

# The ecological tale of Gonyleptidae (Arachnida, Opiliones) evolution: phylogeny of a Neotropical lineage of armoured harvestmen using ecological, behavioural and chemical characters

Daniel S. Caetano<sup>a,b</sup> and Glauco Machado<sup>a,\*</sup>

<sup>a</sup>Programa de Pós-graduação em Ecologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, 05508-900, Brazil; <sup>b</sup>Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, 05508-900, Brazil

Accepted 30 September 2012

---

## Abstract

The large Neotropical family Gonyleptidae comprises nearly 820 species divided into 16 subfamilies. The majority of publications on harvestman ecology, behaviour and scent gland secretion chemistry have focused on this family. We used the information available in the literature and combined it with an intensive search for ecological, behavioural and chemical data to infer the phylogeny of the Gonyleptidae. We included 28 species belonging to 14 of the 16 gonyleptid subfamilies in the ingroup and four species belonging to the families Cosmetidae, Stygnidae and Manaosbiidae in the outgroup. We performed the analyses using equally weighted characters and coded 63 characters comprising 153 states, which makes this the largest non-morphological, non-molecular phylogenetic data matrix published to date. We obtained five most parsimonious trees, and the strict consensus resulted in six collapsed nodes. The results show that the monophyly of Gonyleptidae is equivocal because Metasarcinae is placed at a basal polytomy with the outgroups Cosmetidae and Stygnidae. Gonyleptinae, Pachylinae and Progonyleptoidellinae are polyphyletic groups, but the remaining subfamilies are monophyletic and have several synapomorphies. Based on the resulting topology, we discuss the performance of ecological, behavioural and chemical characters, and map a selected set of characters to discuss their evolutionary patterns in the family.

© The Willi Hennig Society 2013.

---

The order Opiliones is one of the oldest arachnid groups and includes almost 6500 species, divided into four suborders and 47 families (Giribet and Kury, 2007; Giribet et al., 2010; Kury, 2011). Among these families, Gonyleptidae is the second most diverse, representing nearly 13% of all harvestman species (Kury, 2003) and exhibiting a wide geographical distribution, from southern South America to northern Central America (Giribet and Kury, 2007). Like almost all other harvestman families, the systematics of Gonyleptidae was strongly influenced by the taxonomists Carl Friedrich Roewer and Cândido Firmino de Mello-Leitão (Machado et al., 2007). Both authors had a strong typological background, which was responsible

for the definition of a series of artificial groups within Gonyleptidae (see discussion in Giribet and Kury, 2007). This typological approach, which was followed by many other authors during the 20th century, ended at the beginning of the 1990s when the first works using the cladistic method were published.

The first cladistic work addressing gonyleptids was conducted by Kury (1991), who revised the subfamily Mitobatinae. Kury (1994a) also studied the basal gonyleptid lineages and described Cobaniinae, Metasarcinae and Heteropachylinae. In the same year, he transferred Cranainae, Heterocranainae, Prostygminae and Stygnicranainae from Gonyleptidae to Cranaidae (Kury, 1994b). Later, Pinto-da-Rocha (2002) revised Caelopyginae and proposed the only phylogeny of gonyleptid subfamilies published thus far. However, his paper does not include a data matrix or a list of

---

\*Corresponding author:  
E-mail address: glaucom@ib.usp.br

morphological characters. More recently, cladistic analyses and taxonomic reviews were published for Bourguiiinae, Goniosomatinae, Hernandariinae, Heteropachylinae, Mitobatinae and Sodreaninae (Table 1). After this series of studies, Gonyleptidae currently includes 16 subfamilies, approximately 264 genera and 820 species (Table 1). Although the monophyly of several subfamilies has already been tested in cladistic analyses using morphological characters (Table 1), the relationships among these subfamilies remain largely unknown.

Similarly to the majority of animal groups, the potential data sources for constructing phylogenies have not been equally explored in harvestmen (see discussion in Proctor, 1996). Morphological characters were used most intensively in producing phylogenies of the suborder Laniatores (e.g. Kury, 1992, 1993, 1994a, b, 1997; Pinto-da-Rocha and Kury, 2003; see also references in Table 1), whereas molecular characters were employed mainly in phylogenies of the suborder Cypophthalmi (e.g. Boyer et al., 2005; Boyer and Giribet, 2007; Muriene et al., 2010). Although some previous studies mapped reproductive (Machado and Raimundo, 2001; Machado et al., 2004; Nazareth and Machado, 2009) and defensive behaviours (Hara and Gnaspini, 2003; Hara et al., 2005) on phylogenies, behavioural and ecological characters are virtually absent from the data matrices of all harvestman groups. Two exceptions are provided by Kury (1991), who defined one ecological character related to habitat use in the phylogeny of Mitobatinae; and by DaSilva and Pinto-da-Rocha (2010), who included the character “camouflage with debris” in the phylogeny of

Hernandariinae. In addition to the difficulty of acquiring behavioural data for a large number of species, other issues, such as the difficulty of defining behavioural homologies and the supposed plasticity of behavioural characters, are frequently used as reasons to not employ behavioural and ecological data in phylogenetic analyses (see discussion in Proctor, 1996). Wenzel (1992), however, provides a detailed discussion addressing the definition of homologies for ecological and behavioural characters, demonstrating that the same criteria proposed by Remane (1952) to define homologies for morphological characters can also be used to define behavioural and ecological characters.

To formally test the argument that behavioural characters are more homoplastic than morphological characters, De Queiroz and Wimberger (1993) compared the consistency index (CI) of trees based on morphological and behavioural characters. They compared subsets of behavioural and morphological characters from the same data matrices and from data matrices composed only of morphological or behavioural characters. These authors demonstrated that the mean CI values of behavioural and morphological characters within the same data matrix, and the mean CI values of trees constructed using either behavioural or morphological data, do not differ. More recently, Puniamoorthy et al. (2009) compared the CI of one phylogeny based on molecular data with the CI of another phylogeny based exclusively on behavioural characters of the same species of sepsid flies (Diptera). Although these authors used only characters related to courtship and copulation behaviours, which are traditionally considered to be rapidly evolving phenotypes

Table 1

Diversity of subfamilies of Gonyleptidae and list of reviewed subfamilies. The column “Subfamily” includes a list of all subfamilies currently recognized within Gonyleptidae; “Cladistic review” provides the references for taxonomic reviews of these subfamilies (when available). “Described species” and “Sampled species” present the total number of described genera (gen.) and species (spp.) and the number of species sampled for this work, respectively. The numbers of genera and species were obtained from Kury (2003) or from the most recent systematic review for each subfamily

Subfamily	Cladistic review	Described species	Sampled species
Ampycinae	Not available	2 gen.; 3 spp.	0
Bourguyiinae	Yamaguti and Pinto-da-Rocha (2009)	2 gen.; 10 spp.	1 gen.; 1 spp.
Caelopyginae	Pinto-da-Rocha (2002)	9 gen.; 29 spp.	1 gen.; 1 spp.
Cobaniinae	Not available	1 gen.; 2 spp.	1 gen.; 1 spp.
Goniosomatinae	DaSilva and Gnaspini (2009)	6 gen.; 35 spp.	2 gen.; 2 spp.
Gonyassamiinae	Not available	2 gen.; 3 spp.	0
Gonyleptinae	Not available	38 gen.; 142 spp.	4 gen.; 4 spp.
Hernandariinae	DaSilva and Pinto-da-Rocha (2010)	5 gen.; 23 spp.	2 gen.; 2 spp.
Heteropachylinae	Mendes (2011)	4 gen.; 22 spp.	2 gen.; 2 spp.
Metasarcinae	Not available	13 gen.; 25 spp.	1 gen.; 1 spp.
Mitobatinae	Kury (1991), Bragagnolo (2009)	11 gen.; 47 spp.	2 gen.; 2 spp.
Pachylinae	Not available	129 gen.; 400 spp.	4 gen.; 5 spp.
Pachylospeleinae	Not available	1 gen.; 1 spp.	1 gen.; 1 spp.
Progonyleptoidellinae	Not available	10 gen.; 17 spp.	2 gen.; 2 spp.
Sodreaninae	Pinto-da-Rocha and Bragagnolo (2010)	1 gen.; 10 spp.	1 gen.; 2 spp.
Tricommatinae	Kury (1992a)	29 gen.; 51 spp.	2 gen.; 2 spp.

(Eberhard, 2004), the phylogenetic analysis resulted in a tree showing a CI similar to that of the molecular tree. Therefore the argument that behavioural characters are more prone to homoplasy than other types of character is not sustained by empirical evidence. The only intrinsic drawbacks of using behavioural and ecological data in phylogenies are the difficulty of obtaining these data and the fact that, for most described animal species, especially invertebrates, the only information available is their taxonomic description, in which no biological data are generally provided (Kim and Byrne, 2006). Harvestmen are not an exception to this pattern, and the majority of species described thus far are known only from their type specimens. Simple ecological information, such as the habitats and activity periods of these species, is not included in their museum labels or species descriptions.

In the past 15 years, knowledge about the ecology, behaviour and chemical composition of the scent gland secretions of gonyleptid harvestmen has increased tremendously, such that biological information is now available for a large number of taxa (Table 2). Currently, Gonyleptidae is the harvestman family with the largest number of species studied from the most diverse biological aspects (Kury and Pinto-da-Rocha, 2007). Here, we used a subset of the information available in the literature and combined it with an intensive search for ecological, behavioural and chemical data to infer the phylogeny of Gonyleptidae. Based on the resulting topology, we discuss the performance of ecological, behavioural and chemical characters, and map a selected set of characters to discuss their evolutionary patterns in the family.

## Materials and methods

### *Sampled species*

We selected the sampled species of each family or subfamily according to the following criteria: (i) abundance: we preferred species with large populations, such that individuals are easily found in the field; (ii) the facility of maintaining individuals in captivity: we preferred species that have already been successfully maintained in captivity; (iii) the availability of information in the literature: we preferred species for which ecological, behavioural and chemical data have already been published; and (iv) morphological diversity: we selected species representing a wide diversity of body shapes found in each group (Table 2). Our final set of species consisted only of the taxa for which we obtained the most complete dataset, to avoid taxa in which missing entries are concentrated, which could produce poorly resolved consensus topologies by

increasing the number of most parsimonious trees (Prevosti and Chemisquy, 2010).

Table 2 presents a list of 32 species sampled to perform a phylogenetic analysis of gonyleptid subfamilies (see photos of each taxon in Figs 7 and 8). The ingroup comprises 28 species; the remaining four species form the outgroup. We included representatives of all gonyleptid subfamilies, with the exception of Ampycinae, whose member species are rare and are found only in isolated areas of Amazon forest and Gonyassamiinae, which is composed of three species that occur mainly in the mountain region of the state of Rio de Janeiro, Brazil (Kury, 2003). Subfamilies that have already been revised and are composed of relatively few species (30 or fewer) are represented by only one or two species (Table 2). On the other hand, subfamilies that have not been revised and are composed of many species (from 31 to more than 100) are represented by three or four species (Table 2). The outgroup includes two representatives from Cosmetidae, which is the sister group to Gonyleptidae (Kury, 1994a,b; Pinto-da-Rocha, 2002; Yamaguti and Pinto-da-Rocha, 2009; but see Giribet et al., 2010), one representative from Stygnidae and one from Manaosbiidae. Both Stygnidae and Manaosbiidae belong to the superfamily Gonyleptoidea, but are not closely related to Gonyleptidae (Giribet et al., 2010).

We maintained individuals of each species in different terraria, which had a base of 40 × 25 cm and a height of 20 cm. Each terrarium simulated as closely as possible the habitat where the individuals of each species are often found in the field. The conditions in the laboratory were as follows: temperature of 20–25°C, humidity of 80–90%, and a photoperiod of 13L : 11D from April to September and 11L : 13D from October to March. The specimens were fed pieces of dead cockroaches and industrialized cat food three times per week.

### *Character sampling and coding*

We defined character states using behavioural categories following Wenzel (1992), consisting of discrete and easily recognizable acts that could be assigned as present or absent for each terminal. For most characters related to defensive behaviour, we applied a protocol of stimulation of individuals with the objective of standardizing the data recorded for all terminal species (see Appendix 1). As intraspecific variation could occur in the observed behaviours, we performed each behavioural experiment with at least 20 individuals (10 males and 10 females) for each species, unless fewer individuals were available (see sample sizes in Table 2). Each individual was stimulated only once to define a given character state, and the recovery time between

Table 2

Species used as terminals in the phylogenetic analysis of the family Gonyleptidae (number of individuals used in the experiments given in parentheses). The column “Locality” gives the sites where the individuals of each species were collected; “Available information” shows papers that were used as source for behavioural, ecological and chemical data.

