

# Differences in native and introduced chalcid parasitoid communities recruited by the invasive chestnut pest *Dryocosmus kuriphilus* in two Iberian territories

## Research Paper

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### Abstract

*Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) is a global invasive gall wasp and a pest of chestnuts (*Castanea* spp.). A study of the Chalcidoidea parasitoid community of *D. kuriphilus* was undertaken over two years, from March 2017 to March 2019, at 15 sites in south and northwest Spain (Málaga and Galicia regions). More than 18,000 galls were collected, and 1153 parasitoids belonging to 22 species of seven chalcidoid families, plus two individuals of an inquiline Cynipidae, *Synergus facialis*, emerged. Richness was higher in the Málaga region, with 20 species, while 17 parasitoids and one inquiline were identified in Galicia. The parasitism rate of native chalcid parasitoid species in both regions was low. *Eupelmus urozonus* and *Mesopolobus lichtensteini* were the most abundant native species. *Mesopolobus tibialis* was a dominant species in south Spain, while *Ormyrus pomaceus* was a dominant species in northwest Spain. Our results revealed the existence of a sub-community of univoltine, probably host specialized, parasitoids in south Spain, which overwinter in galls, exhibiting a similar life cycle to *Torymus sinensis*. These species were *Torymus notatus*, *Aulogymnus bicolor*, *Aulogymnus obscuripes* and *Aulogymnus balani*. Data on the recovery of *T. sinensis* after release in the south Spain region show it to be well established, but its numbers are still low in northwest Spain.

## Introduction

Non-native species are being introduced into many countries with increasing frequency. These exotic organisms can threaten and damage ecosystems, biodiversity, agriculture and even human health (Wittenberg and Cock, 2001; Pimentel *et al.*, 2005; Pejchar and Mooney, 2009). Biological invasions present important problems in different facets of human society, such as environmental, human health and agricultural production issues. Biological invasions lead to pest problems that can cause considerable economic damage due to losses of agricultural and forestry crops. Additionally, exotic species can disturb ecosystems and trophic networks through the displacement and extinction of native species (Gurevitch and Padilla, 2004), causing environmental damage and biodiversity loss (Vitousek *et al.*, 1996; Pimentel *et al.*, 2005). The damage to biodiversity and the economy produced by non-native species are due to their high propensity for dispersal and explosive production of large populations during the first years of an invasion resulting from their establishment in an environment without their natural enemies; the ‘enemy-release hypothesis’ (Williamson, 1996; Crawley, 1997). In the case of arthropodan biological invasions, one of the most important groups related to natural enemies is the parasitoids (Quicke, 1997; Schönrogge *et al.*, 2006), mostly belonging to the order Hymenoptera that have theoretically co-specified with their hosts through adaptations to overcome their hosts’ defences (top-down relations), and the hosts modifying their defences to avoid being parasitized (bottom-up relations) (Stone *et al.*, 2002).

Invasions by gall-inducing insects such as gall wasps (Hymenoptera: Cynipidae) present difficulties for pest control because these organisms spend most of their life cycle concealed inside a vegetal structure (the gall) which is part of the host plant (Rohfritsch and Shorthouse, 1982). As cynipids are in their larval and pupal stages inside galls, many types of pest control, such as chemical biocides, are ineffective (Cooper and Rieske, 2007). Therefore, knowledge of their association with natural parasitoid enemies, most of which belong to the superfamily Chalcidoidea (Askew *et al.*, 2006, 2013), can indicate which of the parasitoid species have potential as biological control agents and would be candidates for intentional introductions to new regions.

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An example of an invasive gall wasp biological invasion is *Andricus quercuscalicis* Burgsdorf 1783 (Collins *et al.*, 1983; Stone and Sunnucks, 1993; Schönrogge *et al.*, 1995; Schönrogge *et al.*, 2006), which was introduced to western Europe from south-eastern Europe through the planting and human transport of *Quercus cerris*. In its invaded range, *An. quercuscalicis* impedes the development and production of *Quercus* acorns.

Another example, addressed in this paper, is provided by the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, 1951, a parthenogenetic cynipid from China that has colonized *Castanea* Mill. trees in North America (Payne *et al.*, 1975), Japan (Oho and Umeya, 1975) and Europe (Brussino *et al.*, 2002) and is an economic pest of chestnut forests in these newly colonized territories (Payne *et al.*, 1975; EFSA, 2010; Gehring *et al.*, 2017). *D. kuriphilus* is the only taxon of Cynipidae to attack chestnuts in Europe and North America, and there was, therefore, no native parasitoid fauna associated with cynipids on *Castanea* trees when it was introduced (Stone *et al.*, 2002), unlike the situation in China, where this cynipid has natural enemies (Moriya *et al.*, 1989). The best-studied natural enemy of *D. kuriphilus* is *Torymus sinensis* Kamijo, 1982 (Hymenoptera: Torymidae), a chalcid parasitoid that is also native to China and which has been used as a biological control agent in many countries (Moriya *et al.*, 2003; Cooper and Rieseke, 2007; Quacchia *et al.*, 2013; Matošević *et al.*, 2016; Borowiec *et al.*, 2018; Avtzis *et al.*, 2019). This torymid appears to be a host-specific parasitoid of *D. kuriphilus*, and its life cycle is synchronized with that of its host, although there is also evidence that *T. sinensis* can parasitize native oak galls (Ferracini *et al.*, 2015, 2018). As the total attack rates of native parasitoid species remained low for many years after the arrival of *D. kuriphilus* (Quacchia *et al.*, 2012), biological control programmes involving *T. sinensis* appeared to be the most promising method of controlling the pest in chestnut forests worldwide (Moriya *et al.*, 1990; Quacchia *et al.*, 2008). However, the use of *T. sinensis* in biocontrol involves introducing an exotic species, and this could potentially lead, in the absence of adequate screening, to environmental problems, especially non-target impacts (Gibbs *et al.*, 2011; Ferracini *et al.*, 2015, 2018).

The studied *D. kuriphilus* communities showed high parasitoid species recruitment in a short time, and new biological communities were formed in many territories, though parasitism rates were low. This contrasts with the invasion of Western Europe by *An. quercuscalicis* and other *Andricus* cynipid species, in which effective parasitoid recruitment did not begin until after more than 20 years (Schönrogge *et al.*, 2006). The parasitoid communities recruited by *D. kuriphilus* have been studied in many countries such as USA (Cooper and Rieseke-Kinney, 2006), Italy (Aebi *et al.*, 2007; Quacchia *et al.*, 2012; Panzavolta *et al.*, 2013), Croatia (Matošević and Melika, 2013) and Slovenia (Kos *et al.*, 2015). In Spain, the first native recruited parasitoids of *D. kuriphilus* were reported from Catalonia (Jara-Chiquito *et al.*, 2016), and preliminary lists of parasitoids of *D. kuriphilus* in Galicia have also been published (Pérez-Otero *et al.*, 2017; Santolamazza-Carbone *et al.*, 2018). However, there have been no reports from another important chestnut-growing region of Spain where the chestnut crop economy has been badly affected in recent years by *D. kuriphilus*: Málaga Province (Andalusia, southern Spain).

On the Iberian Peninsula, *D. kuriphilus* was first detected in Catalonia in 2012 (EPPPO, 2012; Pujade-Villar *et al.*, 2013), and new *D. kuriphilus* hotspots then appeared rapidly in other

Spanish regions (Gil-Tapetado *et al.*, 2018). The species was recorded in Galicia (north-western Spain) and Andalusia (southern Spain) in 2014, and it was detected in the northwest of Portugal in 2014 (EPPPO, 2014).

The Iberian Peninsula is a heterogeneous territory incorporating different climate and with marked variations in temperature and precipitation across a latitudinal gradient (Hawkins *et al.*, 2003a, 2003b; Cabrero-Sañudo and Lobo, 2006; Mittelbach *et al.*, 2007; Vetaas and Ferrer-Castán, 2008). Considering this climatic diversity, and despite the consistent worldwide patterns of *D. kuriphilus* communities (Aebi *et al.*, 2006), regional differences were expected to be found in the *D. kuriphilus* parasitoid communities in Spain. Differing environmental conditions lead to differences in vegetation, and *Quercus* species diversity close to stands of chestnut trees infested by *D. kuriphilus* is one of the most important sources of dissimilarity between native gall wasp parasitoid communities (Nieves-Aldrey, 2001; Askew *et al.*, 2013). Different oak tree species support different native cynipid species, and these in turn support different chalcid parasitoid species. This could explain the differing composition of parasitoid communities recruited by *D. kuriphilus* in different geographical sites in Spain.

