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Taxonomic diversity and distribution of mites (Acari: Acariformes and Parasitiformes) in nests of the semi-collared flycatcher (*Ficedula semitorquata*)

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Abstract

The mite fauna inhabiting nests of various birds in Bulgaria and the world as a whole not yet been insufficient studied. Data of mites of many species of birds, one of which is *Ficedula semitorquata* are still scarce. The aim of the present paper is to describe the taxonomic diversity and distribution of astigmatic, prostigmatic and mesostigmatic mites in nests of semi-collared flycatcher in North-East Bulgaria. Differences in the species richness and abundance of mites at studied nest-box plots were assessed using Analysis of Variance (ANOVA). Pairwise comparisons were performed using Tukey´s tests. Twelve species, belonging to seven families of mites (Dermanyssidae, Macronyssidae, Laelapidae, Myonyssidae, Macrochelidae, Cheyletidae and Acaridae) were recorded. 157 (83.51%) of investigating 188 nests were infested with mites and the average abundance per nest was 21.24. The distribution of the found species in the nests and nest-box plots is uneven. Comparisons of species richness and abundance at studied nest-box plots show statistically significant differences. The analysis of the results shows that none of the identified 12 species not found at all seven plots. Only two species - *Dermanyssus gallinae* and *Androlaelaps casalis* were present in six of the sites, and three other - *Androlaelaps fahrenholzi, Cheyletus malaccensis* and *Eucheyletia bakeri* were found only at one of the nest-box plots. The mite fauna in the nests of semi-collared flycatcher seem to be influenced from the quantity and quality of the imported from the outside mites, as well as from the microclimate in the different nests and plots.

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Introduction

Semi-collared flycatcher *Ficedula semitorquata* (Homeyer, 1885) is European species which breeding area covers almost the whole of Southeast Europe, Asia Minor and the western parts of Iran. In Bulgaria the main part of the population is concentrated in the Eastern and Central Balkan Mountain, the Strandzha and the forests in the lower streams of the rivers Batova, Kamchiya and Ropotamo. The species is included in the Red Data Book in Bulgaria, category vulnerable. Its international status is TN, near threatened (IUCN), included in Annexes II of the Bern and Bonn Conventions, what makes the study of its biological characteristics, and factors influencing its breeding, developing and distributing essential. In Bulgaria are nesting around 1500-3500 semi-collared flycatcher pairs.

The density of nesting population depends on the abundance or absence of comfortable seats for nests. Semi-collared flycatcher habitats are mainly old natural broadleaf forests composed of oak (*Quercus* spp.), Polish narrow-leafed ash (*Fraxinus oxycarpa*), beech (*Fagus silvatica*, *Fagus orientalis*) etc. It constructs nest in hollows (including artificial nest-boxes), usually at 3-6 m tall, from moss, dry leaves, grass stems, roots, wool, hair, feathers from other birds. In May female lays from 4 to 7 eggs and incubates them from 12 to 14 days (Georgiev and Yankov, 2015).

Data on the mite fauna in the nests of semi-collared flycatcher were found only in the work of Davidova and Vasilev (2011). The authors investigating the gamasids in nest holes of three passerine birds from Kamchia Mountain reported of finding of three species of gamasid mites - *Dermanyssus gallinae, Ornithonyssus sylviarum* and *Androlaelaps casalis* in seven nests of semi-collared flycatcher.The aim of the present paper is to describe the taxonomic diversity and distribution of astigmatic, prostigmatic and mesostigmatic mites in nests of semi-collared flycatcher in North-East Bulgaria. It is the second of a series of author's publications investigating in detail mite fauna inhabiting the nests of *Ficedula semitorquata* in Bulgaria.

Material and methods

Study Area

To study the mite fauna inhabiting the nests of semi-coloured flycatcher we used artificial nest boxes placed in the area to the North-West of Goritsa village, about 50 km south of Varna, with an altitude of 160-205 m and coordinates N 42°55'10,11" E 27°48'56,08", an area characterized by high concentration of birds with conservation significance. The nest boxes were placed in an oak forest, dominated by *Quercus frainetto*, on seven nest-box plots along the Bulgarian route № 9 (E87), which runs between Varna and Burgas (Fig. 1).

