REVIEW ARTICLE

Diversity and surge in abundance of native parasitoid communities prior to the onset of *Torymus sinensis* on the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) in Slovenia, Croatia and Hungary

Katarina Kos[1] · **Nikola Lacković**[4] · **George Melika**[3] ·
Dinka Matošević[2]

Abstract Since the rapid spread of the Asian chestnut gall wasp (ACGW) throughout south-east Europe in the last few years, the possibilities of its control have been increasingly investigated. Due to constraints in available suppression measures in forest stands, biological control is recognized as the most suitable action to lower the abundance of the new invasive pest. *Torymus sinensis*, as a specialized parasitoid chalcid wasp of ACGW, was introduced to suppress the pest in Italy in 2005, and later in Croatia, Hungary (2014) and Slovenia (2015). We investigated the native parasitoid complex associated with ACGW in Slovenia, Croatia and Hungary over 8 years of sampling. We found 41 species of native parasitoids, eight of which are the first records on ACGW in Europe, adding up to a total of 51 species known to parasitize ACGW. These findings represent a large complex of cynipid-parasitoid fauna. Moreover, the abundance of native parasitoid populations on ACGW have demonstrated a temporal surge between the invasion of a new alien host (ACGW) and the onset of its specialized parasitoid, *T. sinensis*. Our results indicate that the introduced parasitoid acts as a very successful biocontrol of ACGW, outcompeting native parasitoids. This outcome should be considered as beneficial through the maintenance of ecological balance in affected forest ecosystems.

Keywords Invasive pest · ACGW · Biological control · Native parasitoids · *Torymus sinensis*

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✉ Katarina Kos
katarina.kos@bf.uni-lj.si

[1] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

[2] Department for Forest Protection and Game Management, Croatian Forest Research Institute, Cvjetno naselje 41, 10450 Jastrebarsko, Croatia

[3] Plant Health and Molecular Biology Laboratory, Directorate of Plant Protection, Soil Conservation and Agri-environment, National Food Chain Safety Office, Budaörsi u. 141-145, Budapest 1118, Hungary

[4] Antuna Mihanovića 4, 10450 Jastrebarsko, Croatia

Introduction

The Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (ACGW) is a member of a diverse group of gall wasps (Hymenoptera, Cynipidae), and is one of the most important pests of the sweet chestnut *Castanea sativa* Mill., the Japanese chestnut *C. crenata* Siebold & Zucc., the Chinese chestnut *C. mollisima* Blume, the American chestnut *C. dentata* (Marshall) Borkh. and their hybrids worldwide (EPPO 2005; Aebi et al. 2006). The ACGW is a parthenogenetic species with one generation per year, and females lay eggs in the buds of chestnuts in the summer. The overwintering stage is the first-instar larvae that induce gall formation the following spring (Ôtake 1989). One of the reasons why this alien species became invasive was the lack of natural enemies in the invaded area (Matošević and Melika 2013). Among the various factors (parthenogenesis, abundant egg laying), the inactivity of native parasitoids occurred during the early stages of infestation, favouring the rapid spread of this new phytophagous insect in Europe. Parasitoid insects are commonly considered to be host-specific and thus represent one

of the most used natural enemies for biological pest control (Godfray 1994). There are numerous parasitoid species that have a wide host range that may not be consistent across the distribution of an entire species, while host adaptation can occur in parasitoid populations and thus restrict the potential host range (Antolin et al. 2006; Henry et al. 2008; Zepeda-Paulo et al. 2013). However, within the range of all potential hosts, not all are equally preferred and/or become susceptible to the development of parasitoids (Desneux et al. 2009). The community of gall wasp parasitoids, especially on oaks, is very abundant owing to the species richness of oak gall wasps (Askew et al. 2013). Parasitoids reared from cynipid galls are usually specific to cynipid wasps, and a certain number can adapt to the new abundant alien host, *D. kuriphilus* (Askew et al. 2013). The reported parasitism rates of native parasitoids were too low, rendering them ineffective in controlling the growing populations of ACGW (Matošević and Melika 2013; Quacchia et al. 2013; Kos et al. 2015; Colombari and Battisti 2016).

In Europe, studies on native parasitoid recruitment were only recorded for a few countries: Italy, Croatia, Hungary, Slovenia and Spain. Within six families (Eulophidae, Eupelmidae, Eurytomidae, Ormyridae, Pteromalidae and Torymidae), 31 species were found in Italy (Aebi et al. 2007; Speranza et al. 2009; Boriani et al. 2013; Panzavolta et al. 2013; Quacchia et al. 2013; Colombari and Battisti 2016). Matošević and Melika (2013) reported 15 species from five families in Croatia, Kos et al. (2017) found 35 native species in Slovenia, Kriston et al. (2014) and Melika et al. (2013) reported 11 species in Hungary and Jara-Chiquito et al. (2016), 14 species in Spain.

