Szent István University

ARBOREAL HUNTING SPIDERS IN APPLE ORCHARDS: TAXONOMIC COMPOSITION, NATURAL PREY AND CIRCADIAN BIOLOGY

Thesis booklet

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1. BACKGROUND AND OBJECTIVES

It seems that in the second half of the 20th-century mankind has nearly overcome world hunger, which is partly due to some innovative processes for intensifying agricultural production which took place mainly between the years 1960 and 1970 and later became known as 'Green Revolution'. Adoption of a series of new inventions and technologies such as high-yielding crop varieties, fertilization, irrigation, and modern pesticides increased worldwide agricultural production satisfying the nutritional needs of humanity and resulting in a steep growth in human population. However, in return for the glut of agricultural products never seen before, humanity has to face global ecological crises. Several studies link features and consequences of modern agricultural practice such as intensive use of pesticides or loss and fragmentation of native habitats to honeybee colony collapse disorder or to arthropod decline and extinction. Therefore, there is an urgent need to implement fundamental changes in the current agricultural practice, which includes, e.g. the invention and introduction of greener and more sustainable production technologies, conservation of biodiversity and maximization of ecosystem services from conservation biological control. In the literature, spiders (Araneae) are often referred to as promising biological control agents as they form abundant and diverse assemblages in agroecosystems such as fruit orchards and consume a remarkable amount of arthropod prey. Although spiders are often considered to be polyphagous and opportunistic predators in general, limited information is available on their natural prey at both species and community levels. Furthermore, many other aspects of the biology of the species with potential economic importance are poorly known. Thus, their ecology and function in agricultural systems require much more research.

This thesis consists of three articles that have been published in level A (Q1) and level B (Q2) peer-reviewed journals relevant to the field, presented on international conferences, and showed on other scientific forums. The general research aims were as follows: (1) to better explore the arachnofauna of apple orchards in Hungary; (2) to characterize the natural prey composition of arboreal hunting spiders (communities, guilds and species) in apple orchards and analyse their role in food-web dynamics and in pest suppression; (3) and finally, to describe the circadian biology of the two most abundant arboreal hunting spider species (*Carrhotus xanthogramma* and *Philodromus cespitum*) in apple orchards in Hungary.

2. MATERIALS AND METHODS

2.1. Exploring the arachnofauna of apple orchards in Hungary

Spiders were collected sporadically in various parts of Hungary, mainly in apple orchards (Bács-Kiskun, Pest, Szabolcs-Szatmár-Bereg and Tolna counties) and city parks (Budapest, Gödöllő) from 2013 to 2016. A variety of collecting methods were used, including hand collecting, beating, cardboard bands and litter sampling. For collecting overwintering spiders from apple trees, we used corrugated cardboard stripes placed around the tree trunks in September. The bands and litter samples were collected during winter months, and for processing the litter samples, we used Winkler extractors. Since proper identification of spiders requires adult specimens, juvenile individuals of certain species were kept alive and fed with *Drosophila hydei* Sturtevant, until their final molt. The collected and reared specimens were stored in 70 % ethanol. Individuals were examined in the laboratory of the Department of Entomology Szent István University.

2.2. Examining the natural prey and biocontrol potential of arboreal hunting spiders in apple orchards

Data on the natural diet (actual prey) of the arboreal hunting spider assemblage was collected between 2013 and 2019 in apple orchards in Hungary. Apple trees were visually inspected regularly in organic orchards, and hunting spiders with prey in their chelicerae were collected during the growing season (from the beginning of April to the end of October). Our *in situ* observations were conducted both day and night (approximate ratio 7:3) to get information not only on the prey of the diurnal hunting spiders but also on the nocturnal ones. Apple trees were examined mainly between 9:00 and 12:00, between 14:00 and 18:00 and between 20:00 and 23:00 (after sunset). After collecting the spiders with their prey, both species were identified to the lowest taxonomic level possible. Moreover, in spiders, the width of the prosoma and in case of the preys, the width of the thorax were measured with 0.1 mm accuracy. In juvenile spiders where the species-level identification was not possible, spiders were raised to the adult stage (on *D. hydei*) in the laboratory.

To obtain information on the potential prey community of arboreal hunting spiders, a D-VAC sampler was used. In the organic apple orchard located at Újfehértó, suction samples were taken at monthly intervals between April and

October in 2016 and 2017 (on 14 sampling dates). On each sampling date, five samples were taken. Each sample consisted of suction samples collected from one (left or right) side of the canopy of four randomly selected apple trees in a randomly selected row. The collected material was sorted and identified in the laboratory.

For most analyses, the spiders were classified into six groups: (1) *Carrhotus xanthogramma* (Latreille), (2) Other salticids, (3) *Philodromus cespitum* (Walckenaer), (4) *Ebrechtella tricuspidata* (Fabricius), (5) *Xysticus* spp. s. lat., and (6) *Clubiona* spp. The main criterion for group formation was that the number of records in a particular group should exceed 5% of the total sample at the lowest possible taxonomic level.

Prey items retrieved from spiders or collected by D-VAC sampling were classified into the following 16 taxonomic groups: Acari, Araneae, Coleoptera, Lepidoptera, Formicidae, Other (non-formicid) Hymenoptera, Brachycera, Nematocera (i.e., all non-Brachycera dipterans), Auchenorrhyncha, Heteroptera, Sternorrhyncha, Ephemeroptera, Neuroptera, Psocoptera, Thysanoptera, and Trichoptera. The prey categories that had relative abundances of less than 1% in the total actual prey of the whole arboreal hunting spider assemblage were pooled into the group of 'Other prey' in certain statistical analyses.

