Feeding Frequency, Prey Acceptance, and Natural Diet of the Mygalomorph Spider *Acanthogonatus centralis* Goloboff 1995 (Araneae: Nemesiidae)

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Gabriel Pompozzi and Sofía Copperi (2018) The family Nemesiidae is the second most diverse family of Mygalomorphae spiders. *Acanthogonatus centralis* (Nemesiidae) is commonly found in hilly areas of central Argentina. These spiders are considered generalists due to their basal position in the phylogeny, but little is known about the trophic ecology of the species. Therefore, the objectives of this study were to study some of the species' predatory traits, such as feeding frequency and prey acceptance, and to assess its natural diet. In addition, two parameters - attack latency and consumption time - were measured for each accepted prey. We found that *A. centralis* feeds every two days and accepts almost all prey offered. The most accepted prey items were beetle larvae and termites. In fact, termites were attacked faster than any other prey, suggesting they are a suitable prey for these spiders. In regard to the species' natural diet, we found remains of seven prey items, ants and beetles being the most frequent. In this study, we found that *Acanthogonatus centralis* is polyphagous since it accepts different kinds of prey.

Key words: Trophic ecology, Weight gain, Polyphagous, Mygalomorphae; South America.

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BACKGROUND

Spiders are considered the most diversified group of terrestrial predators; one reason for this is because they are able to employ a wide variety of strategies to capture their prey (Coddington and

Levi 1991). Most spiders have an euryphagous diet, feeding on a great variety of prey types. However, some species show diet specialization, feeding on only one or a few taxa; mirmecophagy is the best-known specialization in spiders (Pekár et al. 2012). Mygalomorphae spiders are considered generalists, due to their basal position in the phylogeny, as diet specialization is considered a derived state in spiders (Pekár et al. 2012). Moreover, Pekár et al. (2012) hypothesized that stenophagy is completely absent in the Mygalomorphae. Despite being euryphagous, mygalomorph spiders could have some prey preferences or they could avoid some kind of prey, like ants. However, there are only a few studies on their trophic ecology, which mainly focus on the Theraphosidae spiders (Coyle and Ketner 1990; Pinto and Saiz 1997; Pérez-Miles et al. 2005; Dias and Lo-Man-Hung 2009; Nespolo et al. 2011; Kosiba et al. 2012; Souza-Silva et al. 2014).

The family Nemesiidae is distributed worldwide and has more than 300 described species (WSC 2017). In South America they are well distributed and very diverse, being the second most diverse mygalomorph family (Goloboff 1995). Although some nemesiid species are abundant, they are usually found in specific areas, but there is insufficient knowledge about their biology and ecology. *Acanthogonatus centralis* Goloboff 1995 is a mygalomorph spider found in the hilly areas of central Argentina, particularly in the Ventania system (Ferretti et al. 2012). These are medium sized spiders: both males and females measure approximately 12 mm in total body length (Ferretti et al. 2011). In general, these spiders build tunnel-webs with no branching tunnels and only one open entrance (Ferretti et al. 2011). The periods of highest activity recorded for the males of *A. centralis* are spring and end of fall, showing two clear activity peaks (Ferretti et al. 2012). These spiders have a passive strategy for capturing their prey, waiting at or near the entrance of the tunnel-web for prey to pass, similar to other mygalomorph spiders (Coyle 1986). *A. centralis* has been well-studied over the last a few years, but only their sexual and agonistic behaviors have been recorded (Ferretti et al. 2011; 2014). Although some ecological aspects - such as species abundance in a nature reserve - have been reported (Ferretti et al. 2012), trophic ecology and feeding behavior are still unknown. Only one study presents some results on the natural diet of one species of this genus, *A. franckii*, from Chile (Pinto and Saiz 1997). Moreover, there are only observational reports of diet in family Nemesiidae (Decae et al. 2007; Souza-Silva et al. 2014).

Due to this lack of knowledge on this spider group, this study addresses some aspects of *A. centralis'* trophic ecology. We wondered if *A. centralis* has a polyphagous diet with no particular prey preferences, as is common in mygalomorph spiders. Thus, the objectives of this study were to study some predatory traits of this species, such as feeding frequency and prey acceptance, and measure the following parameters: weight gain, attack latency, and consumption time. In addition, we studied the species' natural diet to complement the laboratory experiments. According to

previous studies of natural diet in other Nemesiidae species, we predict that *A. centralis* accepts different kind of prey, a high proportion of which are ants.