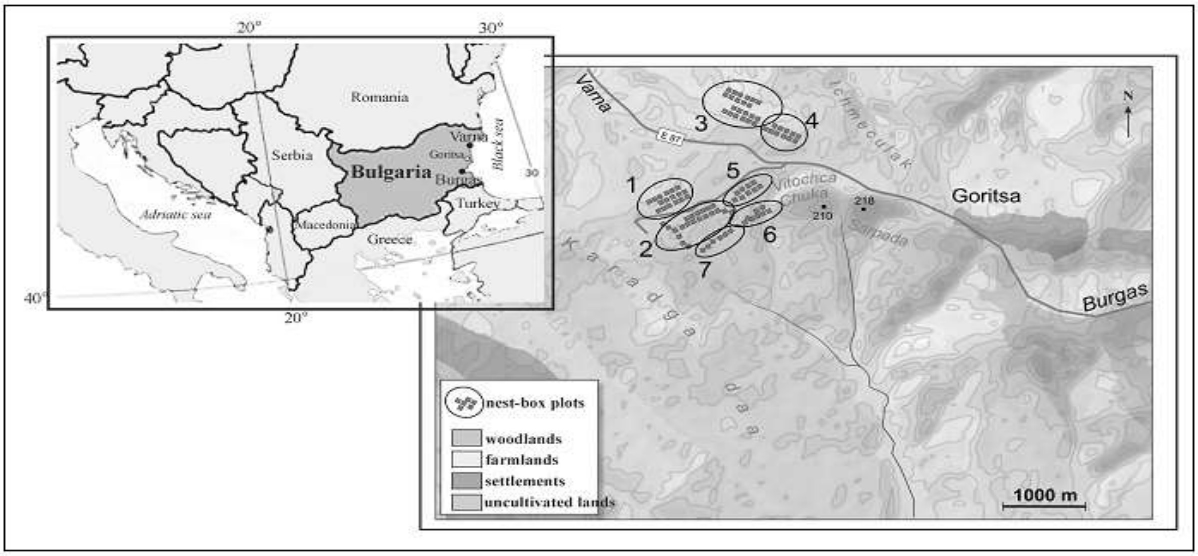

Fig. 1. Map showing the study area and location of the researched nest-box plots.

The nest-box plot I, where have been arranged by the 60 nest boxes for each of the studied years (2012-2015), was situated in a forest area with fairly sparse oaks, without undergrowth.

The nest-box plot II, with at 40 nest boxes for each of the studied years, was placed in a younger oak forest with lower density. The nest-box plot III, with 60 nest boxes for 2012 season, was placed in an area where had dense scrubland and the nest-box plot IV, with 30 nest boxes for 2012, was located in a sparse and tall oak forest and only several areas were overgrown with bushes.

The nest-box plot V, with at 50 nest boxes for the years 2013, 2014 and 2015, was situated in young oak wood with a lower density. The nest-box plots VI and VII, where have been located respectively at 50 for the years 2013, 2014 and 2015 and at 20 nest boxes for the years 2014 and 2015, was placed in a rare and high oak forest in some areas overgrown with bushes.

The artificial nest boxes were made of wood, with dimensions of 18x25x20 cm, and an opening on the front wall measuring 28 mm or 30 mm. The wooden material was natural (mainly fir trees) without outer decoration (painting or varnishing). All nest boxes could be opened from the sides so as to check the broods and clean the nests.

From all 830 artificial nests, put in the studied years (2012-2015), 188 were inhabited by the semi-coloured flycatcher, as the distribution of work plots is as follows: nest-box plot I- 69 nests, nest-box plot II- 44, nest-box plot III- 27, nest-box plot IV- 7, nest-box plot V- 21, nest-box plot VI- 15 and nest-box plot VII- 5.

The remaining boxes were occupied by *Parus major* or *Cyanistes caeruleus*, and in rare cases by the forest dormouse (*Dryomys nitedula*).

Sampling and sample analysis

The nests were collected after the nesting period of birds, placed in polyethylene bags with labels indicating the place and the date of collection, and were taken to the laboratory for a consequent treatment.

The isolation of ectoparasites living in the birds' nests was done by means of Tulgren's funell and by hand with the help of a stereomicroscope. Collected specimens were preserved in 70% alcohol.

Temporary microscope samples were prepared in lactophenol in order to identify the species. Species identification was made according to Bregetova (1956), Till (1963), Fain and Bochkov (2001), Bochkov (2004) and Kontschan (2007).

Data analysis

The dominance (D%) of each taxon was calculated as a percentage of the total count (Margolis *et al.* 1982). According to their dominance the species were divided into 5 groups: eudominant (>10%); dominant (5 - 10%); subdominant (1 - 5%); recedent (0.5 - 1%), and subrecedent (< 0.5%). Differences in the species richness and abundance of mites at studied nest-box plots were assessed using Analysis of Variance (ANOVA) with unequal sample size. Pairwise comparisons were performed using Tukey´s tests. Statistical analysis was carried out using computer programs STATISTICA 9.0 (StatSoft Inc., 2009) and PAST (Hammer *et al.*, 2001).

Results

Taxonomic composition of mite fauna in nests of semi-collared flycatcher

A total of 3994 specimens belonging to twelve species of mites from orders Mesostigmata, Prostigmata and Astigmata were found in the studied nests.

The maximum species and specimens belonged to the Mesostigmata (75.00% and 99.87% respectively), while to the order Prostigmata belonged only 16.67% of the species and 0.08% of the specimens, and to the order Astigmata - 8.33% of the species and 0.05% of the specimens (Table 1).

