ORIGINAL ARTICLE

Tracing the evolution of trophic specialisation and mode of attack behaviour in the ground spider family Gnaphosidae

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Abstract
The evolutionary history of prey specialisation differs among spider species, particularly among active wandering species which have evolved a variety of prey-capture tactics. Here, we conducted a comparative analysis of prey specialisation and prey capture behaviour in Gnaphosidae. We used nine species each representing a different genus and investigated their acceptance of spiders and ants as prey, on which they may specialise, and their attack behaviour. Then we collected such data for another about 20 species from literature. The studied species used only either biting or silk (followed by biting) to constrain prey during attack. For each species, we measured selected morphological characteristics—specifically, the relative sizes of cheliceral fangs and spinnerets as well as the number of spigots on spinnerets—and related them to the ability to catch spiders (araneophagy) and ants (myrmecophagy) and mode of attack behaviour. We found the relative fang size to be significantly shorter for myrmecophagous species. Other traits were not related to prey specialisation or attack behaviour. They used silk particularly for larger prey. Use of silk was a conditional strategy in some species. We reconstructed the phylogenetic relationships among the studied genera using molecular and morphological data. We found that araneophagy was frequent but myrmecophagy was rare among recent taxa. Comparative analysis revealed that araneophagy is an ancestral state, while myrmecophagy was less likely and repeatedly lost. The use of silk for prey immobilisation was also as likely as unlikely for ancestors and has been repeatedly lost.

Keywords Araneophagous · Generalist · Ground spiders · Myrmecophagous · Prey-capture

Introduction

Spiders are the most taxonomically diversified group of terrestrial predators and have evolved a great variety of prey capture strategies (Cardoso et al. 2011). The majority of spider species are euryphagous (Pekár et al. 2012) i.e. they capture a taxonomically diverse prey range. These species have evolved generalized prey-hunting behaviour that includes a variety of hunting tactics which are effective in capturing different prey types (e.g. Harland and Jackson 2006). The use of these prey-capture tactics is often conditional upon the type of prey and its defensive characteristics, such as dangerousness or size (Mukherjee and Heithaus 2013).

Only about 5% of spider species are stenophagous and specialised. Specifically, spiders specialize on the following prey types: spiders, ants, woodlice, termites, moths, and flies (Pekár and Toft 2015). Recent comparative analysis of the breadth of trophic niche in spiders revealed that various prey specialisations have different phylogenetic histories (Pekár et al. 2012). For example, while araneophagy appears to be a trait that has evolved at the base of phylogeny of some spider families, myrmecophagy appears to be a derived state, and present only in some families.

Gnaphosidae, with 159 genera and 2539 species, are the fourth most specious family of spiders (World Spider Catalogue 2019). Gnaphosid spiders are assumed to be generalist, free-hunting, non-web-building spider predators (Cardoso et al. 2011). For a long time, few gnaphosids were known to prey on ants and other spiders (Bristowe 1958; Jarman and Jackson 1986) but only recent studies showed that some species have specialised on these prey types (Michálek

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et al. 2017, 2018; Petráková Dušátková et al. 2020). Both of these prey types represent dangerous prey.

Specialisation on dangerous prey requires the use of specialised adaptations to decrease the chances of retaliation. This includes morphological, behavioural, and venomic traits (Pekár and Toft 2015). For example, enlarged chelicerae, a thick cuticle, and elongated spinnerets (Michálek et al. 2017; Jackson 1992; Wood et al. 2012; Pekár and Toft 2015; Wolff et al. 2017) characterize araneophagy, while shorter chelicerae and spinnerets, and specific venom composition are relevant to myrmecophagy (Kuhn-Nentwig et al. 2011; Michálek et al. 2017, 2018; Pekár et al. 2018a).

To immobilize dangerous prey, gnaphosid spiders use two distinct attack tactics: in the main, they rely either on the use of silk in conjunction with biting, or on the use of venom alone via biting (Wolff et al. 2017). When using the bite tactic without silk, the prey is constrained by the forelegs and then immobilized by a bite (Heller 1974; Michálek et al. 2018). Grasping prey with legs has many disadvantages because the prey can harm the predator. The application of silk, on the other hand, prevents counterattack. When using the silk attack tactic, spiders first extrude sticky silk from piriform glands and apply it on the legs and head of prey to constrain it, and only then deliver a bite.

So far, only a few studies have investigated the trophic ecology of gnaphosid spiders (Bristowe 1958; Grimm 1985; Jäger 2002; Jarman and Jackson 1986; Pekár et al. 2012; Michálek et al. 2017), so very little is known about their trophic specialisation or prey capture behaviour. This is probably because most species occur in litter, appear to have nocturnal activity, are difficult to identify, and are very fast. Also, the taxonomic definition of the family has not yet stabilized (Azevedo et al. 2018).

There is an ongoing controversial discussion on the evolution of foraging webs in spiders. Fernández et al. (2018) found that foraging webs have evolved several times repeatedly from a webless ancestor. But Coddington et al. (2019) found that foraging webs were instead repeatedly lost and the ancestor used silk to catch prey. So, investigation of traits like use of silk in prey capture in such a diversified family has the potential to elucidate evolutionary history also in other spider families.

Our aim here was to trace the evolution of two prey specialisations and the use of silk in attack behaviour in the family Gnaphosidae. We investigated the acceptance of spider and ant prey and the level of prey specialisation in nine species of Gnaphosidae which had not previously been studied and recorded the way these spiders attack prey. Then we combined our data with published data on another gnaphosid and closely related non-gnaphosid species and related them to selected morphological traits in order to reveal whether ability to catch spiders and ants can be predicted by some morphological traits. Finally, we reconstructed phylogenetic relationships among study species using molecular and morphological data. Using comparative methods, we investigated the histories of araneophagy and myrmecophagy, and attack behaviour in Gnaphosidae in order to reveal evolutionary pathway of prey specialisation.

Material and methods

Material

We collected nine species of gnaphosid and closely related spiders at different sites across the globe (Table 1). Specimens at different ontogenetic stages (except for adult males) were collected by hand either under stones or under bark. Collected specimens were placed singly in Eppendorf tubes (2 ml), and together with a piece of wet paper tissue, were put in a plastic bag and transported to the laboratory. Spiders were kept in a chamber at a low temperature (10 °C) and under a short-day regime (LD = 8:16) prior to their use in experiments in order to slow their ontogenetic development and thus be available for a period needed to run the feeding trials. They were fed at least once a week with fruit flies ad libitum or were allowed to consume the prey accepted in laboratory trials to standardize their satiation level (see below).

Spiders were identified using Nentwig et al. (2019) and Dippenaar-Schoeman et al. (2010). After identification, the prosoma length of all individuals was measured to the nearest 0.1 mm using an ocular micrometre using a stereomicroscope. Valid species names were checked against World Spider Catalog (2019).

Prey acceptance

To investigate whether spiders accepted ants and spiders as prey, we conducted feeding experiments with three types of prey: ants, spiders, and flies. For ants, we used *Lasius flavus* Fabricius (mean body length ± SE, 2.64 ± 0.33 mm) and for spiders, we used *Pardosa* sp. and *Xysticus* sp. (mean prosoma length ± SE, 1.68 ± 0.42 mm). As a control prey, we used *Drosophila* flies (*Drosophila melanogaster* Meigen and *Drosophila hydei* Sturtevant) (mean body length ± SE, 2.32 ± 0.17 mm). Ants and prey spiders were collected outside at the university campus. Ants were kept in a plastic container half-filled with soil from the nest. A test tube filled with water and plugged with a cotton ball was placed in the container. Prey spiders were kept in plastic containers/tubes with a piece of moist paper tissue. Both prey animals were held under the following conditions 4 °C, LD = 8:16. Flies came from a breeding culture.

