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DIVERSITY OF CLICK BEETLE ASSOCIATED WITH MAIZE AND SUNFLOWER CROPS FROM SOUTH AND SOUTHEASTERN ROMANIA

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ABSTRACT

Wireworms are considered key pests in agriculture across Europe, and are particularly important in Romania for maize and sunflower crops in favorable years. The present study aimed to evaluate the structure of the click beetle (Coleoptera:Elateridae) fauna associated with corn and sunflower crops in the south and south-east of Romania, based on the specimens collected during 5 years (2015-2019), in 25 plots from 9 localities belonging to 3 counties. The analysed material included a number of over 3000 specimens and the results showed a significantly high click beetles diversity represented by 16 species and a variety assigned to 9 genera and 8 subfamilies. At regional scale, dominant species were found: *Agriotes ustulatus* (22.15%), *Drasterius bimaculatus* (19.17%), *Agriotes sputator* (14.4%), *Agriotes obscurus* (11.45%), *Agriotes lineatus* (9.72%) and *Selatosomus latus* (8.84%). The analysis of the data highlights the similarity between the Elateridae communities in Călăraşi and Ialomița Counties, where the dominant species were *A. ustulatus* and *A. sputator*, and their net difference with the click-beetles communities in Constanța County, where the dominant species were *D. bimaculatus, S. latus* and *A. sputator*. The obtained results represent the starting point of a database on the wireworm fauna associated with maize and sunflower crops in Southern Romania, and bring basic information’s needed for further development of Integrated Pest Management.

Keywords: click beetle diversity, Elateridae, maize, sunflower.

INTRODUCTION

According to the National Statistics Institute, annually, 2.4-2.7 million hectares of maize and 1-1.3 million ha of sunflower were cultivated all over the country (Romanian Statistical Yearbook 2021; MADR data, 2022), which represent around 35-40% of arable land in Romania. This data positions Romania often on the first place in the EU in case of surfaces harvested but not always as the producer of the largest amount of maize or sunflower (FAOSTAT, 2021). In the period 2015-2020, the annually average yield per hectare of maize ranged from 3.42 to 7.64 tons while for sunflower was between 1.76 to 3.04 tons per hectare (MADR data, 2022; Romanian Statistical Yearbook, 2021). Maize and sunflower crop production greatly depend on the agricultural technologies (Dragomir et al., 2022; Brumă et al., 2021), abiotic stresses like prolonged drought and irregular rainfall patterns and biotic stresses exhibited by pest insects (Chávez-Arias et al., 2021). The area of south and south-east of the Romania is particularly susceptible to the attack of the maize leaf weevil (*Tanymecus dilaticollis* Gyll) which is the main pest of maize and sunflower crops (Popov et al., 2007; Georgescu et al., 2016, 2020) and wireworms (Mărgărit et al., 1990, 1998; Manole et al., 1999; Popov et al., 2001) that in favourable conditions might induce significant yield losses or even compromise the crops. Wireworms are soil-dwelling larvae of click-beetles (Coleoptera: Elateridae), being known over ten thousands of species but only a few are considered key pests in agriculture across Europe (Furlan et al., 2017a). In Europe, EU Directive 2009/128/EC (Official Journal of the European Union, 2009) on the sustainable use of pesticides makes it mandatory the implementation of integrated pest management (IPM) for annual

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crops from January 2014. IPM procedures for efficient management of few important wireworm pest species were intensively studied and damage thresholds of *Agriotes ustulatus, A. brevis and A. sordidus* in Europe is already available (Furlan, 2014; Furlan et al., 2017b; Furlan et al., 2020). Development of IPM strategies for wireworms remain a difficult task due to the scarcity of reliable information on key aspects like their biology and dynamic, their long developmental cycle (of 2 to 5 years), their polyphagous feeding, patchy distributions and the simultaneously occurrence of more than one species at the same area. In this context, identifying which wireworm species are present in the area is of tremendous importance, as wireworm damage and threshold (Perju et al., 1971; Furlan, 2014; Poggi et al., 2021) is species dependent. The present study aimed to bring new data about the structure of the click beetle (Coleoptera: Elateridae) fauna associated with corn and sunflower crops in the south and south-east of Romania, based on a long term sampling effort of 5 years, as a basis for further development of Integrated Pest Management.

MATERIAL AND METHODS

The study was carried out during 5 years (2015-2019) in 25 plots (5 each year), cultivated with corn or sunflower, within 9 localities in 3 counties (Călăraşi, Ialomița, Constanța) (Table 1). The sampling was performed monthly, between April and July, each year. At every plot, 4 pitfall traps, containing 4% formaldehyde solution, were installed and the content collected after 7 days. After this period, all samples were transferred to laboratory, sorted at family level and preserved in 70% alcohol. Click beetles were identified to species according to Lohse (2012a, 2012b). For the present study, exclusively the data from the plots established with seeds not treated with insecticides were used.

