

Five New Cases of Paternal Care in Harvestmen (Arachnida: Opiliones): Implications for the Evolution of Male Guarding in the Neotropical Family Gonyleptidae

by

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ABSTRACT

In this paper, we present field observations on paternal care in five species of harvestmen belonging to the family Gonyleptidae: *Gonyleptes saprophilus*, *Neosadocus* sp. (Gonyleptinae), *Iguaepeia melanocephala*, *Iporangaia pustulosa*, and *Progonyleptoidellus striatus* (Progonyleptoidellinae). We also provide a critical reassessment of all cases of paternal care in harvestmen and examine the extent to which the available data can be used to test a recent theory on the evolution of exclusive male care via sexual selection. Eggs of the two Gonyleptinae species are laid inside natural cavities in trunks and in the soil, whereas eggs of the Progonyleptoidellinae are laid on the undersurface of leaves and are covered by a thick mucus coat. Females of the five species are iteroparous and the batches generally consist of eggs in several embryonic stages. This finding suggests that males have many mating opportunities and that they guard eggs laid by more than one female. Data from other four paternal harvestmen (*Zygopachylus albomarginis*, *Lepchana spinipalpis*, *Ampheres leucopheus*, and *Cadeadoius niger*) are quite similar and support most of the predictions of the theory on the evolution of exclusive male care via sexual selection. However, information supporting the prediction that males should be willing to guard unrelated eggs is ambiguous since in at least two species, *I. pustulosa* and *Z. albomarginis*, vagrant males do not hesitate to eat the eggs of unprotected batches. There are several differences in the behavioral patterns of guarding male and female harvestmen. The evolution of maternal care was clearly driven by natural selection, whereas paternal care seems to be a sexually selected trait. Paternal care has apparently evolved independently several times in the order Opiliones and at least twice within the Gonyleptidae: once in the subfamily Gonyleptinae and once in the ancestor of the clade Caelopyginae + Progonyleptoidellinae.

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INTRODUCTION

Numerous factors have been proposed to explain which sex is more likely to provide parental care (Queller 1997). Anisogamy generates the conditions for sexual selection since numerically abundant male gametes compete for access to rare female gametes (Bulmer & Parker 2002). This competition decreases the confidence of paternity, especially among species with internal fertilization (Williams 1975). Moreover, direct male-male competition for access to females and/or female mate choice creates an elite group of males in the population that are more likely to acquire mates (Bateman 1948). Low confidence of paternity and marked variation in mating success (number of mates) may act against the evolution of paternal care relative to female care since they reduce the benefits and increase the costs of caring behavior, respectively (Kokko & Jennions 2003).

Despite these obstacles, exclusive postovipositional paternal care has evolved in many animal groups (Clutton-Brock 1991) and, among arthropods, this behavior is known to occur in at least 14 unrelated taxa (reviewed in Tallamy 2000, 2001). According to Tallamy's 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 (Tallamy 2000, 2001).

All cases of paternal care in the class Arachnida described so far are restricted to the order Opiliones, which comprises nearly 5500 species (see Hara *et al.* 2003). Males of *Zygopachylus albomarginis* (Manaosbiidae) build a dish-like nest made of mud and debris on tree trunks. These mud nests may be visited by several females, which inspect their structure and occasionally lay 1 - 5 eggs during the breeding season. After oviposition, the females leave the nests and the males protect the eggs against fungi infection and attack by potential predators, including ants and conspecifics (Rodriguez & Guerrero 1976; Mora 1990). In *Lepchana spinipalpis* (Assamiidae) and *Ampheres leucopheus* (Gonyleptidae), males guard a large egg-batch containing eggs in different developmental stages and even newly hatched nymphs (Martens 1993, Hara *et al.* 2003). In these two cases, possibly more than

one female contributes to the batches at different times, as observed for *Z. albomarginis*. Males of *Leytpodoctis oviger* (Podoctidae) have a distinctive strategy in which 1 - 13 eggs are attached to their third and fourth femora by females (Martens 1993, A.B. Kury & G. Machado unpublished data).

In this paper, we present field observations on five harvestmen species belonging to the neotropical family Gonyleptidae, *Gonyleptes saprophilus*, *Neosadocus* sp. (Gonyleptinae), *Iguapeia melanocephala*, *Iporangaia pustulosa*, and *Progonyleptoidellus striatus* (Progonyleptoidellinae), in which males care for the offspring. We also map the available information on the forms of parental care in a phylogenetic tree of the family Gonyleptidae in order to discuss the evolution of behavioral traits in a well-resolved clade. Finally, we provide a critical reassessment of all cases of paternal care in harvestmen and examine the extent to which the available data can be used to test a recent theory on the evolution of exclusive male care via sexual selection (Tallamy 2000, 2001).

MATERIALS AND METHODS

Study sites

Populations of *Gonyleptes saprophilus* were studied in two semi-deciduous Atlantic Forest fragments in São Paulo state, southeastern Brazil: one in the Parque Florestal do Itapetinga (23°10' S; 46°25' W; 1000 m alt.), in the municipality of Atibaia, and another in the Serra do Japi (23°17' S, 47°00' W; 1250 m alt.), in the municipality of Jundiá. The climate at both sites is seasonal and has two well-defined seasons. The dry-cold season lasts 4 - 6 months (April to September), has a mean monthly rainfall of 70 mm, and a mean monthly air temperature of 17°C. The wet-warm season lasts from October to March, has a mean monthly rainfall of 180 mm, and a mean monthly air temperature of 21°C (for more details on these sites see Machado *et al.* 2002 and Pinto 1992, respectively).

Populations of *Neosadocus* sp., *Iguapeia melanocephala*, and *Iporangaia pustulosa* were studied in a dense, ombrophyle Atlantic Forest fragment in the Parque Estadual Intervales (24°14' S; 48°04' W; 800 m alt.), close to the municipality of Capão Bonito, southern São Paulo state. The annual rainfall in the region ranges from 2000 to 3000 mm and the mean annual temperature ranges from 17°C to 19°C. Although seasonality is not as marked as in the semideciduous forest mentioned above, there is a dryer, colder period lasting from April to September when frosts are common (for more details on this site see Leonel 1994).

