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Silk *versus* venom: alternative capture traits employed by closely related myrmecophagous spiders

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Abstract

Predators that prey on potentially dangerous species have evolved particularly effective capture traits to restrain prey. In spiders, venom and silk represent alternative traits. However, the utilization of such adaptations comes with a cost, as these substances are metabolically and ecologically costly. Based on a possible trade-off, the utilization of only one effective capture strategy should be optimised if a predator is to specialize on a single prey type.

We investigated silk and venom utilization in two *Callilepis* and one *Nomisia* species, closely related spiders from the family Gnaphosidae, feeding on ants but employing different hunting strategies. We compared their hunting efficacy and hunting strategies with emphasis on the investment in venom versus silk.

Nomisia restrained ants with silk (then bit them), while *Callilepis* relied solely on its venom. This was also reflected in trophic traits connected with silk and venom utilization: *Callilepis* had larger venom glands than *Nomisia*, meanwhile adults of *Nomisia* had more piriform silk glands than *Callilepis*. *Callilepis* was more effective as it subdued prey more quickly, presumably due to ant-specific venom. *Callilepis* and *Nomisia* handled ants from two subfamilies with different degrees of success: *Callilepis* was more successful with Formicinae ants, while *Nomisia* handled better Myrmicinae ants.

We show that investment in venom allows *Callilepis* to be more efficient in overcoming ants than *Nomisia* that uses both silk and venom. However, such specific adaptations may restrict specialised predators from utilising alternative prey.

Introduction

Spiders are the most diverse taxon of terrestrial predators (Coddington & Levi, 1991) and have evolved a great variety of predatory strategies (Cardoso et al., 2011). Approximately half of the species use webs to catch prey while the other half captures prey by gripping it with the forelegs and employing envenomation. Silk and venom thus represent two distinct capture traits, yet both are products of metabolism.

It has been proposed that venom synthesis is metabolically and ecologically costly. Several studies on snakes and one on scorpions showed that venom depletion led to an increase in metabolic rate (McCue, 2006; Pintor et al., 2010; Nisani et al., 2007). Furthermore, venom metering has been reported for snakes, spiders, and scorpions, i.e. taxa with independently evolved venom systems (Morgenstern & King, 2013). In addition, an ecological cost is associated with the time needed to produce venom, or the time spent without adequate venom stores (Young et al., 2002; Hayes, 2008; Young, 2008).

Similarly, silk is also an expensive product. In web-building spiders, the construction of a web represents a considerable initial investment in this predation strategy, as it also leads to an increase in metabolic rate (Ford, 1977). Reductions in costs have been reflected in the evolution of spider web design. For example, modern orb-weaving spiders produce less costly adhesive capture threads compared to the dry, fuzzy cribellate threads of their ancestors. Moreover, some spiders reduce costs by silk recycling (Opell, 1998). The synthesis of dragline silk produced by spiders also requires significantly more ATP than the synthesis of silks produced by herbivorous insects. Although the diets of predatory spiders are in general more protein-rich than the diets of herbivores, they are likely to be energy poor, thus it may be difficult to satisfy silk production needs (Craig et al., 1999).

As both venom and silk are composed of proteins and, therefore, amino-acids, there may be a trade-off in the utilisation of these substances. Indeed, venom has been found to be secondarily lost in uloborid spiders, which use silk to wrap their prey during capture (King, 2004). Other spiders, such as prey-specialized zodariids, rely on potent venom only (Pekár et al., 2014). Yet, most spiders seem to use both venom and silk, though in differing proportions (Olive, 1980).

According to the optimal foraging theory, foraging and prey choice are associated with benefits and costs (Davies, Krebs, & West, 2012). Predators which prey on dangerous prey often expend considerable amounts of energy on overcoming their prey and less energy on search and pursuit (Griffiths, 1980). A predator’s energy should be invested in weaponry efficient at restraining prey. Given a possible trade-off, the utilization of one effective mechanism to subdue prey should be more optimal. We hypothesise that this will be especially pronounced in specialists hunting dangerous prey as a result of greater specialization to increase the precision of an attack and to lower the associated costs.

To test this, we investigated two phylogenetically related spider genera of the family Gnaphosidae. Gnaphosids are bold predators able to subdue large and hazardous prey with the use of piriform silk (Wolff et al., 2017). Here, we focused on *Callilepis* and *Nomisia*, two ant-eating genera that employ different hunting strategies. *Callilepis* spiders are reported to be ant specialists hunting without the use of silk (Heller, 1976; Borovsky, 2012). *Nomisia* spiders hunt ants with the use of silk to immobilize them (Soyer, 1943). As both spider genera employ different strategies to subdue dangerous prey, we investigated the hunting strategies of these spiders in detail; we compared their hunting efficacies and the time investment associated with venom *versus* silk utilization. In addition, we also compared the morphological traits connected with silk and venom production.

