RESEARCH ARTICLE

Torymus sinensis Kamijo, a biocontrol agent against the invasive chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu in Spain: its natural dispersal from France and first data on establishment after experimental releases

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Abstract

Aim of study: The globally invasive gall wasp, *Dryocosmus kuriphilus* Yasumatsu, 1951 (Cynipidae: Cynipini), reached Spain seven years ago and is already regarded as an important pest of chestnuts (*Castanea* spp.) in this country as well as worldwide. In this paper, we present comprehensive data on the establishment in Spain of *Torymus sinensis* Kamijo, 1982 (Chalcidoidea: Torymidae), an effective non-native natural enemy of this pest, as a result of both natural dispersal and settlement after controlled releases since 2015.

Area of study: Sites of the Spanish autonomous communities of Galicia, Asturias, Basque Country, Catalonia, Andalusia and Madrid where *D. kuriphilus* is present.

Material and methods: To study the natural dispersal of *T. sinensis* from France, we selected two sampling sites in Catalonia, six in the Basque Country and two in Navarra known for their heavy Asian chestnut gall wasp (ACGW) infestation; to study *T. sinensis* establishment after authorized controlled releases by the concerned authorities, the field samplings were done mainly in Galicia (35 sites) and Andalusia (8 sites). Additionally an experimental release study was made in Madrid.

Main Results: Our results showed that *T. sinensis* has spread throughout Spain by natural dispersal across the French border and now occurs in Catalonia (two sites), the Basque Country (three sites) and Navarra (one site) but not in the neighbouring region of Cantabria. The percentage of parasitism by *T. sinensis* on *D. kuriphilus* is higher in the Basque Country sites, which are close to the French border, thus indicating that its establishment in these localities is not recent. After controlled releases, *T. sinensis* has been successfully established in five release sites in Andalusia (Valle del Genal and Sierra Blanca, Málaga Province) and one release site in Madrid. However, in the region of Galicia (NW Spain), where the number of authorized releases has been higher, the establishment of *T. sinensis* still appears to be very low.

Research highlights: Established populations of *T. sinensis* may exert a positive buffer against *D. kuriphilus*-driven chestnut infestation in Spain, similar to what is observed in other invaded European countries.

Additional keywords: Controlled releases, Torymidae, invasive species, Cynipidae, natural spread, biological control.

Abbreviations used: Asian chestnut gall wasp (ACGW); *Torymus sinensis* parasitism rate (TsPR); mean of ACGW larval chambers per gall (MACGW).

Introduction

The likely polyphyletic genus *Dryocosmus* Giraud (Hymenoptera: Cynipidae) includes 47 species of Cynipini, commonly referred as 'oak gall wasps', distributed across the Holarctic and Oriental regions (Ács *et al.*, 2010; Melika *et al.*, 2011; Cerasa *et al.*, 2018). Two of these cynipid species induce galls on *Castanea* spp.; one is the well-known Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu, 1951, a species native from China that currently constitutes a severe pest of chestnut trees (*Castanea* spp.) worldwide (EFSA, 2010), while the other is a recently described species, *Dryocosmus zhuili* Liu & Zhu, 2015, also from China (Zhu *et al.*, 2015).

ACGW is one of the few oak gall wasps that are harmful to their *Fagaceae* host plants and thus have economic importance (Kinsey, 1935; Bailey & Stange, 1966; Scutareanu & Roques, 1993; Baldassari & Baronio, 1996; Nieves-Aldrey, 2001). The peculiar life cycle of ACGW (univoltine, with only parthenogenetic females known, whose small first instar larvae are present in overwintering chestnut buds and are thus visually hardly detectable) has undoubtedly contributed to the rapid expansion and settlement of this exotic invasive species, helped by the human-assisted dispersal of infested chestnut from nurseries (Hulme, 2009). The negative effects of ACGW on chestnuts include a reduction in the number of fruits, malformation of the branches and general weakening of the tree (Battisti *et al.*, 2014; Gehring *et al.*, 2018; Avtzis *et al.*, 2019), with consequent chestnut production losses and considerable economic damage to the chestnut tree sector (Brussino *et al.*, 2002; Zhang, 2009; EFSA, 2010).