To evaluate the biological control potential of the hunting spiders, the prey items were also categorized into groups according to their economic status in apple orchards in Central Europe as pests, natural enemies and neutral arthropods. A prey species was considered to be a pest if at least one of its life stages is known to feed on any parts of the apple tree. Natural enemies are defined as species that can feed (at least in one of their life stages) on any stage of arthropods that were previously categorized as pests. Finally, the neutral category was comprised of other (non-pest and non-natural enemy) prey species. For prey that could be identified only to suborder, such as Nematocera, the classification was made according to the dominant characteristics of the taxon (i.e., the vast majority of Nematocera occurring in apple orchard are neutral species).

All statistical analyses were performed within the R (v.3.5.3.) statistical environment. For all analyses, the natural prey data were pooled across orchards and years, except for the comparison of actual and potential prey where only the data

collected at the same place (Újfehértó) and in the same years (2016-2017) were analysed.

A wide variety of statistical methods were used for analysing the data: (**1**) to compare the actual and potential prey Generalised Linear Mixed Models with binomial error structure (GLMM-b) were used, and to assess the degree of selectivity shown by spiders, Ivlev's electivity indices (IE) were computed; (**2**) spider and prey composition compared on temporal and spatial scale with Mantel tests based on Morisita dissimilarity distance; (**3**) food web metrics (specialisation, H_2' and d'), niche width (Levins' B) and niche overlap (based on prey taxonomic composition and prey size) were calculated to compare the trophic characteristics of the six most abundant spider taxa and null models were used for each niche dimension to test the possible differences between the occupied niches of the spider groups; (**4**) the prey composition of spider groups compared with a model based fourth-corner analysis as well; (**5**) the predator-prey size relationship analysed with GLMs with gamma error structure and log-link (GLM-g), while the variation of the taxa-specific body traits (predator prosoma width, prey thorax width and thorax-prosoma ratio) were analysed separately by Linear Models (LMs), and for testing the post-hoc contrasts, Welch's t-test with the Holm's correction was used.

2.3. Studying the circadian biology of *Carrhotus xanthogramma* and *Philodromus cespitum*

Spiders used in the experiments were collected by beating the canopies of apple trees in various orchards in Hungary. The collected individuals were transported to the Department of Entomology at Szent István University, where the study was carried out. 10 males and 11 females of *C. xanthogramma*, and 11 females and 11 males of *Ph. cespitum* were involved in the study. The spiders were housed individually in plastic Petri dishes, and the sides of the Petri dishes were covered with white tape to reduce disturbance from neighboring spiders. The spiders' circadian activity was examined under natural light and photoperiod.

To monitor the spiders' locomotor activity, the activity of all individuals were simultaneously recorded using a video camera for 72 h. During the scotophase, a red LED bulb illuminated the recording area. The activity was recorded from 26 to 29 April in the case of *C. xanthogramma* and 6–9 July in the case of *Ph. cespitum*. The

exact time of sunrise, sunset, nautical and civil twilights were calculated. Spiders were placed in the final layout three days before the tests and from this time to the end of the study food and water were not provided.

Video recordings were analysed with a 10-min resolution. The level of activity was scored on a scale of 0–2 in each interval (0 – zero locomotion; 1 – minimal change in body position or the locomotion was shorter than the body length of the individual; and 2 – the locomotion was longer than the length of the individual). Applying this scoring procedure, we obtained a time series of 432 activity records (72-h long time series) for each individual.

Data analysis procedures comprised several statistical methods: (**1**) to quantify the relative amount of activity during the photophase and twilight periods, we calculated diurnality and crepuscularity indices and Student's t-test was used to compare the means of the index values between sexes; (**2**) circular statistics (Rao's spacing test, Watson's two-sample test etc.) were used to examine the temporal distribution of locomotor activity in different spider groups (females or males within a species) and to compare the diel activities of the sexes; (**3**) to compare the mean activity levels in different temporal windows (nautical dawn, civil dawn, daytime, civil dusk, nautical dusk and night-time) Linear Mixed-Effect Models (LMMs) were used and for testing the post-hoc differences Student's t-tests with the Holm's correction were applied; (**4**) to examine the periodicities in the activity patterns, a discrete Fourier transform (DFT) analysis was applied; (**5**) and finally, cosinor-based rhythmometry was used to quantify and compare the circadian rhythms of activity in males and females, and the potential differences of the extracted model parameters ('Max', 'Mean', 'Min') between sexes within each species were tested using LMMs.

3. RESULTS

3.1. Results of the faunistic survey

Cyclosa sierrae Simon, 1870 (Araneidae) and *Porrhomma oblitum* (O. P.-Cambridge, 1871) (Linyphiidae) proved to be new spider species for the fauna of Hungary. *Cyclosa sierrae* also represents the first record of this species from Central Europe. Furthermore, I provided evidence about the occurrence of *Dysdera lata* Reuss, 1834 and *Philodromus marmoratus* Kulczyński, 1891 in Hungary. I reported new data for six further species: *Brigittea vicina* (Simon, 1873) (Dictynidae), *Iberina*

microphthalma (Snazell & Duffey, 1980) (Hahniidae), *Mermessus trilobatus* (Emerton, 1882) (Linyphiidae), *Pulchellodromus ruficapillus* (Simon, 1885) (Philodromidae), *Lasaeola prona* (Menge, 1868) (Theridiidae) and *Diaea livens* Simon, 1876 (Thomisidae). All these spider species were collected in apple orchards except *D. lata*, *Ph. marmoratus*, *B. vicina* and *D. livens*.

