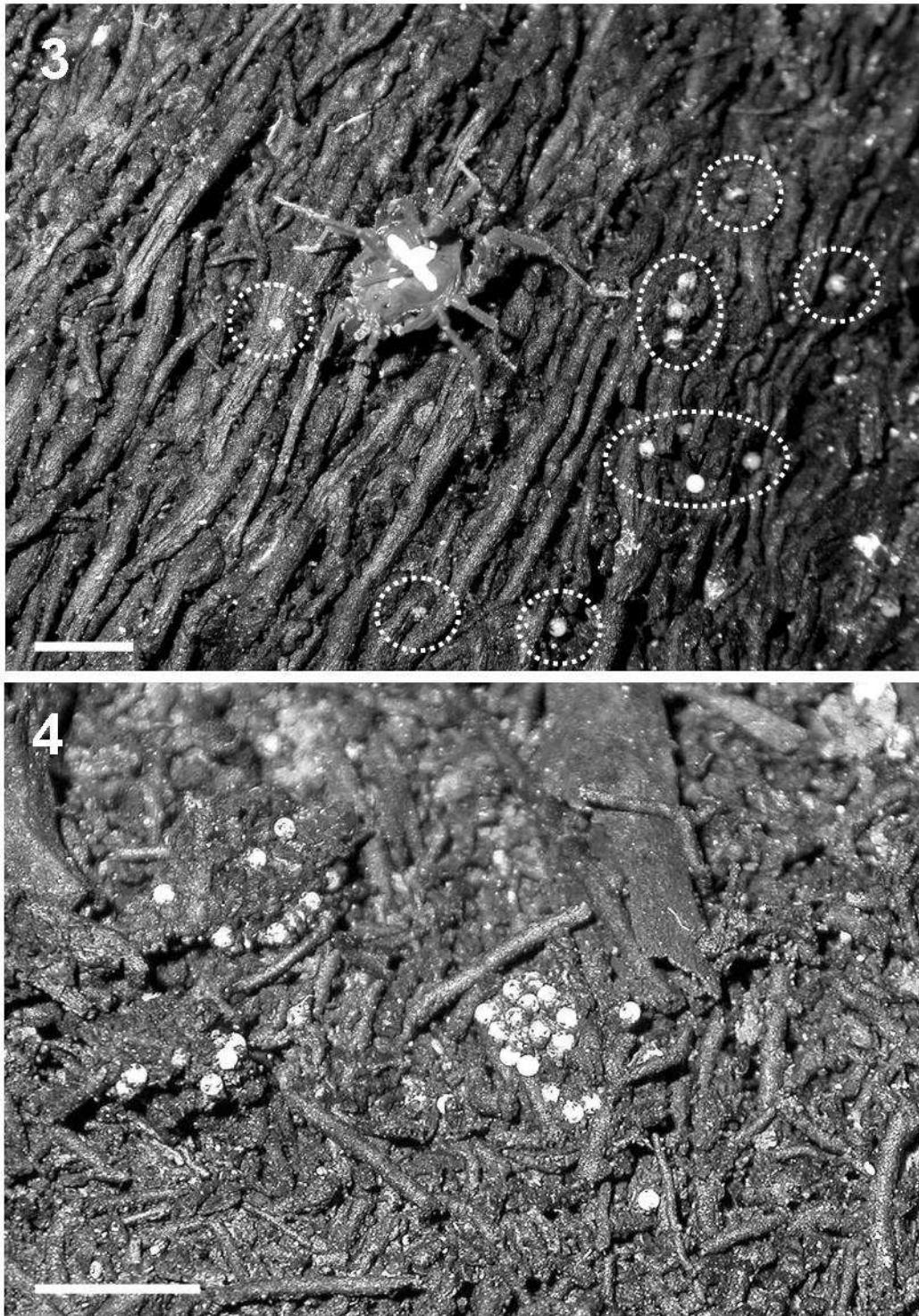
M1 copulated at least five times with four different females, achieving a total of 228 eggs in his nest, and M2 copulated at least three times with three different females, achieving a total of 83 eggs. Most of the females (6 out of 9) were observed copulating at least once. One of them was observed copulating and laying eggs with M1 and M2 and another one was observed laying eggs twice with M1. The mean number of eggs laid in each oviposition was 38.9 (SD = 12.2; $n = 8$), and the intervals between the two oviposition events of each female ranged from 9 to 12 days ($n = 8$). After the hatching of all nymphs inside the artificial nests, M1 left his first nest and established a new nesting site under a piece of tree fern trunk (Figures 3 and 4). Eleven days after, 54 eggs covered by debris and in two different stages of embryonic development (according to Machado *et al.*, 2004) were found attached to the

undersurface of the tree fern trunk. Since males accept eggs only after copulating with the female, the presence of the clutch under the tree fern trunk indicates that M1 copulated with two females or twice with the same female. M1 remained close to the eggs until they hatched 16 days later (Figures 3 - 4).

Just before copulation, the male approached the female frontally and intensely tapped her genital opening with his second pair of legs. Meanwhile, the male also gently touched the dorsum of the female with his first pair of legs ($n = 2$). In one case, touching behavior lasted 30 s and, in the sequence, the male (M1) grasped the female pedipalps with his own pedipalps. The female raised the frontal region of her body, approaching her ventral region to the genital opening of the male. In this position the male everted his penis and penetrated the female's genital opening. The other courtship lasted almost 1 h and, during all this time, the male (M2) touched the female as described above. During most of the courtship, the female bended the frontal region of her body so that it was impossible for the male to accomplish penetration. Occasionally, she also put her venter in contact with the substrate, also preventing the male from touching her genital opening. Eventually, the male managed to grasp the female pedipalps with his pedipalps and then she raised the frontal region of her body allowing penetration. Both copulations lasted nearly 2 min, and during penetration the male performed intense leg tapping on the dorsum of the female using his first pair of legs, and simultaneously on the female's hind legs and venter using his second pair of legs. Penetration was apparently terminated by the female, which promoted a sudden backwards movement of the body, strong enough to release her from the male pedipalpal grasping. Immediately after separation, the male continued to tap the dorsum and venter of the female with his second pair of legs for nearly 2 min.



Figures 1-2. (1) Marked female of the harvestman *Chavesincola inexpectabilis* evertting the ovipositor and manipulating the egg with the chelicerae while scrapping the substrate of the nest with her first pair of legs. (2) Another marked female of the same species covering a recently laid egg with debris. Behind the female it is possible to see the nest entrance (A) and the guarding male walking around while she is ovipositing (B). Both photos were taken throughout the glass wall of the terrarium. Scale bars = 5 mm.



Figures 3–4. (3) Marked male of the harvestman *Chavesincola inexpectabilis* taking care of eggs laid on a piece of tree fern trunk. The dotted circles indicate the position of the eggs. (4) Detail of the clutch after the addition of more eggs. Note that the eggs are covered by debris (photos by: B.A. Buzatto). Scale bars = 5 mm.

