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SHORT COMMUNICATION

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Newly invaded territories by Dryocosmus kuriphilus in Spain and first records of *Torymus sinensis* in the Sistema Central

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Abstract

Aim of study: To update the distribution of *Dryocosmus kuriphilus,* the Asian chestnut gall wasp, focusing on the central area of the country (Sistema Central). Gall samplings of these areas were carried to obtain the first records of parasitoids on *D. kuriphilus* in this area.

Area of study: Spain and, especially, the Sistema Central.

Material and methods: Georeferenced new records were used to produce a map with the updated distribution of *D. kuriphilus* as of 2019. Galls were collected and stored in emergence boxes. Parasitoids that emerge from these galls were collected and identified at the most detailed taxonomic level.

Main results: The alien species *D. kuriphilus* was found in the Spanish Sistema Central. Infestation focal points were detected in the Valle del Jerte (South of Sierra de Gredos), Sierra de Francia and in the mountains southwest of Madrid. In all detected focal points of infestation, the available evidence indicates that introduction was caused by the accidental mobilization of infested chestnut material. Alongside other parasitoids recruited by *D. kuriphilus,* we found individuals of *Torymus sinensis,* constituting the first records of this foreign species in the Sistema Central.

Research highlights: We updated the previously published distribution of *D. kuriphilus* in Spain, an alien species and pest of chestnut forests and orchards. We also reported the first list of recruited parasitoids by *D. kuriphilus* and, more specifically, the first records of *T. sinensis* in the Sistema Central.

Keywords: Asian chestnut gall wasp; *Torymus sinensis;* parasitoids; galls; Iberian Peninsula; pest species; alien species.

Authors' contributions: Conceived and designed the work: DGT, JLNA. Performed the experiments and analyzed the data: DGT, MPRR, JLNA. Contributed materials/data: DGT, MPRR, AV, JLNA. Wrote the paper: DGT, JLNA.

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Introduction

The Asian chestnut gall wasp, *Dryocosmus kuriphilus Yasumatsu,* 1951 (Hymenoptera: Cynipidae) is an alien species from China and a pest of different *Castanea* spp. in Japan, Korea, North America and Europe (Oho & Umeya, 1975; Payne *et al.,* 1976; Brussino *et al.,* 2002; Gehring *et al.,* 2019). This gall-maker and parthenogenetic species is distributed in most of the *Castanea sativa* Mill. forests and timber and chestnut production stands of Europe (EFSA, 2010), including the Iberian Peninsula (IP) (Gil-Tapetado *et al.,* 2018). Although *D. kuriphilus* was introduced in Europe (specifically in Italy) in 2002 (Brussino *et al.,* 2002), this species arrived into the IP in 2012 in Catalonia, in the northeast of the IP, next to the French border of the Pyrenees (Pujade-Villar *et al.,* 2013). In 2014, this species was found in Galicia (northwest IP) (Pérez-Otero & Mansilla, 2014) and Andalusia (specifically in Málaga, southern IP). Considering the natural dispersal distance of *D. kuriphilus* has been established to be an average of 6.6 km/year (Gilioli *et*

al., 2013), it is very unlikely that this species reached these remote territories without continuity among chestnut trees in only two years. This fact might be explained by the main cause of dispersion of this alien species: the movement of plant material, such as seedlings or grafts, from infested chestnuts (Quacchia *et al.,* 2008; Gilioli *et al.,* 2013). In Portugal, *D. kuriphilus* was also introduced in 2014 in the north-western territory (EPPO, 2014) but was separated ~35 km from the nearest border with Spain and was probably an independent focal point.