Table 1. Mite fauna established in nests of *F. semitorquata*. N - number of specimens; Nn - number of nests in which the species was established; Range - minimum-maximum number of specimens per nest; Mean - mean number of specimens per nest; Plots - nest-box plots in which the species was established.

Taxa	N	Nn	Range	Mean	Plots
Mesostigmata					
Laelapidae Berlese, 1892					
Androlaelaps casalis (Berlese, 1887)	195	36	1-45	1.24	I, II, III,V,VI, VII
Androlaelaps fahrenholzi (Berlese, 1911)	2	1	2	0.01	III
Hypoaspis heselhausi (Oudemans, 1812)	11	5	1-4	0.07	I, II, III
Hypoaspis lubrica Oudemans et Voigts, 1904	13	8	1-4	0.08	I, II, V, VII
Dermanyssidae Koleant, 1859	2628	105	1-395	16.74	I, II, III, IV, V, VI
Dermanyssus gallinae (De Geer, 1778)					
Dermanyssus hirundinis (Hermann, 1804)	8	3	1-5	0.05	I, III, VI
Myonyssidae Evans & Till, 1966	55	4	1-36	0.35	III, V
Myonyssus gigas (Oudemans, 1912)					
Macronyssidae Oudemans, 1936	1075	41	1-163	6.85	I, II, III, IV, VI
Ornithonyssus sylviarum (Canestrini et Fanzago, 1877)					
Macrochelidae Vitzthum, 1930	2	2	1	0.01	I, II
Macrocheles glaber (Muller, 1860)					
Prostigmata	2	1	2	0.01	I
Cheyletidae Leach 1815					
Cheyletus malaccensis Oudemans, 1903					
Eucheyletia bakeri Volgin, 1969	1	1	1	0.01	III
Astigmata	2	2	1	0.01	II, VI
Acaridae Latreille, 1802					
Rhizoglyphus echinopus (Fumouze & Robin, 1868)					

Mites were found in 157 out (83.51%) of all studied 188 nests of semi-coloured flycatcher. Their density varied between one and 401 specimens, average of 21.24 specimens per nest. The number of species found in one nest varied from one to four species.

The most frequent and abundant is *D. gallinae*, established at 105 nests of the investigated 188 and the number of specimens of this species varies from 1 to 395 in one nest (Table 1). In 38 (20.21%) of the nests this species was the only mite species observed. The second most frequent and abundant species of mite is *O. sylviarum*, established at 41 nests, the number of specimens ranges from 1 to 163 in one nest. *A. casalis* also present at a different number of specimens in the nests - from 1 to 45 and was found in 36 of the nests. The other mites are less frequent and abundant.

The species *Eucheyletia bakeri*, *Androlaelaps fahrenholzi* and *Cheyletus malaccensis* present only at one nest and the number of specimens found was 1 or 2 per nest.

Distribution of mites in nest-box plots.

The distribution of species found in the investigated nests and plots is uneven.

Comparing species richness and abundance of mite in studied nest-box plots showed that the differences were significant (ANOVA, respectively F= 3.493, df= 31.94, p= 0.009 and F= 4.467, df= 44.72, p= 0.001). Of all seven studied plots, at nest-box plot III we found the highest number of specimens - 1564 or 39.15 % of all 3994 specimens, and species - nine of a total established twelve (Fig. 2). The species *O. sylviarum* and *D. gallinae* were established with the highest relative significance, respectively 51.54% and 47% and belong to the group of eudominants.

All other species belong to the groups of recedents - *A. casalis* (0.77% dominance) or subrecedents - *Dermanyssus hirundinis* (0.32%), *A. fahrenholzi* (0.13%) and *Hypoaspis heselhausi, Myonyssus gigas, E. bakeri* and *Rhizoglyphus echinopus* (by 0.06%) (Fig. 3).

At nest-box plot I was found eight mite species and 1313 specimens or 32.88% of the total found.

The highest dominance was recorded of *D. Gallinae*-77.3%. *O. sylviarum* also belongs to the eudominants but with a significantly lower relative significance-12.26%. According to their dominance the remaining four species were distributed as follows: *A. casalis* (9.14%) was dominant, *H. heselhausi* (0.69%)-recedent and *H. lubrica* (0.23%), *D. hirundinis* (0.15%), *C. malaccensis* (0.15%) and *Macrocheles glaber* (0.08%)- subrecedents (Fig. 2 & 3).