Graphical representation of click beetle species community structure and relative frequency by localities was done using MS Excel. Abundance and relative frequency of each taxon, species richness, dominance and Shannon-Weiner diversity index were calculated using pooled data sets of the click beetle community for each site. A comparison of the click beetle diversities in the different sites and between years was also performed. Graphical representations, dendrograms, determination of similarity indices and distances were performed using the Past 4.03 application (Hammer, 2018). The grouping method was based on the Euclidean distance and the algorithm used was Paired group (UPGMA). The Euclidean distance between two points “x” and “y” having Cartesian coordinates x_i and y_i with i=1,...n, in an n-dimensional space, is given by the formula:

$$\mathbf{d(x,y)} = \sqrt{\sum_{i=1}^{n}(x_i - y_i)^2}$$

The extent to which the dendrogram followed the original data was determined using cophenetic correlation.

RESULTS AND DISCUSSION

Altogether, 3056 Elateridae beetles were collected, belonging to 8 Subfamilies, 9 genera, 16 species and 1 variety (Table 2). Out of 142 species cited for Romanian fauna (Zaharia, 2006), 59 were found to occur in agricultural crops and various spontaneous plant species adjacent to them and only 18 were confirmed for maize and sunflower crops (Manole et al., 1999).

Overall, dominant species were found: *Agriotes ustulatus* (22.15%), *Drasterius bimaculatus* (19.17%), *Agriotes sputator* (14.4%), *Agriotes obscurus* (11.45%), *Agriotes lineatus* (9.72%) and *Selatosomus latus* (8.84%), followed by *A. ustulatus* var. flavicornis (3.24%) and *A. pilosellus* (3.01%) (Table 2).

Table 1. Details about maize or sunflower plots in south-eastern Romania where click-beetles were sampled

Year of study	Locality (County)	Plot ID Code	Crop	Cultivar	Soil name
2015	Săpunari (Călăraşi)	Sa1(CL)	Maize	PHY LEAXX	Cambic chernozem
	Fundulea (Călăraşi)	Fu1(CL)	Maize	Olt	Cambic chernozem
	Borduşelu (Ialomiţa)	Bo1(IL)	Sunflower	P64LE25	Cambic chernozem
	Agigea (Constanța)	Ag1(CT)	Sunflower	P64LE25	Vermi-calcic chernozem
	Mihail Kogălniceanu (Constanța)	Mk(CT)	Maize	Olt	Vermi-calcic chernozem
2016	Săpunari (Călăraşi)	Sa2(CL)	Maize	TEXXEL	Cambic chernozem
	Fundulea (Călăraşi)	Fu2(CL)	Maize	Olt	Cambic chernozem
	Belciugatele (Călăraşi)	Be1(CL)	Sunflower	P64LE99	Cambic chernozem
	Agigea (Constanța)	Ag2(CT)	Maize	DK4795	Vermi-calcic chernozem
	Mihail Kogălniceanu (Constanța)	Mk2(CT)	Maize	Olt	Vermi-calcic chernozem
2017	Belciugatele (Călăraşi)	Be2(CL)	Maize	Alexxandra	Cambic chernozem
	Săpunari (Călăraşi)	Sa3(CL)	Maize	Alexxandra	Cambic chernozem
	Borduşelu (Ialomiţa)	Bo2(IL)	Sunflower	P64LE99	Cambic chernozem
	Agigea (Constanța)	Ag3(CT)	Maize	PO412	Vermi-calcic chernozem
	Mihail Kogălniceanu (Constanța)	Mk3(CT)	Sunflower	P64LE25	Vermi-calcic chernozem
2018	Belciugatele (Călăraşi)	Be3(CL)	Maize	P 9903	Cambic chernozem
	Săpunari (Călăraşi)	Sa4(CL)	Maize	DKC4590	Cambic chernozem
	Borduşelu (Ialomiţa)	Bo3(IL)	Maize	DCK4670	Cambic chernozem
	Agigea (Constanța)	Ag4(CT)	Sunflower	P64LE99	Vermi-calcic chernozem
	Mihail Kogălniceanu (Constanța)	Mk4(CT)	Sunflower	P64LE25	Vermi-calcic chernozem
2019	Coslogeni (Călăraşi)	Co1(CL)	Maize	Olt	Cambic chernozem, limo-argilous type
	Belciugatele (Călăraşi)	Be3(CL)	Maize	RGT Lexxtour	Cambic chernozem
	Borcea (Călăraşi)	Bo1(CL)	Maize	DKC4670	Calcari-gleyic Luvisol
	Agigea (Constanța)	Ag5(CT)	Maize	P64LE99	Vermi-calcic chernozem
	Topraisar (Constanța)	To(CT)	Maize	P9903	Vermi-calcic chernozem