The rapid recruitment of oak gall cynipid parasitoids to ACGW occurred in Europe. Nevertheless, the actual emergence rate of native parasitoids on ACGW remains low, mainly due to a mismatch between the phenology of gall development and emergence time of native parasitoids (Aebi et al. 2006, 2007).

The aim of this research was to provide comprehensive information on the species parasitizing ACGW in Europe and show the species-rich complex of native parasitoids in Slovenia, Croatia and Hungary. As ACGW became established in new environments, it recruited several species of parasitoids associated primary with oak gall wasps.

Materials and methods

A survey for chalcid wasp parasitoid recruitment on ACGW was carried out over an 8-year period (2010–2017) in Slovenia, Croatia and Hungary. In Slovenia, data for the entire 8 years are provided (2010–2017), while in Croatia and Hungary data are available from 2013 to 2015. The differences

in sampling are due to logistical problems. However, we tried to unify and present the data so that valid conclusions could be made.

Sampling of ACGW galls occurred from February to April (overwintering galls), and from late May to mid-June (collecting newly formed galls) from 2010 to 2017. A total of 137,511 galls (Slovenia 98,267, Croatia 22,589 and Hungary 16,655) were collected from 57 locations in all three countries and in all sampling years in the main chestnut orchards and stands in each of the three countries. They were randomly collected by hand and with a tree cutter up to the height of 2.5 m. The number of galls varied among years and locations depending on the rate of infestation. Galls were placed in nylon bags and transported to the entomological laboratories, the Laboratory of Phytomedicine at the Biotechnical Faculty in Ljubljana (Slovenia), Croatian Forest Research Institute in Jastrebarsko (Croatia), and the Plant Health and Molecular Biology Laboratory of the National Food Chain Safety Office in Budapest (Hungary). They were stored at room temperature in plastic mass rearing boxes, checked daily until the wasps emerged and after emergence, wasps from each sample were preserved in 70% ethanol and identified at the Plant Health and Molecular Biology Laboratory in Budapest. Adult parasitoid identification was based on the key for Chalcidoidea families and genera (Goulet and Huber 1993).

In 2016 and 2017, very few galls were collected at certain locations in Slovenia due to severe spring frosts when most of new (yet forming) galls were destroyed. In Croatia and Hungary, no galls were collected in these years.

It is acknowledged that the data is not uniform (not the same sampling periods nor the same number of collected galls) in our research for Slovenia, Croatia and Hungary but we believe that it is justified to present this data as there are several new host-parasitoid records that add to the knowledge of these interactions in Europe. In order to have a complete overview of data on native parasitoids found on alien ACGW, Table 1 includes native parasitoids collected during this research as well as data already published (Matošević and Melika 2013; Melika et al. 2013; Kos et al. 2015). The inclusion of already published data with the original data of this research provides a complete overview of native parasitoid complex on ACGW prior to the arrival of the introduced alien parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) in Slovenia, Croatia and Hungary.

As the number of galls collected varied according to different rates of the infestation within years and in locations, and as the sampling effort was different among countries, the number of emerged specimens of native parasitoids and *T. sinensis* was standardized to 100 galls: standardized emergence rate (er) = (number of parasitoids/total number of galls per sample) × 100 (Quacchia et al. 2013). In addition, using a Chi square test, the sex ratio of emerged parasitoid

Table 1 Number of specimens, sex ratio and gall age ratio of native parasitoid species emerged from ACGW (*Dryocosmus kuripilus*); numbers represent combined data from Slovenia, Croatia and Hungary from 2010 to 2017