A population of *Progonyleptoidellus striatus* was studied in the Estação Biológica Alto da Serra (23°46'S; 46°18'W; 800 m alt.), in the municipality of Santo André, southern São Paulo state. This site is located in the same mountain chain as the Parque Estadual Intervales, and has a similar floristic composition. The climate is also similar, with the annual rainfall ranging from 2000 to 3000 mm, and the mean annual temperature ranging from 17°C to 19°C. There is a wet, warm period lasting from October to March and a dry, cold period lasting from April to September.

Naturalist observations

Males of the five harvestmen species found caring for their offspring were measured (dorsal scutum length) and their eggs were counted. A sample of the eggs was measured with calipers (to the nearest 0.01 mm) in the laboratory. The eggs were classified in four stages of embryonic development according to their size and coloration. The first stage corresponded to recently laid eggs (Fig. 1), which were completely white (in the three *Progonyleptoidellinae* species) or yellowish (in both *Gonyleptinae*), and had not yet absorbed water from the environment. Eggs in the second stage were of the same color as those of the first stage, but were larger because of water absorption. Additionally, in the second stage it was possible to identify the embryonic formation of legs and ocelli (Fig. 1). Eggs in the third stage were similar in size to those of the second stage, but were generally brownish or dark yellow with black spots, which corresponded to the ocelli and tegumentary pigmentation of the embryo. In the fourth and last stage, immediately before hatching, the eggs were almost black (Fig. 1) and were similar in size to the third stage. These stages are easily recognized in the field and are likely to be the result of different oviposition events (see Hara *et al.* 2003).

To assess female fecundity, individuals of each species were collected and preserved in 70% ethanol. The females were dissected in the laboratory and the number of eggs in their reproductive tract was counted. The eggs were classified as mature when they were located in the distal 1/3 of the ovary, had a bright yellow coloration, and were > 1 mm in diameter.

Naturalist observations in the field were done as follows: *Gonyleptes saprophilus* – Eight guarding males were found caring for the offspring in the field (three in Serra do Japi and five in Parque Florestal do Itapetinga) from November 1998 to May 2003. Three of these males were marked with enamel color paint and monitored once a week from October 2002 to May 2003.

Neosadocus sp. – Fourteen males were found caring for the offspring in the field. Three of these males were marked and monitored once a month from May to September 2003. The field observations consisted of three daily inspections (once each morning, afternoon, and evening) for three consecutive days. At each inspection, the presence of the guarding males was monitored continuously and all relevant behavioral events were recorded (cf. Martin & Bateson 1986), including interactions between individuals and predator attacks on the eggs.

Seven non-caring males and 13 adult females were brought to the laboratory and maintained in a terrarium (40 x 90 cm base, 20 cm high) containing soil, pieces of tree trunks, and nine artificial nests made of clay. These shelters were of the same size and format as the natural nests in which the guarding males were observed in the field. The laboratory temperature was 20°C - 25°C, and the humidity ranged from 70% to 95%. The animals were fed three times a week with crushed cockroaches and an artificial diet for ants (Bhatkar & Whitcomb 1970). All individuals were marked and behavioral observations were made during the day and at night using a flashlight covered with a red filter (cf. Machado & Oliveira 1998) from May to September 2003.

Iporangaia pustulosa – Seventy-seven guarding males were marked and monitored once a month from March 2003 to January 2004. During each field trip, the guarding males were monitored six times a day (twice each morning, afternoon, and evening) for four consecutive days. The preliminary results presented here are part of a long-term project on the reproductive biology of this species with a focus on paternal care and sexual selection. Additional information on the reproductive system and detailed quantitative data on paternal care will be presented elsewhere (G.S. Requena *et al.* unpublished data).

Iguapeia melanocephala – Ten guarding males were found in the field between February 2003 and January 2004. Three of these individuals were marked and monitored once a month from March to September 2003. The field protocol was the same as described above for *Neosadocus*.

Progonyleptoidellus striatus – Four guarding males were found in the field and monitored twice a day (once in the morning and evening) on two consecutive days in June 2003.

Voucher specimens of all species were deposited in the Museu de Zoologia da Universidade de São Paulo (MZSP) and the Museu de História Natural da Universidade Estadual de Campinas (ZUEC), São Paulo state, Brazil.

Table 1. Variation in egg diameter during embryonic development, presence of a mucus coat surrounding the egg-batch, and number of mature eggs present in the ovary of five species of gonyleptid harvestmen with paternal care. Measures are in mm.

Species	Egg diameter (Mean \pm SD)		Mucus	Number of mature eggs
	Recently laid	Prior hatching		
<i>Gonyleptes saprophilus</i>	1.24 \pm 0.03 (n = 10)	1.52 \pm 0.05 (n = 10)	absent	26 – 82 (n = 4)
<i>Neosadocus</i> sp.	1.37 \pm 0.04 (n = 10)	1.37 \pm 0.03 (n = 10)	absent	11 – 12 (n = 2)
<i>Iguapeia melanocephala</i>	1.21 \pm 0.03 (n = 10)	1.48 \pm 0.05 (n = 10)	abundant	14 – 37 (n = 6)
<i>Iporangaia pustulosa</i>	1.18 \pm 0.04 (n = 10)	1.36 \pm 0.03 (n = 10)	abundant	7 – 48 (n = 11)
<i>Progonyleptoidellus striatus</i>	1.23 \pm 0.03 (n = 10)	1.50 \pm 0.05 (n = 10)	abundant	12 – 40 (n = 4)

RESULTS

General features

The eggs of all species are laid 0 – 2 mm apart, in a single layer, and are sometimes surrounded by an abundant and transparent mucus coat (Table 1). Egg-batches generally consisted of eggs in several embryonic stages, as well as early-hatched nymphs (Table 2). The time between oviposition and hatching is nearly one month. The number of mature eggs in the female reproductive tract ranged from 7 to 82 (Table 1), which is much less than the total number of eggs found in each batch in the field (Table 2). Guarding males were observed either resting over the eggs or stationary close to their respective egg-batches (within 1 – 20 cm). These males were also found walking or feeding far (one to several meters) from their batches and, during these periods, the eggs were left unattended.

