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Neodryinus typhlocybae, a biological control agent of *Metcalfa pruinosa*, spreading in Hungary and reaching Slovakia

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Abstract

The North American citrus flatid planthopper, *Metcalfa pruinosa* (Say) (Hemiptera Flatidae), introduced in Europe in the late seventies, has become a major pest of a wide range of plant taxa in many countries throughout the continent. In Italy, the early recognition of this rapidly spreading species resulted in the introduction of *Neodryinus typhlocybae* (Hymenoptera Dryinidae), an already known natural enemy of *M. pruinosa* in North America, and was followed by intentional release programmes in several additional countries within southern Europe. However, the features of dispersal and the impact of the parasitoid species on the populations of the pest have been studied only in a few cases. There is a lack of information on the occurrence and biology of *N. typhlocybae* in other regions of the continent, including Hungary, where *N. typhlocybae* was first recorded only in 2014. The results of this study, carried out in Hungary between 2015–2018, revealed that although there had been no official release programmes for *N. typhlocybae* it became widely distributed. The rate of parasitism ranged between 0.66% (in Martonvásár, 2016) and 29.65% (in Kaposvár, 2018). The species was found to develop through one or two generations a year, with a major proportion of the studied populations being bivoltine. These findings suggest *N. typhlocybae* could provide effective and environmentally sound control of *M. pruinosa* in urban habitats. Furthermore, *N. typhlocybae* is reported here for the first time from Slovakia, indicating the natural range expansion of the parasitoid in Central Europe. A hyperparasitoid species, *Cheiloneurus boldyrevi* (Hymenoptera Encyrtidae) is first recorded from Hungary.

Key words: non-native species, natural enemy, dispersal, biology, Central Europe.

Introduction

The citrus flatid planthopper, *Metcalfa pruinosa* (Say) (Hemiptera Flatidae), native to North America, was accidentally introduced in Italy in 1979 (Zangheri and Donadini, 1980; Girolami *et al*., 2002). Since its first record in Europe, it has spread to and established in many other European countries, and this process is expected to continue, as it has been described by Byeon *et al*. (2017). *M. pruinosa* was first found in Hungary in 2004 (Pénzes, 2004), and by now it has become widely distributed throughout the country (Pénzes and Hári, 2016).

The species is highly polyphagous having been recorded on a wide range of woody and herbaceous plants (Bagnoli and Lucchi, 2000; Kahrer *et al*., 2009; Kim *et al*., 2011; Preda and Skolka, 2011; Pénzes and Hári, 2016; Vlad and Grozea, 2016), many of which are used in horticulture and forestry. In a review, Bagnoli and Lucchi (2000) highlight the extreme polyphagy of the pest by presenting a list consisting of more than 300 plant taxa affected by *M. pruinosa*. Direct damage is caused by phloem-feeding by the nymphs, which may weaken the host plant and lead to some leaf distortion. However, the white, flocculent, waxy secretion produced by the juveniles as well as the excreted honeydew, and developing sooty molds, covering the infested plant parts and objects under the affected plants, are usually of major concern, especially in ornamental plant trade and in urban environments (Mead, 1969; Bagnoli and Lucchi, 2000; Lauterer, 2002; Strauss, 2012; Pénzes and Hári, 2016). Moreover, Donati *et al*. (2017) have recently demonstrated the capacity of the pest to transmit *Pseudomonas syringae* pv. *actinidiae*, a pathogenic organism responsible for the bacterial canker of kiwifruit.

There are several problems associated with chemical control of *M. pruinosa*. As it is a polyphagous species, the reinfestation of the sprayed crop with highly mobile individuals from the neighbouring untreated vegetation cannot be excluded. Prolonged hatching of nymphs and the waxy secretion covering their body during development also make chemical treatments difficult (Girolami and Mazzon, 1999; 2001; Bagnoli and Lucchi, 2000; Pénzes and Hári, 2016). Although many insecticides have been shown to be effective against the pest, the majority of them are broad spectrum ones belonging to the groups of organophosphates and pyrethroids (for a review see Girolami and Mazzon, 2001), and therefore they may pose a considerable threat to beneficial arthropods. For example, bees which are attracted to the honeydew produced in large quantities when *M. pruinosa* is abundant may also be threatened if these infested plants were the subject of chemical treatments. Furthermore, the use of chemical insecticides in urban areas, where *M. pruinosa* occurs frequently on ornamental plants, is less acceptable and so more restricted than in agricultural landscapes (Girolami and Mazzon, 1999; Malausa, 1999; Lucchi, 2000; Malausa *et al*., 2006).

