Differences in native and introduced chalcid parasitoid communities recruited by the invasive chestnut pest \textit{Dryocosmus kuriphilus} in two Iberian territories

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\textbf{Abstract}

\textit{Dryocosmus kuriphilus} (Hymenoptera: Cynipidae) is a global invasive gall wasp and a pest of chestnuts (\textit{Castanea} spp.). A study of the Chalcidoidea parasitoid community of \textit{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, \textit{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. \textit{Eupelmus ursonus} and \textit{Mesopolobus lichensteini} were the most abundant native species. \textit{Mesopolobus tibia} was a dominant species in south Spain, while \textit{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 \textit{Torymus sinensis}. These species were \textit{Torymus notatus}, \textit{Aulogymnus bicolor}, \textit{Aulogymnus obscuripes} and \textit{Aulogymnus balani}. Data on the recovery of \textit{T. sinensis} after release in the south Spain region show it to be well established, but its numbers are still low in northwest Spain.

\textbf{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 \textit{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 \textit{et al}., 1996; Pimentel \textit{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 \textit{et al}., 2006), mostly belonging to the order Hymenoptera that have theoretically co-speciated 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 \textit{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 \textit{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.
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 southeastern 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 Rieske, 2007; Quacchia et al., 2013; Matošević et al., 2016; Borowiec et al., 2018; Avtisz 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 Rieske-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 (EPPO, 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 (EPPO, 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-Saúndo 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
70 km², 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 km² 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 km²), 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.

### 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          | Vilarinho Das Touzas  | Galicia | 41.94513    | −7.31340    | 626     |
| 9          | Ríos                  | 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.
**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 de 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 Thuroczy for an update in some parasitoid families. Some of the reared parasitoid species, such as *Eupelmus urozonus* Dalman, 1820, *Ormyrus pomaceus* (Geoffroy, 1785), and *Bootanonymia 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; Gomés 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 (%)s 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 Chao-Jost 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 secondary fauna of Homoptera that use *D. kuriphilus* galls.
Table 2. List of inquilines 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 |      |
| Synergus facialis  | Cynipidae     | 2      | 0       | 2   | 2  |    |    |    |    |    |    |    |    |    |    |    |      |
| Aulogygnum bicolor| Eulophidae    | 6      | 0       | 6   |    |    |    |    |    |    |    |    |    |    |    |    |      |
| Aulogygnum balani | Eulophidae    | 23     | 0       | 23  |    |    |    |    |    |    |    |    |    |    |    |    |      |
| Aulogygnum obscuripes | Eulophidae | 2      | 0       | 2   |    |    |    |    |    |    |    |    |    |    |    |    |      |
| Eupelmus urozonus  | Eupelmidae    | 47     | 100     | 147 | 0  |
| Eupelmus azureus   | Eupelmidae    | 12     | 2       | 14  |    |
| Anastatus sp.      | Eupelmidae    | 0      | 1       | 1   |    |
| Eurytoma brunniventris | Eurytomidae | 2      | 1       | 3   | 24 | 2  | 19 |    |    |    |    |    |    |    |    |    |      |
| Eurytoma pistocia | Eurytomidae   | 9      | 9       | 18  |    |
| Sycophila biguttata| Eurytomidae   | 2      | 9       | 1   | 4  |
| Sycophila variegata| Eurytomidae   | 12     | 16      | 28  | 18 |
| Sycophila flavicollis | Eurytomidae | 1      | 1       | 0   | 1  |
| Ormyrus pomaceus   | Ormyriddae    | 2      | 8       | 8   | 3  | 11 | 33 | 24 | 1  |    |    |    |    |    |    |    |      |
| Mesopolobus mediterraneus | Pteromalidae | 9      | 21      | 30  |    |
| Mesopolobus sericeus | Pteromalidae | 1      | 0       | 1   |    |
| Mesopolobus tibialis | Pteromalidae | 27     | 13      | 30  | 23 | 2  | 11 | 3  | 95 | 14 | 109 |    |
| Mesopolobus lichtensteini | Pteromalidae | 26     | 11      | 4   | 23 | 8  | 1  | 6  | 2  | 1  | 2  | 4  | 2  | 4  | 1  | 73  | 22  | 95  |
| Pachyneuron muscorum* | Pteromalidae | 6      | 6       | 0   | 6  |
| Bootanomyia dorsalis | Megastigmidae | 1      | 5       | 2   | 8  | 4  | 14 | 1  | 2  | 17 | 8  | 46 | 54 |
| Torymus auratus    | Torymidae     | 2      | 2       | 4   |    |
| Torymus flavipes   | Torymidae     | 4      | 48      | 52  |    |
| Torymus sinensis   | Torymidae     | 9      | 6       | 181 | 15 | 1  | 2  | 8  | 7  | 2  |    |    |    |    |    |    | 212 |    | 231 |
| Torymus notatus    | Torymidae     | 10     | 5       | 18  | 1  |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 24  |