Here, we describe and analyse the chalcid parasitoid communities recruited by *D. kuriphilus* in two geographically distant areas of Spain: (1) Málaga province (Andalusia, southern Spain) and (2) the autonomous community of Galicia (northwest Spain). Both geographical areas harbour important *Castanea* forests which have been invaded by *D. kuriphilus* in recent years, causing severe chestnut yield losses. Consequently, controlled releases of *T. sinensis* have been authorized by the Spanish government and implemented in recent years (Nieves-Aldrey *et al.*, 2019). These parasitoid communities are characterized by differences in the abundance and composition of species and by their emergence dates. Our study provides the first information on the species communities formed *de novo* in 2014, which is of great economic interest, given the status of *D. kuriphilus* as an important pest of *Castanea* trees. We examine the role that *T. sinensis* is playing in these novel communities in the two areas where this torymid has been released, and we reveal apparent differences. Finally, we lay the groundwork for future studies on the interactions of the recruited *D. kuriphilus* community with the native parasitoid community associated with cynipids inducing galls on oak hosts (*Quercus* spp., *Fagaceae*).

## Materials and methods

### Sampling area

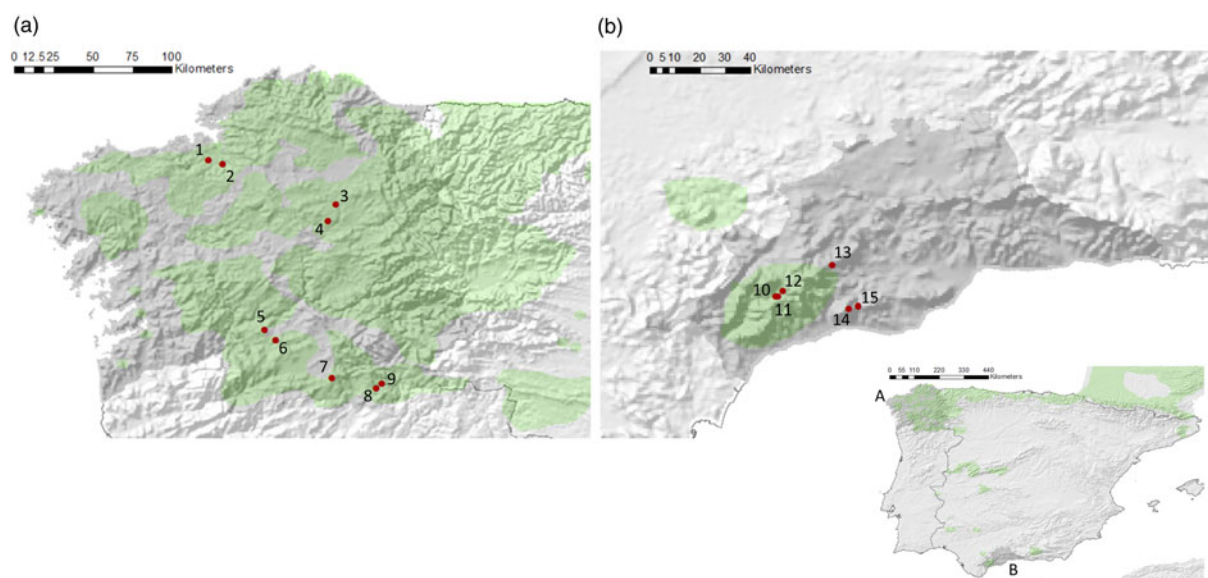
To perform standardized sampling, 15 localities were selected according to the *D. kuriphilus* infestation data across the zones provided by the Spanish regional public administration of Andalusia and Galicia. These localities are in two different regions: (1) Sierra de las Nieves and Valle del Genal (Málaga, south Spain) and (2) Galicia (northwest Spain), the latter divided into two sub-regions: (i) Mediterranean Galicia (southern Galicia) and (ii) Eurosiberian Galicia (northern Galicia) are chosen to represent zones of *D. kuriphilus* hotspots (Gil-Tapetado *et al.*, 2018). Coordinates of the sampling areas are recorded in table 1, and their geographical locations are indicated in fig. 1.

The Málaga region studied in this survey refers only to two different areas: (1) Sierra de las Nieves and Sierra Blanca (localities of Yunquera, El Juanar and Puerto Ojén), with areas of 202 and

**Table 1.** *Dryocosmus kuriphilus* localities sampled in this survey

Map legend	Locality	Region	Latitude	Longitude	N galls
1	Mabegondo	Galicia	43.24449	-8.26882	400
2	Oza de los Ríos	Galicia	43.22089	-8.18733	200
3	Campus Lugo	Galicia	42.99192	-7.54488	200
4	Lousada	Galicia	42.89538	-7.58722	785
5	Trelle	Galicia	42.27925	-7.95125	1526
6	Merouzo Pequeño	Galicia	42.22088	-7.88746	867
7	As Corvaceiras	Galicia	42.00367	-7.56624	654
8	Vilariño Das Touzas	Galicia	41.94513	-7.31340	626
9	Riós	Galicia	41.97346	-7.28377	635
10	Júzcar	Málaga	36.61822	-5.14425	1362
11	Pujerra	Málaga	36.61840	-5.13640	1312
12	Igualeja	Málaga	36.64000	-5.11995	3034
13	Yunquera	Málaga	36.73152	-4.94308	1031
14	El Juanar	Málaga	36.57785	-4.88418	3895
15	Puerto Ojén	Málaga	36.58562	-4.85148	1547

The total number of sampled galls is indicated in the *N* galls column. The map legend shows the relationships of the sampling points that appear in [fig. 1](#) and their corresponding locality.



**Figure 1.** Maps of the locations of the sampling points on the IP. (a) The region of Galicia and (b) the region of Málaga. The names of the localities of each sampling point appear in [table 1](#).

70 km<sup>2</sup>, respectively, and an average altitude of 1000 m a.s.l.; and (2) Valle del Genal (localities of Pujerra, Igualeja and Júzcar), with an area of 485 km<sup>2</sup> and an average altitude of 700 m a.s.l. In the sampling sites of the Málaga region, the predominant vegetation is composed of oak forests of *Quercus suber* L. and *Quercus canariensis* Willd., with *Quercus faginea* Lam., *Quercus ilex* L., and *Quercus coccifera* L. in more xeric areas. The presence of chestnut trees at higher altitudes (up to 1700 m a.s.l.) in the Sierra de las Nieves, in a relict forest of *Quercus alpestris* Boiss., is remarkable. These mixed forests of *Quercus* and *Castanea* nestle in these mountains and valleys, but they are always at an altitude of

~800 m a.s.l. In the Galicia region (with an area of 29,575 km<sup>2</sup>), chestnut trees are distributed more widely, from sea level to a high altitude, because of the more humid conditions and high rainfall throughout the entire territory, but especially in the Eurosiberian subregion. Eurosiberian Galicia is a colder and rainier environment that favours the dominance of *Quercus robur* L. over other *Quercus* species in this area (Amaral Franco, 1990). In Mediterranean Galicia, oak forests of *Quercus pyrenaica* Willd. and *Q. suber* are predominant, while *Q. ilex* is restricted to only a small area of Sierra da Enciña Lastra close to the community of Castilla y León.

### Gall collection and rearing

At each sampling site, a minimum of 200 galls was collected (a total of 18,074 galls, 5893 in Galicia and 12,181 in Málaga) (table 1). *D. kuriphilus* galls were collected from the trees between ground level and a height of 2 m. Galls were collected during multiple sampling campaigns between March 2017 and January 2019 in both regions. Fresh galls were collected in spring and summer samplings, and dry ones were collected in autumn and winter.

*D. kuriphilus* galls were stored for rearing in cardboard boxes equipped with skylight extractors and were maintained indoors in the Museo Nacional de Ciencias Naturales of Madrid (MNCN-CSIC). The boxes were checked daily to collect emerged parasitoids. Specimens were preserved in vials with 99% ethanol and labelled with their emergence date and collection location.

### Parasitoid identification

Parasitoids were identified to species using available morphological taxonomic keys (Nieves-Aldrey, 1983, 1984a, 1984b, unpublished) and unpublished keys from Askew and Thúrocz for an update in some parasitoid families. Some of the reared parasitoid species, such as *Eupelmus urozonus* Dalman, 1820, *Ormyrus pomaceus* (Geoffroy, 1785), and *Bootanomyia dorsalis* (Fabricius, 1798), include complex cryptic lineages that are difficult to distinguish morphologically, as has been revealed in studies with molecular markers and other tools (Al-Khatib *et al.*, 2014; Nicholls *et al.*, 2010; Gómez *et al.*, 2017). A conservative approach to the identification of species belonging to these cryptic species aggregates was chosen, and they were assigned to the binominal combination used for the aggregate or complex (table 2). In addition, all published information was compiled (table 3) to show the absolute numbers of parasitoids sampled from galls of *D. kuriphilus* in Spain.