Indigenous natural enemies of *M. pruinosa* appear to be unable to control the pest sufficiently in Europe (Girolami and Mazzon, 1999; 2001; Frilli *et al*., 2001). The non-native parasitoid wasp *Neodryinus typhlocybae* (Ashmead) (Hymenoptera Dryinidae) has been considered as a potential control agent since the eighties, because it was the only natural enemy of *M. pruinosa* occurring throughout its native range (Girolami and Mazzon, 2001). The first specimens of *N. typhlocybae* were introduced from the USA into Italy in 1987 (Girolami and Camporese, 1994). Cocoons of the parasitoid were found in the Northeast of the USA, and individuals from the Connecticut origin were repeatedly released in northeastern Italy (Veneto and Friuli-Venezia Giulia regions) in the following years. All the populations which have spontaneously spread around Padua (Veneto) derive from the previously mentioned introductions as well as those used subsequently for releases in other places by the Institute of Entomology of Padua and Udine and the Biolab (today Bioplanet) of Cesena (Girolami and Mazzon, 1999). Implementation of extensive release programmes in the late nineties followed by the successful establishment of *N. typhlocybae* in various regions of Italy were reported by several authors (e.g., Frilli *et al*., 2001). Besides many parts of Italy, *N. typhlocybae* was also released in Croatia, France, Greece, the Netherlands, Slovenia, Spain and Switzerland, as reviewed by Girolami and Mazzon (2001) and Strauss (2009; 2012). *N. typhlocybae* individuals originating from the southern USA (Texas) have also been introduced into Italy and released in the region of Tuscany (Girolami and Mazzon, 2001). Malausa *et al*. (2003) reported that in the case of France, stock materials of *N. typhlocybae* released originated partly also from the area of the Finger Lakes (State of New York, USA) and the southern part of the State of Delaware (USA), in addition to those of Connecticut origin. The main purpose of the release programmes carried out in southern Europe were to make the control of *M. pruinosa* effective over a large area in the medium or long term (Cenderello, 2006). Occurrences of *N. typhlocybae* in Montenegro and Serbia (Glavendekic *et al*., 2010; Glavendekic, 2012) as well as in Gibraltar (Guglielmino *et al*., 2013), where coordinated release programmes did not take place, have been reported recently, which indicate the natural dispersal of the species in southern Europe. Similarly, the first record of *N. typhlocybae* (cocoons) in Vienna, Austria, in 2014 was also not associated with deliberate release. In this country one intentional release took place but in another district and soon after the first finding of the parasitoid wasp (Strauss, personal communication). The latest reports on the occurrences of *N. typhlocybae* in Europe came from Hungary (Szöllősi-Tóth *et al*., 2017), where the species had not been released deliberately, and Bulgaria, from where information on its release in 2017, soon after its first record in 2016 (Lapeva-Gjonova *et al*., 2018), is available (Tomov and Vasileva, 2018).

All the known hosts of *N. typhlocybae* belong to the subfamily Flatinae (Hemiptera Flatidae), which include, besides *M. pruinosa*, namely *Anormenis septentrionalis* (Spinola), *Flatormenis chloris* (Melichar), and *Metcalfa regularis* (Fowler) (Guglielmino *et al*., 2013). The morphological description and geographical distribution of *N. typhlocybae* is given by Olmi (1999). Its biology in Italy has been studied by several authors. According to Girolami and Mazzon (2001) and Strauss (2009), females of *N. typhlocybae* parasitise the third, fourth and fifth nymphal stages of *M. pruinosa*, and they also prey on the nymphs. *N. typhlocybae* overwinters as a mature larva in a silky cocoon, usually fixed on the underside of the leaf. Pupation takes place in late spring, and adults emerge in June, the latter period more or less coinciding with the appearance of the fourth nymphal stage of the host, as it has been described by Girolami and Mazzon (2001). The egg of the parasitoid is inserted by the female into the intersegmental membrane below the mesothoracic wingpad of the nymph of *M. pruinosa*. The hatching and developing white-yellowish larva may be observed with its posterior part protruding partly from the body of the host below the wingpad, forming a thylacium. The mature larva enters the host body, devours its organs, and finally leaves the host and prepares the whitish, oval, double-walled cocoon under the residues of the dead host (Olmi, 1999; Guglielmino and Bückle, 2003). Larvae of the first parasitoid generation may either enter diapause or pupate in July-August to give rise to a second generation in the middle of summer. Therefore, *N. typhlocybae* has one or two generations a year (Girolami and Mazzon, 2001; Mazzon *et al*., 2001). The significance of bivoltinism in the case of the parasitoid wasp from the aspect of effective biological control of *M. pruinosa* has been described and highlighted by Mazzon *et al*. (2001). Based on the studies of Malausa (2000), Girolami and Mazzon (2001) and Mazzon *et al*. (2001), the extent of bivoltinism seems to be highly variable in nature, however, the exact factors determining the voltinism of *N. typhlocybae* remain unclear (Mazzon *et al*., 2001). Nevertheless, Mazzon *et al*. (2001) found that the *N. typhlocybae* population of Texas origin showed a decidedly higher tendency to bivoltinism compared to the population of Connecticut origin.

Several hymenopteran species belonging to various families have been reported as hyperparasitoids of *N. typhlocybae* (Olmi, 2000; Viggiani *et al*., 2002; Malausa *et al*., 2006). The occurrence of hyperparasitoids is detrimental to the populations of the beneficial insect, hence they are considered undesirable organisms from a pest control perspective. Therefore, it is important to know whether any natural enemies have accepted *N. typhlocybae* as a host in a specific region, and which species they are.

In this study, documenting the distribution of *N. typhlocybae* in Hungary was primarily to assess the extent of occurrence at locations other than Budapest, where it was first recorded in the country. Both natural spread from the direction of neighbouring countries as well as unintentional introduction and further dispersal with its host on plants for planting might have led to the expansion of its already known area of distribution in Europe. As the presence of the parasitoid in Slovakia might have been attributed to spread either from Budapest (Szöllősi-Tóth *et al*., 2017) or Vienna, Austria (Strauss, personal communication); both towns are located relatively near the Slovak border, attempts to find *N. typhlocybae* in the southern region of Slovakia were also made. Assessing the rate of parasitism in Hungary and Slovakia was considered to be important in revealing the history and impact of the species in the region concerned. Moreover, in order to get further insights into the biology of the dryinid wasp, the number of generations under Hungarian environmental conditions was assessed. Finally, the occurrence of any hyperparasitoid species that may occur in *N. typhlocybae* populations based on the findings of former studies in other European countries was also investigated.