3.2. Natural prey of hunting spiders in the canopy of apple trees

As a result of our high sampling effort, a total of 878 hunting spider individuals (belonging to 29 species and seven families) were collected with identifiable prey in their chelicerae from the canopy of apple trees between 2013 and 2019. The most abundant spider taxa/groups, in decreasing order, were *C. xanthogramma*, *Ph. cespitum*, *Clubiona* spp., the group 'Other salticids', *E. tricuspidata* and *Xysticus* spp., which accounted for 89% of all spiders in the dataset. Approximately 0.8–1.2 spiders with a prey item in the chelicerae were collected per person-hour, and 34, 46 and 20% of the individuals were collected in the morning, afternoon, and after sunset, respectively. Species of Sternorrhyncha, Brachycera and Nematocera together accounted for 66.5% of the total prey of the hunting spider assemblage, and spiders most frequently (54%) preyed upon arthropods that were irrelevant to pest management (neutral prey). Aphids and spiders were preyed upon to the greatest extent within the pest and natural enemy groups, respectively. In contrast, none of the hunting spiders collected in this study preyed on larvae or adults of codling moth [*Cydia pomonella* (L.)], the key pest of apple in Europe. For the total hunting spider assemblage, prey size was significantly and positively related to spider size (exponential relationship), with the average prey thorax width and spider prosoma width being 1.13 and 1.72 mm, respectively, while the average prey-predator size ratio was 0.67 (SD: 0.34).

3.2.1. Comparison of actual and potential prey: In the apple orchard located in Újfehértó in 2016 and 2017 the relative frequencies of actual prey groups differed significantly from those of potential prey (GLMM-b), demonstrating that hunting spiders, as a community, showed selectivity in their diet. Brachycera and Nematocera were captured significantly more, while Coleoptera was captured significantly less frequently than their abundance would suggest. Brachycera had the highest Ivlev's

index value, while Coleoptera had the lowest value of the Ivlev's index being our measure of selective predation.

Considering the economic status of prey species, the proportions of the categories in the actual prey differed significantly from the potential prey (GLMM-b). We found that the actual prey of arboreal hunting spiders consisted of proportionally more neutral prey, and fewer pest individuals, as compared with the relative abundance of potential prey. Natural enemies were preyed proportionally to their availability. The diets of all hunting spider groups show a similar pattern. Based on the Ivlev's index, four out of the six spider taxa selected natural enemies positively.

3.2.2. Food web metrics, niche width, and niche overlap: For further analyses, we focused only on the most abundant hunting spider groups. Figure 1 shows the trophic interactions between the spider groups and the canopy-dwelling arthropod community for the whole growing season.

However, it has to be noted that the seasonal abundance of the spider and potential prey groups, and therefore the food web structure, showed significant seasonal change. While *Ph. cespitum* was the most abundant hunting spider species in spring, *C. xanthogramma* dominated in summer and fall. Brachycera, Nematocera, and Sternorrhyncha were the most abundant prey groups in spring, summer, and fall, respectively.

Food web specialization (H_2') was the highest in spring and the lowest in summer. In general, hunting spiders were found to be generalists as their species-level specialization (d') was low (values are mostly close to 0) and their trophic niche breadth (B) was wide. *Xysticus* spp., followed by *Ph. cespitum,* was the most specialised (most stenophagous) group and, in accordance with this, had the narrowest niche breadth.
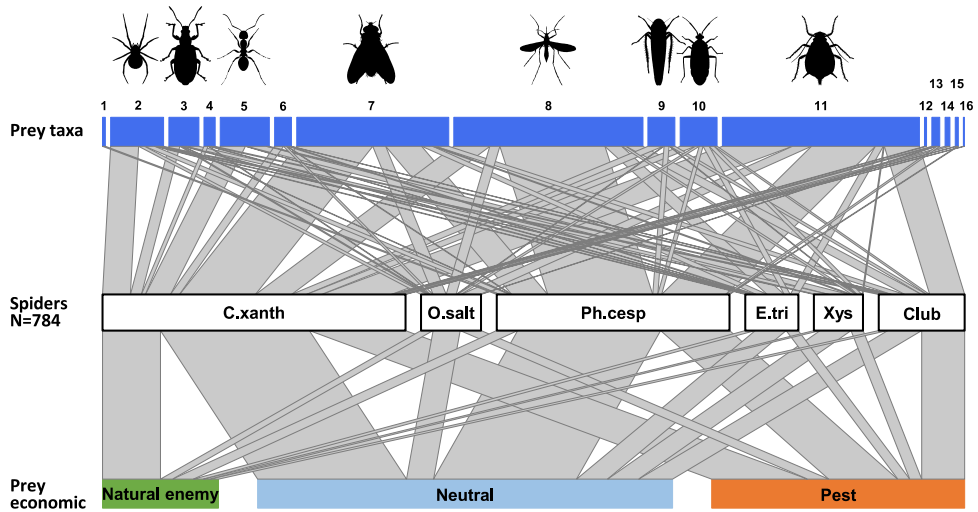
Figure 1: Trophic interactions between the most abundant hunting spider groups and the arthropod community in the canopy of apple trees. Whole growing season, N=784. The middle bars represent spider groups and upper and bottom bars represent the spiders' prey divided taxonomically and according their economic status. The width of the links between the trophic levels depict the frequency of interactions and bar widths indicate the relative abundance of each category. Numbers refer to following prey taxa: **1** Acari, **2** Araneae, **3** Coleoptera, **4** Lepidoptera, **5** Formicidae, **6** Other Hymenoptera, **7** Brachycera, **8** Nematocera, **9** Auchenorrhyncha, **10** Heteroptera, **11** Sternorrhyncha, **12** Ephemeroptera, **13** Neuroptera, **14** Psocoptera, **15** Thysanoptera, **16** Trichoptera; Spiders: **C.xanth** = *C. xanthogramma*, **O.salt** = Other salticids, **Ph.cesp** = *Ph. cespitum*, **E.tri** = *E. tricuspidata*, **Xys** = *Xysticus* spp., **Club** = *Clubiona* spp.