Taxa	Locality	Available information
<b>MANAOSBIIDAE</b>		
<i>Saramacia lucasae</i> * (7♂, 6♀)	Manaus, Amazonas, Brazil	Gnaspini and Hara (2007)
<b>STYGNIDAE</b>		
<i>Protimesius longipalpis</i> * (10♂, 10♀)	Manaus, Amazonas, Brazil	Machado and Macías-Ordóñez (2007a,b), Villareal and Machado (2011)
<b>COSMETIDAE</b>		
Cosmetinae		
<i>Vonones sayi</i>	Not sampled	Eisner et al. (1971), Jones and Cokendolpher (1985), Cokendolpher and Jones (1991) Eisner et al. (2005)
Discosomaticinae		
<i>Gryne coccineoides</i> * (10♂, 10♀)	Campinas, São Paulo, Brazil	Machado and Macías-Ordóñez (2007b)
<b>GONYLEPTIDAE</b>		
Bourguyiinae		
<i>Bourguyia trochanteralis</i> * (10♂, 10♀)	Cananéia, São Paulo, Brazil	Machado and Oliveira (2002), Hara and Gnaspini (2003), Osses et al. (2007), Wouters (2011)
Caelopyginae		
<i>Ampheres leucopheus</i> * (10♂, 10♀)	Ribeirão Grande, São Paulo, Brazil	Hara and Gnaspini (2003), Hara et al. (2003, 2005), Lerche (2009), Gnaspini and Lerche (2010)
Cobaniinae		
<i>Cobania picea</i> * (10♂, 10♀)	Itamonte, Minas Gerais, Brazil	Rocha et al. (2011)
Goniosomatinae		
<i>Acutisoma longipes</i> * (10♂, 10♀)	Atibaia, São Paulo, Brazil	Machado and Oliveira (1998), Machado (2002), Machado et al. (2000, 2005), Hara and Gnaspini (2003)
<i>Serracutisoma proximum</i> * (10♂, 10♀)	Ribeirão Grande, São Paulo, Brazil	Ramires and Giaretta (1994), Machado (2002), Buzatto et al. (2007, 2011), Buzatto and Machado (2008, 2009), Lerche (2009), Chelini et al. (2011), Rocha et al. (2011), Munguía-Steyer et al. (2012)
Gonyleptinae		
<i>Gonyleptes saprophilus</i> * (10♂, 10♀)	Itamonte, Minas Gerais, Brazil	Machado and Raimundo (2001), Machado et al. (2004), Wouters (2011)
<i>Mischonyx cuspidatus</i> * (10♂, 10♀)	Campinas, São Paulo, Brazil	Mestre and Pinto-da-Rocha (2004), Pereira et al. (2004), Willemart and Pellegatti-Franco (2007), Hara et al. (2005), Hara and Gnaspini (2003), Wouters (2011)
<i>Neosadocus maximus</i> * (10♂, 10♀)	Ribeirão Grande, São Paulo, Brazil	Machado and Pizo (2000), Willemart et al. (2007, 2009), Hara et al. (2005), Hara and Gnaspini (2003), Osses et al. (2008), Chelini and Machado (2012), Rocha et al. (2011)
<i>Acanthogonyleptes pulcher</i> * (7♂, 10♀)	Santo André, São Paulo, Brazil	Hara et al. (2005), Hara and Gnaspini (2003), Wouters (2011)
Hernandariinae		
<i>Pseudotrogulus funebris</i> * (10♂, 10♀)	Santo André, São Paulo, Brazil	Firmo and Pinto-da-Rocha (2002), Wouters (2011)
<i>Multumbo terrenus</i> † (10♂, 10♀)	Teresópolis, Rio de Janeiro, Brazil	DaSilva and Pinto-da-Rocha (2010), Wouters (2011)
Heteropachylinae		
<i>Chavesincola inexpectabilis</i> * (10♂, 10♀)	Santa Teresa, Espírito Santo, Brazil	Nazareth and Machado (2009), Wouters (2011)
<i>Magnispina neptumus</i> * (10♂, 10♀)	Linhares, Espírito Santo, Brazil	Nazareth and Machado (2010), Wouters (2011)
Metasarcinae		
<i>Tapacochana insignita</i> * (10♂, 10♀)	Huáscaran, Llaco, Peru	Not available
Mitobatinae		
<i>Longiperna concolor</i> * (10♂, 10♀)	Ribeirão Grande, São Paulo, Brazil	Lerche (2009), Zatz (2010), Zatz et al. (2010)
<i>Promitobates ornatus</i> * (10♂, 10♀)	Ribeirão Grande, São Paulo, Brazil	Willemart (2001), Hara et al. (2005), Hara and Gnaspini (2003), Machado and Macías-Ordóñez (2007a), Lerche (2009), Willemart et al. (2009), Zatz (2010)
Pachylinae		
<i>Acanthopachylus aculeatus</i> * (6♂, 10♀)	Montevideo, Uruguay	Canals (1936), Estable et al. (1955), Fieser and Ardao (1956), Capocasale and Bruno-Trezza (1964), Eisner et al. (2004), Toscano-Gadea (2011)
<i>Discocyrtus oliverioi</i> * (10♂, 10♀)	Campinas, São Paulo, Brazil	Elpino-Campos et al. (2001), Pereira et al. (2004), Wouters (2011)
<i>Discocyrtus prospicuous</i> * (10♂, 10♀)	Montevideo, Uruguay	Canals (1936), Capocasale (1966), Toscano-Gadea (2011)
<i>Pachyloidellus goliath</i> * (10♂, 10♀)	Córboba, Argentina	Acosta et al. (1993, 1995), Acosta and Machado (2007), Machado and Macías-Ordóñez (2007a)

Table 2  
(Continued)

Taxa	Locality	Available information
<i>Pachyloides thorellii</i> * (6♂, 10♀)	Montevideo, Uruguay	Canals (1936), Capocasale and Bruno-Trezza (1964), González et al. (2004), Toscano-Gadea (2011)
Pachylospeleinae		
<i>Pachylospeleus strinati</i> * (10♂, 10♀)	Iporanga, São Paulo, Brazil	Pinto-da-Rocha (1996), Hoenen and Gnaspini (1999), Wouters (2011)
Progonyleptoidellinae		
<i>Iporangaia pustulosa</i> * (10♂, 10♀)	Ribeirão Grande, São Paulo, Brazil	Hoenen and Gnaspini (1999), Hara and Gnaspini (2003), Machado et al. (2004), Hara et al. (2005), Willemart and Chelini (2007), Willemart et al. (2007), Lerche (2009), Requena et al. (2009), Gnaspini and Lerche (2010), Rocha et al. (2011)
<i>Progonyleptoidellus striatus</i> * (10♂, 10♀)	Santo André, São Paulo, Brazil	Hara and Gnaspini (2003), Machado et al. (2004), Hara et al. (2005), Wouters (2011)
Sodreaninae		
<i>Sodreana leprevosti</i> * (10♂, 10♀)	Ribeirão Grande, São Paulo, Brazil	Hara and Gnaspini (2003), Hara et al. (2005), Wouters (2011)
<i>Sodreana barbiellini</i> * (2♂, 8♀)	Ubatuba, São Paulo, Brazil	Wouters (2011)
Tricommatinae		
<i>Camarana flavipalpi</i> * (10♂, 10♀)	Ubatuba, São Paulo, Brazil	Machado and Pomini (2008)
<i>Cryptogeobius crassipes</i> † (10♂, 10♀)	Rio de Janeiro, Rio de Janeiro, Brazil	Not available

\*Vouchers deposited in the Museu de Zoologia da Universidade de São Paulo (MZSP, Brazil).

†Vouchers deposited in the Museu Nacional do Rio de Janeiro (MNRJ, Brazil).

two different experiments for each individual was always longer than 1 day.

There are several techniques for addressing intraspecific variation in cladistic analyses (review in Wiens, 2000), and we used two different approaches, depending on the character. The first approach was the majority method, also known as modal coding, which codes a polymorphic species as exhibiting the state that is most common among the sampled individuals (Wiens, 2000). This method ignores rare variants within species and is commonly used in morphological studies (even if it is not stated explicitly). An emblematic example of this approach in harvestmen is provided by certain sexually dimorphic morphological traits that are defined by their presence in males of the alpha morph, ignoring the existence of males of the beta morph (female-like) in which the trait is absent (e.g. DaSilva and Gnaspini, 2009). In our case, we used modal coding whenever no detailed information on the frequency of each state was available in the literature, or when character coding was based on an unknown sample of individuals observed in the field. The second approach applied in the present study was based on the percentage of individuals in a sample that exhibited a behavioural act upon a standardized stimulation. This percentage may be interpreted as a species-level propensity to express such an act. Similarly to stereotyped monomorphic behaviours, this propensity is most likely the result of a hereditary internal organization (Japyassú and Machado, 2010). This rationale has been employed in other phylogenetic

studies involving behavioural characters, such as character 7 in Stuart and Currie (2001). These authors codified the propensity of caddisfly larvae to reject uncut pieces when building their cases into three states: “never”, “occasionally” and “often”.

To define the chemical composition of the scent gland secretions released by different species, we collected samples that were then analysed in the Laboratory of Natural Product Chemistry, Chemistry Institute, Campinas State University (Unicamp), Brazil. The protocol for storage, identification of chemical compounds, and quantification of their relative frequencies in the scent gland secretions is described by Machado and Pomini (2008) and Rocha et al. (2011). Given that we had information on the relative frequency of each chemical compound, we used a coding procedure similar to the second approach described above.

We assigned equal weights for all characters, and we coded 10 of them as ordered (characters 4, 16, 18, 40, 47, 50, 52, 56, 57 and 63 in Appendix 1). All these ordered characters are the result of the categorization of continuous variables and therefore are clearly connected by intermediate states, allowing the construction of plausible transformation series (Wenzel, 1992).

#### Phylogenetic analyses

We searched for the most parsimonious trees (MPT) using 200 random addition sequences, followed by tree bisection–reconnection and branch-swapping, employ-

ing pre-existing trees as starting points and retaining up to 500 trees at each replication. We performed these analyses with TNT software (Goloboff et al., 2008). Then we computed Bremer support values (Bremer, 1994) for each node of the resulting trees. Although previous studies have measured the CI to compare the degree of homoplasy between distinct character classes (e.g. De Queiroz and Wimberger, 1993; Puniamoorthy et al., 2009), we used the retention index (RI) to infer the relative performance of the characters. Given that the RI is proportional to the evidence of grouping for a character (Kitching et al., 1998), we contrasted the relative contribution of each character class (see Results) to the topologies, rather than their degree of homoplasy. To understand the evolutionary patterns of the ecological, behavioural and chemical characters, we mapped them on the strict consensus tree under equal weights using the parsimony ancestral states reconstruction method available in the TNT software. Finally, we used the Mesquite software (Maddison and Maddison, 2011) to make the illustrations.

## Results

### *Characters*

We coded 63 characters based on various aspects of ecology, behaviour and the chemical composition of the scent gland secretions of harvestman species (Appendices 1 and 2). A detailed description of the characters and their states, as well as the standardized protocols used in the behavioural experiments, is presented in Appendix 1. We divided these characters into five main classes: (i) activity pattern and habitat use, associated with eight characters (1–8) and 23 states (median RI = 0.82); (ii) gregariousness, with four characters (9–12) and 11 states (median RI = 1.0); (iii) reproductive behaviour, with 16 characters (13–28) and 40 states (median RI = 1.0); (iv) defensive behaviour, with 18 characters (29–46) and 39 states (median RI = 0.88); and (v) chemical composition of the scent gland secretions, with 17 characters (47–63) and 40 states (median RI = 0.73).

### *Topology*

Analysis of the equally weighted characters resulted in five MPTs being obtained. Each of these trees has 171 steps, a CI of 0.52 and an RI of 0.76. The strict consensus resulted in only six collapsed nodes and a tree with 173 steps, a CI of 0.52 and an RI of 0.75. Figure 1 shows the strict consensus tree with the characters mapped and the Bremer support values for each node.

## Discussion

### *Use of behavioural characters*

Most studies published to date that have employed behavioural characters in phylogenetic analyses are based mainly on stereotyped behavioural sequences, such as courtship (Scholes, 2008; Puniamoorthy et al., 2009), prey capture (Japyassú and Machado, 2010), calling songs (Robillard et al., 2006; Cap et al., 2008) and case building (Stuart and Currie, 2001); or structures resulting from a behaviour, such as nests and spider webs (Zyskowski and Prum, 1999; Noll, 2002; Kuntner et al., 2008). In the present study, we used a wide range of behavioural, ecological and chemical data related to defensive behaviours, the chemical composition of scent gland secretions, reproduction, activity patterns and habitat use to infer the phylogeny of gonyleptid harvestmen. This report is the largest non-morphological and non-molecular data matrix published to our knowledge, comprising 63 characters and 153 states, among which we defined 17 characters using standardized experiments (characters 29–35 and 37–46). These experiments decreased the time needed to record behavioural data for each terminal species, which is regarded as the main limiting factor in phylogenetic studies using behavioural or ecological characters for a large number of terminals (Proctor, 1996). The use of simple experiments also allowed us to control the context in which the behavioural acts were expressed, thus minimizing possible misinterpretations of homologies.