After the first report of its presence as a pest in 1941 in Japan (Murakami *et al.*, 1980), ACGW became established in several countries, from Korea and United States to Nepal and Canada (Cho & Lee, 1963; Payne *et al.*, 1975; Abe *et al.*, 2007; Huber & Read, 2012). The species arrived in Europe in 2002 (Brussino *et al.*, 2002) and since then spread rapidly throughout neighbouring countries, from Slovenia in 2005 to the Czech Republic in 2012 (Borowiec *et al.*, 2014) and Romania in 2015 (Rádócz *et al.*, 2016). The first published reports of the establishment of ACGW in the Iberian Peninsula (IP) were from Catalonia (northeastern Spain) (DOGC, 2012; Pujade-Villar *et al.*, 2013), although an earlier detection in 2010 is mentioned in Borowiec *et al.* (2014) (as a personal communication from J. H. Delader), and data on heavy chestnut attacks in the Montseny Natural Park (Barcelona and Girona) in 2011 were reported by Cristina Castro Torres (in Rubio, 2014).

Since this first Spanish introduction, ACGW has spread throughout the Cantabrian coast (north of the IP) and was reported from the Basque Country and Cantabria in 2013 and from Asturias in 2014. In May 2014, the species was detected in Galicia (northwestern Spain) and northwestern Portugal (Pérez-Otero & Mansilla, 2014). The species was also recorded at the same time (2014) in the Andalusian provinces of Málaga and Granada (southern Spain) Wong *et al.*, 2015, while important chestnut areas in the central and western IP now seem to be unaffected (as of May 2018) (Gil-Tapetado *et al.*, 2018).

In territories where ACGW has been introduced and represents an important forestry pest, the wasps' protection inside the galls, in addition to potential environmental impacts, makes chemical control ineffective, and efficient natural enemies at the first stages of invasion are lacking. Thus, classical biological control with a parasitoid of *D. kuriphilus* in its native geographic area has been widely used in many invaded countries. This biological control agent is *Torymus sinensis* Kamijo, 1982 (Hymenoptera: Chalcidoidea: Torymidae), a specialist parasitoid with a univoltine life cycle that is synchronised with that of ACGW, which is native to East Asia (Murakami *et al.*, 1980, 2001; Moriya *et al.*, 1989). It should be noted however, that in some invader areas a prolonged life-cycle diapause of *T. sinensis* has been reported as well as the species has shown to be able to adapt to the attack of native cynipid species associated to *Quercus* (*Fagaceae*) (Ferracini *et al.*, 2015a, 2017). Biological control assays with this species were first set up in Japan (Moriya *et al.*, 1989, 2003) and continued in the United States (Cooper & Rieske, 2007) and Europe, where it has been introduced in Italy (Quacchia *et al.*, 2008; Gibbs *et al.*, 2011), France (Borowiec *et al.*, 2014, 2018) and Croatia (Matošević *et al.*, 2014), among other countries (Avtzis *et al.*, 2019). The results of the introduction and effectiveness of ACGW biocontrol were mostly positive (Moriya *et al.*, 2003; Quacchia *et al.*, 2008; Matošević *et al.*, 2014, 2017; Paparella *et al.*, 2016), so that the demand to introduce *T. sinensis* into new areas is growing among the affected chestnut communities (orchards, timber production and natural forests management).

However, the introduction of *T. sinensis* into non-native areas may also represent a possible threat to the native fauna (Gibbs *et al.*, 2011; Ferracini *et al.*, 2018), and studies on balancing the benefits and risks have been carried out (e.g., in Switzerland: Aebi *et al.*, 2011). Accordingly, the two main threats

associated with *T. sinensis* introduction are attacks on non-target cynipid species (Italy: Quacchia *et al.*, 2008; Ferracini *et al.*, 2015b, 2017) and the risk of hybridization and ecological competition with native torymid species (Japan: Moriya *et al.*, 1989, 2003; Yara *et al.*, 2007; Italia: Ferracini *et al.*, 2018; Pogolotti *et al.*, 2018). These two risks are currently being evaluated also in Spain by authors of this paper and will be published elsewhere.

