Estimation of the competitiveness of *Ephedrus plagiator* in relation to other parasitoids from the subfamily Aphidiinae

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Abstract: The parasitoid species *Ephedrus plagiator* (Nees, 1811) (Hymenoptera: Aphidiinae) is one of the most important biological agents against pest aphids. We investigated whether this species was in competition with some other aphidiine species for the same hosts. We thus examined its potential in biological programs to control aphids. We applied an unsupervised artificial neural network, a self-organizing map (SOM), which classified the competitive parasitoids into seven groups. The SOM also visualized the distributional pattern of 31 parasitoid wasps along the neural network, revealing their competitive ability in relation to *E. plagiator*. Indicator value (IndVal) analysis quantified the competitive ability and showed that the most competitive species with regard to *E. plagiator* were *Lysiphlebus testaceipes* (Cresson, 1880), *L. fabarum* (Marshall 1896), *L. cardui* (Marshall 1896) and *Binodoxys angelicae* (Haliday, 1833). These species appeared in four different SOM groups and mostly parasitized the *Aphis fabae* Scopoli, 1763 (Hemiptera: Aphididae) host.

Keywords: Braconidae; Aphididae; competitive species; trophic association; self-organizing map (SOM)

INTRODUCTION

Parasitoid wasps from the subfamily Aphidiinae (Hymenoptera: Braconidae) are solitary endoparasitoids of aphids [1]. Over 400 species have been registered worldwide [2,3]. One of the largest genera from this subfamily is *Ephedrus* Haliday, 1833, with about 55 described species worldwide [4]. *Ephedrus plagiator* (Nees, 1811), as a natural enemy of pest aphids, plays an important role in biological control programs, and the parasitoid has been selected for commercial production by some companies. It has been well studied in many aspects of its morphology [5], as well as its biology and ecology [6,7] and phylogeny [8,9]. *E. plagiator* is involved in tritrophic associations with various aphid species and numerous host plants [10,11]. This parasitoid attacks more than 120 aphid species, predominantly from the genera *Aphis* Linnaeus, 1758, *Macrosiphum* Passerini, 1860 and *Myzus* Passerini, 1860 [11-13]. In addition, Žikić et al. (14) presented even wider host range of *E plagiator*, which attacks 167 species from 51 genera belonging to seven aphid subfamilies. Its host range often overlaps with other polyphagous parasitoids such as *Aphidius ervi* Haliday, 1834, *A. matricariae* Haliday, 1834, *E. persicae* Froggatt, 1904, *Lysiphlebus fabarum* (Marshall 1896), *L. testaceipes* (Cresson, 1880) and *Praon volucre* (Haliday, 1833) [14]. In addition to the Palaearctic region, *E. plagiator* also inhabits the Nearctic and Oriental regions. It was introduced in Brazil to control wheat aphids [15]. Also, *E. plagiator* was introduced from Japan via Australia to New Zealand to control *Acyrthosiphon kondoi* Shinji, 1938, and *A. pisum* (Harris, 1776) [16]. Together with six other parasitoid species, three from the subfamily Aphelininae (Hymenoptera: Aphelinidae) and three from the subfamily Aphidiinae, *E. plagiator* was released in eastern Colorado (USA). The purpose of its introduction was to decrease the number of Russian wheat aphid species, *Diuraphis noxia* (Kurdjumov, 1913), which was also an introduced

species from southwestern Asia [17]. Together with nine other parasitoids, *E. plagiator* was introduced in Chile on two occasions to control three aphid species: *D. noxia*, *Metopolophium dirhodum* (Walker, 1849) and *Sitobion avenae* (Fabricius, 1775) [18]. In addition, Rakhshani et al. [19] showed that *E. plagiator* prefers cereal aphids as its hosts at lower altitudes, while Singh et al. [20] showed that this parasitoid has a broader altitudinal distribution ranging from 601 to 1801 m a.s.l. According to the habitat type, *E. plagiator* spreads over different types of forests (coniferous, deciduous or mixed), parks, gardens and urban areas (cities and villages), as well as crops [21].