Considering the taxonomic composition of their prey, spider groups exhibited a relatively high level of trophic niche overlap ($0.61 < NO$ in all comparisons), except for *Xysticus* spp., which had a relatively distinct prey composition ($NO < 0.39$ in all comparisons) (Fig. 2A). The highest levels of niche overlap ($0.73 \leq NO$) were observed between the following group pairs: *C. xanthogramma* and Other salticids, *Clubiona* spp. and Other salticids, and *E. tricuspidata* and Other salticids (Fig. 2A). Although the overall niche overlap between spider groups remained relatively high (between 0.51 and 0.85), we found significant differences in 11 out of 15 pairwise comparisons (Fig. 2C).

Niche overlap based on prey taxonomic composition
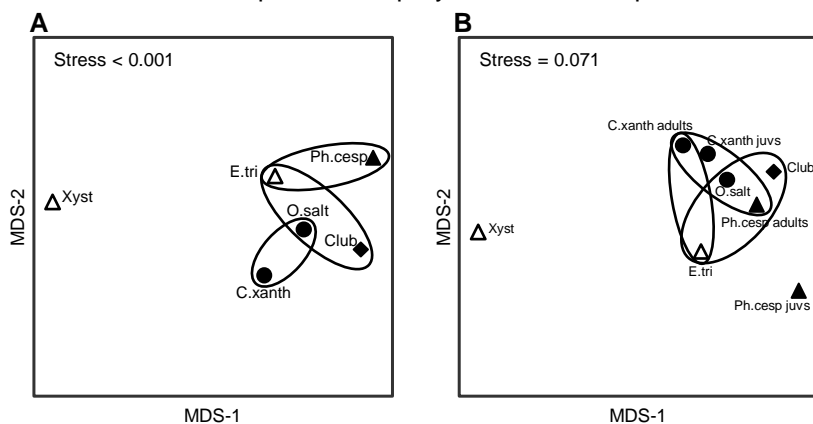
Overall niche overlap
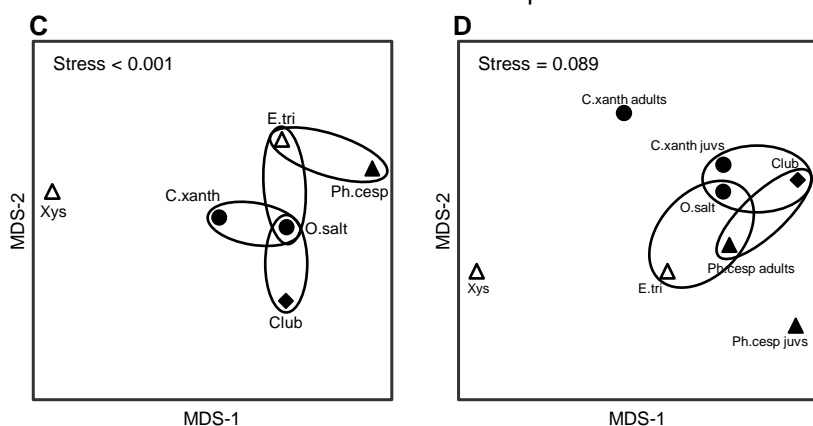
Figure 2: Trophic niche overlap between the most abundant arboreal hunting spider groups in apple orchards. Interspecific similarities in niche overlap based on taxonomic composition of spiders' natural prey (**A** and **B**) and on two functional traits (**C** and **D**): (1) taxonomic composition of natural prey and (2) prey size. (**A**, **C**) the six most abundant spider groups; (**B**, **D**) the same but *C. xanthogramma* and *Ph. cespitum* were split to juveniles (all juvenile stages) and adults (subadults and adults). Similarities are represented graphically as multi-dimensional scaling. Ellipses encircle species occupying niches that were not identified as significantly different using null model tests. Different marks and fill indicate different guilds: circle - stalkers, triangle - ambushers, square - foliage runners (based on guild classification by *Uetz et al., 1999*[1]); empty marks - ambush hunters, solid marks - other hunters (based on guild classification by *Cardoso et al., 2011*[2]). Spiders: **C.xanth** = *C. xanthogramma*, **O.salt** = Other salticids, **Ph.cesp** = *Ph. cespitum*, **E.tri** = *E. tricuspidata*, **Xys** = *Xysticus* spp., **Club** = *Clubiona* spp.

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[1] Uetz et al., 1999. Guild structure of spiders in major crops. *J Arachnol* 27:270-280.

