



RESEARCH PAPER

Multiple Lines of Egg Defense in a Neotropical Arachnid with Temporary Brood Desertion

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Abstract

Egg predation is the one of the main costs of brood desertion in many ectothermic animals. When stressful environmental conditions constrain parental activities to only some periods of the day, the combination of physical or chemical defenses may attenuate the costs related to egg loss during periods of temporary parental absence. Females of the harvestman *Neosadocus maximus* periodically abandon their clutches to shelter or forage. They also cover their eggs with a hygroscopic mucus coat and seem to lose fewer eggs to predation than other syntopic harvestmen whose eggs lack the mucus coat. Using two species of *N. maximus* egg predators, we demonstrate that eggs whose mucus coat was experimentally removed suffered higher predation rate than eggs whose mucus coat was left intact. We argue that this mucus provides physical protection against egg predators, especially small arthropods. A similar mucus coat has independently evolved in other two clades of Neotropical harvestman in which males care for the eggs and typically leave their clutches unattended for several hours a day. We propose that the presence of multiple lines of egg defense may have evolved as a way of lowering the costs imposed by intra- and interspecific egg predation during periods of temporary brood desertion.

Introduction

Parental investment is typically considered one of the main determinants of female reproductive success (Trivers 1972; Gross 2005; Smiseth et al. 2012). Egg attendance, in particular, is often essential to brood survival since eggs of many species suffer strong predation, especially in tropical regions (Buzatto et al. 2013; Wong et al. 2013). However, egg attendance may decrease female future reproductive success as a consequence of two non-exclusive processes: (1) increased mortality risks due to greater exposure to natural enemies (e.g., shorebirds nesting in open areas) or stressful environmental conditions during the caring period (e.g., water pythons incubating eggs in cold areas) and (2) decreased investment in new eggs due to lower foraging activity (e.g., mouth-brooding cichlids) (references in Clutton-Brock 1991; Refsnider & Janzen 2010; Alonso-Alvarez & Velando 2012). Therefore, in species in which egg attendance

compromises foraging and/or survival, females may minimize the costs of maternal care by balancing the time invested in parental activities with the time spent foraging and/or safe from predators and adverse abiotic conditions (e.g., Fontaine & Martin 2006).

Females may also minimize the costs of egg attendance by adopting strategies that keep eggs protected in their absence, such as: (1) camouflaging eggs against the substrate (e.g., harvestmen: Machado & Raimundo 2001), (2) laying eggs in less vulnerable sites (review in Refsnider & Janzen 2010), (3) transferring chemical defenses to the eggs (e.g., insects: Eisner et al. 2000; Dreon et al. 2013), (4) producing physical barriers that hinder predator access to their clutch, such as burrows (e.g., vipers: Greene et al. 2002) or a layer of non-viable eggs (e.g., beetles: Deas & Hunter 2012), or (5) making the embryo itself less vulnerable to attacks, such as a hard layer covering the chorion (e.g., beetles: Ang et al. 2008). Physical and chemical egg protection may be observed even in

species in which females also exhibit egg attendance, so that the eggs are protected against predators by more than one line of defense (e.g., mud turtles: Iverson 1990; long-tailed skink: Huang 2006; earwigs: Kölliker 2007; jumping spiders: Vieira & Romero 2008; birds: Colwell et al. 2011; Stevens 2013).

Considering that egg attendance is not cost free (Clutton-Brock 1991; Alonso-Alvarez & Velando 2012), multiple lines of egg defense may evolve only if they provide an improvement in offspring survival significant enough to counterbalance the females' potential reduction in lifetime fecundity. For instance, when stressful environmental conditions constrain parental activities to only some periods of the day, the combination of physical or chemical defenses may attenuate the costs related to egg loss during periods of temporary parental absence. Moreover, multiple lines of egg defense may protect the eggs from the activity of some natural enemies that are able to overcome female guarding, such as parasitoids. In fact, the combination of egg attendance and physical barriers (such as cocoons and burrows) in several arthropods has been experimentally demonstrated to increase egg survival in comparison with only one line of defense (e.g., spiders: Vieira & Romero 2008; Gonzaga & Leiner 2013; earwigs: Kölliker 2007).