In Spain, *D. kuriphilus* was detected in many other territories since 2012 (Gil-Tapetado *et al.,* 2018). This previous article shows a total of 14 *D. kuriphilus* focal points throughout the Spanish territory and were disjointly distributed, with all of them except the initial one in Catalonia, caused by the dispersal of infested chestnut tree material. These focal points are related to the *D. kuriphilus* of 2018, which were found in all northern and southern mountain enclave areas of Spain, with the absence of this alien cynipid in the centre of the IP, except in the Madrid focal point. This *D. kuriphilus* focal point is located within the city of Madrid in two botanical royal gardens (RJB Alfonso XIII and RJB Madrid). The origin of the infestation of these planted *C. sativa* trees is uncertain because the last chestnut trees that were transported to these royal gardens came from non-infested areas before 2012 (Gil-Tapetado & Nieves-Aldrey, 2018). Omitting this isolated focal point, the mountain system in which chestnut trees are embedded in the central area of Spain, the Sistema Central, did not have any focal point of *D. kuriphilus* presence until 2018. Although it is true that there is a continuity of chestnut trees between Galicia (north-western IP) and the Sistema Central through Portugal (EPPO, 2014), it would take *D. kuriphilus* many years to arrive through its own natural dispersion.

A remarkable fact about the biological invasion of *D. kuriphilus* is the fast process of native chalcid parasitoid (Chalcidoidea: Cynipidae) recruitment that this alien cynipid has (Aebi *et al.,* 2006; Lombardero & Cabaleiro, 2015; Pérez-Otero *et al.,* 2017). Native chalcid parasitoids are the main population controllers of native cynipids; and they seem to be attracted to *D. kuriphilus* galls as hosts (Aebi *et al.,* 2006; Cooper & Rieske, 2007; Matošević & Melika, 2013; Quacchia *et al.,* 2012), making this cynipid-chalcid biological community have greater species richness. In addition, the establishment of these communities is very fast because in the same years in which *D. kuriphilus* is introduced in a territory, the native parasitoids can detect and oviposit in its galls as a host (Lombardero & Cabaleiro, 2015; Pérez-Otero *et al.,* 2017). This fast recruitment is remarkable because of the absence of any other species of cynipid-inducing galls in *C. sativa* in the Western Palearctic. Together, the low regulatory capacity of native fauna of parasitoids on *D. kuriphilus* has led to the need for the releases of *Torymus sinensis* Kamijo, 1982 (Hymenoptera: Torymidae), as a biological control against this pest species (Moriya *et al.,* 2003; Quacchia *et al.,* 2008; Gibbs *et al.,* 2011; Borowiec *et al.,* 2018; Nieves-Aldrey *et al.,* 2019). After experimental releases of *T. sinensis* in large affected areas of Spain, the legal use and release of this biological control has been recently authorised by the Spanish government (November 2019).

In this work, we provide information about the current distribution of *D. kuriphilus* in Spain as of 2019, updating the previous information published in Gil-Tapetado *et al.* (2018), and we report new focal points in the chestnut forest and crops in the mountains of Sistema Central, Spain. Additionally, we provide the first records of chalcid parasitoid fauna associated with this alien cynipid in this area, including the striking presence of *T. sinensis* in one of the study sites.

Materials and methods

Area of study

Through communication with the different Spanish administrations, we were informed of the detection of *D. kuriphilus* in new areas throughout Spain and its expansion for the previous cited focal areas. We have paid a special attention to the Spanish Sistema Central where *D. kuriphilus* and *T. sinensis* were not previously found.

The Sistema Central, located in the centre of the IP, has foothills of 500 to 1200 m a.s.l. and a maximum altitude of 2592 m a.s.l.; these foothills divide the Iberian Plateau. This division forms the North and South Iberian sub-plateaus, two extensive plains that are highly continental. The Sistema Central is a mountain range in the middle of the Iberian territory that preserves cooler and more humid conditions due to its altitude, and it is capable of harbouring flora such as chestnut trees as well as plants linked to humid areas with moderate temperatures. In addition, the Sistema Central is linked to the Portuguese territory of Serra da Estrela, which also has chestnut trees that form a semi-continuous mass of *C. sativa* towards the north-east of the IP (Galicia).

Chestnut trees of the Sistema Central are mainly located in the west of this range, in the southwest of Community of Madrid and Ávila, the north of Toledo (Sierra de Gredos), the north of Cáceres, and the south of Salamanca

(Sierra de Béjar, Sierra de Francia and Sierra de Gata). Additional Spanish chestnut forests were also prospected, as the Sierra de Aracena, in Andalusia (Huelva). All these areas were visited by the authors to confirm the presence of *D. kuriphilus* galls.