After copulation, the female generally walked inside the nest for nearly 3 min ($n = 7$), always followed by the male, probably searching for a proper place for egg laying. In the first step of the oviposition, female everted her ovipositor and placed its tip in contact with her chelicerae for up to 7 min. At the same time, the male, stood behind the female, repeatedly tapped her dorsum using his second pair of legs. Once every 3 min the male also gently tapped the venter of the female ($n = 2$ ovipositions) — it was not possible to see if the male touched the ovipositor. Next, the female released an egg, which was held into the chelicerae while she scraped the nest's wall with her first pair of legs (Figure 1). Every two or three scrapes of the nest's wall, the female brought the leg to the mouth, probably to clean or humidify the tip of the leg — this process lasted from 7 to 13 min. In the sequence, the female put the egg on the scraped area using her chelicerae and rolled it on the substrate using the first pair of legs until the egg got completely covered by debris, a process that lasted up to 1 min (Figure 2). After oviposition of each egg, the male walked around inside the nest until the female started to lay the next egg (Figure 2). At this moment, the male resumed tapping the female using his second pair of legs, as described above. The whole process of oviposition lasted 2 to 4 days (mean $\pm$ SD = 2.6 ± 0.7 ; $n = 8$), and was intercalated by periods of resting (*sensu* Elpino-Campos *et al.*, 2001), when both male and female did not interact with each other. After this period, the female abandoned the nest and the eggs were left under the male protection until they hatched 23 - 24 days later.

Non-guarding males and females were frequently observed walking around in the terrarium at night, and they were observed eating at least 10 times. Guarding males, on the other hand, rarely left their nests to forage at night — when they did ($n = 2$), they remained within 10 cm from the nest entrance. Additionally, unlike females that ate the cockroach pieces on the spot, guarding males and males that were defending nests without eggs took the food to their nests before consumption ($n = 6$). In one case, a non-guarding male was observed entering a nest and seemingly trying to remove some eggs with the pedipalps,

probably as an attempt of cannibalism. The guarding male (M1), which was 2 cm away from the nest entrance, attacked the intruding male using the first pair of legs and pedipalps. The non-guarding male left the nest without cannibalizing any egg, and was chased by the guarding male for nearly 30 s. After that, the guarding male came back to his nest and remained with the fourth pair of legs blocking the nest entrance for nearly one hour.

DISCUSSION

When males are in charge of egg brooding they become a reproductive resource for females and some degree of sex-role reversal may be expected (Owens & Thompson, 1994; Parker & Simmons, 1996). In such cases, male-male competition may be less intense and no sexual dimorphism is expected. Although most gonyleptids show strong sexual dimorphism, males being larger and more armed than females, this dimorphism in paternal species of the subfamilies Caelopyginae and Progonyleptoidellinae is very subtle. Females of many species present spiny legs and apophyses as long as those of males (e.g., Pinto-da-Rocha, 2002), or in other cases neither sex has any leg armature at all (e.g., Kury & Pinto-da-Rocha, 1997). However, strong sexual dimorphism may be found among paternal species of the subfamily Gonyleptinae. In this subfamily, males of some species defend very specific sites (holes in ravines and trunks) as nesting sites, and leg armature seems to be involved in defense of this scarce resource against other males (Machado *et al.*, 2004). Males of *C. inexpectabilis* also defend nesting sites and, as could be expected, males present a strong armature on the fourth pair of legs. They use the spines and apophyses of these legs to fight other males and to repel them from the nesting sites. Similarly to males of *Neosadocus* sp., which also use holes in ravines as nesting sites, males of *C. inexpectabilis* use the heavily armed fourth pair of legs to

block the entrance of their nests and to pinch intruder males (see Figures 2B, C in Machado *et al.*, 2004).

Most descriptions of courtship in harvestmen of the suborder Laniatores lack detailed information, such as which parts of the female body are touched by the male. Even though the courtship behavior of *C. inexpectabilis* follows the general pattern previously recorded for some gonyleptid harvestmen (see Machado & Macías-Ordóñez, 2007), here we provide additional information showing, for instance, that males intensively touch the genital opening of the female. It is possible that these touches stimulate the female to open her genital opening, a pre-requisite for male intromission among Laniatores. Unreceptive females clearly avoid male touches on the genital opening approaching the venter to the substrate. On the other hand, receptive females allow the male pedipalpal grasping and raise the frontal region of the body so that penetration can occur. The end of the copulation is also apparently determined by the females, which seem to be able to release themselves from the intromission and from the pedipalpal grasping. In species of Eupnoi, the female may reject intromission, but grasping seems harder to avoid because the male hooks tightly his long, sexually dimorphic pedipalps to the base of female's legs II, near the trochanter. Apparently, Eupnoi males rely more on the powerful grasping to negotiate with the female, whereas Laniatores rely more on precopulatory courtship (discussion in Machado & Macías-Ordóñez, 2007).

Post-copulatory courtship in *C. inexpectabilis* occurred as males tap on the dorsum and venter of females using their legs. Intense female stimulation both during and after copulation may be viewed as a male strategy to increase the number of eggs fertilized and also increase paternity (Eberhard, 1996). Additionally, the total time spent by ovipositing females inside a male's nest may reach four days, quite a long period when compared to other harvestmen species (e.g., Machado & Oliveira, 1998; Willemart, 2001; Juberthie & Muñoz-Cuevas, 1971; Mora, 1990). In *Pseudopucroliia* sp., other Heteropachylinae species we

are studying in our laboratory, males block the entrance of the nest with their body this preventing females from leaving (Nazareth & Machado, unpub. data). This coercive behavior, associated with repeated copulations, is possibly another male strategy to increase paternity and the number of eggs that one female will lay inside the nest.

The oviposition behavior of *C. inexpectabilis* presents marked differences when compared to other Laniatores, including representatives of the family Gonyleptidae (e.g., Juberthie & Muñoz-Cuevas, 1971; Machado & Oliveira, 1998; Willermart, 2001). A unique behavioral feature is that females hold the eggs on the chelicerae before depositing them on the substrate. It is possible that females use secretions from the mouthparts to cover the eggs before their deposition on the substrate, promoting the attachment of debris on them or moistening them with anti-pathogenic compounds, as some centipedes do (Brunhuber, 1970; Lewis, 1981). Egg covering with debris has been previously described for several harvestman species of the families Cosmetidae and Gonyleptidae that present no care or exclusive maternal care (references in Willemart, 2001). The only case of egg covering reported so far for a paternal species occurs in the tryaenonychid *Karamea* (Machado, 2007), which is not closely related to the Gonyleptidae (Giribet & Kury, 2007). This behavioral trait, therefore, clearly evolved independently in these two families, but in both cases might be related to egg protection by providing camouflage or preventing dehydration (Willemart, 2001; Elpino-Campos *et al.*, 2001).

Maternal egg-guarding is a costly behavioral strategy for iteroparous arthropods because it reduces lifetime fecundity by increasing the risk of death from predation and reducing foraging opportunities for guarding females during the long periods of care (Tallamy & Brown, 1999; see also Buzatto *et al.* 2007). Reduction of foraging is one of the main costs paid by guarding females, and according to the “enhanced fecundity hypothesis”, exclusive post-zygotic paternal care may be viewed as a fitness-enhancing gift from males to females, because it offers females two direct benefits: (1) the cost-free care of their offspring

and (2) the freedom to forage for additional food (Tallamy, 2001). After oviposition, eggs of *C. inexpectabilis* are left under the guard of the male, and females are released to forage and to produce more eggs. The intervals between two consecutive ovipositions ranged from 9 - 12 days, which is almost 10 times shorter than the median interval between two ovipositions in the maternal gonyleptid *Bourguyia hamata* (Machado & Oliveira, 2002), representative of a closely related subfamily, the Bourguyiine (Kury, 1994). Apparently, the reproductive rate of *C. inexpectabilis* females is higher than females of species with maternal care, a likely consequence of their increased foraging rate. Experimental studies are necessary to address this question more carefully.

