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Dryocosmus kuriphilus Yasumatsu, 1951 (Hymenoptera: Cynipidae) in Galicia (NW Spain): pest dispersion, associated parasitoids and first biological control attempts.

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Abstract: *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) is the most important European sweet chestnut *Castanea sativa* pest. It was first detected in Galicia (NW Spain) in 2014 and it represents a major threat for the Galician chestnut production. Since its arrival, its dispersion pattern in the Galician councils and its phenology and native associated parasitoids have been studied. After some field releases in controlled areas, individuals of its parasitoid *Torymus sinensis* Kamiyo, 1982 (Hymenoptera: Torymidae) are also being recovered. Moreover, seventeen cultivars are still under study in order to assess varietal susceptibility. In this paper the first results of these studies are presented.

Key words: Hymenoptera, Cynipidae, Torymidae, *Dryocosmus kuriphilus*, chestnut gall wasp, *Torymus sinensis*, parasitoid, Galicia, NW Spain.

Resumen: *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) en Galicia (NO España): dispersión de la plaga, parasitoides asociados y primeros intentos de control biológico. *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) es la plaga más importante del castaño europeo *Castanea sativa*. Se detectó por primera vez en Galicia (NO España) en 2014 y representa la mayor amenaza para la producción gallega de castaña. Desde su llegada se ha estudiado su patrón de dispersión por los ayuntamientos gallegos, su fenología y los parasitoides autóctonos asociados. También los parasitoides *Torymus sinensis* Kamiyo, 1982 (Hymenoptera: Torymidae) recuperados después de algunas sueltas en áreas controladas. Además, se están estudiando 17 cultivares para conocer su sensibilidad. En esta publicación se presentan los primeros resultados de estos estudios.

Palabras clave: Hymenoptera, Cynipidae, Torymidae, *Dryocosmus kuriphilus*, avispa gallícola del castaño, *Torymus sinensis*, parasitoides, Galicia, NO España.

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Introduction

Chestnut trees are forest species with great economical importance worldwide (BOUNOUS, 2014). Sweet chestnut *Castanea sativa* Mill., 1768 is the only native European species among the six *Castanea* spp., and it covers a total area of 2.5 million ha in Europe (CONEDERA et al., 2016). Economical exploitation of *C. sativa* and its hybrids comprises both timber and fruit production. Spanish sweet chestnut fruit plantations encompass approximately 31000 ha (MAPAMA, 2015), being located in Galicia (NW Spain) the 78% of such plantations. As a consequence, the economical importance of this crop is crucial for the local economy.

The Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae) is considered the most dangerous pest of *Castanea* spp. and their hybrids. It has caused serious damages in its origin area (China) and neighbouring countries (Japan and Korea) to different chestnut tree species (BERNARDO et al., 2013). Typical injuries comprise the formation of galls on the

new spring shoots (GRAZIOSI & SANTI, 2008). As a consequence, twig growth is hindered, and fruit and timber production is severely narrowed (EFSA, 2010). Indeed, pest can even be life-threatening for younger trees in high-pest level locations (EPPO, 2005).

Dryocosmus kuriphilus is present worldwide; it was spread from China to Japan, Korea, USA and Canada (GIBBS et al., 2011). It is added to quarantine pest list (USA) in 1994, and to EPPO A2 list in 2003. European Union established emergency measures in 2006, and it is added to 2000/29/CE Annex I/B in 2014. European dispersion has been delayed by forbidding importation of *Castanea* plants (except fruits and seeds) from non-European countries (EPPO, 2005). However it was finally introduced in Europe through Italy, in 2002, and then dispersed throughout many countries (GIBBS et al., 2011; MATOŠEVIĆ et al., 2014). It was first detected in NE Spain (Catalonia) in 2012 (DOGC, 2012). Two years later the wasp was first spotted in Galicia (PÉREZ-OTERO & MANSILLA, 2014) and in North Portugal (EPPO, 2014).

The cynipid wasp is univoltine and thelytokous (i.e. only females develop from unfertilized eggs). Adult winged wasps emerge from galls and immediately can penetrate and infect new buds. Females can lay over up to 100 eggs (some buds can contain 20-30 eggs, EPPO, 2005).