MATERIALS AND METHODS

Spiders

We collected five adult females of *A. centralis* under stones in Sierra de la Ventana (38°04'21.3"S, 62°03'02.6"W), Buenos Aires province, Argentina. These females constructed egg-sacs in the laboratory and were kept until the spiderlings hatched. We used juveniles of the third instar in both experiments (total length = 7.57 mm ± 0.43 SE). Spiders were placed individually in glass petri dishes (diameter 60 mm) with a small amount of wet cotton wool. The room temperature during breeding and the experiments was 22.7°C ± 1.52°C. The spiders were kept under a natural 16L: 8D photoperiod. We maintained the moisture level by adding drops of water to the cotton wool. Before the experiments, all spiders were weighed using an ACCULAB balance with a precision of 0.0001 mg. We killed all the spiders when we finished with all the experiments. Voucher specimens were deposited in the arachnological collection of the Laboratorio de Invertebrados II, Universidad Nacional del Sur, Bahía Blanca, Argentina.

Feeding frequency

The feeding frequency experiment took nine days overall and used 27 spiders. This experiment consisted of feeding the spiders six different kinds of prey (cockroaches, crickets, beetle larvae, moth larvae, termites, and spiders) every day. All the prey types offered were smaller than the spiders. Some prey used in this experiment were taken from laboratory-reared cultures, such as weevils and moth larvae (*Rhizopertha dominica*, Coleoptera, 3.02 mm ± 0.19 SE, $n = 5$; and *Plodia interpunctella*, Lepidoptera, 5.59 mm ± 0.11 SE, $n = 5$), cockroaches (*Blatella germanica*, Blattodea, 3.52 mm ± 0.85 SE, $n = 5$), and crickets (*Acheta domestica*, Orthoptera, 4.12 mm ± 0.12 SE, $n = 5$). The remaining prey were collected from the field: termites (workers of *Anoplotermes* sp., Isoptera, 3.96 mm ± 0.07 SE, $n = 5$) and spiders (juveniles of different families, Araneae, 3.68 mm ± 0.24 SE, $n = 5$). Five days before beginning the experiment, we fed spiders with cockroaches until satiation in order to standardize their hunger level. We offered the spiders different prey in a random order. The experiment began when one prey was released in a spider's petri dish. If it did not accept the prey after 30 minutes, we offered it a different prey. If it did not accept it again, the

prey was removed. This was repeated until the spiders were completely satiated. When the spider accepted no further prey, the feeding was ended and the body mass of the spider was measured. We followed this method every day until all the spiders were fed in at least four different feeding events.

Prey acceptance

We used prey commonly found crawling in the soil near *A. centralis* burrows (Pompozzi pers. obs.). In this experiment we released the prey in the petri dish occupied by the spider and recorded whether or not the spider consumed the prey. Five days before the experiment started, we fed all spiders with cockroaches in order to standardize their hunger levels. Overall, we used nine kinds of prey from eight arthropod orders. From laboratory-reared cultures, we took adult and larvae beetles (*Tribolium castaneum*, Coleoptera, tl: 3.41 ± 0.1 SE, $n = 5$), crickets (*Acheta domestica*, Orthoptera), and cockroaches (*Shelfordella tartara*, Blattodea, 4.96 ± 0.14 SE, $n = 5$). From the field, we collected spiders (*Steatoda* sp., *Metaltella* sp., Araneae), millipedes (Julidae, Diplopoda, 6.52 ± 0.19 SE, $n = 5$), ants (workers of *Acromyrmex striatus*, Hymenoptera, 4.42 ± 0.15 SE, $n = 5$), termites (workers of *Anoplotermes* sp., Isoptera), and woodlice (*Armadillidium* sp., Isopoda, 5.9 ± 0.22 SE, $n = 5$). For this experiment we used 32 spiders. Each spider was offered all prey types in a randomized order. If the spider did not attack the prey within 30 minutes, we removed the prey from the dish and we offered it a different prey. If the spider consumed the prey, we offered it a new prey two days later. We measured the attack latency (i.e., the time from the moment in which the spider orients itself towards the prey to the time of the first attack) and the consumption time for each spider after beginning to feed on each prey item.