[2] Cardoso et al., 2011. Global patterns of guild composition and functional diversity of spiders. *PloS ONE* 6:e21710.

3.2.3. Fourth-corner analysis of spider-prey associations: Fourth-corner analysis revealed that spider groups and seasons significantly contributed to the prey selection by spiders. Taking into account the differences in total and seasonal abundances we found that, as compared to the other spiders, *C. xanthogramma* was positively associated (PA) with the prey groups Formicidae and Coleoptera and negatively associated (NA) with Nematocera, Lepidoptera, and Heteroptera. Similar selectivity was observed in other spider groups as well: Other salticids (PA: Other Hymenoptera, Sternorrhyncha; NA: Formicidae, Nematocera), *Ph. cespitum* (PA: Nematocera, Sternorrhyncha, Auchenorrhyncha; NA: Formicidae, Coleoptera, Lepidoptera), *E. tricuspidata* (PA: Other Hymenoptera, Heteroptera, Lepidoptera; NA: Araneae, Auchenorrhyncha), *Xysticus* spp. (PA: Formicidae, Coleoptera, Heteroptera; NA: Sternorrhyncha, Brachycera, Nematocera), *Clubiona* spp. (PA: Sternorrhyncha, Lepidoptera; NA: Coleoptera). The coefficient matrix also indicates significant seasonal variation in predation of certain prey taxa (e.g., Araneae, Coleoptera, Nematocera, Auchenorrhyncha) throughout the season.

3.2.4. Intraguild differences and interguild similarities: Based on the guild classification of *Uetz et al. (1999)*, marked intraguild differences and high interguild similarities were found in the composition (taxonomic or taxonomic with size) of natural prey (Fig. 2). Prey preferences could also differ within a guild. Based on the guild classification of *Cardoso et al. (2011)*, certain species also showed significant differences in their diet (Fig. 2) or preferences within the guilds of other hunters or ambush hunters. Furthermore, despite belonging to different guilds, some taxa showed no difference in trophic niche occupancy (Fig. 2).

3.2.5. Predator-prey size relationship: A moderately strong exponential relationship was found between the spider and prey size (GLM-g) for all six hunting spider groups. Spider size differed between spider groups, seasons, and also between prey taxa (LMs, Table 1). Prey size differed between spider groups, seasons, and between prey taxa (LMs, Table 1). The thorax-prosoma ratio differed among the spider groups, the seasons, and the different prey groups (LMs). Compared to their own size, *Ph. cespitum* and *C. xanthogramma* caught the smallest whereas *Xysticus* and *Clubiona* spp. caught the relatively largest prey items (Table 1).

Table 1. Spider prosoma and prey thorax widths, and thorax-prosoma ratios (mean ± SD) for hunting spider groups and seasons.

		Spider taxa				Season		
C. xantho-gramma	Other salticids	*Ph. cespitum*	*E. tricuspidata*	*Xysticus* spp.	*Clubiona* spp.	Spring	Summer	Fall
N*								
275	46	105	44	46	55	161	293	117
Spider prosoma width (mm)								
1.86	1.53	1.59	1.78	1.86	1.20	1.92	1.53	1.89
(0.56) C	(0.36) B	(0.42) B	(0.35) C	(0.68) BC	(0.43) A	(0.59) b	(0.39) a	(0.66) b
Prey thorax width (mm)								
1.18	0.99	0.97	1.28	1.39	0.88	1.30	1.06	1.03
(0.64) B	(0.49) AB	(0.61) A	(0.85) AB	(0.88) B	(0.39) A	(0.69) b	(0.66) a	(0.56) a
Thorax-prosoma ratio								
0.64	0.66	0.62	0.70	0.77	0.77	0.68	0.69	0.59
(0.28) A	(0.29) AB	(0.36) A	(0.41) AB	(0.50) AB	(0.30) B	(0.33) ab	(0.35) b	(0.27) a

Different capital letters indicate significant differences between spider groups, while different lowercase letters indicate significant differences between seasons at *P* < 0.05 level.

*Spiders with no prosoma or prey thorax width data were excluded.

Analysing separately, the prey size was significantly and positively related to spider size for all three main prey groups (Brachycera, Nematocera, Sternorrhyncha). However, the thorax-prosoma size ratio was significantly different, with Brachycera being the largest prey caught by a same-sized spider.

3.2.6. Life stages of C. xanthogramma and Ph. cespitum: Comparing the prey of the spider life stages, the following results were obtained: *C. xanthogramma* adults had the widest trophic niche breadth (*B* = 6.76), followed by *C. xanthogramma* juveniles (*B* = 5.85), *Ph. cespitum* adults (*B* = 4.32) and *Ph. cespitum* juveniles (*B* = 2.51). Considering the taxonomic composition of their prey, these four groups showed a high level of niche overlap (0.70 < *NO*) except for the lower overlap (*NO* < 0.54) between *Ph. cespitum* juveniles and both adults and juveniles of *C. xanthogramma* (Fig. 2B). *Ph. cespitum* adults occupied a trophic niche different from that of juvenile conspecifics, indicating an ontogenetic niche shift (Fig. 2B). Taking into account both niche dimensions, adults and juveniles of both species differed from each other in niche occupancy (Fig. 2D).

3.3. Locomotor activity rhythm of *C. xanthogramma* and *Ph. cespitum*

3.3.1. Activity indices: According to the calculated indices, *C. xanthogramma* was strictly diurnal. Females and males showed similar diurnal and twilight activity. *Ph. cespitum* was active during the whole 24 h period, and thus cannot be considered either strictly diurnal or nocturnal and cannot be characterized as crepuscular, although it was relatively more active during twilight than *C. xanthogramma*.