The lack of natural enemies when an allochthonous species is introduced in a new ecosystem is a fundamental factor for its fast expansion (QUACCHIA et al., 2008). Nevertheless, some local parasitoids are capable of parasitizing cynipid wasps (see, for example, KOS et al., 2015; COLOMBARI & BATTISTI, 2016). Thus indigenous and potential *D. kuriphilus* parasitoids have received much attention in the last years (e.g. ASKEW & NIEVES-ALDREY, 2004; ZEROVA & SERYOGINA, 2006; ABE et al., 2007; SANTI & MAINI, 2011; FERRACINI et al., 2015) in order to know if they are suitable biological control agents capable to protect chestnut tree plantations and ecosystems.

Some Chinese *D. kuriphilus* native populations are kept at low densities, probably due to the presence of natural enemies (GIBBS et al., 2011). *Torymus sinensis* Kamiyo, 1982 (Hymenoptera: Torymidae) seems to be the major Chinese population control responsible. Indeed, it has a good synchronization with *D. kuriphilus* life cycle (AEBI et al., 2011) and it is highly host specific (MORIYA et al., 2003). Hence, *T. sinensis* was released and successfully established as a biocontrol agent in Japan (reviewed in AEBI et al., 2006). Afterwards, it was applied in USA and Italy (see GIBBS et al., 2011, for a review).

However, some issues have been posed regarding *T. sinensis* introduction. For instance, local parasitoids displacement, host shift (it was already proved by FERRACINI et al., 2015) and hybridization between *T. sinensis* and native *Torymus* species (GIBBS et al., 2011). Actually, the study of both host shift and hybridization with local *Torymus* spp. is nowadays mandatory in order to obtain official permission by the Spanish Ministry of Agriculture and Fisheries, Food and Environment (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, MAPAMA) to perform controlled release assays.

The establishment of new biological control strategies should take into account additional putative damages and threats by *D. kuriphilus* to other species. In fact, the attacks of *Dryocosmus* spp. to other Fagaceae genus (e.g. *Chrysolepis*, *Castanopsis* and *Quercus*) are well documented (MELIKA, 2006; ABE et al., 2007). In Galicia, *Quercus robur* L. is a fundamental species since it has a key role forming the indigenous Atlantic Forest core. Besides, *Quercus suber* L. is a species in regression with great ethnographic and high conservation value. Therefore *D. kuriphilus* eventual host shift should be carefully considered.

Furthermore, integrated pest management strategies should consider as well differential natural responses among chestnut varieties in order to promote less susceptible cultivar plantations to effectively control the pest. Indeed field observations indicate that with similar pedoclimatic conditions different sweet chestnut varieties present dissimilar susceptibility to *D. kuriphilus* attacks (see as an example PANZAVOLTA et al., 2012). Actually some hybrids (e.g. "Bouche de Bétizac", a *C. sativa* and *C. crenata* hybrid) show strong responses against gall wasp attacks (DINI et al., 2012).

The spread of *D. kuriphilus* in Galicia seriously threatens the sweet chestnut timber and especially the fruit production. Since its arrival in 2014, we have been studying its dispersion, phenology, associated indigenous parasitoids, *Castanea* spp. hybrids susceptibility and *T. sinensis* effectiveness in order to slow down the pest. The aim of this publication is to present our first results and current research status.

Material and methods

D. kuriphilus dispersion

Sampling campaigns were carried out in Galicia since 2014 by Estación Fitopatológica Areeiro (EFA, Deputación de Pontevedra) in collaboration with SEAGA, a public company related to Consellería do Medio Rural (Xunta de Galicia). Main initial criteria for campaign design were *D. kuriphilus* personal and authority reports (i.e. infested and neighbouring areas) and high chestnut fruit production locations. Sampling radius and transected areas were progressively increased as more infested areas were detected. We have as well taken advantage of ongoing sampling campaigns of different plant species to sample *Castanea* spp. too.

D. kuriphilus phenology and indigenous parasitoids

Galician chestnut buds (sampled in winter) and galls, especially from Central Ourense and from controlled enclosures (see next sections for further explanation) were monthly analyzed and *D. kuriphilus* phenological stages were recorded. Sampling intensity was variable among different campaigns according to qualified staff availability. Different gall numbers were sampled from 2014 (256, 1428, 14408 and 2768 galls in 2014, 2015, 2016 and 2017 respectively; current sampling campaign is still not closed).

Larvae phenological stages were named according to VIGGIANI & NUGNES (2010). Samples were sliced and studied under magnifier and some parasites were examined right after gall emergency. The occurring parasitoids were identified and recorded as well.