Materials and methods

Spiders

Two species of *Callilepis* were collected on the forest edge at two sites. *Callilepis nocturna* (Linnaeus, 1758) and a few *C. schuszteri* (Herman, 1879) spiders of various stages (prosoma length 1.61 ± 0.37 mm) were collected in the valley of the Gröβer Dürrenbach river, between Villach and Klagefurt, Austria in June 2015. *Nomisia exornata* (C. L. Koch, 1839) spiders of various stages (prosoma length 2.06 ± 0.45 mm) were collected near Serpa, southern Portugal in October 2015 and 2017.

In laboratory experiments, juveniles were occasionally used as the number of adults was too low; thus, identification to species level was not possible and some data were pooled as *Callilepis* spp. Spiders used in laboratory experiments were kept in plastic vials containing moisturized gypsum and placed in a chamber at a constant temperature (22 ± 1 °C) and under a LD regime (16:8). Spiders were fed at least once a week with an ant or were allowed to consume the prey accepted in laboratory trials. Experiments were performed from July 2015 to October 2017.

All statistical analyses were performed within the R environment (R Core Team, 2017).

Capture behaviour

To compare the hunting strategies of both species, capture sequences were recorded using a high speed camera (IDT MotionXtra N3), utilizing 500 fps for *Callilepis* spp. and a lower frame rate (100 or 200 fps) for *N. exornata* in order to record the whole hunting sequence. A high speed camera was used, as the hunting actions of both *Callilepis* spp. and *N. exornata* were very quick:

prey capture took only a few seconds. Ants of the genus *Tapinoma* were used as prey for *Callilepis* spp. and ants of the genus *Messor* for *N. exornata*. The capture strategy did not vary for different ant genera in either spider (Michálek, pers. obs.). Spiders were placed individually in plastic cups (diameter 3.5 cm, height 5 cm) with gypsum on the bottom and a layer of butter on the walls to prevent escape. Each prey was introduced after 1 hour of acclimation. In total, 27 hunting videos involving *Callilepis* spp. and 22 videos involving *N. exornata* were obtained. In these videos, the following types of behaviour were distinguished: **approach** – the prey or the predator moved towards the other; **touching** – the predator gently touched the prey with its first pair of legs; **orientation** – the predator turned to face the direction in which the prey was situated; **immobile** – the predator stopped on the spot and remained without performing any other activity; **wrapping** – the predator ran around the prey and released silk, immobilizing the prey in the process; **biting** – the predator delivered a bite to the prey; **release** – the prey was released from the chelicerae; **feeding** – the predator started to consume the prey. Using this ethogram, transition matrices were created with JWatcher software (Blumstein, Evans & Daniels, 2006). Then, flow diagrams for each spider genera were made. The frequencies of bites on different body parts (leg or antenna) were compared between spiders using GLM with binomial distribution and the logit link function (Pekár & Brabec, 2016). The type of predator was used as the factor and the relative size of the prey was the covariate. The duration of contact with the prey (from the first approach to the release of the ant) was compared between both spiders using a GLM model with the Gamma distribution and a logarithmic link. Here, the type of predator and the bite site were used as factors, and the relative size of the prey was a covariate. We also compared the time that ants were held in chelicerae using GLM with the Gamma distribution. Time measurements were obtained from recorded hunting sequences using Kinovea software

(Kinovea; Version 0.8.15; Kinovea open source project, https://www.kinovea.org). To measure the stereotypy of hunting behaviour, we used Shannon entropy. Entropy estimates along with 95% confidence intervals were calculated from the transition matrices by bootstrapping with 1000 replicates for both *Callilepis* spp. and *N. exornata*.

Capture efficiency

To compare the hunting efficiencies of *Callilepis* spp. and *N. exornata* for differently sized prey, *Formica* and *Messor* ants of various sizes were offered to both spiders in a similar manner as in the acceptance trials. Individuals of *Callilepis* spp. and *N. exornata* were placed singly in Petri dishes and after acclimation the prey was offered. If the ant was not accepted within 1 hour it was replaced by a smaller one. The size of the prosoma of all spiders and the total body lengths of ants were measured under a LEICA EZ5 stereomicroscope with an ocular micrometer before experiments. In total, 30 trials (17 *Formica* ants, 13 *Messor* ants) with 23 individuals of *Callilepis* spp. and 37 trials (17 *Formica* ants, 20 *Messor* ants) with 19 individuals of *N. exornata* were performed. The difference in hunting success was analysed using Generalised Estimating Equations (GEE) from the geepack package (Halekoh, Højsgaard & Yan, 2006). GEE is an extension of the Generalised linear model (GLM) for correlated data. It was used because there were repeated measurements on each individual spider (Pekár & Brabec, 2018). GEE with binomial distribution and the logit link function was used. An AR1 correlation matrix was used to account for the temporal replications.