Gonyleptes saprophilus

Seven egg-batches were found in tree holes and one was found in a rock breach near a cave entrance (Table 2). All of the batches were guarded by an adult male (Fig. 2A), which was commonly found over the eggs with the fourth pair of legs directed towards the hole entrance. In this position, the male is able to use the sharp spines of the femur and coxa IV to pinch any potential predator that enters the hole. The number of eggs per batch ranged from 24 to 409, and 50% of the batches contained eggs in more than one stage of embryonic development (Table 2).

Adult females were observed at least once inside all nests but did not remain with the guarding males far more than one week. On two occasions, females were seen laying eggs, during which the guarding males remained behind the ovipositing female and continuously touched the female with legs I and II. One of the nests monitored was visited by

Table 2. Main features of the egg-batches of four harvestmen species with paternal care. The data for *Neosadocus* sp. include only field observations.

Male size (mm)	Oviposition site	Total number of eggs	Number of egg stages	Number of nymphs
<i>Gonyleptes saprophilus</i>				
9.5	In a rock fissure	328	2	0
8.6	In a tree hole	24	1	0
9.8	In a tree hole	281	3	24
10.1	In a tree hole	409	3	12
10.0	In a tree hole	49	1	0
10.1	In a tree hole	49	1	0
9.3	In a tree hole	59	2	3
9.7	In a tree hole	50	1	5
<i>Neosadocus</i> sp.				
6.7	In a mud hole	157	3	12
7.7	In a mud hole	215	4	5
7.2	In a mud hole	9	1	0
7.2	In a mud hole	146	1	0
7.8	In a mud hole	82	1	0
8.1	In a mud hole	212	4	10
7.1	In a mud hole	153	4	0
7.5	In a mud hole	165	3	0
7.6	In a mud hole	315	4	0
7.7	In a mud hole	8	1	0
8.0	In a mud hole	401	4	0
7.9	In a mud hole	557	4	0
7.5	In a mud hole	177	3	0
7.3	In a mud hole	518	3	0
<i>Iguapeia melanocephala</i>				
6.8	Undersurface of a leaf	89	3	9
6.9	Undersurface of a leaf	84	2	0
6.8	Undersurface of a leaf	96	2	1
7.0	Undersurface of a leaf	34	2	0
6.7	Undersurface of a leaf	53	3	3
6.9	Undersurface of a leaf	27	1	0
7.1	Undersurface of a leaf	81	2	0
<i>Progonyleptoidellus striatus</i>				
7.2	Undersurface of a leaf	31	2	0
7.0	Undersurface of a leaf	133	2	0
6.9	Undersurface of a leaf	65	2	4
7.0	Undersurface of a leaf	121	2	0

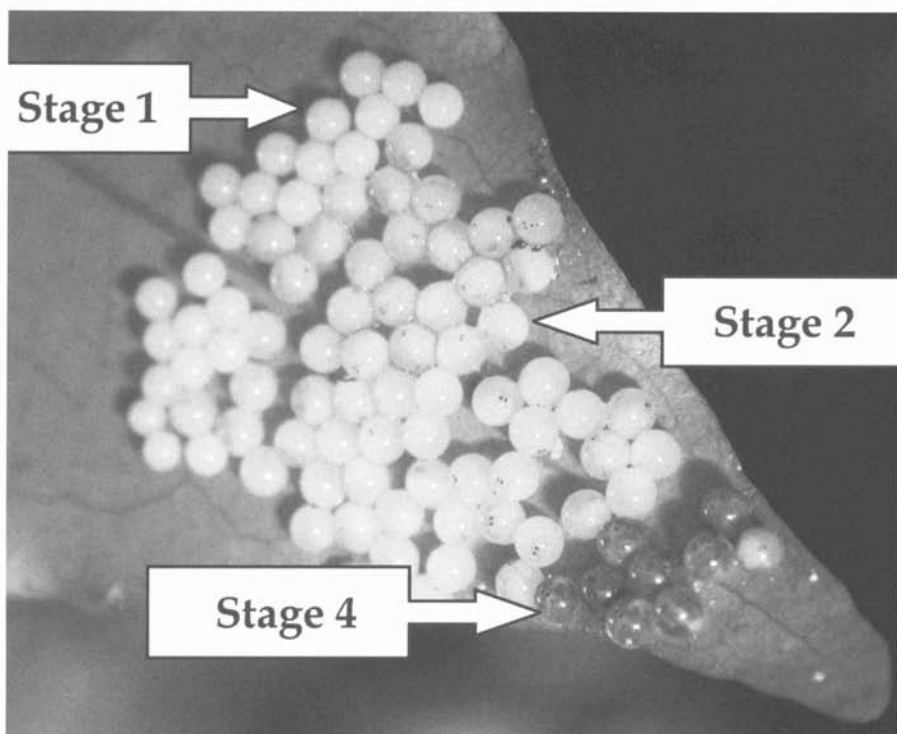


Fig. 1. Egg-batch of the harvestman *Iporangaia pustulosa* on the undersurface of a leaf. Dark eggs at the leaf apex correspond to the fourth stage and are nearly ready to hatch. Eggs in the first stage have been recently laid and are located at the periphery of the batch. These eggs are smaller than those in other stages and it is not possible to distinguish the ocelli in the embryo. Eggs in the second stage are larger than those in the first stage and the ocelli of the nymphs can be seen inside the eggs.

three females during a 7-month period, and two of them added eggs to the batch.