Biological control by *Torymus sinensis*

Precedents of serious damages in the other European countries where ACGW was introduced (see as an example BERNARDO *et al.*, 2013) and density of galls in chestnut tress in some locations constrained us to initiate immediate *Torymus sinensis* importation to try to control pest. EFA started the required procedure for the importation of *T. sinensis* with experimental aims, through the appropriate authorization applications to MAPAMA. We finally acquired import permission in September 2014, and *Torymus sinensis* samples were supplied by GreenWood Service S.r.l. (Italy).

T. sinensis field releases were performed in 2015, 2016 and 2017 in controlled areas by Xunta de Galicia by means of SEAGA. Number of released vials was 105 (19 individuals in each vial, 7♂♂ and 12♀♀), 2100 (32 individuals, 12♂♂ and 20♀♀) and 4200 (36 individuals, 14♂♂ and 22♀♀) respectively. After the first release we have periodically received samples from SEAGA to confirm the occurrence of *T. sinensis*.

Fagaceae and *Castanea* hybrids resistance

Seventeen Spanish (especially Galician and Andalusian) *Castanea* hybrids cultivars were planted in two controlled enclosures sealed with anti-trips net and double entrance. Cultivars were exposed to *D. kuriphilus* to assess their natural resistance. Considered cultivars were "Famosa", "Inxerta", "Judia R", "Longal", "Martaiña", "Pareda", "Amarelante", "Chamberga", "Comisaria", "Helechal", "Luguesa", "Negral", "Pilonga", "Planta Alajar", "Temprana", "Ventura" and "Verata" (see CUENCA VARELA &

MAJADA GUIJO, 2014 for more varietal information). "Bouche de Bétizac", a resistant hybrid between *Castanea sativa* and *C. crenata* (BOTTA et al., 2010) was planted as well. Seven cultivars were planted in January 2015 in one enclosure and 13 were planted in April 2016 in another one. The first one was exposed to a release of 1594 female wasps between June and August 2015 whereas the latter was semiforced defiled by communicating it with a contaminated enclosure during the *D. kuriphilus* flight period in 2016. Ten replicates were planted for each cultivar. Gall presence was biweekly studied in the chestnut vegetative period, although the results are expressed monthly.

We have included in our experiments three extra Fagaceae species (*Quercus robur* L., *Quercus ilex* L. and *Quercus suber* L.), which were exposed to *D. kuriphilus* in a separated enclosure to assess gall formation feasibility.

Results and discussion

D. kuriphilus dispersion

Current wasp distribution and dispersion pattern is shown in Fig. 1. Its meteoric expansion entails the perfect adaptation of the gall wasp to the Galician climatic conditions. In 2014 *D. kuriphilus* was detected in 13 out of 314 Galician councils, whereas three years later it is present in 151, almost half of the territory. The highest detection year was 2015, in which 71 new councils were affected. Dispersion data are unequal among the four Galician provinces: Ourense is the most affected (the wasp was detected in 93% of the councils) whilst Pontevedra has only 18%. Lugo and A Coruña have a condition percentage of 51 and 22% respectively. This inequality could be a consequence of the different chestnut tree planted area and density and putative arrival areas of *D. kuriphilus*.