Morphological trophic traits

The venom glands from nine adult female individuals of *C. schuszteri* and *N. exornata* were dissected. Spiders were first anesthetised by CO_2 and the glands were placed into a drop of the physiological solution NaCl 0.9% on a glass slide. The dimensions of the glands – the widths ($2r$) and the lengths (d) – were measured using an ocular micrometer attached to an Olympus SX stereomicroscope. The volume of the gland (V) was estimated by assuming a cylindrical shape ($V = d\pi r^2$). The length of the prosoma was measured for each individual.

The anterior lateral spinnerets and silk glands from four adult female individuals of *C. schuszteri* and five juvenile individuals of *N. exornata* of similar body size to adults of *C. schuszteri* were dissected. The number of piriform glands, the number of major ampulate glands, and the length and width of the secretory part of the piriform glands were measured. The volume of the piriform glands was estimated similarly as for the venom glands. The volume of the piriform glands and not the volume of the major ampulate glands was estimated as only the piriform glands are used to restrain prey in gnaphosid spiders (Wolff et al., 2017). The length of the prosoma was also measured for each individual. The relative volumes of venom and silk glands were compared between spiders using linear model (LM).

Results

Capture behaviour

The predatory behaviour of *Callilepis* spp. began with a brief tapping of the ant's antennae with its first pair of legs, followed by a rapid bite to the antenna base and release (Fig. 1A-D, Video S1). *Nomisia exornata* used a very different tactic: first, it wrapped the prey in silk to immobilize it, and then delivered a bite (Fig. 1E-H, Video S2). *Callilepis* spp. was slightly more consistent in selecting the location of the bite than *N. exornata* (GLM, $F_{1,48} = 40.2$, $P = 0.05$): the prey was bitten more often on the antenna (93%, N = 27) than on the leg (7%). When the ant was bitten on the antenna, it was always on its base. *Nomisia exornata* also bit the prey on the antenna in most cases. However, unlike *Callilepis* spp., it bit the ant on the distal part of the antenna and, in 27% of cases, the ant was also bitten on the distal part of the leg (N = 22). The prey size did not affect selection of the bite site (GLM, $F_{1,47} = 39.3$, $P = 0.33$).

The Shannon entropy of behavioural sequences (Fig. 2) differed significantly between *Callilepis* spp. and *N. exornata*: the entropy estimate for *Callilepis* spp. sequences was 2.39 (CI_{95} = 2.07, 3.08), while for *N. exornata* it was 5.59 (CI_{95} = 4.89, 7.38); therefore, the behaviour of *Callilepis* spp. was more stereotypical.

The duration of total hunting activity was significantly shorter for *Callilepis* spp. (GLM, $F_{1,47} = 142.6$, $P < 0.0001$): the mean hunting time was 1.18 s (CI_{95} = 0.99, 1.43) for *Callilepis* spp., while it was 6.66 s (CI_{95} = 5.45, 8.25) for *N. exornata*. The mean duration of prey wrapping for *N. exornata* was 1.34 s (CI_{95} = 1.00, 1.86). The mean duration of the bite was also significantly shorter for *Callilepis* spp. (GLM, $F_{1,47} = 294.5$, $P < 0.0001$): it took 0.24 s (CI_{95} = 0.20, 0.30) for *Callilepis* spp., and 3.95 s (CI_{95} = 3.20, 4.97) for *N. exornata* (Fig. 3). Furthermore, the duration of the bite was significantly influenced by the interaction between the type of predator and the bite site (GLM, $F_{1,45} = 4.1$, $P < 0.05$). *Callilepis* spp. spent less time biting the leg (0.10 s, CI_{95} = 0.05, 0.23) than biting the antenna (0.25 s, CI_{95} = 0.21, 0.31), while *N. exornata* spent more time biting the leg (4.28 s, CI_{95} = 2.90, 6.70) than biting the antenna (3.83 s, CI_{95} = 3.00, 5.00).

Capture efficiency

The capture success on ants changed differently in *Callilepis* spp. and *N. exornata* with the relative prey/predator size ratio and type of ant prey (GEE, $\chi^2_1 = 5.0$, $P < 0.05$). *Callilepis* spp.

was more successful in handling larger Formicinae (*Formica*) ants than *N. exornata* (Fig. 4A). *Callilepis* spp. captured *Formica* ants with a 50% success rate at an ant body length/spider prosoma length ratio equal to 8.52, while *N. exornata* achieved a similar success at a ratio of 2.17. However, *N. exornata* was more effective in handling large Myrmicinae (*Messor*) ants (Fig. 4B): it captured *Messor* ants with a 50% success rate at an ant body length/spider prosoma length ratio equal to 8.08, while *Callilepis* spp. achieved similar success at a ratio of 3.87.

Morphological trophic traits

The relative sizes of venom glands differed significantly between *N. exornata* and *C. schuszteri* (LM, $F_{1,16} = 35.8$, $P < 0.0001$): venom glands of *C. schuszteri* were 1.65 times larger than those of *N. exornata* (Fig. 5). As for the spinning apparatus, *C. schuszteri* and *N. exornata* did not differ in their numbers of piriform glands (LM, $F_{1,16} = 3.8$, $P = 0.07$), which varied between two and four. Both *C. schusteri* and *N. exornata* had one functional major ampulate gland on each spinneret. There was also no significant difference in the relative volume of piriform glands between *C. schuszteri* and *N. exornata* (LM, $F_{1,44} = 0.5$, $P = 0.5$, Fig. 5).