Gall sampling and parasitoid identification

Three areas were visited and sampled: Sierra de Francia, Valle del Jerte, and mountains southwest of Madrid. Samples of *D. kuriphilus* galls were collected between the ground and a height of two metres along a half hour transect in each sampled locality. Collected *D. kuriphilus* galls were stored in plastic bags and transported to the Museo Nacional de Ciencias Naturales of Madrid (MNCN). A fraction of collected galls were stored in emergence cardboard boxes, equipped with skylight extractors, under indoor room conditions in the MNCN. The other fraction of galls was dissected and examined, and the adult and immature stages of the individuals within them were identified at the most detailed level possible.

Adult *D. kuriphilus* and parasitoids that were collected were stored in 70% ethanol vials and dry mounted for morphological examination and identification under light microscopy. The larvae collected from gall dissections were stored in 96% ethanol vials for examination under scanning electron microscopy (SEM) with the low vacuum technique as described in Nieves-Aldrey *et al.* (2005) and Gómez *et al.* (2008). Samples of adults and larvae were stored in the Entomological Collection of the MNCN. Chalcid parasitoids were identified by the senior author using some taxonomic key references (Graham & Gisjwist, 1998; Nieves-Aldrey, 1984; Gómez *et al.,* 2008) as well as more recent published and unpublished information (Al-Khatib *et al.,* 2014, Askew and Thúroczy (unpublished); Nieves-Aldrey (unpublished)).

Map building

The map of this short article was elaborated by modifying the map of Gil-Tapetado *et al.* (2018) and including the new *D. kuriphilus* focal points appearing before this published article. These new focal points and the *D. kuriphilus* expansion data come from different official sources: the *Ministerio para la Transición Ecológica* of Spain, *Servicio de Defensa del Medio Natural* of Junta of Castilla y León, *Dirección General de Agricultura, Ganadería y Alimentación* of Community of Madrid, *Cooperative Association Valle del Jerte* and Junta of Andalusia.

The map was elaborated using ArcGIS v 10.3 (ESRI, 2014).

Results and discussion

Distribution update of D. kuriphilus

The distribution of *D. kuriphilus* has been modified in some areas of Spain (Fig. 1), expanding throughout Galicia to cover almost the entire territory of this region (1). The adjoining infested areas to Galicia (1), the El Bierzo (León) and Alta Sanabria (Zamora), have also been extended. The infested area of Serranía de Ronda and Valle del Genal (12), south of the IP, has been expanded. The Catalonian region (5 and 6) has not been modified with the data we have, and the entire chestnut territory is invaded by *D. kuriphilus*. We do not have general data on the rest of the infested areas: Asturias (2), Cantabria-West Basque Country-North Burgos (3) and Navarra-East Basque Country (4). However, in the last surveys in the Navarra territory (4), near the French border, we observed an apparent decrease in the populations of *D. kuriphilus*. This observed *D. kuriphilus* population decrease may be related to the natural dispersion of *T. sinensis* into Navarra from France (Nieves-Aldrey *et al.,* 2019), across the ecological corridor of the west of the Pyrenees, through the mass of chestnuts trees and the *D. kuriphilus* in them. The focal point of Madrid (11) has not changed due to its isolation from chestnut forests.

There were four newly detected focal points of *D. kuriphilus*. All were prospected by different official institutions, a priori discarding the presence of *D. kuriphilus* in previous years, either for not having been detected or for having successfully destroyed the detected infested chestnut material without possibility of propagation. One of these new focal points was detected in the south region in the Tejeda-Almijara (13), between the focal points of Lanjarón (14) and Serranía de Ronda and Valle del Genal (12). This focal point is relatively isolated and separated from the other two nearest focal points, and its infestation by *D. kuriphilus* is likely due to the movement of infested chestnut material from other areas to this one.