During experiments, spiders were kept at room temperature (23 ± 1 °C). Spiders were deprived of prey for 1 week before being used in behavioural trials. Individuals were placed

Table 1 List of spider species used in the prey capture experiment, the number of specimens used, and their origin

Species	Number of individuals	Country: site
Asemesthes ceresicola Tucker	9	South Africa: Ndumo Nature Reserve
Drassodes lapidosus (Walckenaer)	19	Czechia: Mohelno
Gnaphosa lucifuga (Walckenaer)	15	Czechia: Mohelno
Haplodrassus silvestris (Blackwall)	10	Czechia: Senorady
Hemicloea sundevalli Thorell	13	Australia, NSW: Sydney
Pterotricha sp.	13	Israel: Sde Boker
Scotophaeus scutulatus (L. Koch)	6	Czechia: Dolní Kounice
Trichothyse hortensis Tucker	10	South Africa: Ndumo Nature Reserve
Zelotes latreillei (Simon)	18	Czechia: Senorady

singly in Petri dishes (diameter 30 and 50 mm, depending on their body size) and the trial began after at least 1 h of acclimation. If the prey was not attacked within 1 h, it was considered rejected and was replaced by another prey. The trial ended when a spider killed and consumed a prey. If a spider did not accept any prey type, it was considered unmotivated to eat (i.e. satiated or preparing to moult). For each individual, trials were performed approximately in 1-week intervals. Each prey type was offered to each spider individual only once so the design was randomised blocks. Before each trial, we measured the prosoma length of the spider predator and the total body length of each prey using an ocular ruler under a Leica stereomicroscope. Acceptance/rejection was recorded and the proportion of accepted prey was estimated for each species.

The hunting sequence was recorded using a video camera (Canon Legria HF R606). From the video recordings, predatory behaviour, particularly the immobilization tactic (use of silk and/or bite), was recorded. The relative frequency of using an attack tactic was estimated for each species.

The use of a tactic was modelled to the relative prey/predator body size by means of generalized linear mixed models (Pekár and Brabec 2019) with binomial setting and logit link (GLMM-b). To account for measurements nested within species, we set species as random effects and fitted GLMM with a semiparametric generalized additive model from the mgcv package (Wood 2006).

We searched the literature for information on the prey acceptance and attack behaviour of gnaphosid and non-gnaphosid genera from closely related families (Ammoxenidae, Gallieniellidae, Lamponidae, Phrurolithidae, and Trochanteriidae) and found such data for another 14 gnaphosid and five non-gnaphosid species (Table 2).

Morphology

For 21 species each representing a different genus (*Aphantaulax seminigra*, *Asemesthes lineatus*, *Callilepis nocturna*, *Cesonia bilineata*, *Drassodes lapidosus*, *Drassodex heeri*, *Eilica bicolor*, *Gnaphosa lucifuga*,

Haplodrassus hiemalis, *Hemicloea sundevalli*, *Herpyllus ecclesiasticus*, *Kishidaia conspicua*, *Micaria fulgens*, *Nomisia exornata*, *Poecilochroa senilis*, *Pterotricha elegans*, *Scotophaeus quadripunctatus*, *Sergiolus capulatus*, *Trichothyse hortensis*, *Zelanda erebus*, and *Zelotes ungula*), we measured three morphological traits that are used during prey capture and thus potentially related to prey specialisation: (1) relative fang length i.e. length of cheliceral fang divided by the width of the basal cheliceral segment; (2) number of spigots on anterior lateral spinnerets (ALS), and (3) relative length of ALS i.e. length of spinneret divided by the width of spinnerets area (Table 3). All measurements were performed on illustrations presented by Murphy (2007) which depict females.

First, we subjected all three morphological traits to multivariate redundancy analysis (RDA) from the vegan package (Oksanen et al. 2017) in order to explain their variation using three predictors: myrmecophagy, araneophagy, and silk attack. Measurements were column-scaled prior to analysis. Then, we tested the relationship between the three traits and most closely related predictor using generalized least squares (GLS) with a phylogenetic correlation structure (Pekár and Brabec 2016). The phylogenetic correlation structure, constructed from updated phylogenetic relationship (see below), was based on the Brownian motion model of character evolution (Paradis 2006). The measurements were logarithmically transformed prior to analysis in order to meet GLS assumptions.

Phylogeny

Currently, a single phylogeny of the family Gnaphosidae, based on morphological characters, is available (Azevedo et al. 2018). As it is lacking some genera that we used in our study, we aimed to reconstruct an updated phylogenetic relationship hypothesis of gnaphosid spiders combining molecular and morphological data. For this purpose, we used 20 gnaphosid species and five non-gnaphosid representatives from closely related families (Azevedo et al. 2018) which

Table 2 List of species for which data on prey and capture behaviour were found in the literature

Family/species	Source
Ammoxenidae	
Ammoxenus amphalodes Dippenaar et Meyer	Petráková et al. (2015)
Gallieniellidae	
Galianoella leucostigma (Mello-Leitão)	Goloboff (2000)
Gnaphosidae	
Aphantaulax sp.	Van der Berg & Dippenaar Schoeman 1991
Callilepis nocturna (Linnaeus)	Heller (1974), Michálek et al. (2018), Petráková Dušátková et al. (2020)
Cesonia bilineata (Hentz)	Platnick and Shadab (1980)
Drassodex cf. *heeri*	Wolff et al. (2017)
Eilica sp.	Nanoon (1982), Goloboff (2000)
Eilica albopunctata (Hogg)	Atkinson (2015)
Herpyllus ecclesiasticus Hentz	Guarisco (2007)
Kishidaia conspicua (L. Koch)	Pekár (unpublished)
Micaria sociabilis Kulczyński	Platnick and Shadab (1988), Pekár and Jarab (2011)
Molycria quadricauda (Simon)	Atkinson (2015)
Nomisia exornata (C. L. Koch)	Michálek et al. (2018)
Poecilochroa senilis (O. P-Cambridge)	Whitehouse and Lubin (1998), Michálek et al. (2019)
Prodidomus rufus Hentz	Pekár (unpublished)
Sergiolus capulatus (Walckenaer)	Guarisco (2007)
Scotophaeus scutulatus (L. Koch)	Jäger (2002), Wolff et al. (2017)
Zelanda erebus (L. Koch)	Jarman and Jackson (1986), Levy (1999)
Wydundra carinda Platnick & Baehr	Atkinson (2015)
Lamponidae	
Lampona murina L. Koch	Michálek et al. (2017)
Phrurolithidae	
Phrurolithus festivus (C. L. Koch)	Pekár and Jarab (2011)
Trochanteriidae	
Hemicloea sp.	Anonymous (2006), Pekár et al. (2017)

have sequences available in GenBank (mitochondrial 12S, 16S and COI, nuclear H3, 18S and 28S; Table S1). Two species (*Drassodex simoni*, *Pterotricha* sp.) were barcoded de novo during this study (see below) and for one species (*Galianoella leucostigma*, Gallieniellidae), sequences were not available, therefore, we used sequences of a different species (*Gallieniella betroka*) of this family. For morphological data, we used 248 characters extracted from the matrix used by Azevedo et al. (2018). For a single species, *Kishidaia conspicua*, only morphological data were available.

All the material used for DNA analysis for this study were alcohol-preserved (70% ethanol). DNA was extracted using E.Z.N.A.® Tissue DNA Kit (OMEGA BIO-TEK) following the manufacturer's protocol. Individual specimens were rinsed in PBS buffer, placed in sterile tubes, and incubated overnight at 56 °C with proteinase K. PCRs (total volume = 20 μL) were

performed using barcoding primers for COI by Folmer et al. (1994).

Amplified products were purified using the QIAquick PCR Purification Kit (QIAGEN). Sequencing was carried out with BigDye Terminator ver.3.1 (Applied Biosystems, Foster, CA) on an ABI 3100 genetic analysis sequencer (Perkin Elmer Applied Biosystems, Norwalk, CT). All sequences were assembled and edited in SEQUENCHER 4.8 (Gene Codes Corporation, Ann Arbor, MI). GenBank accession numbers for the sequences are listed in Table S1.