Discussion

Both *Callilepis* and *Nomisia* subdued ants from two subfamilies (Formicinae, Myrmicinae), but with different degrees of success: *Callilepis* handled Formicinae ants more efficiently, while *Nomisia* was more successful with Myrmicinae ants. As the defences of these two ant subfamilies differ markedly (Formicinae use agility and formic acid, Myrmicinae use stings and powerful mandibles), the hunting strategies of the two spider genera in question seem to be adapted to overcome the defences of the preferred prey. The hunting strategy of *Callilepis* spiders may be specially tuned to subdue Formicinae ants, which were more effectively captured than Myrmicinae ants. Cuticle thickness varies among ants; Myrmicinae ants (e.g. *Messor, Tetramorium*) have on average relatively thicker cuticles than Formicinae ants (e.g. *Lasius, Camponotus*) (Peeters et al., 2017). Perhaps it is difficult for *Callilepis* to penetrate such thicker cuticles with its swift bite; therefore, it has higher success with less sclerotized ants. The use of silk may be a more efficient strategy against Mymricinae ants, which were subdued by *N. exornata* more efficiently than Formicinae ants. Also, the use of silk appears to be safer. We observed at least two attacks on *Callilepis* spiders by *Formica* and *Camponotus* ants resulting in the loss of a leg or even death (Video S3). Meanwhile, no *N. exornata* spiders were killed by ants.

It took *N. exornata* a relatively long time to subdue ants. Most apparently, the ant was held in chelicerae for a considerable period. Spiders can adjust the amount of venom injected (Wigger, Kuhn-Nentwig & Nentwig, 2002) while holding prey in chelicera (Morgenstern & King, 2013; Boevé, 1994). However, long envenomation represents a greater risk, particularly when subduing a dangerous prey as it has a longer time to retaliate. Predators can minimize this risk behaviourally by minimizing contact or shortening the handling time and also by selecting the direction and position of an attack (Mukherjee & Heithaus, 2013). For example, ant-specialized *Zodarion* spiders bite ants on the most extended leg (Pekár, 2004). This behaviour may lower the risk even more, as the spider keeps a greater distance from a dangerous prey. *Callilepis* and *Nomisia* dealt with this task in a different way. *Nomisia exornata* reduced the risk by first restraining the prey with silk, then biting the ant on the distal part of the antenna or leg. Yet, silk production is an additional cost. Furthermore, envenomation still plays a significant role in *N. exornata*, as the time spent biting was longer than the time spent wrapping. In contrast, *Callilepis* spiders use only venom.

As the bite delivered by *Callilepis* spiders was very short, we suppose its venom to be especially potent towards ant prey. It is possible that the venom of specialist spiders is tailored more closely to their specific prey taxon (Kuhn-Nentwig, Stocklin & Nentwig, 2011). The venom of specialists is less diversified in its composition (Pekár et al., 2018), thus the synthesis of such venom may be less costly. It has been confirmed that the venom composition of *Conus* snails is connected to the level of specialization, as the venom of specialized *Conus* snails contains fewer conotoxins than that of generalist species of the same genus (Remigio & Duda, 2008).

The bite of *Callilepis* spiders was delivered to the base of the ant's antenna. This bold behaviour probably also facilitates quicker immobilization, as the venom is injected close to nerve ganglions in the head capsule of the ant. The spider *Oecobius annulipes* Lucas, 1859 also bites ants at the base of the antenna, but in this case the ants are first immobilized with silk (Glatz, 1967). *Callilepis* spiders tapped approaching ants on the head or antennae before biting them, presumably to identify the bite site. Biting the antennae had, in particular, a significant effect on the response of Formicinae, which are more agile than Myrmicinae. The bitten Formicinae ant moved in circles so that it could not escape after release by the spider (Video S3). Wrapping in silk also prevents the escape of prey. Although similar touching behaviour was observed in *N. exornata* in several cases, this spider also touched the ant on other body parts.

The hunting strategy of *Callilepis* spiders was very conservative and stereotyped when compared to *N. exornata*. Heller (1976) noted that *Callilepis* spiders are not able to envenomate ants with removed antennae, although, here, we observed two cases of leg biting. However, in one case, the ant's leg was in close proximity to the ant's antenna and in the second case the *Callilepis* spider almost immediately changed the bite site to the antenna. We observed a similar pattern in hunting precision in araneophagous spiders (Michálek et al., 2017). When the prey is dangerous, any mistakes could have a significant impact on predator survival (Mukherjee & Heithaus, 2013). As a result, specialization may lead to greater accuracy in prey capture (Ferry-Graham et al., 2002) and subsequently to overall stereotypy. Evidence gathered in this study shows that *Callilepis* spiders are more specialized, as their hunting strategy is ant-specific. Also, *Callilepis* spiders need to be more precise, as ants are not immobilized with silk and thus remain dangerous during the bite. On the other hand, *N. exornata* is less specialised, as its hunting strategy is more complex and thus generalized.