Since *E. plagiator* is usually characterized as being broadly oligophagous [22] and also as a polyphagous parasitoid [14], it has the opportunity to come into contact with various parasitoids, particularly with other aphidiine species. Consequently, these potentially competitive parasitoids of *E. plagiator* share many common hosts. These interactions have been confirmed in previous studies [12,13]. Unlike most predators, parasitoids usually have narrow or limited host ranges, so contact between them leads to competition [23]. However, knowledge of the autecology of *E. plagiator* is quite limited, especially information on its competitive ability, defined as the ability of one species to exclude another [24], which is the main constraint to the successful implementation of this species in biocontrol programs. Having all this in mind, the central aim of the present study was to determine how powerful *E. plagiator* is as a parasitoid in competition with other Aphidiinae species. To estimate the competitive ability of *E. plagiator,* an artificial neural network modelled all of the trophic associations in which *E. plagiator* was directly or indirectly (via its aphid host) involved.

MATERIALS AND METHODS

Study area and sampling

In this analysis we used material of world Aphidiinae fauna that was sampled from 1989 to 2018. The territories investigated belong to the following geographical regions: Afrotropical (Algeria, Libya), Nearctic (USA), Neotropical (Chile, Costa Rica), Oriental (India), Eastern Palaearctic (China, Iran, Russia, Turkey)

and Western Palaearctic (Austria, Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Lithuania, Malta, Montenegro, North Macedonia, Poland, Serbia, Slovenia, Spain, Sweden, Switzerland, Wales). Together with plant parts, live and mummified aphids were placed in plastic containers covered with muslin cloth and transferred to the laboratory where they were kept in a growth cabinet until emergence of the parasitoids. In order to identify the host aphids, several live adults were placed in small plastic tubes filled with 75% ethyl-alcohol.

The parasitoids were deposited in the collection of the Faculty of Biology, Institute of Zoology, University of Belgrade, Serbia. Aphid samples were deposited in the collection belonging to the Faculty of Agriculture, Institute for Phytomedicine, University of Belgrade, Serbia. Plant vouchers were deposited at the Faculty of Sciences and Mathematics, Department of Biology and Ecology, University of Niš, Serbia. The nomenclature of aphids followed Remaudière and Remaudière [25].

Data analysis

For this analysis we constructed a data matrix based on the trophic associations established between *E. plagiator* and aphid hosts found in the samples. We also analyzed the samples with the same aphid hosts when there was no occurrence of *E. plagiator* and we found other parasitoids from the subfamily Aphidiinae. In Supplementary Table S1, each row of the data matrix represents one field sample. The sample contains one aphid species and all parasitoid specimens detected therein (one to several). Out of 560 samples, 15 contained aphids attacked only by *E. plagiator*. Moreover, in 55 samples, *E. plagiator* was found together with one or more other parasitoid species (maximum 4). Finally, 490 samples encompassed only aphids and potential *E. plagiator* competitors.

To estimate the competitive ability of parasitoid *E. plagiator* and its competitors, as well as to pattern any trophic associations established, an unsupervised artificial neural network (ANN) self-organizing map (SOM) was applied [26]. The SOM, as an unsupervised learning algorithm in which the output values are not provided for the neural network, is a multivariate exploratory technique that processes and visualizes the linear and nonlinear variability of huge data

sets. As its output, this method ordinates and classifies the samples in a 2-dimentional neural network. ANNs have already been applied in many aspects of ecological studies: for clustering, classification, estimation, prediction, and data mining at different ecological levels [27-44]. Since the data set in the present study is multivariate, large and nonlinear, and as the abundance of many parasitic species broadly varies over different samples (aphids), the SOM method is a suitable approach for testing the main hypothesis and describing the complex interactions within the trophic associations.