All the sequences were aligned using MAFFT version 7 (Katoh and Standley 2013) on the MAFFT server (http://mafft.cbrc.jp/alignment/server/). The method (L-INS-I) was automatically selected by the software according to the size of sequences being aligned. The resulting alignments were visually inspected and manually refined in MEGA 7 (Kumar

Table 3 Measures of three morphological traits for 21 species used in the analysis. Data are based on Murphy (2007)

Species	Relative	ALS	
	Fang length	No. of spigots	Relative length
Aphantaulax trifasciata (O. Pickard-Cambridge)	1.31	4	0.93
Asemesthes lineatus Purcell	1.06	5	0.86
Callilepis nocturna	1.09	5	0.73
Cesonia bilineata	1.14	6	0.72
Drassodes lapidosus	2.32	12	1.07
Drassodex heeri (Pavesi)	1.00	18	0.63
Eilica bicolor Banks	1.01	4	1.12
Gnaphosa lucifuga	1.08	14	0.92
Haplodrassus hiemalis (Emerton)	1.20	9	0.81
Hemicloea sundevalli Thorell	1.13	6	0.73
Herpyllus ecclesiasticus	1.21	8	0.30
Kishidaia conspicua	1.35	8	1.25
Micaria fulgens (Walckenaer)	1.39	3	1.06
Nomisia exornata	1.01	8	0.64
Poecilochroa senilis	1.39	6	0.75
Pterotricha elegans (C. L. Koch)	0.93	4	0.73
Scotophaeus quadripunctatus (Linnaeus)	1.18	15	0.92
Sergiolus capulatus	1.92	6	0.77
Trichothyse hortensis	1.49	8	0.35
Zelanda erebus	1.24	10	0.56
Zelotes natalensis Tucker	1.28	8	0.80

et al. 2016) when necessary. The resulting dataset included 5808 characters, which were combined and concatenated with 248 morphological characters.

We simultaneously tested the concatenated dataset using Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) approaches. The matrix was rooted using *Gallieniella betroka*.

To evaluate the best fit model for the model-based analyses, all the gene partitions (including the third positions of COI) were tested separately and then evaluated in MrModeltest v.2.2 (Nylander 2004) using both hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC). For all the partitions (unlinked), we used GTR + Γ + I as the best fitting evolutionary model (Rodriguez et al. 1990) in model-based analyses.

Bayesian inference as implemented in MrBayes version 3.2.6. (Huelsenbeck and Ronquist 2001) was carried out on the CIPRES computer cluster (Cyber-infrastructure for Phylogenetic Research; San Diego Supercomputing Center, Miller et al. 2010), with nucmodel = 4by4, ngen = 15mil, samplefreq = 1000, nruns = 2, and nchains = 4. Burn-in was set to 30%. All parameters were unlinked across partitions. The convergence of the runs was assessed by checking the potential scale reduction factor (PSRF) values of each parameter (in all cases, approaching 1.000) and the standard deviation of split frequencies (< 0.01) in MrBayes.

ML analyses were conducted in Garli v.2.0 (Zwickl 2006). Two independent runs of five million generations were carried out using the default automated stopping criterion. Nodal support was assessed using a non-parametric bootstrap with 500 replicates.

MP analysis was performed using TNT v. 1.5 (Goloboff and Catalano 2016; Goloboff et al. 2008) with the following parameters: new technology search, level 50, initial addseqs = 9, and find minimum tree length five times. Nodal support was assessed by jackknife resampling (JK, 1000 replicates with 36.8% character deletion).

The phylogenetic trees were visualized using Interactive Tree Of Life (Letunic and Bork 2016).

Comparative analysis

Binary data (presence/absence) on the spider and ant acceptance (araneophagy, myrmecophagy) as well as the attack tactic (use of silk) of 25 species were mapped on the phylogenetic tree to investigate the evolutionary history of these selected discrete characters. For few species, these data were missing. To estimate the ancestral states for these characters, we used rayDISC function from the corHMM package

(Beaulieu et al. 2013) because there were missing data and the tree was incompletely resolved. At first, we fitted the data (separately for all three traits) to the final phylogeny using equal, symmetric, and all rates different models to find the best fit based on AIC values. Then we applied the best model to 100 randomly selected trees taken from the posterior of Bayesian inference to estimate 95% confidence intervals of the ancestral state estimates using normal approximation.

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

Results

Prey

In the feeding experiment, three prey types were offered to nine species. Flies were killed by all nine species at a high frequency (> 50%) (Fig. 1a). Spiders were killed at an even higher frequency (> 62%) by all, except for *Hemicloea sundevalli*. Ants, however, were killed at variable frequencies across the species: *Gnaphosa lucifuga*, *Haplodrassus silvestris*, *Hemicloea sundevalli*, and *Pterotricha* sp. captured them with a high frequency; *Drassodes lapidosus* and *Zelotes latreillei* captured them with a low frequency; and *Asemesthes ceresicola*, *Scotophaeus scutulatus*, and *Trichotyse hortensis* completely avoided ants. Most prey that were captured were also consumed (Fig. 1a). *Zelotes latreillei*, however, consumed very few flies, *Gnaphosa lucifuga* consumed only some spiders, and *Drassodes lapidosus* consumed only some spiders and ants.
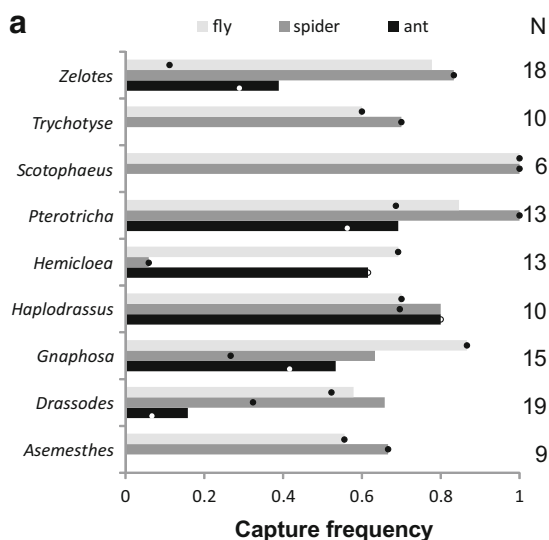
Data from literature (Table 2) showed that the majority of species (12 of 19) are araneophagous, while only seven species are myrmecophagous (*Callilepis nocturna*, *Eilica* sp., *Eilica albopunctata*, *Galianeolla leucostigma*, *Molycria quadricauda*, *Nomisia exornata*, and *Wydundra carinda*) (Fig. 1b). Available data on a number of species—namely, *Sergiolus capulatus*, *Cesonia bilineata*, *Aphantaulax* sp., *Herpyllus ecclesiasticus*, *Zelanda erebus*, and *Drassodex* cf. *heeri*—do not contain sufficient information on the acceptance of prey other than spiders. Available data on the species *Micaria sociabilis*, *Kishidaia conspicua*, *Poecilochroa senilis*, and *Prodidomus rufus* show that these species rejected ants but accepted other prey types.

Capture behaviour

We recognized three distinct prey immobilization strategies: either the spider (1) grasped the prey with its forelegs, pulled it towards its chelicera, and immobilized it using a bite, or (2) bent the distal part of its abdomen and applied silk from a short distance towards the prey, before grabbing it with its chelicera, or (3) pulled the prey with its forelegs below the predator's abdomen and swathed the prey's body in silk, before biting it.