Table 2. Structure of click-beetle fauna collected in 5 years (2015-2019) in various plots cultivated with maize or sunflower from 9 localities in south and south eastern Romania

Crt. No.	Subfamily	Taxon	ID Code	Adult incidence in pitfall traps			R %
				No. of localities	No. of county	No. of specimens	
1	Adrastinae	*Synaptus filiformis* (Fabricius, 1781)	*Sy. filiformis*	3	2	24	0.79
2	Agriotinae	*Agriotes gurgistanus* (Faldermann, 1835)	*A. gurgistanus*	6	3	47	1.54
3		*Agriotes lineatus* (Linnaeus, 1767)	*A. lineatus*	8	3	297	9.72
4		*Agriotes obscurus* (Linnaeus, 1758)	*A. obscurus*	9	3	350	11.45
5		*Agriotes pilosellus* (Schonherr, 1817)	*A. pilosellus*	5	3	92	3.01
6		*Agriotes sputator* (Linnaeus, 1758)	*A. sputator*	6	2	440	14.40
7		*Agriotes ustulatus* (Schaller, 1767)	*A. ustulatus*	9	3	677	22.15
8		*Agriotes ustulatus var. flavicornis* (Panzer, 1799)	*A. ustulatus v. flavicornis*	6	2	99	3.24
9	Athoinae	*Athous haemoroidallis* (Fabricius, 1781)	*At. haemoroidallis*	3	2	18	0.59
10		*Athous (Hemicrepidius) niger* (Linnaeus, 1758)	*At. niger*	4	2	40	1.31
11		*Cidnopus (Limonius) pilosus* (Leske, 1785)	*Ci. pilosus*	3	2	55	1.80
12	Cardiophorinae	*Cardiophorus rufipes* (Goeze, 1777)	*Ca. rufipes*	1	1	5	0.16
13	Conoderinae	*Drasterius bimaculatus* (Rossi, 1790)	*D. bimaculatus*	9	3	592	19.37
14	Ctenicerinae	*Selatosomus latus* (Fabricius, 1801)	*S. latus*	4	2	270	8.84
15	Elaterinae	*Ampedus sinuatus* (Germar, 1844)	*A. sinuatus*	3	2	28	0.92
16	Melanotinae	*Melanotus crassicollis* (Erichson, 1841)	*M. crassicollis*	2	1	7	0.23
17		*Melanotus rufipes* (Herbst, 1784)	*M. rufipes*	3	2	15	0.49
TOTAL				**9**	**3**	**3056**	**100.00**

Our data partly confirm data from previous study performed in 1994-1999 by Popov et al. (2001) considering the prevalence of main click beetle in field crops as follow: *Agriotes ustulatus* (40.1%), *A. obscurus* (17.3%), *A. sputator* (11.3%), *A. flavicornis* (10.9%), *A. pilosus* (6.9%), *A. lineatus* (3.8%), *Limonius pilosus* (1.8%), *Synaptus filiformis* (1.6%) and *Selatosomus latus* (1.5%). Also, we found 17 out of 18 species confirmed for maize and sunflower crops by Manole et al. (1999) in a study examining specimens collected over a long period of time at national scale, but scarcely covering the area investigated here.

Throughout the study period, the most abundant species were found: *A. ustulatus* (677 specimens), *D. bimaculatus* (592 specimens), *Agriotes sputator* (440 specimens) (Table 2, Figure 1 and 2), the same species being the only ones sampled in all the 9 localities studied (Figure 3).