### Sampling effort curves

To calibrate the completeness of the inventory of *D. kuriphilus* parasitoid communities, an evaluation of the sampling of the 15 studied areas was performed with sampling effort curves (SECs). SECs can estimate the degree of sampling of the different *D. kuriphilus* communities by sampling area and locality, to observe any possible bias that exists in the description of the community according to the data obtained, and to indicate the strength of the analyses.

To perform this evaluation, the software EstimateS (Colwell, 2018) was used to calculate the Chao2 estimator of species incidences and CurveExpert (Hyams, 2007) to obtain the Clench adjustment curve (Jiménez-Valverde and Hortal, 2003). The parameters of the curve slope (slope), the percent of registered species (%rs) and the percent of realized effort (%re) were calculated using the *a* and *b* parameters of the Clench curve. These last analyses were performed in R Studio (RStudio Team, 2019) (table 4).

### Analyses of communities

To describe biodiversity patterns in *D. kuriphilus* communities, three different parameters were analysed: (1) species richness (sS), (2) parasitoid emergence dates, and (3) species nestedness and structure.

To calculate sS (1), the identified individuals from each locality were counted by species, as shown in table 2. The numbers of the Hill series or true diversity (Jost, 2006) of predicted sS ( $q_0$ ),

effective sS ( $q_1$ ), dominant sS ( $q_2$ ) and accessory sS ( $q_0-q_1$ ) were calculated using R Studio (RStudio Team, 2019) with the Spade R library (Chao *et al.*, 2016). The ChaoJost Hill series of sS indicates a predicted sS according to the data for each biological community, showing the theoretical number of possible species that could constitute each community (table 5).

Emergence date graphs of the parasitoid species (2) were generated using the relative abundance of species, considering the total abundance per parasitoid divided by the number of *D. kuriphilus* galls per sample (table 1). In this way, the differences among the absolute numbers of parasitoids are reduced and standardized, producing a graph that is better adjusted to the biological community. These graphs were generated for different Chalcidoidea families associated with *D. kuriphilus*, separated by region, and were created using R Studio with the library *ggplot2* (Wickham, 2018). The graphs show the presence of parasitoid adults between May 2017 and March 2018 in both regions (fig. 2).

Nestedness analyses of *D. kuriphilus* community (3) showed the structure and species evenness of both communities. These analyses were performed with the Nestedness Temperature Calculator v. 2.0 software (Ulrich, 2006; Ulrich *et al.*, 2009) using 10,000 random runs. Additionally, system temperatures were compared with temperatures obtained at random, while Monte Carlo-derived probabilities were generated to confirm the nestedness of communities, and the ordering of the species in the locality richness matrix was obtained together with the state occupancy percent (fig. 3). Differences between the 2017–2018 and 2018–2019 were not calculated due to the difference in sampling numbers between these periods. The probability of state occupancy probability for each species was also calculated by the program. Nested distributions imply that the most ubiquitous species will virtually always appear on the most hospitable locality, whereas the most marginal species will appear on the least hospitable locality; the other species vary in a specifiable manner between these two extremes, permitting us to calculate the probability of their occupancy in the other localities (Atmar and Patterson, 1993). This is represented by colours, with black being a 100% occupancy percentage and the other colours in fig. 3 being a lower percentage, with red being the lowest percentage between 0 and 10%.

## Results

### Native parasitoid species recruited by *D. kuriphilus* on the Iberian Peninsula

The parasitoids identified in this survey included a total of 22 species of Chalcidoidea, 17 of which were present in the Galicia region and 19 in the Málaga region (table 2). Almost all obtained species exhibit a generalist biology with a wide host range (Askew *et al.*, 2013; Noyes, 2019), and many of them are multivoltine. The absence of the family Eulophidae in the samples from the Galicia region can be highlighted. *Pachyneuron muscarum* (Linnaeus, 1758), reared in the Málaga samples, was probably present due to the secondary fauna of Homoptera that use *D. kuriphilus* galls as a refuge. This species almost certainly plays no part in the *D. kuriphilus* parasitoid community as a natural enemy of the gall insect.

Four species, *Torymus notatus* (Walker, 1833), *Aulogymnus bicolor* (Askew, 1975), *Aulogymnus balani* (Pujade-Villar, 1991) and *Aulogymnus obscuripes* (Mayr, 1877) stand out among the identified native parasitoids because of their biological characteristics. The three species of *Aulogymnus* are recorded for the first

**Table 2.** List of inquiline and native and non-native chalcidoid parasitoids reared from galls of *D. kuriphilus* from sampling sites in Galicia and Málaga

Species	Family	Málaga						Galicia						Sum					
		Ig	Pu	Jz	Ju	PO	Yu	Ri	VT	AC	MP	Tr	CL	Lo	Ma	OR	Málaga	Galicia	Total
<i>Synergus facialis</i>	Cynipidae	-	-	-	-	-	-	-	-	-	-	-	2	-	-	0	2	2	
<i>Aulogymnus bicolor</i>	Eulophidae				6											6	0	6	
<i>Aulogymnus balani</i>	Eulophidae	1			10	12										23	0	23	
<i>Aulogymnus obscuripes</i>	Eulophidae					2										2	0	2	
<i>Eupelmus urozonus</i>	Eupelmidae	20	2	5	3	17		16	3	13	30	26	1	8	3	47	100	147	
<i>Eupelmus azureus</i>	Eupelmidae				1	11						2				12	2	14	
<i>Anastatus sp.</i>	Eupelmidae									1						0	1	1	
<i>Eurytoma brunniventris</i>	Eurytomidae			2						1	3	24	2	19		2	49	51	
<i>Eurytoma pistaciae</i>	Eurytomidae	3			4	2				1	2	6				9	9	18	
<i>Sycophila biguttata</i>	Eurytomidae			2						9	1	4				2	14	16	
<i>Sycophila variegata</i>	Eurytomidae	12	2	12	28	18				16	16	70		2	3	6	72	113	185
<i>Sycophila flavicollis</i>	Eurytomidae						1										1	0	1
<i>Ormyrus pomaceus</i>	Ormyridae		1	1				8	3	11	33	24		1			2	80	82
<i>Mesopolobus mediterraneus</i>	Pteromalidae	2		5	1		1			10	5	3	2	1			9	21	30
<i>Mesopolobus sericeus</i>	Pteromalidae													1			0	1	1
<i>Mesopolobus tibialis</i>	Pteromalidae	27	13	30	23		2			11	3						95	14	109
<i>Mesopolobus lichtensteini</i>	Pteromalidae	26	11	4	23	8	1	6	2	1	2	4	2	4	1		73	22	95
<i>Pachyneuron muscarum</i> <sup>a</sup>	Pteromalidae	6															6	0	6
<i>Bootanomyia dorsalis</i>	Megastigmidae			1	5	2		8			4	14		1	2	17	8	46	54
<i>Torymus auratus</i>	Torymidae				2						2						2	2	4
<i>Torymus flavipes</i>	Torymidae	3			1				3	5	3	25		12			4	48	52
<b><i>Torymus sinensis</i></b>	<b>Torymidae</b>	<b>9</b>		<b>6</b>	<b>181</b>	<b>15</b>	<b>1</b>	<b>2</b>		<b>8</b>	<b>7</b>	<b>2</b>					<b>212</b>	<b>19</b>	<b>231</b>
<i>Torymus notatus</i>	Torymidae			1	5	18		1									24	1	25
N Individuals		109	29	69	293	105	6	41	11	86	112	204	7	51	9	23	611	544	1155
N Species		10	5	11	14	10	5	6	4	11	14	12	4	10	4	2	20	18	23

Ig, Igualeja; Pu, Pujerra; Jz, Júzcar; Ju, Juánar; PO, Puerto Ojén; Yu, Yunquera; Ri, Riós; VT, Vilariño das Touzas; AC, As Corvaceiras; MP, Merouzo Pequeño; Tr, Trelle; CL, Campus Lugo; Lo, Lousada; Ma, Mabegondo; OR, Oza de los Rios. The inquiline species is underlined, and the released non-native species is indicated in bold. The sum columns show the total individuals of a species, indicating the most abundant species in blue and the rarest species from the sampling campaigns in red.

<sup>a</sup>Probably related to secondary fauna that inhabits *D. kuriphilus* galls.

time from the *D. kuriphilus* parasitoid community in Spain. Unlike most of the native parasitoids recruited by *D. kuriphilus*, these four chalcids are similar to *T. sinensis* in being univoltine species that develop only on the cynipid host and hibernate as pharate adults inside old galls remaining on the tree in winter. Their early spring emergence dates are synchronized with the early development of their host galls.