In *Asemesthes ceresicola*, both spiders and flies were immobilized more often by biting than by the use of silk (Fig. 2a). Both prey were simply grabbed and swathed in silk. In *Drassodes lapidosus*, ants were immobilized solely by biting, while flies and spiders were immobilized more often by silk applied over them while being grasped. In *Gnaphosa lusifuga*, flies and ants were mostly immobilized by biting, while spiders were immobilized either by their legs being swathed in silk or by biting. In *Haplodrassus silvestris*, all
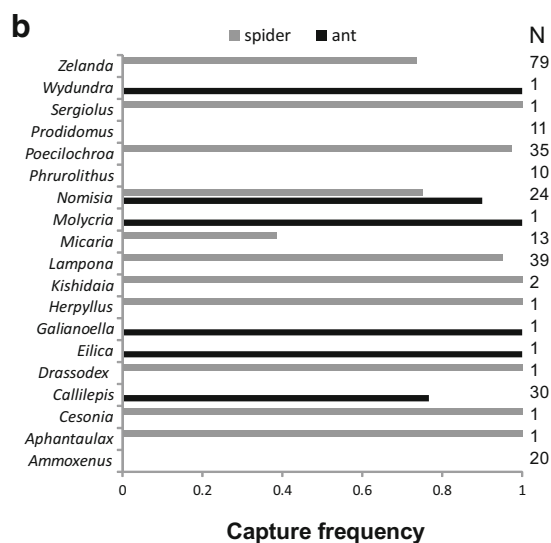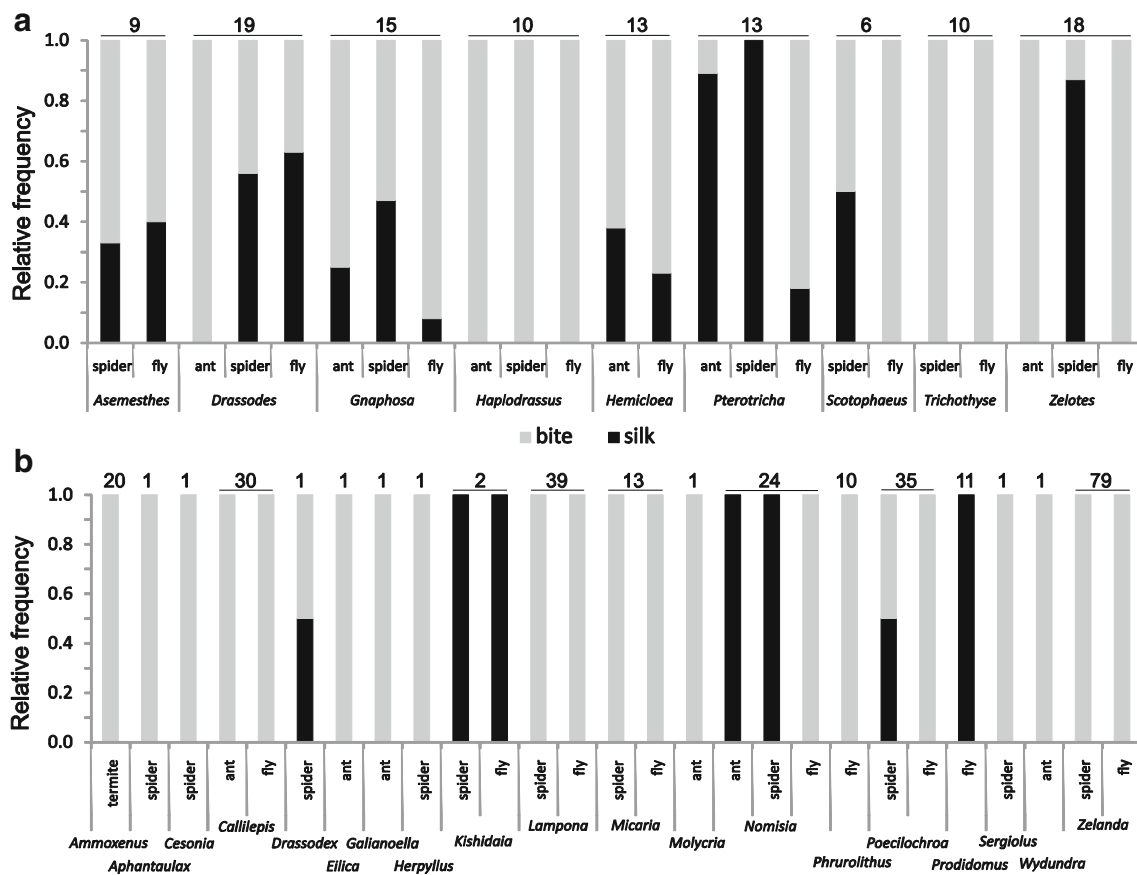

Fig. 1 Comparison of the relative frequencies of the capture (i.e., killing) of ants, spiders, and flies among gnaphosid and several non-gnaphosid genera (see Tables 1 and 2 for species names) observed in this study (**a**) and extracted from the literature (**b**). Numbers after bars represent numbers of observations. The proportion of araneophagy in *Hemicloea* (**a**) was not observed in this study but in Pekár et al. (2017). Points in **a** show the proportion of prey consumed after being attacked

Fig. 2 Comparison of the relative frequencies of the use of immobilization strategies (by silk or biting) for flies, spiders, and ants among gnaphosid and several non-gnaphosid genera (see Tables 1 and 2 for species names) observed in this study (**a**) and extracted from the literature (**b**). Numbers above bars represent numbers of observations

offered prey types were caught solely by biting and being immobilized by venom. *Hemicloea sundevalli* attacked both flies and ants mainly by using biting. Unfortunately, we were not able to observe how *Hemicloea sundevalli* hunted spiders. *Pterotricha* sp. immobilized spider and ant prey most frequently by swathing it in silk, firstly, the legs, then the whole body. Subsequently, the prey was bitten on the abdomen or prosoma. Flies were captured predominantly by biting. In *Scotophaeus scutulatus*, flies were always immobilized by biting, whereas spiders were immobilized equally by being swathed in silk and by biting alone. Prey capture by *Trichothyse hortensis* was achieved by biting both spiders and flies. *Zelotes latreillei* immobilized flies and ants solely by biting and by holding the prey in chelicerae until it was completely paralysed. Attacks on spider prey consisted of grabbing and applying silk.

In *Asemesthes ceresicola*, *Drassodes lapidosus*, *Gnaphosa lusifuga*, *Hemicloea sundevalli*, and *Pterotricha* sp., the use of silk for immobilization was significantly related to the relative size of prey (GLMM-b, $F_{1,173} = 13.7$, $P = 0.0003$): the use of silk increased with prey size so that 50% probability of attack occurred when the prey was about 1.5 times larger than the prosoma of the spider (Fig. 3). In *Haplodrassus silvestris*,
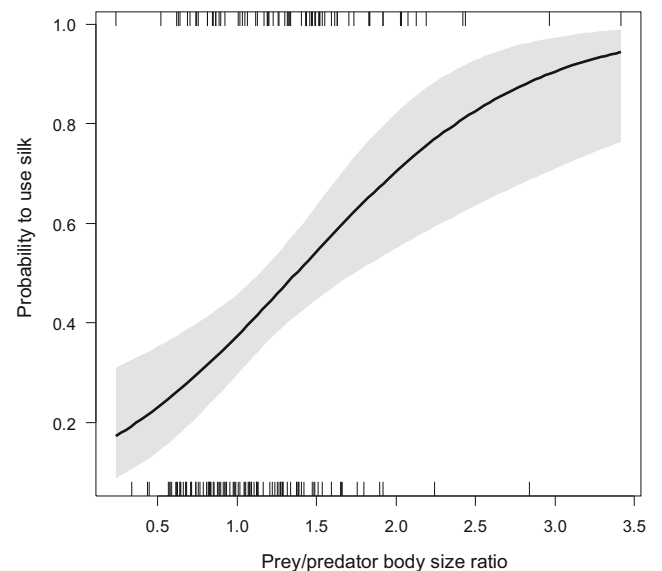
Fig. 3 Relationship between the probability of using silk (rather than biting) for the immobilization of prey and the prey-to-predator body size ratio (ratio of total body size of prey to prosoma length of spider). Data were based on five species (*Asemesthes ceresicola*, *Drassodes lapidosus*, *Gnaphosa lusifuga*, *Hemicloea sundevalli*, and *Pterotricha* sp). The estimated logit model is shown with a 95% confidence band (grey area)

Trychothyse hortensis, and *Zelotes latreillei*, the use of silk for immobilization was not significantly related to the relative size of prey (GLMM-b, $F_{1, 69} = 0.1$, $P = 0.77$).