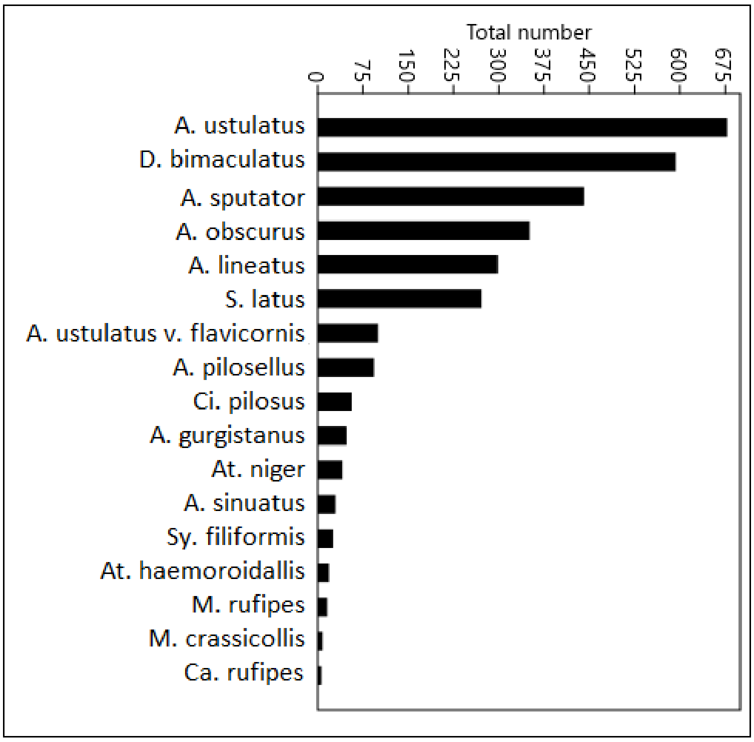

Figure 1. Hierarchy of species according to their abundance throughout the study period

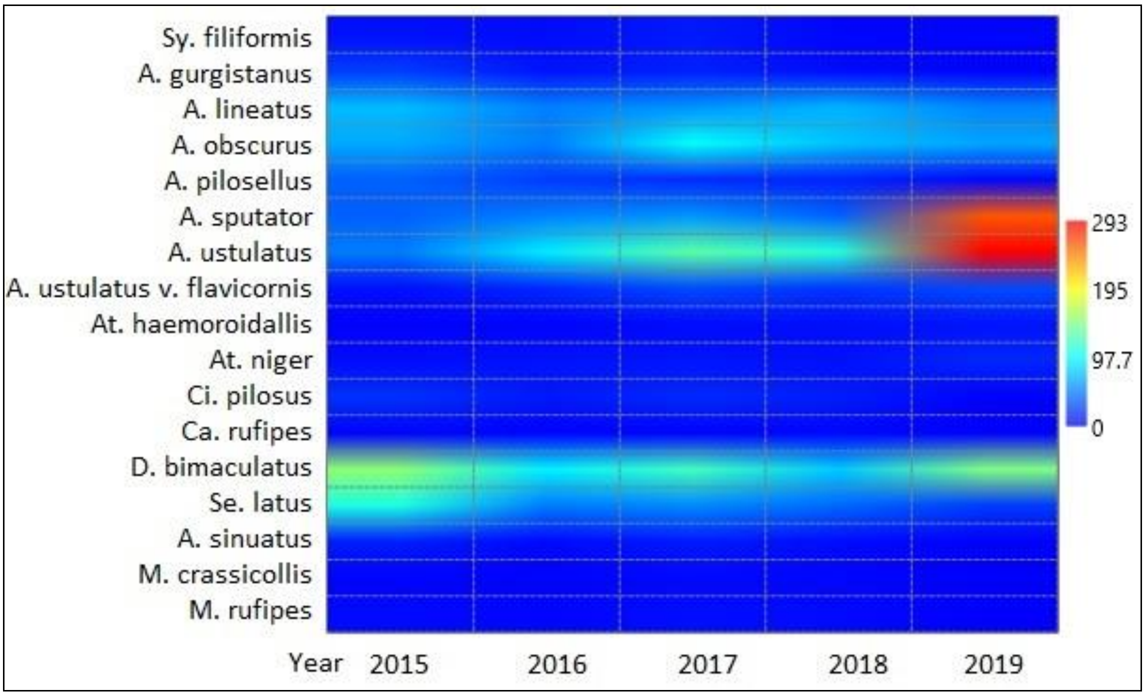

Figure 2. Matrix plot for describes the number of individuals captured from each species