No.	Parasitoid species	Sum specimens	sum m	sum f	F RATIO	chi2	exp 50/50	*p* value	SUM old	SUM new
1	*Aprostocetus biorrhizae*	4	0	4	4	2.00	2	0.1573	4	0
2	*Aprostocetus aethiops*	2	2	0	−2	1.00	1	0.3173	2	0
3	*Aprostocetus glandicola*	1	1	0	−1	0.50	0.5	0.4795	1	0
4	*Aulogymnus skianeuros*	25	7	18	11	2.42	12.5	0.1198	19	6
5	*Baryscapus* sp.	29	0	29	29	14.50	14.5	**0.0001*****	29	0
6	*Bootanomyia dorsalis*	1573	538	1035	497	78.52	786.5	**0.0000*****	54	1519
7	*Bootanomyia dumicola*	1	0	1	1	0.50	0.5	0.4795	0	1
8	*Bootanomyia stigmatizans*	1	1	0	−1	0.50	0.5	0.4795	0	1
9	*Cecidostiba fungosa*	23	3	20	17	6.28	11.5	0.0122*	1	22
10	*Cecidostiba semifascia*	3	2	1	−1	0.17	1.5	0.6831	3	0
11	*Closterocerus trifasciatus*	1	0	1	1	0.50	0.5	0.4795	0	1
12	*Eupelmus annulatus*	1435	545	890	345	41.47	717.5	**0.0000*****	404	1031
13	*Eupelmus splendens*	16	2	14	12	4.50	8	0.0339*	14	2
14	*Eupelmus urozonus*	2149	695	1454	759	134.03	1074.5	**0.0000*****	393	1756
15	*Eupelmus vesicularis*	4	0	4	4	2.00	2	0.1573	0	4
16	*Eurytoma brunniventris*	333	162	171	9	0.12	166.5	0.7273	180	153
17	*Eurytoma pistaciae*	266	103	163	60	6.77	133	0.0093**	86	180
18	*Mesopolobus albitarsus*	7	0	7	7	3.50	3.5	0.0614	3	4
19	*Mesopolobus amaenus*	14	1	13	12	5.14	7	0.0233*	0	14
20	*Mesopolobus dubius*	13	3	10	7	1.88	6.5	0.1698	0	13
21	*Mesopolobus fasciiventris*	42	15	27	12	1.71	21	0.1904	5	37
22	*Mesopolobus sericeus*	688	204	484	280	56.98	344	**0.0000*****	1	687
23	*Mesopolobus tarsatus*	28	4	24	20	7.14	14	0.0075**	7	21
24	*Mesopolobus tibialis*	722	248	474	226	35.37	361	**0.0000*****	5	717
25	*Ormyrus nitidulus*	137	67	70	3	0.03	68.5	0.8562	0	137
26	*Ormyrus pomaceus*	390	296	94	−202	52.31	195	**0.0000*****	1	389
27	*Pediobius rotundatus*	1	0	1	1	0.5	0.5	0.4795	0	1
28	*Pediobius saulius*	4	3	1	−2	2	2	0.4795	4	0
29	*Reikosiella rostrata*	5	0	5	5	2.50	2.5	0.1138	2	3
30	*Sycophila biguttata*	60	7	53	46	17.63	30	**0.0000*****	1	59
31	*Sycophila flavicollis*	315	36	279	243	93.73	157.5	**0.0000*****	1	314
32	*Sycophila iracemae*	2	1	1	0	0.00	1	1.0000	0	2
33	*Sycophila variegate*	55	10	45	35	11.14	27.5	**0.0008*****	0	55
34	*Torymus auratus*	171	50	121	71	14.74	85.5	**0.0001*****	4	167
35	*Torymus fastuosus*	15	6	9	3	0.30	7.5	0.5839	0	1
36	*Torymus flavipes*	1917	608	1309	701	128.17	958.5	**0.0000*****	34	1897
37	*Torymus formosus*	84	16	68	52	16.10	42	**0.0001*****	0	84
38	*Torymus geranii*	1166	311	855	544	126.90	583	**0.0000*****	2	1164
39	*Torymus nobilis*	3	1	2	1	0.17	1.5	0.6831	0	3
40	*Torymus notatus*	8	2	6	4	1.00	4	0.3173	0	8
41	*Torymus scutellaris*	3	1	2	1	0.17	1.5	0.6831	0	3
	Sum specimens	11716	3951	7765					1260	10456

The observed sex ratios were tested against the expected 50:50 ratio (all DF = 1; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

Symbology: sum specimens = specimens of one species collected in our study; sum m—male specimens collected; sum f—female specimens collected; F-ratio—difference of number in favor of female specimens; chi2—Chi square test; exp 50/50—expected 50:50 sex ratio; sum old—specimens collected from old (overwintered) ACGW galls; sum new—specimens collected from new (fresh) ACGW galls; *p* value—statistical significance

specimens was tested against a null hypothesis of a 50:50 sex ratio (Matošević and Melika 2013) to determine whether significant sex bias to any gender (e.g., more males or more females) existed in the sampled populations.

Results

Forty-one native parasitoid species emerged from the Asian chestnut gall wasp in Slovenia, Croatia and Hungary (Table 1). Eight of the 41 species were recorded for the first time on the ACGW in the countries in this study, and also represent the first records in Europe. Table 2 is a synoptic table of all ACGW parasitoid species in Europe with references to provide a complete list of all native parasitoids found on this new alien host. New records are in bold.