The SOM is composed of two layers, an input and an output, each consisting of processing units, neurons. The construction of the input layer depends on the input matrix, which in our study was composed of 32 species of parasitic wasps (given in columns, Supplementary Table S1) and 560 samples of aphid hosts (given in rows, Supplementary Table S1). Before engaging the SOM in the training process, the data, showing parasitoid abundances, were log-transformed and then normalized and scaled from 0 to 1. During the training process, all samples (in our case, aphids) go through the network successively, being attached to a particular neuron (best matching unit; BMU) from the SOM. This is done by comparing the input pattern of the data matrix with each of the SOM neurons, using Euclidean distance as the criterion. All samples from the same neuron have a similar model of data (in our case, a similar suite of parasitic wasps). As the distance between the neurons on the map increases, the similarity of the patterns that these neurons carry decreases. Once the samples (aphids) have been attached to the appropriate neuron, the training process is completed. The k-means method [45] was applied for grouping the neurons that carry similar information (model of trophic associations).

The size of the output layer (2-dimensional neural network) is very important for achieving a reliable interpretation of the results, since the wrong resolution of a 2-dimensional network can bias the variability models of the data. If the network is too small (with an insufficient number of neurons), some important variability data patterns can be concealed. On the other hand, if the network is too large (with many neurons without assigned samples), the differences in the ordinational patterns will increase and, consequently,

plausible interpretation of the results will be more difficult. Therefore, we determined the map resolution *a priori*, using the appropriate methods [46,38]. In the first method, 5√n is the optimal number of neurons in the map, where *n* is the number of training samples [46]. In the second method, proposed by Park et al. [38], the size of the neural network is set by following the local minimum quantization error (QE) and topographic error (TE). Using these two approaches and trying to avoid a large number of empty neurons in the map [47], we determined the resolution of 10X9 neurons to be the most appropriate for our study.

The component plane technique was applied in order to define the competitive ability of each parasitoid species from the SOM model [42]. This method visualizes the distributional model of each of the parasitoid wasps across all ordinated samples (aphids) on the trained neural network. The distribution of each parasitoid species is presented through a gradient of greyness in which shades of black indicate their high abundance in particular samples (aphids). The SOM analysis does not provide any statistical indications on species responsible for the SOM groups, and so the IndVal of the indicator species was used to quantify the competitive ability of the parasitoid species [48]. If a parasitoid species appeared in a particular group of aphids (samples) defined by the SOM, with a relative frequency and abundance of at least 50%, that parasitoid species was determined as the representative of that group, with an IndVal greater than 25% and $p<0.05$. If a species still appears significantly only in a single SOM group ($p<0.05$) but sporadically with low frequency, its IndVal will be lower than 25% and such species will be considered as a rare taxon in the sample. In this study, all parasitoid species, representative of SOM groups where *E. plagiator* presence was not recorded and with IndVal≥25% were considered as potential competitors of *E. plagiator*.