Mapping the available data about reproductive biology on the internal phylogeny of the Gonyleptidae, it is possible to infer that paternal care has evolved at least three times independently in the family: once in the ancestor of the subfamilies Progonyleptoidellinae and Caelopyginae, at least once in the subfamily Gonyleptinae (see discussion in Machado *et al.*, 2004), and once in the subfamily Heteropachylinae, which occupies a basal position within the Gonyleptidae (Figure 5). This is a conservative scenario because the two Gonyleptinae genera that present paternal care, *Gonyleptes* and *Neosadocus*, do not seem to be closely related (A.B. Kury, pers. comm.). Thus, no detailed analysis can be accomplished until an internal phylogeny of the subfamily Gonyleptinae is available. Since there is no published information on any aspect of the biology of the Andean subfamily Metasarsinae, it is not possible to understand if paternal care in Heteropachylinae is derived from no care or from maternal care. Additionally, there is also no information on the basal monotypic subfamily Cobaniinae. Data on the reproduction of these two subfamilies are crucial to infer the plesiomorphic form of egg assistance in gonyleptids and to provide a more complete scenario of the transitions between different forms of parental care in the family Gonyleptidae.

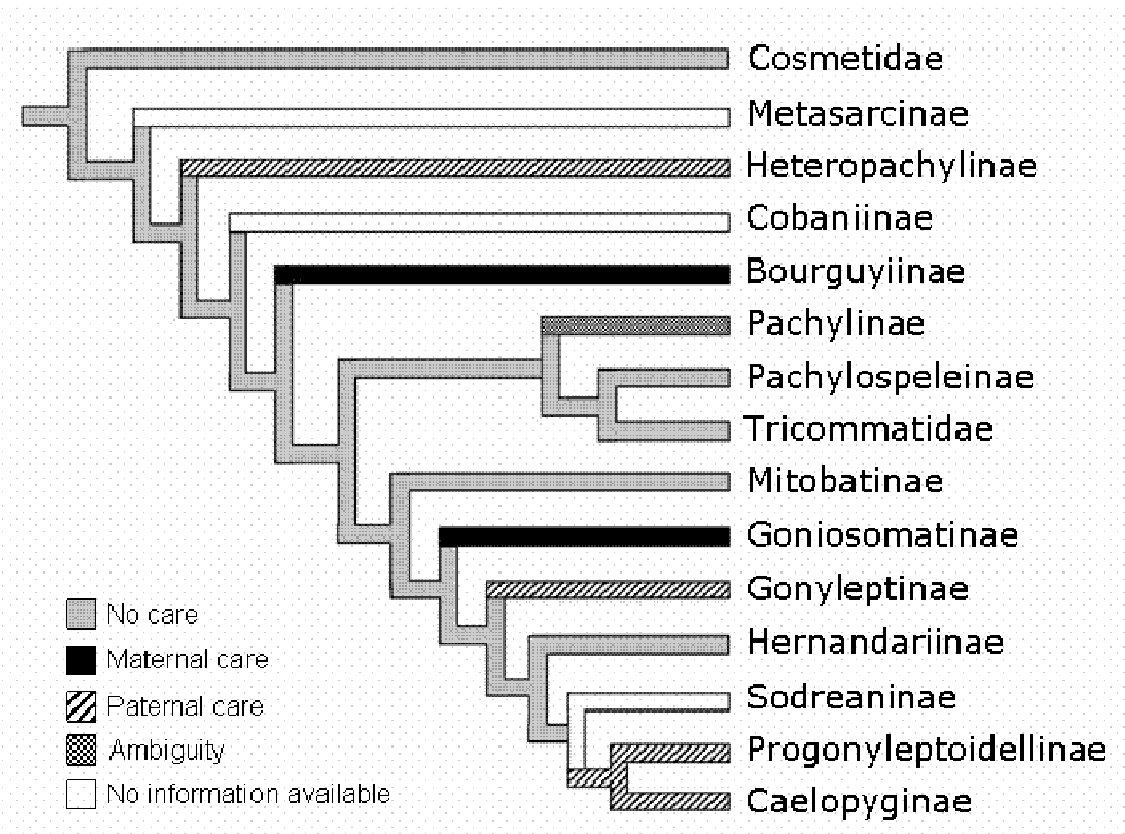


Figure 5. — Internal phylogeny of the family Gonyleptidae (modified from Kury 1994 and Pinto-da-Rocha 2002) showing the forms of parental care presented by each subfamily.

Although there is one case of maternal care in the Cosmetidae (Goodnight & Goodnight 1976), the plesiomorphic state for the family is probably no care (see Machado & Raimundo 2001). The information for the Pachylinae is considered polymorphic because there are both cases of no care and maternal care. Since there is no data on the internal phylogeny of this subfamily, it is not possible to infer that the plesiomorphic state of the character. Behavioral data were mapped using the program Mesquite (Maddison & Maddison 2001).

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CAPÍTULO 2

Evolution of exclusive paternal care in a neotropical harvestman (Arachnida: Opiliones)*

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ABSTRACT

The aim of this study was to test the predictions of the theory of the evolution of paternal care via sexual selection, using the neotropical harvestman *Pseudopucrolia* sp. as model organism. Females use natural cavities in ravines as nesting sites, which are defended by males against other males. After oviposition, females leave the nests and all postzygotic parental care is accomplished by males, which protect eggs and nymphs from predators. We provided artificial mud nests to individuals in the laboratory and conducted observations on the reproduction of the species. Male size did not influence the chances of achieving copulations. However, male reproductive success was directly related to nest ownership time: the longer a male holds a nest, the higher his chances of obtaining copulations. All clutches were composed of eggs laid by more than one female, suggesting that guarding males have several mating opportunities. Experimental manipulations also demonstrated that guarding males are more attractive to females than non-guarding males. Moreover, males accepted to guard unrelated eggs. Finally, we demonstrated that females spent more time foraging than guarding and non-guarding males. This study is the first to formally test all predictions from the theory of evolution of paternal care via sexual selection. Our results suggest that exclusive postzygotic paternal investment in *Pseudopucrolia* has evolved by sexual selection and not by natural selection.

Key-words: Egg adoption, female preference, Gonyleptidae, mating system, *Pseudopucrolia*, resource defense polygyny, sexual selection.

INTRODUCTION

Numerous factors have been proposed to explain which sex is more likely to provide parental care (Queller, 1997). Direct male-male competition for access to females coupled with female mate choice result in low confidence of paternity, especially among species with internal fertilization (Williams, 1975). Moreover, internal fertilization creates a physical and temporal isolation between males and the eggs they have fertilized (Gross & Shine, 1981). Low confidence of paternity and marked variation in mating success may act against the evolution of paternal care because they reduce the benefits and increase the costs males pay for caring (Trivers, 1972; Kokko & Jennions, 2003). However, it is important to stress that the certainty of paternity *per se* can not directly affect the evolution of paternal care because a male can not increase his paternal confidence by adopting a caring role.

One of the most robust hypotheses to explain the evolution of paternal care has been proposed by Williams (1975). According to him, females would be attracted to suitable oviposition sites, which males would defend against other males in an attempt to acquire mates. Moreover, males that defend a territory would further increase their fitness because they also indirectly defend eggs against conspecific predators. In this case, paternal care does not necessarily decrease the probability of a caring male to acquire additional matings because several females may visit his territory. The territoriality hypothesis presupposes that paternal care has evolved under the pressure of natural selection and predicts that (1) eggs do not increase male attractiveness and (2) males do not guard unrelated eggs. Even though this hypothesis does not account for the evolution of paternal care in all animal groups, it has been proposed as the main explanation for groups such as fishes (Ridley, 1978) and anurans (Lehtinen & Nussbaum, 2003).