Natural diet

In order to complement the laboratory experiments, we assessed the species' natural diet in the field. During one day we meticulously inspected tunnel-webs of *A. centralis* in the field and collected all the prey remains that we could find. We carried out this sampling during winter because spiders are more active in that season (Ferretti et al. 2012). The prey remains were identified in the laboratory to order and family levels when possible.

Data analysis

We compared the initial and final weights in the feeding frequency experiment using the Student's T test for paired samples. We tested the normality using the Shapiro-Wilk test. Data that

did not fit a normal distribution were logarithmically transformed. We compared the attack latency and consumption time between the most accepted prey using the non-parametric Kruskal-Wallis test because data did not fit a normal distribution or were heteroscedastic. We tested the normality using the Shapiro-Wilk test. We performed these analyses in INFOSTAT (Di Rienzo et al. 2016). We analyzed the results prey acceptance using Generalized Estimating Equations (GEE), which is a linear method that handles correlations resulting from repeated usage of the same individuals. We used GEE with binomial errors (GEE-b) to compare the acceptance of prey. We used the ''AR1'' correlation structure due to the sequential offering of prey. We performed this analysis in R (R Development Core Team 2010).

RESULTS

Feeding frequency

We found that *A. centralis* feeds every 2.02 days on average (SE = 0.11). Spiders fed on all prey types, but with a higher frequency on beetle larvae and termites. We found a significant increase in their weight after the feeding ended ($t = 8.42$; $p < 0.0001$; $n = 27$). On average, the weight of the spiders increased by 20.03% (SE = 2.37) and they fed on 0.65 prey individuals per day (SE = 0.04).

Prey acceptance

We found that the acceptance of prey differed significantly between prey types (GEE-b, $\chi^2_8 = 1 \times 10^{19}$, $p < 0.001$). The most accepted prey types were beetle larvae and termites (Fig. 1). These two prey types were accepted significantly more often than the other prey types (contrasts, $P < 0.005$). The least accepted prey were ants and beetles, acceptance of which did not differ significantly from that of cockroaches, crickets, spiders and woodlice (contrasts, $P > 0.05$). Millipedes were not accepted at all. In relation to the traits measured, we found that spiders attacked termites significantly faster than the other prey types (Fig. 2; $H = 7.85$, $p = 0.0491$), and the consumption time of termites was significantly shorter than the rest of the captured prey (Fig. 3; $H = 21.35$, $p = 0.0001$).

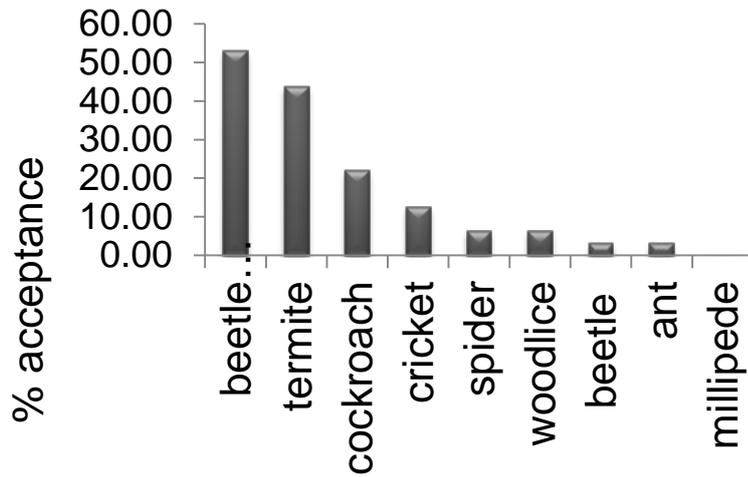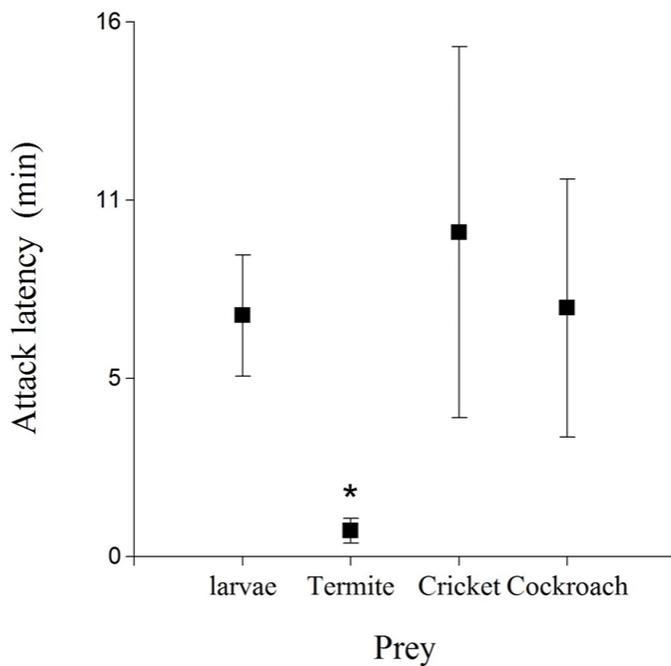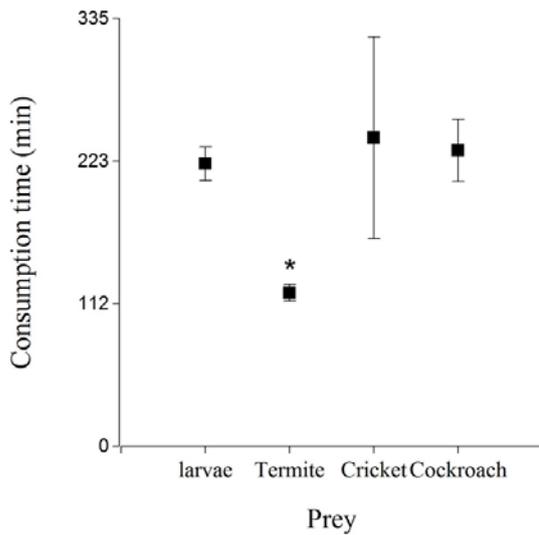**Fig. 1.** Percent acceptance for different kinds of prey by *A. centralis* in captivity.

