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# Recruitment of native parasitoids to a new invasive host: first results of *Dryocosmus kuriphilus* parasitoid assemblage in Croatia

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## Abstract

Research on recruitment of native parasitoids on the recently introduced invasive species *Dryocosmus kuriphilus* has been carried out on four sites in 2011 and 2012 in Croatia. In total 15 species of native parasitoids were reared which belong to 5 of 6 chalcid families attacking native oak cynipid gallwasps (Eupelmidae - 2, Eurytomidae - 4, Ormyridae - 1, Pteromalidae - 4 and Torymidae - 4 species). This research has shown that the time lag between introduction of new host and recruitment of native parasitoids is short. Sex ratios for the most abundant parasitoid species appeared to be female-biased and the parasitoid emergence rate for all sites was relatively low. *Torymus flavipes* was recorded from *D. kuriphilus* at all sites and in both years and was the most abundant species exploiting the new host. Among other species, especially *T. flavipes* and *Megastigmus dorsalis*, which have been reared from *D. kuriphilus* galls, could provide a good possibility for biological control but further research is needed.

**Key words:** invasive species, Cynipidae, host-shift, Chalcidoidea, *Torymus flavipes*.

## Introduction

Invasive species are considered a major threat to biodiversity (Williamson, 1996; Walker and Steffen, 1997; Kamata and Gottschalk, 2007; Scalera, 2010) as well as an increasing economic concern (Vitousek *et al*., 1996; Wilcove *et al*., 1998; Pimentel *et al*., 2000). Alien species often become invasive due to the lack of natural enemy attack in the invaded territory, which is also known as the Enemy Release Hypothesis (Sakai *et al*., 2001; Lockwood *et al*., 2006; Roy *et al*., 2011). *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae) is one of the most recent invasive species in European forest ecosystems (Aebi *et al*., 2006; Seljak, 2006; Csóka *et al*., 2009; Forster *et al*., 2009), particularly in Croatia (Matošević *et al*., 2010) threatening sweet chestnut trees (*Castanea sativa* Mill.) and their hybrids. The invasion history of *D. kuriphilus* has already been well documented (Aebi *et al*., 2006; Graziosi *et al*., 2008; Gibbs *et al*., 2011). It is considered to be one of the most important pests of chestnut trees worldwide inducing galls on leaves and shoots, causing decline in vigour of trees and significantly reducing fruit yield (EFSA, 2010). *D. kuriphilus* has one generation each year, and to our knowledge entirely parthenogenetic females lay eggs in buds of chestnuts during the summer. The first instar larva overwinters in buds and the following spring induces galls on newly emerged leaves and shoots (Brussino *et al*., 2002; Bosio *et al*., 2010; Santi and Maini, 2012).

New invaders may act as foci for assembly of native species that may use them as additional resources (Cornell and Hawkins, 1993). Over the years following the introduction and spread of *D. kuriphilus* in Japan, Korea, USA and Europe, the new host has quite rapidly been colonized by a rich parasitoid assemblage mainly by native generalist chalcid species (Hymenoptera Chalcidoidea) which attack galls induced by cynipid gallwasps (Hymenoptera Cynipidae) on oaks (*Quercus* spp.) (Aebi *et al*., 2006; 2007; Cooper and Rieske, 2007; 2011; Gibbs *et al*., 2011). Generalist parasitoids can shift hosts more easily and thus are more successful in colonizing invasive host (Cornell and Hawkins, 1993; Hawkins, 2005). About one hundred morphospecies of Chalcidoidea are parasitoids in oak cynipid galls in the Western Palaearctic (Askew *et al*., 2013), however many of them cannot shift onto new invasive hosts due to life cycle and biological constraints (Bailey *et al*., 2009). Thirty-two native parasitoid species in six chalcid families (Eurytomidae, Pteromalidae, Torymidae, Eupelmidae, Ormyridae and Eulophidae) parasitise *D. kuriphilus* in Italy (Quacchia *et al*., 2013) and *Torymus sinensis* Kamijo (Hymenoptera Torymidae), a native parasitoid from China, is being released in Italy (Quacchia *et al*., 2008; Gibbs *et al*., 2011). The attack rates of indigenous parasitoids in Japan, South Korea, USA and Italy vary from 2% (Cooper and Rieske, 2007; Gibbs *et al*., 2011; Quacchia *et al*., 2013) to 32% in some regions of Italy (Santi and Maini, 2011).

As various measures to control *D. kuriphilus* populations have proven unsuccessful or too labour intensive (use of chemical pesticides, pruning, mechanical protection, selection of resistant varieties) (Aebi *et al*., 2007), native and introduced parasitoids have been recognized as a potential solution *via* biological control (Aebi *et al*., 2006; 2007; Cooper and Rieske, 2007; 2011; Gibbs *et al*., 2011; Quacchia *et al*., 2013).