The emergence rates are provided for the seven most abundant species in Slovenia from 2010 to 2017 (Table 3), in Croatia (Table 4) and in Hungary (Table 5) from 2013 to 2015. The highest emergence rate in Slovenia was 5.0 by *Torymus flavipes* in 2011, followed by *E. annulatus* in 2014 (3.2) and *E. urozonus* in 2013 (2.5) (Table 3). Abundance indices peaked between 2011 and 2014. Based on the data from Slovenia, the absolute number of emerged specimens of native parasitoids fell from 1720 in 2014 to only 20 in 2017 (Fig. 1), while that of *T. sinensis* increased rapidly in this period (Table 3, Fig. 1).

Considering the effect of formational stage or age of the galls, 1,260 specimens of native parasitoids emerged from old, overwintered galls, while 10,456 specimens emerged from new, freshly formed galls (Table 1). This suggests that the first generation of native parasitoids, emerging in April–May, was successful in parasitizing ACGW in small but freshly formed galls, while the second generation (emerging in June–July) was far less successful in parasitizing ACGW in fully formed galls.

Overall, the most abundant species, with a number of emerged specimens over 1000, were *E. urozonus* (2149), *T. flavipes* (1917), *B. dorsalis* (1573), *E. annulatus* (1435), and *T. geranii* (1166) (Table 1). Several other species were present in lower amounts though most of the study period; e.g., *E. brunniventris*, *E. pistaciae*, *M. sericeus*, *M. tibialis*, *O. nitidulus*, *O. pomaceus*, and *S. flavicollis*. There were 19 species with only few recorded specimens (Table 1).

The most abundant native parasitoid in Slovenia and Croatia was *T. flavipes*, at 958 and 809 specimens, respectively, collected during the sampling period (2010–2017) which is similar to neighboring Italy (Santi and Maini 2011; Panzavolta et al. 2013, 2018; Francati et al. 2015; Ferracini et al. 2018), while the most abundant species in Hungary was *E. urozonus* (666), followed by *B. dorsalis* (375) and *S. flavicollis* (198) over all 3 years of collection (Table 5). Furthermore, in Slovenia the second highest number was *E.*

annulatus (922), followed by *E. urozonus* (907), *B. dorsalis* (560) and *O. pomaceus* (337). On the other hand, in Croatia, over 5 years of collection (2013–2015 and based on data from Matošević and Melika (2013), the second most abundant species was *T. geranii* (786), followed by *E. urozonus* (571), *M. sericeus* (533) *B. dorsalis* (401), and *E. annulatus* (384). Interestingly, populations of the most common native parasitoids varied substantially over the years. In particular, populations of *T. flavipes* and *E. urozonus* in Slovenia fluctuated, while *M. tibialis* and *O. pomaceus* were abundant in the first years, but in the following years there were only a few specimens present (Table 3). In contrast, the population of *E. annulatus* increased through the years until the onset and expansion of the alien *T. sinensis*.

Our results show that the most abundant parasitoid species, *Eupelmus urozonus*, *E. annulatus*, *Bootanomyia dorsalis*, *T. flavipes*, and *T. geranii* also demonstrated female-biased ratios with only one species having a male-biased sex ratio (Table 1).

The results of this study show the surge in abundance of native parasitoids and female-biased sex ratios during the study years (Tables 1 and 6). The bias was statistically significant between the years 2011 and 2015, marginally significant in 2016 and not significant from 2010 to 2017. Similar temporal patterns were observed regarding total yearly abundance.

Discussion

In the last two decades, a number of taxonomic revisions were made in different Chalcidoidea families, and new valid names and new synonymies established. Although they were neglected in the majority of papers on native parasitoids of ACGW, they were followed in this study: Eurytomidae, *Eurytoma* Illiger (Zerova and Seryogina 2009; Fusu 2013); Eupelmidae, *Eupelmus* Dalman (= *Macroneura*) (Fusu 2017), *Reikosiella* Yoshimoto (Al Khatib et al. 2014, 2016; Gibson and Fusu 2016); Torymidae, *Torymus* Dalman and Megastigmidae, *Bootanomyia* Girault (Doğanlar 2011; Janšta et al. 2017).

Our research shows that a rich parasitoid community has been formed on Asian chestnut gall wasp in Europe, which coincides with earlier studies (e.g., Matošević and Melika 2013; Melika et al. 2013). In the three countries covered by this study; 42 native parasitoid species, including *Mesopolobus mediterranneus* discovered by Jurc et al. (2013), from 13 genera and the alien *T. sinensis* were found on ACGW. Of these, eight are new records (Table 5), adding to the total of 51 native and one introduced species from 13 genera and six families known to parasitize Asian chestnut gall wasp in Europe.