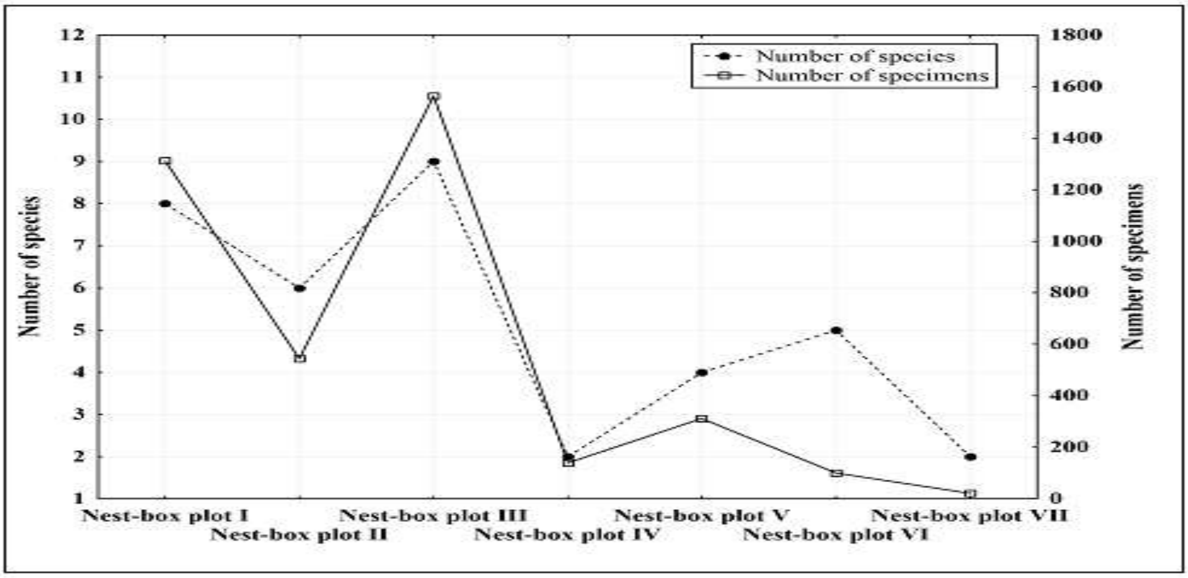

Fig. 2. Number of species and specimens in different nest-box plots.

At nest-box plot II was found 544 specimens or 13.62 % of a total established specimens and six mite species. The species *D. gallinae* (82.54%) and *O. sylviarum* (12.69%) were eudominants again. The subdominants were two as well - *A. casalis* (2.94%) and *H. lubrica* (1.47%). *H. heselhausi* (0.18%) and *M. glaber* (0.18%) were established with at one specimens and belong to the group of subrecedents (Fig. 2 & 3).

At nest-box plot VI was found 100 specimens or 2.5 % of a total established and five mite species. With the highest number of specimens was *D. gallinae* (85.00%), followed by *A. casalis* (11.00%). The other three species belong to the subdominants- *O. sylviarum* (2.00%), *D. hirundinis* (1.00%) and *R. echinopus* (1.00%) (Fig. 2 & 3).

At nest-box plot V was found four mite species and 312 specimens or 7.81% of the total found. The number of the eudominants was two again, but these were: *D. gallinae* with dominance 77.56% and *M. gigas* with 17.31%. *A. casalis* belongs to the subdominants and *H. lubrica* is subrecedent (Fig. 2 & 3).

At nest-box plots IV and VII only two species were found. At plot IV and both species were eudominants-*D. gallinae* (73.38% dominance) and *O. sylviarum* (26.62%). The number of specimens in the plot IV is 139 and represents 3.48% of the total found. At plot VII the dominant structure is significantly different compared to other nest-box plots, as the highest number of specimens has *A. casalis* (95.45% dominance) who is also the only eudominant. The second species *H. lubrica* with dominance 4.55% belongs to the subdominants. The number of specimens in the plot VII is 22 and represents only 0.56% of the total found.

Considering the number of specimens per nest we found that at nest-box plot III it is much higher (57.92) compared to other nest-box plots, respectively, 19.86 at the nest-box plot IV, 19.03 at the nest-box plot I, 14.86 at the nest-box plot V, 12.39 at the nest-box plot II, 6.67 at the nest-box plot VI and is significantly higher in comparison with 4.4 at the nest-box plot VII (Fig. 4). There is a statistically significant differences in terms of species abundance per nest at nest-box plot III and nest-box plot VII (Tukey's Pairwise comparison, $p < 0.05$).