More recently, Tallamy (2000, 2001) analyzed all described cases of exclusive paternal care in arthropods and proposed that male care has evolved under the pressure of sexual selection. According to his hypothesis, paternal care in arthropods minimizes the physiological costs related to egg-guarding by females, allowing them to forage immediately after oviposition. Moreover, paternal care may also provide an honest signal of a male's quality as offspring defender. Under such assumptions, males providing paternal care would be chosen by females and should obtain a greater number of copulations than males that are unable and/or unwilling to provide care. This hypothesis generates at least two different predictions in relation to the territoriality hypothesis: (1) eggs increase male attractiveness to females and (2) males are willing to guard unrelated eggs. Other important predictions from the hypothesis of evolution of paternal via sexual selection are: (3) paternal care creates more opportunities for females to forage and, therefore, they could feed more and produce more eggs; (4) females are iteroparous, and (5) guarding males have many mating opportunities during brood caring.

Exclusive postovipositional paternal care is the rarest form of parental investment in arthropods, and has evolved in no more than 15 unrelated *taxa* (Tallamy, 2000, 2001; Machado, 2007). All cases of exclusive paternal care in the class Arachnida described so far are restricted to the order Opiliones, which comprises nearly 6,000 species (review in Machado & Macías-Ordóñez, 2007). Observational data obtained for many harvestman species with paternal care support some of the predictions of the hypothesis of evolution of paternal via sexual selection (revision in Machado *et al.*, 2004). However, experimental studies are necessary for a formal test of this hypothesis, and to better understand the evolution of paternal care in harvestman. The objective of this study was to test all predictions from the hypothesis of evolution of the paternal care via sexual selection (Tallamy, 2000, 2001) using the harvestman *Pseudopucrolia* sp. as study model.

MATERIALS AND METHODS

Study animal

Pseudopucrolia sp. is a harvestman with 6-7 mm of body length and dark brown coloration, which belongs to the subfamily Heteropachylinae, a basal group of the family Gonyleptidae (Kury, 1994). The systematics of the Heteropachylinae is unsatisfactory and the generic boundaries are confusing. The species studied herein is certainly a new taxa that was tentatively placed in the genus *Pseudopucrolia*. A systematic review of the subfamily is currently in progress, and the study species is being formally described (A.C. Mendes, pers. comm.). Voucher specimens of males and females were deposited in the arachnological collections of the Museu de Zoologia da Universidade de São Paulo (MZSP, São Paulo state) and Museu Nacional do Rio de Janeiro (MNRJ, Rio de Janeiro state), Brazil.

Preliminary field observation showed that individuals of *Pseudopucrolia* sp. are active only during the night, when they were observed in the field walking around (mainly females) or standing still at the entrance of natural holes in ravines (mainly males). These natural holes had an entrance of 8 - 41 mm of diameter and 33 - 92 mm of depth, and were always occupied by adult males. In some of the observed holes, there were also clutches with eggs covered by a thick layer of debris. The clutches were composed of eggs in different stages of embryonic development, suggesting that several females contributed with eggs at different times.

Maintenance in captivity

The individuals used in this study were collected in an Atlantic Forest fragment, close to the municipality of Linhares (19°06' S; 39°45' W), Espírito Santo state, southeastern Brazil. Harvestmen were found at night on a 50 m long ravine located at the edge of the forest

fragment. Two field trips were conducted, one in June 2003 and another one in June 2005, and the total number of individuals collected was 113 (49 males and 64 females). All harvestmen were brought to our laboratory in the Museu de História Natural of the Universidade Estadual de Campinas, Brazil. To test the prediction that females are iteroparous, 15 ovigerous individuals were preserved in 70% ethanol and dissected. The eggs in their reproductive tract were counted and classified as mature when they were located in the proximal 1/3 of the ovary, had a bright yellow coloration, and were > 1 mm in diameter (following Machado *et al.*, 2004). The remaining 98 individuals (49 males and 49 females) were maintained alive in three communal terraria (40 x 90 cm base, 20 cm height) with similar number of individuals and sex ratio 1:1. One week before the beginning of behavioral observations, the individuals had the width of the dorsal scute measured with calipers (to the nearest 0.01 mm) and were individually marked on their dorsal scute with colored dots of enamel paint. Three times a week, they were fed pieces of dead cockroaches and an artificial diet for ants (Bhatkar & Whitcomb, 1970).

Each terrarium contained soil, small pieces of tree fern trunks, and 12 artificial nests built in clay blocks (with 6 x 2 cm base, 3 cm height). Each artificial nest had a central hole (1 cm in diameter and 2 cm depth) crossing the clay block from side to side. These blocks were placed against the glass wall of the terrarium so that it was possible to observe the harvestmen behavior inside the nests. These nests successfully simulated the natural cavities in ravines in which males of *Pseudopucrolia* sp. were found taking care of eggs in the field, thus artificial nests were immediately used by captive individuals. All observed copulations (n = 36) and oviposition events (n = 38) occurred inside the artificial nests. During the study period, the abiotic conditions in the laboratory were (mean $\pm$ SD): temperature of 25.5 ± 1.2 °C, humidity of $82.0 \pm 5.4\%$, and photoperiod of 13L:11D.

Behavioral observations

The nests of terrarium 1, which contained 17 males and 17 females, were inspected daily ever 3 hours: 08:30, 11:30, 14:30, 17:30, and 20:30 h. Each time, the identity of the individuals inside each nest was recorded. Continuous recording (*sensu* Martin & Bateson, 1994) was made of all relevant behavioral events, such as fights between males, copulations, and ovipositions. Once a day, the number of eggs in each nest was counted and classified in stages of embryonic development according to their size and coloration (following Hara *et al.*, 2003; Machado *et al.*, 2004). As previously recorded in other harvestmen, recently laid *Pseudopucrolia* sp. eggs are white and small, becoming larger and darker during embryonic development. Daily inspections lasted from May to July 2004, comprising 60 days of sampling. These data were used to provide descriptive information on the reproductive behavior of the species and to characterize its mating system.

The number per census of visited nests, the number of nest changes, and the number of records inside the nests were compared between males and females using t tests. The size of guarding and non-guarding males was compared also using a t test. Pearson correlations were used to test for relationships between the size of the guarding males and the total number of eggs they achieved in their clutches and the number of females they copulated with. Logistic regressions were used to investigate what factors influence the probability of males to acquire a clutch. The independent variables were the number of visited nests by each male, the number of nest changes of each male, and the total number of times each male was recorded inside the same nest (in this case, the nest with longest period of permanence was used). The dependent variable was the presence (1) or absence (0) of a clutch in the nests of the 17 males after 60 days of observations.