Table 2 List of parasitoids (native and non-native) on the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) in Europe, with references of first records

	Parasitoid species	Family	First record (author(s), year)
1	*Aprostocetus biorrhizae* (Szelényi, 1941)	Eulophidae	Kos et al. (2015)
2	*Aprostocetus aethiops* (Zetterstedt, 1838)	Eulophidae	Kos et al. (2015)
3	*Aprostocetus glandicola (*Graham, 1987)	Eulophidae	Kos et al. (2015)
4	*Aulogymnus arsames* (Walker, 1838)	Eulophidae	Quacchia et al. (2013)
5	*Aulogymnus skianeuros* (Ratzeburg, 1844)	Eulophidae	Kos et al. (2015)
6	*Baryscapus pallidae* (Graham, 1991)	Eulophidae	Aebi et al. (2006)
7	**Closterocerus trifasciatus (Westwood, 1833)**	Eulophidae	**First report Croatia**
8	*Pediobius chilaspidis* (Bouček, 1965)	Eulophidae	Quacchia et al. (2013)
9	**Pediobius rotundatus (Fonscolombe, 1832)**	Eulophidae	**First report Hungary**
10	*Pediobius saulius* (Walker, 1839)	Eulophidae	Quacchia et al. (2013)
11	*Eupelmus annulatus* Nees, 1834	Eupelmidae	Quacchia et al. (2013)
12	*Eupelmus azureus* Ratzeburg, 1844 (= *Eupelmus spongipartus* (Förster, 1860))	Eupelmidae	Quacchia et al. (2013)
13	*Eupelmus fulvipes* Förster, 1860	Eupelmidae	Quacchia et al. (2013)
14	*Eupelmus splendens* Giraud, 1872	Eupelmidae	Quacchia et al. (2013)
15	*Eupelmus urozonus* Dalman, 1820	Eupelmidae	Aebi et al. (2006)
16	*Eupelmus* (=*Macroneura*) *vesicularis* (Retzius, 1783)	Eupelmidae	Kos et al. (2015)
17	**Reikosiella rostrata** (Ruschka, 1921)	Eupelmidae	Boriani et al. (2013)
18	*Eurytoma adleriae* (Zerova, 1995)	Eurytomidae	Quacchia et al. (2013)
19	*Eurytoma brunniventris* (Ratzeburg, 1852)	Eurytomidae	Aebi et al. (2006)
20	*Eurytoma pistaciae* (Rondani, 1877) (= *E. setigera* (Mayr, 1878)	Eurytomidae	Aebi et al. (2006)
21	*Sycophila biguttata* (Swederus, 1795)	Eurytomidae	Aebi et al. (2006)
22	*Sycophila flavicollis* (Walker, 1834)	Eurytomidae	Colombari and Battisti (2016)
23	*Sycophila iracemae* (Nieves Aldrey, 1984)	Eurytomidae	Aebi et al. (2006)
24	*Sycophila variegata* (Curtis, 1831)	Eurytomidae	Aebi et al. (2006)
25	*Ormyrus nitidulus* (Fabricius, 1804)	Ormyridae	Quacchia et al. (2013)
26	*Ormyrus pomaceus* (Geoffroy, 1785)	Ormyridae	Aebi et al. (2006)
27	**Cecidostiba fungosa (Geoffroy, 1785)**	Pteromalidae	**First report Croatia and Slovenia**
28	*Cecidostiba semifascia* (Walker, 1835)	Pteromalidae	Kos et al. (2015)
29	*Mesopolobus albitarsus* (Walker, 1834)	Pteromalidae	Kos et al. (2015)
30	*Mesopolobus amaenus* (Walker, 1834)	Pteromalidae	Quacchia et al. (2013)
31	*Mesopolobus dubius* (Walker, 1834)	Pteromalidae	Matošević et al. (2013)
32	*Mesopolobus fasciiventris* (Westwood, 1833)	Pteromalidae	Panzavolta et al. (2013)
33	*Mesopolobus lichtensteini* (Mayr, 1903)	Pteromalidae	Jara-Chiquito et al. (2016)
34	*Mesopolobus mediterraneus* (Mayr, 1903)	Pteromalidae	Aebi et al. (2006)
35	*Mesopolobus sericeus* (Förster, 1770)	Pteromalidae	Aebi et al. (2006)
36	*Mesopolobus tarsatus* (Nees, 1834)	Pteromalidae	Aebi et al. (2006)
37	*Mesopolobus tibialis* (Westwood, 1833)	Pteromalidae	Quacchia et al. (2013)
38	*Mesopolobus xanthocerus* (Thomson, 1878)	Pteromalidae	Pérez-Otero et al. (2017)
39	*Bootanomyia almusiensis* (Doganlar, 1989)	Torymidae	Colombari and Battisti (2016)
40	*Bootanomyia dorsalis* (Fabricius, 1798)	Torymidae	Aebi et al. 2006
41	**Bootanomyia dumicola (Bouček, 1982)**	Torymidae	**First report Slovenia**
42	**Bootanomyia stigmatizans (Fabricius, 1798)**	Torymidae	**First report Croatia**
43	*Torymus auratus* (Müller, 1764)	Torymidae	Aebi et al. (2006)
44	*Torymus erucarum* (Schrank, 1781)	Torymidae	Speranza et al. (2009)
45	**Torymus fastuosus (Boheman, 1834)**	Torymidae	**First report Hungary**
46	*Torymus flavipes* (Walker, 1833)	Torymidae	Aebi et al. (2006)
47	*Torymus formosus* (Walker, 1833)	Torymidae	Kos et al. (2015)
48	*Torymus geranii* (Walker, 1833)	Torymidae	Matošević et al. (2013)