Croatia has natural sweet chestnut (*C. sativa*) forests in the mainland and in the Istria peninsula, usually mixed with oak species [*Quercus petraea* (Mattuschka) Liebl., *Quercus pubescens* Willd., *Quercus cerris* L.]. In Istria old chestnut trees and their fruits are an important part of local heritage (so called "maruni").

The main objectives of our study are to address the following questions: i) Have parasitoids started to shift to this new invasive host, assuming that the invading *D. kuriphilus* has recruited a native parasitoid complex from the cynipid oak gall community? ii) Does the number of parasitoid species shifting to the new host, and their abundance, correlate positively with the duration of new host presence? The prediction is that both will, following previous work on parasitoid recruitment to invading oak gallwasps in western Europe (Schönrogge *et al*., 1995; 1996a; 1996b). iii) Have any cynipid inquilines (Synergini) colonised *D. kuriphilus* galls in Croatia? Cynipid inquilines are a dominant component of oak cynipid communities (Ács *et al*., 2010), but have not yet been recorded from any samples of *D. kuriphilus*.

Gall wasps are good models for investigating ecological community interactions including parasitoid attack because these interactions occur within gall and are easily quantifiable (Stone *et al*., 2002). The significance of host tree taxon on parasitoid community structure has also been studied in the oak gall system (Bailey *et al*., 2009). This is the first research of this kind in Croatia.

## Materials and methods

Parasitoids were reared from galls of *D. kuriphilus* collected in 2011 and 2012. The galls were collected in spring (newly formed galls) starting in the second half of May until the first half of June. Prior to collection, all galls were inspected in the field for the developmental stage of larvae so that the galls are not collected too early and to check for any exit holes of parasitoids (to be sure that the parasitoids have not start emerging). In this study the overwintered galls were not checked for parasitisation and thus the total parasitisation rate of *D. kuriphilus* might be higher due to the possible parasitoid infestation that occurs after the galls were collected.

### Site description

Galls were collected at 4 sites in Croatia (figure 1) chosen to present different first record (colonisation) dates of *D. kuriphilus* and different site conditions (parkland with no oak trees, continental and coastal chestnut forests).

Medvednica (North western continental Croatia, a mountain ridge near Zagreb and an area highly popular with visitors) has the highest infestation rate in Croatia. *D. kuriphilus* was first recorded there in 2010. It is very likely to be the first introduction site in Croatia [estimated 2008 or earlier (Matošević *et al*., 2010)]. Medvednica is a mixed natural sessile oak (*Q. petraea*) and sweet chestnut forest with an admixture of Turkey oak (*Q. cerris*), common beech (*Fagus sylvatica* L.), common hornbeam (*Carpinus betulus* L.) and deciduous shrub species.