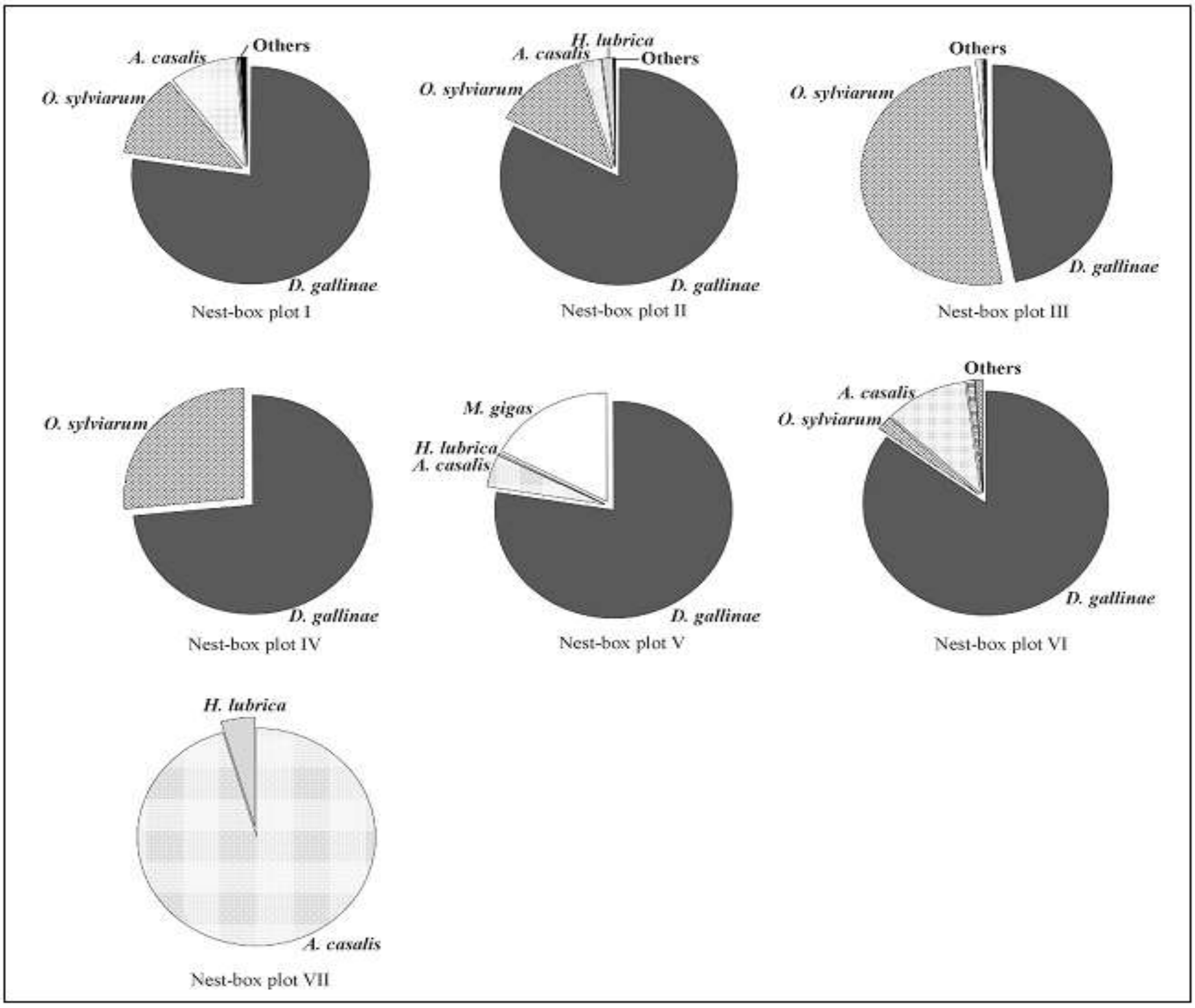

Fig. 3. Pie charts showing the dominance of mite species in different nest-box plots.

The mean number of species per nest is also the highest at nest-box plot III – 1.78 (Fig. 5), but observed differences were not significant (Tukey's Pairwise comparison, $p > 0.05$). Most of the nests of nest-box plot III were infested with two mite species while in all other plots was highest the number of nests in which is found only one species (Fig. 6).

Discussion

Taxonomic composition of mite fauna

The results indicate that the mites in nests of *F. semitorquata* may be highly diverse in terms of both species composition and degree of infestation of nests of different species.

Dominating among them are three species from order Mesostigmata- *D. gallinae*, *O. sylviarum* and *A. casalis* while the other nine species and especially those from the orders Prostigmata and Astigmata are present with relatively smaller number or found in single specimens.