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.

*a*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 state occupancy percentage indicated the most-accessory 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 $<0.1$, 75%rs and 75%re), 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 $q_0 = 19$, Chao1 $q_0 \approx 19.1$, and a mean per locality of $q_0 = 9.2$ (table 5). The Galicia region exhibited an empirical $q_0 = 17$, Chao1 $q_0 \approx 17.5$ 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,a and 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*, *T. 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 *Sympophysa flavicollis* (Walker, 1834), *Au. obscuripes*, *Sympophysa bicutata* (Swederus, 1795) and *Eur. brunniventris* in Málaga and *T. notatus*, *Eupelmus azureus* Ratzeburg, 1844 (=*Eus. spongipartus* Förster, 1860 (Al Khatib et al., 2014)), *Mesopolobus sericeus* (Förster, 1770), and *Anastatus* sp. in Galicia, with the addition of the cynipid inquiline (*Synergus*) *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, *Symp. 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 et al., (Catalonia) (2016) | Pérez-Otero et al., (Galicia) (2016–2017) | Gavira et al., (Andalusia) (2015–2016) | Current article (Málaga, Andalusia) (2017–2019) | Current article (Galicia) (2017–2019) | Total Individuals/Species – Spain |
|--------------------------|--------------|-----------------------------------------|------------------------------------------|----------------------------------------|------------------------------------------------|----------------------------------|---------------------------------|
| Synergus facialis*       | Cynipidae    |                                          |                                          |                                        |                                                |                                  | 2                               |
| Eupelmus urozonus        | Eupelmidae   | 57                                      | 17                                       | 97                                     | 47                                             | 100                              | 318                             |
| Eupelmus azureus         | Eupelmidae   | 1                                       |                                          | 12                                     | 2                                              | 2                                | 15                              |
| Anastatus sp.            | Eupelmidae   |                                          |                                          |                                        |                                                | 1                                | 1                               |
| Eurytoma brunniventris  | Eurytomidae  | 4                                       | 49                                       | 7                                      | 2                                              | 49                               | 111                             |
| Eurytoma pistociae       | Eurytomidae  |                                          |                                          | 6                                      | 9                                              | 9                                | 24                              |
| Sycophila variegata      | Eurytomidae  | 32                                      | 13                                       | 114                                    | 72                                             | 113                              | 344                             |
| Sycophila biguttata      | Eurytomidae  | 2                                       | 7                                        | 2                                      | 14                                             | 14                               | 25                              |
| Sycophila flavicollis    | Eurytomidae  |                                          |                                          | 1                                      | 1                                              | 1                                | 2                               |
| Ormyrus pomoceus         | Ormyridae    | 65                                      | 8                                        | 2                                      | 2                                              | 80                               | 155                             |
| Ormyrus nitidulus        | Ormyridae    | 14                                      |                                          |                                        |                                                |                                  | 14                              |
| Mesopolobus mediterraneus| Pteromalidae | 4                                       | 2                                        | 50                                     | 9                                              | 21                               | 86                              |
| Mesopolobus amaenus      | Pteromalidae |                                          |                                          | 1                                      |                                                |                                  | 1                               |
| Mesopolobus tibialis     | Pteromalidae | 4                                       | 5                                        | 127                                    | 95                                             | 14                               | 245                             |
| Mesopolobus lichtensteini| Pteromalidae | 4                                       |                                          | 85                                     | 73                                             | 22                               | 184                             |
| Mesopolobus xanthocerus  | Pteromalidae |                                          |                                          | 1                                      |                                                |                                  | 1                               |
| Mesopolobus albitarsis   | Pteromalidae | 7                                       |                                          |                                        |                                                |                                  | 7                               |
| Mesopolobus fasciventris | Pteromalidae | 10                                      |                                          |                                        |                                                |                                  | 10                              |
| Mesopolobus sericeus     | Pteromalidae | 42                                      |                                          |                                        |                                                |                                  | 43                              |
| Pachyneuron muscarum     | Pteromalidae |                                          |                                          | 6                                      |                                                |                                  | 6                               |
| Cecidostiba fungosa      | Pteromalidae | 3                                       |                                          | 2                                      |                                                |                                  | 5                               |
| Bootanomyia dorsalis     | Megastigmidae| 128                                     | 7                                        | 78                                     | 8                                              | 46                               | 267                             |
| Bootanomyia dumicola     | Megastigmidae| 1                                       |                                          | 1                                      |                                                |                                  | 2                               |
| Monodontomerus aeneus    | Torymidae    | 1                                       |                                          |                                        |                                                |                                  | 1                               |
| Torymus notatus          | Torymidae    | 3                                       |                                          | 24                                     | 1                                              | 28                               | 28                              |
| Torymus erucarum         | Torymidae    | 7                                       |                                          |                                        |                                                |                                  | 7                               |
| Torymus flavipes         | Torymidae    | 152                                     | 48                                       | 21                                     | 4                                              | 48                               | 273                             |
| Torymus formosus         | Torymidae    | 4                                       |                                          |                                        |                                                |                                  | 4                               |
| Torymus nobilis          | Torymidae    | 1                                       |                                          |                                        |                                                |                                  | 1                               |
| Torymus auratus          | Torymidae    | 6                                       | 10                                       | 128                                    | 2                                              | 2                                | 148                             |