Materials and methods

Survey on the distribution of *N. typhlocybae* in Hungary and on its occurrence in Slovakia

Following the early record of *N. typhlocybae* cocoons in the Buda Arboretum, Budapest, 17 March 2015 (see Szöllősi-Tóth *et al*., 2017), the members of the Hungarian Entomological Society, the members of the Section of Agrozoology of the Hungarian Plant Protection Society and further entomologists as well as botanists were asked by the authors in April-July 2016 to provide information on locations in the country where they had observed considerable infestation of plants with *M. pruinosa* in recent years. These data requests helped the authors find sites where the presence of *N. typhlocybae* could be expected because of the mass occurrence of its host. Based on the reports received, ten locations, including two sites within Budapest, were visited between 20 and 28 July, 2016, to search for parasitised *M. pruinosa* individuals. Plants at twenty-two additional locations throughout the country, with twelve sites within the capital, where *M. pruinosa* was found in 2015-2017, were also examined to determine occurrence of *N. typhlocybae*.

In July 2018, the survey was focused on the northern parts of Hungary, because no information had been received on considerable infestation of plants with *M. pruinosa* from this region previously. In this latter case, seven locations were visited either randomly or based on specific data requests from regional agricultural entomologists on the mass occurrence of the pest.

Two towns in Slovakia, close to the Hungarian border, where the parasitoid species had not yet been reported were also visited on 10 July 2018 to assess the presence of *N. typhlocybae*.

The coordinates of each studied location have been recorded.

Assessment of the rate of parasitism

Between 20-28 July 2016, leaves of various plant taxa (e.g., *Acer*, *Celtis*, *Hibiscus*, *Ulmus*) infested with *M. pruinosa* were collected randomly at twelve locations, including four sites within Budapest, from the thirty-nine locations visited during the survey on the distribution of *N. typhlocybae* in Hungary. This period was chosen for sampling for the following reasons: adults of the pest had been reported to begin to emerge from July in Hungary (Pénzes and Hári, 2016), and so live juveniles and also exuviae of the fifth (last) instar of *M. pruinosa* are readily visible on leaves. Furthermore, the symptoms of parasitism, if *N. typhlocybae* is present at the specific location, may also be observed in July (Mazzon *et al*., 2001). The collected material was put in plastic bags together with a piece of cotton wool impregnated with ethyl acetate as an insect killing agent. The samples were then carried to the laboratory of the Department of Entomology, Szent István University, where they were examined by using Alpha STO-4LED zoom stereo microscopes. The insect individuals were categorised as follows: the specimens of *M. pruinosa* were considered 'live' if the nymph found was apparently intact, and 'survived' if the exuviae of the last instar was observed, which indicated that the pest escaped from having been parasitised and could reach the adult stage. The fifth instar nymphs, and also their exuviae, which remain attached to the leaf for a long time (Malausa, 1999), were distinguished from the previous instars by the mesonotum with each wingpad broadly expanded, extending to apex of metanotal wingpad (Wilson and McPherson, 1981). Parasitised specimens of *M. pruinosa* could be recognised by the thylacium of *N. typhlocybae* protruding laterally from the thorax of the nymph, and by the cocoons of the parasitoid wasp on the underside of the studied leaves. The calculation of the rate of actual parasitism was based on the formula described by Malausa (1999), but, in our case, all apparently intact nymphal stages found were recorded as live individuals.

In July 2018, the level of parasitism was assessed again at three southwestern Hungarian sites studied already two years before so as to reveal potential temporal changes. Furthermore, samples were taken at the two Slovak locations to estimate the rate of parasitism there. The sampling and assessment methods used were the same as in 2016.

Determination of the number of generations

In order to reveal the number of generations *N. typhlocybae* produces under the climatic conditions in Hungary, leaves of deciduous trees with cocoons of the parasitoid wasp were collected at three locations between 20 and 22 July, 2016. One sample was taken in a cemetery (47.4438°N 19.1958°E), and one in a public park (47.4764°N 19.0330°E) in Budapest. The third sample was collected also in a cemetery but at Szekszárd (46.3360°N 18.7022°E), southwestern Hungary. Based on the study of Mazzon *et al*. (2001), mid-July is optimal for collecting the cocoons to evaluate the bivoltine portion of the whole population. The leaves with cocoons were put in plastic bags without adding any killing agents. In the laboratory of the Department of Entomology, Szent István University, the cocoons were gently cut out of the leaves, and they were placed in labelled plastic vials (three cocoons in each vial) closed with cotton wool allowing for ventilation. The material was then kept at ambient temperature in the laboratory. Appearance of adults was recorded on 28 August 2016, i.e. when adults of the bivoltine portion of the populations could be expected to have finished emergence (Mazzon *et al*., 2001). The vials were checked once again on 28 October 2016 to see if any further adults emerged after late August. Those larvae which remained in the cocoon (diapausing individuals) were considered as representatives of the univoltine portion. The diapausing larva in the cocoon could be distinguished from those which continued development to reach the adult stage and emerge in the same year by examining the morphological characteristics given by Girolami and Mazzon (2001). The identifiable individuals of *N. typhlocybae* which continued their development but did not emerge from the cocoons for any reasons in 2016 were considered to belong to the bivoltine part of the population. The completely formed specimens, either emerged or not, were separated by the sex. For each of the samples originating in the three different locations in Hungary, the ratio of the individuals of the bivoltine and univoltine proportions of the population was calculated.

Rearing for finding hyperparasitoid species

More than fifty cocoons of *N. typhlocybae* were collected from *Acer platanoides* L. and *Acer pseudoplatanus* L. trees in a private garden in Budapest (47.4881°N 19.2542°E) on 24 October 2015. The cocoons were gently cut out of the leaves and were kept in an open plastic box (300 × 200 × 200 mm) under field conditions but protected from rain. In early March 2016, the box was covered so as to prevent the adults from escaping when emergence began. The individuals emerging in April-May were collected, and the species were identified.