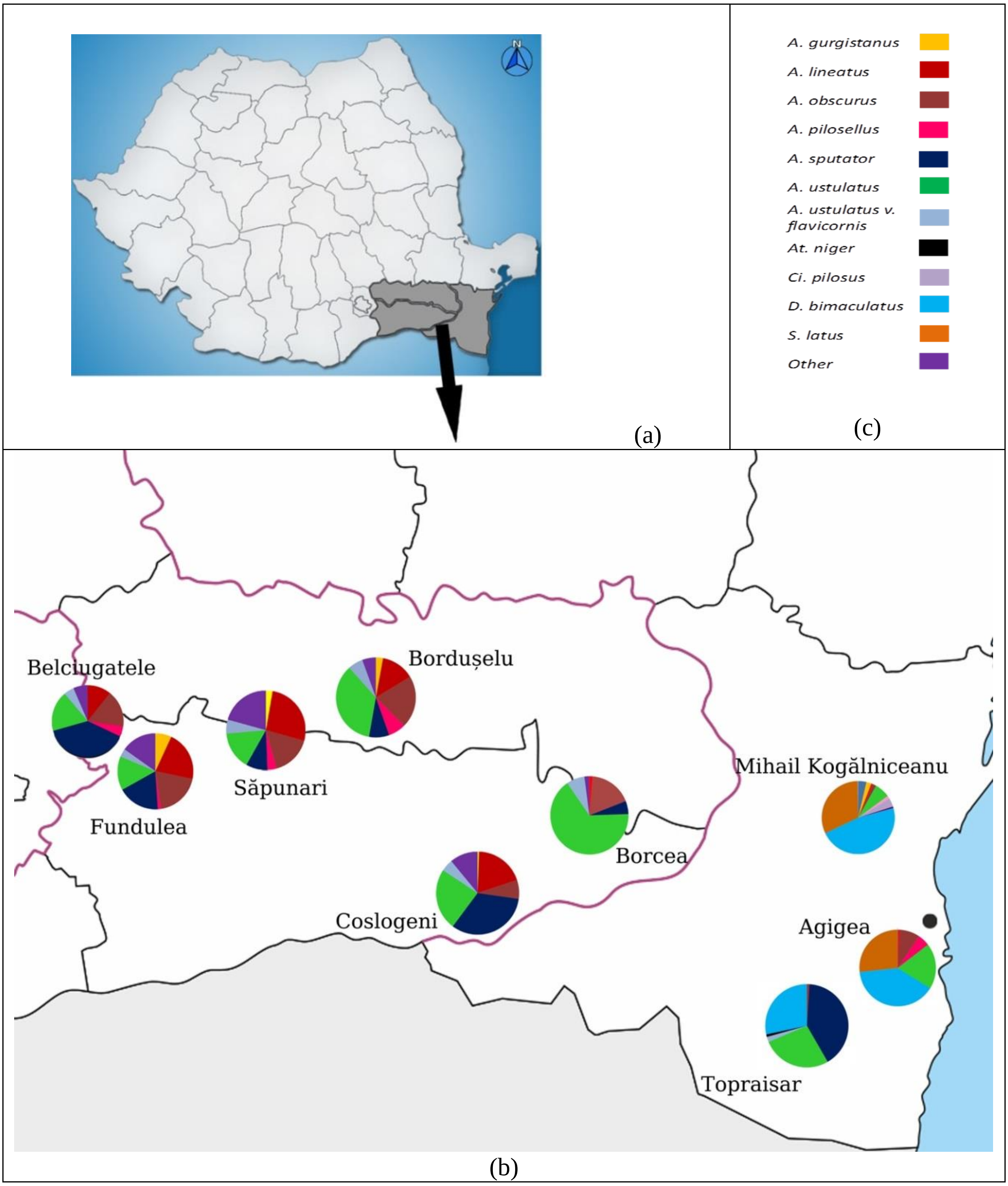

Figure 3. Map of the study area from south and south-eastern Romania (a), with diagrams of structure and relative frequency of the communities of click-beetle collected in 5 years (2015-2019) cultivated with maize or sunflower from 9 localities (b), and legend (c).
The Elateridae species corresponding to each of the codes in the legend are listed in Table 2.

When the diversity indices were compared for all 25 sites together (Table 3), we found the highest species richness at Săpunari (13-14 species) and Fundulea (12 species), both localities also reached the highest values of Shannon Diversity (2.03 to 2.24).

Table 3. Abundance and diversity indices of analysed samples depending on plot

	PLOT ID												
	Sa1 (CL)	Fu1 (CL)	Bo1 (IL)	Ag1 (CT)	Mk1 (CT)	Sa2 (CL)	Fu2 (CL)	Be1 (CL)	Ag2 (CT)	Mk2 (CT)	Be2 (CL)	Sa3 (CL)	Bo2 (IL)
S	14	11	11	5	9	14	12	7	6	10	7	14	10
A	99	86	123	102	185	128	59	74	71	117	100	138	155
D	0.13	0.18	0.18	0.39	0.41	0.16	0.17	0.22	0.28	0.31	0.22	0.14	0.23
H	2.24	1.97	1.90	1.18	1.13	2.14	2.04	1.63	1.43	1.51	1.67	2.22	1.78
	Ag3 (CT)	Mk3 (CT)	Be3 (CL)	Sa4 (CL)	Bo3 (IL)	Ag4 (CT)	Mk4 (CT)	Co1 (CL)	Be4 (CL)	Bo1 (CL)	Ag5 (CT)	To1 (CT)	
S	5	10	10	13	9	7	10	10	8	6	4	6	
A	103	141	81	113	101	85	102	191	129	147	120	306	
D	0.26	0.30	0.19	0.18	0.29	0.23	0.24	0.22	0.32	0.48	0.35	0.31	
H	1.47	1.58	1.91	2.03	1.51	1.61	1.69	1.77	1.48	1.07	1.14	1.30	

S - Species richness; A - Total no. of specimens; D – Dominance; H - Shannon-Weiner diversity index.
The localities name corresponding to each of the plot ID are listed in Table 1.