According to the Student's t-test *Ph. cespitum* males were more active during daytime than the females, which were significantly more crepuscular than the males.
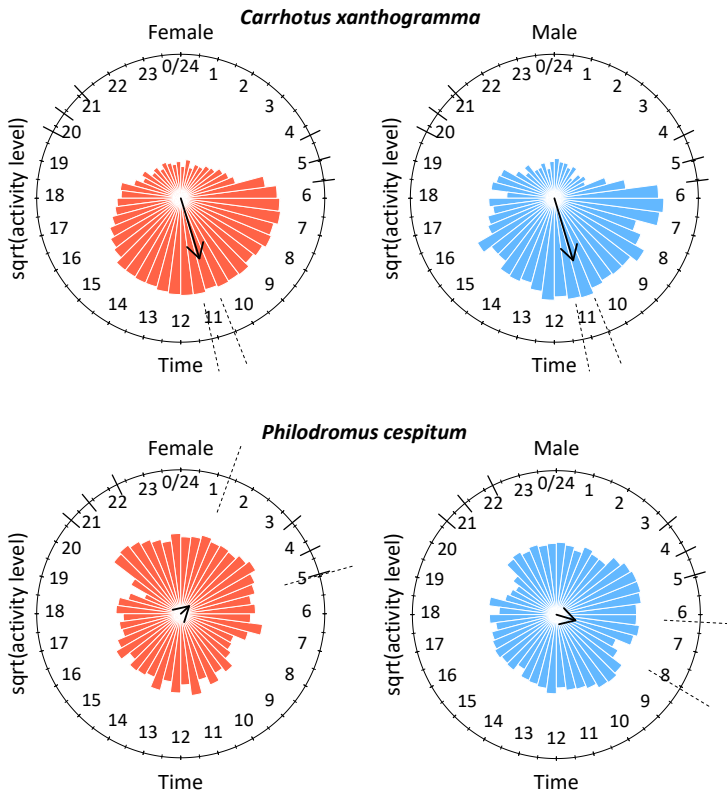

Figure 3: The activity pattern of *C. xanthogramma* (above) and *Ph. cespitum* (below) females (left) and males (right). The mean activity peak (circular mean) is indicated by the arrow and its length is related to the mean resultant length (R). The square-root of the level of activity was plotted at 30 min intervals. Dashed lines indicate the 95% confidence intervals of the mean peak activity and the six shorter solid lines indicate the different twilight periods as follow: nautical dawn, civil dawn, sunrise, sunset, civil dusk and nautical dusk.

3.3.2. Diel distribution of activity: Circular analysis revealed in *C. xanthogramma*, that the mean peak of locomotor activity of females occurred at 10:51 and of males at 10:53 (Fig. 3). Activity patterns of the sexes did not differ (Watson's two-sample test). In case of *Ph. cespitum*, the mean activity peak of the females occurred at 03:09 while that of males was at 07:07. The activity patterns (Fig. 3) of females and males of *Ph. cespitum* differed significantly (Watson's two-sample test).

Comparing the mean activity level of females and males in the same temporal windows using Student's t-test revealed that *C. xanthogramma* females were significantly more active than males during civil dawn and daytime. In contrast to *C.*

xanthogramma, *Ph. cespitum* females were less active during civil dawn and daytime, and significantly more active during civil dusk than males.

    3.3.3. Circa- and ultradian rhythmicity: Fourier analysis revealed that the locomotor activity rhythms of *C. xanthogramma* and *Ph. cespitum* are circadian, as both species exhibited a single cycle per day as the main frequency component. Furthermore, for both species, higher-frequency (ultradian), statistically significant components of activity were detected.
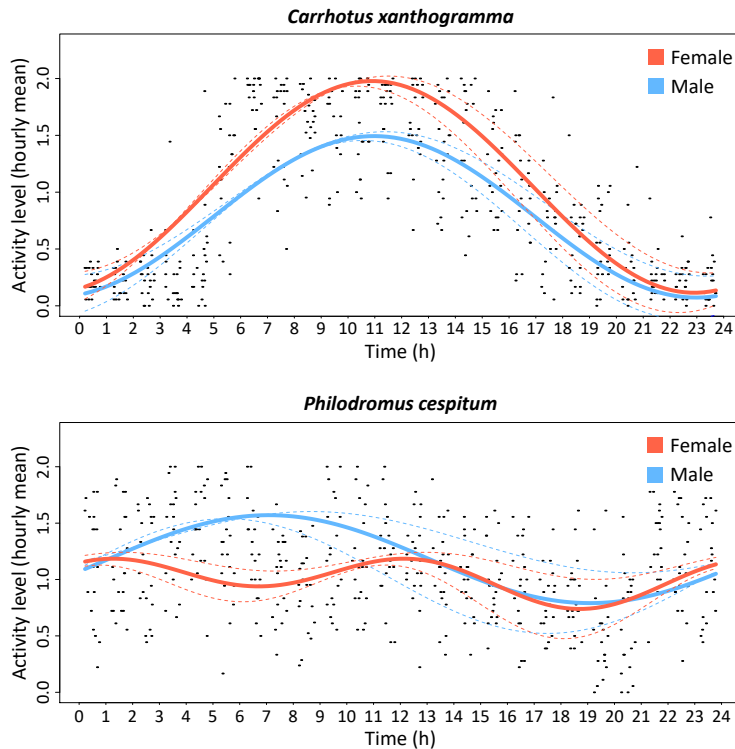
Figure 4: Daily or 12 h locomotor activity rhythms recorded for *C. xanthogramma* (above) and *Ph. cespitum* (below). Cosine-fitted curve based on the model parameters obtained using a fixed 24-h period. Each dot is the hourly average activity level (jittered) of one individual. Dashed lines indicate the 95% confidence intervals of the sex-specific fitted curves.