The other three new focal points are related to the Sistema Central mountain range: Sierra de Francia (7), Valle del Jerte (8) and southwest of Madrid (10). The appearance of these new areas might be due to 1) the natural dispersal of *D. kuriphilus* from neighbouring focal points or 2) the artificial dispersal caused by the movement of infested material to distant areas with no local presence of this invasive cynipid. *A priori,* the Sistema Central should has a low probability of the arrival of *D. kuriphilus* by natural dispersal due to this area is far away from the other infested areas of the Iberian Peninsula. These focal points are separated up to ~50 km, meaning it is unlikely that they were infested by each other or by the focal point of the city of Madrid through the natural dispersion of

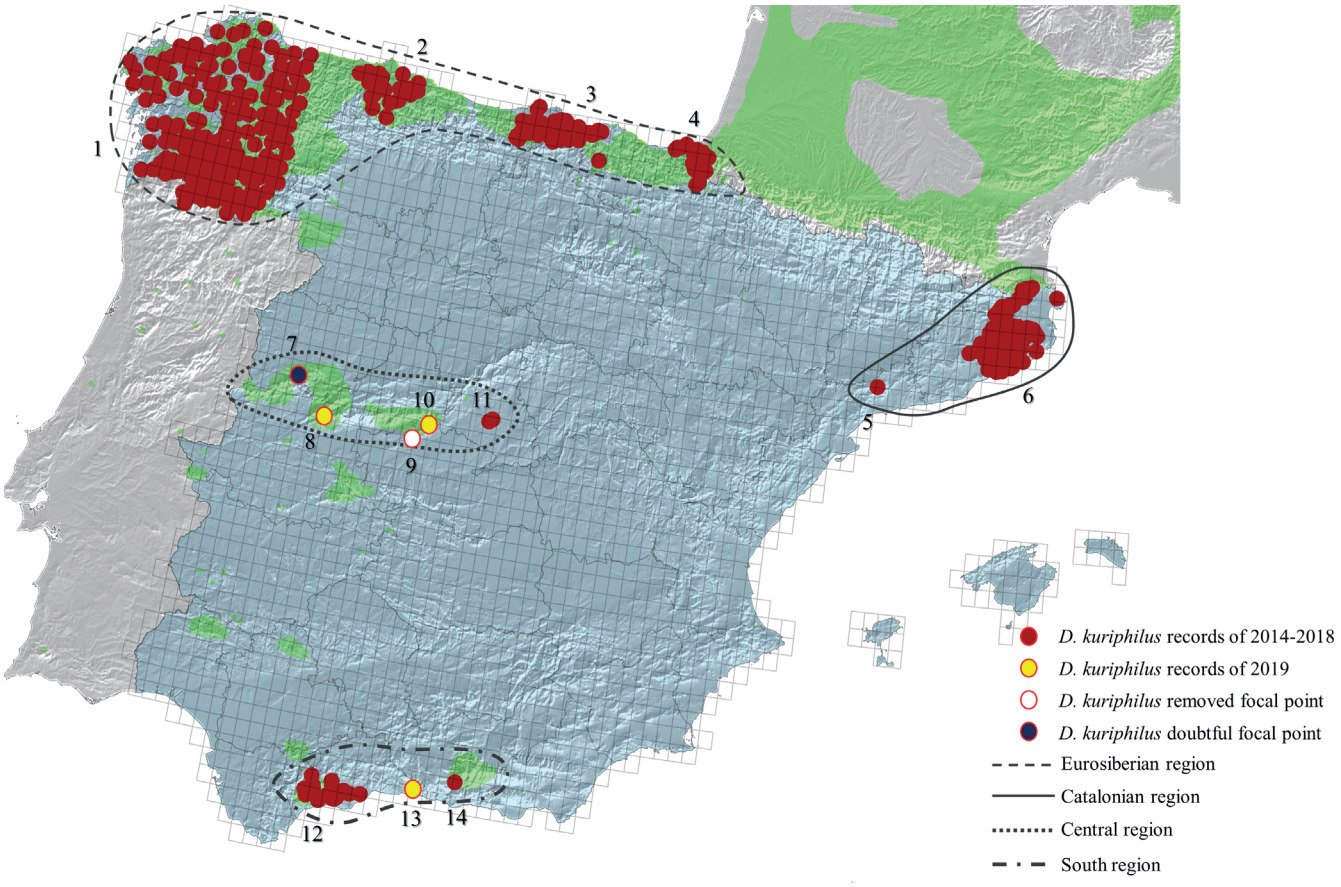

Figure 1. Distribution of *D. kuriphilus* in Spain until 2019. The red dots show an updated distribution of *D. kuriphilus* with respect to the focal points described in Gil-Tapetado et al. (2018). The yellow dots indicate the new *D. kuriphilus* focal points described in this brief communication. Different line types indicate the infested areas by *D. kuriphilus* in Spain. The colour green indicates the presence of *C. sativa* in the Iberian Peninsula and south of France. The names of each updated focal point related to each geographical area are in the Eurosiberian region: 1) Galicia-El Bierzo-Alta Sanabria; 2) Asturias; 3) Cantabria-West Basque Country-North Burgos; and 4) Navarra-East Basque Country. Catalonian region: 5) Prades; 6) Montseny. Central region: 7) Sierra de Francia; 8) Valle del Jerte; 9) Sierra de San Vicente; 10) Southwest of Madrid; 11) Madrid. South región: 12) Serranía de Ronda-Valle del Genal; 13) Sierra de Tejera-Almijara; 14) Lanjarón.

D. kuriphilus. In addition, the nearest focal point to the Portuguese frontier is ~50 km away. Thus, these recently produced *D. kuriphilus* focal points are probably due to the accidental mobilization of infested chestnut material, which caused the new introductions. As Gil-Tapetado *et al.* (2018) indicated in their survey, without quarantine measures, the chestnut forests at the centre of the IP were only vulnerable to *D. kuriphilus* dispersal mediated by human transport and could be colonized only in short-medium terms in this manner. In this brief communication, we inform about this statement, with the beginning of the *D. kuriphilus* problems forming from chestnut production in the Sistema Central.