A total of 1153 chalcid parasitoids and two inquiline cynipid individuals was reared from the galls collected in all standardized sampling campaigns in Málaga and Galicia (table 2). The total relative abundances of the recruited parasitoids (total parasitoids divided by the total collected galls by region) were similar in the Mediterranean areas of Galicia and Málaga (0.09 and 0.05, respectively). However, despite the number of galls collected in both regions, the relative abundance was greater in Galicia. The most abundant native parasitoid species in both regions were *Mesopolobus tibialis* (Westwood, 1833) (109), *Mesopolobus lichtensteini* (Mayr, 1903) (95), *Eup. urozonus* (147), *Sycophila variegata* (Curtis, 1831) (185) and *O. pomaceus* (82). However, while *M. tibialis* was a dominant species in Málaga and an accessory species in Galicia, *O. pomaceus* was dominant in Galicia and accessory in Málaga (fig. 3). If both native and non-native parasitoids are considered within the recruited parasitoid community of *D. kuriphilus* in the two study areas, then the dominant species is the alien released species *T. sinensis*, of which a total of 231 individuals were recovered, including 212 from Málaga and 19 from galls collected in Galicia.

### Sampling effort curves

The sampling effort was sufficient at all the collecting sites except for two, Yunquera and Campus Lugo (table 4). On average, excepting for these two localities, the SECs per locality showed a slope of 0.03, 85%rs and 93.2%re. Considering the results for the Galicia and Málaga regions, the SECs showed a mean slope of 0.00 (the SECs have reached the asymptote), with 85.7 and 84.8%rs and 97.3 and 97.5%re, respectively. As the values of these parameters met the usual evaluation criteria (slope <0.1, %rs>75 and %re>75), the sample collected was theoretically representative of the real biological community, thus possible bias was minimized and the data were sufficient to support the following analyses.

### Species richness

sS varied between the regions, being higher in Málaga, with an observed or empirical  $q_0 = 19$ , ChaoJost  $q_0 \approx 19.1$ , and a mean per locality of  $q_0 = 9.2$  (table 5). The Galicia region exhibited an empirical  $q_0 = 17$ , ChaoJost  $q_0 \approx 17.5$  and a mean per locality  $q_0 = 7.4$ . The empirical effective species  $q_1$  was higher in Galicia than in Málaga (9.094 and 7.212, respectively). The dominant species values of the communities in the Galicia and Málaga regions were different (7.333 and 4.916, respectively), and the  $q_2$  value of the Málaga community was very similar to that reported in Catalonia (5.184) (Jara-Chiquito *et al.*, 2016). The number of accessory species ( $q_0 - q_1$ ) also varied between the regions, being much higher in Málaga (11.79) than in Galicia (7.91).

### Emergence date and relative abundance of native and non-native parasitoids

The emergence date charts showed a high relative abundance of *T. sinensis* in the Málaga region and low representation in Galicia

(fig. 2A, B). They also emphasized the abundance difference between *T. sinensis* and the native *Torymus* species in the Málaga region (212 vs. 30 total individuals, respectively) (fig. 2A.g). Together with *T. sinensis*, other species that constitute a biological sub-community with an emergence date at the beginning of the year (February–March) appear when new galls are starting to develop. This overwintering sub-community includes three species of the genus *Aulogymnus* (fig. 2A.c) and *T. notatus* (figs 2A.g and 4). The emergence date charts also show the existence of multi- or bivoltine species, such as *Eup. urozonus* (fig. 2A.a and B.a) and *Eurytoma brunniventris* Ratzeburg, 1852, only in the Galicia region (fig. 2B.a). The emergence date of *B. dorsalis* (fig. 2A.d and B.c) is later than that of other native parasitoids.

There are a relatively large number of accessory species in the Málaga community (fig. 3), but they are present in small numbers of individuals and their impact in the recruited community is probably insignificant. The highest relative abundance among all parasitoids in the sample was found for *Syc. variegata* in the locality of Trelle, in the Mediterranean subregion of Galicia, at 0.14%. The relative abundance of *T. sinensis* reared from all the sampled *D. kuriphilus* galls was very low (<0.1%).

### Species nestedness and community structure

Comparison of the nestedness diagram of both communities together with sS (fig. 3) estimates how many species were categorized as the most dominant species ( $q_2$ ), effective species ( $q_1$ ) and most-accessory species ( $q_0 - q_1$ ) of each *D. kuriphilus* parasitoid community. In the Málaga region, the most abundant species were *M. lichtensteini*, *Eup. urozonus*, *Syc. variegata*, *M. tibialis* and *T. sinensis*, while in Galicia, the dominant species were *Eup. urozonus*, *M. lichtensteini*, *Syc. variegata* and *O. pomaceus*. The state occupancy percentage indicated the most-accessory species of each community. These accessory species were *Sycophila flavicollis* (Walker, 1834), *Au. obscuripes*, *Sycophila biguttata* (Swederus, 1795) and *Eur. brunniventris* in Málaga and *T. notatus*, *Eupelmus azureus* Ratzeburg, 1844 (= *Eupelmus spongipartus* Förster, 1860 (Al Khatib *et al.*, 2014)), *Mesopolobus sericeus* (Förster, 1770), and *Anastatus* sp. in Galicia, with the addition of the cynipid inquiline (Synergini) *Synergus facialis* Hartig, 1840.

In both cases, tests show that the system nesting temperature was different from the mean nesting temperature generated at random (Galicia 17.26° vs. 49.31°(±7.72°) and Málaga 27.68° vs. 46.19° (±8.33°), respectively; Monte Carlo-derived bootstrap results: Galicia:  $P < 0.001$  and Málaga:  $P = 0.01$ ). The system nesting temperatures were colder than those generated at random, confirming the presence of a pattern that can be studied and analysed in detail.

## Discussion

### Cynipid inquiline species

Together with identified species from previously reported *D. kuriphilus* community surveys (table 3), the total number of native parasitoids associated with this alien gall wasp in Spain is 34 species (Jara-Chiquito *et al.*, 2016, 2020; Pérez-Otero *et al.*, 2017), in addition to one inquiline Cynipidae species.

This cynipid inquiline species, *Syn. facialis*, appeared in the *D. kuriphilus* community of Galicia. This is the second record in Europe of an inquiline species recruited by *D. kuriphilus*; the

**Table 3.** Compilation of the native parasitoids and inquilines (\*) reared in all reports addressing the *D. kuriphilus* communities of Spain

Species	Family	Jara-Chiquito <i>et al.</i> , (Catalonia) (2016)	Pérez-Otero <i>et al.</i> , (Galicia) (2016–2017)	Gavira <i>et al.</i> , (Andalusia) (2015– 2016)	Current article (Málaga, Andalusia) (2017–2019)	Current article (Galicia) (2017– 2019)	Total Individuals/ Species – Spain
<i>Synergus facialis</i> *	Cynipidae					2	2
<i>Eupelmus urozonus</i>	Eupelmidae	57	17	97	47	100	318
<i>Eupelmus azureus</i>	Eupelmidae	1			12	2	15
<i>Anastatus</i> sp.	Eupelmidae					1	1
<i>Eurytoma brunniventris</i>	Eurytomidae	4	49	7	2	49	111
<i>Eurytoma pistaciae</i>	Eurytomidae			6	9	9	24
<i>Sycophila variegata</i>	Eurytomidae	32	13	114	72	113	344
<i>Sycophila biguttata</i>	Eurytomidae	2	7		2	14	25
<i>Sycophila flavicollis</i>	Eurytomidae			1	1		2
<i>Ormyrus pomaceus</i>	Ormyridae	65	8		2	80	155
<i>Ormyrus nitidulus</i>	Ormyridae	14					14
<i>Mesopolobus mediterraneus</i>	Pteromalidae	4	2	50	9	21	86
<i>Mesopolobus amaenus</i>	Pteromalidae			1			1
<i>Mesopolobus tibialis</i>	Pteromalidae	4	5	127	95	14	245
<i>Mesopolobus lichtensteini</i>	Pteromalidae	4		85	73	22	184
<i>Mesopolobus xanthocerus</i>	Pteromalidae		1				1
<i>Mesopolobus albitarsis</i>	Pteromalidae	7					7
<i>Mesopolobus fasciiventris</i>	Pteromalidae	10					10
<i>Mesopolobus sericeus</i>	Pteromalidae	42				1	43
<i>Pachyneuron muscarum</i>	Pteromalidae				6		6
<i>Cecidostiba fungosa</i>	Pteromalidae	3		2			5
<i>Bootanomyia dorsalis</i>	Megastigmidae	128	7	78	8	46	267
<i>Bootanomyia dumicola</i>	Megastigmidae	1			1		2
<i>Monodontomerus aeneus</i>	Torymidae			1			1
<i>Torymus notatus</i>	Torymidae			3	24	1	28
<i>Torymus erucarum</i>	Torymidae	7					7
<i>Torymus flavipes</i>	Torymidae	152	48	21	4	48	273
<i>Torymus formosus</i>	Torymidae	4					4
<i>Torymus nobilis</i>	Torymidae	1					1
<i>Torymus auratus</i>	Torymidae	6	10	128	2	2	148

(Continued)

Table 3. (Continued.)