To test the prediction that paternal care creates more opportunities for females to forage, quantitative behavioral observations on the foraging activities of individuals living in the terrarium 1 were done during the night, when they were in activity of exploration and

feeding. These samples were done in 13 nights from June 25 to July 17, 2004. Each sample consisted of 1 h of observation randomly selected from 18:00 to 07:00 h. Each time slot was sampled only once. During each sample, scans were conducted at 3-minute intervals, in which the behavior of all individuals was recorded (“fixed-interval time point sampling” *sensu* Martin & Bateson, 1994). Individuals were scored as foraging if they were: (1) walking with legs II extended sideways or frontward touching or not the substrate (“exploration” *sensu* Elpino-Campos *et al.*, 2001); (2) feeding, or (3) walking carrying food with the chelicerae and/or pedipalps. Any other behavior was scored as “not foraging”. When an individual was not visible in the terrarium, probably hiding under pieces of tree fern trunks, it was scored as not foraging as well. A flashlight with red filter was used for the nocturnal observations (following Elpino-Campos *et al.*, 2001; Pereira *et al.*, 2004). Food (pieces of dead cockroaches) was always placed in the terrarium 1 h before the beginning of the sampling. The proportions of observations in which females (n = 17), guarding (n = 6) and non-guarding males (n = 11) were observed foraging during the 13 h of samples were compared using an one-way ANOVA. The proportion of time each individual (P_i) spent foraging was calculated as $P_i = \left(\frac{n_i}{T} \right)$, where n_i is the total number times an individual i was recorded foraging and T is total number of scans, which was 273 for all individuals (21 scans per one hour-sample x 13 one hour-samples).

Experimental manipulation 1: Female preference

To test the prediction that eggs increase male attractiveness to females, a choice experiment was conducted between June and September 2005. Two males from the terraria 2 or 3 were placed in a smaller terrarium (20 x 10 cm of base, 15 cm of height) together with their respective nests. One of these males had eggs previously deposited in its nest, and the

other male was an individual of similar size (paired t test = 0.430; n = 14; p = 0.674), holder of a nest for more than 20 consecutive days, but that did not obtain a clutch yet. In the sequence, an ovigerous female, which had not copulated or laid eggs with any of the experimental males, was placed inside the terrarium. The female was continuously followed during the first 2 h after their introduction in the experimental terraria. If no copulation and oviposition happened during this period, the observations were finished and the nests were inspected in the next day to verify in which of them the female had oviposited. If no oviposition occurred within one week, the experiment was staged again with a different ovigerous female.

After this first phase, the experimental males were placed again in the communal terrarium where they came from. Since females avoid laying eggs in nests where many nymphs have already hatched (unpub. data), the males with a clutch did not receive additional eggs, and within two months their nests were empty. During this period, all males without clutches in the first phase of the experiment acquired eggs. Thus, in the second phase of the experiment, the same males were once again paired, but this time the males without a clutch in the first phase had eggs now, whereas males that had a clutch in the first phase, did not. Once more, an ovigerous female was placed in each experimental terrarium, and the same data record described above was performed. This experiment was replicated 14 times and the number of males with and without clutches that copulated and acquired eggs in each phase was compared using a Fisher exact test.

Experimental manipulation 2: Egg adoption

To test the prediction that males are willing to guard unrelated eggs, an experiment was conducted in terraria 2 and 3 (both containing 16 females and 16 males) in July 2005. These individuals were left to copulate and oviposit freely during 30 days and, after this period, 16 males had eggs in their nests. Guarding males were removed from their nest and

maintained in small vials. Only one guarding male was removed at each time and replaced in the terrarium where it came from after four days. During this period, the experimental nest was monitored *ad libitum* during daylight, and at 30 minutes intervals for 4 h from 18:00 to 22:00 h. The presence, behavior, and time of permanence of males and females inside the empty nests were recorded at each census. To measure the time of permanence inside the nests, it was assumed that individuals did not leave them if they were recorded inside the same nest in two consecutive censuses. The same procedure was adopted if one individual was recorded in the following day inside the same nest where it was recorded the previous night.

The frequency of visits in which males and females that ate or not eggs from unattended clutches was compared using a chi-square test. A Fisher exact test was used to compare the proportion of visiting males and females that defend unattended clutches. Clutch defense was regarded as any aggressive behavior, such as attacks with the pedipalps or intense leg tapping, exhibited towards another individual. The time of permanence and the number of eggs consumed by visiting males and females were compared with using a Mann-Whitney nonparametric test. According to the hypothesis of evolution of paternal care via sexual selection, the behavior of males and females in relation to unprotected clutches should be markedly different. Males should adopt unattended eggs and thus remain longer periods (some hours or days) inside the nests when compared with females. Males should also be less prompt than females to cannibalize eggs, and only males should exhibit defensive behaviors against potential egg predators, even if the eggs were not genetically related to them.

RESULTS

Mating system

All nests in the terrarium 1 were visited at least once during the study period. Females visited more nests than males (Table 1). Males were recorded inside the nests more frequently than females, and moved among different nests less frequently than females (Table 1). The same nest could be occupied by more than one individual, but males were less tolerant to conspecifics of the same sex than females. Fights were observed in 58% of the cases in which two males were recorded inside the same nest ($n = 12$). Females, on the other hand, were never observed fighting other individuals (either males or females) found in the same nest ($n = 16$).

Table 1. Comparison of three parameters (median and range) of nest utilization between males ($n = 17$) and females ($n = 16$) of the harvestman *Pseudopucroliia* sp. reared in captivity. Data are based on 298 point samples taken during 60 days.

	Males	Females	Statistics
Number of visited nests	4.12 ± 2.57	7.50 ± 1.90	$U = 40.5$, d.f. = 31, $p > 0,001$
Number of nest changes	5.88 ± 5.67	13.31 ± 5.46	$U = 38.3$, d.f. = 31, $p > 0,001$
Number of records inside nests	176.2 ± 99.7	71.0 ± 29.8	$U = 56.0$, d.f. = 31, $p > 0,004$

Copulations occurred only inside the nests ($n = 14$). Courtship before, during, and after copulations follows a pattern very similar to the one described for the close related *Chavesincola inexpectabilis* (Nazareth & Machado, submitted). There was no size difference between guarding (mean $\pm$ SD = 7.17 ± 0.14 mm; $n = 6$) and non-guarding males (7.02 ± 0.51 mm; $n = 11$) ($t = 0.910$; d.f. = 12; $p = 0.382$). Asimilar result was found when all males from

the other two terraria were included in the analysis ($t = 1.280$; d.f. = 46; $p = 0.207$). All males that obtained clutches ($n = 25$) copulated with more than one female, which contributed with eggs for multiples clutches. There was no correlation between male size and the total number of eggs they achieved in their clutches ($r = 0.060$; $n = 6$; $p = 0.900$) or between male size and the number of females they copulated with ($r = -0.062$; $n = 6$; $p = 0.910$). Again, the same pattern was found when all males from the other two terraria were included in the analyses ($p > 0.5$ for both correlations). However, the greater the nest tenure and the permanence of males inside the same nest, the higher their chances of mating (Figure 1).

Prediction 1: Females are iteroparous

The number of mature eggs in the reproductive tract of the dissected females ranged from 20 to 124 (mean $\pm$ SD = 67.1 ± 33.2 eggs; $n = 15$ females). The number of immature eggs was generally much higher, reaching up 150 eggs (mean $\pm$ SD = 80.0 ± 46.3 eggs). In the terrarium 1, based on observations seven females copulated with one male, five with two males, and four females copulated with three different males. A similar pattern was observed in the other two terraria. The mean number of eggs laid in each oviposition event was 39.0 (SD = 19.7 eggs; $n = 90$ events in the three terraria), and the mean interval between consecutive ovipositions was 2.1 days (SD = 1.1 days; $n = 70$ events).