After the introduction and spread of *D. kuriphilus* into the main chestnut production areas of Spain, concerned authorities in the regions (autonomous communities) of Galicia and Andalusia began controlled release assays of *T. sinensis*, authorised by the Spanish Ministry of Agriculture and Fisheries and Food (MAPA). Releases of *T. sinensis* in the affected areas of the Province of Málaga were authorized in 2015, 2016 and 2017, and in the same years, releases were undertaken in large areas of the Autonomous Community of Galicia (Pérez-Otero *et al.*, 2017) and also in Asturias (north of the IP). However, the presence of *T. sinensis* in France since 2011 posed the possibility of natural dispersal of the species into Spain across the French border. The dispersal ability of *T. sinensis* has been discussed by Colombari & Battisti (2016) for the case of Italy suggesting a dispersal rate up to 70 km per year. In the case of Japan, the first country in which *T. sinensis* was introduced as an ACGW control, the estimation of dispersal rates was of less than 1 km/year during the first few years, followed for more rapid and gradual spread in the next years (equal to 12 km) and finally reaching a constant rate of ca 60 km per year (Moriya *et al.*, 1989, 2003). Among the trans-boundary dispersal events of *T. sinensis* that have been published, we can mention the passage of the species from Italy to Switzerland (Aebi *et al.*, 2011), from Italy or Switzerland to France (Borowiec *et al.*, 2014), and from Croatia to Bosnia (Matošević *et al.*, 2014). In the case of Spain, we recently found *T. sinensis* in Catalonia (NE of the IP) (unpublished data) and it has also been independently detected by other authors (Jara-Chiquito & Pujade-Villar, 2018). Given that no *T. sinensis* releases were authorised in Catalonia, its presence in the region is more likely due to dispersal from France. It is thus necessary to see if this parasitoid also occurs in Navarra and the Basque Country (both next to France), where no releases of *T. sinensis* have occurred.

The aim of this paper is twofold: (i) first, we report the dispersal of *T. sinensis* from France into Spain throughout the Basque Country and Navarra and confirm the same path across the eastern border into Catalonia; (ii) second, we provide the first data on the establishment of *T. sinensis* in Andalusia, Galicia and central Spain after experimental releases.

Materials & Methods

Study sites

Natural dispersion

Sampling and field work were conducted within the framework of three scientific projects focused on ACGW in Spain. The main project, developed at the level of the entire state, had the twofold aim of studying the recruitment of native parasitoids and assessing the environmental risks associated with the introduction of *T. sinensis*. At the same time, similar projects were conducted in the autonomous communities of Andalusia and Galicia.

The chestnut forests of the northern IP form a "continuum" from SW France along the Cantabrian Coast to Galicia. To study the natural dispersal of *T. sinensis* from France, we selected two sampling sites in Catalonia, six in the Basque Country and two in Navarra known for their heavy ACGW infestation, at the east and west corners, respectively, of the border with France, where the Pyrenees are not an effective barrier and both form biological corridors (Table 1, Fig. 1). These areas are isolated and very distant from the Spanish territories where the *T. sinensis* 2015-2017 releases were performed. An additional locality was also sampled in the community of Cantabria, close to the Basque Country, but further away (approximately 200 km) from the French border and at about the same distance from Asturias, where controlled releases were carried out in 2017 (Table 1, Fig. 1).

Establishment after authorized controlled releases

To study *T. sinensis* establishment after controlled releases by the concerned authorities, the field samplings were done mainly in Galicia (NW of the IP) and Andalusia, the Spanish regions were the first experimental releases of *T. sinensis* were authorized and performed. In Andalusia, 8 sites were repeatedly sampled from 2016 to 2018 while in Galicia we sampled 35 sites in 2018. In addition, 2 sites of the Autonomous Community of Asturias were also sampled for this purpose (Table 1).

Experimental release in Madrid

An experimental release of *T. sinensis* was performed in 2017 on infested chestnuts of the Royal Botanical Garden Alfonso XIII of the Universidad Complutense de Madrid (Madrid, Central Spain).

Table 1. Summary of collection sites, numbers of reared *Torymus sinensis* and estimated parasitism rates. AC = autonomous community, Lat = latitude, Long = longitude, ACGW = *Dryocosmus kuriphilus*, Ts = *Torymus sinensis*, TsPR = parasitism rate by *T. sinensis* A dashed line separates the data corresponding to natural dispersal from France from those regarding establishment after releases and experimental infections. The value of 5.2 is an estimated mean of number chambers/gall and not a direct calculation by dissection.