The performance of each of the five character classes, measured in terms of the RI, was slightly different: gregariousness and reproduction showed the highest medians, followed by defensive behaviour, activity pattern and habitat use, and finally by the chemical composition of scent gland secretions. The number of missing entries also differed between these classes. The activity pattern and habitat use class does not have any missing entries, most likely because this information is easily obtained in the field and in the literature. The defensive behaviour, gregariousness and scent gland secretion chemical composition classes have only 2.3, 3.1 and 7.6% missing entries, respectively. Finally, the class reproduction has 29% missing entries, and there is no information for almost all of the characters in this class for seven species included in our matrix (Appendix 2). Reproductive characters are certainly the most difficult to obtain, as several species did not breed in captivity or were not observed performing reproductive behaviours, such as courtship, copulation, oviposition, or parental care in the field. Although reproduction is one of the most intensively studied subjects among gonyleptid harvestmen, basic information for species of the subfamilies Metasarcinae,

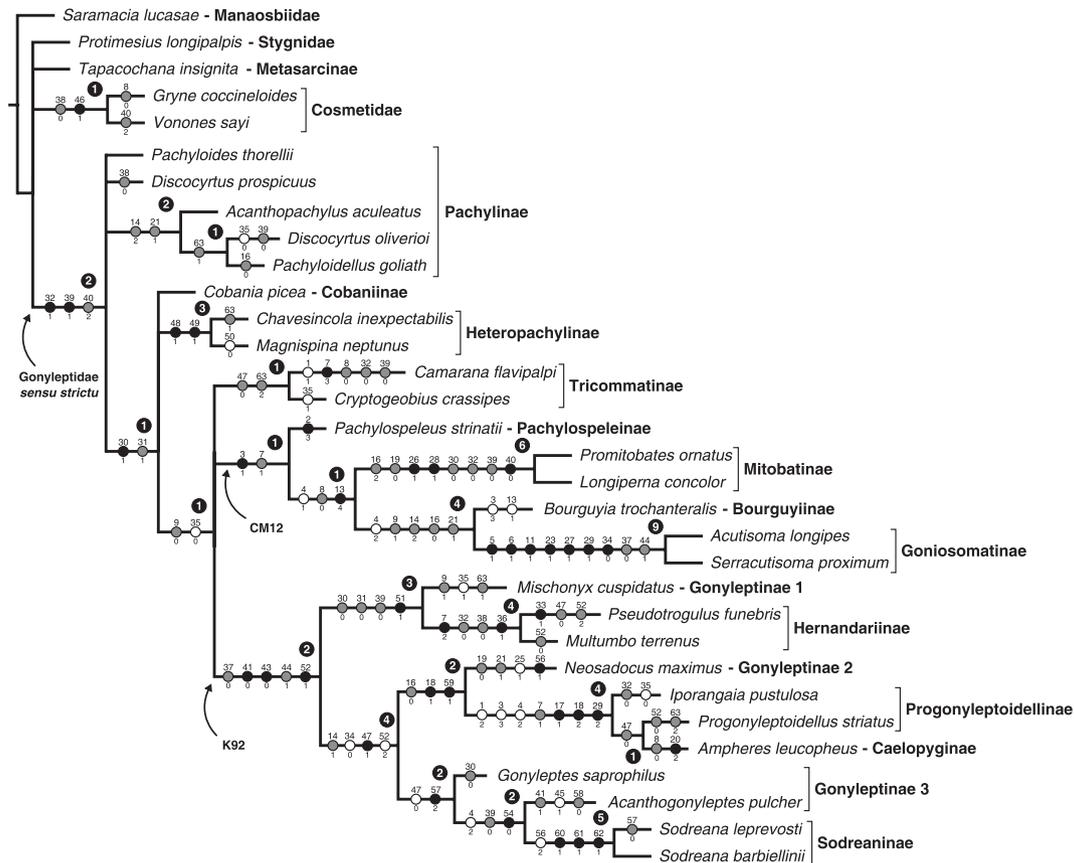


Fig. 1. Phylogeny of the subfamilies of Gonyleptidae based on ecological, behavioural and chemical characters using equal weights. Strict consensus of the five most parsimonious trees (steps = 173, CI = 0.52 and RI = 0.75). Numbers inside black circles are Bremer support values for the clades. Black circles are synapomorphies; grey circles are unambiguous homoplasies; white circles are ambiguous homoplasies. Only the characters of the clades present in the consensus tree were mapped using the “apo” command of TNT software (Goloboff et al., 2008). The list of characters is presented in Appendix 1 and the data matrix is presented in Appendix 2.

Cobaniinae and Sodreaninae is lacking in the literature and could not be obtained in this study. Given the strong phylogenetic signal of reproductive characters, future behavioural investigations should focus on species of these three subfamilies.

### Phylogeny of the Gonyleptidae

Although we used only two species belonging to the large family Cosmetidae, they appeared as a monophyletic group. A behavioural synapomorphy of this family is the unique defensive behaviour known as leg dabbing (character 46), in which droplets of scent gland secretions are gathered by the tarsus of leg I and directly delivered to the aggressor (Eisner et al., 1971). Our results also show that the monophyly of Gonyleptidae is equivocal because *Tapacochana insignita* (Metasarcinae) is placed in a basal polytomy with the outgroups Cosmetidae and Stygnidae (Fig. 1). A phylogenetic hypothesis based on morphological data shows a similar topology, with Metasarcinae included in a basal polytomy with representatives of Cosmeti-

dae and Stygnidae (Pinto-da-Rocha, 2002; Fig. 2). However, both the results obtained here and those obtained with morphological data show a monophyletic group formed by “Gonyleptidae”, with the exception of Metasarcinae, which will hereafter be referred to as *Gonyleptidae sensu strictu* (Fig. 1). This clade has two behavioural synapomorphies (character 32: nipping, and character 39: individuals that emit scent gland secretions when seized by the femur of leg IV), and shares one unambiguous homoplasy (character 40: a high proportion of individuals that emit scent gland secretions when pressed dorso-ventrally).

We recovered the monophyly of Goniosomatinae, Hernandariinae, Heteropachylinae, Mitobatinae and Sodreaninae, all of which are subfamilies that have already been revised using morphological characters (Table 1). Heteropachylinae, *Cobania picea* (Cobaniinae) and the rest of *Gonyleptidae sensu strictu* (except the “Pachylinae”) form a polytomy at the base of *Gonyleptidae sensu strictu* (Fig. 1). A previous phylogeny based only on morphological characters (Fig. 2) shows Heteropachylinae as a monophyletic group sis-

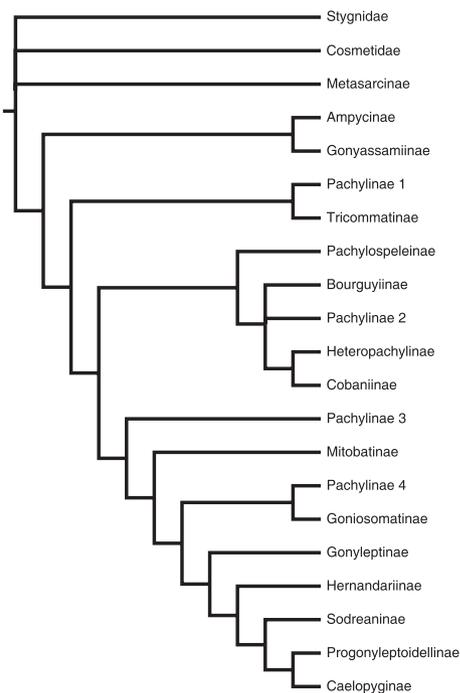


Fig. 2. Phylogeny of the subfamilies of Gonyleptidae using morphological data. Modified from Pinto-da-Rocha (2002).

ter to *C. picea*, but there is no information on which synapomorphies support this clade (Fig. 2). In the case of the Tricommatinae, morphological data suggest that they are related to *Huralvoiooides*, which is formally included in Pachylinae (Kury, 1995). Although Tricommatinae has never been formally revised, it was the focus of a phylogenetic analysis in which this group was removed from the family Phalangodidae and elevated to the rank of family based on morphological characters (Kury, 1992). Only later was the family “Tricommatidae” transferred to Gonyleptidae and given the status of a subfamily (Kury, 2003). However, the monophyly of Tricommatinae, as well as the position of this group within Gonyleptidae, has never been investigated using a phylogenetic approach. Our results show, for the first time, that Tricommatinae is indeed a monophyletic group, but its position within Gonyleptidae *sensu strictu* still deserves further study.

Among the subfamilies that have never been revised (Table 1), our results indicate that Pachylinae, whose taxonomic definition is problematic (e.g. Kury, 1995; Pinto-da-Rocha, 2002; Hara and Pinto-da-Rocha, 2010), is not a monophyletic group (Fig. 1), in agreement with the phylogeny based on morphological data (Fig. 2). In the present study, however, “Pachylinae” appears in a polytomy at the base of Gonyleptidae *sensu strictu*, whereas in the topology proposed by Pinto-da-Rocha (2002), species currently included in this subfamily appear at four positions: as the sister

group to Tricommatinae; in a polytomy with Bourguyiinae and the clade Heteropachylinae + Cobaniinae; as the sister group to the clade composed of Mitobatinae, Goniosomatinae, Gonyleptinae, Hernandariinae, Sodreaninae, Caelopyginae and Progonyleptoidellinae; and as the sister group to Goniosomatinae (Fig. 2). In fact, results of ongoing phylogenetic analyses clearly show that Pachylinae is a polyphyletic group that will be split into several small subfamilies (A.B. Kury and R. Pinto-da-Rocha, pers. commun.).

Gonyleptinae is another subfamily that has never been revised, and it is difficult to define using morphological characters. We sampled four very distinct species from morphological, ecological and behavioural perspectives. Our results corroborate the hypothesis of DaSilva and Pinto-da-Rocha (2010) that shows Gonyleptinae as a polyphyletic group. In a recent paper, Werneck et al. (2012) argued that although the forms of parental care are highly conservative within the subfamilies of Gonyleptidae (see Nazareth and Machado, 2009), “Gonyleptinae” includes species exhibiting egg hiding (Pereira et al., 2004; Stanley, 2011), maternal care (Machado and Vital, 2001; Werneck et al., 2012) and also paternal care (Machado et al., 2004). Assuming that the forms of parental care are indeed conservative within the clades of Gonyleptidae, it is possible that characters 21, 23 and 24 could be used to define monophyletic units among the polyphyletic group currently known as “Gonyleptinae”.

The clade comprised of *Mischonyx cuspidatus*, *Gonyleptes saprophilus*, *Acanthogonyleptes pulcher*, *Neosadocus maximus* (“Gonyleptinae”), *Hernandariinae*, *Sodreaninae*, *Iporangaia pustulosa*, *Progonyleptoidellus striatus* (“Progonyleptoidellinae”) and *Ampheres leucopheus* (Caelopyginae) was designated K92 in the present work (Fig. 1), after the seminal work of Kury (1992a), who first recognized two potential synapomorphies for this group related to penis anatomy: a distal margin of the ventral plate with a deep parabolic cleft, and a piriform ventral plate with the basal lobes directed dorsally. Here, we add three further synapomorphies to K92 (Fig. 1): individuals do not emit scent gland secretions in droplets (character 41), do not accumulate droplets of scent gland secretions between the bases of legs I and II (character 43), and emit scent gland secretions containing more than 10% vinyl-ketones (character 52). The clade K92 also shares two unambiguous homoplasies (Fig. 1): individuals may emit scent gland secretions without previous emission of enteric fluid (character 37), and emit scent gland secretions in a jet (character 44). Therefore the morphological, behavioural and chemical data are highly congruent and show unequivocal evidence for this major gonyleptid clade.

Although our results do not support the monophyly of “Progonyleptoidellinae”, this subfamily forms a

monophyletic group with *Ampheres leucopheus* (Caelopyginae), in agreement with previous studies using morphological characters (Figs 1 and 2). The clade formed by Caelopyginae and “Progonyleptoidellinae” has three synapomorphies (characters 17, 18 and 29) and one shared unambiguous homoplasy (character 7). Furthermore, according to the topology obtained with morphological characters, Sodreaninae, represented here by *Sodreana barbiellinii* and *S. leprevosti*, is the sister group to the clade formed by Caelopyginae and “Progonyleptoidellinae” (Fig. 2; see also Pinto-da-Rocha and Bragagnolo, 2010). However, the topology obtained herein shows Sodreaninae not as a sister group to Caelopyginae and “Progonyleptoidellinae” (Fig. 1), but rather as a representative of a clade that also includes *G. saprophilus*, *A. pulcher* and *N. maximus*, all of which belong to “Gonyleptinae” (Fig. 1). The position of the subfamily Hernandariinae is also markedly different between the morphological phylogeny and the results reported here (compare Figs 1 and 2). It is therefore clear that the relationships between the subfamilies that form clade K92 are far from obvious, and combined analyses in the future may shed some light on this subject.

We also found another major clade within Gonyleptidae *sensu strictu* that is composed of *Pachylospeleus strinatii* (Pachylospeleinae), Mitobatinae, *Bourguyia trochanteralis* (Bourguyiinae) and Goniosomatinae (Fig. 1). This clade, herein designated CM12 after its first appearance in this work, has one synapomorphy (character 3: individuals hide among boulders or on rock walls), and shares one unambiguous homoplasy (character 7: females cross the femora of legs IV at mid-length when prostrated). Apparently, the clade CM12 shares a single conspicuous morphological trait: slight to great elongation of male femur IV (A.B. Kury, pers. commun.). However, cladistic analyses using morphological characters never show these subfamilies as a monophyletic clade (Fig. 2). Bourguyiinae is frequently reported as a basal lineage of Gonyleptidae (Kury, 1994a,b; Yamaguti and Pinto-da-Rocha, 2009), while Goniosomatinae is a sister group to K92 (Pinto-da-Rocha, 2002), and Mitobatinae either is related to lineages of “Pachylinae” (Bragagnolo, 2009) or is the sister group to the clade Goniosomatinae + K92 (Pinto-da-Rocha, 2002). In conclusion, the topology of CM12 based on behavioural, ecological and chemical characters is not congruent with topologies based only on morphological characters, and combined analyses may again help to solve this problem.