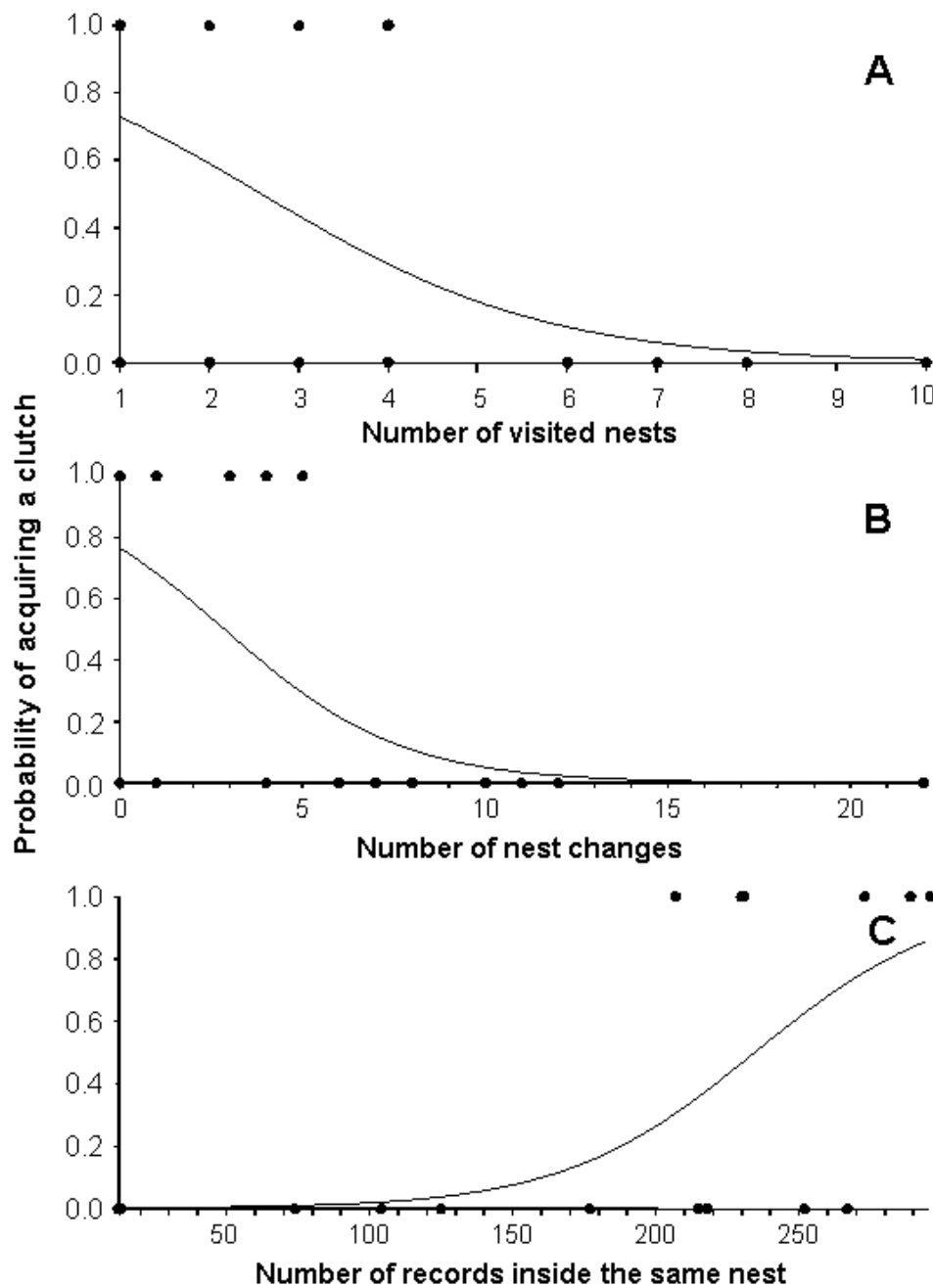


Figure 1. Logistic regressions used to investigate the factors that influence the probability of *Pseudopucroliia* males acquiring a clutch. The independent variables were: (A) the number of visited nests by each male ($\chi^2 = 4.903$; d.f. = 1; Odds ratio = 0.534; $p = 0,027$); (B) the number of nest changes of each male ($\chi^2 = 6.517$; d.f. = 1; Odds ratio = 0.661; $p = 0,011$), and (C) the total number of times each male was recorded inside the same nest ($\chi^2 = 8.690$; d.f. = 1; Odds ratio = 1.030; $p = 0.003$). The dependent variable was the presence (1) or absence (0) of a clutch in the nest of each of the 17 males after 60 days of observations.

Prediction 2: Males have many mating opportunities

Only six out of the 17 males in the terrarium 1 achieved copulations: two males copulated seven times, three males copulated four times, and one male copulated three times. Thus, clutches of all these males were composed of eggs laid by more than one female. The total number of eggs in the nests were 100, 164, 195, 249, 265, and 291. In two occasions, a guarding male was observed with two females inside his nest. In these cases, while one female was ovipositing, the male courted the other female. When both females were ovipositing, the male alternated touches on the dorsum between each of the females ($n = 1$ observation) or touched both simultaneously using both legs from the second pair ($n = 1$ observation). Males were never seen repelling females from their nests. Conversely, they generally blocked the entrance of the nest with their body this preventing females from leaving after oviposition ($n = 5$ observations).

Prediction 3: Paternal care creates more opportunities for females to forage

The proportion of time females foraged and fed was higher than guarding males, but similar to non-guarding males ($F_{2,31} = 10.846$; $p < 0.001$; Figure 2). In the great majority of the records (94.2%) females consumed the food items where they had found them. In 58.3% of the records, non-guarding males exhibited a similar behavior, but in the remaining cases (41.7%), they took the food items to their nests. Guarding males were recorded feeding only four times and in all cases the food item was consumed at the nest entrance, but not inside as non-guarding males did. They kept the posterior part of the body (including the third and fourth pairs of legs) inside the nest and left the food item outside — probably avoiding contact between the eggs and the food. In all records of guarding males in exploration activities inside the terrarium ($n = 84$), they never walked more than 10 cm away from their nests. Females and most non-guarding males, on the other hand, generally left their shelters (nests or pieces of trunks) and walked through the entire terrarium seeking for food.

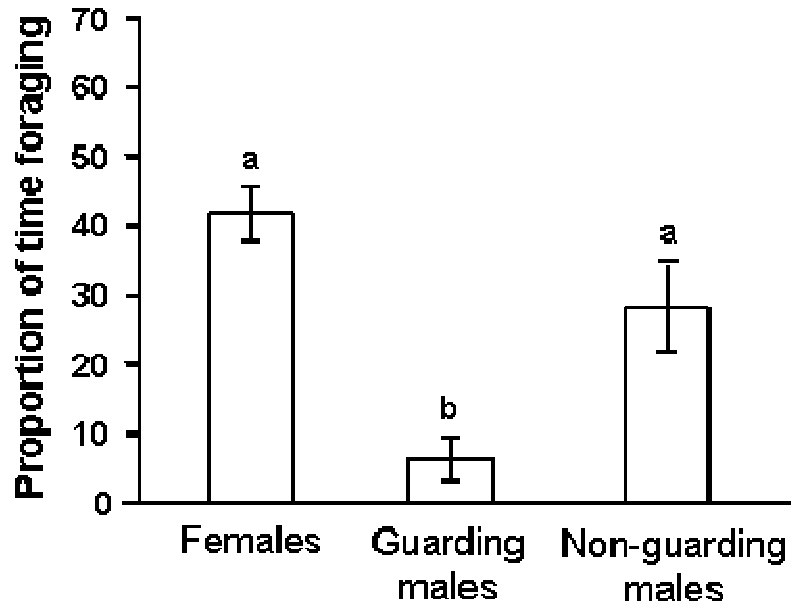


Figure 2. Mean proportion of records ($\pm$ standard deviation) in which females ($n = 17$), guarding ($n = 6$), and non-guarding males ($n = 11$) of the harvestman *Pseudopucrolia* sp. were observed foraging during 13 one hour-samples (total number of records per individual = 273). Different letters above the bars indicate significant difference (Tukey, $p < 0.05$).