Locality	Province	AC	Lat.	Long.	Collection Date	Emergence Date
Bera de Bidasoa	Navarra	CF Navarra	43.2833	-1.7029	23/02/2018	03/18
Santisteban	Navarra	CF Navarra	43.1435	-1.6671	23/02/2018	-
Lezo	Guipúzcoa	Basque C.	43.3326	-1.9011	24/02/2018	03/18
Hondarribia	Guipúzcoa	Basque C.	43.3678	-1.8187	24/02/2018	03/18
Jaizkíbel	Guipúzcoa	Basque C.	43.3554	-1.8245	24/02/2018	03/18
Urdúliz	Vizcaya	Basque C.	43.3523	-2.9485	24/02/2018	-
Berango	Vizcaya	Basque C.	43.3558	-2.9812	24/02/2018	-
Orozco	Vizcaya	Basque C.	43.0762	-2.9235	24/02/2018	-
Sant Marçal	Barcelona	Catalonia	41.8029	2.4239	30/07/2017	02/18
Sant Hilari	Girona	Catalonia	41.8838	2.4969	31/07/2017	02/18
Botánico UCM	Madrid	Madrid	40.4471	-3.7270	09/02/2018	02/18
Juanar	Málaga	Andalusia	36.5779	-4.8842	29/09/2017	01/18
Juanar	Málaga	Andalusia	36.5779	-4.8842	14/12/2017	01/18
Juanar (2)	Málaga	Andalusia	36.5782	-4.8843	01/02/2017	02-03/17
Juanar (2)	Málaga	Andalusia	36.5782	-4.8843	09-12/2017	01-03/18
Juanar (2)	Málaga	Andalusia	36.5782	-4,8843	01-03/2018	01-04/18
Igualeja	Málaga	Andalusia	36.6292	-5.1111	15/12/2017	02/18
Igualeja (La Sala)	Málaga	Andalusia	36.6194	-5.1348	03/11/2016	01/17
Igualeja (La Sala)	Málaga	Andalusia	36.6194	-5.1348	12/03/2018	03/18
Júzcar (Cerro C)	Málaga	Andalusia	36.6303	-5,1674	06/10/2016	02/17
Júzcar (Cerro C)	Málaga	Andalusia	36.6303	-5,1674	04/12/2017	01/18
Júzcar (Cerro C)	Málaga	Andalusia	36.6303	-5.1674	02-03/2018	02-03/2018
Júzcar	Málaga	Andalusia	36.6182	-5.1442	28/09/2017	02/18
Yunquera	Málaga	Andalusia	36.7213	-4.9538	11-12/2017	01/2018
Yunquera	Málaga	Andalusia	36.7213	-4.9538	13/02/2018	02/2018
Puerto Ojén	Málaga	Andalusia	36.5864	-4.8319	14/12/2017	01/18
Premoño	Asturias	Asturias	43.3949	-5.9961	31/03/2018	-
Las Ablanosas	Asturias	Asturias	43.4237	-6.0221	31/03/2018	-
S. Roque de R.	Cantabria	Cantabria	43.2359	-3.7035	25/02/2018	-
Santo Estevo	Ourense	Galicia	42.4173	-7.6855	21/02/2018	-
Vilouxe	Ourense	Galicia	42,3790	-7.6240	21/02/2018	-
+33 sites	Galicia	Galicia	-	-	01/04/2018	-
TOTALS						

Introduced *T. sinensis* were provided by "Agrobio enterprise" that imported the specimens from Italy, the same commercial source that supplied the individuals released in Andalusia. On 16th May, 120 *T. sinensis* (48 males and 72 females), females mated about 10 days old, were released on four branches infested with ACGW galls isolated with gauze sleeves. In February, the isolated galls were collected, moved to the laboratory and kept under indoor conditions until the emergence of *T. sinensis*.

Field samplings: collecting galls and rearing of parasitoids

With the aim of rearing *T. sinensis* (as well as native parasitoids with a similar life-cycle), whose emergence

Table 1. Continued.