One batch was invaded by ants (*Pheidole* sp.) while the guarding male was not present. The ants swarmed on the eggs and consumed two of them. The guarding male returned to the nest nearly 2 h later and repelled the ants, grasping the ants one by one with the chelicerae and dropping them outside the nest. Another unprotected batch was visited by an adult male that remained over the eggs for 3 h without consuming any eggs. When the guarding male returned to the nest, he aggressively repelled the conspecific.

***Neosadocus* sp.**

The 14 guarding males were found in cavities in ravines (Fig. 2B). These cavities had a circular entrance 8 - 25 mm in diameter and a

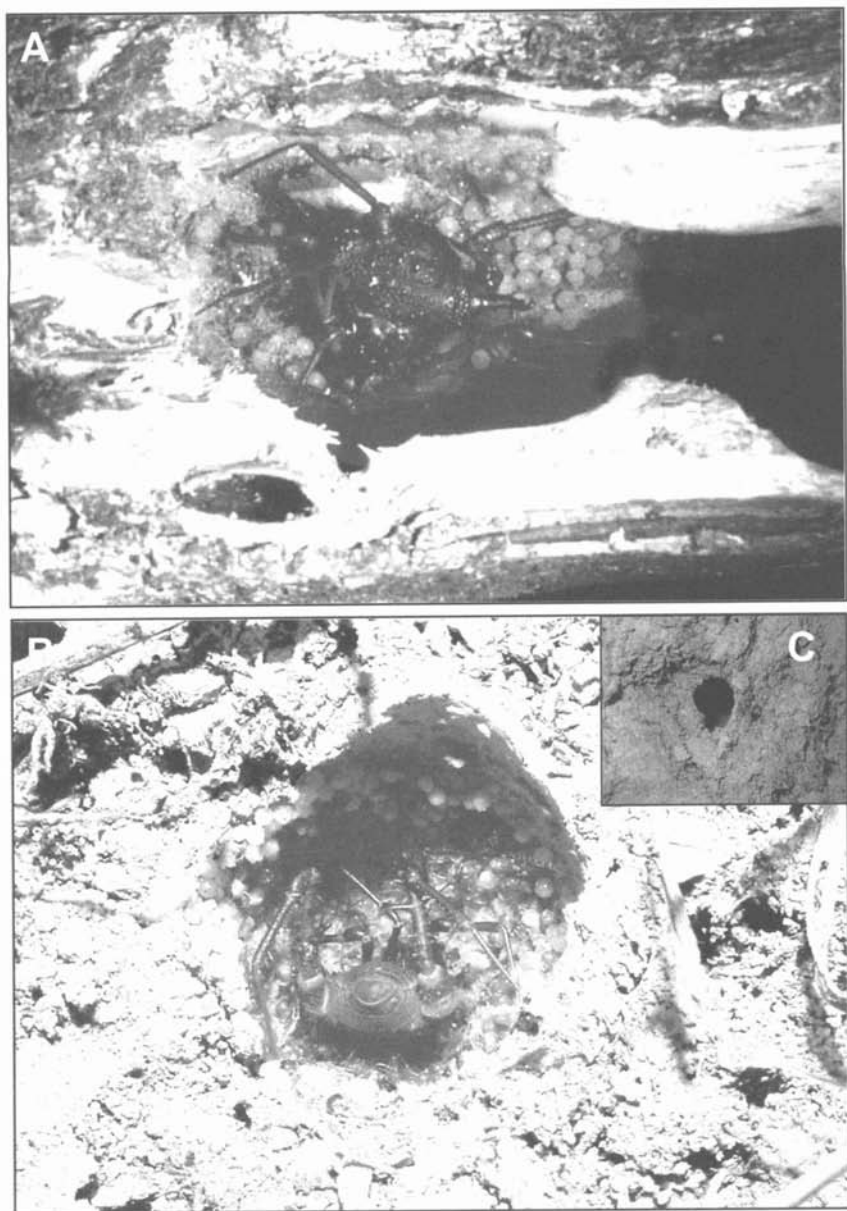


Fig. 2. (A) A male of the harvestman *Gonyleptes saprophilus* guarding his nest in a tree hole. The trunk was cut to show the eggs and the male on the batch. (B) A male of the harvestman *Neosadocus* sp. guarding his mud nest. The entrance of the hole was destroyed to show the eggs and the characteristic defensive position of male with the fourth pair of legs directed towards the entrance. The intact entrance is shown in (C).