#### *Evolutionary patterns*

For the sake of conciseness, we will not discuss all of the 63 characters used in the phylogenetic analysis. Instead, we have chosen certain characters from each

character class to be discussed in greater detail. Regarding habitat use, the basal lineages of Gonyleptidae and all of the species in the outgroup live mainly on the ground and can also be found in lower vegetation or on tree trunks. Among the taxa we sampled, only five subfamilies of Gonyleptidae have representatives that can typically be observed in high vegetation (between 1 and 3 m above ground): Goniosomatinae, Bourguyiinae, “Progonyleptoidellinae”, Caelopyginae and Sodreaninae. Among these subfamilies, three independent events of specialization occurred, allowing them to climb on high vegetation (Fig. 3). In all three cases, the transition to high vegetation was preceded by the use of lower vegetation (up to 1 m) to hide and forage, as is recorded for the sister groups of these clades (Fig. 3). The use of high vegetation by harvestmen is frequently associated with morphological modifications, such as an increase in the number of tarsal segments of legs I–IV and elongation of their femora and tibiae (Guffey et al., 2000; Shultz and Pinto-da-Rocha, 2007). However, these modifications have been recorded only in the two clades belonging to K92 (Fig. 3), in which species exhibit a large number of tarsal segments, suggesting specialization for locomotion on vegetation (Curtis and Machado, 2007).

Gregariousness is the plesiomorphic state of Gonyleptidae *sensu strictu* (Fig. 4). In three species of the outgroup, as well as many lineages of Gonyleptidae *sensu strictu*, aggregations occur mainly under rocks or tree trunks (character 10; Fig. 4), are rarely composed of more than 20 individuals (character 11; Fig. 4), and are generally found throughout the year (character 12). An independent event of evolution of gregariousness supports the clade formed by Goniosomatinae and Bourguyiinae (Fig. 4). In these two subfamilies, the observed aggregations are indeed different from those of the other subfamilies because they occur only during the non-reproductive season (winter for Goniosomatinae and summer for Bourguyiinae). Moreover, among the Goniosomatinae, individuals form large, exposed aggregations on rocky walls (Machado, 2002) comprising up to 200 individuals, whereas in the Bourguyiinae, aggregations occur inside tank bromeliads and rarely exceed 20 individuals (Machado and Oliveira, 2002).

Many gonyleptid lineages exhibit postzygotic parental care; depending on the group, either the male or the female can care for the eggs (Machado and Macías-Ordóñez, 2007a). Nevertheless, the plesiomorphic state of parental care in the family is the deposition of debris on the surface of the eggs by females, which subsequently desert the offspring (character 16), as previously proposed by Machado and Raimundo (2001). Maternal care (characters 21 and 22) has evolved independently three times (Fig. 5), which contrasts with the results reported by Nazareth and Machado (2009), who found four independent origins of

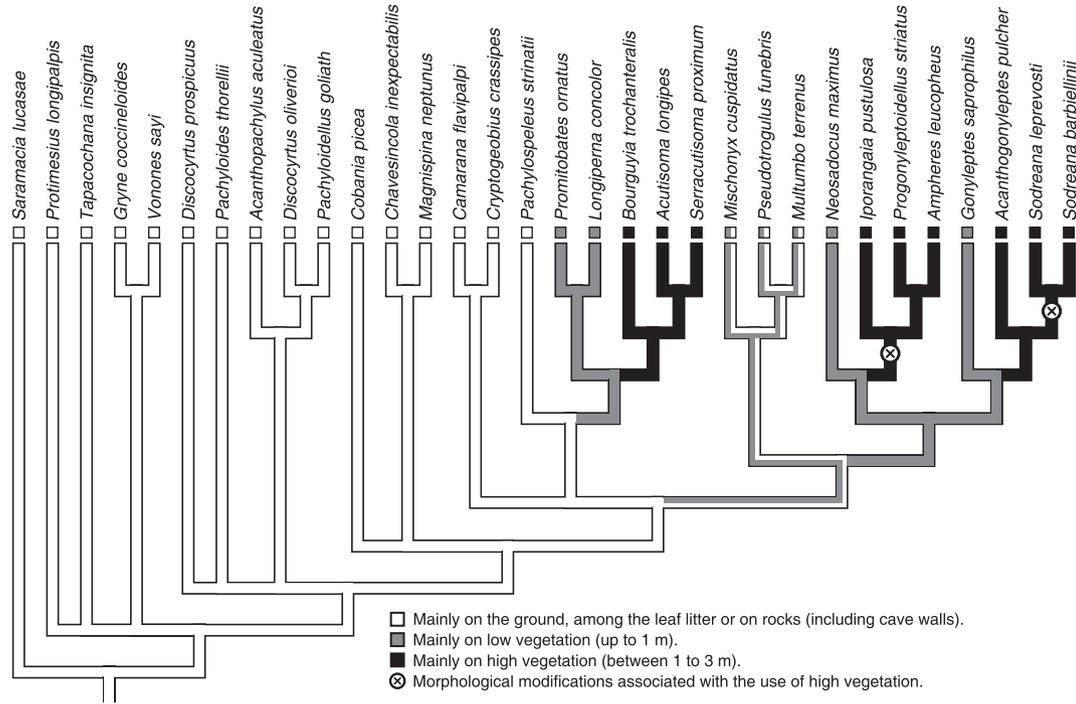


Fig. 3. Phylogeny of the subfamilies of Gonyleptidae showing the evolution of the use of vegetation as a foraging substrate (character 4). The squares on the tips of the phylogeny show the character state of each terminal. Ambiguous optimizations are shown as such. The topology is based on the strict consensus shown in Fig. 1. The morphological modifications associated to the use of high vegetation include both increase in the number of tarsal segments of legs I–IV and elongation of their femora and tibiae.



Fig. 4. Phylogeny of the subfamilies of Gonyleptidae showing the evolution of gregariousness (characters 9–11). The squares on the tips of the phylogeny show the character state of each terminal. Question marks identify species for which we do not have information (missing entries). Ambiguous optimizations are shown as such. The topology is based on the strict consensus shown in Fig. 1.

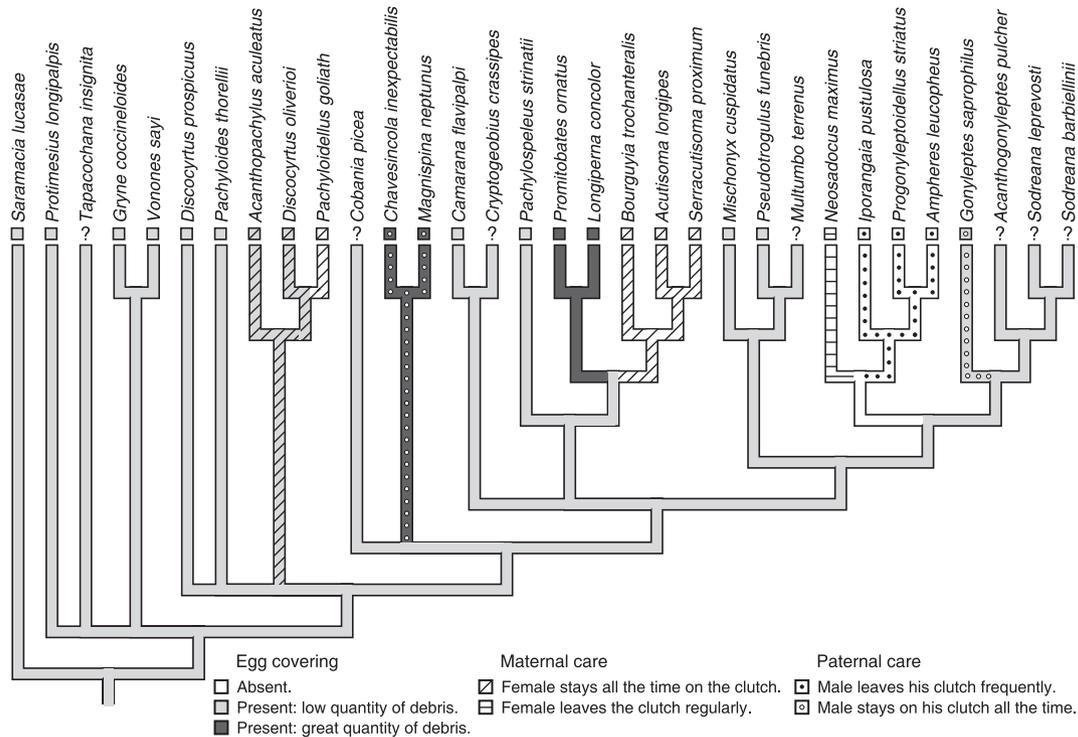


Fig. 5. Phylogeny of the subfamilies of Gonyleptidae showing the evolution of parental care. Three characters are represented in the topology: “deposition of debris on the eggs by the females” (character 16), “time the female stays with the clutch” (character 22) and “time the male stays with the clutch” (character 24). All characters, with the exception of “time the male stays with the clutch” (mapped here with the DELTRAN method), have unambiguous optimizations. Given that the existence of parental care is independent of egg covering, maternal and paternal care were represented by lines and circular patterns, respectively, and the shading of the branches represents the states of the character “deposition of debris on the eggs”. Question marks identify species for which we do not have reproductive information (missing entries). The topology is based on the strict consensus shown in Fig. 1.

this behaviour using the topology proposed by Pinto-da-Rocha (2002). This difference most likely occurs because Bourguiiinae is a sister group to Goniosomatinae in our study, instead of a basal subfamily close to Cobaniinae and Heteropachylinae (compare Figs 1 and 2). With respect to exclusive paternal care (characters 23 and 24), there are two equally parsimonious reconstructions. Under the ACCTRAN method, this behaviour has evolved twice independently: in Heteropachylinae, and in the clade formed by K92 (except *M. cuspidatus* and *Hernandariinae*), where it has been lost once in *N. maximus* (“Gonyleptinae”). On the other hand, under DELTRAN method, exclusive paternal care has evolved three times independently: in Heteropachylinae, in *G. saprophilus* (“Gonyleptinae”) and in the clade formed by *A. leucopheus* (Caelopyginae) and “Progonyleptoidellinae” (Fig. 5). Results from the DELTRAN method are in agreement with the results previously reported by Nazareth and Machado (2009). Only in the Heteropachylinae is it possible to say that male care has evolved from a non-caring condition. In the two other cases, the absence of reproductive information available for the sister clades makes it impossible to produce any inference regarding

the ancestral condition that originated exclusive male care (Fig. 5).

The main defensive behaviours reported for gonyleptids are thanatosis, fleeing, emission of scent gland secretions, and retaliation with chelicerae, pedipalps and spines on the legs (Gnaspini and Hara, 2007). Thanatosis (character 34) is plesiomorphic in the family, and it was lost only once, as a synapomorphy of Goniosomatinae (Fig. 1). Individuals usually exhibit thanatosis immediately after being disturbed, but they can emit scent gland secretions if the attack persists (Machado and Pomini, 2008). The emission of droplets of scent gland secretions that flow to the posterior portion of the body (character 41) is also plesiomorphic in the family (Fig. 1). The loss of this behaviour occurred only once, simultaneously with the evolution of jet secretion in K92 (Fig. 1; see also Obs. of character 44 in Appendix 1). Most gonyleptid species can also use leg IV to pinch their aggressors, a behaviour known as nipping (character 32). Nipping is a synapomorphy of Gonyleptidae *sensu strictu* (Fig. 1) and was not recorded for *T. insignita* (Metasarcinae). This result is curious because *T. insignita* and other species of Metasarcinae have a strong armoured leg IV; thus individuals would be expected to exhibit nipping. If an

absence of nipping is confirmed for the Metasarcinae, it means that the evolution of leg spines preceded the evolution of this defensive behaviour, suggesting a case of exaptation.

One of the most conspicuous characters of species belonging to the order Opiliones is the emission of volatile substances through a pair of gland openings (Shultz and Pinto-da-Rocha, 2007). The main role attributed to these substances is protection against predators (Gnaspini and Hara, 2007), but they can also play an important role in intraspecific communication, working as alarm pheromones (Machado et al., 2002). Among species of the suborder Laniatores, the volatile compounds produced can be categorized into three main chemical groups (Gnaspini and Hara, 2007): benzoquinones (character 47), vinyl-ketones (character 52) and alkyl-phenols (character 63). The production of benzoquinones is plesiomorphic in *Gonyleptidae sensu strictu*, with four independent losses of these compounds being observed (Fig. 6). In contrast, the production of vinyl-ketones is a synapomorphy of K92 (Fig. 6). Finally, alkyl-phenols evolved at least five times, including one evolution event as a synapomorphy of Tricommatinae (Fig. 6). The identity of individual molecules was also highly informative, resulting in synapomorphies to two subfamilies (Heteropachylinae and Sodreaninae), as well as three more inclusive clades (*Mischonyx cuspidatus* + *Hernandariinae*, *Neosadocus maximus* + *Caelopyginae* + *Progonyleptoidellinae* and *Gonyleptes saprophilus* + *Acanthogonyleptes pulcher* + *Sodreaninae*) (Fig. 6).

### Concluding remarks

The large amount of ecological, behavioural and chemical information available for several species of *Gonyleptidae* enabled us to infer a well resolved phylogeny of this family. Moreover, we identified several new synapomorphies for the majority of subfamilies we sampled and for major clades within *Gonyleptidae*, such as K92, which was previously proposed based on only two morphological traits (Kury, 1992). In the future, integration of ecological, behavioural and chemical information with morphological and molecular data may provide better comprehension of the relationships among the subfamilies of *Gonyleptidae*. Given the strong phylogenetic signal of ecological and behavioural data, we suggest that basic information on natural history should be incorporated into specimen labels upon collection. Data such as diel activity patterns, foraging and sheltering microhabitats, the existence and type of post-zygotic parental care, and the emission mode of the scent gland secretions are easily recorded in the field and could reduce the number of missing entries in data matrices, increasing the number of species sampled in future phylogenetic analyses.