Species	Family	Jara-Chiquito <i>et al.</i> , (Catalonia) (2016)	Pérez-Otero <i>et al.</i> , (Galicia) (2016–2017)	Gavira <i>et al.</i> , (Andalusia) (2015– 2016)	Current article (Málaga, Andalusia) (2017–2019)	Current article (Galicia) (2017– 2019)	Total Individuals/ Species – Spain
<i>Torymus geranii</i>	Torymidae	6		1			7
<i>Aulogygmus arsamensis</i>	Eulophidae			4			4
<i>Aulogygmus bicolor</i>	Eulophidae			6			6
<i>Aulogygmus balani</i>	Eulophidae			23			23
<i>Aulogygmus obscuripes</i>	Eulophidae			2			2
Total		22	11	20	17	16	34

Table 4. Results of sampling effort curves (SECs) by locality, region and subregion of Málaga and Galicia

Locality	Region	Slope	%rs	%re
Mabegondo	Galicia	0.09	74.6	82.1
Oza de los Ríos	Galicia	0.00	94.2	96.3
Campus Lugo	Galicia	0.20	55.6	72.6
Lousada	Galicia	0.04	65.2	83.5
Trelle	Galicia	0.00	92.5	98.5
Merouzo Pequeño	Galicia	0.00	83.0	97.4
As Corvaceiras	Galicia	0.00	88.5	97.8
Vilariño das Touzas	Galicia	0.05	72.9	88.3
Ríós	Galicia	0.01	95.1	94.5
Júzcar	Málaga	0.01	81.9	93.6
Pujerra	Málaga	0.02	80.8	87.8
Igualeja	Málaga	0.00	94.7	96.9
Yunquera	Málaga	0.86	–	–
Juanar	Málaga	0.00	90.7	97.0
Puerto Ojén	Málaga	0.00	90.6	98.5
Galicia	–	0.00	85.7	97.3
Málaga	–	0.00	84.8	97.5
Catalonia	–	0.00	94.2	99.3

The slope represents the Clench curve slope of the SEC; %rs is the percentage of species observed; and %re is the percentage of the realized sampling effort by area. Localities with insufficient data are indicated by – for each unfulfilled parameter. Data from the Catalonia region were reported by Jara-Chiquito *et al.*, (2017) to show the communities studied in detail on the Iberian Peninsula.

first came from Greece, where a new species of *Saphonecrus* Dalla Torre and Kieffer was described by Melika *et al.* (2018). Prior to these records, only one inquiline species, *Synergus castaneus* Pujade-Villar, Bernardo and Viggiani, 2013, was recorded from cynipid galls on *Castanea* in China, although these were not galls of *D. kuriphilus* (Bernardo *et al.*, 2013). We found *Syn. facialis* in a single locality (Lousada), and it should be emphasized that we failed to rear additional individuals either in the second year of the sampling campaigns at this locality or at other collection sites in Galicia. Given that *Syn. facialis* is one of the cynipid inquiline species associated with the widest range of host galls (Nieves-Aldrey, 2001; Askew *et al.*, 2013), it is quite possible that its rearing from *D. kuriphilus* represents only an incidental presence and that this species is not established as a stable component of the associated community of this invasive species in Galicia.

#### Parasitoid overwintering sub-community

While most of the recruited parasitoids by *D. kuriphilus* (table 2) exhibit generalist behaviour and phenologies with two or more emergence dates, we found four species with similar life cycles to that of *T. sinensis* in Málaga: *T. notatus*, *Au. bicolor*, *Au. obscuripes* and *Au. balani* (fig. 4). In contrast to the generalist species, these four parasitoids are specialist species exhibiting univoltine life cycles, and they are more synchronized with the life cycle of *D. kuriphilus*, attacking the early spring stage of the host gall. For this reason, these species might regulate *D. kuriphilus* more

**Table 5.** Species richness (sS) diversity of parasitoids of *D. kuriphilus* communities of Málaga and Galicia by locality and region

Locality	Region	$q_0$	Empirical				ChaoJost		
			$q_1$	$q_2$	$q_0-q_1$	$q_0$	$q_1$	$q_2$	
Mabegondo	Galicia	4	–	–	–	–	–	–	
Oza de los Ríos	Galicia	2	1.775	1.628	0.225	2	1.816	1.675	
Campus Lugo	Galicia	4	–	–	–	–	–	–	
Lousada	Galicia	10	5.818	4.357	4.182	13.922	6.712	4.67	
Trelle	Galicia	12	7.278	5.451	4.722	12	7.484	5.574	
Merouzo Pequeño	Galicia	14	7.484	5.279	6.516	14.661	7.998	5.491	
As Corvaceiras	Galicia	11	8.608	7.868	2.392	13.965	9.347	8.56	
Vilariño Das Touzas	Galicia	4	3.947	3.903	0.053	4	4.572	5.5	
Riós	Galicia	6	4.591	3.955	1.409	6.488	4.941	4.271	
Júzcar	Málaga	11	6.132	4.115	4.868	13.217	6.771	4.313	
Pujerra	Málaga	5	3.361	2.813	1.639	5.241	3.648	3.007	
Igualeja	Málaga	10	6.83	5.687	3.17	10.495	7.155	5.945	
Yunqueira	Málaga	5	–	–	–	–	–	–	
El Juanar	Málaga	14	4.276	2.465	9.724	18.485	4.412	2.478	
Puerto Ojén	Málaga	10	8.038	7.335	1.962	10	8.403	7.811	
Region		$q_0$	$q_1$	$q_2$	$q_0-q_1$	$q_0$	$q_1$	$q_2$	
Galicia		17	9.094	7.333	7.906	17.499	9.249	7.424	
Málaga		19	7.212	4.916	11.788	19.1	7.332	4.95	
Catalonia		14	7.456	5.184	6.544	16.994	7.579	5.226	

Empirical sS indicates the sampled species diversity in each area, while ChaoJosts S provides a prediction of possible diversity in each zone. The sS diversity of the Catalonia region reported by Jara-Chiquito *et al.* (2017) is presented to show all the communities studied in detail on the IP.  $q_0$  = total sS; in empirical  $q_0$  = observed sS;  $q_1$  = sS of effective species;  $q_2$  = sS of dominant species; and  $q_0-q_1$  = sS of accessory species.

efficiently than other native parasitoids and may represent a possible alternative as a biocontrol agent to the alien *T. sinensis*. However, it should be emphasized that the low numbers of these species, at least in the first years of recruitment, suggest that these native chalcids may not be effective biological control agents of *D. kuriphilus* (Quacchia *et al.*, 2012). Because of their possible potential to control *D. kuriphilus*, relevant biological information is discussed below.