The prey-capture behaviour of species obtained from literature (Fig. 2b) showed that a great majority of species capture prey by biting. *Kishidaia conspicua*, *Nomisia exornata*, and *Prodidomus rufus* predominantly used swathing in silk to catch prey. For 11 species (*Aphantaulax* sp., *Cesonia bilineata*, *Drassodex* cf. *heeri*, *Eilica* sp., *Eilica albopunctata*, *Galianoella leucostigma*, *Herpylus ecclesiaticus*, *Kishidaia conspicua*, *Molycria quadricauda*, *Sergiolus capulatus*, and *Wydundra carinda*), the available data were based on observations of just one prey type.
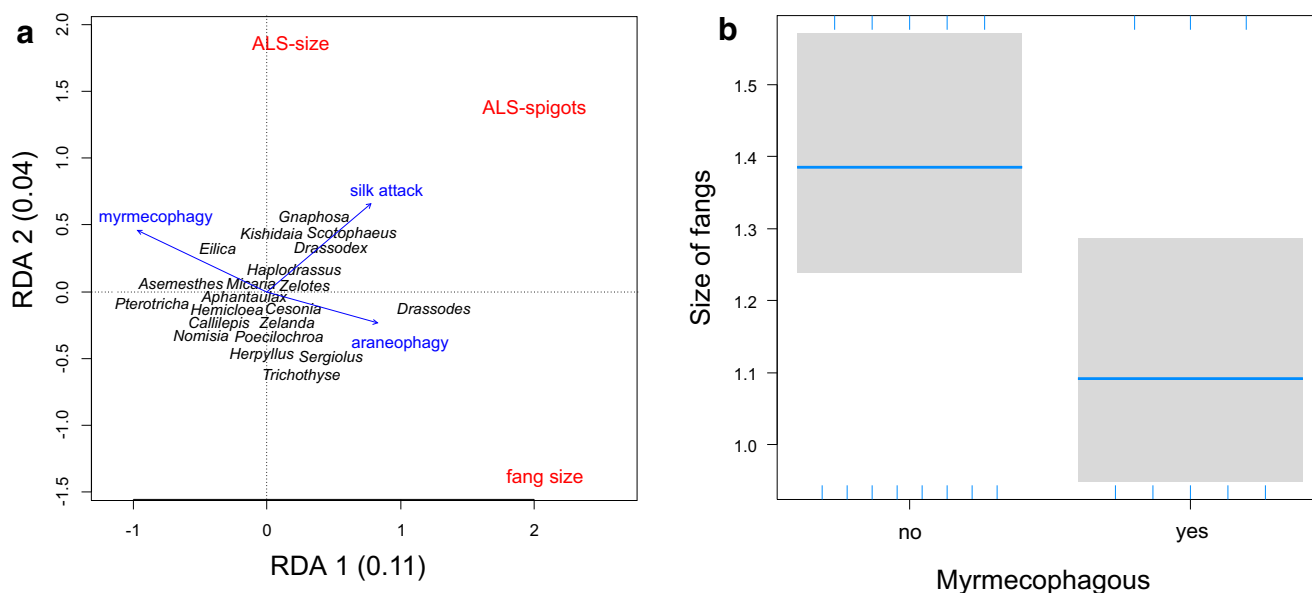
Morphology

RDA failed to reveal significant effects of either araneophagy, myrmecophagy, or silk attack on the studied traits (RDA, $F_{3,17} = 1.2$, $P = 0.34$, Fig. 4a). The ordination plot showed that myrmecophagy was negatively associated with fang size. Indeed, myrmecophagous species had significantly relatively shorter fangs than non-myrmecophagous species (GLS, $F_{1,19} = 5.0$, $P = 0.04$, Fig. 4b). The number of spigots on ALS was most closely associated with araneophagy and was marginally significantly different between araneophagous and non-araneophagous species (GLS, $F_{1,19} = 4.5$, $P = 0.05$): araneophagous species had 1.8 times more spigots than non-araneophagous species. The size of ALS was most closely associated with silk attack but was not

significantly different between species using silk or biting (GLS, $F_{1,19} = 0.4$, $P = 0.53$).

Phylogeny

The non-coding mitochondrial and nuclear gene fragments were aligned using MAFFT v7 and the partial COI sequence was checked on the basis of amino acid translations and yielded a 1075-bp-long indel-free alignment. The resulting concatenated dataset contained 5808 characters (COI—1075 bp, 12S—333 bp, 16S—457 bp, H3—318 bp, 18S—1731 bp, 28S—1894 bp) representing 20 gnaphosid species and five outgroup species. In the next step it was combined with 248 morphological features.

We tested the concatenated dataset using Bayesian inference (BI) and maximum likelihood (ML) approaches. Maximum parsimony analysis yielded a tree with multiple polytomies (TL = 4498, CI = 0.5507, HI = 0.4493, RI = 0.2879), where no reliable resolution was achieved (not shown). BI was used to calculate the alternative phylogenetic hypothesis based on 6056 characters of the 25 terminal taxa. BI hypothesis suggested the most reliable topology; the standard deviation of split frequencies was, in all cases, < 0.01 and the log-likelihood value for the best tree was − 25,917.77 After testing the hypothesis with ML analysis, we obtained a resulting topology rather similar to that for BI. Some of the clades received low support after resampling (bootstraps = 500), and the inter-generic relationships across both analyses (BI and ML) were rather inconsistent. However, some clusters with

Fig. 4 **a** Ordination RDA plot showing the relationship between three morphological traits (red) and myrmecophagy, araneophagy, and silk attack (arrows) in 21 gnaphosid and non-gnaphosid genera (see Tables 1 and 2 for species names). The first two axes explained 15% of the data variation. **b** Comparison of the relative fang size (length divided by width) between species feeding and not feeding on ants. Horizontal lines are estimates, grey bars are 95% confidence intervals

high posterior probabilities were similar across both analyses (BI/ML): *Cesonia bilineata* + *Herpyllus ecclesiaticus* (0.9/58), *Aphantaulax* sp. + *Kishidaia conspicua* (88/66), *Drassodex simoni* + *Hemicloea* sp. (0.72/46), *Prodidomus rufus* + *Zelanda* sp. (0.94/50), and (*Asemesthes* sp. + *Nomisia exornata*) + *Pterotricha* sp. (1.0/61). The species *Ammoxenus amphalodes* was consistently placed within the clade covering gnaphosid species (*Gnaphosa lucifuga*, *Callilepsis nocturna*, *Eilicia* cf. *trilineata*, *Asemesthes* cf. *corticola*, *Nomisia exornata*, and *Pterotricha elegans*) (1.0/88).

Comparative analysis

The best model of the discrete character evolution for all three traits was the one with equal rates. Among the 25 species used in the analysis, myrmecophagy has been recorded in ten terminals (Figs. 5, S1A). It is clustered particularly in a central clade which contains myrmecophagous specialists (*Callilepis*, *Eilica*). The ancestral state estimate of the probability of myrmecophagy at the base of the tree was 0.5 (CI$_{95}$ = 0.49, 0.51), thus myrmecophagy being seemingly ancestral condition as likely as non-myrmecophagy.

Araneophagy has been recorded in 19 terminals (Figs. 5, S1B) among 25 taxa, lacking particularly in myrmecophagous specialists. The ancestral state estimate of the probability of araneophagy at the base of the tree was 0.86 (0.83, 0.89), thus seemingly ancestral condition.

The use of silk for prey immobilization has been recorded in 12 terminals (Figs. 5, S1C) evenly dispersed across the tree. The ancestral state estimate of the probability of the use of silk near to the base of the tree was 0.49 (0.48, 0.5), thus seemingly ancestral condition as likely as biting.