In order to evaluate whether during the period 2015-2019 the species keep their place in a hierarchy of species abundance, indicated by the total number of captured individuals, Kendall correlation coefficient of ranks was used. The data in Table 4 positioned below the main diagonal indicate the correlation coefficient values, and show that with the exception of 2019 compared to 2015 all other values for the successive pairs of years analysed revealed strong correlations, higher than the value of 0.7, statistically ensured with $p<0.001$. The year 2019 appears to be atypical compared to 2015, bringing a change in the hierarchy given the abundance of species captured. This is explained by the trend of dominant click beetles evolution in 2019 compared to 2015, species *A. ustulatus* and *Agriotes sputator* having best conditions for their population development in 2019, related to climatic favorability, respectively temperatures in soil layer of 0-10 cm above 12 degrees accompanied by soil humidity over 30%, as a result of frequent rains (Perju and Mare, 1984), and their multiannual cycle of 3-5 years (Table 4, Figure 3). From the point of view of the total number of individuals collected each year, 2019 was particularly favourable for click beetles, followed by 2017 and 2015, and less favourable were 2016 and 2018 (Figure 4 and 5, Table 5).

Table 4. Kendall tau correlation coefficient / p - value

	Year 2015	Year 2016	Year 2017	Year 2018	Year 2019
Year 2015		<0.001	<0.001	<0.001	0.11271
Year 2016	0.82922		<0.001	<0.001	<0.001
Year 2017	0.7663	0.96671		<0.001	<0.001
Year 2018	0.70786	0.94556	0.96647		<0.001
Year 2019	0.39891	0.80335	0.77837	0.75503	

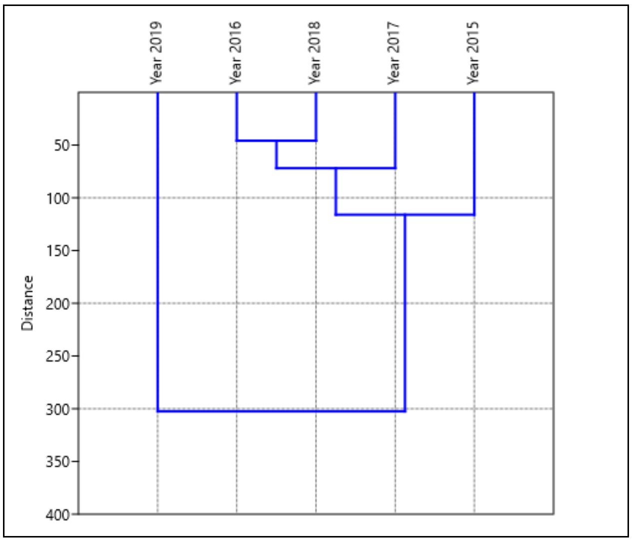

Figure 4. The dendrogram for groups determined by years. Cophen. Corr. = 0.9841 (Cophenetic correlation coeficient)

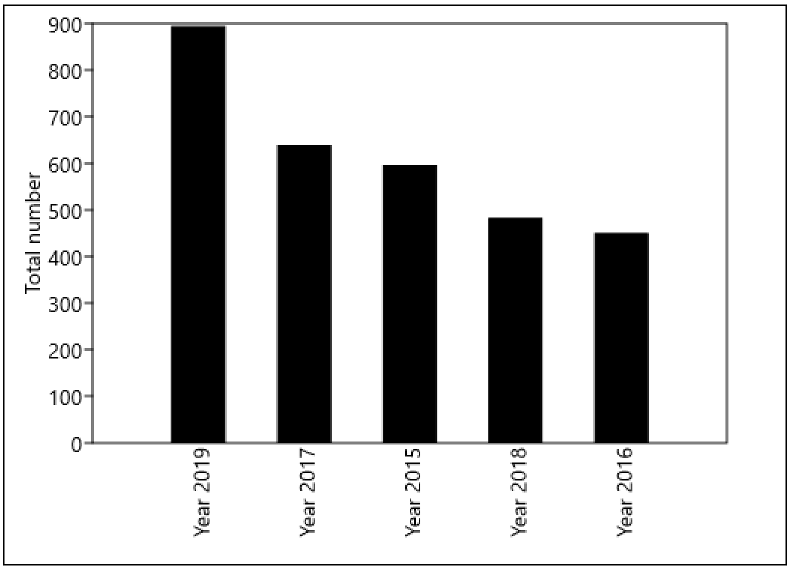

*Figure 5***.** Ranking of years by total number of individuals captured

Table 5. Similarities and distance indices (euclidean) between years

	Year 2015	Year 2016	Year 2017	Year 2018	Year 2019
Year 2015	0				
Year 2016	104.14	0			
Year 2017	118.58	77.89	0		
Year 2018	125.37	45.83	65.96	0	
Year 2019	347.07	300.06	265.51	297.64	0