The presence of mucus coats surrounding eggs is common among marine invertebrates (Wilson et al. 1994) and amphibians (Wells 2007). In these organisms, which usually spawn in the water and rarely exhibit egg attendance, the mucus can reduce egg dehydration (Strathmann & Hess 1999), absorb UVB radiation (Licht 2003), and protect the eggs against predators, parasites, and infections (Benkendorff et al. 2001; Wells 2007). Mucus coats can also be found around the eggs of some terrestrial organisms, such as gastropods (Baur 1994), earthworms (Oumi et al. 1996), and arthropods (Machado et al. 2004), but the functions of these coats are not well understood. The harvestman *Iporangaia pustulosa*, for instance, surrounds its eggs by a thick mucus coat laid by females during oviposition. An experiment with this species has shown that the removal of caring males increases predation rate in eggs without mucus coat, but less so in eggs with mucus (Requena et al. 2009). Although the mucus coat may serve other functions, such as those cited above for aquatic animals, the experiment with *I. pustulosa* indicates that at least one function of the mucus is to prevent predation or decrease predation rates when guarding males temporarily abandon their clutches to forage or shelter.

A mucus coat around the eggs, similar to that of *I. pustulosa*, has been recently reported for the

harvestman *Neosadocus maximus*, a species exhibiting exclusive female attendance of eggs (Chelini & Machado 2012). In a previous study focused on the costs and benefits of temporary brood desertion in this species, we demonstrated that egg predation was inversely related to the time spent by the female caring for her eggs (Chelini & Machado 2012). The number of eggs consumed by predators during periods of female absence was considerably lower than other syntopic harvestmen whose eggs lack the mucus coat (e.g., Buzatto et al. 2007). In the present study, therefore, we use a combination of field observations and laboratory experiments to investigate if the mucus coat protects the eggs against predators in the absence of the guarding female. We hypothesize that the mucus coat protects the clutches, reducing predation rate in periods of female absence. In the discussion, we explore the correlated evolution of temporary brood desertion and presence of mucus coat around the eggs in three clades of Neotropical harvestmen.

Material and Methods

Sources of Egg Mortality in the Field

We conducted our fieldwork at Intervalles State Park (24°14'S, 48°04'W; 800 m above sea level), a large Atlantic Forest fragment in the state of São Paulo, southeastern Brazil. The region's weather is mainly subtropical, with a warm-wet season from October to March and a cold-dry season from April to September (Chelini et al. 2011). We gathered data on egg predation in seven field trips regularly spread between October 2009 and February 2010, corresponding to the reproductive season of *N. maximus* (Chelini & Machado 2012). These field trips lasted from 8 to 14 d each and were spaced out by one- to 3-wk intervals. In each trip, we conducted diurnal (between 0600 and 1800) and nocturnal (between 1800 and 0600) hourly rounds of observations of all clutches we found along a 450-m-long transect. In each round of observation, we monitored each clutch for 5 min and recorded the presence and behavior of any egg predator (see Chelini & Machado 2012 for more details on the observation rounds).

To determine how many eggs were consumed during day and night, we photographed each clutch daily at 0700 h and at 1900 h. By superimposing and comparing subsequent photos of the same clutch, we were able to identify how many eggs had been consumed in each period. The dataset is the same used by Chelini & Machado (2012) to examine how female presence may increase egg survival in *N. maximus*. In the

present study, however, we took into consideration only clutches whose guarding female had been absent for at least eight consecutive hours to investigate the protective role of the mucus coat during long periods of parental absence under field conditions. We also excluded all clutches showing signs of egg hatching in order to avoid overestimation of egg mortality. We used the number of eggs consumed during the periods of female absence to discuss how the intensity of predation on unattended eggs in *N. maximus* compares to other syntopic harvestman species that lack a mucus coat on the eggs.