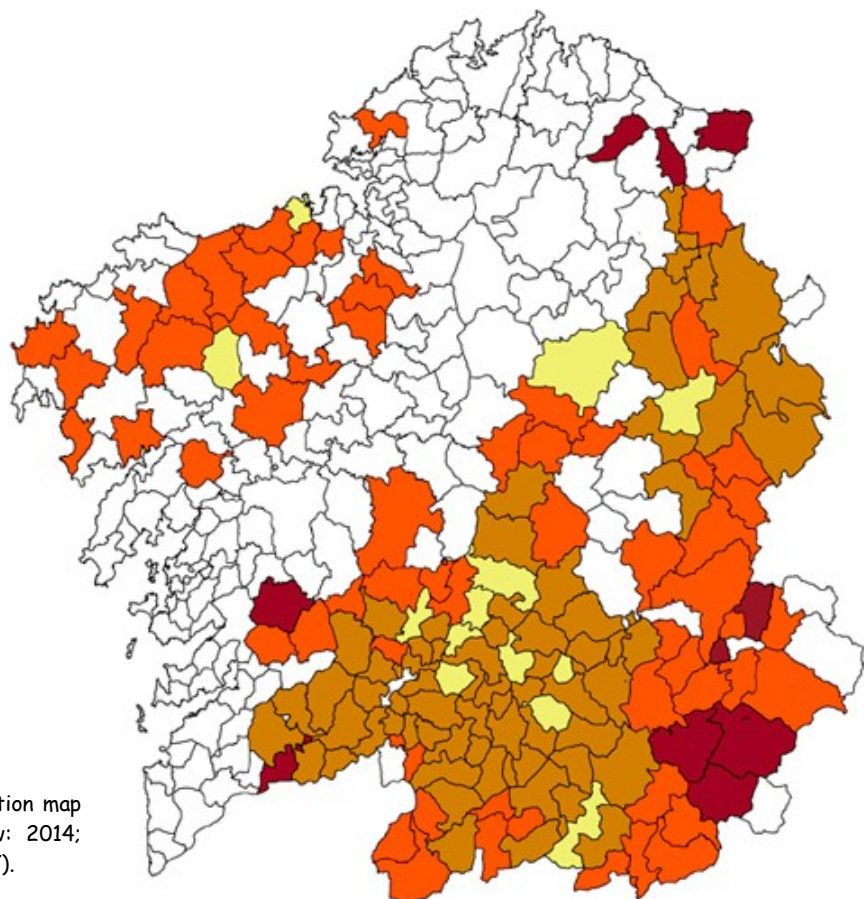


Fig. 1.- *D. kuriphilus* Galician distribution map by council and detection year (yellow: 2014; brown: 2015; orange: 2016; garnet: 2017).

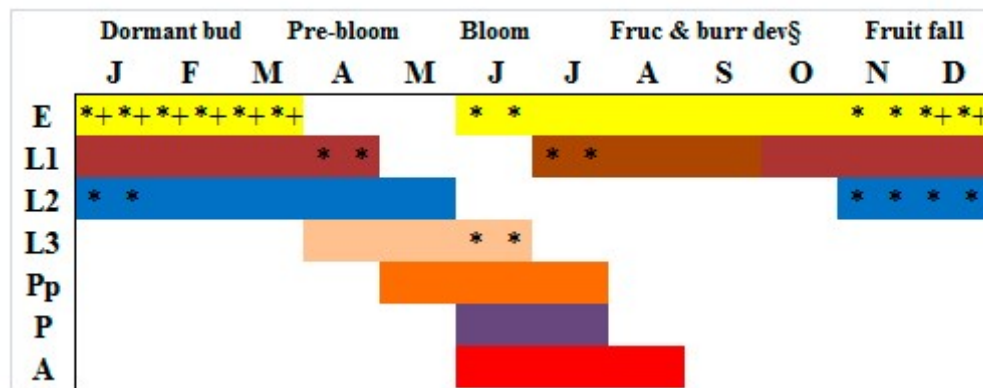


Fig. 2.- Monthly *D. kuriphilus* phenology (E: egg; L1: first-instar larvae; L2: intermediate instar larvae; L3: terminal-instar larvae; Pp: pre pupae stage; P: pupae stage; A: adult). *: punctual presence; +: sterile eggs. Sweet chestnut fruit phenology is given for orientation (§: fructification and burr development).

D. kuriphilus phenology and parasitoids

Phenological results are summarized in Fig. 2. Eggs were found all year long excluding April and May (although in January, February, March, June, November and December egg presence was sporadic). Wintering eggs presence was punctual and eggs were considered non viable (actually some of them could be exuviae). Larvae presence is more time-concentrated as larval stage becomes higher. First-instar larvae were found the whole year excluding May, and adults flight on June, July and August.

Our results slightly differ from those obtained by BERNARDO *et al.* (2013) and JARA (2015), although we could just contrast similar phenological stages (notice in both different larvae notation). Main divergence was found in egg presence. Those researchers found a narrower egg presence period, albeit we have considered our recorded egg presence for half a year as punctual.

We have interpreted JARA's (2015) first larvae stage as L1 and second larvae stage as L2 and L3 following his pictures. Our first-instar larvae results match with those obtained by JARA (2015) although are quite dissimilar to those found by BERNARDO *et al.* (2013). Interestingly we have punctually detected L2 larvae in November and December 2016, explained by particular Galician climatic conditions that year. Adults flight period was similar between all performed and considered research.

Different gall provenances (with different climatic conditions) could provide a feasible explanation for phenological differences. In addition Galician climatic conditions in 2016 were specially warm and dry and, as a consequence, L1 larvae hatching could begin earlier.