| Locality	Number of ACGW galls	Number chambers/gall	Number of Ts ♀	Number of Ts ♂	Total n of Ts	TsPR
Bera de Bidasoa	464	5.2	17	7	24	0.99%
Santisteban	112	-	-	-	-	-
Lezo	256	5.2	43	22	65	4.88%
Hondarribia	244	5.2	10	5	15	1.11%
Jaizkíbel	269	5.2	77	39	116	8.29%
Urdúliz	183	-		-	-	-
Berango	147	-	-	-	-	-
Orozco	182	-	-	-	-	-
Sant Marçal	200	5.2	10	-	10	0.96%
Sant Hilari	200	5.2	2	-	2	0.19%
Botánico UCM	130	5.2	13	12	25	3.69%
Juanar	175	8.7	2	5	7	0.45%
Juanar	944	8.7	28	13	41	0.49%
Juanar (2)	1,583	4.8	7	1	8	0.10%
Juanar (2)	1,743	4.8	26	30	56	0.66%
Juanar (2)	2,851	4.8	70	48	118	0.86%
Igualeja	548	4.3	3	2	5	0.21%
Igualeja (La Sala)	116	3.7	1	1	2	0.60%
Igualeja (La Sala)	624	3.7	2	-	2	0.09%
Júzcar (Cerro C)	294	5.1	4	7	11	0.73%
Júzcar (Cerro C)	900	5.9	-	6	6	0.11%
Júzcar (Cerro C)	952	5.9	3	-	3	0.05%
Júzcar	175	8.1	4	1	5	0.35%
Yunquera	756	3.6	2	5	7	0.25%
Yunquera	278	3.6	1	-	1	0.09%
Puerto Ojén	279	7.7	1	2	3	0.13%
Premoño	300	-	-	-	-	-
Las Ablanosas	300	-	-	-	-	-
S. Roque de R.	800	-	-	-	-	-
Santo Estevo	75	-	-	-	-	-
Vilouxe	86	-	-	-	-	-
+33 sites	4,871	4.8	-	1	1	0
TOTALS	21,037		326	207	533	

occurs from dry galls after winter, ACGW galls were preferentially collected during the winter and then transferred to indoor laboratory conditions until emergence of the insects. This usually occurred one or two months before the natural outdoor emergence because of the forced conditions of the laboratory. Collected galls were located into cardboard emergence boxes equipped with light extractors and kept in normal indoor laboratory conditions.

For an accurate estimation of the real emergence date of *T. sinensis* in the field, an additional experiment was performed in Juanar (Málaga, Andalusia). Three infested chestnuts were selected, and the galled branches bagged. A total of 30 bags were placed (10 per tree), encaging an average of 8, 6 and 7 galls per bag. The emergence of *T. sinensis* was checked weekly.

Taxonomic identification

A careful morphological examination of the reared specimens attributed to *T. sinensis* is essential to prevent any possible misidentification with other native torymid parasitoid species. Confusion is particularly possible with three species that are
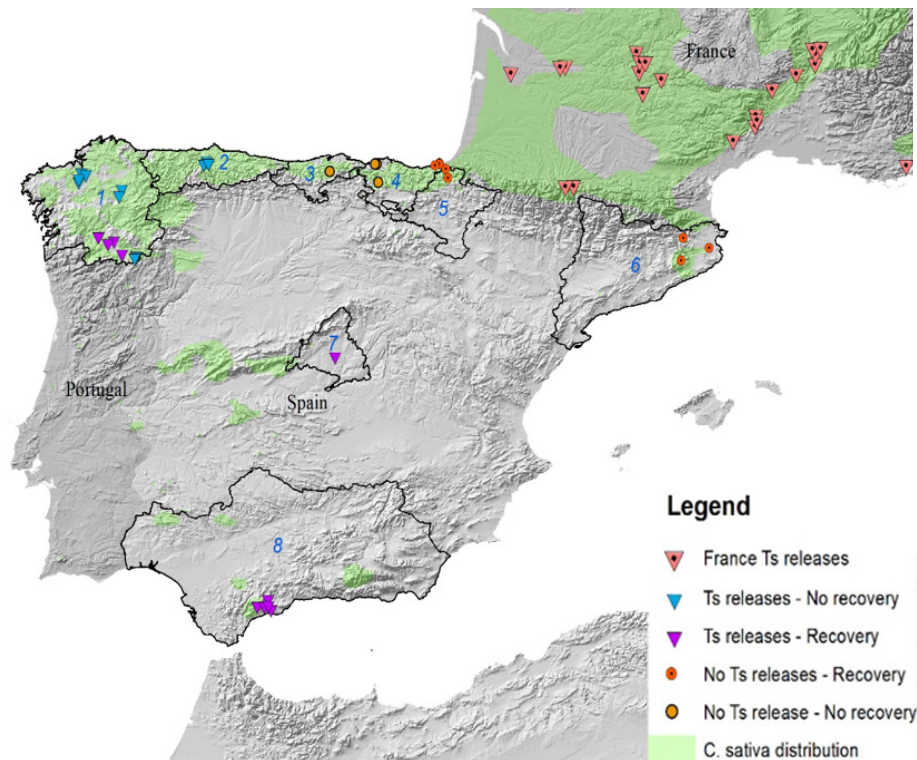
Figure 1. Map of the study area indicating the distribution of *Castanea sativa* in the Iberian Peninsula and southern France. The sites where *T. sinensis* was either released or not and where it was either recovered or not are shown. Autonomous communities: 1, Galicia; 2, Asturias; 3, Cantabria; 4, Basque Country; 5; CF Navarra; 6, Catalonia; 7, Madrid; 8, Andalusia.