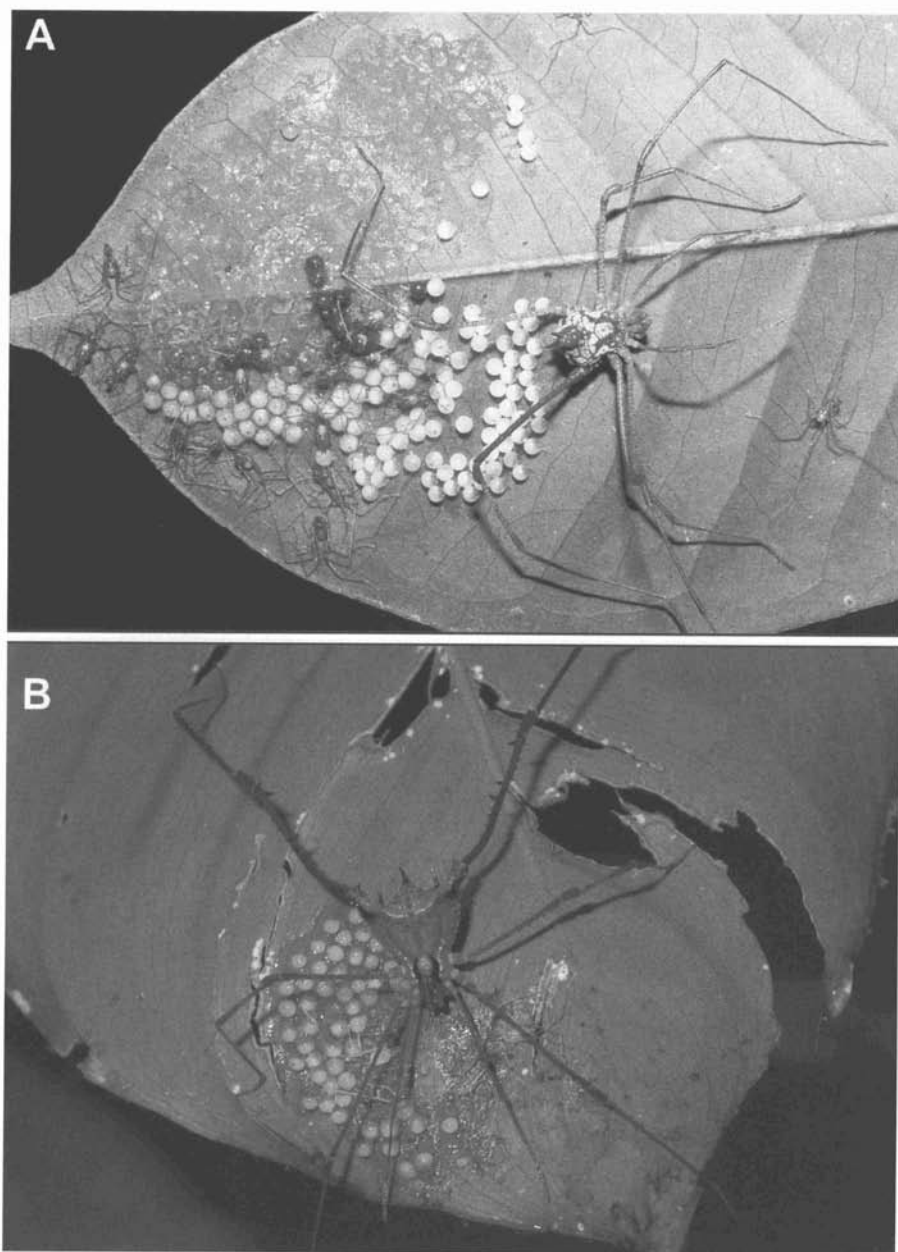


Fig. 3. (A) A male of the harvestman *Iporangaia pustulosa* guarding his egg-batch on the undersurface of a leaf. (B) A male of the harvestman *Iguapeia melanocephala* guarding his egg-batch on the undersurface of a leaf. Note that both batches consist of eggs in at least two stages, as well as early hatched nymphs.

depth of 32 - 45 mm (Fig. 3C). Digger wasps or spiders probably built some of the holes, and the others were natural cavities in the soil. Guarding males were observed inside the nests in a position very similar to that described for *G. saprophilus* (Fig. 2B). The fourth pair of legs was used to repel a nest invader, a female of the harvestman *Mischonyx* sp. (Gonyleptidae). The mean number of eggs per batch was 224.4 ± 168.7 (range = 8 to 557 eggs; $n = 14$) and 71.4% of the batches consisted of eggs in more than one stage of embryonic development (Table 2). Three batches contained newly hatched nymphs (5 - 12 individuals). There was no correlation between the length of the dorsal scutum of the guarding males and the number of eggs in their respective batches (Pearson correlation, $r = 0.29$; $p = 0.32$; $n = 14$).

The artificial mud nests offered in the laboratory were promptly used by the individuals as nest sites. Between June and September, five egg-batches were created, with four of them containing eggs in more than one stage of embryonic development. The number of eggs per batch ranged from 74 to 204 (Table 2). One female was observed copulating with three guarding males. In two nests, the guarding males were replaced by conspecific males, but it was unclear whether the guarding males had first abandoned these nests or whether they had been usurped by the new males. There was no reduction in the number of eggs in either of these two nests. On the contrary, in one of these cases, the new male copulated with two females and quadrupled the previous number of eggs in the batch.

Iporangaia pustulosa

This species lays eggs on the undersurface of the leaves of shrubs growing at the margin of streams, and the eggs are covered by an abundant transparent mucus coat (Table 1). A general pattern of egg distribution on the leaf was observed: older eggs are placed closer to the leaf apex, suggesting that eggs are laid from this portion of the leaf towards its base (Fig. 1). The mean number of eggs per batch was 88.6 ± 46.1 (range = 14 - 215 eggs; $n = 54$), and this value does not include batches containing hatched eggs (as shown in Fig. 3A). Nearly 20% of all batches had eggs in only one stage of embryonic development, 44% had eggs in two stages, 31% had eggs in three stages, and 5% had eggs in four stages. Nymphs (1 - 10 individuals) were found in 15.6% of the batches. There was a positive correlation between the number of different stages of embryonic development and the total number of eggs in the batches (Spearman correlation, $r_s = 0.632$; $p < 0.0001$; $n = 54$). All egg-batches were guarded at least once by an adult male found near the eggs (Fig. 3A) or on the upper surface of the leaf containing the

batch. The length of the dorsal scutum of the guarding males was not correlated with the number of eggs in their batches (Pearson correlation, $r = -0.09$; $p = 0.50$; $n = 54$). There was also no correlation between the size of the males and the number of embryonic developmental stages in their batches (Spearman correlation, $r_s = 0.02$; $p = 0.89$; $n = 54$); the latter is probably related to the number of copulations achieved by these males.

During the colder months (May to September), most males were found hidden in natural cavities in the ground or under fallen trunks at the base of the shrub containing their respective egg-batches (Table 3). In this period, unattended egg-batches were not attacked by predators or visited by conspecifics. Guarding males became active at night, when they were observed close to their egg-batches or foraging on the vegetation (Table 3). Adult females showed the same activity schedule and were observed walking on the vegetation exclusively at night (Table 3).