Recorded parasitoids and abundances are shown in Table 1. A total number of 201 parasitoid individuals belonging to at least 9 species were present in approximately 16100 galls analyzed between 2014 and 2016. In 2014 the dominant species were *Ormyrus pomaceus* Geoffroy, 1785, *Mesopolobus mediterraneus* Mayr, 1903 and *Torymus flavipes* Walker, 1833. The first two species were present in higher numbers as well in 2015 whereas in 2016 *Eupelmus urozonus* Dalman, 1820 was the most abundant species. The situation depicted in the last two years is quite different from 2014 since species abundance is by far more distributed and there is not a clear dominance. Dominant parasitoids seem to be quite characteristic depending on the studied site, albeit *T. flavipes* is prevalent in previous European research (see Table 2).

All recorded parasitoids were previously found and associated to *D. kuriphilus* galls in previous works except *Mesopolobus xanthocerus* Thomson, 1878, which is mainly found associated to *Andricus* spp. galls (see ASKEW *et al.*, 2013 for a review). This is, to our knowledge, the first record of *M. xanthocerus* associated to *D. kuriphilus* galls. Parasitoid results are quite complex to interpret in bibliography (see Table 2 and references therein) because they are greatly variable with time. In the

first place, parasitoid records vary according to sampling effort. However differences between 2014 and 2016 (a total number of 123 and 67 recovered parasitoids respectively) cannot be explained by sampling issues. In addition it has been documented that adaptation process between new hosts and native parasitoids requires time and it is very complex (see as an example FRANCATI et al., 2015: they have not obtained a regular increment of parasitoid abundance, but erratic results through consecutive sampling campaigns). A constant rise in the number and diversity of parasitoids could be hypothesized but further research is needed to assess the ongoing host-parasitoid adaptation process along the next years.

Biological control by *Torymus sinensis*

Sampling reception and processing is not still closed, and thus these preliminary results could significantly change (particularly if we consider annual variations). A total number of 220395 *T. sinensis* (135660 females) were release in three different campaigns and only 12 were recovered in Ourense within SEAGA's release sampling sites. *T. sinensis* fecundity is noteworthy (females can lay over up to 71 eggs, PIAO & MORIYA, 1992) but our results are not reflecting it. We hypothesize that the poor results could be a consequence of a bad release schedule and time span choice. Optimal releases should be performed in a very specific and short span periods concurring with low gall and concrete developmental stage (BERNARDO et al., 2013). Therefore an inadequate releasing choice could lead to a mismatch between two naturally synchronized species. In addition both species have different biotic potential (*T. sinensis* potential is smaller than that of *D. kuriphilus*) and this could lead to a slower interspecific population balance.

Cultivar resistance

Results are shown in Fig. 3. First results revealed "Inxerta" and "Judia R" as the most susceptible varieties (they presented a total amount of 183 and 162 galls respectively, therefore a mean by tree of 18 and 16 galls) whereas "Pareda" and "Martaiña" were the less attacked cultivars. "Famosa" and "Longal" presented 99 and 66 galls respectively. "Bouche de Bétizac" did not present galls at all as expected.

The existence of indigenous resistant or tolerant varieties could partially reduce gall wasp problem in Galicia. Indeed "Pareda" preliminary results were truly promising with a complete absence of galls. Unfortunately one gall finally appeared in 2 trees in 2017, but it is still the less susceptible cultivar to the pest. Fortunately, none of the three additional Fagaceae species studied