RESULTS

The self-organizing map ordinated and classified 560 samples into seven groups, based on the qualitative and quantitative composition of the parasitoid species (Fig. 1). In addition, via component planes, the SOM visualized the distribution patterns of each parasitoid species along the trained neural network to reveal
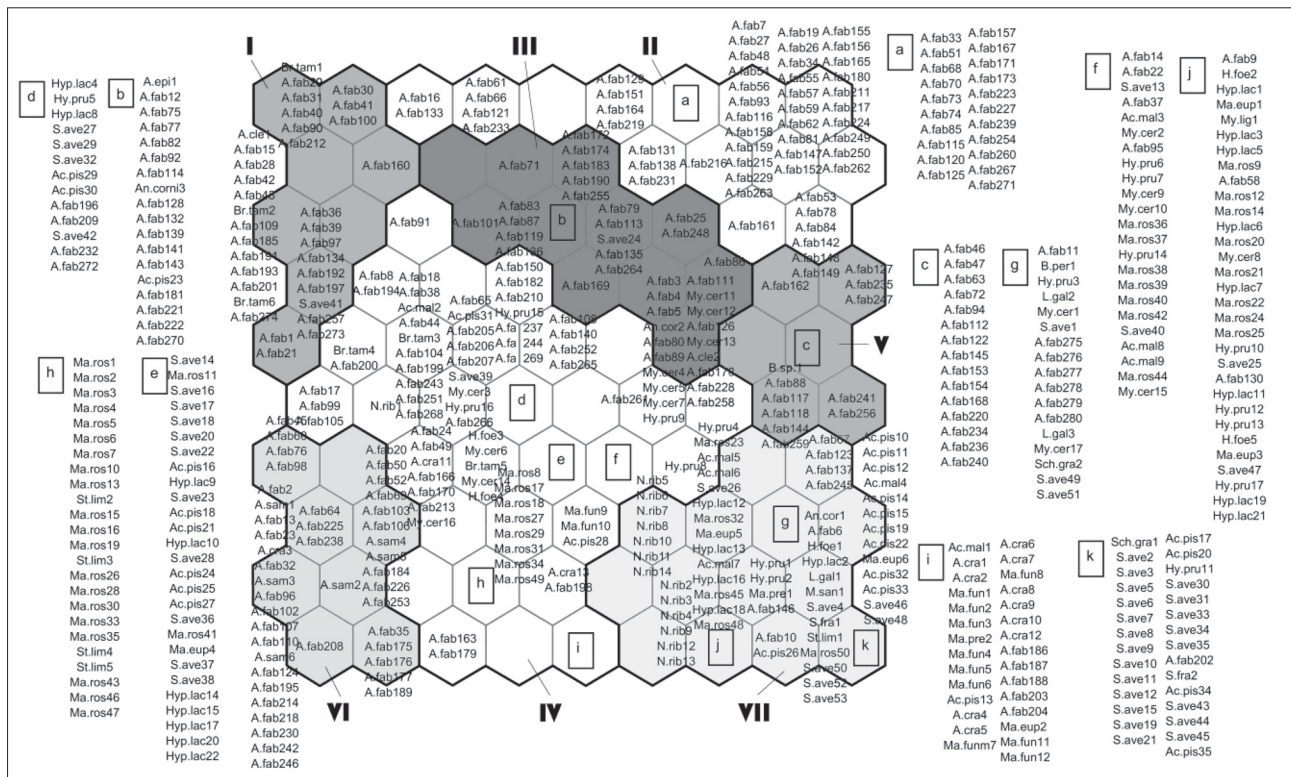
Fig. 1. The self-organizing map (SOM) presenting the ordination and classification pattern of aphid hosts based on their parasitoid qualitative and quantitative structure. Different shades of black indicate 7 subgroups (I-VII) with a similar suite of parasitoids, classified by the k-means method [45]. The labels attached to the neurons in the map stand for a particular aphid species (sample).

their competitive preferences. Following the degree of overlap in the distribution of aphidiine species, the IndVal method listed 32 parasitoid species and quantified their competitive ability. Besides *E. plagiator*, of the 31 parasitoid species analyzed here, 16 showed statistically significant differences (p<0.05) (Fig. 2B). Nevertheless, only five of them could be considered as potential competitors of *E. plagiator*, since their IndVals exceeded 25% and their distributional patterns did not overlap with that of *E. plagiator* over the SOM. Two species, *Praon volucre* and *Aphidius ervi*, showed IndVals>25% and had the same distributional pattern over the SOM as *E. plagiator* (Fig. 2B). Species with an IndVal lower than 25% and p<0.05 appeared sporadically in particular SOM groups as rare taxa with a restricted distribution and consequently low competitive ability (Fig 2B).