morphologically quite similar and taxonomically closely related: *Torymus notatus* (Walker, 1833), *Torymus cyaneus* Walker, 1847 and *Torymus affinis* (Fonscolombe, 1832), and particular attention should be taken with males, which are less easily distinguished. For the identifications, we used the unpublished keys kindly provided by R. R. Askew and C. Thuroczy, as well as additional diagnostic morphological characters (Nieves-Aldrey, unpublished data).

Estimation of parasitism

All the galls collected during the study together with their collection dates, the number of *T. sinensis* individuals recovered per locality, their emergence dates, and parasitism percentages (TsPR) appear in Table 1. To estimate parasitism rate of ACGW by *T. sinensis* we used the following equation

$$\frac{N_{Tsinensis}}{N_{galls} \cdot MACGW} \cdot 100$$

where $N_{Tsinensis}$ is the number of *T. sinensis* individuals per sample; N_{galls} is the number of ACGW galls per sample, and MACGW is the mean of ACGW larval chambers per gall in each locality (see Table 1). This

number was calculated after dissecting at least ten galls for each sampling locality in Andalusia and Galicia. For the samples collected in the Basque Country, Navarra, Madrid and Catalonia, where galls were not dissected, 5.2 chambers per gall was used as an estimated mean number for the remaining Spanish localities (Table 1).

Results

Natural dispersal of T. sinensis from France into Spain

Navarra, Basque Country and Cantabria (northern IP)

From the samples collected in Navarra and the Basque Country, we recovered 147 females and 73 males of *T. sinensis*. The species was present in one of the sampled sites from Navarra (Bera de Bidasoa) and in three of the sampling sites in Guipúzcoa Province (Basque Country): Hondarribia, Jaizkíbel and Lezo. Compared with the relatively low figures of parasitism to date, from the Spanish sites where were *T. sinensis* was artificially released, the parasitism percentages in

the Basque Country and Navarra were variable but were generally moderate to high (from 0.99% to 8.29%), the relatively high rates at Lezo (4.88%) and especially at Jaizkíbel (nearly 8.5%) being remarkable. These data unequivocally show the successful establishment of *T. sinensis* in these two autonomous communities, likely as a result of its natural dispersal from France. All the localities with *T. sinensis* are very close to the border with France, Lezo being the farthest away (approximately 14 km). In contrast, in the samples collected in three sites in Vizcaya Province (Basque Country) (Orozco, Berango and Urdúliz), which are substantially farther from France (approximately 130 km), *T. sinensis* was not present. In addition, samples of ACGW galls collected in S. Roque de Riomiera, in the neighbouring Autonomous Community of Cantabria (225 km from France), did not yield any *T. sinensis* individuals.

Catalonia (northeast IP)

Twelve *T. sinensis* females were reared from ACGW galls collected during the summer in two sites, Sant Marçal and S. Hilari Sacalm, in the Natural Park of Montseny (Barcelona and Girona, Catalonia). The calculated parasitism rate was 0.96% at the first site and 0.19% at the second (Table 1). The specimens successfully emerged in the laboratory from fresh galls collected eight months before (see Table 1), despite the galls being collected in summer and not subsequently enduring a 'cold' period for larval diapause before pupation.

Establishment of T. sinensis after releases

Andalusia (southern IP)

A total of 275 individuals of *T. sinensis* (154 females and 121 males) were recovered from 12,218 galls collected in eight sampling sites of Málaga Province, where experimental releases of *T. sinensis* were made in 2015-2017 (Table 1; Fig. 1). The settlement of *T. sinensis* was especially successful in Juanar, where 230 individuals were recovered, with an estimated mean parasitism rate of 0.51% (range 0.10-0.86, n = 5), despite the fact that experimental releases were made only in 2015 at this site, with 720 individuals (480 females and 240 males) being released. In addition, 34 *T. sinensis* individuals were recovered from another two sites, Igualeja and Júzcar, in the Valle del Genal, while we also recovered 3 specimens from Puerto de Ojén, which is not too close to Juanar, both being in the Sierra Blanca, and 8 from Yunquera in the Sierra de las Nieves.

