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Letter to the Editor

The assassination of a hypothesis by non-critical interpretation of molecular data: A comment on Sharma et al. (2017)

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ABSTRACT

In a recent paper, Sharma et al. (2017) tested the hypothesis that eggs attached to males' legs in podoctid harvestmen are laid by conspecifics. Using molecular methods, they falsify the "paternal care hypothesis" and suggest that the eggs belong to spiders. Here we raise several criticisms to the authenticity of this finding and present arguments supporting the hypothesis that eggs belong to harvestmen and are not accidentally attached to the males. We argue that the falsification of the paternal care hypothesis in podoctids is premature and based on non-critical interpretation of molecular data.

In a recent paper, Sharma et al. (2017) provide the first phylogenetic approach to the harvestman family Podoctidae (Opiliones: Laniatores). This family comprises nearly 150 species (Kury, 2007), and as with many other harvestman groups, the biology of podoctids is poorly understood. Perhaps the most striking information available about the biology of podoctids is that males of some species carry large eggs attached to the femur of their hind legs (Martens, 1993; Kury and Machado, 2003; Sharma et al., 2017). Martens (1993) assumed "that the eggs attached to the legs of the males of *Leytpodoctis oviger* n.sp. are correctly identified as eggs of that species" (page 101). Although no further investigation has been made on this species, the hypothesis that males were carrying conspecific eggs as a form of paternal care was embraced in subsequent papers on paternal care in arthropods (e.g. Tallamy, 2001; Requena et al., 2013).

Using modern molecular methods, Sharma et al. (2017) tested the paternal care hypothesis in males of *Borneojapetus* Özdikmen, 2006 (incorrectly cited as *Japetus* Roewer, 1949) and *Ibalonius* Karsch, 1880, both with eggs attached to the metatarsus of the third legs. Surprisingly, the authors show "that eggs carried on the legs of male Podoctidae are not conspecific to the males" (p. 164). Even more surprising was the finding that the "best BLASTn hits against the obtained sequences [from the eggs] were those of araneoid spiders (eggs carried by *Japetus* cf. *longipes*) and of haplogyne spiders (eggs carried by *Ibalonius* sp.)". The authors conclude that their results falsify the hypothesis of paternal care and suggest that the "eggs attached to the specimens [...] may alternatively be attributable to accidental attachment of debris (e.g., during disruptive bouts of sifting), or may represent a strategy among ground-dwelling spider females to lay eggs on a group of mobile, spined harvestman species that are equipped with repugnatorial glands" (p. 168). According to our view, the falsification of the paternal care hypothesis is premature and based on non-critical interpretation of molecular data. Moreover, the alternative suggestions provided by the authors are not compatible with other information available about podoctids that we will elaborate on below.

In the original paper by Martens (1993), the author states that the "identification of the eggs as opilionid eggs is not difficult. At least in one case, the translucent cover allows inspection of the interior of the egg and the developing embryo, especially the knobs of the appendages, are to be seen" (p. 101). Although this statement refutes the possibility that the eggs originate from a parasitoid wasp, one may argue that embryos of different arachnid orders look rather similar, especially at an early stage. However, spider eggs have an overall appearance different from harvestman eggs. Spider eggs have a granular surface, due to a coating of proteinaceous spheres that are insoluble in ethanol and can be recognized even at low magnification under a light microscope (Grim and Slobodchikoff, 1982; Humphreys, 1995). In contrast, harvestman eggs exhibit a thick vitelline envelope with a surface lacking

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sphere-like structures, and are often coated with mucus (Witaliński and Żuwała, 1981; Humphreys, 1995). Furthermore, to our knowledge, there is no report of any spider species laying eggs without attached silk threads. Therefore, simple morphological features of the egg surface, which are not mentioned by Sharma et al. (2017), could have been used to test whether these eggs could have been laid by spiders. Martens' (1993) observations indicate that the morphology of the eggs is consistent with harvestman eggs.

A second information about podoctid eggs provided by Martens (1993) is that the eggs were firmly attached to the males' legs by a cementing secretion. According to the author, the "attachment of the eggs to the leg seems to be strong and not at all accidental. I did not try to remove the eggs, but they did not fall during usual handling of the specimens" (p. 101). If the eggs were accidentally attached to the legs by dislocation during litter sifting, as suggested by Sharma et al. (2017), only weak adhesion would be expected because strong, durable adhesion requires some curing mechanism. The use of cementing secretions to attach eggs on the substrate, such as rocks, bark, and leaves, is widespread in harvestmen (Wolff et al., in press). In spiders, however, only small amounts of an adhesive secretion are added to the eggs, which exclusively serve the adhesion to other eggs in the clutch (Foelix, 2011). We performed a search in *Web of Science* using the following combination of keywords: (spider* OR Araneae) AND (egg* OR chorion OR clutch*) AND (mucus OR cement* OR glue OR attach*). This search returned 48 papers, and there was no record of spiders gluing eggs directly onto the substrate without silk cover. Silken egg sacs are a unifying feature of spiders (Foelix, 2011), and it has repeatedly been shown that the silk cover is important to protect eggs and larvae against predators, parasitoids, and dehydration (e.g. Austin, 1985; Hieber, 1992; Vieira and Romero, 2008). In some haplogyne spiders, such as Pholcidae and Scytodidae, the egg sac can be greatly reduced, but eggs are carried by the mother, which may reduce the risk of predation and parasitism and render a dense protective sheet unnecessary (Sedey and Jakob, 1998; Li et al., 1999).