Discussion

We found that only some gnaphosid species accepted ants. Ants represent very dangerous prey, as many ant species can bite, perform communal attack, and even sting or use chemical defence (Hölldobler and Wilson 1990). Therefore, it was not surprising to see ant avoidance in some species. A few species, such as *Drassodes lapidosus*, killed ants but consumed them at much lower frequency suggesting that ants are not preferred prey of these species but were rather attacked as a result of defence. Among the gnaphosid species that captured ants, a few are strictly myrmecophagous (e.g. *Callilepis nocturna*), while others are rather euryphagous (e.g. *Nomisia exornata*) (Michálek et al. 2018). Myrmecophagy has been reported for species from a number of families not studied here, such as Clubionidae, Gallieniellidae, Salticidae,
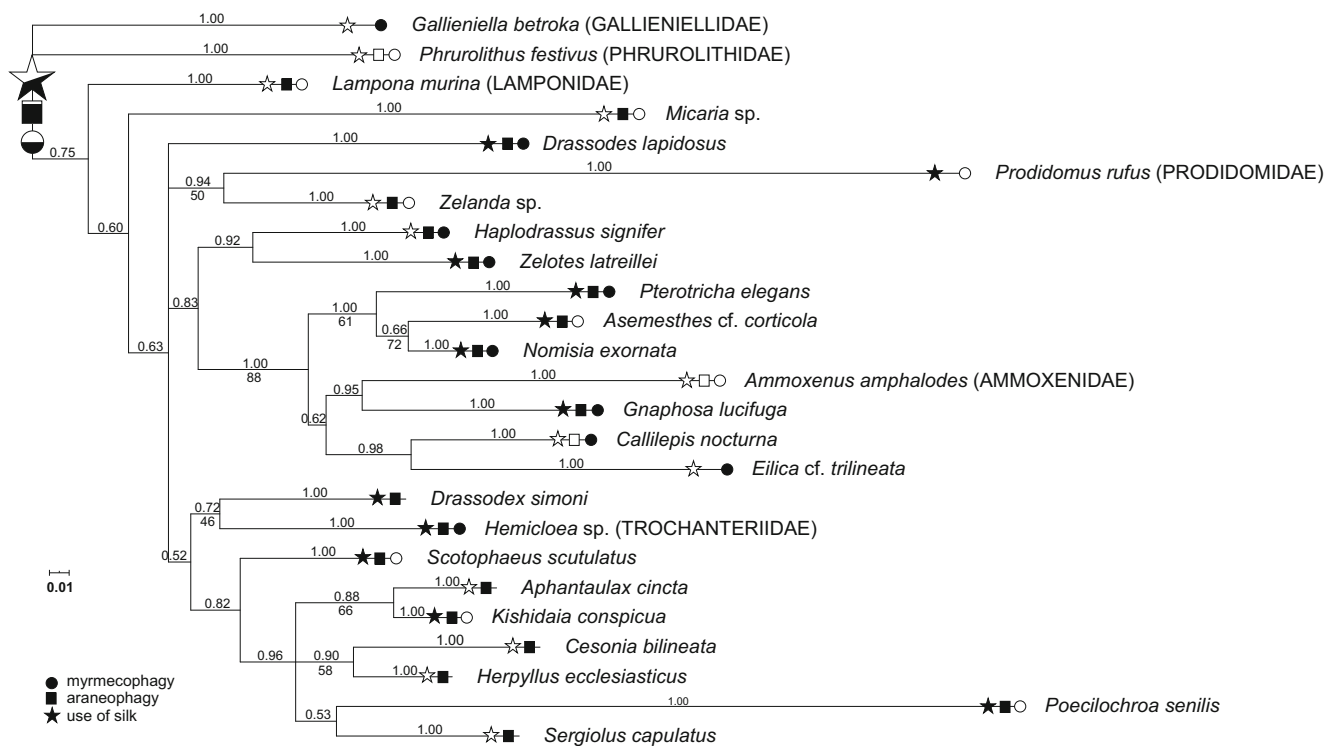
Fig. 5 Bayesian hypothesis for the phylogenetic relationships of the study species based on the combined dataset of 6056 characters (5808 bp + 248 morphological characters). Posterior probabilities are shown above nodes; bootstrap values (> 50) are below nodes. Non-gnaphosid species are displayed with their family names (in capitals).

Three characters, myrmecophagy, araneophagy, and use of silk for immobilization, are projected at terminals. Full symbols represent presence, empty symbols absence of the state character. Symbols at the base of tree represent ancestral state estimates of the probabilities of myrmecophagy, araneophagy, and the use of silk

Theridiidae, Thomisidae, and Zodariidae (Goloboff 2000; Pekár and Toft 2015). Similarly to gnaphosids, some species within these families are strictly myrmecophagous (Pekár 2004; Pekár et al. 2014), others are not (e.g. Líznarová and Pekár 2019).

In contrast, spiders were captured at a high frequency. Like ants, spiders also represent a dangerous prey, as they possess venom and silk as effective weapons (Pekár and Toft 2015). Indeed, during experiments, gnaphosid spiders were killed by other spiders in 7% of cases. Yet, all tested species captured and consumed spiders at high frequencies indicating that spiders might be a common prey of gnaphosids, except for those that have specialised on ants (e.g. *Callilepis nocturna*). The fact that araneophagy always occurred together with dipterophagy suggests that none of the known species is a strict spider specialist. Araneophagy is known from many spider families (Pekár et al. 2012) including closely related ones, namely Lamponidae (Platnick 2000; Michálek et al. 2017) and Cithaeronidae (Edwards and Stiles 2011), but none of these species is strictly specialised on spiders.

The ancestral state estimation for myrmecophagy revealed that it is ancestral but has been lost repeatedly. This is consistent with results for other spider families, namely Theridiidae (Liu et al. 2016), Zodariidae (Pekár et al. 2013), and Salticidae (Pekár et al. 2012). On the other hand, the ancestral state for araneophagy was high for all nodes. This is also consistent with results for other spider families, namely Salticidae and Zodariidae (Pekár et al. 2012). Araneophagy in our study included only instances of capturing non-gnaphosid spiders. However, it may also include cannibalism, which might not be uncommon in gnaphosids, though evidence is rare (Sentenská and Pekár 2013, 2014).

Being solitary cursorial species, gnaphosids use silk or biting when catching prey. The function of these two tactics seems very similar: (1) to restrain active prey so it cannot escape, and (2) to protect spiders from dangerous prey. Yet, there must be differences between these tactics. The production of both silk and venom is costly, so there should be a trade-off in using them. These two immobilization tactics seem to be optimal for different prey. Silk immobilizes prey more quickly than venom and requires only a superficial contact with prey. Biting and the injection of venom, on the other hand, require full contact (manipulation) with prey and this can result in retaliation. While venom causes mortality, silk must be followed by biting not only to achieve this state. It is, however, not known whether in biting silk-constrained prey, the spider injects venom (composed of toxins) or only enzymes that are necessary to initiate extra-oral digestion. Silk is also a more universal capture tactic than biting, as venom can be tailored to a certain prey (Pekár et al. 2018a). For example, strictly specialised myrmecophagous *Callilepis nocturna* possesses venom that is more potent on ants than the venom of myrmecophagous but less specialised *Nomisia exornata* (Pekár et al. 2018b).

The capture of dangerous prey is associated with various morphological, behavioural, and venomic adaptations that increase the efficacy of capture (Pekár and Toft 2015; Pekár et al. 2017). Thus, we expected that myrmecophagy and araneophagy in gnaphosids can be related to the size of some morphological traits which are used during prey capture, such as the chelicera and anterior spinnerets. It has been shown that in oniscophagous dysderid spiders, the shape of the chelicera (both basal and distal segments) predicts their prey specialisation (Řezáč et al. 2008). We found that myrmecophagous gnaphosid species have relatively shorter cheliceral fangs than araneophagous species. Smaller chelicerae are also known for strictly myrmecophagous species of the genus *Zodarion* from Zodariidae (Pekár et al. 2013). In contrast, Goloboff (2000) attributed the elongated chelicerae of ant-preying *Galianoella leucostigma* (Gallieniellidae) to play a special role in holding the ant away from the spider body after attack. We expected this to be true for araneophagous species, as in the case of archaeid spiders (Wood et al. 2012). However, this was not supported by our sample of gnaphosid species.

The other trait, namely the size of spinnerets, was not found to be significantly different, though our analysis indicated that silk attack is associated with longer ALS, which contain piriform glands. It is the piriform glands that produce the silk used during silk attack (Wolff et al. 2017). A comparison of these glands between one species that uses biting and another that uses silk attack showed that the latter species had a higher number of glands than the former (Michálek et al. 2019). A higher number of glands, however, may not be reflected in the size of the spinnerets but in the number of spigots. Indeed, our analysis indicated that araneophagous species had higher number of spigots on ALS. But data on more species, particularly non-araneophagous ones, are needed to support this observation.