Prey immobilization with silk is a common strategy of gnaphosid spiders. Morphological and functional modification of the spinning apparatus allows them to subdue large and dangerous prey, such as spiders (Wolff et al., 2017). However, it appears that the use of silk for immobilization is not advantageous for specialist spiders. Araneophagous *Lampona murina* L. Koch, 1873 does not use silk but venom for prey capture (Michálek et al., 2017). Wolff et al. (2017) argue that araneophagy may have evolved earlier than spinneret modification in Gnaphosidae. However, ant-specialized *Callilepis* spiders do not use silk at all, while less specialized *N. exornata* spiders do. As *Callilepis* spiders rely only on venom, its venom glands are larger than in *N. exornata*. Alternative capture strategies or dietary shifts may lead to morphological and physiological alterations, such as reduced venom glands in some snakes or uloborid spiders (Fry et al., 2008; King, 2004). Similarly, *Callilepis* spiders may have evolved atrophied spinning apparatus in order to allow greater investment in the venom system. Here, we found that the number and volume of piriform glands do not differ between *C. schuszteri* and *N. exornata* and that the number of piriform glands is lower compared to other gnaphosids (Wolff et al., 2017). However, we compared juveniles of *Nomisia* with adults of *Callilepis*. In adults of

Nomisia there are at least four active piriform glands according to the number of piriform spigots on anterior lateral spinnerets (Platnick, 1990). Therefore, considering adult stages, the piriform glands of *Callilepis* are reduced in number compared to those of *Nomisia*. Swathing with silk probably represents an efficient generalized hunting strategy towards dangerous prey in gnaphosid spiders, but it is not used on harmless prey as it is too costly (Wolff et al., 2017). Predators specialized exclusively on dangerous prey may thus prefer investment in other means of prey capture. Although a study on wandering and web-building *Tetragnatha* spider species has shown that they do not differ in the amount of venom (Binford, 2001), here we discovered that *C. schuszteri* has larger venom glands than silk-utilizing *N. exornata*.

Overall, both spider genera were able to subdue ants, but *Callilepis* was more efficient, as it required less time to overcome an ant and it only relied on its venom, in contrast to *N. exornata*, which utilized both venom and silk. Yet, the strategy of *N. exornata* is safer, as silk-restricted ants cannot retaliate. Strict specialization on a certain prey type may enhance the pronounced utilization of one strategy (and subjugation mechanism), allowing a reduction in the energy needed to subdue prey. However, such specific adaptations restrict a predator from utilizing alternative prey. Indeed, *Callilepis* was not so successful at subduing Myrmicinae ants compared to Formicinae ants. *Nomisia exornata* maintained the ability to capture alternative prey, with or without the use of silk depending on the prey's dangerousness (Wolff et al., 2017).

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Competing interests

The authors declare no competing interests.