Protective Role of the Mucus Coat

To determine the protective role of the mucus coat, we designed an experiment in which we offered eggs with and without the mucus coat to the two most frequent egg predators observed in the field (see Results): conspecifics (body length: $\bar{x} \pm SD = 4.78 \pm 0.32$ mm, $n = 80$) and the harvestman *Promitobates ornatus* (9.66 ± 0.80 mm, $n = 80$). Besides the difference in body size between the two species, we selected *N. maximus* because cannibalism is the most frequent cause of egg mortality in harvestmen with parental care (e.g., Mora 1990; Buzatto et al. 2007; Requena et al. 2009) and because this species was the only predator observed consuming eggs both during day and night (see Results). Regarding *P. ornatus*, individuals of this species are very abundant in the study area and are known to be important predators of harvestman eggs (Buzatto et al. 2007; see also Results). The other predators observed in the field either did not consume a large number of eggs or were too rare to be used in our experiment.

We collected 40 individuals of each predator species (20 males and 20 females, totaling 80 individuals) at Intervales State Park and took them to the laboratory, where we housed them in plastic boxes (17 × 13 cm base, 11 cm high) with water, leaves for shelter, and branches for perching. We also collected 20 egg clutches outside of our study transect, containing between 70 and 150 eggs each. These clutches were transported to the laboratory in large cylindrical plastic vials (10 cm diameter × 15 cm height) with a piece of wet cotton to maintain high humidity levels. Climatic conditions in the laboratory were as follows: 21–24°C, 61–80% air humidity, and 12:12 h (light: dark) photoperiod.

Before we began the experiment, we fed all individuals a small amount of low-protein food (a grain of cooked rice) and allowed them to eat it for 24 h. We

then removed all the remaining food and kept the individuals in starvation for 5 d. After this period, we separated the individuals into two experimental groups, each one composed of 10 females and 10 males of each predator species (totaling 40 individuals per treatment). The experimental groups were as follows: (1) 'without mucus,' in which we offered to each individual 10 *N. maximus* eggs whose mucus coat had been previously removed by gently rolling them in a clean piece of filter paper, and (2) 'with mucus,' in which we offered to each individual 10 *N. maximus* eggs that were manipulated, but still had their mucus coat intact. This method was proved efficient in previous studies (Requena et al. 2009) and did not impair egg viability (approx. 80% of eggs hatched after 16–20 d in both experimental groups). We placed eggs of both experimental groups on a small plastic lid at the bottom of each cage and moistened them with a water spray on a daily basis. We recorded the number of eggs consumed by each individual at 1000 h during five consecutive days. We also conducted 5 min long focal observations at different times of the day to describe predatory attacks to the eggs. After the experiment, we released all the experimental individuals in the field.

To test the effect of the mucus coat and of the predator species on the proportion of eggs consumed after 5 d, we used a generalized linear model (GLM) with a beta-binomial distribution of errors. The beta-binomial distribution is suitable for proportion data with variance higher than that fitted by a binomial distribution due to high frequency of zero values, which promotes overdispersion of the data (Bolker 2008). We compared models including the effect of: (1) the experimental group (with and without mucus), (2) the predator species (*N. maximus* and *P. ornatus*), (3) the additive effect of these two factors, (4) the interaction of these two factors, and (5) no effect of these two variables (Table 1). We selected the most plausible competing model through AICc values comparison, selecting the one with the smaller $\Delta AICc$ (Burnham & Anderson 2002).