Interestingly, silk immobilization was estimated as an ancestral condition with a similar probability as use of biting. It is apparently less frequent than the modification of the piriform glands. Thus, it appears that use of silk in prey capture has been repeatedly lost during evolution of gnaphosids alike the use of foraging webs in spiders (Coddington et al. 2019). According to the recent phylogeny (Wheeler et al. 2017), Gnaphosidae is closely related to other families which do not use silk in prey capture, such as Phrurolithidae (this study) or Anyphaenidae (Pekár, unpublished). It is important to bear in mind that the use of silk is, in many species, a conditional strategy, used only for a certain prey.

The use of silk attack, particularly swathing silk, was more frequently used for large prey, namely spiders. Taking together the facts that silk attack must be followed by biting and that the use of silk attack is a conditional strategy suggests that biting is a primary capture tactic, while silk attack is a secondary one. This is also consistent with the mode of capture in related hunting spiders, such as Lamponidae (Michálek et al.

2017) and Ammoxenidae (Petráková et al. 2015), which do not use silk to immobilize prey but simply grab and hold the prey in their chelicerae.

Only some gnaphosid genera showed versatility in using both silk and bite attack tactics. Strict prey specialists, such as *Callilepis*, used only biting. Such stereotyped predatory behaviour must work as a filter in prey selection and restrict the prey range. In contrast, the ability to use the silk attack tactic in addition to biting may extend the prey breadth and lead to euryphagy.

Unfortunately, phylogenetic treatments of Gnaphosidae are rare. The only available recent broad family analysis was based on morphological data only (Azevedo et al. 2018). The updated phylogenetic analysis we performed did not aim to reveal the evolutionary history of the whole family, only that of the species under focus. Given the small selection of species, the resolution of the phylogeny was very limited. Yet, it showed some interesting patterns. For example, *Ammoxenus* appears to be real gnaphosid, which is in contrast to Azevedo et al. (2018) but in agreement with Wheeler et al. (2017), who used molecular data. Nevertheless, the tree topology essentially supports the subfamily ranks given by Azevedo et al. (2018).

We conclude that gnaphosid spiders are bold predators. They frequently catch other spiders but less likely ants. They have adapted to dealing with such prey by using three different attack tactics, of which bite and immobilisation by venom is most frequent. Beside behavioural adaptations, myrmecophagous species exhibit the morphological adaptation of shorter cheliceral fangs. Given the fact that Gnaphosidae are very species-rich and that we investigated here only a fraction of the genera (12%), our conclusions might be subject to change once data on many more species are available.

Contribution of authors NB performed the experiments and took measurements; SP conceived the study, designed the study, collected spiders, and analysed data; and AT sequenced specimens and performed phylogenetic analyses. All authors wrote the manuscript.

Data accessibility Sequences of *Drassodex simoni* and *Pterotricha* sp. were submitted to the GenBank sequence database and are available in Online Supporting Information (Table S1).

References

Anonymous (2006). https://natureitems.blogspot.com. Accessed 1 July 2019.

Atkinson, R. (2015). The find-a-spider guide for the spiders of Southern Queensland. http://www.findaspider.org.au/find/family.htm. Accessed 1 August 2019.

Azevedo, H. F. G., Griswold, C. E., & Santos, A. J. (2018). Systematics and evolution of ground spiders revisited (Araneae, Dionycha, Gnaphosidae). *Cladistics, 34*, 579–626.

Beaulieu, J. M., O'Meara, B. C., & Donoghue, M. J. (2013). Identifying hidden rate changes in the evolution of a binary morphological character: the evolution of plant habit in campanulid angiosperms. *Systematic Biology, 62*(5), 725–737.

Bristowe, W. S. (1958). *The world of spiders*. London: William Collins Press.

Cardoso, P., Pekár, S., Jocqué, R., & Coddington, J. A. (2011). Global patterns of guild composition and functional diversity of spiders. *PLoS One, 6*(6), e21710.

Coddington, J. A., Agnarsson, I., Hamilton, C. A., & Bond, J. E. (2019). Spiders did not repeatedly gain, but repeatedly lost, foraging webs. *PeerJ, 7*, e6703.

Dippenaar-Schoeman, A. S., Haddad, C. R., Foord, S. H., Lyle, R., Lotz, L. N., Helberg, L., Mathebula, S., Van den Berg, A., Van den Berg, A. M., Van Niekerk, E., & Jocqué, R. (2010). First atlas of the spiders of South Africa. South African National Survey of Arachnida. Technical Report, 1.

Edwards, G., & Stiles, J. T. (2011). The first North American records of the synanthropic spider *Cithaeron praedonius* OP-Cambridge (Araneae:Gnaphosoidea:Cithaeronidae), with notes on its biology. *Insecta Mundi, 187*, 1–7.

Fernández, R., Kallal, R. J., Dimitrov, D., Ballesteros, J. A., Arnedo, M. A., Giribet, G., & Hormiga, G. (2018). Phylogenomics, diversification dynamics, and comparative transcriptomics across the spider tree of life. *Current Biology, 28*(9), 1489–1497.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294–297.

Goloboff, P. A. (2000). The family Gallieniellidae (Araneae, Gnaphosoidea) in the Americas. *Journal of Arachnology, 28*, 1–6.

Goloboff, P. A., & Catalano, S. A. (2016). TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics, 32*, 221–238.

Goloboff, P. A., Farris, S., & Nixon, K. (2008). TNT, a free program for phylogenetic analysis. *Cladistics, 24*, 774–786.

Grimm, U. (1985). Die Gnaphosidae Mitteleuropas (Arachnida, Araneae). *Abhandlungen des Naturwissenschaftlichen Vereins in Hamburg (NF), 26*, 1–318.

Guarisco, H. (2007). *Checklist of Kansas ground spiders*. Emporia: The Kansas School Naturalist.

Harland, D. P., & Jackson, R. R. (2006). A knife in the back: use of prey-specific attack tactics by araneophagic jumping spiders (Araneae: Salticidae). *Journal of Zoology, 269*, 285–290.

Heller, G. (1974). Zur Biologie der ameisenfressenden Spinne *Callilepis nocturna* Linnaeus 1758 (Araneae, Drassodidae). PhD Thesis. Mainz: Johannes Gutenberg Universitat.

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Berlin: Springer-Verlag.

Huelsenbeck, J. P., & Ronquist, F. R. (2001). MrBayes: Bayesian inference of phylogeny. *Biometrics, 17*, 754–755.

Jackson, R. R. (1992). Eight-legged tricksters: spiders that specialize at catching other spiders. *Bioscience, 42*, 590–598.

Jäger, P. (2002). Über eine bemerkenswerte Verhaltensweise von *Scotophaeus scutulatus* (Araneae: Gnaphosidae). *Arachnologische Mitteilungen, 24*, 72–75.

Jarman, E. A. R., & Jackson, R. R. (1986). The biology of *Taieria erebus* (Araneae, Gnaphosidae), an araneophagic spider from New Zealand: silk utilisation and predatory versatility. *New Zealand Journal of Zoology, 13*, 521–541.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution, 30*, 772–780.

Kuhn-Nentwig, L., Stöcklin, R., & Nentwig, W. (2011). Venom composition and strategies in spiders: is everything possible? *Advances in Insect Physiology, 40*, 1–86.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution, 33*, 1870–1874.

Letunic, I., & Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research, 44*, 242–245.

Levy, L. (1999). Spiders of six uncommon Drassodine genera (Araneae: Gnaphosidae) from Israel. *Israel Journal of Zoology, 45*, 427–452.

Liu, J., May-Collado, J. L., Pekár, S., & Agnarsson, I. (2016). A revised and dated phylogeny of cobweb spiders (Araneae, Araneoidea, Theridiidae): a predatory *Cretaceous lineage* diversifying in the era of the ants (Hymenoptera, Formicidae). *Molecular Phylogenetics and Evolution, 94*, 658–675.

Líznarová, E., & Pekár, S. (2019). Trophic niche and capture efficacy of an ant-eating spider, *Euryopis episinoides* (Araneae: Theridiidae). *Journal of Arachnology, 47*(1), 45–51.

Michálek, O., Lubin, Y., & Pekár, S. (2019). Nest usurpation: a specialised hunting strategy used to overcome dangerous spider prey. *Scientific Reports, 9*, 5386.