A further sample containing more than one hundred cocoons was collected from *A. platanoides* and *Ficus carica* L. plants in another private garden next to Buda Arboretum, the location of the first record of *N. typhlocybae* in Hungary (29 June 2014), in Budapest (47.4814°N 19.0369°E) on 3 July 2016. The cocoons cut out of the leaves were kept in a covered plastic box, and emergence of adults was recorded daily following the appearance of the first adult on 28 July 2016. Specimens of species other than *N. typhlocybae*, which emerged in August-September, were collected and identified. Many of the *N. typhlocybae* individuals were released immediately after emergence in order to save the beneficial organism and contribute to its local establishment.

The hyperparasitoids of *N. typhlocybae* were identified by one of the authors (M. Olmi) by comparison with specimens reared from *N. typhlocybae* in Italian and French localities and identified by E. Guerrieri (Italian National Research Council, Portici, Napoli, Italy) and G. Delvare (CIRAD, Montferrier-sur-Lez, France), who are specialists of Encyrtidae and Pteromalidae, respectively.

Results

Distribution of *N. typhlocybae* in Hungary

The records of *N. typhlocybae* based on the data collection through 11 July 2018 in Hungary are shown in figure 1. Parasitised *M. pruinosa* individuals were found at thirty-eight sites out of the thirty-nine examined. The new *N. typhlocybae* records are as follows (in alphabetical order): Balassagyarmat, 48.0745°N 19.3034°E (cemetery), 11 July 2018; Balatonszemes, 46.7945°N 17.7700°E (tree lane bordering a vineyard), 27 October 2015; Balatonvilágos, 46.9723°N 18.1788°E (tree lane between vineyards), 22 July 2016; Budaörs, 47.4629°N 18.9618°E (cemetery), 22 October 2015; Budapest,

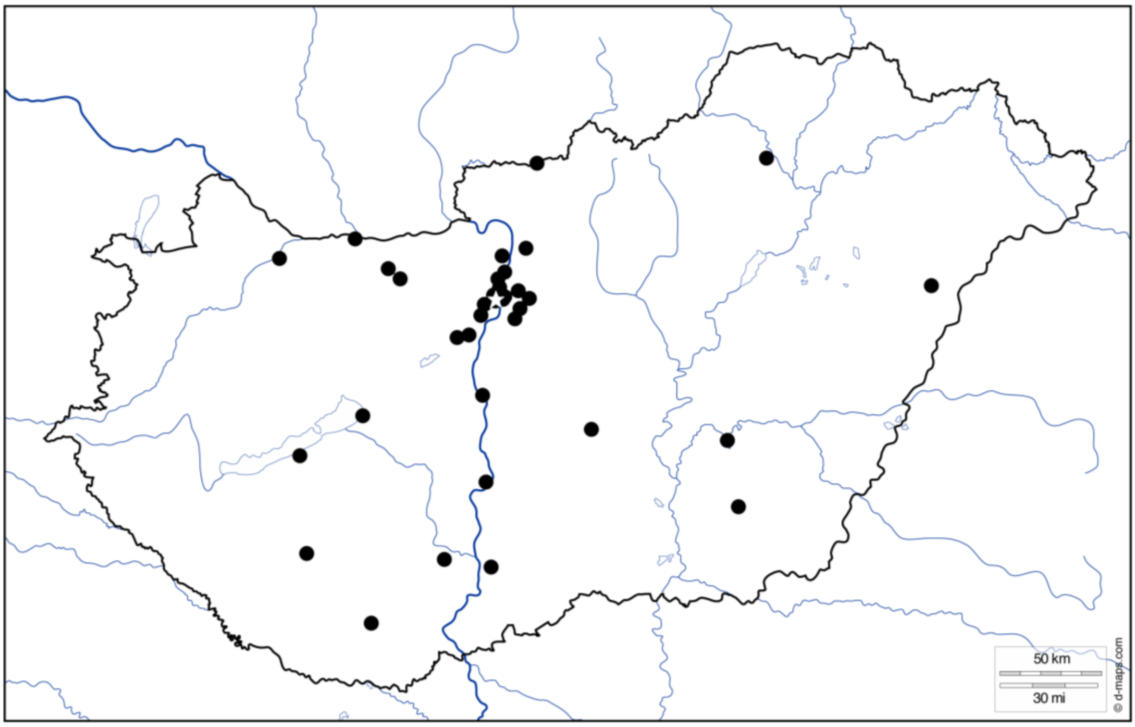

Figure 1. Distribution of *N. typhlocybae* in Hungary known by 11 July 2018. The white star indicates the location of the first record. Source of the original blank map https://d-maps.com/carte.php?num_car=2287&lang=en. The figure was prepared by Balázs Károlyi (IBM Cloud Video, Hungary).