In SOM group I, the aphid host *Aphis fabae* was dominant, while three other species (*A. clematidis* Koch, 1854, *Brachyunguis tamaricis* (Lichtenstein, 1885) and *S. avenae*) were registered with fewer

samples in the group. All these hosts were parasitized by *A. colemani* Viereck, 1912 and *L. testaceipes* parasitoids, whereby *L. testaceipes* was characterized as the most powerful competitor to *E plagiator* with IndVal=98.4 and p<0.01 (Fig. 2B). In addition, SOM group II encompassed only the species *A. fabae*, which was parasitized by *L. fabarum*. For this parasitoid the SOM distribution pattern revealed its competitive preferences (IndVal=80.6; p<0.01). The species *Lipolexis gracilis* Förster, 1862, which belongs to SOM group III, significantly attacked aphids *A. fabae* and *Myzus cerasi* (Fabricius, 1775) (IndVal=34.4; p<0.01). There was also a low frequency (13%) of six other hosts in SOM group III (Fig. 1). In addition, the parasitoid *L. cardui* attacked *A. fabae* and *Brachycaudus* sp., and is the representative (IndVal=80; p<0.01) of SOM group V. The species *A. fabae* was also dominant in SOM group VI, and was parasitized by three species: *Binodoxys angelicae*, *L. confusus* Tremblay & Eady, 1978 and *P. abjectum* (Haliday, 1833). Among them, *B. angelicae* was dominant with a high frequency and abundance among the samples
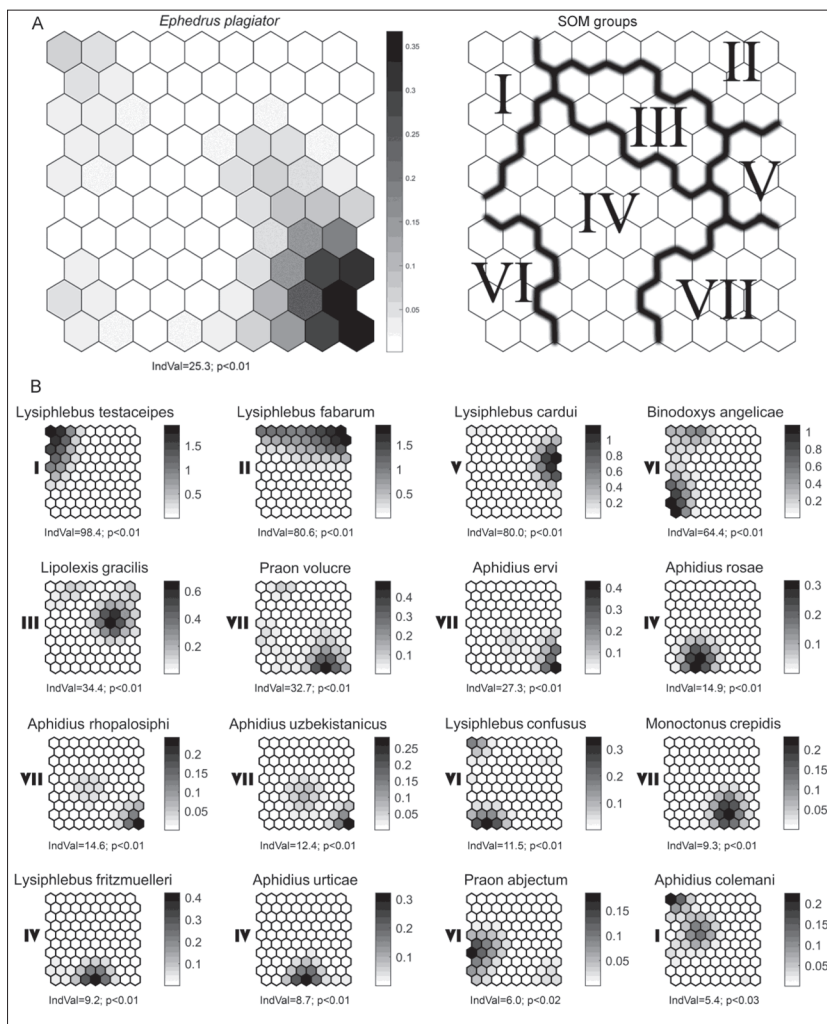
Fig. 2. Distributional patterns of IndVal taxa (with p<0.05) across SOM groups revealing the competitive ability of (**A**) *Ephedrus plagiator* and (**B**) the studied Aphidiinae wasps. The shade of black for each taxon is highly correlated with its maximum abundance recorded in the study. Roman numerals stand for the SOM group at which the taxa is representative.