Results

Egg Mortality in the Field

We found a total of 53 clutches that showed no sign of egg hatching at the moment of our observations. In 36 clutches, guarding females were absent for more than eight consecutive hours, providing a total of 91 photographic records of unattended clutches. These unattended clutches contained from 38 to 296 eggs

Table 1: AICc (second-order Akaike's information criteria) table for the GLMs of the number of eggs consumed ('predation') by *Neosadocus maximus* and *Promitobates ornatus* ('species') in a 5-d-long experiment in which the mucus coat that covers *N. maximus* eggs was either removed or left intact ('experimental group'). k = number of parameters of the models; Δ AICc = difference between the current model and the best model

Model	k	AICc	Δ AICc	AICc weight
Predation is influenced by the additive effect of the experimental group and species	3	206.96	0	0.5852
Predation is influenced by the interaction between experimental group and species	4	209.00	2.04	0.2379
Predation is influenced by experimental group only	2	209.13	2.17	0.1763
Predation is influenced by species only	2	221.46	14.5	0.0004
Predation is not influenced by experimental group or species	1	223.06	16.1	0.0002

(median = 147 eggs). We found evidence of diurnal predation in 15 of the 63 photographs representing clutches left unattended during the day (average number of eggs consumed = 9.9, min–max = 1–43). We found evidence of nocturnal predation in 16 of the 28 photographs representing clutches left unattended during the night (average number of eggs

consumed = 20.1, min–max = 1–135). Moreover, we witnessed 15 attacks to the clutches during our field observations (Fig. 1). We summarize the identity of the predators and the number of eggs consumed in Table 2. In general, diurnal predators consumed a smaller number of eggs than nocturnal predators. The most frequent egg predator was the harvestman *P. ornatus*, and only conspecifics consumed eggs both during day and night.

Protective Role of the Mucus Coat

In the laboratory, both *N. maximus* and *P. ornatus* consumed more eggs without mucus than eggs with mucus (Fig. 2). We also observed individuals of both predator species manipulating the mucus coat with their pedipalps, but leaving the eggs intact. The AICc ranking indicates that the model best fitted to our data combines the additive effects of both predator species and experimental group (Table 1). This model indicated that *N. maximus* had a higher predation rate than *P. ornatus* (coefficient for predator species = 0.957) and that the mucus presence negatively affected egg consumption (coefficient for experimental group = -1.917). Indeed, the proportion of eggs consumed was higher for *N. maximus* than for *P. ornatus* in both experimental groups (Fig. 2).

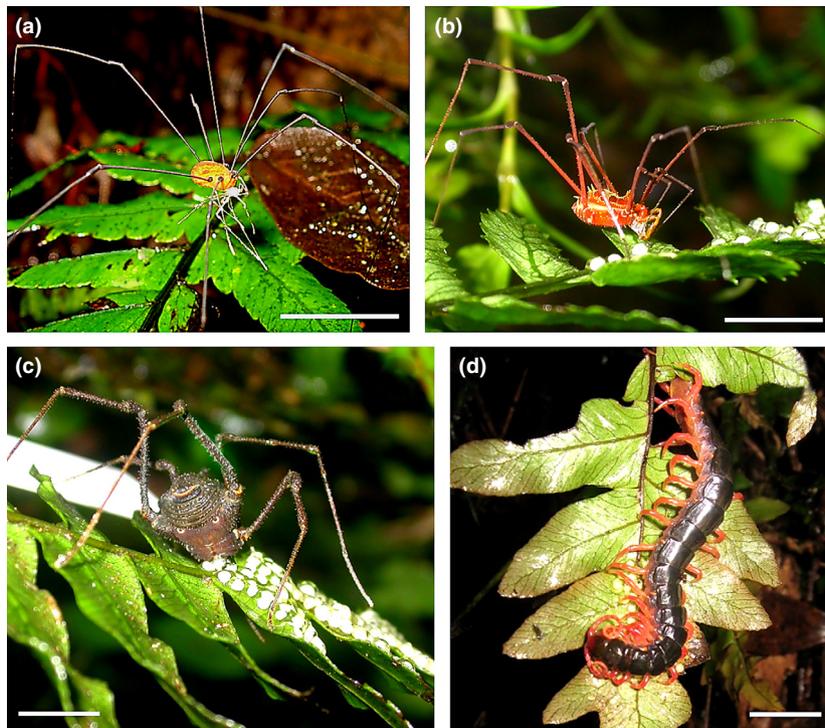


Fig. 1: (a) *Neosadocus maximus* nymph being consumed by the harvestman *Jussara* sp. (Sclerosomatidae). (b) *Promitobates ornatus* female preying upon an *N. maximus* egg. (c) *N. maximus* female preying upon a conspecific clutch. (d) *N. maximus* clutch being attacked by a centipede (Scolopendridae) that displaced the guarding female. Scale bars = 1 cm.