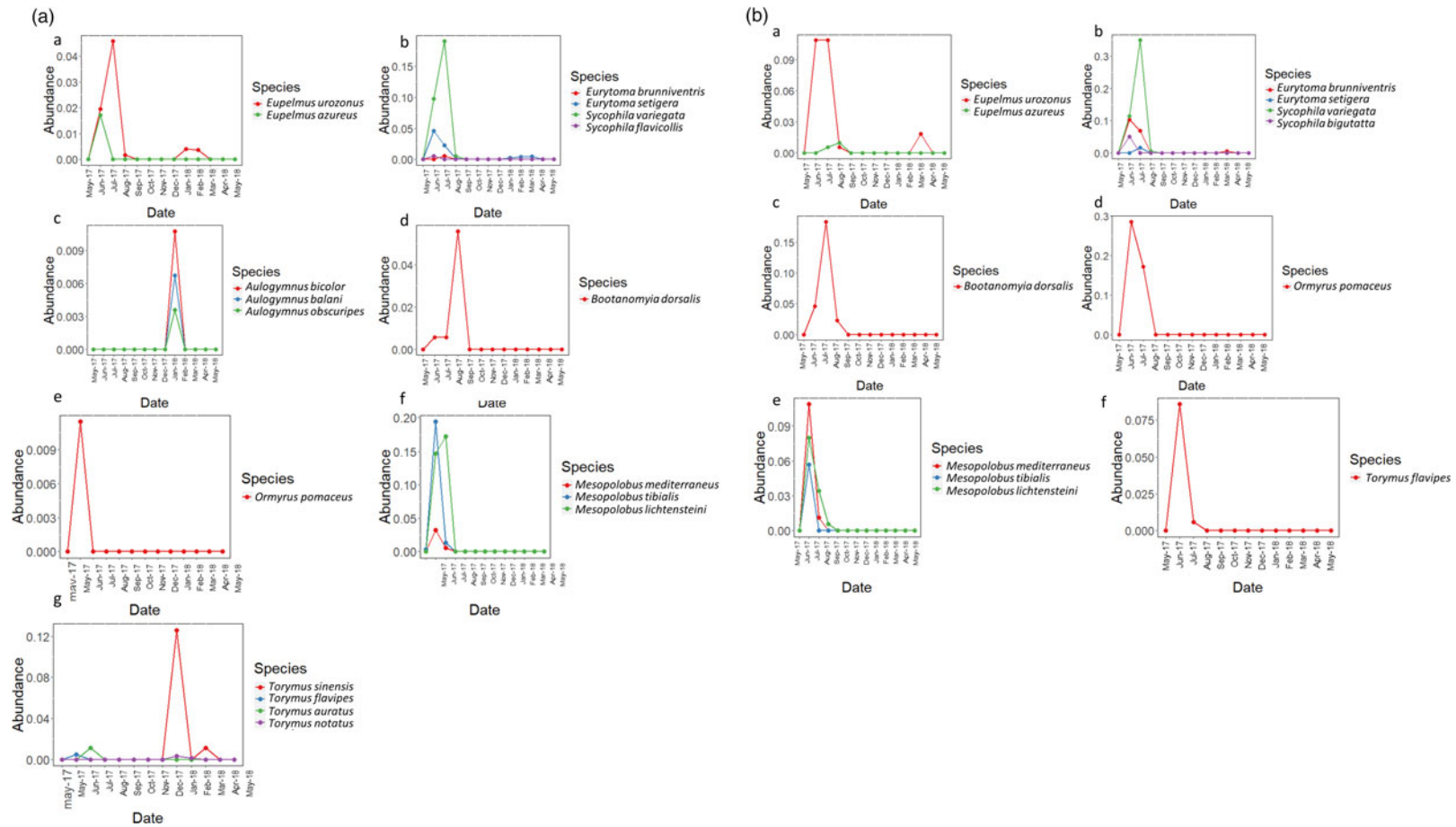
*T. notatus* is a native species that is morphologically closest to *T. sinensis* and it has a univoltine life cycle synchronized with the phenology of its host galls. Given this closeness to *T. sinensis*, *T. notatus* could be a candidate for hybridization with *T. sinensis* (Pogolotti *et al.*, 2018). *T. notatus* has been reared in Europe from a limited number of galls of the genera *Andricus*, *Neuroterus* and *Plagiotrochus* (Askew *et al.*, 2013, but see also Nieves-Aldrey, 2001 and Gómez *et al.*, 2006, for Spanish records), but its most common hosts in Spain are galls of the sexual generations of *Andricus curvator* Hartig, 1840 (in the United Kingdom this is the only known host) and *Plagiotrochus australis* (Mayr, 1882). Since these two species (*T. notatus* and *T. sinensis*) emerge from the galls at the same time and given their morphological similarity, much care is necessary to avoid confusing them.

From the sampling sites in Spain (table 2), 23 individual *T. notatus* (10 males and 13 females) were identified from three sites in the Málaga region: El Juanar, Júzcar and Puerto Ojén. One female of *T. notatus* was also found in Riós in the Mediterranean Region of Galicia. In addition, one male and

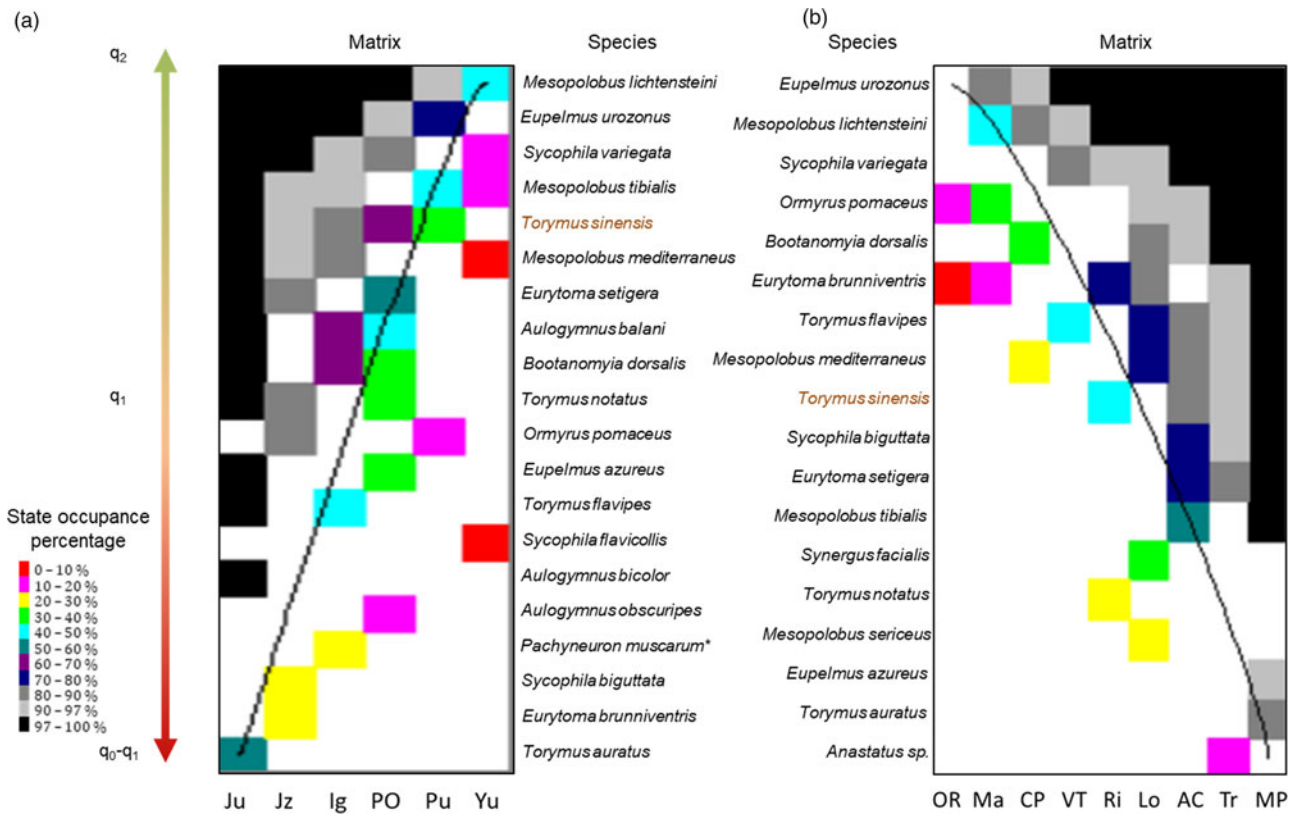
four females were reared from galls collected at one of our sampling sites in Catalonia (Sant Hilari). In all cases, *T. sinensis* was also reared from the same *D. kuriphilus* gall samples. Our data indicate similar establishment rates for the two species in at least one sampling site in the Málaga region (Puerto Ojén). At this site, we reared 18 *T. notatus* and 15 *T. sinensis* individuals, suggesting that, given their similar life cycle and taxonomic closeness, the native *T. notatus* might supplement the biocontrol effect of *T. sinensis*, at least at this site.

Six females of *Au. bicolor* were reared from samples collected in El Juanar (Málaga). This is a specialist parasitoid species only associated with galls of *Plagiotrochus* species on Mediterranean evergreen *Quercus* (*Q. ilex* and *Q. coccifera*). It is recorded only from Spain and Jordan where it is associated mainly with *Plagiotrochus quercusilicis* (Fabricius, 1798) and *P. australis* (Nieves-Aldrey, 1983; Pujade-Villar and Ros-Farré, 1998; Gómez *et al.*, 2006; Rizzo and Askew, 2008; Askew *et al.*, 2013).