Prediction 4: Females prefer to copulate with guarding males

In the first phase of the experiment, 12 males (86%) with clutches were selected by the ovigerous females and received additional eggs in their clutches. Only two males (14%) without eggs copulated and acquired eggs. In the second phase of the experiment, males with clutches were again preferred by the ovigerous females: 13 (93%) of them received new eggs in their clutches whereas only one male (7%) without eggs copulated and acquired eggs. Thus, regardless of the males' identity or of their morphological and behavioral features (such as courting skills), females select them based on the presence of eggs inside their nests (Fisher exact test, $p < 0.001$).

Prediction 5: Males are willing to guard unrelated eggs

The behavior of males and females upon unattended clutches was markedly different. Females remained less time inside the nests than males, and most of this time was devoted to cannibalizing eggs (Table 2). Only 20.4% of the visiting males cannibalized eggs from the nests whereas 71.1% of the females did so (Table 2). The number of consumed eggs per visit was also different between visiting individuals from the two sexes: males ate up to eight eggs whereas females ate up to 42 eggs, promoting great reductions in clutch size (Table 2). Finally, only visiting males exhibited egg-guarding activities, such as repelling intruders (both males and cannibal females) from the nests (Table 2). Females did not present such behavior and were commonly seen cannibalizing eggs in the company of other individuals, especially females (n = 40 observations). However, it is important to stress that females never consumed eggs of nests where they had previously laid eggs. They were seen entering the nest, inspecting the clutch with the second pair of legs, and leaving the place without consuming any egg (n = 5 observations).

Table 2. Behavior of males and females of the harvestman *Pseudopucrolia* sp. that visited 12 experimentally unattended clutches in the laboratory.

Behaviour	Males (n = 50 visits)	Females (n = 83 visits)	Statistic
% of individuals that cannibalized eggs	20.4	71.1	$\chi^2= 547.59$; $p < 0.001$
Median number of cannibalized eggs (min. – max.)	2.5 (1 – 8)	7 (1 – 42)	U = 141.0; $p < 0.009$
Median time (h) of permanence inside the nest (min. – max.)	11.8 (0.02 – 72)	1.4 (0.02 – 34.7)	U = 1,496.0; $p < 0.009$
% of individuals that repelled intruders while inside the nest	34	0	Fisher exact test, $p < 0.001$

DISCUSSION

Resource defense polygyny occurs when males control females indirectly by defending territories or resources against conspecific males (Emlen & Oring, 1977). Even though arachnids present a great diversity of reproductive strategies (Thomas & Zeh, 1984), there are only two cases of resource defense polygyny reported so far, both for the order Opiliones (Macías-Ordóñez, 1997, 2000; Buzatto & Machado, submitted). In the present study, we provided a brief description of the polygynous mating system of the paternal harvestman *Pseudopucrolia* sp., whose females use natural cavities in ravines as oviposition sites. Males defend these cavities against other males and care for eggs laid by several females. In the laboratory, we were able to emulate the oviposition site of the species using artificial nests built in clay blocks, which allowed us to investigate details of the species reproductive biology. Our results suggest that male size does not influence their chances of achieving copulations. However, male reproductive success is directly related to ownership time of a nest: the longer a male holds a nest, the higher his chances of obtaining copulations. Similar data were obtained for the paternal harvestman *Zygopachylus albomarginis*, whose males build and defend mud nests that females seek for egg laying (Mora, 1990). In this species, no morphological trait of the males or of their nests accounted for their reproductive success, but the ownership of a nest was crucial for the males' to achieve copulations.

In the present study, we have also tested all predictions from the theory of evolution of paternal care via sexual selection, using *Pseudopucrolia* sp. as model organism. The first prediction is that the species should be iteroparous because females can not benefit from males that care for the offspring unless they have the physiological capability and the ecological opportunity to lay more than one clutch during a breeding season (Tallamy, 2000, 2001). The freedom to continue foraging and producing eggs after the first clutch is laid

provides no advantages for females constrained to semelparity (Tallamy & Brown, 1999). We found that *Pseudopucrolia* females are iteroparous since they produce eggs continuously. The same pattern has been found in other harvestman species presenting paternal care, such as *Zygopachylus albomarginis* (Mora, 1990), *Ampheres leucopheus* (Hara *et al.*, 2003), *Gonyleptes saprophilus*, *Iporangaia pustulosa*, *Iguapeia melanocephala*, *Neosadocus* sp., and *Progonyleptoidellus striatus* (Machado *et al.*, 2004). Moreover, in at least one of them, *Iporangaia pustulosa*, a longterm study in the field also showed that there is no seasonal interruption in the oviposition activity in the population (Requena *et al.*, in prep.). This phenological pattern is remarkably different from species presenting maternal care, in which females generally produce only one clutch per reproductive season and the oviposition activity is markedly seasonal (see Machado & Macías-Ordóñez, 2007). Field studies with *Pseudopucrolia* are needed in order to know if the oviposition activity under natural conditions occurs continuously throughout the year or if females are somehow constrained to a clearly defined reproductive season.

Postovipositional maternal care in arthropods is a costly life-history trait because it reduces lifetime fecundity and reduces foraging opportunities for guarding females during long periods of care, especially in predatory species (Tallamy & Denno, 1982; Fink, 1986; Gundermann *et al.*, 1997; Tallamy & Schaefer, 1997; Stegmann & Linsenmair, 2002; Zink, 2003; Buzatto *et al.*, 2007). However, care-related reductions in feeding activities are predicted to be less costly to the reproductive potential of males than to that of females because sperm and other seminal products generally require fewer nutrients and thus are cheaper to produce than eggs (Parker *et al.*, 1972). The “enhanced fecundity hypothesis” (Maynard-Smith, 1982) proposes that in species in which postzygotic care is crucial for offspring survival and such care may be provided as effectively by one parent as by two, paternal care may minimize the physiological costs related to egg-guarding by females, allowing them to forage immediately after oviposition without sacrificing offspring

survivorship (Tallamy, 2000, 2001). Our behavioral observations showed that *Pseudopucrolia* females are free to forage just after oviposition and quantitative data demonstrated that their foraging rate is much higher than that of males (especially those that are guarding eggs). Additionally, the interval between two consecutive ovipositions was only two days, which is almost 40 times shorter than the median interval between two ovipositions in the maternal gonyleptid *Bourguyia hamata* (Machado & Oliveira, 2002), representative of a closely related subfamily, the Bourguiinae (Kury, 1994). Apparently, the reproductive rate of *Pseudopucrolia* females is in fact higher as a consequence of their also higher foraging rate. However, since the mean number of mature eggs in the ovary of the dissected females was almost two fold greater than the mean number of eggs laid in each oviposition event, this difference reproductive rate may be in part explained by the fact that ovigerous females do not lay all their mature eggs at once. The number of eggs laid in each oviposition may depend on the courtship skills of the males; the better the courting behavior of a male, the more eggs laid by the female with this male (Eberhard, 1996). This hypothesis is currently being investigated in our laboratory using an experimental approach.