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Figures

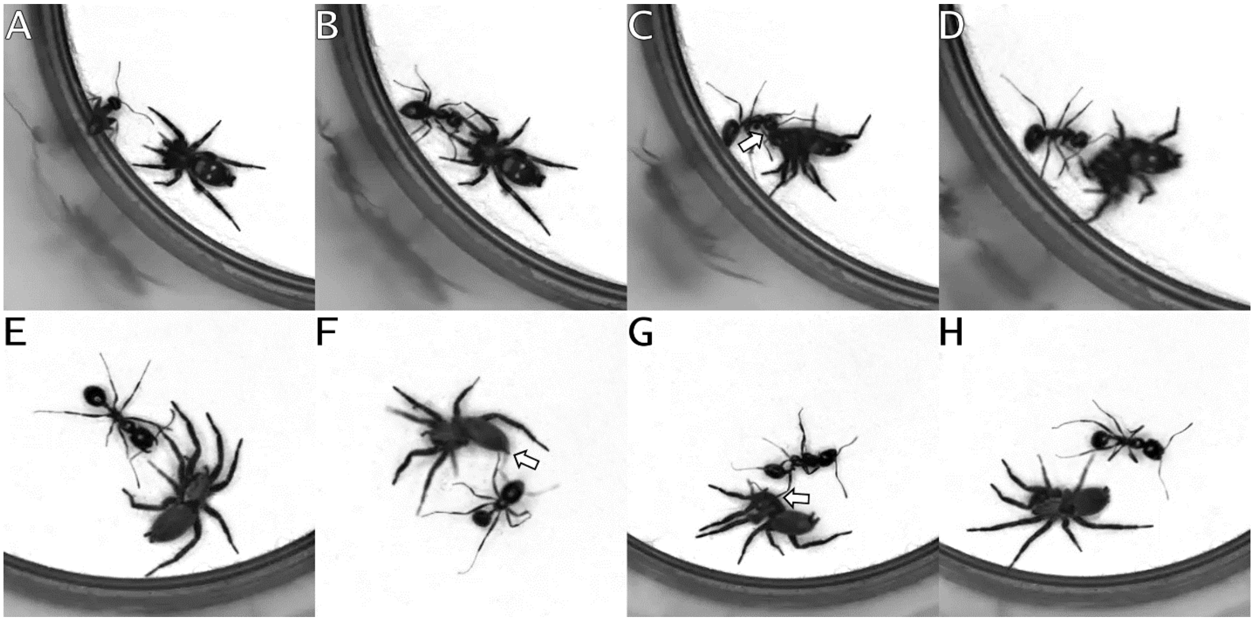

Figure 1. Elements of the predatory behaviour of *Callilepis* spp. (**A-D**) and *Nomisia exornata* (**E-H**) in detail. **A.** *Callilepis* approaches the ant and raises its forelegs. **B.** It gently touches the antennae of the ant with the first pair of legs. **C.** It lunges forward and bites the ant at the base of antenna (arrow). **D.** The prey is released and *Callilepis* waits nearby until the ant is paralyzed. **E.** *Nomisia* approaches the ant. **F.** It runs around the ant, turning its abdomen and spinnerets toward the ant (arrow), and releases silk, immobilizing the ant in the process. **G.** It bites the immobilized ant on the leg (arrow). **H.** The prey is released and *Nomisia* waits until the ant is paralyzed.

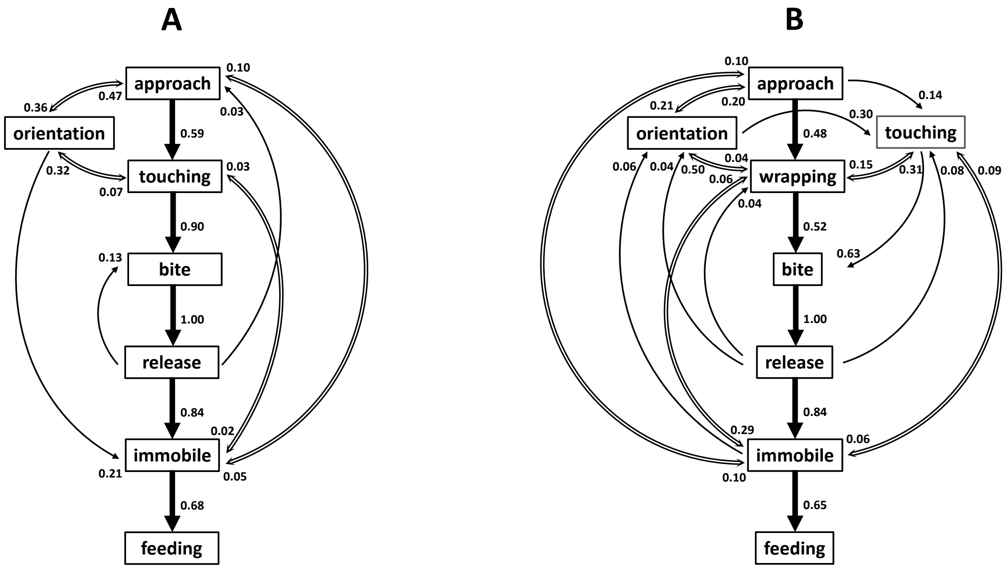

Figure 2. Flow diagrams of the prey capture behaviour of *Callilepis* spp. (**A**) and *Nomisia exornata* (**B**). Transition probabilities are shown for each transition.