(Continued)
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. obscures* 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...
from a limited number of galls of the genera *Andricus* (this is the only known host) and *Plagiotrochus* (Askew 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 generation 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 Ríos 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 reared 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*

| Locality          | Region | q₀ | q₁ | q₂ | q₀–q₁ | q₀ | q₁ | q₂ |
|-------------------|--------|----|----|----|--------|----|----|----|
| Mabegondo         | Galicia| 4  | –  | –  | –      | 2  | –  | –  |
| Oza de los Ríos   | Galicia| 2  | 1.775 | 1.628 | 0.225 | 2  | 1.816 | 1.675 |
| Campus Lugo       | Galicia| 4  | –  | –  | –      | –  | –  | –  |
| Lousada           | Galicia| 10 | 5.618 | 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  |
| Ríos              | 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 |
| Igualaje          | Málaga | 10 | 6.83  | 5.687 | 3.17  | 10.495 | 7.155 | 5.945 |
| Yunquera          | 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            |        | q₀ | q₁ | q₂ | q₀–q₁ | q₀ | q₁ | q₂ |
| 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 ChaoJost 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. q₀ = total sS; in empirical q₀ = observed sS; q₁ = sS of effective species; q₂ = sS of dominant species; and q₀–q₁ = sS of accessory species.
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.

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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 bicolour; (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; Matosévić 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 (Goméz *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, 2006; Al Khatabi *et al.*, 2014) and *Eur. brunniventris* (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 $q_0$S 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 Malaga, 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 *Dryocosmus kuriphilus* on the Iberian Peninsula and in other parts of the world.

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**References**

Aebi A, Schönrogge K, Melika G, Alma A, Bosio G, Quacchia A, Picciau L, Abe Y, Moriya S, Yara K, Seljak G and Stone GN (2007) Native and introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*. *EPPO Bulletin* 37, 166–171.

Askew RR (1997) The organisation of chalcid-dominated parasitoid communities centred upon endophytic hosts. In Vinson SB and Price PW (eds), *Evolutionary Strategies of Parasitic Insects and Mites*. Boston, MA: Springer, pp. 130–153.

Askew RR, Nieves-Aldrey JL and Cooper WR (2009) The genus *Eupelmus*: a viable management option for the biological control of the Asian chestnut gall wasp *Dryocosmus kuriphilus*. *Journal of Pest Science* 8, 35–46.