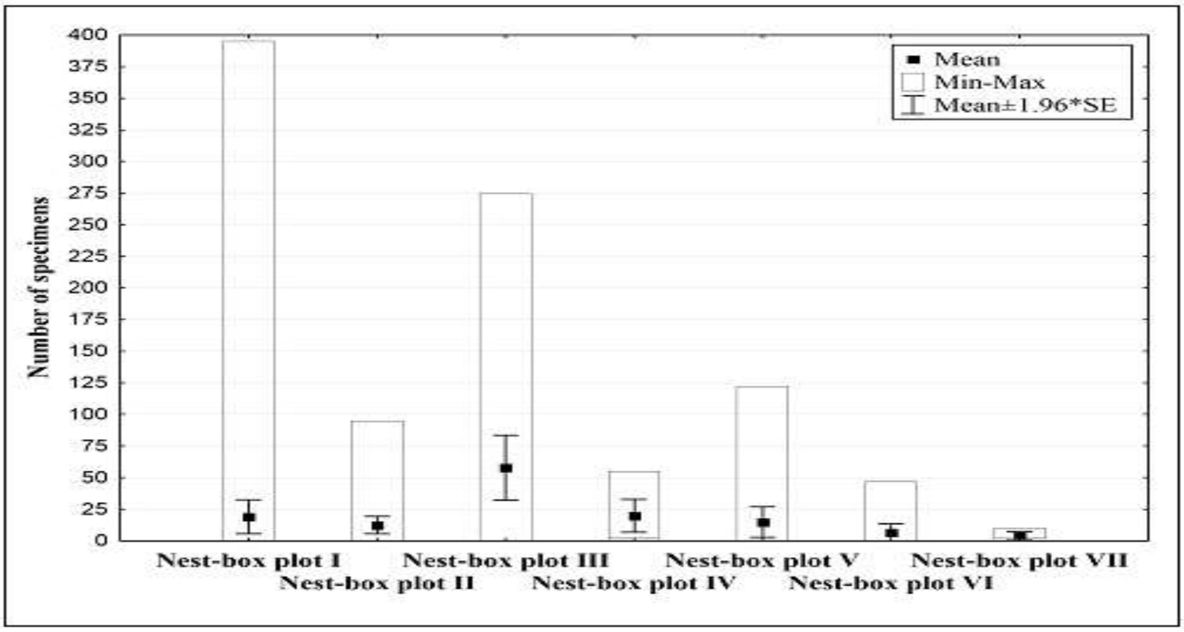

Fig. 4. Box plots comparing mean number of specimens per nest and minimum to maximum specimens per nest in different nest-box plots. Species abundance per nest was significantly higher in nest-box plots III in comparison with it's in nest-box plot VII (Tukey´s pairwise comparison, $p<0.05$ ($p=0.04$).

The our results agree with observations of other authors who indicated that the main elements of nesting fauna of birds are *D. gallinae*, *O. sylviarum* and *A. casalis* occurring with high frequency and abundance (Ambros *et al.*, 1992; Salmane, 2001; Tryjanowski *et al.*, 2001; Krištofík *et al.*, 2003, 2005, 2007; Švaňa *et al.*, 2006; Fenďa, 2009).

Distribution of mites in nest-box plots.

Although the seven nest-box plots are located relatively close to each other and are separated only by the road, concerning the species composition and abundance in them some differences were found. On the one hand this is evident in the differences in taxonomic richness and number of specimens in the nests and plots, as well as comparatively low faunal similarity between some of the nest-box plots.

By cluster analysis of data for qualitative and quantitative composition of communities of mites is made analysis the degree of similarity of the studied communities in the seven nest-box plots (Fig. 7). Nest-box plots I, II, IV, V and VI are separated in one group. With the highest degree of similarity in the group are plots I and II.

This is likely due to the almost equal number of species in worksites (8 at plot I and 6 at plot II) and the largest number of the same species - 6. All species found in plot II are established and in plot I. At degree of similarity 0.9 individually is separated nest-box plot III.

This plot has a different number of the same species with nest-box plots I, II, IV, V and VI, and therefore cannot be added to them. Significantly higher taxonomic diversity (9 species, 2 of which are found only in it) and abundance (number of specimens per one nest 57.92) of mite fauna found in the nests of plot III probably due to the different conditions in it.

The forest area in which the plot III was located is characterized by a higher density of trees and with significant development of bush vegetation compared to all other plots.

This contributes to the maintenance of the humidity in the nests for an extended period of time, to reduce the power of the wind, which creates favorable conditions for the development of a more abundant nest fauna.