One may ponder that the eggs attached to males of the two podoctid species studied by Sharma et al. (2017) may belong to a highly specialized spider species that is able to produce a cementing secretion to glue their eggs on a protective host. Following the argumentation of Sharma et al. (2017), the spider eggs would benefit from presumed defensive mechanisms of the harvestman, including spines and repugnatorial glands. This could explain the lack of silk around the eggs. However, there is a key problem with this argument: spiders lack an ovipositor or any structure that allows them to precisely place their eggs on a thin and short structure, such as the leg segments of podoctid males. Harvestman females, on the other hand, have a muscular ovipositor that is known to perform precise maneuvers to deposit eggs in narrow rock cracks, deep inside snail shells, or on small moss leaflets (Machado and Macías-Ordóñez, 2007). Moreover, egg attachment by cement would also demand some time to rest securely and undisturbed on or next to the leg of the harvestman. Hence, certain cooperation of the harvestman would be necessary, especially if the harvestman has efficient defensive mechanisms against spiders.

The third information available on podoctid eggs is that they are consistently attached to males (Martens, 1993; Kury and Machado, 2003; Sharma et al. (2017)). Sharma et al. (2017) do not mention sample sizes, but they clearly state that eggs were attached to males in both studied species. Martens (1993) reported information on two individuals of *L. oviger* carrying eggs attached to their legs and both were unequivocally males. Kury and Machado (2003) reported that six out of eight males of an undescribed *Ibalonius* species from Solomon Islands had eggs on their legs, but none of the seven females in the assemblage had them. This sexual bias is not to be expected if the eggs were attached to the legs accidentally during litter sifting, as proposed by Sharma et al. (2017). The hypothesis that eggs are attached by spider females also seems unable to explain the sexual bias. Why should spider females select exclusively males, and how could they? Sexual dimorphism in podoctids is not well-marked (Kury, 2007), and both males and females have spines distributed all over the carapace and legs, as in *Borneojapetus* cf. *longipes*. On the other hand, both males and females of *Ibalonius* sp. and the undescribed Ibaloninae species mentioned above lack spines on the carapace and legs. Furthermore, males and females are known to produce similar amounts of repugnatorial secretion in Gonylepidae (Nazareth and Machado, 2015). If the same pattern also occurs in other harvestman families, such as Podoctidae, male hosts would not provide better protection to the eggs when compared to females.

In the discussion, Sharma et al. (2017) argue that the strategy of laying eggs on the body of other species is common among marine arthropods. They mention the example of spider crabs of the genus *Loxorhynchus*, "which sometimes bear micro-communities composed of multiple animal phyla on the dorsum of their cephalothorax" (p. 172). Although no reference is provided by the authors, we found a review on the so-called decorating behavior, which is taxonomically widespread in spider crabs. According to this review, individuals of many species deliberately attach pieces of debris, algae, or sessile marine organisms to their exoskeleton (Wicksten, 1993). Thus, the example provided by Sharma et al. (2017) is misleading for three main reasons. First, the "micro-communities" are formed by the active action of the spider crabs as a form of camouflage against predators (Wicksten, 1993). Second, an intensive search in *Google Scholar* resulted in no paper reporting spider crabs with eggs from other species attached to their bodies. Third, none of the organisms attached to the exoskeleton by spider crabs can potentially harm or prey on them—in fact, some hydroids can even provide additional defense for their hosts (Wicksten, 1993). Spiders, on the other hand, are predators and harvestmen are their potential prey (Cokendolpher and Mitov, 2007). If spiders are using male harvestmen of a single family as hosts for their eggs, it would be important to understand how females immobilize their hosts, how and why they lay eggs in very specific positions of the host's body, and why male hosts do not remove or eat these eggs because they are laid on a segment that can be easily placed in their mouthparts. Ectoparasitoids of spiders, for instance, glue their eggs on the body of live hosts, but these eggs are usually laid on places where the hosts are unable to remove or eat them, such as the cephalothorax and abdomen (e.g. Gonzaga and Sobczak, 2007).

Instances of individuals with eggs attached to their bodies are well-reported among terrestrial arthropods. Besides the parasitoids mentioned above, there are some cases of conspecific eggs attached to the body of coreid bugs (Panizzi and Santos, 2001). The most intensively studied coreid species is *Phyllomorpha laciniata*, in which eggs are deposited on the dorsum of females and males. Molecular data show that 87% of the eggs carried by males are not genetically related to them (Tay et al., 2003). Thus, the behavior of *P. laciniata* may be regarded as an example of parental-care parasitism, defined as an "interaction in which an individual (the parasite) obtains reproductive benefits while reducing or completely eliminating its own costs of parenting by exploiting any type of offspring care provided by other individuals (the hosts)" (Roldán and Soler, 2011). This is a relatively rare behavior among arthropods and all known examples of interspecific parental-care parasitism occur among nest-provisioning species (Roldán and Soler, 2011). Although we cannot rule out the possibility that eggs carried by podoctid males are not a case of *intra-specific* parental-care parasitism, all arguments presented

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here suggest that they are not a case of *inter-specific* parental-care parasitism.

In conclusion, although the molecular analyses performed by Sharma et al. (2017) indicate that the eggs attached to podoctid males do not belong to conspecifics, we raised several criticisms to the authenticity of this finding and also to the alternative hypotheses raised by the authors. We argue that, until convincing behavioral evidence is available, their falsification of the paternal care hypothesis in podoctids is premature and almost certainly wrong. According to our view, the most plausible and parsimonious explanation is that podoctid males are caring for their offspring, like waterbugs of the subfamily Belostomatinae (Smith, 1997). The strong belief in molecular data as the most trustworthy source of information is symptomatic for modern biology. However, like any other method, molecular methods are not infallible and can have multiple sources of errors. Molecular methods have unequivocally brought a new quality of data acquisition and problem solving in nearly all fields of biology, enabling the investigation of numerous previously untestable questions. Still, these techniques do not render classical behavioral, morphological, and natural history approaches useless. The issue discussed here is a strong example for this.

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