Michálek, S., Petráková, L., & Pekár, S. (2017). Capture efficiency and trophic adaptations of a specialist and generalist predator: a comparison. *Ecology and Evolution, 7*, 2756–2766.

Michálek, O., Řezáč, M., Líznarová, E., Symondson, W. O. C., & Pekár, S. (2018). Silk versus venom: alternative capture strategies employed by closely related myrmecophagous spiders. *Biological Journal of the Linnean Society, 126*, 545–554.4.

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, 1–8.

Mukherjee, S., & Heithaus, M. R. (2013). Dangerous prey and daring predators: a review. *Biological Reviews, 88*, 550–563.

Murphy, J. A. (2007). *Gnaphosid genera of the world* (Vol. 1, 2). St Neots: British Arachnological Society.

Nanoon, G. R. (1982). Notes on interactions between the spider *Eilica puno* (Gnaphosidae) and the ant *Camponotus inca* in the Peruvian Andes. *Biotropica, 14*(2), 145–148.

Nentwig, W., Blick, T., Gloor, D., Hänggi, A., & Kropf, C. (2019). Araneae: spiders of Europe, Version 10. 2019. https://www.araneae.nmbe.ch. Accessed 15 October 2019.

Nylander, J. A. A. (2004). *MrModeltest v 2.2 (online).* Program distributed by the author. Uppsala University: Evolutionary Biology Centre.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2017). Vegan: community ecology package. R Package Version 2.4–2. http://CRAN.Rproject.org/package=vegan.

Paradis, E. (2006). *Analysis of phylogenetics and evolution with R.* New York: Springer.

Pekár, S. (2004). Predatory behavior of two European ant-eating spiders (Araneae, Zodariidae). *Journal of Arachnology, 32*(1), 31–41.

Pekár, S., & Brabec, M. (2016). Marginal models via GLS: a convenient yet neglected tool for the analysis of correlated data in the behavioural sciences. *Ethology, 122*, 621–631.

Pekár, S., & Brabec, M. (2019). *Modern analysis of biological data. 3.* Brno: Non-linear Models in R. Masaryk University Press [in Czech].

Pekár, S., & Jarab, M. (2011). Life-history constraints in inaccurate Batesian myrmecomorphic spiders (Araneae: Corinnidae, Gnaphosidae). *European Journal of Entomology, 108*, 255–260.

Pekár, S., & Toft, S. (2015). Trophic specialisation in a predatory group: the case of prey-specialised spiders (Araneae). *Biological Reviews, 90*, 744–761.

Pekár, S., Coddington, J. A., & Blackledge, T. A. (2012). Evolution of stenophagy in spiders (Araneae): evidence based on the comparative analysis of spider diets. *Evolution, 66*(3), 776–806.

Pekár, S., Bočánek, O., Michálek, O., Petráková, L., Haddad, C. R., Šedo, O., & Zdráhal, Z. (2018a). Venom gland size and venom complexity—essential trophic adaptations of venomous predators: a case study using spiders. *Molecular Ecology, 27*, 4257–4269.

Pekár, S., Líznarová, E., Bočánek, O., & Zdráhal, Z. (2018b). Venom of prey-specialized spiders is more toxic to their preferred prey: a result of prey-specific toxins. *Journal of Animal Ecology, 87*, 1639–1652.

Pekár, S., Michalko, R., Korenko, S., Šedo, O., Líznarová, E., Sentenská, L., & Zdráhal, Z. (2013). Phenotypic integration in a series of trophic traits: tracing the evolution of myrmecophagy in spiders (Araneae). *Zoology, 116*(1), 27–35.

Pekár, S., Petráková, L., Bulbert, M. W., Whiting, M. J., & Herberstein, M. E. (2017). The golden mimicry complex uses a wide spectrum of defence to deter a community of predators. *eLife, 6*, e22089.

Pekár, S., Šedo, O., Líznarová, E., Stanislav, K., & Zdráhal, Z. (2014). David and Goliath: potent venom of an ant-eating spider (Araneae) enables capture of a giant prey. *Naturwissenschaften, 101*, 533–540.

Petráková, L., Líznarová, E., Pekár, S., Haddad, C. R., Sentenská, L., & Symondson, W. O. C. (2015). Discovery of a monophagous true predator, a specialist termite-eating spider (Araneae: Ammoxenidae). *Scientific Reports, 5*, 14013.

Petráková Dušátková, L., Pekár, S., Michálek, O., Líznarová, E., & Symondson, W. O. C. (2020). Estimation of trophic niches in myrmecophagous spider predators. *Scientific Reports, 10*, 8683.

Platnick, N. I. (2000). A relimitation and revision of the Australasian ground spider family Lamponidae (Araneae: Gnaphosoidea). *Bulletin of the American Museum of Natural History, 245*, 1–328.

Platnick, N. I., & Shadab, M. U. (1980). A revision of the spider genus *Cesonia* (Araneae, Gnaphosidae). *American Museum of Natural History, 155*, 1–66.

Platnick, N. I., & Shadab, M. U. (1988). A revision of the American spiders of the genus *Micaria* (Araneae, Gnaphosidae). *American Museum Novitates, 2916*, 1–64.

R Core Team. (2017). *A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing http://www.R-project.org.

Řezáč, M., Pekár, S., & Lubin, Y. (2008). How oniscophagous spiders overcome woodlouse armour. *Journal of Zoology, 275*(1), 64–71.

Rodriguez, F., Oliver, J. L., Marin, A., & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology, 142*, 485–501.

Sentenská, L., & Pekár, S. (2013). Mate with the young, kill the old: reversed sexual cannibalism and male mate choice in the spider *Micaria sociabilis* (Araneae: Gnaphosidae). *Behavioral Ecology and Sociobiology, 67*, 1131–1139.

Sentenská, L., & Pekár, S. (2014). Eat or not to eat: Reversed sexual cannibalism as a male foraging strategy in the spider *Micaria sociabilis* (Araneae: Gnaphosidae). *Ethology, 120*, 511–518.

Van den Berg, A., & Dippenaar-Schoeman, A. S. (1991). Ground-living spiders from an area where the harvester termite *Hodotermes mossambicus* occurs in South Africa. *Phytophylatica, 23*, 247–253.

Wheeler, W. C., Coddington, J. A., Crowley, L. M., Dimitrov, D., Goloboff, P. A., Griswold, C. E., Hormiga, G., Prendini, L., Ramírez, M. J., Sierwald, P., Almeida-Silva, L. M., Álvarez-Padilla, F., Arnedo, M. A., Benavides, L. R., Benjamin, S. P., Bond, J. E., Grismado, C. J., Hasan, E., Hedin, M., Izquierdo, M. A., Labarque, F. M., Ledford, J., Lopardo, L., Maddison, W. P., Miller, J. A., Piacentini, L. N., Platnick, N. I., Polotow, D., Silva-Dávila, D., Scharff, N., Szűts, T., Ubick, D., Vink, C., Wood, H. M., & Zhang, J. X. (2017). The spider tree of life: phylogeny of Araneae

based on target-gene analyses from an extensive taxon sampling. *Cladistics, 33*(6), 574–616.

Whitehouse, M. E. A., & Lubin, Y. (1998). Relative seasonal abundance of five spider species in the Negev desert: intraguild interactions and their implications. *Israel Journal of Zoology, 44*, 187–200.

Wolff, O. J., Řezáč, M., Krejčí, T., & Gorb, S. N. (2017). Hunting with sticky tape: functional shift in silk glands of araneophagous ground spiders (Gnaphosidae). *Journal of Experimental Biology, 220*, 2250–2259.

Wood, S. N. (2006). Generalized additive models. An Introduction with R. Chapman & Hall/CRC.

Wood, H. M., Griswold, C. E., & Gillespie, R. G. (2012). Phylogenetic placement of pelican spiders (Archaeidae, Araneae), with insight into evolution of the "neck" and predatory behaviours of the superfamily Palpimanoidea. *Cladistics, 28*(6), 598–626.

World Spider Catalog. (2019). Version 20.5. Natural History Museum Bern. http://wsc.nmbe.ch. Accessed 14 October 2019.

Zwickl, D. J., (2006). Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. thesis. Austin: University of Texas.