In the focal area of Valle del Jerte, our prospections indicate that the presence of *D. kuriphilus* was earlier than 2019. In this area, the gall abundance did not coincide with a first-year invasion scenario, and it is probable that the infestation occurred in 2017 or 2018 and went undetected.

The Government of Castilla y León informed us that the different infested chestnut trees found in the Sierra de Francia (7) were eliminated by cutting off the tree canopy and burning it. However, these measures were taken in May and June, when the *D. kuriphilus* adult can emerge, and they may have oviposited and infested nearby chestnut tree buds. Therefore, the settlement of *D. kuriphilus* in the Sierra de Francia area remains in doubt in 2019. The infested chestnuts of this area came from Asturias and Galicia, confirming its introduction by transport through human forestry activities. A similar event occurred in the Sierra de San Vicente (9), near the Sierra de Gredos in Toledo in 2016. In this area, infested chestnut trees were reported and eliminated by burning galls. *A priori,* this control measure seems to be effective because no new presence of *D. kuriphilus* has been reported in this area since 2016 (Gil-Tapetado *et al.,* 2018).

In 2019, at the time this distribution update was written, the Sierra de Aracena and Picos de Aroche Natural Park (Huelva, Andalusia) was the last most important Spanish chestnut forest without *D. kuriphilus*, after our own prospecting. This area is a priority area of control, and chestnut trees and *Castanea* forestry material should be quarantined to prevent new introductions

Table 1. Sampling localities. The geographical location, province and focal area name, collection date and number of collected galls and number of female individuals of *D. kuriphilus* and emergency date, number of individuals by sex and identification to species level of the parasitoids of each sample are indicated. * Data from gall dissection.