Table 2: List of arthropods found consuming eggs of the harvestman *Neosadocus maximus* in the field during day and night

Taxon (number of observations)	Number of eggs/nymphs consumed per observation	
	Day	Night
Class Arachnida		
Araneae (1)	–	1 (egg)
<i>Jussara</i> sp. (Sclerosomatidae) (1)	1 (nymph)	–
<i>Neosadocus maximus</i> (2)	5 (eggs)	7 (eggs)
<i>Promitobates ornatus</i> (7)	1–2 (eggs)	–
Velvet mite (Trombididae) (1)	Mucus only	–
Class Chilopoda		
Scolopendridae (1)	–	90 (eggs)
Class Insecta		
<i>Lutosa</i> sp. (Anostostomatidae) (1)	–	50 (eggs)

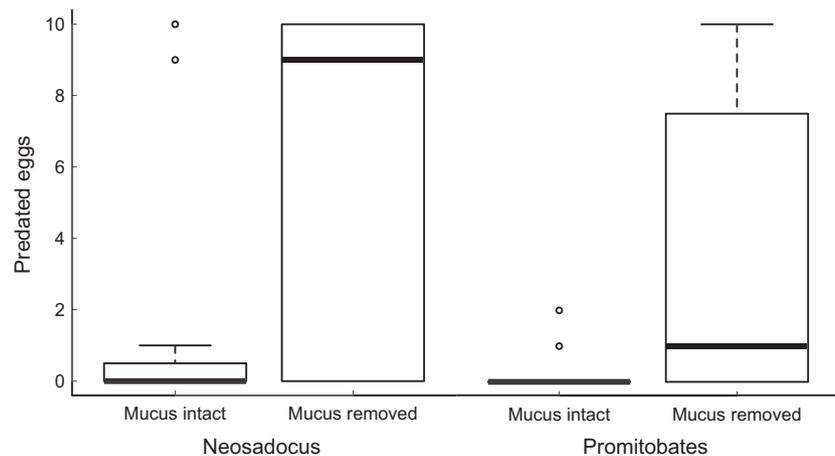
Discussion

Our field observations showed that several arthropod species attack *N. maximus* eggs while guarding females temporarily abandon their clutches. Diurnal predators, overall smaller than *N. maximus* (Fig. 1), were generally ineffective in overcoming the protection afforded by the mucus coat and their attacks resulted in small or no egg loss (Table 2). Although large nocturnal predators were more effective in overcoming the mucus protection and eating a larger number of eggs than diurnal predators (Table 2), few clutches lost more than 10% of their eggs over their entire embryonic development, which lasts nearly 40 d (Chelini & Machado 2012). These results are strikingly different from those obtained with the syntopic harvestman *Serracutisoma proximum*, whose females also lay eggs on the vegetation, but do not cover the eggs with mucus. Contrary to *N. maximus*, unattended clutches of *S. proximum* are entirely consumed in a few hours or days (Buzatto et al. 2007).

A comparative study between *S. proximum* and *I. pustulosa* showed that predation rates on unattended clutches were significantly lower in the latter, probably because of the mucus coat around the eggs (Requena et al. 2009). It seems, therefore, that the mucus coat keeps eggs of both *I. pustulosa* and *N. maximus* relatively protected when parents (either males or females) temporarily abandon their clutches, which happens mostly during the day (Machado et al. 2004; Chelini & Machado 2012).