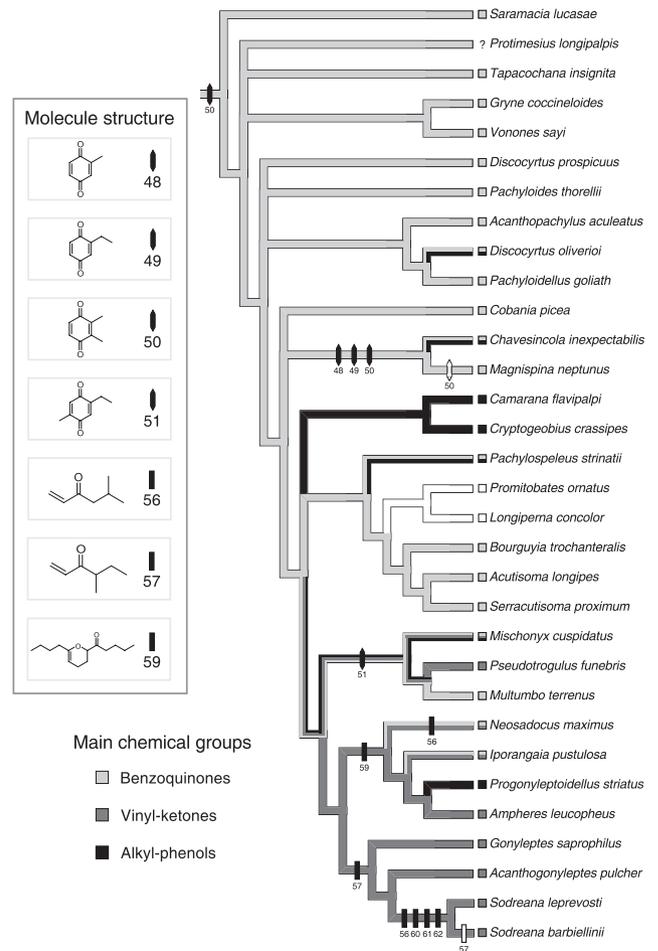


Fig. 6. Phylogeny of the subfamilies of *Gonyleptidae* showing the common synapomorphies of all MPTs related to the chemical composition of scent gland secretions. The shading of the branches represents the three main chemical groups: benzoquinones (character 47), vinyl-ketones (character 52) and alkyl-phenols (character 63). The symbols indicate specific molecules; the number below each symbol identifies the character number. The molecules of benzoquinone from 48 to 51 are represented by diamonds; the molecules of vinyl-ketones from 56 to 59 are represented by rectangles. Black symbols identify gains; white symbols identify losses of character states. The box on the left shows the detailed structure of each molecule, except for the undescribed molecules of the characters 60–62 (adapted from Rocha et al., 2011 and Wouters, 2011). Ambiguous optimizations are shown as such. The topology is based on the strict consensus shown in Fig. 1.

### Acknowledgements

We thank B.A. Buzatto, G.S. Requena, C. Bragagnolo, J. Ochoa, A.L. Guil, R. Munguía-Steyer, D. Funny, P.M. Omena, E. Stanley and C. Toscano-Gadea for valuable assistance in the laboratory or fieldwork; R. Pinto-da-Rocha for constant support in collecting data and helping in the identification of the sampled species; A.J. Marsaioli, D.F. Rocha, F.C. Wouters and C. Rossini for providing the identification of the chemical compounds; B.A. Buzatto for providing many photos used

in Appendix 1; B.S. Medeiros, A.B. Kury, G.S. Requena and D.J. Machado for fruitful discussions on systematics and evolution; and R. Pinto-da-Rocha, A.B. Kury, F.B. Noll, J. Wenzel, T. B. Quental, T. Grant, M.J. Ramírez and an anonymous reviewer for comments on early versions of the manuscript. The study was supported by fellowships from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2008/55871-8, 2008/06604-7 and 2009/50031-4) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

## References

- Acosta, L.E., Machado, G., 2007. Diet and foraging. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 309–338.
- Acosta, L.E., Poretti, T.I., Mascarelli, P.E., 1993. The defensive secretions of *Pachyloidellus goliath* (Opiliones, Laniatores, Gonyleptidae). *Bonn. Zool. Beit.* 44, 19–31.
- Acosta, L.E., Pereyra, F.E., Pizzi, R.A., 1995. Field observation on *Pachyloidellus goliath* (Opiliones, Gonyleptidae) in Pampa de Achala, province of Córdoba. *Bull. Br. Arachnol. Soc.* 10, 23–28.
- Boyer, S.L., Giribet, G., 2007. A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. *Cladistics* 23, 337–361.
- Boyer, S.L., Karaman, I., Giribet, G., 2005. The genus *Cyphophthalmus* (Arachnida, Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Balkan Peninsula biogeography. *Mol. Phylogenet. Evol.* 36, 554–567.
- Bragagnolo, C., 2009. Revisão sistemática de Promitobates Roewer 1913 e análise filogenética de Mitobatinae Simon 1879 (Arachnida, Opiliones, Gonyleptidae). PhD thesis, Instituto de Biociências, Universidade de São Paulo, Brazil.
- Bremer, K., 1994. Branch support and tree stability. *Cladistics* 10, 295–304.
- Buzatto, B.A., Machado, G., 2008. Resource defense polygyny shifts to female defense polygyny over the course of the reproductive season of a Neotropical harvestman. *Behav. Ecol. Sociobiol.* 63, 85–94.
- Buzatto, B.A., Machado, G., 2009. Amphisexual care in *Acutisoma proximum* (Arachnida, Opiliones), a Neotropical harvestman with exclusive maternal care. *Insect. Soc.* 56, 106–108.
- Buzatto, B.A., Requena, G.S., Martins, E.G., Machado, G., 2007. Effects of maternal care on the lifetime reproductive success of females in a Neotropical harvestman. *J. Anim. Ecol.* 76, 937–945.
- Buzatto, B.A., Requena, G.S., Lourenço, R.S., Munguia-Steyer, R., Machado, G., 2011. Conditional male dimorphism and alternative reproductive tactics in a Neotropical arachnid (Opiliones). *Evol. Ecol.* 25, 331–349.
- Canals, J., 1936. Observaciones biológicas en arácnidos del orden Opiliones. *Rev. Chil. Hist. Nat.* 40, 61–63.
- Cap, H., Deleporte, P., Joachim, J., Reby, D., 2008. Male vocal behavior and phylogeny in deer. *Cladistics* 24, 917–931.
- Capocasale, R., 1966. Opiliones del Uruguay. *Discocyrtus prospicuum* Holmberg, el alotipo hembra de *Pygophalangodus gemignanii uruguayensis* Ringuet (Gonyleptidae) y *Metalibitia rosascostai* sp. nov. (Cosmetidae). *Bull. Mus. Nat. D'Hist. Nat.* 37, 631–644.
- Capocasale, R., Bruno-Trezza, L., 1964. Biología de *Acanthopachylus aculeatus* (Kirby, 1819) (Opiliones: Pachylinae). *Rev. Soc. Uruguaya Entom.* 6, 19–32.
- Chelini, M.C., Machado, G., 2012. Costs and benefits of temporary broad desertion in a Neotropical harvestman (Arachnida: Opiliones). *Behav. Ecol. Sociobiol.* 66, 1619–1627.
- Chelini, M.C., Willemart, R.H., Gnaspini, P., 2011. Caves as a winter refuge by a Neotropical harvestman (Arachnida, Opiliones). *J. Insect Behav.* 24, 393–398.
- Cokendolpher, J.C., Jones, S.R., 1991. Karyotype and notes on the male reproductive system and natural history of the harvestman *Vonones sayi* (Simon) (Opiliones, Cosmetidae). *P. Entomol. Soc. Wash.* 93, 86–91.
- Curtis, D.J., Machado, G., 2007. Ecology. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 280–308.
- DaSilva, M.B., Gnaspini, P., 2009. A systematic revision of Goniosomatinae (Arachnida: Opiliones: Gonyleptidae), with a cladistic analysis and biogeographical notes. *Invertebr. Syst.* 23, 530–624.
- DaSilva, M.B., Pinto-da-Rocha, R., 2010. Systematic review and cladistic analysis of the Hernandariinae (Opiliones: Gonyleptidae). *Zoologia* 27, 577–642.
- De Queiroz, A., Wimberger, P.H., 1993. The usefulness of behavior for phylogeny estimation: levels of homoplasy in behavioral and morphological characters. *Evolution* 47, 46–60.
- Eberhard, W.G., 2004. Rapid divergent evolution of sexual morphology: comparative tests of antagonistic coevolution and traditional female choice. *Evolution* 58, 1947–1970.
- Edmunds, M., 1974. *Defence in Animals: A Survey of Antipredator Defences*. Longman, Harlow, UK.
- Eisner, T., Kluge, A.F., Carrel, J.E., Meinwald, J., 1971. Defense of phalangid: liquid repellent administered by leg dabbing. *Science* 173, 650–652.
- Eisner, T., Rossini, C., González, A., Eisner, M., 2004. Chemical defense of an opilionid (*Acanthopachylus aculeatus*). *J. Exp. Biol.* 207, 1313–1321.
- Eisner, T., Eisner, M., Siegler, M., 2005. Secretions of opilionids, whip scorpions and pseudoscorpions. In: Eisner, T., Eisner, M., Siegler, M. (Eds.), *Secret Weapons: Defenses of Insects, Spiders, Scorpions, and Other Many Legged Creatures*. Harvard University Press, Cambridge, MA, pp. 7–10.
- Elpino-Campos, A., Pereira, W., Del-Claro, K., Machado, G., 2001. Behavioural repertory and notes on natural history of the neotropical harvestman *Discocyrtus oliverioi* (Opiliones: Gonyleptidae). *Bull. Br. Arachnol. Soc.* 12, 144–150.
- Estable, C., Ardao, M.I., Brasil, N.P., Fieser, L.F., 1955. Gonyleptidae. *J. Am. Chem. Soc.* 77, 4942.
- Fieser, L., Ardao, M., 1956. Investigation of the chemical nature of Gonyleptidae. *J. Chem. Ecol.* 78, 774–781.
- Firmo, C.L., Pinto-da-Rocha, R., 2002. A new species of *Pseudotrogulus* Roewer and assignment of the genus to the Hernandariinae (Opiliones, Gonyleptidae). *J. Arachnol.* 30, 173–176.
- Giribet, G., Kury, A., 2007. Phylogeny and biogeography. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 62–87.
- Giribet, G., Vogt, L., González, A.P., Sharma, P., Kury, A.B., 2010. A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with emphasis on biogeography and the systematics of Laniatores. *Cladistics* 26, 408–437.
- Gnaspini, P., Lerche, C.F., 2010. Embryonic development of *Ampheres leucopheus* and *Iporangata pustulosa* (Arachnida: Opiliones: Gonyleptidae). *J. Exp. Zool.* 314B, 489–502.
- Gnaspini, P., Hara, M.R., 2007. Defense mechanisms. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 374–399.
- Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24, 774–786.
- González, A., Rossini, C., Eisner, T., 2004. Mimicry: imitative depiction of discharged defensive secretion on carapace of an opilionid. *Chemecology* 14, 5–7.