47.3988°N 19.1580°E (botanical garden), 27 April 2015; Budapest, 47.4935°N 19.0939°E (cemetery), 18 May 2015; Budapest, 47.4859°N 19.0475°E (urban forest), 8 July 2015; Budapest, 47.4839°N 19.0280°E (tree lane along a road), 19 August 2015; Budapest, 47.4976°N 19.0243°E (green belt between a road and a railway station), 19 August 2015; Budapest, 47.6008°N 19.0941°E (tree lane along a road), 30 September 2015; Budapest, 47.4881°N 19.2542°E (private garden), 22 October 2015; Budapest, 47.4843°N 19.0839°E (botanical garden), 1 June 2016; Budapest, 47.4438°N 19.1958°E (cemetery), 20 July 2016; Budapest, 47.4764°N 19.0330°E (public park), 20 July 2016; Budapest, 47.5014°N 19.0255°E (public park), 20 July 2016; Budapest, 47.5193°N 19.1823°E (public park), 20 July 2016; Budapest, 47.5357°N 19.0632°E (public park), 20 July 2016; Budapest, 47.5712°N 19.0517°E (public park), 20 July 2016; Debrecen, 47.5432°N 21.8520°E (adventure park in a forest), 8 July 2018; Diósd, 47.4145°N 18.9409°E (group of trees within the residential area), 24 October 2015; Érd, 47.3295°N 18.8636°E (tree lane bordering a public park surrounded by agricultural area), 27 August 2016; Győr, 47.6613°N 17.6405°E (cemetery), 10 July 2018; Kaposvár, 46.3606°N 17.8126°E (cemetery), 22 July 2016; Kecskemét, 46.9135°N 19.6539°E (arboretum), 21 July 2016; Komárom, 47.7450°N 18.1253°E (public park), 10 July 2018; Madocsa, 46.6806°N 18.9756°E (apple orchard), 12 September 2017; Makád, 47.0626°N 18.9536°E (private garden), 2 October 2016; Martonvásár, 47.3158°N 18.7852°E (castle garden), 21 July 2016; Miskolc, 48.0981°N 20.7868°E (parking lot), 8 July 2018; Orosháza, 46.5715°N 20.6060°E (private garden), 15 August 2016; Pécs, 46.0504°N 18.2345°E (cemetery), 22 July 2016; Sükösd, 46.2997°N 19.0058°E (organic apple orchard), 7 December 2017; Szarvas, 46.8641°N 20.5350°E (public park), 28 July 2016; Szekszárd, 46.3360°N 18.7022°E (cemetery), 22 July 2016; Szentendre, 47.6729°N 19.0802°E (parking lot), 1 October 2017; Tata, 47.6172°N 18.3404°E (rest area on a highway), 10 July 2018; Tatabánya, 47.5734°N 18.4170°E (urban footpath between private gardens), 10 July 2018; Vácrátót, 47.7074°N 19.2309°E (botanical garden), 9 August 2016.

There was no parasitism by *N. typhlocybae* evident from a sample of 327 *M. pruinosa* specimens at Újfehértó, 47.8193°N 21.6673°E (tree lane between orchards), 15 August 2016.

The results indicate that *N. typhlocybae* is widely distributed in Hungary, though it was first recorded in the country, in Budapest, only in 2014 (Szöllősi-Tóth *et al*., 2017). However, considering the fact that *N. typhlocybae* is known to have been present in the neighbouring countries of Croatia and Slovenia for approximately two decades (Ciglar *et al*., 1998; Žežlina *et al*., 2001), its occurrence in the southwestern part of Hungary might be explained by its natural dispersal from these invaded regions, at least based on the studies by Girolami and Mazzon (1999; 2001) carried out in Italy. Furthermore, in 2003, adults of the parasitoid species were released in a public park in Ljubljana, Slovenia (Žežlina, personal communication), which might have reduced the time needed by the dryinid wasp to reach Hungary. However, recent studies in the region to confirm these theories are missing.

The numerous records of *N. typhlocybae* from Budapest and the occurrence of the species in other big towns (e.g., Győr, Kaposvár, Kecskemét, Miskolc, Pécs, Szekszárd) might have resulted, partly, also from human-mediated dispersal. Pénzes and Hári (2016) as well as Lauterer (2002), Strauss (2010), Kim *et al*. (2011) and Preda and Skolka (2011) highlight the potential role of imported ornamental nursery stock in the unintentional introduction of the host, *M. pruinosa* (i.e. its overwintering eggs laid in the bark may remain undetected in plant trade). It is possible that *N. typhlocybae* has arrived in Budapest, and the other big towns, on plants for planting as cocoons or parasitised *M. pruinosa* nymphs concealed on the underside of leaves. Once established, *N. typhlocybae* could have spread unaided from the donor regions (e.g., Budapest) to the neighbouring locations. The multiple potential introduction routes and processes could account for the rapid range expansion (Girolami and Mazzon, 1999; Malausa *et al*., 2003) and make it difficult to quantify the directions and speed of spread of *N. typhlocybae* within newly invaded regions.

The records from sites outside of urban areas (e.g., in orchards) of relatively small and isolated locations, such as Madocsa and Sükösd, evidently indicate the successful establishment and active dispersal of the species in Hungary. Based on the southeastern Hungarian records (Orosháza and Szarvas) as well as the report by Glavendekic (2012) on the occurrence of *N. typhlocybae* in Belgrade, Serbia, the beneficial wasp species could reach western Romania by natural dispersal soon. Indeed, it is possible that it has already expanded its range to Romania, but remains undetected there.

Confirmation of the occurrence of the dryinid wasp throughout the northern region of Hungary in 2018 completed the picture on the distribution of *N. typhlocybae*. Nevertheless, it is worth noting that only a single cocoon was found at each of the northeastern locations visited (Balassagyarmat, Debrecen and Miskolc). The results suggest that *N. typhlocybae* might be in low prevalence and perhaps only reached northeastern Hungary recently.

Although the pathways of introduction of both the host and the parasitoid in Hungary remain uncertain, the results on the distribution of *N. typhlocybae* clearly show the successful establishment and spread of this natural enemy of *M. pruinosa* in the country.

First record of *N. typhlocybae* in Slovakia

The survey on the occurrence of *N. typhlocybae* carried out in two towns located in southern Slovakia confirmed the presence of the dryinid species in the country. The records are as follows: Komárno, 47.7645°N 18.1168°E (cemetery), 10 July 2018; Šamorín, 48.0442°N 17.3171°E (tree lane at the edge of the town), 10 July 2018. These are the first records of *N. typhlocybae* from Slovakia. The record in Komárno was taken in the middle of the town, while the other one at Šamorín came from the edge of the residential area. In both cases, several parasitised specimens (*M. pruinosa*

Table 1. Rates of *M. pruinosa* individuals parasitised by *N. typhlocybae* based on a survey carried out in Hungary between 20-28 July 2016.