Askew RR (2007) Community associates of an exotic gall-maker, *Dryocosmus Kuriphilus* (Hymenoptera: Cynipidae), across the heterogeneity of the Iberian Peninsula. *Entomologia Generalis* 4, 1–18.

Askew RR and Shaw MR (1986) Parasitoid communities: their size, structure, and development. In Waage J and Greathed D (eds), *Insect Parasitoids*. London, UK: Academic Press, pp. 225–264.

Askew RR, Plantard O, Gómez JF, Hernández-Nieves M and Nieves-Aldrey JL (2006) *Dryocosmus Kuriphilus* (Hymenoptera: Cynipidae), across the heterogeneity of the Iberian Peninsula. *Entomologia Generalis* 4, 1–18.
agallas de cinipidos (Hymenoptera, Cynipidae) en la Comunidad de Madrid. Graelis 62, 293–331.

Goméz JF, Hernández-Nieves M, Gayubo SF and Nieves-Aldrye JL (2017) Terminal-instar larval systematics and biology of west European species of Ormyridae associated with insect galls (Hymenoptera, Chalcidoidea). ZooKeys 644, 51–88.

Gurevitch J and Padilla DK (2004) Are invasive species a major cause of extinctions? Trends in Ecology and Evolution 19, 470–474.

Hawkins BA, Porter EE and Diniz-Filho JAF (2003a) Productivity and history as predictors of the latitudinal diversity gradient of terrestrial birds. Ecology 84, 1608–1623.

Hawkins BA, Field R, Cornell HV, Currie DJ, Gaégan JF, Kaufman DM, Kerr JT, Mittelbach GG, Oberdorff T, O’Brien EM, Porter EE and Turner JRG (2003b) Energy, water, and broad-scale geographic patterns of species richness. Ecology 84, 3105–3117.

Hyams DG (2007) CurveExpert Basic v1.4. Hyams Development.

Jara-Chiquito JL, Hera J and Pujade-Villar J (2016) Primeros datos de reclutamiento de hemipteros parasitoides autóctonos para Dryocosmus kuriphilus Yasumatsu (Hymenoptera: Cynipidae) en Cataluña (Península Ibérica). Boletín de la SEA 59, 219–226.

Jara-Chiquito JL, Askew RR and Pujade-Villar J (2020) The invasive ACGW Dryocosmus kuriphilus (Hymenoptera: Cynipidae) in Spain: native parasitoid recruitment and association with oak gall inducers in Catalonia. Boletín de la SEA 8, 47–54 (in Spanish).

Jara-Chiquito JL, Heras J and Pujade-Villar J (2020) The invasive ACGW Dryocosmus kuriphilus (Hymenoptera: Cynipidae) in Spain: its natural dispersal from France and the first data on establishment after experimental releases. Forest Systems 28, 1–11. https://doi.org/10.5424/fs/2019281-14361.

Nieves-Aldrye JL (1984a) Sobre las especies de Sycophila Walker, asociadas con agallas de cinipidos en la Península Ibérica, con descripción de una nueva especie. Eos 59, 179–191 (in Spanish).

Nieves-Aldrye JL (1984b) Observaciones sobre los torímidos (Hym., Chalcidoidea, Toremidae) asociados con agallas de cinipidos (Hym., Cynipidae) sobre Quercus Sp. en la zona centro-occidental de España. Boletín de la AEE 8, 121–134 (in Spanish).

Nieves-Aldrye JL (2001) Hymenoptera: Cynipidae, vol. 16. Madrid, España: Editorial CSIC-CSIC Press (in Spanish).

Nieves-Aldrye JL, Gil-Tapetado D, Gavira O, Boyero JR, Polidori C, Lombardero MJ, Blanco D, Rey Del Castillo C, Rodriguez-Rojo MP, Vela JM, Gascón R and Wozny E (2019) Torymus sinensis Kamijo, a biocontrol agent against the invasive chestnut gall wasp Dryocosmus kuriphilus Yasumatsu in Spain: its natural dispersal from France and the first data on establishment after experimental releases. Forest Systems 28, 1–11. https://doi.org/10.5424/fs/2019281-14361.