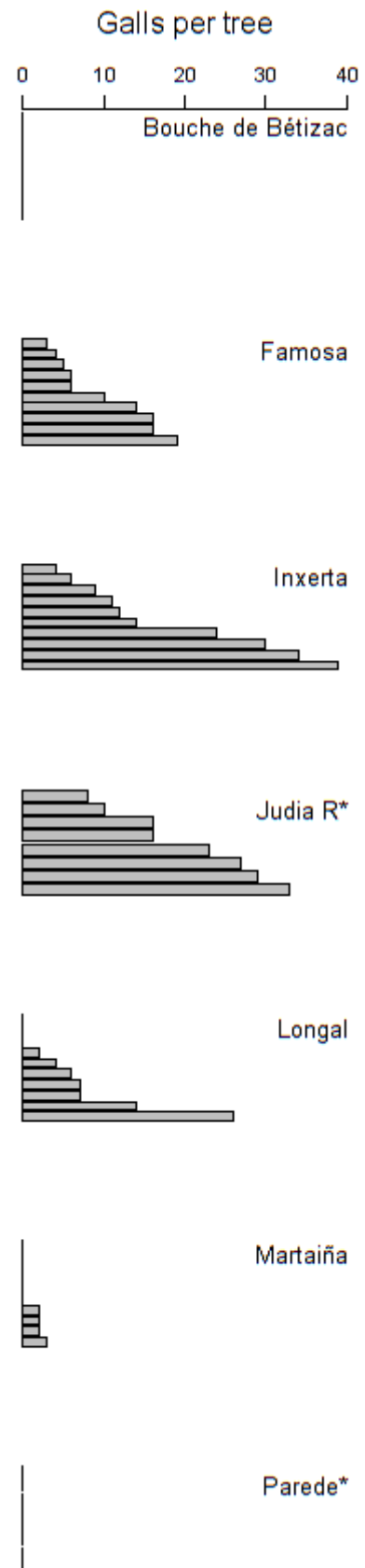


Fig. 3.- *D. kuriphilus* galls per chestnut tree barplot by variety in 2016. Each bar represents one tree. Ten individuals were considered for each variety except for "Judia R" (n=8) and "Pareda" (n=8). *: two individuals were supplied misidentified by the nursery.

presented gall formation so far. However these are preliminary results and therefore they should be treated with caution. Actually breeding of resistant chestnut varieties was carried out successfully for approximately 20 years in Japan (BERNARDO *et al.*, 2013). Nevertheless a specific ecotype of *D. kuriphilus* was able to overcome chestnut resistance and it became the dominant ecotype (MORIYA *et al.*, 2003). Thus further pest-plant association research is needed to design a successful and integrate control strategy.

Conclusions

1. *Dryocosmus kuriphilus* is present in almost half of Galician territory, and it is seriously threatening chestnut tree populations.
2. The presence of native parasitoids is still low, but their population is expected to be increased in the next years.
3. "Pareda" could be the less susceptible variety to *D. kuriphilus* so far.
4. *Torymus sinensis* releases were not successful so far, but we expect to obtain better yields in next releasing campaigns by refining releasing time.

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Year	2014	2015	2016	2017*	Total
<i>Eupelmus urozonus</i> Dalman, 1820	3	3	17		
<i>Eupelmus</i> sp.			2		
<i>Eurytoma brunniventris</i> Ratzeburg, 1852			5	44	
<i>Eurytoma</i> sp.			2		
<i>Megastigmus</i> sp.			7		
<i>Mesopolobus mediterraneus</i> Mayr, 1903	13	4		2	
<i>Mesopolobus tibialis</i> Westwood, 1833				5	
<i>Mesopolobus xanthocerus</i> Thomson, 1878	5		1		
<i>Mesopolobus</i> sp.			1	1	
<i>Ormyrus pomaceus</i> Geoffroy, 1785	85	4	8		
<i>Sycophila biguttata</i> Swederus, 1795				7	
<i>Sycophila variegata</i> Curtis, 1831			1	12	
<i>Sycophila</i> sp.			2	1	
<i>Torymus auratus</i> Müller, 1764				10	
<i>Torymus flavipes</i> Walker, 1833	17		8	40	
<i>Torymus sinensis</i> Kamiyo, 1982			12		
<i>Torymus</i> sp.			1	2	
Total	123	11	67	124	325

Table 1. - Presence and abundance of *D. kuriphilus* parasitoids in chestnut galls.

*: First emergences from samples collected until June 2017.

Research	S	Dominant species	P / gall	Country
Matošević & Melika (2013)	15	<i>Mesopolobus tibialis</i> , <i>Torymus flavipes</i> and <i>Megastigmus dorsalis</i>	0,04	Croatia
Panzavolta et al. (2013)	10	<i>Torymus flavipes</i>	0,05	Italy (Tuscany)
Francati et al. (2015)	10	<i>Torymus flavipes</i>	0,16	Italy (Emilia Romagna)
Jara (2015)	14	<i>Megastigmus dorsalis</i> , <i>Torymus flavipes</i>	0,13	Spain (Cataluña)
Current publication	12	<i>Ormyrus pomaceus</i> , <i>Torymus flavipes</i> and <i>Eupelmus urozonus</i>	0,01	Spain (Galicia)

Table 2. - Summary of previous native parasitoids studies in Europe versus the present research. S: Number of species (when genus was given, it was reckoned as one species); P / gall: ratio between recovered parasitoid individuals and number of studied galls.