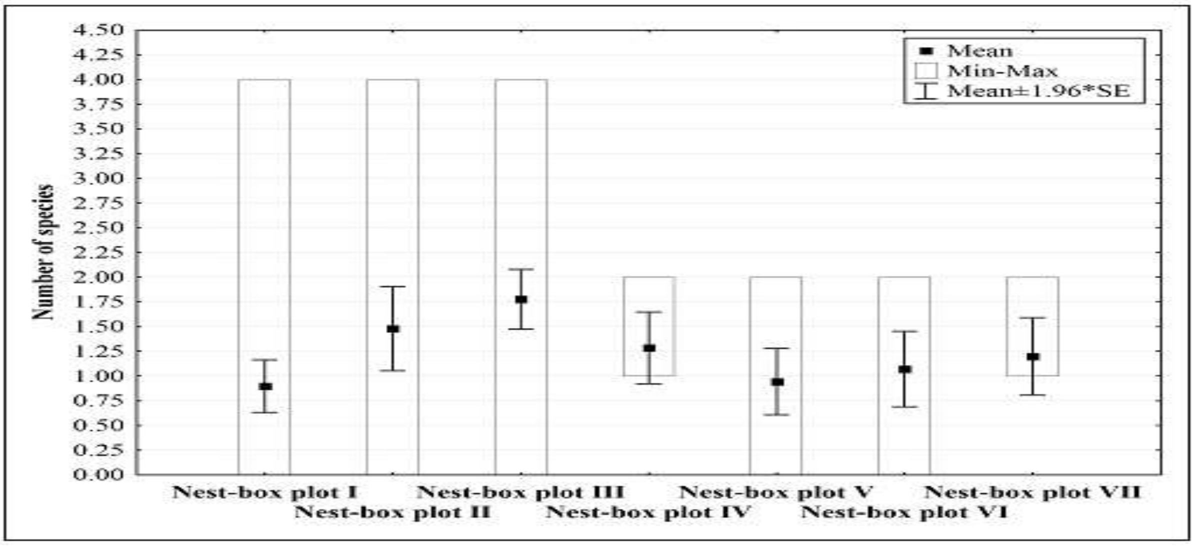

Fig. 5. Box plots comparing mean number of species per nest and minimum to maximum species per nest in different nest-box plots.

The most different is mite fauna at nest-box plot VII, as the degree of its similarity to the fauna of other plots is very low.

On the one hand, this plot is characterized by lowest abundance of mites - the number of specimens per one nest was only 4.4.

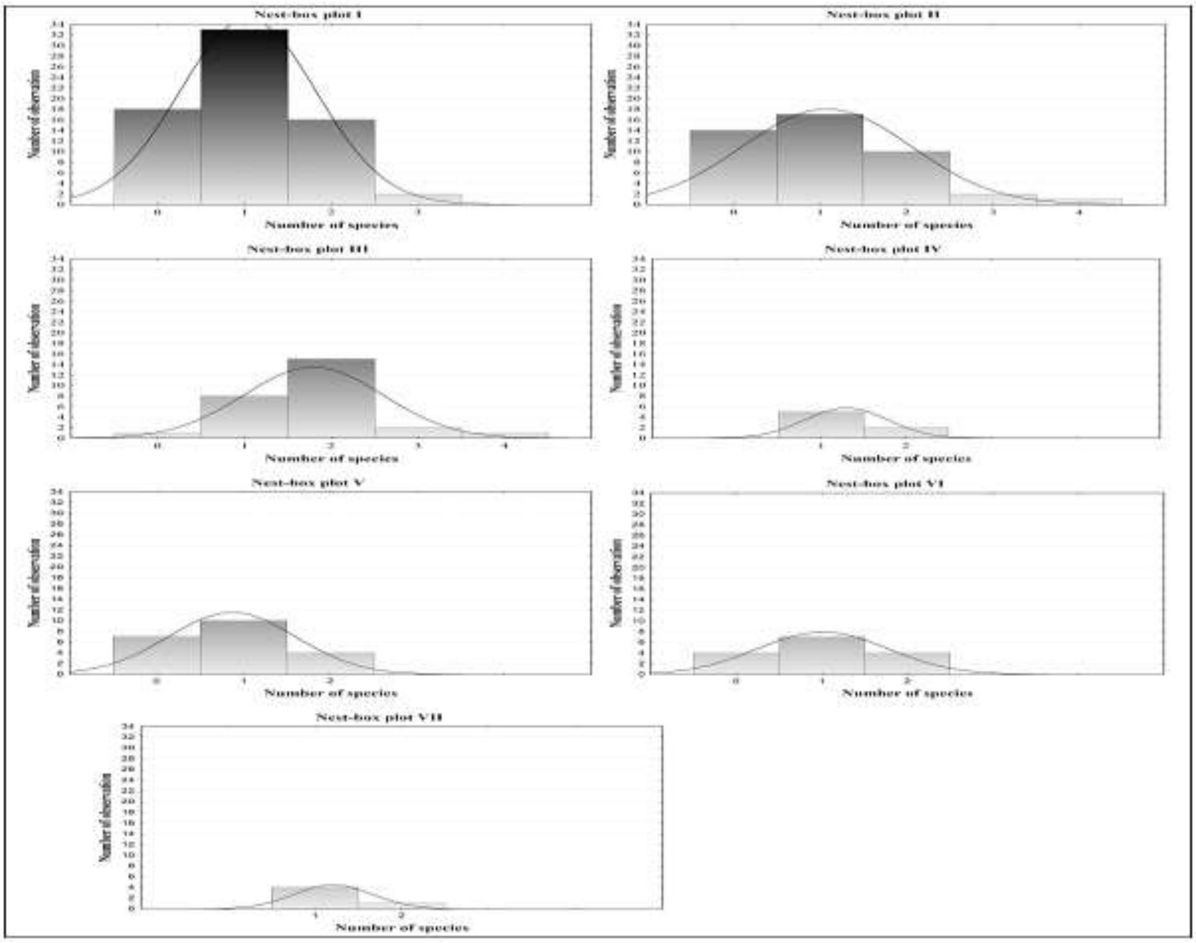

Fig. 6. Histograms comparing the number of species observed in the nests in different nest-box plots.