- Guffey, C., Townsend, V.R. Jr, Felgenhauer, B.E., 2000. External morphology and ultrastructure of the prehensile region of the legs of *Leiobunum nigripes* (Arachnida, Opiliones). *J. Arachnol.* 28, 231–236.
- Hara, M., Gnaspini, P., 2003. Comparative study of the defensive behavior and morphology of the gland opening area among harvestmen (Arachnida, Opiliones, Gonyleptidae) under a phylogenetic perspective. *Arthrop. Struct. Dev.* 32, 257–275.
- Hara, M.R., Pinto-da-Rocha, R., 2010. Systematic review and cladistic analysis of the genus *Eusarcus* Perty 1833 (Arachnida, Opiliones, Gonyleptidae). *Zootaxa* 2698, 1–136.
- Hara, M.R., Gnaspini, P., Machado, G., 2003. Male egg guarding behavior in the Neotropical harvestman *Ampheres leucopheus* (Mello-Leitão 1992) (Opiliones, Gonyleptidae). *J. Arachnol.* 31, 441–444.
- Hara, M.R., Cavalheiro, A.J., Gnaspini, P., Santos, D.Y.A.C., 2005. A comparative analysis of the chemical nature of defensive secretions of Gonyleptidae (Arachnida: Opiliones: Laniatores). *Biochem. Syst. Ecol.* 33, 1210–1225.
- Hoenen, S., Gnaspini, P., 1999. Activity rhythms and behavioral characterization of two epigeal and one cavernicolous harvestmen (Arachnida, Opiliones, Gonyleptidae). *J. Arachnol.* 27, 159–164.
- Japyassú, H.F., Machado, F.A., 2010. Coding behavioural data for cladistic analysis: using dynamic homology without parsimony. *Cladistics* 26, 625–642.
- Jones, S.R., Cokendolpher, J.C., 1985. Spermatogenesis in the harvestman *Vonones sayi* (Simon) (Opiliones: Laniatores: Cosmetidae). *Bull. Br. Arachnol. Soc.* 6, 403–413.
- Kim, K.C., Byrne, L.B., 2006. Biodiversity loss and the taxonomic bottleneck: emerging biodiversity science. *Ecol. Res.* 21, 794–810.
- Kitching, I.J., Forey, P.L., Humphries, C.J., Willians, D.M., 1998. *Cladistics: Theory and Practice of Parsimony Analysis*. Oxford University Press, New York.
- Kuntner, M., Coddington, J.A., Hormiga, G., 2008. Phylogeny of extant nephilid orb weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. *Cladistics* 24, 147–217.
- Kury, A.B., 1991. Análise filogenética de Mítobatinae (Opiliones, Laniatores, Gonyleptidae). MSc thesis, Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil.
- Kury, A.B., 1992. The false Cranainae of the Brazilian Atlantic forest (Opiliones: Gonyleptidae). *Trop. Zool.* 5, 279–291.
- Kury, A.B., 1993. Análise filogenética de Gonyleptoidea (Arachnida, Opiliones, Laniatores). PhD thesis, Universidade de São Paulo, São Paulo, Brazil.
- Kury, A.B., 1994a. The genus *Yania* and other presumed Tricommatidae from South American highlands (Opiliones, Cranidae, Prostyginae). *Rev. Arachnol.* 10, 137–145.
- Kury, A.B., 1994b. Early lineages of Gonyleptidae (Arachnida, Opiliones, Laniatores). *Trop. Zool.* 7, 343–353.
- Kury, A.B., 1995. A review of *Huralvioides* (Opiliones, Gonyleptidae, Pachylinae). *Amazoniana* 13, 315–323.
- Kury, A.B., 1997. A new subfamily of Agoristenidae, with comments on suprageneric relationships of the family (Arachnida, Opiliones, Laniatores). *Trop. Zool.* 10, 333–346.
- Kury, A.B., 2003. Annotated catalogue of the Laniatores of the New World (Arachnida, Opiliones). *Rev. Iber. Aracnol. (vol. especial monográfico)* 1, 1–325.
- Kury, A.B., 2011. Order Opiliones Sundevall, 1833. In: Zhang, Z.Q. (Ed.), *Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness*. *Zootaxa* 3148, 112–114.
- Kury, A.B., Pinto-da-Rocha, R., 2007. Gonyleptidae Sundeval, 1833. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 196–203.
- Lerche, C.F., 2009. Desenvolvimento embrionário em Gonyleptidae (Arachnida: Opiliones: Laniatores): um estudo comparativo. PhD thesis, Instituto de Biociências, Universidade de São Paulo, Brazil.
- Machado, G., 2002. Maternal care, defensive behavior, and sociality in Neotropical *Goniosoma* harvestmen (Arachnida, Opiliones). *Insect. Soc.* 49, 388–393.
- Machado, G., Macías-Ordóñez, R., 2007a. Reproduction. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 414–454.
- Machado, G., Macías-Ordóñez, R., 2007b. Social behavior. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 400–413.
- Machado, G., Oliveira, P.S., 1998. Reproductive biology of the Neotropical harvestman *Goniosoma longipes* (Arachnida, Opiliones: Gonyleptidae): mating and oviposition behaviour, brood mortality, and parental care. *J. Zool.* 246, 359–367.
- Machado, G., Oliveira, P.S., 2002. Maternal care in the Neotropical harvestman *Bourguyia albiornata* (Arachnida: Opiliones): oviposition site selection and egg protection. *Behaviour* 139, 1509–1524.
- Machado, G., Pizo, M.A., 2000. The use of fruits by the Neotropical harvestman *Neosadocus variabilis* (Opiliones, Laniatores, Gonyleptidae). *J. Arachnol.* 28, 357–360.
- Machado, G., Pomini, A.M., 2008. Chemical and behavioral defenses of the Neotropical harvestman *Camarana flavipalpi* (Arachnida: Opiliones). *Biochem. Syst. Ecol.* 36, 369–376.
- Machado, G., Raimundo, R.L.G., 2001. Parental investment and the evolution of subsocial behaviour in harvestmen (Arachnida: Opiliones). *Ethol. Ecol. Evol.* 13, 133–150.
- Machado, G., Vital, D.M., 2001. On the occurrence of epizoic algae and liverworts on the harvestmen *Neosadocus* aff. *variabilis* (Opiliones: Gonyleptidae). *Biotropica* 33, 535–538.
- Machado, G., Warfel, J., 2006. First case of maternal care in the family Cranidae (Opiliones, Laniatores). *J. Arachnol.* 34, 269–272.
- Machado, G., Raimundo, R.L.G., Oliveira, P.S., 2000. Daily activity schedule, gregariousness, and defensive behaviour in the Neotropical harvestman *Goniosoma longipes* (Opiliones: Gonyleptidae). *J. Nat. Hist.* 34, 587–596.
- Machado, G., Bonato, V., Oliveira, P.S., 2002. Alarm communication: a new function for the scent-gland secretion in harvestmen (Arachnida: Opiliones). *Naturwissenschaften* 89, 357–360.
- Machado, G., Requena, G.S., Buzatto, B.A., Osses, F., Rosseto, L.M., 2004. Five new cases of paternal care in harvestmen (Arachnida: Opiliones): implications for the evolution of male guarding in the Neotropical family Gonyleptidae. *Sociobiology* 44, 577–598.
- Machado, G., Carrera, P.C., Pomini, A.M., Marsaioli, A.J., 2005. Chemical defense in harvestmen (Arachnida, Opiliones): do benzoquinone secretions deter invertebrate and vertebrate predators? *J. Chem. Ecol.* 31, 2519–2539.
- Machado, G., Pinto-da-Rocha, R., Giribet, G., 2007. What are harvestmen? In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 1–13.
- Maddison, W.P., Maddison, D.R., 2011. *Mesquite: A Modular System for Evolutionary Analysis*. Version 2.75. Available at: <http://mesquiteproject.org> (accessed 16 January 2012)
- Mendes, A.C., 2011. Phylogeny and taxonomic revision of Heteropachylinae (Opiliones: Laniatores: Gonyleptidae). *Zool. J. Linn. Soc.* 163, 437–483.
- Mestre, L.A.M., Pinto-da-Rocha, R., 2004. Population dynamics of an isolated population of the harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae), in Araucaria Forest (Curitiba, Paraná, Brazil). *J. Arachnol.* 32, 208–220.
- Misslin, R., 2003. The defense system of fear: behavior and neurocircuitry. *Neurophysiol. Clin.* 33, 55–66.
- Munguía-Steyer, R., Buzatto, B.A., Machado, G., 2012. Male dimorphism of a Neotropical arachnid: harem size, sneaker opportunities, and gonadal investment. *Behav. Ecol.* 23, 827–835.

- Murienne, J., Karaman, I., Giribet, G., 2010. Explosive evolution of an ancient group of Cyphophthalmi (Arachnida: Opiliones) in the Balkan Peninsula. *J. Biogeogr.* 37, 90–102.
- Nazareth, T.M., Machado, G., 2009. Reproductive behavior of *Chavesincola inexpectabilis* (Opiliones, Gonyleptidae) with description of a new and independently evolved case of paternal care in harvestmen. *J. Arachnol.* 37, 127–134.
- Nazareth, T.M., Machado, G., 2010. Mating system and exclusive post-zygotic paternal care in a Neotropical harvestman (Arachnida: Opiliones). *Anim. Behav.* 79, 547–554.
- Noll, F.B., 2002. Behavioral phylogeny of corbiculate Apidae (Hymenoptera: Apinae), with special reference to social behavior. *Cladistics* 153, 137–153.
- Osses, F., Martins, E.G., Machado, G., 2007. Oviposition site selection by the Neotropical bromeliad dweller harvestman *Bourguyia hamata* (Arachnida: Opiliones). *J. Ethol.* 26, 233–241.
- Osses, F., Nazareth, T.M., Machado, G., 2008. Activity pattern of the Neotropical harvestman *Neosadocus maximus* (Opiliones, Gonyleptidae): sexual and temporal variations. *J. Arachnol.* 36, 518–526.
- Patterson, C., 1982. Morphological characters and homology. In: Joysey, K.A., Friday, A.E. (Eds.), *Problems of phylogenetic reconstruction*. Academic Press, London, pp. 21–74.
- Pereira, W., Elpino-Campos, A., Del-Claro, K., Machado, G., 2004. Behavioral repertoire of the Neotropical harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae). *J. Arachnol.* 32, 22–30.
- de Pinna, M.C.C., 1991. Concepts and tests of homology in the cladistic paradigm. *Cladistics* 7, 367–394.
- Pinto-da-Rocha, R., 1996. Biological notes on and population size of *Pachylosteus strinati* Silhavy, 1974 in the Gruta das Areias de Cima, Iporanga, south-eastern Brazil (Arachnida, Opiliones, Gonyleptidae). *Bull. Br. Arachnol. Soc.* 10, 189–192.
- Pinto-da-Rocha, R., 2002. Systematic review and cladistic analysis of the Brazilian subfamily Caelopyginae (Opiliones: Gonyleptidae). *Arquiv. Zool.* 36, 357–464.
- Pinto-da-Rocha, R., Bragagnolo, C., 2010. Systematic revision and cladistic analysis of the Brazilian subfamily Siodreaninae (Opiliones: Gonyleptidae). *Invertebr. Syst.* 24, 509–538.
- Pinto-da-Rocha, R., Kury, A.B., 2003. Phylogenetic analysis of *Santinezia* with description of five new species (Opiliones, Laniatores, Cranidae). *J. Arachnol.* 31, 173–208.
- Prevosti, F.J., Chemsiquy, M.A., 2010. The impact of missing data on real morphological phylogenies: influence of the number and distribution of missing entries. *Cladistics* 26, 326–339.
- Proctor, H.C., 1996. Behavior characters and homoplasy: perception versus practice. In: Sanderson, M., Huford, L. (Eds.), *Homoplasy, the Recurrence of Similarity in Evolution*. Academic Press, San Diego, pp. 131–149.
- Puniamoorthy, N., Ismail, M.R.B., Tan, D.S.H., Meier, R., 2009. From kissing to belly stridulation: comparative analysis reveals surprising diversity, rapid evolution, and much homoplasy in the mating behaviour of 27 species of sepsid flies (Diptera: Sepsidae). *J. Evol. Biol.* 22, 2146–2156.
- Ramires, E.N., Giaretta, A.A., 1994. Maternal care in a Neotropical harvestman, *Acutisoma proximum* (Opiliones, Gonyleptidae). *J. Ethol.* 22, 179–180.
- Remane, A., 1952. Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und der Phylogenetik. Königstein-Taurus, Koeltz.
- Requena, G.S., Buzatto, B.A., Munguía-Steyer, R., Machado, G., 2009. Efficiency of uniparental male and female care against egg predators in two closely related syntopic harvestmen. *Anim. Behav.* 78, 1169–1176.
- Robillard, T., Legendre, F., Desutter-Grandcolas, L., Grandcolas, P., 2006. Phylogenetic analysis and alignment of behavioral sequences by direct optimization. *Cladistics* 22, 603–633.
- Rocha, D.F.O., Hamilton, K., Gonçalves, C.C.S., Machado, G., Marsaioli, A.J., 2011. 6-Alkyl-3,4-dihydro-2H-pyrans: chemical secretion compounds in Neotropical harvestmen. *J. Nat. Prod.* 74, 658–663.
- Scholes, E., 2008. Evolution of the courtship phenotype in the bird of paradise genus *Parotia* (Aves: Paradisaeidae): homology, phylogeny, and modularity. *Biol. J. Linn. Soc.* 94, 491–504.
- Shultz, J.W., Pinto-da-Rocha, R., 2007. Morphology and functional anatomy. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 14–61.
- Stanley, E., 2011. Egg hiding in four harvestman species from Uruguay (Opiliones: Gonyleptidae). *J. Arachnol.* 39, 495–496.
- Stuart, A.E., Currie, D.C., 2001. Using caddisfly (Trichoptera) case-building behaviour in higher level phylogeny reconstruction. *Can. J. Zool.* 79, 1842–1854.
- Toscano-Gadea, C.A., 2011. Opiliones en Uruguay. In: Aisenberb, A., Toscazo-Gadea, C.A., Ghione, S. (Eds.), *Guía de Arácnidos de Uruguay*. Ediciones de la Fuga, Montevideo, pp. 212–221.
- Villareal, O.M., Machado, G., 2011. First record of paternal care in the family Stygnidae (Opiliones: Laniatores). *J. Arachnol.* 39, 500–502.
- Wenzel, J., 1992. Behavioral homology and phylogeny. *Ann. Rev. Ecol. Syst.* 23, 361–381.
- Werneck, R.M., Caetano, D.S., Machado, G., 2012. Maternal care in the Neotropical harvestman *Liogonyleptoides tetracanthus* (Opiliones: Gonyleptidae). *J. Arachnol.* 40, 135–137.
- Wiens, J.J., 2000. Coding morphological variation within species and higher taxa for phylogenetic analysis. In: Wiens, J.J. (Ed.), *Phylogenetic analysis of morphological data*. Smithsonian Series in Comparative Evolutionary Biology, Smithsonian Institution, Washington, DC, pp. 115–145.
- Willemart, R.H., 2001. Egg covering behavior of the Neotropical harvestman *Promitobates ornatus* (Opiliones, Gonyleptidae). *J. Arachnol.* 29, 249–252.
- Willemart, R.H., Chelini, M.C., 2007. Experimental demonstration of close-range olfaction and contact chemoreception in the Brazilian harvestman *Iporangaia pustulosa*. *Entomol. Exp. Appl.* 123, 73–79.
- Willemart, R.H., Pellegatti-Franco, F., 2007. The spider *Enoploctenus cyclothorax* (Araneae, Ctenidae) avoids preying on the harvestman *Mischonyx cuspidatus* (Opiliones, Gonyleptidae). *J. Arachnol.* 34, 649–652.
- Willemart, R.H., Chelini, M.C., De Andrade, R., Gnaspini, P., 2007. An ethological approach to a SEM survey on sensory structures and tegumental gland openings of two Neotropical harvestmen (Arachnida, Opiliones, Gonyleptidae). *Ital. J. Zool.* 74, 39–54.
- Willemart, R.H., Osses, F., Chelini, M.C., Macías-Ordóñez, R., Machado, G., 2009. Sexually dimorphic legs in a Neotropical harvestman (Arachnida, Opiliones): ornament or weapon? *Behav. Proc.* 80, 51–59.
- Wouters, F.C., 2011. Semioquímicos de opiliões da família Gonyleptidae (Arachnida: Opiliones). MSc thesis, Instituto de Química, Universidade de Campinas, Brazil.
- Yamaguti, H.Y., Pinto-da-Rocha, R., 2009. Taxonomic review of Bourguyiinae, cladistic analysis, and a new hypothesis of biogeographic relationships of the Brazilian Atlantic Rainforest (Arachnida: Opiliones, Gonyleptidae). *Zool. J. Linn. Soc.* 156, 319–362.
- Zatz, C., 2010. Seleção sexual e evolução do dimorfismo sexual em duas espécies de opiliões (Arachnida: Opiliones). MSc thesis, Instituto de Biociências, Universidade de São Paulo, Brazil.
- Zatz, C., Werneck, R.M., Macías-Ordóñez, R., Machado, G., 2010. Alternative mating tactics in dimorphic males of the harvestman *Longiperna concolor* (Arachnida: Opiliones). *Behav. Ecol. Sociobiol.* 65, 995–1005.
- Zyskowski, K., Prum, R.O., 1999. Phylogenetic analysis of the nest architecture of Neotropical ovenbirds (Furnariidae). *Auk* 116, 891–911.