Fig. 2. Comparison of the mean time to the first attack (± SE) for the most accepted prey: beetle larvae (*n* = 17), termites (*n* = 14), crickets, (*n* = 4) and cockroaches (*n* = 7). * Significant differences.

Fig. 3. Comparison of the consumption time (± SE) for the most accepted prey: beetle larvae (*n* = 17), termites (*n* = 14), crickets, (*n* = 4) and cockroaches (*n* = 7). * Significant differences.

Natural diet

We found 15 tunnel webs with prey remains out of the 22 tunnel-webs inspected. All the tunnel-webs found belonged to females of *A. centralis*. We also found some males, but we did not find any prey remains in their shelters. The prey was mostly destroyed in almost all cases, but some pieces - such as heads, thorax, legs, wings and spider's palp - allowed us to identify them. We identified seven prey items, ants and beetles being the most frequently found (Table 1). We also found remains of spiders in two shelters, one of which was a juvenile *A. centralis*. In one case, we found a spider female feeding on an earthworm.

Table 1. Relative frequencies of the prey items found in the tunnel-webs of *Acanthogonatus centralis*

Prey item	Relative frequency (%)
Ant	60
Beetle	40
Woodlice	20
Cockroach	13.3
Lepidoptera larvae	13.3
Spider (Lycosidae)	6.7
Spider (*A. centralis*)	6.7
Earthworm	6.7

DISCUSSION

We found that *Acanthogonatus centralis* is polyphagous, as we had predicted, because it accepts different kinds of prey. Our findings of its natural diet also corroborate this. These spiders fed very often, accepting prey every two days, and their weight showed a significant increase of 20% by the end of the experiment. In general, spiders that have a polyphagous diet feed with great frequency and consume different prey, using different nutrients from each prey (Mayntz et al. 2005). Indeed, these frequencies are similar to another mygalomorph spider, as Canals et al. (2012) found in the Andean tarantula of the genus *Paraphysa*. The authors found that, even though these spiders fed every three days, the weight gained was close to zero. In this study we found not only a more frequent capture behavior, but also a greater weight gain. Canals et al. (2012) only used one kind of prey. However, other factors could be influencing the foraging behavior in this spider species. More studies are necessary to enhance the knowledge of *A. centralis* foraging pattern.