Location (coordinates)	Date of collection (2016)	Number of live and survived *M. pruinosa* individuals		Total (live and survived *M. pruinosa*)	Number of parasitised *M. pruinosa* individuals		Total (parasitised *M. pruinosa*)	Rate of parasitism (%)
		Live nymphs	Exuviae of 5th instar nymphs		*M. pruinosa* nymphs with thylacium	Cocoons of *N. typhlocybae*		
Budapest (47.4438°N 19.1958°E)	20 July	378	72	450	35	57	92	16.97
Kaposvár (46.3606°N 17.8126°E)	22 July	430	39	469	4	16	20	4.09
Martonvásár (47.3158°N 18.7852°E)	21 July	421	32	453	0	3	3	0.66
Balatonvilágos (46.9723°N 18.1788°E)	22 July	308	9	317	1	3	4	1.25
Kecskemét (46.9135°N, 19.6539°E)	21 July	602	16	618	1	4	5	0.80
Szekszárd (46.3360°N 18.7022°E)	22 July	363	89	452	5	65	70	13.41
Pécs (46.0504°N 18.2345°E)	22 July	535	103	638	7	14	21	3.19
Budapest (47.5193°N 19.1823°E)	20 July	256	28	284	2	9	11	3.73
Budapest (47.5712°N 19.0517°E)	20 July	402	358	760	5	21	26	3.31
Szarvas (46.8641°N 20.5350°E)	28 July	525	91	616	4	6	10	1.60
Budapest (47.4764°N 19.0330°E)	20 July	303	7	310	2	2	4	1.27

nymphs with thylacium as well as cocoons of *N. typhlocybae*) on plants infested with *M. pruinosa* were revealed. Voucher specimens have been deposited in the Hymenoptera Collection of the Hungarian Natural History Museum, Budapest.

Rate of parasitism

The rates of parasitised *M. pruinosa* individuals, based on the evaluation of infested leaves collected at eleven different locations in Hungary between 20-28 July 2016, are shown in table 1. The rate of parasitism varied between 0.66-16.97%. The highest rates, exceeding 10%, were from two samples originating in cemeteries, one located in Budapest (16.97%) and the other at Szekszárd (13.41%). The wide occurrence of *M. pruinosa* in the capital combined with the presence of *N. typhlocybae* in Budapest since at least 2014 (Szöllősi-Tóth *et al*., 2017) might have resulted in a considerable increase of the parasitoid population at some specific locations, including the cemetery concerned, and may explain the relatively high rate of parasitism found. Szekszárd, a town situated in southwestern Hungary, might have been colonised by *N. typhlocybae* through natural dispersal from the directions of Croatia and Slovenia but time of arrival is uncertain because it could have remained unnoticed for several years before it became detected during our survey in 2016. However, there may be other factors which might lead to the result of finding a high rate of parasitism at some sites; for example, how sampling is performed. The availability of favoured host plants for the pest combined with the leaf samples which were taken for analysis might have influenced the results: as in several cases a major proportion of the infested leaves collected at a location were picked from the same plant (part) and/or nearby plants, the rate of parasitism might have been overestimated for the wider area inspected if *N. typhlocybae* was only a recent invader there forming a point source of invasion. This idea may be supported by the results of Malausa (1999). The relatively high chance for the (repeated) unintentional introduction of not only the pest but also its natural enemy on nursery stock in the case of towns compared to isolated and less populated locations should also be taken into consideration, and this also makes the interpretation of the measured rate of parasitism complicated. The time of the first occurrence of *M. pruinosa* may be difficult or impossible to determine for each location, but this might also have had an effect on the rate of parasitism found. For example, it may be assumed that the time lag between the arrival of the pest and its parasitoid might be the reason for the fact that *N. typhlocybae* remained almost undetected in the National Botanical Garden, Vácrátót, in 2016. Here the pest was first observed only in 2013 (Kósa, personal communication), and although *N. typhlocybae* was accidentally found by the authors (as shown in figure 1) during a visit on 9 August 2016, in a preceding survey on 21 July to assess the rate of parasitism based on a large sample of infested leaves containing 421 nymphs of *M. pruinosa*, the symptoms of parasitism had not been revealed (and therefore the data of Vácrátót are not shown in table 1). In general, with the exception of the two considerably high values discussed above, the rate of parasitism did not reach 5% at any sites in Hungary studied in July 2016. Malausa (1999) assessed a rate of parasitism of 6% in Antibes, France, also in July, in the year of release (1996), but in this case infested leaves were taken only from a single tree.

The results of the survey carried out at three southwestern Hungarian sites, already sampled in 2016, showed an increase at each location in July 2018 (table 2). At Kaposvár, for example, this prevalence was almost 30%, which may be regarded a considerable increase in the level of parasitism compared to that of less than 5% two years before. The relatively small numbers of the total live/survived individuals of *M. pruinosa* which were found in two (Kaposvár and Pécs) out of the three samples in 2018 compared to those in 2016 might be indicative of the positive effects of the parasitoid on the populations of the pest. Malausa *et al*. (2006) observed a considerable population decrease of *M. pruinosa* in Antibes, the place of first introduction of *N. typhlocybae* in France in 1996, and reported an increasing rate of parasitism exceeding 70% by the end of summer of 2005. They found a similarly high level of parasitism at other sites of the coastal region of southern France, where the dryinid wasp had been released three years later than in Antibes. Nevertheless, it is noted that other factors might have also contributed to their finding, such as the exceptional drought of the last three years. Our results also clearly show the successful establishment of *N. typhlocybae* and indicate its effectiveness in the control of *M. pruinosa* in Hungary. However, further research is needed to follow the long-term effects of the dryinid wasp on the pest and assess the impacts of environmental factors (e.g., variable climatic conditions, influence of plant taxa on insect development) (Mazzon *et al*., 2000; Byeon *et al*., 2017) as well as anthropogenic effects (e.g., plant trade, gardening techniques, pesticide application) (Dradi, 2002; Strauss, 2010) on the population changes of both non-native species.