Mayr colectadas en el Nordeste de la Península Ibérica. Boletín de la SEA 11, 101–134 (in Spanish).

Moriya S, Shiga M and Adachi I (2003) Classical biological control of the chestnut gall wasp in Japan. In Van Driesche RG (ed.), Proceedings of the 1st International Symposium on Biological Control of Arthropods. Washington, DC, USA: USDA Forest Service, pp. 407–415.

Nicholls JA, Preuss S, Hayward A, Melika G, Čoška G, Nieves-Aldrye JL, Askew RR, Tavakoli M, Schönnógk K and Stone GN (2010) Concordant phylegogenetics and cryptic speciation in two Western Palaearctic oak gall parasitoid species. Molecular Ecology 19, 592–609.

Nieves-Aldrye JL (1983) Contribución al conocimiento de los eulófidos (Hym., Chalcidoidea, Euploidae) parasitidos en las agallas de cinipidos producidas sobre especies de Quercus. Boletín de la AEE 7, 43–54 (in Spanish).

Pérez-Otero R, Crespo D and Mansilla JP (2013) Native parasitoids associated with Dryocosmus kuriphilus Yasumatsu in Spain: its natural dispersal from France and the first data on establishment after experimental releases. Forest Systems 28, 1–11. https://doi.org/10.5424/fs/2019281-14361.

Noyes JS (2019) Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidooids.

Oho N and Umeya K (1975) Occurrence of the chestnut gall wasp in the People’s Republic of China. Shokubutsu Boeki 29, 463–464 (in Japanese).

Panzavolta T, Bernardo U, Bracalini M, Cascone P, Croci F, Gebiola M, Iodice L, Tiberi R and Guerrieri E (2013) Native parasitoids associated with Dryocosmus kuriphilus in Tuscany, Italy. Bulletin of Insectology 66, 195–201.

Payne JA, Menke AS and Schroeder PM (1975) Dryocosmus kuriphilus Yasumatsu (Hymenoptera: Cynipidae), an oriental chestnut gall wasp in North America. Cooperative Economic Insect Report 25, 903–905.

Pechar L and Mooney HA (2009) Invasive species, ecosystem services and human well-being. Trends in Ecology and Evolution 24, 497–504.

Pérez-Otero R, Crespo D and Mansilla JP (2017) Dryocosmus kuriphilus Yasumatsu, 1951 (Hymenoptera: Cynipidae) in Galicia (NW Spain): pest dispersion, associated parasitoids and first biological control attempts. Arquivos Entomológicos 17, 439–448.

Pimentel D, Zuniga R and Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52, 273–288.

Pogolotti C, Cuesta-Porta V, Pujade-Villar J and Ferrari C (2018) Seasonal flight activity and genetic relatedness of Torymus species in Italy. Agricultural and Forest Entomology 21, 159–167.

Pujade-Villar J (1991) Contribuíció al coneixement dels cinípsids cecidògenos dels arbres i arbusts de Catalunya, dels cinípsids associats a quèstius dels seus parasits (Unpublished PhD Thesis), University of Barcelona, Spain.

Pujade-Villar J and Ros-Farré P (1998) Inquinolinos y parasitoides de las agallas del género Plagiotrochus Mayr colectadas en el Nordeste de la Península Ibérica. Boletín de la AEE 22, 115–143 (in Spanish).

Pujade-Villar J, Askew RR, Grami M and Janáa MLB (2010) On Synophrus olivierni (Hymenoptera, Cynipidae) and its parasitoids (Hymenoptera, Chalcidoidea) found in the Khmir mountains (Tunisia). Boletín de la SEA 47, 383–387.

Pujade-Villar J, Torrell A and Rojo M (2013) Primeres troballes a la peninsula Ibèrica de Dryocosmus kuriphilus (Hym., Cynipidae), una espècie de cinipí d'origen asiàtic altament perillosa per a l'arbolat de Quercus. Revista de la SEA 387, 171–186 (in Spanish).

Quachcia A, Moriya S, Bosio G, Scapin I and Alma A (2008) Rearing, release and settlement prospect in Italy of Torymus sinensis, the biological control agent of the chestnut gall wasp Dryocosmus kuriphilus. BioControl 53, 829.