A. craccae Linnaeus, 1758, *M. funestum* (Macchiati, 1885) and *A. pisum* with 145 recorded samples, while the rest appeared with fewer than nine samples per host species.

DISCUSSION

According to the SOM distribution, the native Palaearctic species *E. plagiator* falls into group VII. The SOM showed that its primary hosts are *S. avenae*, *A. fabae* and *A. pisum*. In the same SOM group, *E. plagiator* shares these hosts with *A. ervi* (*E. plagiator* IndVal=25.3%, *A. ervi* IndVal=27.3). There is some evidence that these two parasitoid species attack the same host, e.g. *A. pisum*, and that *E. plagiator* successfully competes with *A. ervi* [49]. The same author revealed that during the act of oviposition most *Ephedrus* species kill *Aphidius* Nees, 1818 embryos in the host body using venom which they insert into the host. This strategy makes *E. plagiator* a more successful competitor in relation to *A ervi*. This information is very important since the pea aphid, *A. pisum,* is specialized to feed on and transmit viruses to about 20 plant genera from the family Fabaceae, e.g.,

Trifolium L., *Lotus* L., *Melilotus* Mill. and *Dorycnium* Mill. [50], and it is an extremely destructive pest in alfalfa fields. However, in the present study, it seems that these two parasitoid species can coexist, being recorded in the same samples and sharing the same hosts. This may be explained by the fact that aphid parasitoids can be polyphagous, with a wide host range. In addition to the two parasitoids mentioned above, the parasitoid range of *A. pisum* includes *A. eadyi* Stary, Gonzalez & Hall, 1980, *A. banksae* (Kittel, 2016), *A. smithii* Sharma & Subba Rao, 1959 and several other species from the genera *Ephedrus* and *Praon* Haliday, 1833 [12,51].

from group VI (IndVal=64.4, p<0.01). SOM group VII included a large number of aphid and parasitoid species. The most dominant hosts from this group were *S. avenae*, *A. fabae*, *A. pisum*, *Nasonovia ribisnigri* (Mosley, 1841)*, Macrosiphum rosae* (Linnaeus, 1758) and *Hyalopterus pruni* (Geoffroy, 1762). These species were parasitized by 11 parasitoids, three of which were significant: *P. volucre* (IndVal=32.7; p<0.01), *A. ervi* (IndVal=27.3; p<0.01) and *E. plagiator* (IndVal=25.3; p<0.01) (Fig. 2A,B). Finally, group IV was composed of 16 aphid hosts and no significant parasitoid representatives (IndVal; p>0.05). The dominant aphids in this SOM group were *A. fabae*, *M. rosae*, *S. avenae*,

Another frequently present parasitoid in SOM group VII is *P. volucre*. It appears to be a weak competitor of the first two parasitoids for *S. avenae*, *A. fabae* and *A. pisum*, since the results show that *P. volucre* predominantly parasitizes *Hyperomyzus lactucae* (Linnaeus, 1758) and *M. rosae*. The rose aphid, *M. rosae*, is the main host for *A. rosae* Haliday, 1834, but according to our results there is no strong competition between *P. volucre* and *E. plagiator* with *A. rosae*.