On the other hand there is established an entirely different and unusual structure - dominant species is *A. casalis*, and the species *D. gallinae* is not established. The observed differences in community structure in nest-box plot VII compared to other sites probably due to the antagonistic relationship between species *D. gallinae* and *A. casalis*.

The strong development of cosmopolitan predatory mite *A. casalis* suppresses the development of *D. gallinae*, which is observed by Lesna *et al.* (2009) in nests of starlings. At all other plots, except plot VII, although the number of identified species and specimens is very different, there is a strong development of species *D. gallinae* - typical blood sucking ectoparasite on birds (Ambros *et al.*, 1992; Gwiazdowicz *et al.*, 1999; Salmane, 2001; Tryjanowski *et al.*, 2001; Krištofík *et al.*, 2003, 2005, 2007; Švaňa *et al.*, 2006; Fenďa, 2009).

On the other hand the differences are confirmed having in mind the structure of mite communities. The analysis of the results shows that none of the identified 12 species not found at all seven plots. Only two species are present in six of the sites - *D. gallinae* and *A. casalis*, and three other species are found extremely rare - only at one of the nest-box plots. These are *A. fahrenholzi*, *C. malaccensis* and *E. bakeri*.

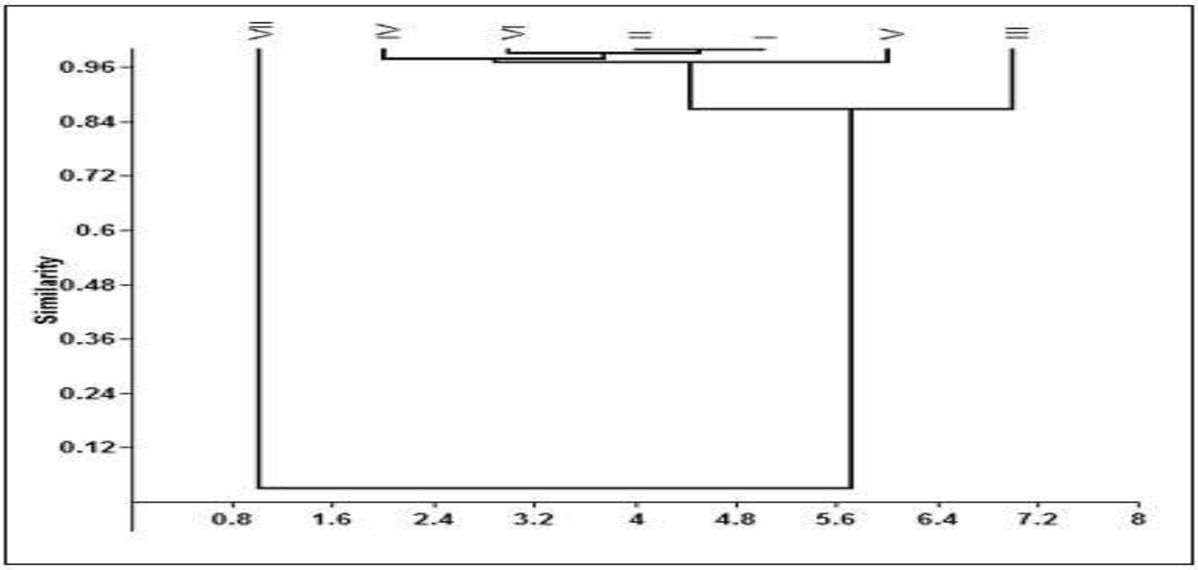

Fig. 7. Cluster dendrogram, showing the similarity of the mites communities in different nest-box plots.

The mite fauna in the nests of semi-collared flycatcher is influenced from the quantity and quality of the imported from the outside mites - from bird itself, along with nesting material and food, as well as from the microclimate in the different nests and plots. Results from the present study confirm to the observation of Fenda and Schniererova, 2004, who studying mesostigmatic mites in nests of *Acrocephalus* spp. found that the nest localisation and nest material structure are more important factors for the composition of nest fauna than the species of nesting bird.

Conclusions

The nests of semi-collared flycatcher are inhabited by the varied mite fauna represented by twelve species from orders Mesostigmata, Prostigmata and Astigmata.

The mites are unevenly distributed in the investigated nests and plots. The taxonomic diversity and abundance in studied nest-box plots is different which leads to the conclusion that each nest and nest-box plot is characterized by specific mite fauna.The highest number of specimens and species were found at nest-box plot III, located in an area covered with dense shrubs.

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