    In *C. xanthogramma*, ultradian rhythmic components were generally more frequent in males than females, although their relative powers were negligible compared to the relative power of the main 24-h period. *Philodromus cespitum* showed more high-frequency oscillations than *C. xanthogramma*, and in contrast to *C. xanthogramma*, the high-frequency components were more common in *Ph. cespitum* females than males. In addition, the means of the relative power of ultradian

components recorded for females of *Ph. cespitum* was relatively higher than that recorded for conspecific males or *C. xanthogramma*. The vast majority of *Ph. cespitum* females had particularly strong secondary components of activity, of two cycles per day (12 h).

As the 24-h period had a major role in determining the pattern in motor activity, a simple cosine curve or double cosine curve (in *Ph. cespitum* females, due to the presence of a strong 12-h period component) with a 24-h fixed main period was fitted to the diel pattern of locomotor activity rhythm of both sexes for both species (Fig. 4). Comparison of the different parameters obtained from the individually fitted curves revealed that *C. xanthogramma* females were significantly more active at the maximum of the fitted curve, and females also showed a significantly higher mean activity than males, while *Ph. cespitum* males were more active at the maximum of the curve than conspecific females.

4. NEW SCIENTIFIC RESULTS

–As a result of my faunal studies in apple orchards, I reported *Cyclosa sierrae* and *Porrhomma oblitum* as new spider species for the fauna of Hungary (and *C. sierrae* also for Central Europe). *C. sierrae*, *P. oblitum*, *Iberina microphthalma*, *Mermessus trilobatus*, *Pulchellodromus ruficapillus* and *Lasaeola prona* were reported from apple orchards for the first time, and *I. microphthalma* was proved to be not strictly associated with the ground level.

–By analysing a total of 878 hunting spider prey items collected from the canopy of apple trees, I proved that although highly polyphagous, arboreal hunting spiders forage selectively and therefore cannot be considered as entirely opportunistic predators.

–I found that arboreal hunting spider assemblages show positive selection for neutral prey, neutral selection for natural enemies, and negative selection for pests. Therefore, although hunting spiders consume a large number of apple pests, this beneficial effect is strongly constrained by intraguild predation and by a propensity to switch from pests to alternative prey.

–Trophic web dynamics and trophic interactions between the most abundant hunting spider groups and the arthropod community were characterised at the canopy level in fruit orchards for the first time.

–Based on the natural prey, I described the trophic ecology of the most abundant arboreal hunting spider groups inhabiting apple orchards. Ontogenetic shifts in the trophic niche of *Carrhotus xanthogramma* and *Philodromus cespitum* were reported.

–Locomotor activity rhythms of *C. xanthogramma* and *Ph. cespitum* were characterized. I found that the females of *C. xanthogramma* are more active than the conspecific males, which phenomenon is rare in spiders. The activity rhythm of *Ph. cespitum* proved to be cathemeral, and the different sexes have different activity patterns. First time was found that spider sexes might have different sets of ultradian components and their relative powers or importance are differ between sexes. Activity rhythms of *Ph. cespitum* females was proved to be bimodal rather than unimodal.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions regarding the faunistic survey

Given their presence in neighbouring countries and distribution in Europe, the occurrence of the new records (*C. sierrae* and *P. oblitum*) for Hungary is not surprising. Probably the two above mentioned species were naturally spread to Hungary, because human-mediated dispersal is less typical for Araneidae and Linyphiidae species. At the moment, the Spiders of Europe database lists 800 spider taxa for Hungary, but the spiders reported here, and the many other recently described and first recorded species, indicate that the list is still far from complete. Therefore, in Hungary, the number of spider species can be estimated to be much higher than 800. International trade and climate change are the major factors that facilitate the spread and establishment of alien spider species. Currently, one alien spider species per year is introduced to Europe, but this rate will inevitably increase in the future. Therefore, it is important to continue the arachnological exploration of Hungary.

5.2. Conclusions regarding the natural prey and biocontrol potential of arboreal hunting spiders

By analysing a total of 878 hunting spider prey items collected from the canopy of apple trees in apple orchards in Hungary, we concluded the following: Although spiders are characterized as polyphagous predators with a high level of functional redundancy, they exert different predation pressure on different arthropod groups, have their own preferences towards certain prey taxa and select prey by its taxonomic identity and size, which means that the degree of pest suppression depends on the taxonomic composition of the hunting spider assemblage and on the taxonomic identity of the key pests. In other way, promoting particular species or particular pest-consuming functional groups might be more effective in biological control rather than enhancing predator biodiversity, as the effect of increased diversity is highly context-dependent. From an economic point of view, *Ph. cespitum* and *Clubiona* spp. were found to be the most effective natural enemies because of their high level of aphid (*Ph. cespitum* and *Clubiona* spp.) and Lepidoptera (*Clubiona* spp.) consumption and low level of intraguild predation.