In regard to the prey acceptance experiments, we found that the spiders accepted almost all the prey offered with the exception of millipedes. In a field study in the *A. centralis* habitat, Schwerdt et al. (2012) found that Coleoptera, Hymenoptera and Araneae were the most abundant orders of epigial arthropods. These orders were more abundant during summer, when the activity of *A. centralis* is lower (Ferretti et al. 2012). Instead, Julidae was markedly abundant during winter (Schwerdt et al. 2012), a season when *A. centralis* spiders are also active (Ferretti et al. 2012). Even though millipedes could be a potential prey to *A. centralis*, none of the spiders attacked millipedes. Termites and beetle larvae were preferred significantly by *A. centralis*. This preference may be due to the soft tissue of the bodies of these two prey items, along with their defensive passivity. Indeed, termites were captured significantly faster than the other prey. This short attack latency of termites could indicate a certain kind of preference for this prey. Unfortunately, we did not find any remains of termites among the prey remains of *A. centralis* to corroborate this preference. However, we cannot discard it as natural prey, as termites have a soft body, and so we could be underestimating termites as prey because it is more difficult to find their remains in the shelters.

Spiders spent a significantly less time consuming termites, even though they attacked it the quickest of all prey; it seems that spiders exploited termites only partially. On the other hand, ants, woodlice, adult beetles, and spiders were accepted in very low proportions (less than 5% each). This result was opposite to what we had predicted. This could be because these kinds of prey are difficult to subdue and kill, either because they are dangerous and/or present good defensive abilities (ants, beetles, spiders, and woodlice) or because they are unpalatable (woodlice and millipedes) (Carrel and Eisner 1984; Deslippe et al. 1996; Nelson et al. 2004; Pekár et al. 2011 2015). Indeed, millipedes were never attacked; these arthropods have very potent chemical defenses, being able to

induce sedation in their predators after attack (Carrel and Eisner 1984). However, Coyle and Ketner (1990) mentioned ants as the main prey item in the natural diet of the mygalomorph funnel-web genus *Ischnotele* (Dipluridae). Likewise, Pinto and Saiz (1997) found ants and spiders among the natural diet of *Acanthogonatus franckii* in Chile. Moreover, they found cockroaches, beetles, crickets and co-specific spiders as the most frequent prey. In addition, Decae et al. (2007) found remains of different prey in burrows of *Nemesia* spiders from Europe. These authors mentioned ants, beetles, and woodlice as regular prey of these spiders. Souza-Silva et al. (2014) found different prey items in another nemesiid species, *Prorachias bristowei*, mentioning ants, beetles, grasshoppers, spiders, and woodlice as prey. We also found ants and beetles as the most frequent prey item in *A. centralis* webs, indicating that females of this species accept ants in their diet, although the juveniles avoid them.

Pinto and Saiz (1997) recorded very frequent cannibalism in juveniles and adults of *A. franckii*. A previous study on agonistic behavior between females of *A. centralis* also shows very aggressive intraspecific behavior (Ferretti et al. 2014). Despite this, we did not find any clear preference for other *A. centralis* individuals, but we did not offer conspecific individuals. However, we did find spiders as prey remains of *A. centralis* females, and one of them was a juvenile of the same species. This could indicate that cannibalism occurred in the field, probably females attacking immature specimens. Cannibalism is frequent in the diet of some spider species, as feeding on conspecifics gives them a high nutritional quality food (Mayntz and Toft 2006). Nevertheless, further studies focusing on this interesting behavior are needed to try to elucidate whether cannibalism plays an essential role in the *A. centralis* diet.

CONCLUSIONS

This study is the first to examine the diet and different trophic traits in *Acanthogonatus centralis*, and one of the few studies on this topic in the family Nemesiidae. We found, as expected, that this mygalomorph spider species has a polyphagous diet. However, the analysis of the species' predatory traits, such as feeding frequency and prey acceptance, allowed us to assess some prey preferences. Despite being polyphagous, juveniles of the first instars could have a certain preference for termites, and they avoid ants, millipedes and woodlice as prey. Our findings in the field indicated that ants and beetles, and even woodlice, are regular prey of *A. centralis* females. In addition, juveniles showed no preference for attacking or consuming spiders. However, different studies and our results concerning its natural diet suggest that this spider species may capture other spiders.

Authors' contributions: GP designed the study and wrote the manuscript. GP and SC performed all the experiments. GP performed the data analysis. All authors participated in revising the manuscript. All authors read and approved the final manuscript.

Competing interests: GP and SC declare that they have no conflict of interest.

Availability of data and materials: Specimens here used are deposited in a public collection (LZI-UNS).

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Ethics approval consent to participate: Not applicable.

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