Table 3. Seasonal comparison of the frequency in which guarding males of the harvestman *Iporangaia pustulosa* were recorded close to their batches, and the percentage of active females in three periods of the day: morning (09:00 – 12:00 h), afternoon (15:00 – 18:00 h), and night (21:00 – 00:00 h).

Season	Period of the day		
	Morning	Afternoon	Night
Cold dry (July to September)			
Guarding males ($n = 47$ egg-batches)	2.1%	4.8%	59.8%
Adult females ($n = 44$ observations)	0	0	100%
Warm wet (November to January)			
Guarding males ($n = 83$ egg-batches)	48.2%	53.0%	79.1%
Adult females ($n = 353$ observations)	32.5%	36.1%	31.4%

During the warmer months (October to April), males and females were active throughout the day (Table 3). However, even during this period, guarding males were not always recorded over their respective batches. Some left their eggs unattended for up to 48 h (more commonly between 3 – 6 h) and were found as far as 5 m from their batches. Unattended batches were attacked by conspecific females and males, which consumed 1 – 7 eggs.

In the presence of guarding males, no successful predation was observed in the field. On one occasion, a guarding male chased a conspecific male in the proximity of the egg-batch. Three guarding

males were seen attacking other harvestmen (a subadult male of *Ampheres leucopheus* and two adult females of *Promitobates ornatus*) that approached their respective batches. All guarding males raised their pedipalps and the first two pairs of legs when attacking the intruders and repelling them from the leaf containing the batch.

Iguapeia melanocephala* and *Progonyleptoidellus striatus

As with *I. pustulosa*, egg-batches of *I. melanocephala* and *P. striatus* were found on the undersurface of leaves and showed the same spatial pattern of egg distribution on the leaf. The batches were found mainly along the margins of streams and the eggs were covered by an abundant transparent mucus coat (Table 1). Guarding males were found resting at the leaf base or on the upper surface of the leaf containing the egg-batch (Fig. 3B). For *I. melanocephala*, the number of eggs per batch ranged from 27 to 96, and 84.3% of the batches consisted of eggs in more than one stage of embryonic development (Table 2). Three batches contained newly-hatched nymphs (Table 2). For *P. striatus*, the number of eggs in the three batches recorded in the field ranged from 31 to 133, and all consisted of eggs in more than one stage of embryonic development (Table 2). One of these batches contained newly-hatched nymphs (Table 2).

The pattern of seasonal activity of *I. melanocephala* was similar to that of *I. pustulosa*: during the cold months individuals (including guarding males) were active only at night, and during the warm months, males and females were active throughout the day. Unattended egg-batches were not attacked by predators or visited by conspecifics. Since field observations on *P. striatus* were conducted only during the cold season, it was not possible to make seasonal comparisons. However, like the species described above, individuals of *P. striatus* (including the guarding males) were active only at night.

DISCUSSION

Theory applied to Opiliones

Mora (1990) was the first to propose a hypothetical scenario for the evolution of male care in Opiliones, using *Zygopachylus albomarginis* as a model organism. According to her theory, females are attracted to appropriate oviposition sites that are defended by males in order to achieve copulations. Males defending territories increase their reproductive success since they indirectly protect the eggs against predation. In the gonyleptid *Goniosoma longipes* (Goniosomatinae), a male may defend a territory on a cave wall on which females lay eggs and care for their batches (Machado & Oliveira 1998). When a female is experimen-

Table 4. Predictions of exclusive paternal care as a sexually selected male trait (Tallamy 2000, 2001) applied to all species of harvestmen known to exhibit male egg-guarding.

Species	Females are iteroparous	Many mating opportunities for the males	Care interferes with female foraging	Eggs increase male attractiveness	Males guard eggs laid by several females	Males are willing to guard unrelated eggs	Local female population is high
ASSAMIIDAE							
<i>Lepchana spinipalpis</i> ¹	Yes (?)	Yes	Yes (?)	?	Yes	Yes	No
GONYLEPTIDAE							
<i>Ampheres leucopheus</i> ²	Yes	Yes	Yes (?)	?	Yes	?	?
<i>Cadeadoius niger</i> ³	?	?	Yes (?)	?	?	?	?
<i>Gonyleptes saprophilus</i> ⁴	Yes	Yes	Yes (?)	?	Yes	Yes (?)	No
<i>Neosadocus</i> sp. ⁴	Yes	Yes	Yes (?)	?	Yes	Yes (?)	?
<i>Iguapeia melanocephala</i> ⁴	Yes	Yes	Yes (?)	?	Yes	?	No
<i>Iporangaia pustulosa</i> ⁴	Yes	Yes	Yes (?)	?	Yes	No	Yes
<i>Progonyleptoidellus striatus</i> ⁴	Yes	Yes	Yes (?)	?	Yes	?	Yes
MANAOSBIIDAE							
<i>Zygopachylus albomarginis</i> ⁵	Yes	Yes	Yes (?)	?	Yes	No	Yes
PODOCTIDAE							
<i>Leytpodoctis oviger</i> ¹	?	?	?	?	?	?	?

¹ Martens (1993); ² Hara *et al.* (2003); ³ Stefanini-Jim *et al.* (1987); ⁴ This study; ⁵ Rodrigues & Guerrero (1976) and Mora (1990, 1991)

tally removed from her egg-batch, the male takes over egg-guarding for up to two weeks. These observations suggest that males of *G. longipes* may care for the brood when the egg-guarding females desert or die. Indeed, males of *G. albiscriptum*, a congeneric species that also reproduces in caves and shows maternal care, were found in the field guarding eggs of abandoned batches (Willemart & Gnaspini 2004). These cases of temporary and conditional egg-guarding by males are remarkably different from the exclusive postovipositional paternal care of other harvestmen species, but provide support for the idea that male guarding in Opiliones may evolve when males defend a territory that is also an oviposition site (Machado & Raimundo 2001).