5.3. Conclusions regarding the circadian biology of *C. xanthogramma* and *Ph. cespitum*

To summarize the results, we found the followings: (1) females of *C. xanthogramma* are more active than males, (2) whereas in *Ph. cespitum* the sexes have different patterns of activity, (3) based on the data for the two species studied the presence of ultradian components possibly does not depend on the total amount of activity, (4) sexes differ in their set of ultradian rhythmic components, (5) strictly diurnal species (*C. xanthogramma*) have fewer high-frequency oscillations in their locomotor activity rhythm with weaker relative power than in the cathemeral species (*Ph. cespitum*) and (6) for *Ph. cespitum*, in addition to one cycle per day (24-h) oscillation two cycles per day (12-h) oscillation can also play an important role in the temporal pattern in its locomotor activity. Further studies are needed to determine the exact ecological functions of ultradian components and the mentioned sexual differences.

6. PUBLICATIONS RELATED TO THE TOPIC OF THE THESIS[3]

6.1. Publications in scientific journals

Mezőfi L, Markó G, Nagy Cs, Korányi D, Markó V. 2020. Beyond polyphagy and opportunism: natural prey of hunting spiders in the canopy of apple trees. *PeerJ* 8:e9334 (SJR 2019: Q1, IF: 2.379) DOI: 10.7717/peerj.9334.

Mezőfi L, Markó G, Kovács P, Markó V. 2019. Circadian rhythms in the locomotor activity of the spiders *Carrhotus xanthogramma* (Salticidae) and *Philodromus cespitum* (Philodromidae): Temporal patterns and sexual differences. *European Journal of Entomology* 116:158-172 (SJR 2019: Q2, IF: 1.051) DOI: 10.14411/eje.2019.017.

Mezőfi L, Markó V. 2018. Some rare and remarkable spider species from Hungary (Arachnida: Araneae). *Arachnologische Mitteilungen* 55:1-9 (SJR 2018: Q2) DOI: 10.30963/aramit5501.

Mezőfi L, Nagy Cs, Markó V. 2015. Adatok almaültetvények lombozatlakó vadászpók együttesének összetételéről és kártevő korlátozó szerepéről. *Növényvédelem* 51:409-416.

6.2. Publications in other journals

Hoppál D, **Mezőfi L**. 2017. Almaültetvények pókfaunisztikai felmérése a peszticidterhelés függvényében. *Biokultúra* 28:30-31.

6.3. Conference abstracts

Mezőfi L, Markó G, Kovács P, Markó V. 2019. Két agrobiont pókfaj lokomotoros aktivitási mintázatának összehasonlító vizsgálata. XIX. Magyar Pókász Találkozó. 2019. október 4–6. Balatongyörök - Keszthely. Program, Előadások összefoglalói, Résztvevők. p. 26.

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[3]For the rest of the publications of the candidate, see the database of MTMT (https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10052764)

Mezőfi L, Markó G, Nagy Cs, Korányi D, Markó V. 2019. Vadászó pókfajok almaültetvények táplálékhálózatában. 7. Szünzoológiai Szimpózium. 2019. március 29. Magyar Természettudományi Múzeum. Budapest. Absztrakt kötet. p. 10.

Mezőfi L, Markó G, Kovács P, Markó V. 2019. Ivari különbségek és aktivitási mintázatok két agrobiont pókfajnál. 65. Növényvédelmi Tudományos Napok. Agrozoológiai Szekció. 2019. február 19–20. MTA. Budapest. Növényvédelmi Tudományos Napok 2019 kiadványa (ISSN 0231 2956). p. 28.

Mezőfi L, Nagy Cs, Korányi D, Markó V. 2018. Preliminary data on the natural prey of arboreal hunting spiders (Araneae) in apple orchards. 31st European Congress of Arachnology. 8–13 July, 2018. Vác, Hungary. Final Program & Abstracts (**Mezőfi L**, Szita É. eds.). p. 96.

Mezőfi L, Nagy Cs, Markó V. 2018. Mivel táplálkoznak almaültetvények lombozatlakó vadászpókjai (Araneae)?. 64. Növényvédelmi Tudományos Napok. Agrozoológiai Szekció. 2018. február 20–21. MTA ATK – TAKI/NÖVI. Budapest. Növényvédelmi Tudományos Napok 2018 kiadványa (ISSN 0231 2956). p. 34.

Mezőfi L, Gyóni D, Markó G, Bársony K, Markó V. 2017. *Carrhotus xanthogramma* viselkedési bélyegeinek vizsgálata és zsákmányspektruma almaültetvényekben. XVIII. Magyar Pókász Találkozó. 2017. szeptember 22–24. Fertőújlak. Program, Előadások összefoglalói, Résztvevők. p. 23.

Mezőfi L, Nagy Cs, Markó V. 2017. Almaültetvények lombozatlakó vadászpókjainak táplálkozási preferenciái. In: Nagy N, Tóbi I. (szerk.): A Pro Scientia Aranyérmesek XIII. Konferenciájának Tanulmánykötete (ISBN 978-963-88289-3-4). Pro Scientia Aranyérmesek Társasága Egyesület, Pécs. pp. 182-183.

Mezőfi L, Nagy Cs, Markó V. 2016. Adatok almaültetvényekben élő vadász stratégiájú pókok zsákmányspektrumának összetételéhez. XVII. Magyar Pókász Találkozó. 2016. szeptember 16–18. Gárdony. Program és Összefoglalók. p. 10.