The abundance of sampled individuals of each species, obtained by summing the data from the analysed locations, with data grouped for each species by year, were used to create the cluster diagram in figure 6. Cluster analysis data shows interesting groups determined by species; for example there is a group formed by *Cardiophorus rufipes* and *Melanotus crassicollis*, located at a small distance from *Athous haemoroidallis*, respectively, *M. rufipes*. Also at a short distance are *A. sinuatus* and *Synaptus filiformis*.

Their group diverges to a level of about 100-150 units from the group formed by *A. lineatus*, *A. obscurus* or *Selatosomus latus*. The entire group determined by the previously mentioned species is significantly different from group of the species *A. sputator, A. ustulatus*, *D. bimaculatus* (Figure 6).

The differences are also confirmed by the hierarchy shown in Figure 2, which indicates the positioning of the last three mentioned species in the top of the species ranking according with their abundance. The cophenetic correlation coefficient has a high value of 0.95, indicating that the dendrogram revealed by Figure 6 preserves the real distances given by the values obtained from the field. These are in line with our personal observation that click beetles might form mixt communities in agricultural crops for example *A. ustulatus* with *A. sputator* or *A. lineatus* with *A. obscurus*.

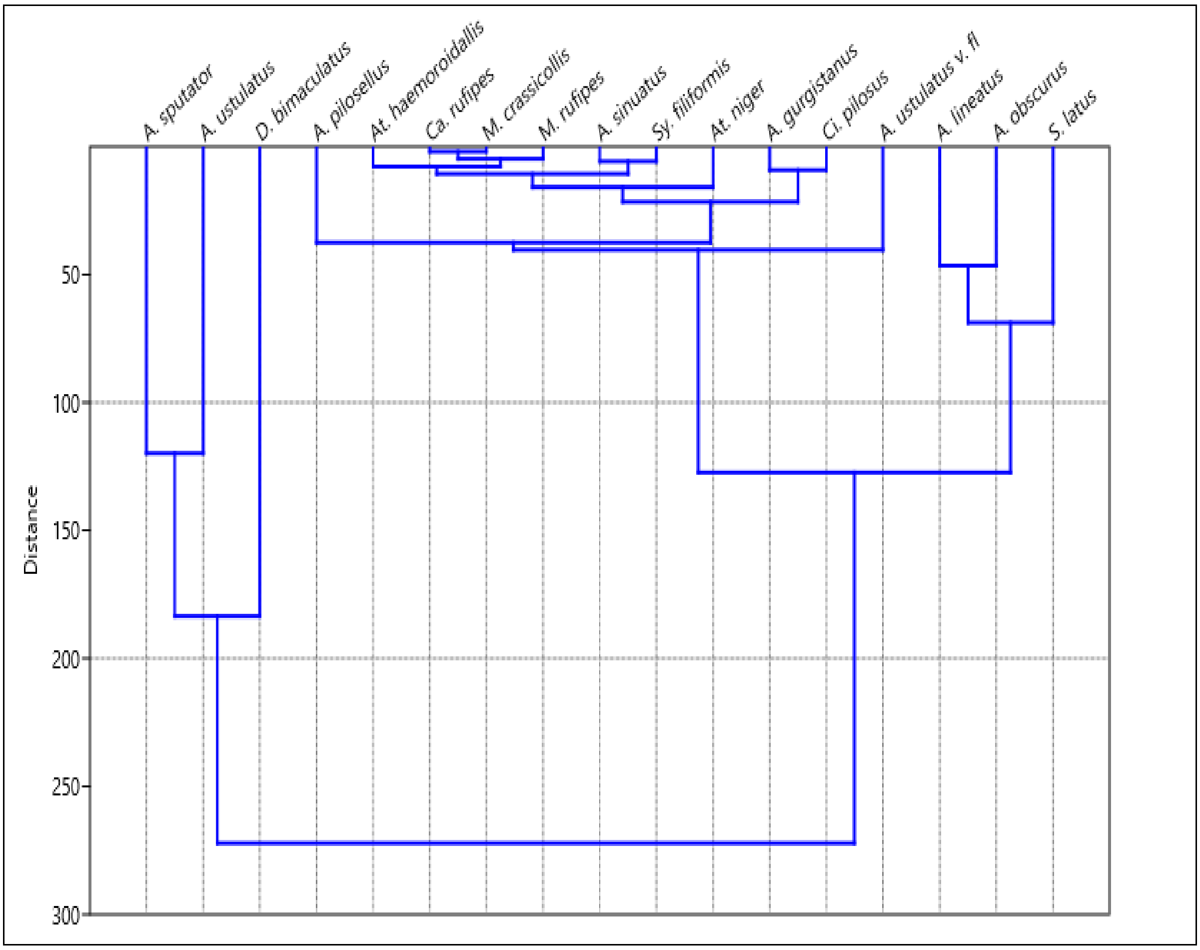

Figure 6. The dendrogram for groups determined by species. Cophen. Corr. = 0.9587

The average values of the abundances of click beetles for five localities (Borduşelu, Săpunari, Belciugatele, Agigea and Mihail Kogălniceanu) were used to create the cluster diagram in Figure 7, made using the Euclidean distances values found in Table 6. The high value of the cophenetic correlation coefficient 0.85 indicates the fidelity of the dendrogram in describing the original data. When analysed the general fauna structure by location, we found a cluster formed by Borduşelu and Săpunari, slightly different from Belciugatele but together