The recent hypothesis by Tallamy (2000, 2001) to explain paternal care in arthropods argues that the main selective force driving the evolution of this rare behavioral pattern is sexual selection rather than territorial defense. According to this hypothesis, some life-history and behavioral features can be predicted if the evolution of paternal care is a sexually selected trait: (a) females are iteroparous, (b) there are many mating opportunities for the males, (c) care interferes with female foraging, (d) eggs increase male attractiveness, (e) males may guard eggs laid by several females, (f) males are willing to guard unrelated eggs, and (g) the local female population is high (Tallamy 2000, 2001).

Data from ten paternal harvestmen (seven of them belong the family Gonyleptidae) provide support for most of these predictions (Table 4). A general feature is that females of all species are indeed iteroparous. In at least eight of these species, males have many mating opportunities and simultaneously guard eggs laid by more than one female. Information on the density of females in natural populations is scarce, and the data presented in Table 4 are based on the number of females found and/or collected during our fieldwork. When this number was less than 10 females, or when the sex ratio was highly male biased, we considered that the density of females in natural populations was low. Field data obtained for some species in this study, as well as for species previously studied (Martens 1993), do not support the prediction that females should be locally abundant (Table 4).

Finally, the evidence that males should be willing to guard unrelated eggs is ambiguous. In at least two species, *Zygopachylus albomarginis* and *Iporangaia pustulosa*, vagrant males do not hesitate to eat eggs of unprotected batches (Mora 1990; this study). In *Zygopachylus*, males eat eggs of unattended nests, but vagrant males are fighting for the ownership of the mud nest itself, which is probably the most important signal of male quality (Mora 1990, 1991). In *Iporangaia*, however, the eggs are the only attractive to females and males should be willing to

guard unrelated batches, even if they eat a few eggs. Contrary to this prediction, vagrant males ate some unprotected eggs and abandoned the batches. Egg adoption is a crucial point of Tallamy's hypothesis and future studies on paternal harvestmen should investigate this aspect more thoroughly. The decision to cannibalize an unprotected batch may depend on the number of eggs present (which may reflect the attractiveness of the site) or on ecological factors such as food abundance, operational sex ratio of the population, and availability of oviposition sites.

Male versus female care

The amount of information on parental care in harvestmen has increased considerably in the last two decades. Since 1990, more than 20 new cases have been described, most of them dealing with maternal care (see reviews in Machado & Raimundo 2001 and Machado 2002). The data available so far indicate that there are several differences in the behavioral patterns exhibited by guarding females and guarding males. Perhaps the most striking difference is that females care for batches containing eggs in only one stage of embryonic development (e.g. Gnaspini 1995; Machado & Oliveira 1998, 2003), while males care for batches containing eggs in several stages of embryonic development that are likely to have come from different oviposition events (Martens 1993; Hara *et al.* 2003).

Additionally, the total time spent by females guarding eggs and newly-hatched nymphs varies among and within species, but usually does not exceed 60 days (see Gnaspini 1995; Machado & Oliveira 1998, 2003). Among paternal species, however, females continually add eggs to the batches and parental activities can, therefore, last up to 8 months. Moreover, during the caring period, females do not leave the egg-batch to forage or undertake any other activity (e.g. Gnaspini 1995; Machado & Oliveira 1998, 2003). In contrast, guarding males frequently leave their egg-batches and may be found as far as 5 m from the oviposition site (Hara *et al.* 2003; this study). At least three hypotheses that are not mutually exclusive may account for the males' forays: (1) males may be unable to accumulate enough energy reserves for the long period of egg-guarding so that they need to leave the batch to forage, (2) males may be patrolling the batches at a distance in order to repel predators and other competing males, and (3) during their walks, males may increase their chances of finding an additional mate.

Most of the behavioral differences mentioned above may be a consequence of the different selective pressures leading to the evolution of paternal or maternal care (Trivers 1972). Among harvestmen and many other arthropods, maternal care is likely to have evolved as a

result of natural selection (Zeh & Smith 1985; Tallamy 2000, 2001). The great majority of maternal harvestmen seem to be constrained to a single reproductive event (Machado & Raimundo 2001; Machado & Oliveira 2003). There are a few exceptions, such as *Goniosoma longipes*, *G. albiscriptum*, and *Bourguyia albiornata*, but in these cases the percentage of females that produced more than one batch in the same breeding season was less than 20% (Machado & Oliveira 1998, 2003; Willermart & Gnaspini 2004). It is possible that females of these species are opportunistically iteroparous and constraints such as availability of food and/or nest sites makes them functionally semelparous (*sensu* Tallamy & Brown 1999). If environmental constraints reduce the chance of subsequent reproductive events during a breeding season, the fecundity-related costs of maternal care are minimized (e.g. Kight 1997; Eggert & Müller 1997). Females could, therefore, increase their lifetime reproductive success by guarding the eggs that they have already laid (Tallamy & Brown 1999). The loss of a single egg to predators may represent a decrease of 5% or more in the global reproductive success of a harvestman female (for information on clutch size see Machado & Raimundo 2001). Thus, guarding females may be selected to remain close to their batches throughout the caring period.

In contrast to females, male care in harvestmen seems to have evolved as a result of sexual selection. A strong suggestion that paternal care in harvestmen is related to sexual rather than natural selection is observed among the Progonyleptoidellinae. In the winter, guarding males of three species studied here leave their batches unattended during the day, despite the presence of egg predators such as ants, wasps, and other harvestmen. Guarding males were found close to their eggs only at night, when females were active on the vegetation. Since females are not active during the day, there is no opportunity for the males to copulate, which decreases the benefit of egg-guarding. Moreover, remaining close to the eggs during the day would expose guarding males to a number of visually oriented predators and to the stressful climatic conditions of the dry-cold season, and would increase the costs of caring. Since the number of eggs obtained during a single breeding season by one guarding male may reach more than 500 (see Table 2), the value of an individual egg is probably much lower for guarding males than for guarding females.