To have available accurate data on the phenology of *T. sinensis* in the areas where it has been introduced is important to determine the best release date for a successful biological control programme with this species. Accordingly, in the experiment performed in 2018 at the site of Juanar, the emergence of *T. sinensis* under field conditions began in mid-March and ended in mid-April, with an emergence peak in late March (Fig. 2). Therefore, this date may be the most appropriate for releasing *T. sinensis* in Andalusia region.

Galicia (northweast IP)

Given the extent and economic importance of the chestnut in Galicia, authorized controlled releases of *T. sinensis* have been important in this region. Up to 220,395 individuals were released in three campaigns (2015-2017) according to Pérez-Otero *et al.* (2017), and even higher numbers were released in the 2018 campaign (704,270 individuals from data of the Xunta de Galicia). However, only 12 individuals have been recovered according to published data, all from release sites in Ourense Province (Pérez-Otero *et al.*, 2017). Our own data even showed null settlement values at different localities. Indeed, from 4,871 galls collected in the winter of 2017 and early spring of 2018 from 33 sites in Galicia, only one male *T. sinensis* was recovered at one site (Riós, Ourense) where controlled release began in 2017. In addition, the species was not recovered from galls collected in winter (2017) from several sites of the Ribeira Sacra region (Central Galicia).

Madrid (central IP)

The experimental release of 120 individual *T. sinensis* on ACGW galls in Madrid returned 25 emerged *T. sinensis* individuals (13 females and 12 males). The success of the experimental infection was high, with a resulting parasitism rate of 3.69%.

Discussion

Natural spread from France

In this work, we report the presence of *T. sinensis* in northern Spain in three autonomous communities at the eastern and western ends of the border with France: the Basque Country, Navarra and Catalonia. Authorized releases of this biological control agent have been made in recent years in Galicia, Asturias and Andalusia, and thus in geographically distant regions from those mentioned above, but no releases have been made to date in communities close to the border with France. Discounting the possibility that the presence of *T. sinensis* in these northern autonomous communities is a result of many illegal or uncontrolled
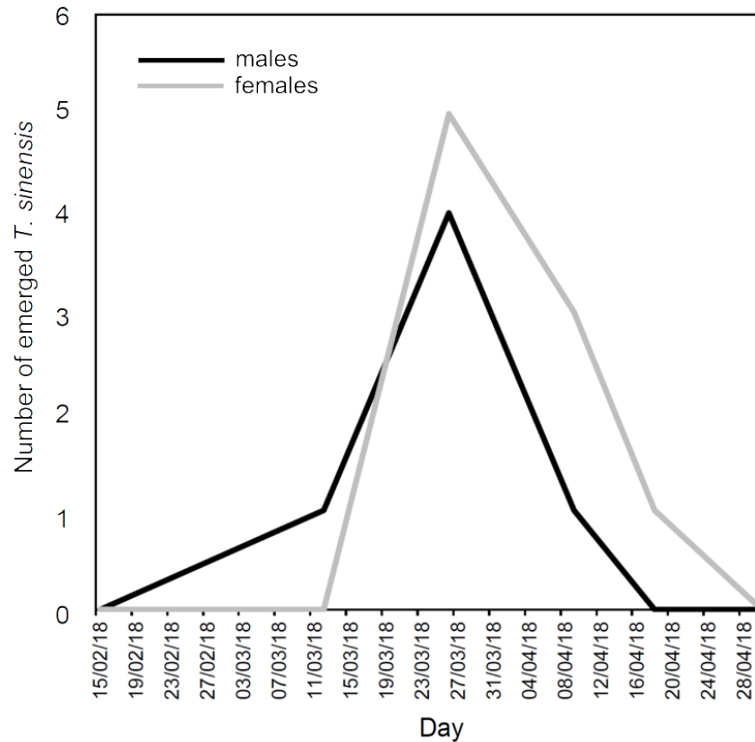
Figure 2. Phenology of *T. sinensis* in the site of Juanar (Málaga) under natural field conditions.

releases, the most likely explanation is that *T. sinensis* has naturally dispersed to these territories from France across the southern border with Spain. Published data of *T. sinensis* releases in France (Borowiec *et al.*, 2014, 2018) show that the closest release sites to Spain are Gironde, Lot, and Hautes-Pyrénées, 172-237 km away from the northern Spanish sampling sites. Taking into account that *T. sinensis* was released in France in 2013 and that the estimated dispersal capacity of this parasitoid is approximately 70 km per year (Colombari & Battisti, 2016), we suggest that *T. sinensis* may have reached the ACGW-infested Spanish areas in 2016. Fast natural dispersal of *T. sinensis* was observed also in Hungary (Melika *et al.*, 2017).