Table 2 (continued)

	Parasitoid species	Family	First record (author(s), year)
49	***Torymus nobilis* (Boheman, 1834)**	Torymidae	**First report Hungary**
50	***Torymus notatus* (Walker, 1833)**	Torymidae	**First report Slovenia**
51	*Torymus scutellaris* (Walker, 1833)	Torymidae	Aebi et al. (2006)
	*Torymus sinensis (Kamijo, 1982)	Torymidae	Aebi et al. (2007)

Table 3 Seven most common native parasitoids and alien *T. sinensis* on ACGW (*Dryocosmus kuripilus*) with standardized emergence rate in Slovenia in the period from 2010–2017

Slovenia	2010		2011		2012		2013		2014		2015		2016		2017	
	np	er	np	er	np	er	np	er	np	er	np	er	np	er	np	er
E.annulatus	0	0.0	18	0.2	75	0.3	162	1.6	555	3.2	102	0.6	9	0.1	1	0.0
E. urozonus	0	0.0	139	1.4	73	0.3	254	2.5	340	2.0	87	0.5	10	0.1	4	0.1
B. dorsalis	1	0.0	83	0.8	66	0.3	14	0.1	240	1.4	152	0.8	1	0.0	3	0.0
M. tibialis	0	0.0	119	1.2	9	0.0	0	0.0	77	0.4	0	0.0	0	0.0	1	0.0
O. pomaceus	0	0.0	171	1.7	61	0.3	14	0.1	75	0.4	15	0.1	1	0.0	0	0.0
T. flavipes	6	0.1	504	5.0	262	1.1	33	0.3	132	0.8	16	0.1	3	0.0	2	0.0
T. geranii	2	0.0	20	0.2	13	0.1	32	0.3	43	0.3	42	0.2	0	0.0	0	0.0
other natives	7	0.1	253	2.5	131	0.6	44	0.4	258	1.5	140	0.8	9	0.1	9	0.1
All native	**16**	**0.3**	**1307**	**13.0**	**690**	**3.0**	**553**	**5.5**	**1720**	**10.0**	**554**	**3.0**	**33**	**0.4**	**20**	**0.3**
T. sinensis	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**647**	**3.5**	**3162**	**40.0**	**Approx 10000**	**146.9**
ngy	5072		10030		22841		10002		17131		18473		7909		6809	

Symbology: np=number of emerged parasitoids, er—standardized emergence rate, ngy—total number of analyzed galls per year

Table 4 Seven most common native parasitoids and alien *T. sinensis* on ACGW (*Dryocosmus kuripilus*) with standardized emergence rate in Croatia in the period from 2013 to 2015

Croatia	2013		2014		2015	
	np	er	np	er	np	er
E.annulatus	14	0.3	150	5.1	149	1.03
E. urozonus	255	4.9	122	4.2	161	1.12
B. dorsalis	26	0.5	46	1.6	260	1.81
M. sericeus	101	1.9	384	13.1	38	0.26
M. tibialis	177	3.4	15	0.5	0	0.00
T. flavipes	295	5.6	68	2.3	316	2.19
T. geranii	366	7.0	71	2.4	214	1.49
other native	171	3.3	531	18.1	395	2.74
All native	**1405**	25.1	**1387**	34.7	**1553**	10.78
T. sinensis	**0**	**0**	**0**	**0**	**1041**	7.23
ngy	5252		2936		14401	

Symbology: np=number of emerged parasitoids, er—standardized emergence rate, ngy—total number of analyzed galls per year

The most abundant species usually act as generalists, e.g., hosts of *E. urozonus* involve 32 families from five orders (Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera), and *E. annulatus* and *M. tibialis* can parasitize hosts from three orders. In addition, *P. saulius* found in our research on ACGW galls can use hosts from 28 families of six different orders (Noyes 2018). It was also reported as most abundant parasitoid found in south-eastern Europe in the horse chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae), another invasive and abundant species (Grabenweger et al. 2010).