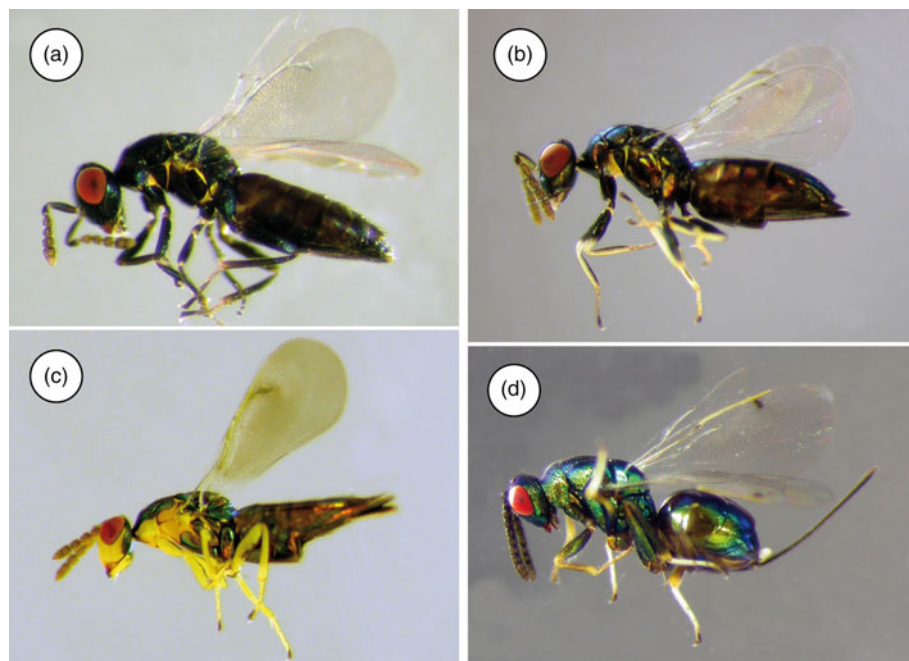
Ten females of *Au. balani* were recovered from galls collected in El Juanar and Puerto Ojén (Málaga). Previously this species was known only from the Catalonia region (northeast Spain), where it has been reared from galls of the sexual generation of *Pseudoneuroterus saliens* (Kollar, 1857) (Pujade-Villar, 1991), and from Tunisia, where it has been reared from *Synophrus olivieri* Kieffer, 1898 (Pujade-Villar *et al.*, 2010), both cynipid hosts being associated with cork oak (*Q. suber*). This is its first record from the parasitoid community of *D. kuriphilus*. It should be noted that *Au. balani* has been recovered from *D. kuriphilus*



**Figure 2.** Emergence date charts of parasitoid species of *D. kuriphilus* obtained in this survey, grouped by family. The y-axis represents the relative abundance of each species of the corresponding family, and the x-axis represents their emergence date from May 2017 to March 2018. (A) The Málaga region. (a) Eupelmidae; (b) Eurytomidae; (c) Eulophidae; (d) Megastigmidae; (e) Ormyridae; (f) Pteromalidae; (g) Torymidae; and, (B) represents the region of Galicia (a) Eupelmidae; (b) Eurytomidae; (c) Megastigmidae; (d) Ormyridae; (e) Pteromalidae; (f) Torymidae.



**Figure 3.** Nestedness diagram and structure of both communities of *D. kuriphilus*. (a) The *D. kuriphilus* community of Málaga and (b) the community of Galicia. Rows indicate the parasitoid species, and columns represent each sampling locality. The order of species from top to bottom is related to their corresponding strong or weak role in the community, as related to  $q_2$ ,  $q_1$  and  $q_1-q_0$  diversity. Species at the top are related to dominant species and species at the bottom to the accessory species of the community. The coloured squares of the matrix indicate the state occupancy percentage or the abundance degree of each parasitoid in each locality. A high state occupancy percentage indicates high abundances and stronger roles in each locality and the whole community, while low percentages indicate low abundances and weaker roles in each locality and community.



**Figure 4.** Habitus of native species of Chalcidoidea representatives of the overwintering sub-community associated with *D. kuriphilus* in Spain. Females of (a) *Aulogymnus obscuripes*; (b) *Aulogymnus balani*; (c) *Aulogymnus bicolor*; (d) *Torymus notatus*.

galls at a site in southern Spain where chestnut species share habitat with natural Mediterranean forests of evergreen oaks such as *Q. suber* and *Q. ilex*, among other species of *Quercus*. These evergreen *Quercus* are the host plants of the cynipid species recorded as hosts of the above-mentioned *Aulogymnus* species. This is the first time that they have been recorded as parasitoids of *D. kuriphilus*.

Two females of *Au. obscuripes* were reared in March from *D. kuriphilus* galls collected in winter in Puerto Ojén (Málaga). This species has been recorded as a parasitoid of ten oak gall wasp species of the genera *Andricus*, *Chilaspis*, *Cynips* and *Pseudoneuroterus*. However, the more common host species galls are those of the sexual generation of *An. quercuscalicis* (Burgsdorf, 1783) (Askew et al., 2013). There is only one previously available record of this *Aulogymnus* on the Iberian Peninsula; it was identified as a parasitoid in sexual galls of *Andricus pseudoinflator* Tavares, 1902 in the Catalonia region (Pujade-Villar, 1991). This is the first time that *Au. obscuripes* has been recorded in the native parasitoid community of *D. kuriphilus*.

The four species mentioned above belong to a native parasitoid sub-community that can be referred to as the ‘overwintering’ parasitoid community, in contrast to the so-called ‘summer’ parasitoid community referred to in most other published studies (Quacchia et al., 2012; Kos et al., 2015). The *Aulogymnus* species recorded in this study have historically been associated with *Q. suber* cynipid gall inducers (Askew et al., 2013), proximity or the presence of cork oaks probably dictating whether they enter *D. kuriphilus* communities. In Eurosiberian Galicia, *Q. suber* is scarce, preferring more thermophilic areas such as the Mediterranean (Gil and Varela, 2008), and the mentioned *Aulogymnus* species have not been found in the *D. kuriphilus* community. A similar case is provided by *T. notatus*, a species that in Spain is predominantly associated with galls of species of *Plagiotrochus* on *Q. ilex*, an evergreen oak that is very scarce in the predominantly Eurosiberian Galicia. The different roles of *T. notatus* in the two regions are noteworthy (fig. 3), as this parasitoid is a potentially effective species for the control of *D. kuriphilus* in Málaga, whereas it may be an accessory or marginal species in Galicia.

Studies addressing the overwintering *D. kuriphilus* parasitoid sub-community are uncommon compared with works focused on the summer sub-community (Aebi et al., 2006; Matošević and Melika, 2013; Jara-Chiquito et al., 2016, 2020; Bonsignore and Bernardo, 2018). The summer community has higher species richness, is easier and more accessible to study and is related to the collection and observation of green and fresh *D. kuriphilus* galls.

### The role of *T. sinensis* in the Iberian communities

As *T. sinensis* was found to be a well-established, dominant species in the Málaga community (fig. 3) and was the most abundant reared parasitoid, it is highly probable that this species can also influence the native parasitoid fauna in the *D. kuriphilus* community. This effect was not quantified in this study; however, it has been measured in other works, such as that of Ferracini et al. (2018) or Bonsignore and Bernardo (2018). In these studies, the authors found a negative relationship between the entry of *T. sinensis* into the community and the species richness of native parasitoids in *D. kuriphilus* galls. It is possible that highly abundant *T. sinensis*, linked to the overwintering sub-community, will parasitize galls in the early stages of formation, and thus decrease the number of available non-parasitized galls for the

summer sub-community. However, the summer sub-community is composed mainly of polyphagous parasitoids that can act as hyperparasitoids, such as *Eup. urozonus* and *Eur. bruniventris*, and these would not be affected. Considering the abundance of native parasitoids and *T. sinensis* that was found in this study (table 2), no negative direct effect of this foreign parasitoid on the indigenous fauna has been detected. Considering the region of Galicia and the relatively low number of individuals in the *T. sinensis* population, it does not seem that *T. sinensis* can have an effect as strong as indicated by Ferracini et al. (2018). However, in the case of the Málaga region, given the relatively large and growing population of *T. sinensis* in proportion to the native parasitoids, it is possible that this foreign parasitoid could greatly affect the native species if it were to invade the native oak cynipid communities in the future. These differences could be considered an effect and bias of the sampling, since less galls were collected in Galicia than in Málaga. However, the species accumulation curves (table 4) support that the samplings by region are correctly carried out and that both areas are comparable.

No specimens of *T. sinensis* were collected in the sampling campaigns of 2017–2018 in Galicia, and only 19 individuals were collected in 2018–2019, indicating a low level of establishment in this territory, confirming preliminary data from Nieves-Aldrey et al. (2019). However, in later years, the number of *T. sinensis* will probably increase, as has occurred in other countries such as Italy (Quacchia et al., 2008).