Our laboratory experiment provided additional support for the role of the mucus coat in minimizing both intra- and interspecific egg predation in *N. maximus* and showed that the protection is more effective against the harvestman *P. ornatus* than against conspecifics (Fig. 2). For many predatory arthropods, the size of the trophic apparatus (mandibles or chelicerae) greatly influences the types of prey that can be successfully captured or consumed (Schaefer & Leschen 1993). Given that individuals of *P. ornatus* are nearly half the size of *N. maximus* (see Material and methods) and have considerably smaller chelicerae and pedipalps (Figs. 1b and 1c), they are probably less efficient at removing eggs from the mucus layer and consuming them. This is also probably true for other small predators that are locally abundant on the vegetation (such as the harvestman *Jussara* sp.) and were never observed consuming eggs. The high efficiency of the mucus coat as a protection against small predators during daylight hours reinforces the notion that the presence of multiple lines of egg defense attenuates the costs related to egg loss during periods of female absence. Moreover, by allowing females to temporarily abandon the clutch to forage or shelter, multiple lines of egg defense may also reduce the physiological costs of egg attendance and minimize the trade-off between current and future reproduction.

Fig. 2: Median, first, and third quartiles (box) and range (whiskers) of the number of *Neosadocus maximus* eggs consumed by conspecifics and the harvestman *Promitobates ornatus* in a 5-d long experiment in which the mucus coat that cover the eggs was either removed or left intact. Outliers are plotted separately.



Although multiple lines of egg defense are relatively common among terrestrial arthropods exhibiting maternal care (review in Costa 2006), there are few experimental demonstrations of their protective role. In the case of *N. maximus*, all predators that consumed eggs also consumed mucus, and many individuals consumed only mucus from the clutches (both in the field and in the laboratory). Therefore, it is unlikely that the mucus contains chemical defenses, like those reported for several marine invertebrates (Benkendorff et al. 2001). Although the exact mechanism through which the mucus coat protects the eggs against predators remains unclear, we hypothesize that this coat acts as a physical barrier, hampering the access of potential predators to the eggs or increasing the manipulation time of the eggs, which ultimately decreases consumption rate (see also Requena et al. 2009). One

could also suggest that egg coating camouflages the eggs, as described for other harvestman species (e.g., Willemart 2001), but it seems unlikely for both *N. maximus* and *I. pustulosa* given that the mucus coat is completely transparent (Fig. 1), and harvestmen do not rely on vision to find food (Acosta & Machado 2007). Considering that harvestman eggs are highly sensitive to dehydration and fungi attack (e.g., Mora 1990; Machado & Oliveira 1998), the mucus coat could also have other protective functions, reducing egg dehydration and preventing egg infection, but to test these possibilities, further experiments are necessary.

In addition of being present in many species of the subfamily Progonyleptoidellinae (Gonyleptidae), including *N. maximus*, *I. pustulosa*, *Iguapeia melanocephala*, and *Progonyleptoidellus striatus* (Machado et al. 2004; Fig. 3), a mucus coat covering the eggs has