## Appendix 1

### Character descriptions

The character descriptions follow the pattern below:

1. Character: [0] State 0; [1] State 1 (Number of steps, CI = consistency index, RI = retention index; these indexes have the same value in each one of the five most parsimonious trees from the equally weighted character analysis).

Exp: Description of the standardized protocol for the manipulation of individuals.

Obs: Important observations regarding character definition.

### Activity pattern and habitat use

1. Diel activity: [0] Mainly nocturnal; [1] Diurnal and nocturnal only during the reproductive season; [2] Diurnal and nocturnal over the course of most of the year (except in the peak of winter) (4 steps, CI = 0.50, RI = 0.66).

2. Mesohabitat: [0] Forested areas with high canopy, clear vegetational stratification and low solar incidence in the understorey; [1] Transitional areas, including forest edges and secondary forests; [2] Open areas with low vegetation and high solar incidence; [3] Caves (5 steps, CI = 0.60, RI = 0.77).

3. Hiding/resting place: [0] Among the leaf litter, under rotten logs or rocks (Figs A1A, C and A2C–G); [1] On or among boulders at river margins (including caves crossed by streams, Figs A1G and A2H); [2] Inside natural cavities in trunks; [3] On vegetation (under or on leaves or inside tank bromeliads) (4 steps, CI = 0.50, RI = 0.80).

Obs: The hiding place is a portion of the microhabitat that is used by individuals as shelter during the periods of inactivity.

4. Foraging substrate: [0] Mainly on the ground, among the leaf litter or on rocks (including cave walls) (Figs A1F, J and A2M); [1] Mainly on low vegetation (up to 1 m); [2] Mainly on high vegetation (between 1 and 3 m) (Figs A1H and A2I, L) (5 steps, CI = 0.40, RI = 0.85).

Obs: Ordered character.

5. Posture of the pedipalps when individuals are foraging: [0] Always retracted (Figs A1B, J, L and A2I, L, M); [1] Often extended forward (Fig. A1H) (1 step, CI = 1.00, RI = 1.00).

6. Sit-and-wait foraging posture in which the body hangs on leaves or sticks, while legs I and II freely flutter about: [0] Absent; [1] Present (see fig. 8.4b in Acosta and Machado, 2007) (1 step, CI = 1.00, RI = 1.00).

Obs: This character is inapplicable in species that do not forage on vegetation (see character 4).

7. Posture of femora IV when females are prostrated: [0] Tips of femora IV convergent, often touching each other (Fig. A1A, see also fig. 1 in Osses et al., 2008); [1] Femora IV crossing each other at approximately midlength (see figs 1 and 2 in Machado and Warfel, 2006); [2] Femora IV approximately parallel to each other, with their tips never touching or crossing each other (4 steps, CI = 0.75, RI = 0.88).

Obs: The prostration posture is exhibited when individuals are completely inactive with at least some of their front legs retracted and the venter in contact with the substrate. We used only females to record characters 7 and 8 because the wide variation in the morphology of male leg IV makes comparisons among species very difficult. In cases when intraspecific variation occurs, we considered the more frequent posture, i.e. that observed in more than 50% of the sampled individuals.

8. Angle of femora IV in relation to the substrate when females are prostrated: [0] Less than 45° (Fig. A1G, see also Fig. 3a in Machado and Oliveira, 1998; [1] Greater than 45° (Figs A1A and A2D, F; see also fig. 1 in Osses et al., 2008) (4 steps, CI = 0.25, RI = 0.57).

Obs: Same as character 7.

### Gregariousness

9. Gregariousness: [0] Absent; [1] Present, forming groups of three or more prostrated individuals, 0–2 cm apart from each other and with legs overlapping (Machado et al., 2000) (4 steps, CI = 0.25, RI = 0.78).

Obs: Although *Multumbo terrenus*, *Neosodocus maximus* and *Gonyleptes saphophilus* form aggregations in captivity, this behaviour has never been recorded in the wild. Therefore these species were codified as exhibiting state [0].

10. Aggregation site: [0] Under rotten logs or rocks (Fig. A1C and A2D); [1] On rock walls, usually inside caves or on boulders close to streams (Fig. A1G, see also fig. 11.1a in Machado and Macías-Ordóñez, 2007b); [2] Inside tank bromeliads (2 steps, CI = 1.00, RI = 1.00).

Obs: Character inapplicable to species that do not form aggregations (see character 9).

11. Number of individuals in aggregations: [0] Few: the number of individuals in an aggregation is rarely greater than 20; [1] Many: the number of individuals in an aggregation is generally greater than 30, sometimes reaching more than 200 (1 step, CI = 1.00, RI = 1.00).

Obs: Character inapplicable to species that do not form aggregations (see character 9).

12. Temporal distribution of the aggregations: [0] Aggregations occur throughout the year; [1] Aggregations occur only in the non-reproductive season (1 step, CI = 1.00, RI = 1.00).

Obs: Character inapplicable to species that do not form aggregations (see character 9).

### Reproductive behaviour

13. Oviposition substrate: [0] On or under rotten logs or stones; [1] On vegetation, including the undersurface of leaves (Figs A1E, K and A2J) or inside tank bromeliads (Fig. A1D); [2] Inside natural cavities in trunks (alive or dead; Fig. A1I); [3] Inside natural cavities on the ground or roadside banks; [4] On tree trunks (Fig. A2B), boulders, or among the moss growing on tree trunks or boulders (5 steps, CI = 0.80, RI = 0.87).

14. Oviposition pattern: [0] Females lay from 1 to 3 eggs individually at time, spreading many eggs throughout the reproductive season (Fig. A2B); [1] Females lay several clutches of 10–50 eggs each throughout the reproductive season (Figs A1E, I and A2J); [2] Females lay one or two clutches of 50–200 eggs each throughout the reproductive season (Fig. A1D) (4 steps, CI = 0.50, RI = 0.81).

Obs: *Neosadocus maximus* females keep adding batches of 10–30 eggs to their clutches for up to 2 weeks after the first oviposition bout (Fig. A1K). Although their clutches may contain more than 100 eggs, they are laid asynchronously, so that the clutches are composed of several small batches of eggs in different stages of embryonic development. For this reason, we scored this species as exhibiting state [1].

15. Egg deposition on the substrate using the chelicerae: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

Obs: See detailed description of this behaviour in Nazareth and Machado (2009).

16. Egg covering: [0] Absent (Figs A1D, E, K and A2J); [1] Present: females deposit a small quantity of debris on their eggs, partially



Fig. A1. Photographs of species used as terminals in the phylogenetic analysis of the family Gonyleptidae. (A) *Saramacia lucasae*: prostrated female; (B) *Protimesius longipalpis*: female foraging on the leaf litter; (C) *Gryne coccineloides*: aggregation with males and females; (D) *Bourguyia trochanteralis*: female guarding eggs inside a bromeliad; (E) *Ampheres leucopheus*: male (below) holding the female (above) while she oviposits; (F) *Cobania picea*: male foraging on a rock; (G) *Acutisoma longipes*: aggregation with males and females; (H) *Serracutisoma proximim*: male foraging on the vegetation; (I) *Gonyleptes saprophilus*: male guarding eggs inside a tree trunk; (J) *Mischonyx cuspidatus*: female foraging on the leaf litter; (K) *Neosadocus maximus*: female guarding eggs on a leaf; (L) *Acanthogonyleptes pulcher*: male foraging on a tree trunk; (M) *Pseudotrogulus funebris*: male with debris attached to the dorsum and legs; (N) *Multumbo terrenus*: female with debris attached to the dorsum; (O) *Magnispina neptunus*: female in thanatosis with legs retracted; (P) *Chavesincola inexpectabilis*: male guarding eggs under a trunk; (Q) *Tapacochana insignita*: prostrated male. Scale bars ca. 1 cm.

covering the egg surface (Fig. A1I); [2] Present: females deposit a great quantity of debris on their eggs, completely covering the egg surface (Figs A1P and A2B) (5 steps, CI = 0.40, RI = 0.70).

Obs: Ordered character.

17. Disposition of eggs on the undersurface of leaves: [0] Eggs laid without a clear pattern (see fig. 1A in Buzatto et al., 2007); [1] Eggs laid from the tip to the petiole of the leaf (Figs A1E and A2J; see also fig. 1 in Machado et al., 2004) (1 step, CI = 1.00, RI = 1.00).

Obs: This character is inapplicable to species that do not lay eggs on vegetation (see character 13).

18. Transparent hygroscopic mucus coat covering the eggs: [0] Absent; [1] Present as a thin layer (Fig. A1K); [2] Present as a thick

layer (Fig. A2J, see also fig. 3 in Requena et al., 2009) (2 steps, CI = 1.00, RI = 1.00).

Obs: Ordered character.

19. Mate guarding after copulation: [0] Absent: the male immediately deserts the female after copulation; [1] Present: the male remains behind (Fig. A1E) or in front of (Fig. A2J) the female while she oviposits (3 steps, CI = 0.33, RI = 0.75).

20. Mate guarding behaviour: [0] The male remains behind the female and slowly touches her hind legs (occasionally her dorsal scute) with his leg II (see fig. 12.5c in Machado and Macías-Ordóñez, 2007a); [1] The male remains behind the female and rapidly touches her hind legs, dorsal scute and venter with his leg II



Fig. A2. Photographs of species used as terminals in the phylogenetic analysis of the family Gonyleptidae. (A) *Longiperna concolor*: fighting males using legs IV to hit legs IV of the opponent; (B) *Promitobates ornatus*: female covering egg with debris; (C) *Acanthopachylus aculeatus*: prostrated male; (D) *Discocyrtus oliverioi*: aggregation with males and females; (E) *Discocyrtus prospicius*: prostrated male; (F) *Pachyloidellus goliath*: prostrated female; (G) *Pachyloides thorellii*: prostrated male; (H) *Pachylospeleus strinatii*: prostrated male; (I) *Progonyleptoidellus striatus*: male foraging on the vegetation; (J) *Iporangaia pustulosa*: male (below on the clutch) and female (above) after copulation; (K) *Sodreana barbiellinii*: male resting on a dead leaf; (L) *Sodreana leprevosti*: male foraging on the vegetation; (M) *Cryptogeobius crassipes*: male foraging on the leaf litter; (N) *Camarana flavipalpi*: male releasing droplets of secretion. Scale bars ca. 1 cm except for (B), (M) and (N), where scale bars ca. 0.5 cm.

(see description of this behaviour in Nazareth and Machado, 2009); [2] The male stands behind the female and grasps her body with his pedipalps (Fig. A1E); [3] The male remains on the clutch in front of the female (Fig. A2J) (3 steps, CI = 1.00, RI = 1.00).

Obs: This character is inapplicable to species in which males do not exhibit mate guarding (see character 19).

21. Maternal care: [0] Absent; [1] Present (3 steps, CI = 0.33, RI = 0.66).

22. Time the female stays with the clutch: [0] Female stays on the clutch all the time (Fig. A1D); [1] Female leaves the clutch regularly, often during the day (Fig. A1K) (1 step, uninformative).

23. Paternal care: [0] Absent; [1] Present, but facultative; [2] Present and obligatory (4 steps, CI = 0.50, RI = 0.66).

Obs: State [1] refers to amphisexual care, in which males care for clutches whose caring females have deserted them or died (Machado and Oliveira, 1998; Buzatto and Machado, 2009).

24. Time the male stays with the clutch in species with obligatory paternal care: [0] Male leaves his clutch frequently and can be seen more than 5 m away from the eggs (Figs A1E and A2J). The period

away from the eggs can be long (>48 h), and the male can leave his clutch not only to forage, but also to hide from stressful climatic conditions; [1] Male stays on his clutch during almost all the period of embryonic development, leaving the eggs only sporadically and for short periods (<1 h) to forage (Figs A1I, P) (1 step, CI = 1.00, RI = 1.00).

25. Defence of harems by males: [0] Absent; [1] Present (2 steps, CI = 0.50, RI = 0.80).

26. Types of harem: [0] Harems containing caring females (see fig. 12.5d in Machado and Macías-Ordóñez, 2007a); [1] Harems containing ovigerous females that do not care for the eggs (see fig. 1D in Zatz et al., 2010) (1 step, CI = 1.00, RI = 1.00).