### *D. kuriphilus* communities’ composition and structure

Considering the nestedness analysis (fig. 3) and the community composition together with the theory of island biogeography (MacArthur and Wilson, 2001; Lomolino et al., 2016), *D. kuriphilus* communities can be considered to act as sink or ‘insular communities’ including fewer species than the source or ‘mainland communities’. These *D. kuriphilus* communities in their early stages are composed almost exclusively of generalist species (table 2), exhibiting high plasticity and adaptability, and a greater colonization potential (Cornell and Hawkins, 1993). The mature ‘mainland communities’ from which the *D. kuriphilus* communities would be nourished are the native cynipid communities found on *Quercus* species, which harbour the native parasitoid fauna (Askew and Shaw, 1986; Nieves-Aldrey, 2001) and constitute a relatively well-organized and structured biological system (Askew, 1975). Thus, generalist species from the mature *Quercus* cynipid communities are the first species to conform to a new biological community (‘insular communities’ in which *D. kuriphilus* enters) since they exhibit a wider ecological plasticity, greater adaptability and an easier fit to a newly available niche (Cornell and Hawkins, 1993), with a greater host diversity, and adaptability to more potential hosts (Askew, 1975).

Theoretically, as there are no apparently known physical barriers between ‘insular’ and ‘mainland communities’ (*Castanea* and *Quercus* trees form mixed forests in Málaga and Galicia regions), the colonization, recruitment of parasitoids and ecological succession in these two communities, both being initiated in 2014 with the arrival of *D. kuriphilus*, should be rapid (Ferracini et al., 2018). The recruitment to the communities of a specialist species such as *T. notatus* (table 2) during the study period, a species not detected in previous years could represent a step in the development of the biological community to a state of greater maturity. In Málaga, *T. notatus* was detected in 2017–2018 in three localities together with the three species of

the genus *Aulogymnus*, while in the Galician communities only one individual of *T. notatus* was found at a single locality in 2018–2019. This is the first difference in *D. kuriphilus* community composition that was identified between Málaga and Galicia; ecological succession in the first region may *a priori* be more advanced than in the second.

The composition differences in beta diversity between the Málaga and Galicia *D. kuriphilus* communities showed a similarity of Jaccard = 0.65 and Sorensen = 0.79, indicating high similarity between them. The composition differences between the Galicia and Málaga *D. kuriphilus* communities were probably due to the different distributions and abundances of the native chalcid species on the Iberian Peninsula. For example, *Mesopolobus* species appear to be more abundant in Málaga than in the Galicia region (table 2), appearing as dominant species in the community of Málaga. This situation is especially noticeable for *M. tibialis*, which is a dominant species in the Málaga region but nearly an accessory species in Galicia. The species *M. lichtensteini* and *Mesopolobus mediterraneus* (Mayr, 1903) seem to represent a peculiarity of the Iberian *D. kuriphilus* communities (tables 2 and 3), as they are scarce or absent in other published European surveys of recruited parasitoids (Aebi *et al.*, 2007; Matošević and Melika, 2013; Panzavolta *et al.*, 2013; Quacchia *et al.*, 2013; Francati *et al.*, 2015; Kos *et al.*, 2015; Bonsignore and Bernardo, 2018). The presence and abundance of reared *M. lichtensteini* and *M. mediterraneus* in the Spanish *D. kuriphilus* communities (95 and 30, respectively, in this study (table 2) and 184 and 86 for the whole Iberian Peninsula) (table 3) could be due to their abundance generally on the Iberian Peninsula, contrasting with just a few records from other European countries, especially in northern Europe (Askew, 2013; Askew *et al.*, 2013). This finding could reinforce the idea that these species exhibit an Iberian distribution affinity.

In contrast, *O. pomaceus* appears to be dominant in the community of Galicia, replacing *Mesopolobus* species (fig. 3b). In fact, *O. pomaceus* occurs as one of the few effective or even accessory species in the Málaga community (fig. 3a) where it is rare (two individuals) with a mean occupancy percentage (45–55) and appearing in only two localities (Júzcar and Pujerra). The scarcity of *O. pomaceus* in the Málaga region (table 2) is difficult to explain, considering a large number of potential cynipid native hosts available here. However, as *O. pomaceus* is a cryptic species complex (Gómez *et al.*, 2017), it is possible that there are two different biological entities from this complex, in Galicia and Málaga, and that these have different roles in the native cynipid communities and, by extension, their recruitment by the new host *D. kuriphilus*. This may also be the case for other cryptic species complexes, such as *Torymus flavipes* (Walker, 1833) (Kaartinen *et al.*, 2010), *Eup. urozonus* (Askew and Nieves-Aldrey, 2000; Al Khatib *et al.*, 2014) and *Eur. bruniventris* (Lotfalizadeh *et al.*, 2007). Together with *Eurytoma pistaciae* Rondani 1877 (= *Eurytoma setigera* Mayr, 1878), these species are reported to have multivoltine cycles that could actually mask different biological units with the same or very similar morphology. *Eur. pistaciae* also seems to represent a peculiarity of the Iberian *D. kuriphilus* communities because, like *M. lichtensteini* and *M. mediterraneus*, more reared individuals were found in Spain (table 3) than were reported in previous papers on *D. kuriphilus* parasitoids in Europe. However, unlike the previously noted *Mesopolobus* species, *Eur. pistaciae* seems to exhibit a primarily circum-Mediterranean distribution (Askew *et al.*, 2013; Delvare *et al.*, 2019).

The nestedness diagram (fig. 3) and sS indices (table 5) show that the *D. kuriphilus* communities in Málaga and Galicia have different structures. The Málaga community exhibits the highest  $q_0$  sS index (total richness of species), although the Galicia region has the highest  $q_1$  and  $q_2$ sS indices (effective and dominant richness). Additionally, the relative abundance is higher in this region than in Málaga. The higher  $q_0$  diversity in Málaga might be explained by the proximity of the *D. kuriphilus* community to a more diverse community of native cynipids and their *Quercus* hosts, given that 5–7 *Quercus* species are present at most collection sites in Málaga, while 2–3 were the typical numbers in the Galicia communities (Stone *et al.*, 2012; Ferracini *et al.*, 2018). Overall, the region having the most disparate cynipid tree hosts was Eurosiberian Galicia, with a predominance of *Q. robur* and scarcity or the absence of *Q. ilex* and *Q. suber*, which is present in both Mediterranean Galicia and the Málaga region. The only cynipids associated with these *Quercus* species on the Iberian Peninsula are *Plagiotrochus* or the sexual generations of heteroecic *Andricus* (associated with *Q. suber*). In Central Europe, they are associated with *Quercus cerris* L. (Nieves-Aldrey, 2001). The co-occurrence of *Q. suber* and *Quercus* species of the *Quercus* section could possibly contribute to the success of heteroecic species of *Andricus*, making the rich parasitoid communities associated with native cynipids more stable and favouring the recruitment of more parasitoids by the *D. kuriphilus* community. The native cynipids, *Quercus* hosts and parasitoid diversity of native gall wasps are three factors that should be studied to better understand these ‘insular communities’ derived from components of the ‘mainland community’. In addition, the distances between the donor and sink communities and, more specifically, between the native cynipids and *D. kuriphilus* galls, are likely to also modulate community composition and species abundance associated with this invasive species.

Accessory species in *D. kuriphilus* communities ( $q_0$ – $q_1$ , fig. 3) are probably incidental species that are irregularly associated with *D. kuriphilus* galls and play no important role in these communities. These accessory species are more abundant in the Málaga region than in Galicia, probably due to the high global parasitoid richness in the former region, which is also related to the high diversity of *Quercus* species, which can harbour more diverse cynipid hosts.

The results of this study can be compared with previously published studies from Spain (table 3). For example, the most abundant species in Catalonia (data of 2013–2014) were *B. dorsalis* and *T. flavipes*, and these species played an important, though not dominant, role in the communities of Galicia and Málaga. The scarcity of *B. dorsalis* in the Galicia region was also indicated by Pérez-Otero *et al.* (2017). However, *T. flavipes* and *B. dorsalis* are among the most abundant species in the *D. kuriphilus* parasitoid community in other European territories (Aebi *et al.*, 2006; Quacchia *et al.*, 2012; Matošević and Melika, 2013; Bonsignore and Bernardo, 2018). It is possible that the roles of these parasitoids are very plastic and vary over time as these biological communities evolve into a more mature state. After all, these community analyses provide a current record of the state of these *D. kuriphilus* parasitoid communities which will undoubtedly change in the future. The realization of biological community studies and their monitoring over the years can show how *de novo* communities, such as the *D. kuriphilus* communities on the Iberian Peninsula evolve. Nevertheless, it will probably take many years for ecological succession to attain maturation. In fact, following the estimate of Cornell and Hawkins (1993), it

will take more than 150 years for a *de novo* community to reach its equilibrium; this is too long to wait for a solution to the problem imposed by *D. kuriphilus* on the Iberian Peninsula and in other parts of the world.

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