Table 5 Total number of seven most common native parasitoids and alien *T. sinensis* on ACGW (*Dryocosmus kuripilus*) with standardized emergence rate in Hungary in the period from 2013 to 2015

Hungary	2013		2014		2015	
	np	er	np	er	np	er
E. annulatus	0	0.0	0	0.0	57	0.4
E. urozonus	296	18.5	238	28.0	132	0.01
B. dorsalis	138	8,6	2	0.2	235	1.6
T. flavipes	0	0.0	0	0.0	9	0.01
T. geranii	7	0.4	38	4.5	121	0.8
Eurytoma brunniventris	15	0.9	0	0.0	2	0.01
Sycophila flavicollis	23	1.4	6	0.7	169	1.2
other native	28	1.7	25	0.1	143	1.0
All native	**507**	31.6	**306**	36	**868**	6.1
T. sinensis	**0**	0.0	**0**	0.0		0.0
Ngy	1602		850		14203	

Symbology: np = number of emerged parasitoids, er—standardized emergence rate, ngy—total number of analyzed galls per year
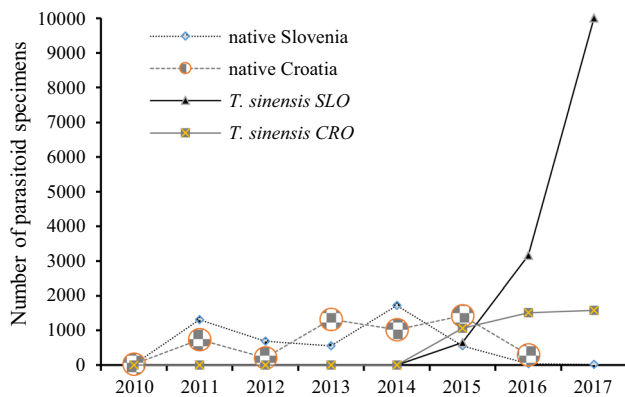
Fig. 1 Number of native parasitoids and alien *T. sinensis* specimens on ACGW found in Slovenia (2010–2017) and Croatia (2010–2016)

It has been shown that seasonal asynchrony between ACGW and native parasitoids could have an indirect impact on the native hosts that share the same parasitoids with abundant alien pests (Budroni et al. 2018). Although parasitism rates of ACGW by native parasitoids generally remain low (Aebi et al. 2011), some studies have shown rates of over 30% (Santi and Maini 2011; Panzavolta et al. 2018). In cases where these two coincide, significantly higher pressure of native parasitoids on their primary native hosts (e.g., oak cynipids) can occur since the second generation of parasitoids, now more numerous due to additional progeny from the newly adopted host, can parasitize only the asexual generation of its primary native host (e.g., oak gall wasps) (Budroni et al. 2018).

A similar scenario was noted in the case of another invasive pest, the horse chestnut leaf-miner (*C. ohridella*), where over 30 species of native parasitoids were found, although in low parasitizing rates (Stojanović and Marković 2004; Ferracini and Alma 2007; Grabenweger et al. 2010). However,

Table 6 Number of native parasitoids emerged from ACGW from Slovenia, Croatia and Hungary from 2010 to 2017, with sex ratio and sex bias test

Year	sum	m	f	F-ratio	exp 50/50	chi2	*p* value
2010	42	13	29	16	21	3.05	0.0809
2011	2041	731	1310	579	1020.5	82.13	0.0000***
2012	895	353	542	189	447.5	19.96	0.0000***
2013	2233	725	1508	783	1116.5	137.28	0.0000***
2014	3108	1086	2022	936	1554	140.94	0.0000***
2015	2997	860	2137	1277	1498.5	272.06	0.0000***
2016	331	126	205	79	165.5	9.43	0.0021**
2017	20	6	14	8	10	1.60	0.2059

The observed sex ratios were tested against the expected 50:50 ratio (all DF = 1; *P < 0.05; **P < 0.01; ***P < 0.001)

Symbology: sum = number of specimen collected; m—number of males; f—number of females; F-ratio—difference of number in favor of female specimens; exp 50/50—expected 50:50 sex ratio; chi2—Chi square test; *p* value—statistical significance

many species of native parasitoids can build up their numbers on a new host early in the spring, resulting in exposure of later-occurring native leaf-miners to a greater pressure of parasitoids (Péré et al. 2010).