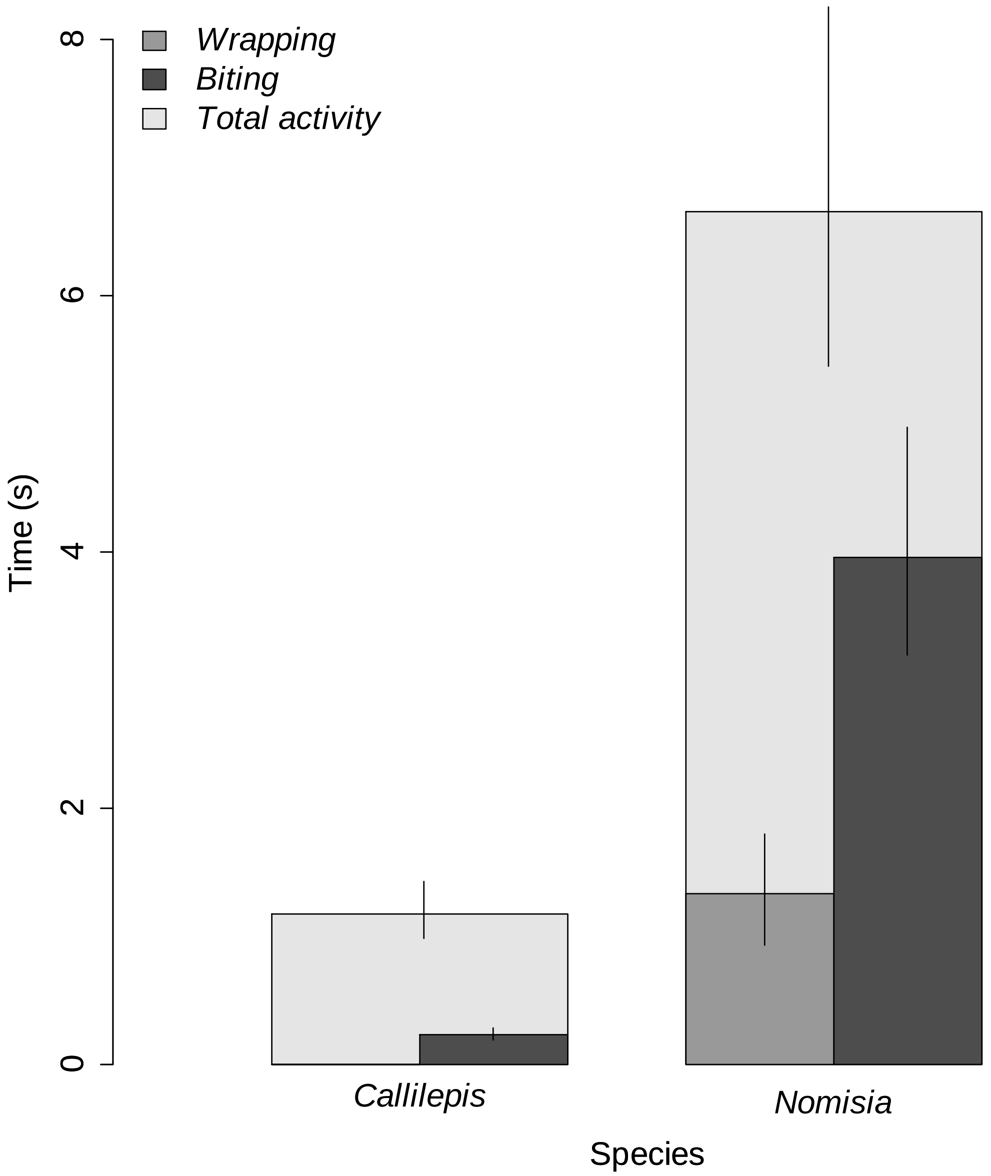

Figure 3. Comparison of the total hunting activity by *Callilepis* spp. and *Nomisia exornata*, including the time spent wrapping and biting an ant. Bars are means, vertical lines represent 95% confidence intervals.

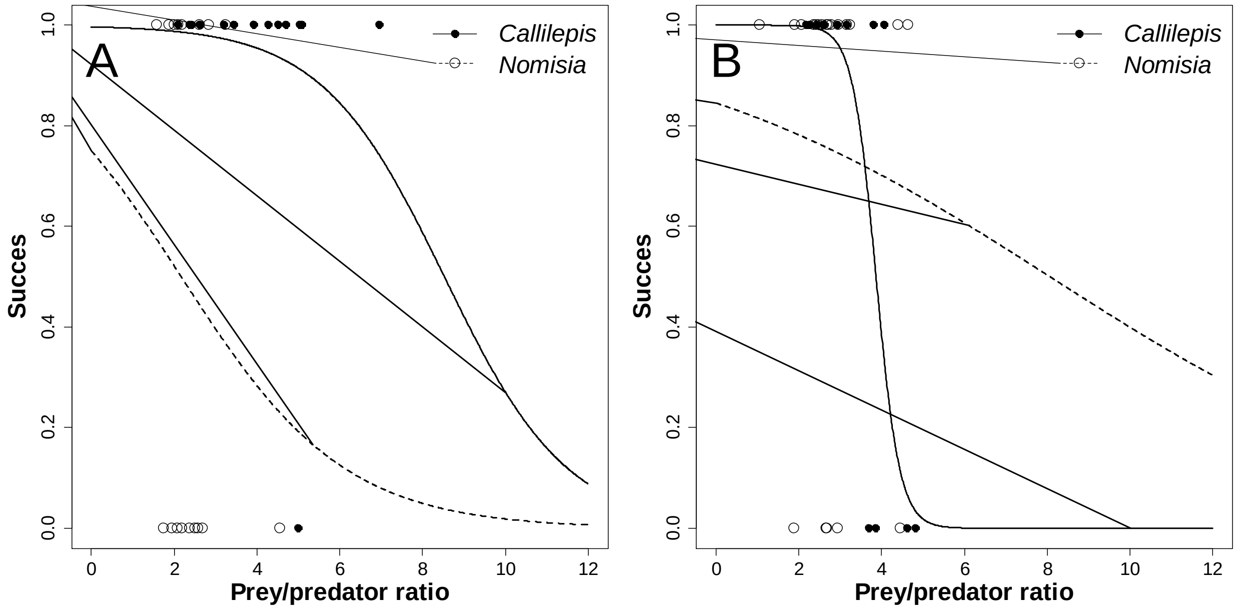

Figure 4. Comparison of the capture success of *Callilepis* spp. and *Nomisia exornata* on *Formica* ants (**A**), and *Messor* ants (**B**) of various relative sizes (prey to predator body size ratio). Estimated logit models are shown.