With an IndVal of 98.4%, from SOM group I the parasitoid *L. testaceipes* appears to be the most important competitor for *E. plagiator*. The species *L. testaceipes* was introduced in Europe (France) from Cuba in 1973 [52] as a biological agent against *Toxoptera aurantii* (Boyer de Fonscolombe 1841) and *A. spiraecola* Patch 1914, which attack citrus fruit. For a relatively short period, it was established through the whole Mediterranean. The situation where *L. testaceipes* suppressed the native aphidiine species in new areas, albeit they were polyphagous, such as the native *E. plagiator* in the Palaearctic, has led to the concern of some braconologists [53,54]. For example, when *L. testaceipes* was registered for the first time in Serbia in 2013, which is part of continental Europe, it was identified in 21 trophic associations encompassing 17 host plants and 16 aphid species [54]. Analyzing the neurons on the SOM map it seems most likely that the main reason for the competition between *E. plagiator* and *L. testaceipes* is their common host, *A. fabae*. The second target aphid species for both parasitoids is *B. tamaricis*. This is a specialized aphid from the family Tamaricaceae, such as *Tamarix africana* Poir., *T. canariensis* Willd., *T. gallica* L., and *T. tetrandra* Pall ex M. Bieb. [55]. In spite of the fact that *L. testaceipes* has overlapping host ranges with *E. plagiator*, which was introduced and spread in other zoogeographic regions, there are no reports regarding their competitive relations.

Two other important competitors come from the genus *Lysiphlebus*: *L. fabarum* and *L. cardui*. These two species are morphologically and genetically very close, belonging to the same "*fabarum*" complex of species [56]. After *L. testaceipes*, the species *L. fabarum* (IndVal=80.6) from SOM group II appeared as a second, very strong competitor of *E. plagiator*. It is also notable that *L. cardui* (IndVal=80.0) is the key competitor from SOM group V. Both *Lysiphlebus* species, *L.*

fabarum and *L. cardui*, are significant competitors of *E. plagiator* since they appear in three different SOM groups. Also, there is strong competitive relationship between *L. fabarum* and *L cardui*. Together with *L. testaceipes*, the *Lysiphlebus* species from the "*fabarum*" complex share the same aphid host, *A. fabae*. The black bean aphid, *A. fabae* is the most common and the most investigated aphid species. It is an extremely polyphagous pest on various crops and weeds [57,58]. *A. fabae* is also a suitable host for several other Aphidiinae parasitoids: *A. colemani, A. matricariae, B. acalephae* (Marshall, 1896)*, B. angelicae, E. persicae, E. nacheri* Quilis Perez, 1934, *L. gracilis, L. confusus, P. abjectum* and *P. volucre* [12,13]. Since *E. plagiator*, *L. fabarum*, *L. cardui* and *L. testaceipes* appeared in four different SOM groups, the results confirm that direct competition is one of the factors responsible for decoupling these wasps in the same type of trophic associations.

B. angelicae, also parasitizes *A. fabae* to a great extent. It is also recorded in *A. sambuci* Linnaeus, 1758, a specialized aphid of the black elder. *B. angelicae* is highlighted as an important species from SOM group VI with an IndVal of 64.4%. This parasitoid has a very broad host range [14], mostly attacking various species from the genus *Aphis*, then *Brachycaudus* van der Goot, 1913, *Dysaphis* Börner, 1931, *Lipaphis* Mordvilko, 1928, *Myzus*, *Ovatus* van der Goot, 1913, *Toxoptera* Koch, 1856 or *Uhlmannia* Börner, 1952 [12]. As mentioned, besides four species from the genus *Lysiphlebus*, *B. angelicae* also competes with *E. plagiator* for the same host, *A. fabae*. It is interesting that *B. angelicae* tolerates several other aphidiines from SOM group VI (primarily *L. confusus* and *P. abjectum*), sharing the same hosts at the same time.