The results of the assessment of the level of parasitism carried out at two locations in Slovakia are shown in table 3. The rates calculated in the cases of Komárno and Šamorín are comparable to those found at the Hungarian sites, and, similarly to Hungary, indicate the recent invasion and the successful establishment of the dryinid species in this central European country as well. Further investigations are recommended to reveal the distribution and biology of *N. typhlocybae* in Slovakia.

Number of generations of *N. typhlocybae* in Hungary

The survey on the life cycle of *N. typhlocybae* carried out at three locations in 2016 revealed that the species is able to develop through two generations in Hungary (table 4). The bivoltine portion of the population predominated in each case. The greatest ratio of bivoltinism (5.7) was calculated in one of the two sites examined in Budapest, where almost six times more individuals (120 specimens, representing 85% of the studied population) continued/finished their development until the end of summer than remained in the cocoon in the diapausing stage (21 individuals, 15%) to overwinter. Regarding the sex-ratio determined for the bivoltine part of the populations (i.e. for individuals which continued/finished development by the end of summer), males were predominant in all the three cases, which is in line with the findings of Mazzon *et al*. (2000; 2001). Whereas the predominance of bivoltinism found at all the Hungarian locations examined is highly favourable from the aspect of the effectiveness of biological control of *M. pruinosa* (Mazzon *et al*., 2001), the factors determining voltinism and sex allocation of *N. typhlocybae* remain to be further investigated.

Hyperparasitoid species

Two hyperparasitoid species could be found in the materials examined. Altogether fourteen individuals of *Pachyneuron muscarum* (L.) (Hymenoptera Pteromalidae) were identified amongst the emerged *N. typhlocybae* adults originating from two sites in Budapest (47.4881°N 19.2542°E and 47.4814°N 19.0369°E). In the case of the sample collected in the private garden located on Gellért Hill, Budapest (47.4814°N 19.0369°E), three specimens of *Cheiloneurus boldyrevi* Trjapitzin et Agekyan (Hymenoptera Encyrtidae), emerged in August-September 2016, were also found. *P. muscarum* is widely distributed in Europe and Asia (Noyes, 2018). It is one of the most common hyperparasitoids of *N. typhlocybae* in Italy (Viggiani *et al*., 2002), but it is not host-specialised and parasitises also other insects belonging to a wide range of taxonomic groups (Noyes, 2018). *C. boldyrevi* occurs in many European and Asian countries, but only *N. typhlocybae* is known as its parasitoid host (Guerrieri and Viggiani, 2005; Noyes, 2018). It is the other most common hyperparasitoid of *N. typhlocybae* in Italy. Both species are indigenous in the country, and have adapted to the host (Viggiani *et al*., 2002). *P. muscarum* and *C. boldyrevi* have been reported as the dominant hyperparasitoids of *N. typhlocybae* in France as well. These species were

Table 2. Rates of *M. pruinosa* individuals parasitised by *N. typhlocybae* based on a survey carried out at three locations in southwestern Hungary between 12-14 July 2018.

Location (coordinates)	Date of collection (2018)	Number of live and survived *M. pruinosa* individuals		Total (live and survived *M. pruinosa*)	Number of parasitised *M. pruinosa* individuals		Total (parasitised *M. pruinosa*)	Rate of parasitism (%)
		Live nymphs	Exuviae of 5th instar nymphs		*M. pruinosa* nymphs with thylacium	Cocoons of *N. typhlocybae*		
Kaposvár (46.3606°N, 17.8126°E)	14 July	102	19	121	17	34	51	29.65
Szekszárd (46.3360°N, 18.7022°E)	12 July	378	37	415	17	58	75	15.31
Pécs (46.0504°N, 18.2345°E)	12 July	113	36	149	4	27	31	17.22

Table 3. Rates of *M. pruinosa* individuals parasitised by *N. typhlocybae* based on a survey carried out at two locations in southern Slovakia on 10 July 2018.

Location (coordinates)	Date of collection (2018)	Number of live and survived *M. pruinosa* individuals		Total (live and survived *M. pruinosa*)	Number of parasitised *M. pruinosa* individuals		Total (parasitised *M. pruinosa*)	Rate of parasitism (%)
		Live nymphs	Exuviae of 5th instar nymphs		*M. pruinosa* nymphs with thylacium	Cocoons of *N. typhlocybae*		
Komárno (47.7645°N, 18.1168°E)	10 July	220	23	243	19	30	49	16.78
Šamorín (48.0442°N, 17.3171°E)	10 July	274	101	375	9	15	24	6.02

Table 4. Ratios of the individuals of the bivoltine and univoltine proportions of the populations of *N. typhlocybae* in samples collected at three locations in Hungary in July 2016.