very different by the cluster formed by Agigea and Mihail Kogălniceanu from Constanța County (Figure 7). The list of dominant species is similar; but their prevalence differs depending on the location, so that in the first 3 localities species of *Agriotes* predominate, while *Drasterius bimaculatus* is dominant in Constanța localities.

Table 6. Similarities and distance indices (euclidean) between locations using the average of the values

	Sa(CL)	Bo(IL)	Ag(CT)	Mk(CT)	Be(CL)
Sa(CL)	0.00	-	-	-	-
Bo(IL)	32.92	0.00	-	-	-
Ag(CT)	56.63	56.90	0.00	-	-
Mk(CT)	81.57	85.61	32.82	0.00	-
Be(CL)	36.10	40.96	57.18	81.84	0.00

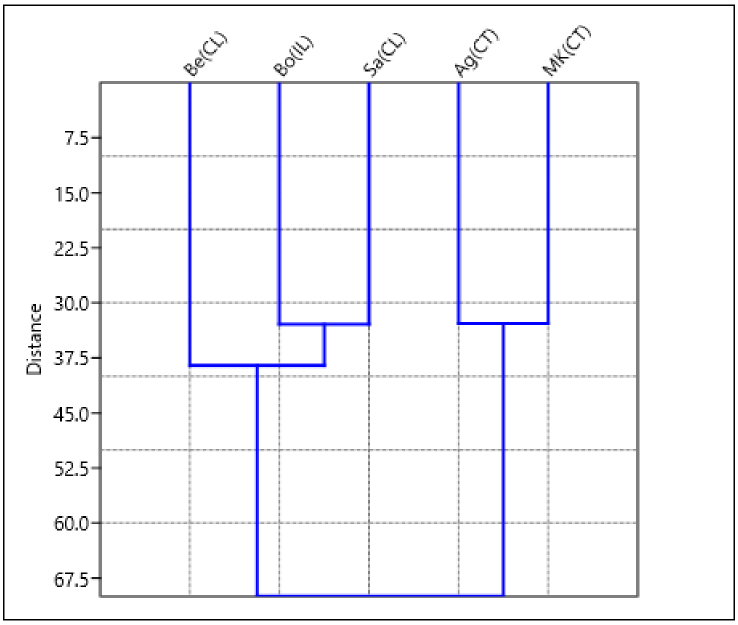

Figure 7. The dendrogram for groups determined by locations using the average of the values. Cophen. Corr. = 0.8554

CONCLUSIONS

The paper brings actual data on the specific composition of click beetle associated with maize and sunflower crops in the main agricultural basin from south and south-eastern Romania. In total samples, 16 species and a variety assigned to 9 genera and 8 subfamilies were identified. This diversity of click beetle in the maize and sunflower studied area is significantly high, and put in evidence the importance of research studies for the areas where the fauna is poorly studied.

At regional scale, core species were found: *Agriotes ustulatus*, *Drasterius bimaculatus*, *Agriotes sputator*, *Agriotes obscurus*, *Agriotes lineatus* and *Selatosomus latus*. Variation of their abundance depend on the year conditions and species biology but specific assemblage of those species can be found at every location. *Agriotes ustulatus* and *A. sputator* were dominant in Călăraşi and Ialomița County while for Constanța County *Drasterius bimaculatus* was preponderant, followed by *S. latus* and *A. sputator*.

The obtained results represent the starting point of a database on the wireworm fauna associated with maize and sunflower crops in Southern Romania, and bring basic information's needed for further development of Integrated Pest Management.

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