Implications for the evolution of paternal care in the family Gonyleptidae

Both *Gonyleptes saprophilus* and *Neosadocus* sp. belong to the large subfamily Gonyleptinae, in which cases of maternal care and no care have been recorded (Machado & Vidal 2001; Pereira *et al.* 2004).

Iguapeia, *Iporangaia*, and *Progonyleptoidellus* are representatives of the subfamily Progonyleptoidellinae, for which there is only a brief note describing a possible case of paternal care in *Cadeadoius niger* (Stefanini-Jim *et al.* 1987).

According to Kury & Pinto-da-Rocha (1997), the Gonyleptinae is the sister group of a monophyletic clade that includes the Hernandariinae, Sodreaninae, Caelopyginae, and Progonyleptoidellinae (Fig. 4). A subsequent analysis corroborated the hypothesis that the Progonyleptoidellinae are the sister group of the Caelopyginae (Pinto-da-Rocha 2002), in which there is also one case of paternal care described for *Ampheres leucopheus* (Hara *et al.* 2003). There is no

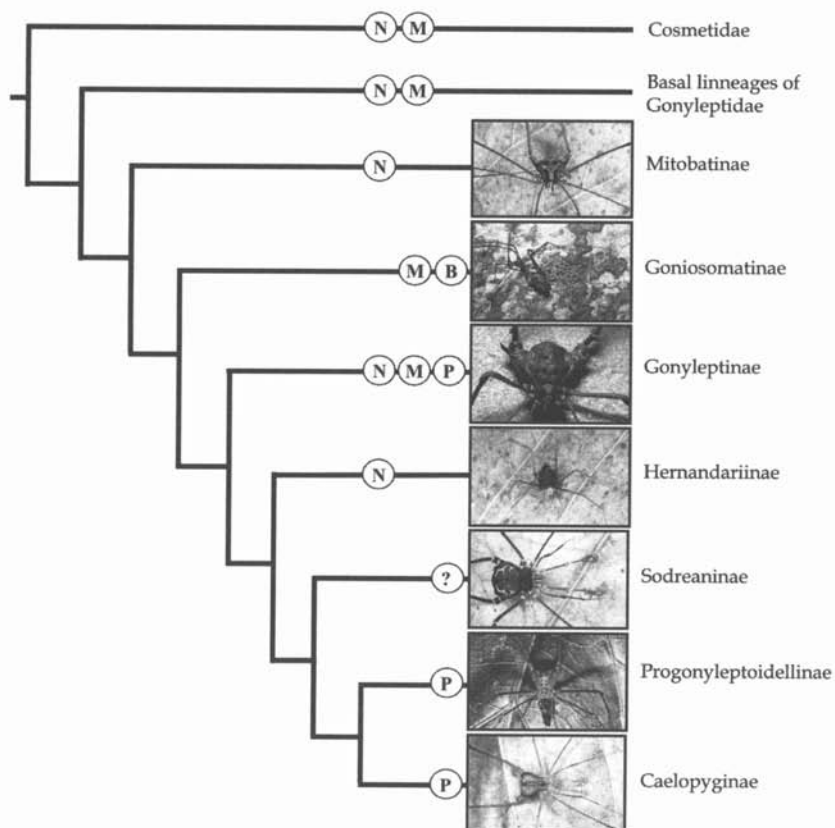


Fig. 4: Phylogeny of a well-resolved clade within the family Gonyleptidae (cf. Kury & Pinto-da-Rocha, 1997; Pinto-da-Rocha, 2002) showing the forms of parental investment presented by each subfamily. N = no care (eggs are generally laid singly in hidden places or covered with debris), M = maternal care, P = paternal care, B = biparental care (males may take care of the eggs if females desert or die), ? = no information available.

biological information on species of the subfamily Sodreaninae, but among the Hernandariinae, females hide their eggs in the substrate and/or cover them with debris (Canals 1934; G. Machado *unpublished data*).

There are still many gaps in our knowledge of gonyleptids, i.e. many species need to be studied and detailed phylogenies are required to increase our understanding of the relationships among the subfamilies. For the moment, we can infer that paternal care has evolved at least twice within the Gonyleptidae: once in the subfamily Gonyleptinae and again in the ancestor of the clade Caelopyginae + Progonyleptoidellinae. This is quite a conservative scenario since the genera *Gonyleptes* and *Neosadocus* appear not to be closely related (A.B. Kury *personal communication*). However, until an internal phylogeny of the subfamily Gonyleptinae is available, no detailed analysis can be accomplished.

In the clade Caelopyginae + Progonyleptoidellinae, besides paternal care, there are at least three other features supporting the homology of the behavior exhibited by species of these two subfamilies: (1) eggs are laid on the undersurface of leaves, (2) females lay eggs from the apex to the base of the leaf, and (3) eggs are covered by a thick mucus coat. The use of leaves as an oviposition site is also found in some Goniosomatinae (Buzatto *et al.* 2003), but in these cases it is the female that guards the eggs. Therefore, the features described above are likely to constitute behavioral sinapomorphies of the clade Caelopyginae + Progonyleptoidellinae. This is an interesting finding since in the previous phylogenetic analyses of Gonyleptidae only a few morphological characters were recognized to support this clade.

Behavioral data on species of Sodreaninae and an internal phylogeny of the subfamily Gonyleptinae are crucial to answer the question of whether male guarding in gonyleptid harvestmen has evolved from no care or from female care. Among all other arthropod groups with paternal care, this question can be answered only for water bugs of the family Belostomatidae for which there is a phylogenetic hypothesis (Mahner 1993). In the water scorpions of the family Nepidae, the sister group of the belostomatids, parental individuals do not provide any additional care after oviposition. Because care is not expressed in the belostomatid sister groups, it is possible to infer that paternal care among water bugs evolved from a plesiomorphic state of no care (Smith 1997). Such a behavioral transition has been described for several vertebrate groups (review in Reynolds *et al.* 2002), and harvestmen may provide additional and phylogenetically independent tests of this pattern among arthropods.

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