Location (coordinates)	Date of collection (2016)	Total number of collected cocoons containing an intact *N. typhlocybae* individual	Number of *N. typhlocybae* adults emerged until 28 August 2016		Number of *N. typhlocybae* individuals emerged between 28 August and 28 October 2016, or developed but not emerged		Number of diapausing larvae of *N. typhlocybae*	Ratio of the individuals of the bivoltine and univoltine proportions of the *N. typhlocybae* population
			males	females	males	females		
Budapest (47.4438°N 19.1958°E)	20 July	168	55	46	11	1	55	1:0.49
Budapest (47.4764°N 19.0330°E)	20 July	141	81	31	5	3	21	1:0.18
Szekszárd (46.3360°N 18.7022°E)	22 July	67	29	10	2	2	24	1:0.56

found to be responsible for a rate of hyperparasitism regularly changing between 5-15% at Antibes, a site sampled throughout several years. It is noteworthy that this level reached an exceptionally high value of 78% at this location in 2003. Further research in the next years extended to other sites in southern France revealed, however, a relatively low rate of hyperparasitism (Malausa *et al*., 2006).

Although the rate of hyperparasitism has not been examined during our survey, further investigation is recommended to reveal the range of hyperparasitoid species and assess their impact on the populations of the beneficial insect not only in Hungary but also in other countries where *N. typhlocybae* has become established. As *C. boldyrevi* has not been reported from Hungary yet, this is the first record of the species in the country.

Conclusions and implications for biocontrol programmes

After its accidental introduction in Italy about four decades ago, the polyphagous *M. pruinosa* has become a pest of major concern in a wide range of habitats, especially in urban environments, in many European countries. The rapid recognition of the increasing problem led to intensive research to obtain information on the biology and sustainable control of *M. pruinosa*. Following the identification and introduction of its natural enemy, *N. typhlocybae*, extensive release programmes of the dryinid wasp have been initiated in a range of countries in southern Europe with the aim of enhancing the control of *M. pruinosa* over a large area in the medium or long term. Although *N. typhlocybae* could establish and control the populations of the pest successfully in many areas, its dispersal towards other regions of the continent has hardly been studied, and its impact on *M. pruinosa*, which is still a problem in several countries, has not been assessed. The wide distribution of *N. typhlocybae* in Hungary and the relatively high rate of parasitism identified at specific sites clearly indicate the successful acclimatisation of this beneficial insect. That *N. typhlocybae* is able to develop through two generations under Hungarian environmental conditions and that bivoltinism was found to be predominant compared to univoltinism at the studied locations are highly favourable from the aspects of effective and environmentally friendly control of *M. pruinosa*. Nevertheless, further research is suggested to follow the spread, reveal the pathways of introduction and assess the impacts of *N. typhlocybae*, and of its hyperparasitoids. The environmental and anthropogenic factors influencing the populations of the non-native species should be studied in all the countries where they occur and *M. pruinosa* still remains or has become a problem.

In those countries where the dryinid wasp has not yet been recorded but its host is present and has established, targeted search to find *N. typhlocybae* is highly recommended. The identification of its occurrence perhaps suggests that a full risk assessment, which should otherwise be a prerequisite before the intentional introduction of an exotic beneficial organism into a region, is unnecessary. This may largely help save money, time and any efforts invested in risk analyses of introducing *N. typhlocybae*. Although in the case of *N. typhlocybae* host range tests had not been conducted prior to its introduction in Europe (Strauss, 2009), its restricted host range was later supported by several studies (Strauss, 2009; Guglielmino *et al*., 2013). These data may contribute to the assessment of the potential ecological risks posed by either the intentional introduction or natural dispersal of the species in new areas. In Hungary, one additional flatid species, *Phantia subquadrata* (Herrich-Schaeffer), is known to occur, represented only by a single record, and similarly to *M. pruinosa*, it is not native to Hungary (Orosz, personal communication). Therefore, direct negative effects posed by *N. typhlocybae* to non-target species are unlikely to occur in the field, and it can be suggested that unacceptable risks for non-target species will not arise in this region. On the contrary, from the aspect of nature conservation, *P. subquadrata* as well as other species closely related to *M. pruinosa* were suggested to be target organisms in host range trials with *N. typhlocybae* in those countries where flatid species are autochthonous to and the dryinid wasp might be intended to use in classical biological control (Strauss, 2009). Preliminary host range trials should be especially recommended if there are native flatids in the potential target area of release already infested with *M. pruinosa*, but *N. typhlocybae* is not present in the neighbouring regions, as it was the case with Korea in 2009 (Kim *et al.*, 2011), hence there is no or minor chance of active or passive dispersal of the parasitoid species towards the area in concern. Thorough studies prior to introduction in a new area as well as post-release monitoring may both provide valuable information for a complex risk assessment, and may help reveal the risks and benefits of biocontrol programmes. For instance, due to the current extensive research on (potential) biological control agents, and based on lessons learnt, the harlequin ladybird (*Harmonia axyridis* Pallas) may serve as an example of a non-native species which may cause adverse effects to species within its new environment if introduced without proper care and regulations (Koch, 2003; Roy and Wajnberg, 2008; van Lenteren *et al.*, 2008; Honek *et al.*, 2016). On the other hand, the case of *Torymus sinensis* Kamijo, introduced in Europe as a natural enemy of the Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu), may serve as one of the most successful stories in classical biological control, as confirmed by long-term post-release studies (Picciau *et al*., 2017; Avtzis *et al.*, 2018; Ferracini *et al.*, 2018). *N. typhlocybae* will hopefully expand the list of effective biocontrol agents posing no real risk to the environment.

Once the dryinid species has spread over a region, it may be able to control the populations of *M. pruinosa* efficiently. The chance of survival and permanent establishment of the beneficial insect may be increased and its further dispersal might be enhanced by avoiding the use of broad spectrum insecticides in urban green areas and/or by not removing fallen leaves from sites (e.g., parks, cemeteries, gardens) where there is a high density of cocoons of the parasitoid. These are simple, easy-to-apply and cost-effective methods by which not only pest management experts but also citizens could easily contribute to the sustainable control of *M. pruinosa* by the conservation of *N. typhlocybae*.

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