Lipolexis gracilis is the only species that has a significant role as a competitor from SOM group III, with IndVal 34.4%. This species parasitizes *A. fabae* and *M. cerasi*. There is great doubt as to whether *L. gracilis* should be considered as a complex of species. A detailed investigation will explain how many species are actually within the *L. gracilis* complex. Potential cryptic species hidden in this complex will probably refute the traditional statement that it is a polyphagous species.

Regarding the aphid host distribution on the SOM, *A. fabae* pervades all SOM groups. This spe-

cies is cosmopolitan, being the most frequent in the samples in this study. The parasitoid range of *A. fabae* is 14 out of 32 species. The SOM map shows that *A. fabae* is the only host in SOM group II, and that it is 99% present in group V, putting *L. fabarum* and *L. cardui* in direct competition. Here we can conclude that these two phylogenetically very close species exclude each other when they are in the same trophic association. In the largest SOM group, IV, *A. fabae* shares these neurons with the rose aphid, *M. rosae*, in a mutual ratio of about 50:50%. *M. rosae* is a cosmopolitan aphid that attacks many species from the genus *Rosa* L., *Dipsacus* L., *Ilex* L., *Knautia* L., *Scabiosa* L., *Valeriana* L. [59]. *M. rosae* can also be found in SOM group VII, where it has a low distribution. This SOM group has a high presence of the currant-lettuce aphid *N. ribisnigri*. The primary hosts for this aphid come from the genus *Ribes* L., but also many species from the family Asteraceae (*Cichorium* L., *Crepis* L., *Hieracium* L., *Lapsana* L. and *Leontodon* L.), Orobanchaceae (*Euphrasia* L.), Plantaginaceae (*Veronica* L.) and Solanaceae (*Nicotiana* L. and *Petunia* Juss.) [60].

The English grain aphid *S. avenae* occupies several neurons in SOM group VII and it is sporadically present in SOM group IV. However, the SOM analysis showed that this aphid is not the host over which 4 parasitoids, *E. plagiator*, *P. volucre* and two phylogenetically sibling species, *A. rhopalosiphi* and *A. uzbekistanicus*, compete.

Generally, as a polyphagous species, three parasitoids of *Lysiphlebus* – *L. testaceipes*, *L. fabarum* and *L. cardui* – together with *B. angelicae*, are the most powerful competitors of *E. plagiator* in this analysis, establishing the highest number of tritrophic associations. Since they all appear in four different SOM groups (I, II, V and VI), and *E. plagiator* is from group VII, they are frequently found alone in the samples, somehow not allowing *E. plagiator* to oviposit or develop in aphid colonies.

Finally, *E. plagiator*, as a native Palaearctic species that has been successively introduced worldwide, has artificially become a cosmopolitan species. However, due to its wide geographical distribution, the study area is also wide, including samples from 29 countries. This fact partly influences the results since some of the parasitoid species with limited areal of distribution have poor chances to compete directly with *E. plagiator* in trophic associations. Nevertheless, the statistical design used here substantially diminished the influence of such species on the main SOM model. More precisely, parasitic wasps with IndVal<25 appeared in only a few samples. In this specific case, such taxa have limited geographic distribution, which leveled off their frequency and consequently decreased their IndVal score [42]. Moreover, since these taxa could not significantly affect the main trends in the SOM model, they were not considered in further analyses of the study. However, in order to completely eliminate this effect from the model, the next step in investigating the competitive ability of *E plagiator* should be to include the spatial dimension in the SOM model.

Author contributions: All authors were involved in data collection. MIM and VŽ designed the study and wrote the manuscript. OPO, SSS, ŽT contributed to the discussion of the results and revised the manuscript. ML, AT prepared the data for statistical analysis.

Conflict of interest disclosure: The authors declare no conflict of interest.

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Supplementary Material

The Supplementary Material is available at: http://serbiosoc.org.rs/NewUploads/Uploads/Ilic%20Milosevic%20et%20al_4663_Supplementary%20Table%20S1.xlsx