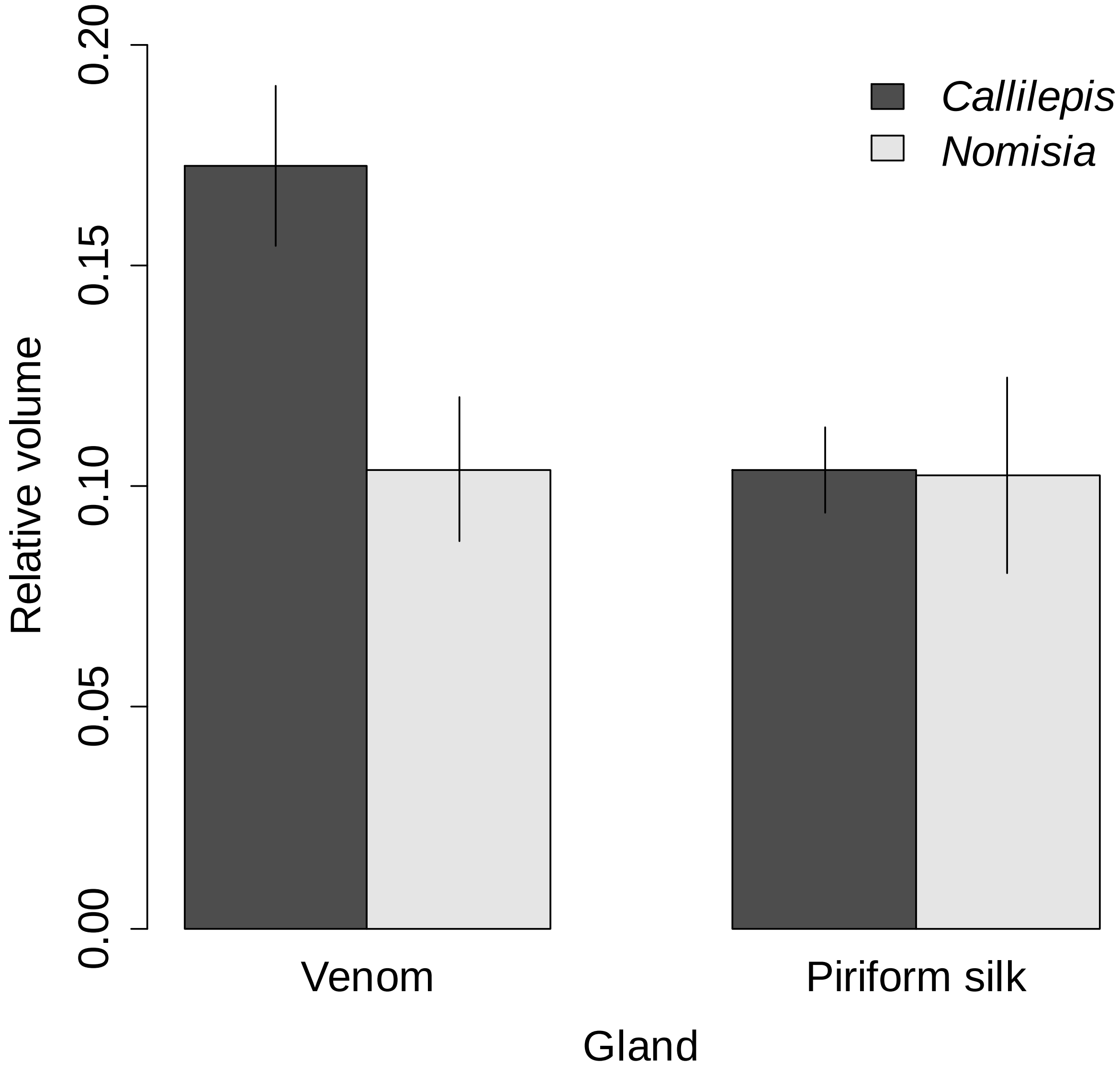

Figure 5. Comparison of the relative volumes of the venom glands and piriform silk glands of *C. schuszteri* and *N. exornata*. Bars are means, vertical lines represent 95% confidence intervals.

Supplementary material

Video S1. Capture of an ant by *Callilepis* sp. recorded using a high speed camera (IDT MotionXtra N3) at 500 fps.

Video S2. Capture of an ant by *Nomisia exornata* recorded using a high speed camera (IDT MotionXtra N3) at 100 fps.

Video S3. Prey capture by *Callilepis* sp.