The hypothesis of an early arrival of *T. sinensis* in northern Spain by natural dispersal from France is reinforced by the relatively high parasitism rates shown in Lezo and Jaizkíbel (4.8% and 8.2%, respectively). It is largely accepted that *T. sinensis* needs 5-7 years after the first release and or settlement to reach such high rates of parasitism (Quacchia *et al.*, 2008; Borowiec *et al.*, 2014; Matošević *et al.*, 2014, 2017; Ferracini *et al.*, 2018). However, despite this early arrival in the Basque Country and Navarra, our data indicate that the progression into Spain throughout the Cantabrian coast was slow, given the apparent absence of *T. sinensis* in the sampled sites of Vizcaya Province and the neighbouring community of Cantabria. The spread of *T. sinensis* from France towards Spain takes

advantage of the existence of two broad ecological corridors connecting both sides of the eastern and western Pyrenees. As a comparison between the two zones, the dispersal of *T. sinensis* across the western corridor, from France across the Basque Country and Navarra, would be easier than along the eastern corridor connecting France and Catalonia, since the first route has a lower altitude and a milder climate.

With regard to Catalonia, our data confirm the recent publication of the presence of *T. sinensis* in this autonomous region (Jara-Chiquito & Pujade-Villar, 2018). These authors reported a total of 45 individuals (15 males and 30 females) from three localities in Girona Province, close to the sites sampled by us. However, they do not provide data on the parasitism levels.

Establishment after controlled releases

The release programmes with *T. sinensis* carried out from 2015 to 2017 in two large areas of Spain, the whole Galicia region and areas of the Andalusian Province of Málaga yielded different results about its settlement. Whereas in Andalusia, the first successful results came after three years of experimental releases (275 *T. sinensis* were recovered out of 41,420 released, present results), in Galicia (where releases were even more numerous), only 12 out of 220,395 released individuals were recovered in post-release controls

(Pérez-Otero *et al.*, 2017), and only one in our study. Up to now, all available data have shown a low rate of *T. sinensis* implantation in this autonomous community. It is expected however, given the massive releases of *T. sinensis* made by the Xunta of Galicia in 2018 that the situation could significantly change after update the recovery data of 2019. We had not still access to the unpublished data of recoveries from the Xunta of Galicia, but our own data have started to show emergences of *T. sinensis* from galls collected in additional samplings in 2018-2019 (3 females and 2 males recovered to date (February 2019) from galls collected at the site of Merouzo pequeno (Ourense)).

The settlement seems to be particularly successful in Juanar, the first site in Málaga Province where *T. sinensis* was experimentally released in 2015. Despite the lack of further releases in the area since then, more than 200 individuals were recovered from test-release controls in 2018, with an estimated parasitism rate of 0.51%, thus suggesting successful establishment. It is important emphasize however, that the release area in Galicia is much wider than the release area in Andalusia, and the relative sampling effort was much lower in the first one, which may be one of the reasons of the low establishment success in that region.

It is interesting to compare the parasitism rate recorded from the different sampling sites. Values were null or low (≈1%) at the release points (Table 1), while they were high at sites in the Basque Country (8.2%), i.e., where *T. sinensis* populations occur because of natural spread from France. The reasons behind this difference is difficult to explain using collected data. It seems not probable that settlement success depends on the time from the releases, since these were done starting back in 2015, possibly even before the arrival of the parasitoid in the northern regions by natural dispersal from France. On the other hand, we cannot exclude an effect of some unknown environmental and climatic conditions (which are extremely variable across the IP, and particularly between the northern and southern coastal areas) on the settlement success (Gil-Tapetado *et al.*, 2018). Additional studies are necessary to understand whether the release of *T. sinensis* is more likely to fail under certain conditions and thus possibly not be useful against ACGW.

The data from the experimental release in Madrid showed successful establishment of the insect in the field, on ACGW galls, which is a promising starting point towards future controlled releases in this and nearby regions where *C. sativa* is present but infection with ACGW have been not detected yet.

We predict that *T. sinensis* will continue its spread over the northern part of the IP, following *C. sativa* forests infested by ACGW, possibly reaching in a few years many more areas than where it actually occurs today.

Established populations of *T. sinensis* may exert a positive buffer against *D. kuriphilus*-driven chestnut infestation in Spain, similar to what is observed in other invaded European countries.

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