Similarly prezygotic nuptial gifts, exclusive postzygotic paternal care may be favored through sexual selection because it increases male mating opportunities by simultaneously offering females the direct, fitness-enhancing gift of cost-free care of their offspring and the freedom to forage for additional food (Trivers, 1972). Moreover, paternal care may also provide an honest signal of a male's quality as offspring defender. Under such assumptions, males providing paternal care should be chosen by females and hence should obtain a greater number of copulations than males that are unable and/or unwilling to provide care (Tallamy, 2000, 2001). Here, we showed that *Pseudopucrolia* males have many mating opportunities, and all clutches obtained in the laboratory were composed of eggs laid by several females. Moreover, we demonstrated that guarding males are preferred by ovipositing females, independently of their morphological features (such as size) or their

courting skills. Mora (1990) has already shown that females of the paternal harvestman *Zygopachylus albomarginis* copulate only with males that have a mud nest, where eggs are laid. However, this is the first experimental demonstration for arthropods that the presence of eggs in the males' nests is a sexually selected trait used by females in order to discriminate their mating pairs.

Since the possession of an egg mass seems to enhance male attractiveness, intense selection should pressure all males to guard because this is the route to additional matings. In this context, males should not only accept unrelated eggs into their brood, but also should compete with other males for egg ownership (Tallamy, 2000, 2001). Paternal behavior that arose through natural selection, in contrast, should not include caring for any eggs with questionable paternity (Trivers, 1972; Williams, 1975). Our experiment with unattended clutches of *Pseudopucroliia* demonstrated that the behavior of visiting males and females is markedly different. As should be expected, the great majority of visiting females cannibalized eggs from the clutches, sometimes promoting a drastic reduction in the total number of eggs. Additionally, no female exhibited aggressive behaviors against other nest visitors. Males, on the other hand, rarely cannibalized eggs and when they did so, the total number of eggs consumed by males was much lower than the number of eggs consumed by females. The most impressive result, however, was that 34% of the visiting males presented aggressive behaviors towards nest visitors, on a clear attempt to protect the clutch from potential predator. Even though egg adoption by *Pseudopucroliia* males could be regarded as cheating, when a male guards unrelated eggs, he is in fact providing an honest signal of his paternal intent for females.

This study is the first to formally test predictions from the theory of evolution of paternal care via sexual selection. Our results suggest that exclusive postzygotic paternal care in *Pseudopucroliia* has evolved by sexual selection and not by natural selection. Therefore paternal care is probably the plesiomorphic state in the subfamily Heteropachylinae. In fact,

we suggest that the existence of a defense related polygynous mating system, allied with a generalist diet, are key points to understand why paternal care has evolved so many times independently within the order Opiliones, and not in the remaining arachnid orders. Among predatory arachnids, prey availability limits egg production, and the limited number of females that succeed in gathering enough resources to produce eggs and become sexually receptive limits mating opportunities for males. Consequently, the majority of male arachnids end up not mating at all, only a few mate once, and very few or none mate more than once (Elgar, 1998). Most harvestmen, however, present an omnivorous/scavenger diet (Acosta & Machado, 2007), and males have a much wider potential to mate several times over their lifetime than predatory arachnids do (Machado & Macías-Ordóñez, 2007). Therefore, the ample opportunity for polygyny, even during extended periods of exclusive male care, decreases the costs of paternal care and probably relaxes the selective pressure against the evolution of this behavioral trait in harvestmen.

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CONCLUSÃO GERAL

Nesta dissertação, dois novos casos de cuidado paternal foram descritos e informações básicas sobre a biologia reprodutiva da subfamília Heteropachylinae foram apresentadas. Fêmeas de *Chavesincola inexpectabilis* e de *Pseudopucroliia* sp. utilizam cavidades naturais em barrancos como sítios de oviposição. Essas cavidades são monopolizadas por alguns machos através de brigas com outros machos, permitindo o acesso apenas de fêmeas ovígeras. O sucesso reprodutivo dos machos está diretamente associado à posse de um ninho e, por esse motivo, o sistema de acasalamento das espécies se encaixa na definição de poliginia por defesa de recursos.

No primeiro capítulo, interações entre machos e fêmeas de *C. inexpectabilis* foram observadas e os rituais de cortejo, acasalamento e oviposição foram descritos. Nessa espécie, são os machos que cuidam da prole, protegendo os ovos do ataque de co-específicos até a sua eclosão. Em espécies nas quais todo o investimento pós-zigótico está sob a responsabilidade dos machos, algum grau de reversão de papéis sexuais é esperado (Trivers, 1972). Machos, portanto, devem ser criteriosos, selecionando suas parceiras sexuais e, eventualmente, recusando cópulas, tal como ocorre no opilião *Zygopachylus albomarginis* (Mora, 1990). Nessa espécie, machos constroem ninhos de barro em troncos caídos onde as fêmeas ovipõem. Os machos são cortejados pelas fêmeas e são eles que decidem se haverá cópula ou não (Mora, 1990). Embora em *C. inexpectabilis* as fêmeas também coloquem seus ovos em ninhos, estes não são construídos pelos machos. Ao contrário de *Z. albomarginis*, a fêmea é quem recebe o cortejo do macho. Portanto, não parece ocorrer uma reversão de papéis sexuais em *C. inexpectabilis* e as fêmeas ainda parecem desempenhar o papel de sexo criterioso.

Apesar dos dados apresentados no Capítulo 1 não terem sido obtidos para testar a hipótese de evolução do cuidado paternal via seleção sexual (Tallamy, 2000, 2001), duas das previsões desta hipótese foram corroboradas. Fêmeas de *C. inexpectabilis* maturam ovos constantemente e ovipõem várias vezes em curtos intervalos de tempo sendo, portanto, consideradas iteropáricas. Adicionalmente, as duas desovas obtidas eram compostas por ovos depositados por mais de uma fêmea, o que sugere que machos guardiões têm muitas oportunidades de cópula.

Testes formais de todas as previsões da hipótese de evolução do cuidado paternal via seleção sexual foram realizados no Capítulo 2, usando *Pseudopucroliia* sp. como modelo de estudo. Foi demonstrado que, como as fêmeas de *Pseudopucroliia* não exercem nenhum cuidado à prole após a oviposição, elas têm mais tempo para obtenção de alimento, o que provavelmente possibilita a maturação constante de ovos. Outra previsão importante da hipótese, a de que a presença de ovos nos ninhos aumentaria a atratividade dos machos, também foi corroborada. Depois que um macho consegue sua primeira cópula e, conseqüentemente, sua primeira oviposição, este macho tem uma probabilidade muito maior de adquirir novas cópulas do que machos que não possuem ovos em seus ninhos. Finalmente, demonstrou-se experimentalmente que machos defendem ovos fecundados por outros machos, provavelmente porque a presença de ovos aumenta sua atratividade para as fêmeas. Dessa maneira, é possível concluir que o cuidado paternal em *Pseudopucroliia* evoluiu por seleção sexual.

Finalmente, o cuidado paternal provavelmente é o estado plesiomórfico na subfamília Heteropachylinae. A associação entre o macho e a sua prole através da defesa de um sítio de oviposição deve ter sido a base para a evolução da guarda paterna dos ovos não apenas nessa subfamília, mas também em diversas outras espécies da ordem Opiliones.

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