Locality	Latitude	Longitude	Province	Focal Area	Sampling Date	N galls	N *D. kuriphilus*	Emergency Date	Parasitoid species	♀	♂	indet
	40.213	-5.790	Cáceres	Valle del Jerte	09/06/2019	60	11	13/06/2019	*Sycophila biguttata*	1	-	-
Cabezuela del Valle	40.214	-5.798	Cáceres	Valle del Jerte	09/06/2019	38	0	-	-	-	-	-
	40.214	-5.796	Cáceres	Valle del Jerte	09/06/2019	53	19	-	-	-	-	-
El Cabaco	40.563	-6.129	Salamanca	Sierra de Francia	29/09/2019	0	0	-	-	-	-	-
Sequeros	40.513	-6.027	Salamanca	Sierra de Francia	29/09/2019	0	0	-	-	-	-	-
Rozas de Puerto Real	40.310	-4.505	Madrid	Southwest of Madrid	26/06/2019	1	0	-	-	-	-	-
Cadalso de los Vidrios	40.298	-4.463	Madrid	Southwest of Madrid	26/06/2019	154	6	26/06/2019*	*Eupelmus* sp. (larva)	-	-	1
									Sycophila sp. (larva)	-	-	1
									Ectoparasitoid (larva)	-	-	1
									Pteromalidae (pupa)	-	-	1
									Torymus sinensis (larva)	-	-	2
								01/07/2019	*Mesopolobus tibialis*	2	-	-
									Ormyrus pomaceus	3	-	-
								08/07/2019	*Sycophila variegata*	1	1	-
									Eupelmus urozonus	5	5	-
								25/09/2019	*Eupelmus urozonus*	1	-	-
									Cecidostiba fungosa	-	2	-
								15/07/2019	*Sycophila flavicollis*	1	-	-
								27/11/2019	*Eurytoma pistaciae*	-	1	-
								09/01/2020	*Torymus sinensis*	6	4	-

of this alien species and an almost complete invasion of Spanish chestnut forests.

Parasitoid collection

Reared parasitoids from samples of galls of *D. kuriphilus* collected in the site of Cadalso de los Vidrios (Community of Madrid) confirm the fast recruitment of native chalcid parasitoid fauna by *D. kuriphilus*, accounting for a minimum of nine different species. This recruitment of parasitoids by this alien cynipid would have occurred after only one year of presence in the chestnut forests of the Sistema Central. All emerged chalcid parasitoids were native and generalists and belonged to different native cynipid communities (Gómez *et al.,* 2006) (Table 1). The species *Cecidostiba fungosa* (Geoffroy, 1785), *Sycophila biguttata* (Swederus, 1795) and *Sycophila flavicollis* (Walker, 1834) were rare in our *D. kuriphilus* gall samples throughout the IP from 2016 to 2018, with the focal point mainly located in Galicia (1) and Serranía de Ronda-Valle del Genal (12), and in the parasitoid list of Catalonia region (5-6) in the work by Jara-Chiquito *et al.* (2020) (Fig. 1). Although the data are very fragmented and scarce, it is possible there are differences in the communities of parasitoids in different areas of the IP as well as in the recruitment of native fauna (Gil-Tapetado *et al.,* unpublished).

Considering the dissection data of *D. kuriphilus* galls, the early finding of *Torymus (Syntomaspis)* larvae in Cadalso de los Vidrios, preliminarily identified as *T. sinensis*, was intriguing and must be emphasized. This fact was later confirmed by the rearing of emerging *T. sinensis* adults from this sample (Table 1). The unexpected and surprising presence of this species in this locality of the Community of Madrid would be explained with two hypotheses: 1) the natural dispersal of *T. sinensis* from the nearest area where this biological control agent was experimentally released, the RJB Alfonso XIII in the city of Madrid; or 2) a possible unauthorized release of *T. sinensis* were made in this site, given that there are no records of such releases being authorized or made by the Community of Madrid. We think the first hypothesis is highly unlikely. In fact, although Madrid is 63 km distant from Cadalso de los Vidrios and, therefore, has a similar range about of 60 km/year of dispersal capacity for the species reported by Moriya *et al.* (2003), the date of establishment in Madrid is quite recent and with a low percentage (spring of 2018, 3.69%, Nieves-Aldrey *et al.,* 2019). This would imply a very fast dispersal event, only one year, to colonize the distant apart chestnut trees of Cadalso de los Vidrios, due to our rearing of *T. sinensis* in this last site are dated in 2019 and the species needs an entire year for its establishment.

In conclusion, *D. kuriphilus* has continued to expand through the IP, both by natural dispersal and dispersal facilitated by human activities. This alien species has recruited as a minimum of nine parasitoid species at one site of the Sistema Central in the same year as *D. kuriphilus* was reported in this area and with the simultaneous presence of its biological control, *T. sinensis*, an alien species that is for the first time recorded from a natural area of the Community of Madrid.

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