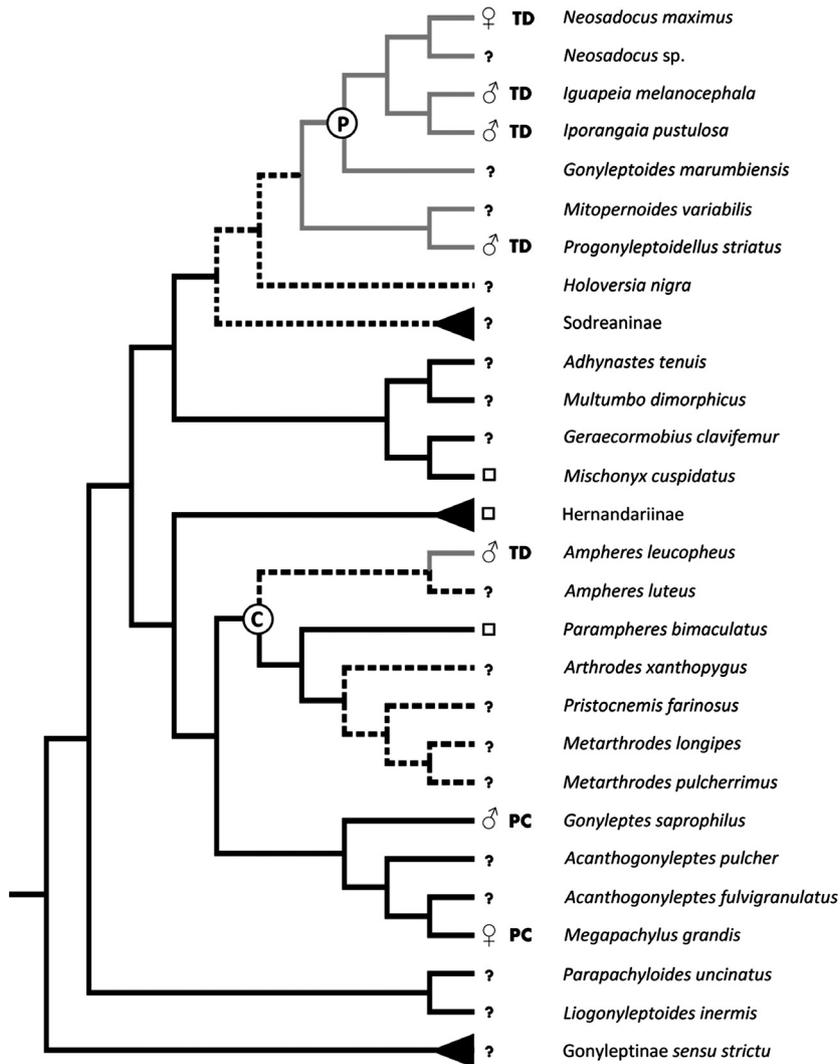


Fig. 3: Phylogeny of a clade (known as K92) within the family Gonyleptidae that includes the subfamilies Caelopyginae (C), Gonyleptinae *sensu strictu*, Hernandariinae, Progonyleptoidellinae (P), and Sodreaninae, as well as scattered species without subfamilial assignment (after Pinto-da-Rocha et al. 2014). The character 'mucus coat around the eggs' was optimized on the tree using parsimony in the program Mesquite (Maddison & Maddison 2011): gray = present, solid black = absent, dashed black = ambiguous due to lack of information. At the tip of the branches, two behavioral traits were mapped: (1) parental care: ♀ = maternal, ♂ = paternal, □ = no care; (2) parent-clutch association: TD = parents (either males or females) temporarily desert the clutch, PC = parents (either males or females) rarely abandon the clutch. Although it is still not possible to infer whether the mucus coat follows or precedes temporary brood desertion, there seems to be a correlation between these two traits (regardless of the sex of the parent).

independently evolved in species belonging to two clades of Neotropical harvestmen in which males care for the eggs: the gonyleptid *Ampheres leucopheus* (Caelopyginae) (Hara et al. 2003; Fig. 3) and the cosmetid *Cryptopocilaema almipater* (Proud et al. 2011). Individuals of all species mentioned above lay eggs on the vegetation and parents (either males or females) periodically abandon their clutches to shelter or to forage (reviewed in Requena et al. 2013; Fig. 3). On the other hand, there is no report of mucus coat among harvestman species in which parents rarely abandon their clutches during the caring period (Machado & Macías-Ordóñez 2007; Caetano & Machado 2013; see also Fig. 3). Thus, the correlation between presence of mucus coat and temporary brood desertion (regardless the sex of the parent) strongly suggests that these two traits coevolved. However, based on the most recent and comprehensive phylogenies, as well as on the available data on the forms of parental care in harvestmen, it is still not possible to infer whether the mucus coat follows or precedes temporary brood desertion.

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