27. Male fights using leg II to hit leg II of the opponent: [0] Absent; [1] Present (see fig. 3a in Buzatto and Machado, 2008) (1 step, CI = 1.00, RI = 1.00).

Obs: For those species for which no behavioural information was available, we scored the character as state [0] when the species does not exhibit sexual dimorphism in the length of leg II. We based this criterion on behavioural and morphological information available

for *Serracutisoma proximum* (Buzatto and Machado, 2008; Buzatto et al., 2011).

28. Male fights using leg IV: [0] Absent; [1] Present: males use leg IV to hit leg IV of the opponent (Fig. A2A, see also fig. 1A in Zatz et al., 2010); [2] Present: males use their leg IV to pinch the leg IV of the opponent (see fig. 2c in Willemart et al., 2009) (2 steps, CI = 1.00, RI = 1.00).

Obs: For detailed description of states [1] and [2], see Zatz (2010) and Willemart et al. (2009), respectively.

### Defensive behaviour

29. Falling from elevated substrates (rock walls or vegetation) when individuals are disturbed: [0] Absent; [1] Present, immediate voluntary falling; [2] Present, rapid movement, followed by falling (2 step, CI = 1.00, RI = 1.00).

Exp: We touched the legs of motionless individuals (prostrated or not) on rock walls, trunks, or leaves and observed their immediate reaction.

Obs: This character is inapplicable to species that hide or forage exclusively on the ground (see characters 3 and 4).

30. Males attack with chelicerae and pedipalps when handled: [0] Absent or present in a low proportion (<20% of sampled individuals); [1] Present in a high proportion (>30% of sampled individuals) (4 steps, CI = 0.25, RI = 0.76).

Exp: We seized the individuals on the median portion of femora IV and touched their mouthparts with the tip of one finger. When handling small specimens, such as *Cryptogeobius crassipes* and *Chavesincola inexpectabilis*, we used a rubber cylinder (1–2 mm in diameter) to touch their mouthparts. We recorded whether the individuals attacked the finger or the rubber cylinder with their chelicerae and/or pedipalps in a 20 s interval.

Obs: For all species in which fewer than five specimens were sampled, we coded the character state as missing (?).

31. Females attack with chelicerae and pedipalps when handled: [0] Absent or present in a low proportion (<20% of the sampled individuals); [1] Present in a high proportion (>30% of the sampled individuals) (3 steps, CI = 0.33, RI = 0.77).

Exp/Obs: Same as character 30.

32. Nipping: [0] Absent; [1] Present (5 steps, CI = 0.20, RI = 0.60).

Exp: We seized males on the apical portion of their right femur IV and placed the tip of a pair of forceps between the apophysis of the coxa and femur of the seized leg. We also gathered information for this character when sampled individuals exhibited nipping behaviour during other laboratory experiments, such as those described for characters 30–46.

Obs: We considered the state to be [1] when a male brought femur IV close to his body with a sudden upward movement, pinching the forceps between the apophysis and either the tubercles of the trochanter or the spines of the femur on the same leg (sensu Willemart et al., 2009).

33. Stridulation: [0] Absent; [1] Present (1 step, uninformative).

Exp: Same as character 32.

Obs: Individuals vibrate and produce a low, but audible sound when their body is gently pressed dorso-ventrally in the median portion of the dorsal scute. Although we searched the external body surface of some individuals of *Pseudotrogulus funebris* under a stereomicroscope, we did not find any stridulatory apparatus on their bodies.

34. Thanatosis: [0] Absent; [1] Present under stimuli (a) and (b) (see description of the experiments below); [2] Present only under stimulus (a) (3 steps, CI = 0.66, RI = 0.88).

Exp: We stimulated individuals (males and females) in two different ways: (a) we seized them on the median portion of their femur IV; and (b) we gently pressed them between our palms, and 5–10 s later we opened our hands slowly. We scored the character as present, independent of the posture of the individuals during thanatosis.

Obs: There are various definitions of thanatosis (or death feigning) in the literature (e.g. Edmunds, 1974; Misslin, 2003). Herein, we scored the character as present when individuals remained completely still after being exposed to at least one of the stimuli described above, regardless of how long they remained motionless or whether their posture was stereotyped.

35. Thanatosis with legs retracted: [0] Absent; [1] Present (Fig. A10, see also fig. 10.1e in Gnaspini and Hara, 2007) (7 steps, CI = 0.14, RI = 0.45).

Exp: Same as character 34.

Obs: In some species, individuals do not retract their legs during thanatosis but instead keep them fully or partially extended laterally (see fig. 2 in Machado and Pomini, 2008). In these cases, we scored the character as [0]. This character is inapplicable to species that do not exhibit thanatosis (see character 34).

36. Deposition of debris on the body: [0] Absent: the individuals are never covered with debris on any portion of their body; [1] Present: debris covers the entire dorsal scute and, occasionally, part of the legs, pedipalps and chelicerae (Fig. A1M) (1 step, CI = 1.00, RI = 1.00).

Obs: Although individuals of some species of *Mischonyx* may be found with debris on their dorsal scute, we did not find debris on any individual of *M. cuspidatus* we sampled.

37. Emission of enteric fluid prior to scent gland secretions: [0] Facultative: individuals can secrete scent gland secretions without previous emission of enteric fluid; [1] Obligatory: individuals always emit enteric fluid prior to scent gland secretions (2 steps, CI = 0.50, RI = 0.91).

Exp: We seized individuals (males and females) on the median portion of femora IV and recorded the emission and displacement of enteric fluid and/or scent gland secretions. If individuals did not emit enteric fluid and/or scent gland secretions, we used a pair of forceps to press their body gently, dorso-ventrally at the median portion of the dorsal scute (area immediately posterior to the ocellarium).

Obs: See detailed description of the two states provided in Gnaspini and Hara (2007).

38. Displacement of enteric fluid along tegumentary channels on the edges of the dorsal scute: [0] Absent: enteric fluid does not flow along dorso-lateral tegumentary channels but can accumulate between the bases of legs I and II (see fig. 1A in Eisner et al., 1971); [1] Present (Fig. A2N, see also fig. 10.5a in Gnaspini and Hara, 2007) (3 steps, CI = 0.33, RI = 0.50).

Exp/Obs: Same as character 37.

39. Emission of scent gland secretions when seized on femur IV: [0] Absent; [1] Present (6 steps, CI = 0.16, RI = 0.64).

Exp: We seized individuals (males and females) on the median portion of femora IV and recorded the emission of scent gland secretions.

Obs: In the case of Mitobatinae species (*Longiperna concolor* and *Promitobates ornatus*), which do not produce scent gland secretions, other characters that refer to scent gland secretions (41–63) are inapplicable.

40. Emission of scent gland secretions when gently pressed dorso-ventrally: [0] Never: no sampled individual ever emitted scent gland secretions ( $n > 200$  individuals of each species manipulated in the field); [1] Low proportion: fewer than 20% of the sampled individuals emitted scent gland secretions; [2] High proportion: more than 30% of the sampled individuals emitted scent gland secretions (4 steps, CI = 0.50, RI = 0.66).

Exp: We immobilized individuals (males and females) by seizing the median portion of femora IV and used a pair of forceps to gently press their body dorso-ventrally on the median portion of the dorsal scute. In a sample of at least three individuals, we recorded the emission of scent gland secretions under a stereomicroscope.

Obs: Ordered character.

41. Emission of scent gland secretions in droplets: [0] Absent; [1] Present (Fig. A2N) (2 steps, CI = 0.50, RI = 0.88).

Exp: Same as character 40.

Obs: Individuals of many sampled species emit scent gland secretions in droplets that mix with the previously secreted droplets of enteric fluid accumulated near the ozopore. The droplet containing enteric fluid and scent gland secretions generally runs along tegumentary channels on the edges of the dorsal scute to the posterior area of the body. See detailed description of this behaviour in Gnaspini and Hara (2007).

42. Displacement of droplets of scent gland secretions: [0] Along tegumentary channels between the bases of legs I and II (see fig. 1B in Eisner et al., 1971); [1] Along tegumentary channels on the lateral margins of the dorsal scute (Fig. A2N, see also fig. 10.5b in Gnaspini and Hara, 2007) (1 step, CI = 1.00, RI = 1.00).

Exp: Same as character 40.

Obs: This character is inapplicable to species that do not emit scent gland secretions in droplets (see character 41).

43. Accumulation of droplets of scent gland secretions on the ventral part of the body, between the bases of legs I and II: [0] Absent; [1] Present (see fig. 1B in Eisner et al., 1971) (1 step, CI = 1.00, RI = 1.00).

Exp: Same as character 40.

44. Release of scent gland secretions in a jet: [0] Absent; [1] Present (see fig. 10.5c in Gnaspini and Hara, 2007) (2 steps, CI = 0.50, RI = 0.91).

Exp: Same as character 40.

Obs: Droplets and jet secretion occur independently from each other and all combinations of presence/absence of these two characters can be found among the terminals (see Appendix 2). Individuals of *Serracutisoma proximum*, *Acutisoma longipes* and *Acanthogonyleptes pulcher*, for instance, emit scent gland secretions both in droplets and in a jet in different moments. According to the “conjunction test”, variation at the individual level is evidence that the processes that originate each one of the phenotypes have independent expression and thus are not homologues (Patterson, 1982 reviewed in de Pinna, 1991).

45. Release of a jet of scent gland secretions directed to the aggressor: [0] Absent: the jet of scent gland secretions is released only in one direction (antero-posterior), frequently toward the posterior portion of the body; [1] Present: the direction of the jet of scent gland secretions varies according to the area of the body where the individual is stimulated (see fig. 10.5c in Gnaspini and Hara, 2007) (2 step, CI = 0.50, RI = 0.50).

Exp: Same as character 40.

46. Leg dabbing: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

Exp: Same as character 40.

Obs: According to Eisner et al. (1971), leg dabbing occurs when droplets of scent gland secretions are gathered by the tarsus of the leg I and directly delivered to the aggressor (see figs 1D and 1G in Eisner et al., 1971).

### Chemical composition of scent gland secretions

47. Relative frequency of 1,4-benzoquinones in the scent gland secretions: [0] Absent; [1] Present at a relative frequency of <10%;

[2] Present at a relative frequency of >80% (7 steps, CI = 0.28, RI = 0.72).

Obs: When it was not possible to collect scent gland secretions in a sufficient quantity to identify the presence/relative frequency of their chemical constituents, we used information available in the following sources: Hara et al. (2003), Eisner et al. (2004), González et al. (2004), Machado et al. (2005), Machado and Pomini (2008), Rocha et al. (2011) and Carmen Rossini (pers. commun.). Ordered character.

48. Molecules of 2-ethyl-1,4-benzoquinone: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

Obs: Same as character 47. See structure of the molecule in Fig. 6.

49. Molecules of 2-methyl-1,4-benzoquinone: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

Obs: Same as character 47. See structure of the molecule in Fig. 6.

50. Relative frequency of molecules of 2,3-ethyl-1,4-benzoquinone: [0] Absent; [1] Present at a relative frequency of <10%; [2] Present at a relative frequency of >30% (5 steps, CI = 0.40, RI = 0.40).

Obs: Same as character 47. See structure of the molecule in Fig. 6. Ordered character.

51. Molecules of 5-ethyl-2-methyl-1,4-benzoquinone: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

Obs: Same as character 47. See structure of the molecule in Fig. 6.

52. Relative frequency of vinyl-ketones in the scent gland secretions: [0] Absent; [1] Present at a relative frequency of <10%; [1] Present at a relative frequency of >80% (6 steps, CI = 0.33, RI = 0.73).

Obs: Same as character 47. Ordered character.

53. Vinyl-ketone molecular chain with six carbons: [0] Absent; [1] Present (2 steps, CI = 0.50, RI = 0.50).

Obs: Same as character 47.

54. Vinyl-ketone molecular chain with seven carbons: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

Obs: Same as character 47.

55. Molecules of 1-hexen-3-one: [0] Absent; [1] Present (2 steps, CI = 0.50, RI = 0.50).

Obs: Same as character 47. See structure of the molecule in Fig. 6.

56. Molecules of 5-methyl-1-hexen-3-one: [0] Absent; [1] Present at a relative frequency of <10%; [1] Present at a relative frequency of 30% (3 steps, CI = 0.66, RI = 0.66).

Obs: Same as character 47. See structure of the molecule in Fig. 6. Ordered character.

57. Molecules of 4-methyl-1-hexen-3-one: [0] Absent; [1] Present at a relative frequency of <10%; [1] Present at a relative frequency of >30% (4 steps, CI = 0.50, RI = 0.33).

Obs: Same as character 47. See structure of the molecule in Fig. 6. Ordered character.

58. Dimerization of vinyl-ketone molecules: [0] Absent; [1] Present (2 steps, CI = 0.50, RI = 0.50).

Obs: Same as character 47. See detailed explanation of the dimerization process in Rocha et al. (2011).

59. Molecules of 1-(6-butyl-3,4-dihydro-2H-pyran-2-yl)pentanone: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

Obs: Same as character 47. This molecule has recently been described as a dimer of the ketone 1-hepten-3-one released by *Neosadocus maximus* and *Iporangaia pustulosa* (Rocha et al., 2011). See structure of the molecule in Fig. 6.

60. Dimer 1 of the molecules 5-methyl-1-hexen-3-one and 1-hexen-3-one: [0] Absent; [1] Present (1 step, CI = 1.00, RI = 1.00).