Our data shows the surge in population numbers of native parasitoids attacking Asian chestnut gall wasp from 2010 to 2015. This suggests that native parasitoids were gradually adapting to their new host, and perhaps could have led to ecological consequences described by Budroni et al. (2018). However, the abundance of native parasitoids on ACGW dropped dramatically after the onset of *T. sinensis* and its outbreak since 2016 (Fig. 1). This highlights the success of this specialized parasitoid in attacking the Asian chestnut gall wasp (Matošević et al. 2017), and indicates that it possibly outcompeted the native parasitoids which were adapting to the new host. The differences in the number of *T. sinensis* in Slovenia and Croatia in 2016 and 2017 are due to rapid, natural spread in Slovenia and the vicinity to Italy, and a slower natural spread in Croatia with a larger distance to Italy (Matošević et al. 2017).

The only effective parasitoid from the native range of *T. sinensis* is phenologically well synchronized with its specific host ACGW. It is also univoltine and may undergo prolonged diapause, and females emerge from overwintered galls of ACGW in spring, right in the time of gall formation (Moriya et al. 1990). It quickly establishes populations, reaches high parasitism rates and has good dispersal abilities, so it effectively controls the ACGW a few years after the release (Matošević et al. 2015, 2017; Avtzis et al. 2019; Ferracini et al. 2019).

Recent studies demonstrated that the introduction of parasitoid *T. sinensis*, along with the absence of oak, caused the loss of about 14% of native parasitoid species on Asian chestnut gall wasp, commonly associated with oak gall wasps, and 32% of their population density (Ferracini et al. 2018). Their results indicate that the introduction of *T. sinensis* affects the richness of native parasitoids on ACGW. The results from Slovenia show that parasitism rates of native parasitoid species were high from 2010 to 2014 compared to the years before and after this period. Rapid decrease in parasitism rates of native parasitoid species corresponded with the onset of *T. sinensis* in 2015 and its massive population growth in later years (Table 3, Fig. 1). In addition, the number of native species was reduced from a maximum 23 in 2014 to 9 in 2017. In Slovenia, the exotic *T. sinensis* reached standardized emergence rates of 3.5 in 2015, 39.9 in 2016, and over 140 in 2017 (Table 3). It may be hypothesized that *T. sinensis* has already spread before first releases in Slovenia in 2015 (Jurc et al. 2017; Matošević et al. 2017). *T. sinensis* had also the highest emergence rate in 2015 in Croatia (Table 4), which corresponds with its rapid spread from Italy to Slovenia (Matošević et al. 2017). No specimens of *T. sinensis* were found in samples from Hungary, which may be due to no established populations prior to 2015. The first release of this biocontrol agent were carried out in 2014 and 2015, and a slower natural spread due to geographical distance from Italy (Matošević et al. 2017).

The presence of abundant populations of *T. sinensis* emerging from overwintered galls renders the fresh ACGW galls effectively unavailable for native parasitoids, thus impairing their opportunity to grow.

The female-biased sex ratio (Tables 1 and 6) is in agreement that parasitoids with female-biased sex ratios are more valuable for biological control, as only females are directly responsible for killing pests by oviposition, so the parasitoid population growth primarily depends on the number of females (Hall 1993; Ode and Heinz 2002).

Only one species, *Ormyrus pomaceus*, demonstrated significant male-biased sex ratios (75% of all specimens were males). This differs from equal ratios reported for Croatia in 2011 (Matošević and Melika 2013) altough in that study the researchers collected only four specimens of these parasitoids. The reasons for male-biased ratios of *O. pomaceus* found in this research remain unanswered, and it would be useful to investigate the reproductive traits of this parasitoid.

Considering the impact of the Asian chestnut gall wasp on the stability of forest ecosystems in Europe in light of the potential direct and indirect consequences of invasions (Ives and Carpenter 2007), the influence of well-synchronized and effective parasitoid *T. sinensis* should be considered for the maintenance of ecological balance which are directly and/or indirectly affected by ACGW.

In a long term perspective, and considering the dynamics of native parasitoids' cycles, native parasitoids could be effective as integrative agents to *T. sinensis* which emerges during a single period (Heimpel and Mills 2017).

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