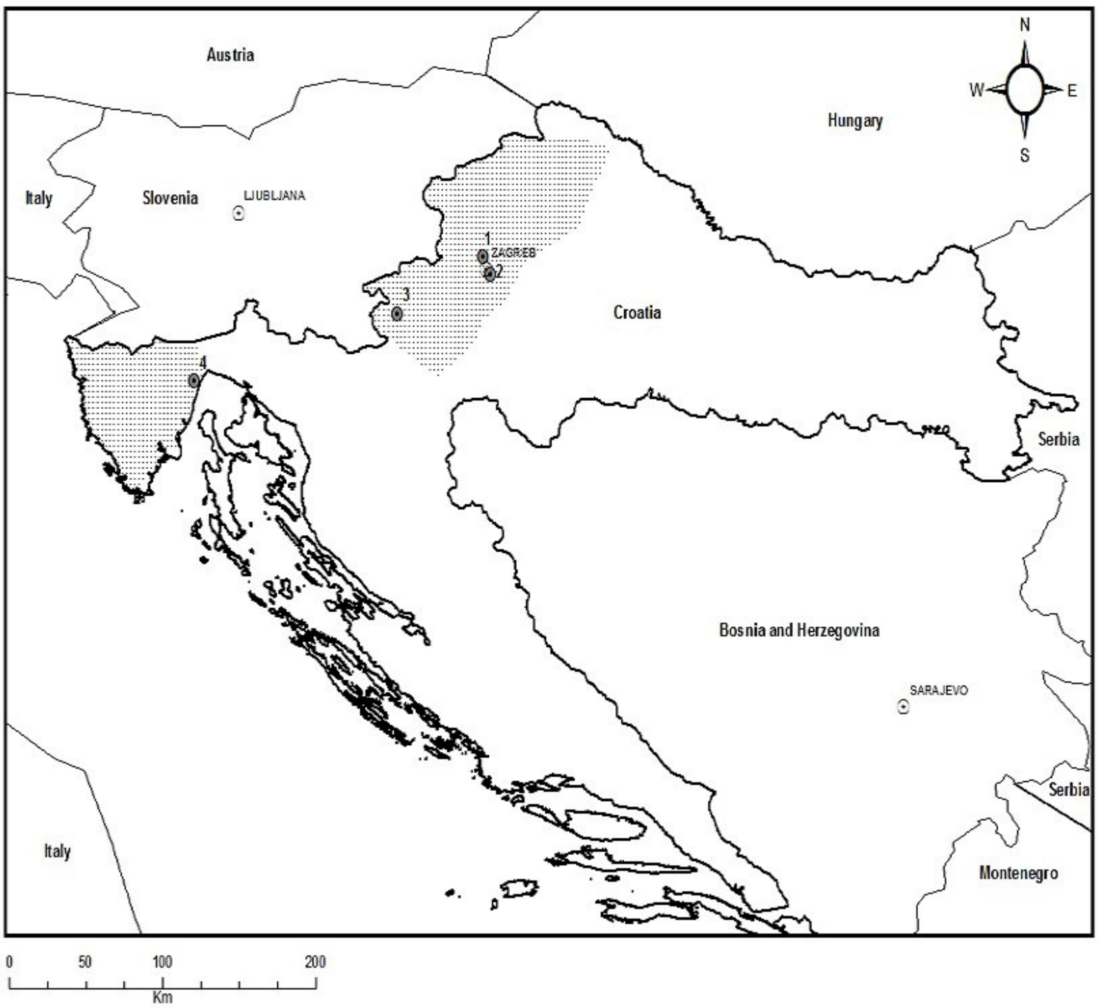


**Figure 1.** Distribution map of *D. kuriphilus* in Croatia: infested area (shaded) - Istrian Peninsula, North-western Croatia; sampling sites (numbered): 1 Medvednica; 2 Bundek; 3 Ozalj; 4 Lovran.

Bundek is a park in Zagreb (mainland Croatia) where sweet chestnuts were artificially planted as solitary ornamental trees. Other trees are mainly *Populus* and *Salix* species. The date of *D. kuriphilus* introduction is estimated to be 2009.

Ozalj was chosen because the first galls were found in 2010 and the estimated date of arrival of the pest is 2009. This site supports a mixed natural forest of sessile oak and sweet chestnut forest is with some Turkey oak and common hornbeam.

Lovran is on the Istrian peninsula (coastal region of Croatia) where the pest was discovered in 2010 and the estimated date of arrival is 2008 or 2009. Samples were collected in fragmented forests with old chestnut trees mixed with downy oak (*Q. pubescens*), Turkey oak and hop-hornbeam (*Ostrya carpinifolia* Scop.).

In 2010 a very small sample was collected (about 10 galls) on Sljeme (also Medvednica region) and in 2011 in Pregrada and Macelj and the parasitoids were reared and identified.

### Gall collection and parasitoid rearing

Galls were randomly collected by hand from low branches at heights up to 2 m. All galls were transferred on the same day to the entomological laboratory of the Croatian Forest Research Institute in Jastrebarsko. The galls were put in cardboard rearing boxes (30 × 22 × 40 cm) with extractable skylights (transparent plastic containers of 150 ml) with 500 galls per box. They were stored in the laboratory at room temperature.

Rearing boxes were checked daily for emerging parasitoids, which were stored in 96% ethanol before identification. All the parasitoids were identified according to their morphological characteristics at the Plant Health and Molecular Biology Laboratory (PHMBL), National Food Chain Safety Office, Budapest, Hungary. For the parasitoids identification an unpublished key generated by R.R. Askew (Manchester, UK) was used, which is a basic identification tool prolong decades in the research of parasitoid communities of oak gallwasps and the catalogue of oak gallwasp parasitoids (Askew *et al*., 2013) was based exclusively on this key. The parasitoid specimens are deposited at the Croatian Forestry Research Institute, Department for Forest Protection and PHMBL.

The number of emerged adults for each parasitoid species found was recorded. For each species the standardized emergence rate (er) was calculated according to Quacchia *et al*. (2013) so that the data could be compared: er = (number of parasitoids/total number of galls per sample)*100. As *D. kuriphilus* induces multilocular galls and several parasitoids can emerge from the same gall, the obtained values are relative parasitoid abundance per unit of sampling effort (multilocular gall).

Sex ratio of emerging adults was tested against a null hypothesis of a 50:50 sex ratio using a chi square test.

## Results

In total 20,598 galls were collected from 4 sites in Croatia, from which 922 parasitoid specimens were reared (table 1) in 2011 and 2012. In total 15 species of native parasitoids emerged: Eupelmidae 2; Eurytomidae 4; Ormyridae 1; Pteromalidae 4 and Torymidae 4 species.

Over the sampling period, 14 species of parasitoids were recorded in Medvednica compared to only 3 in Ozalj (table 1). In 2011 a high emergence rate was observed in Medvednica (when compared to Quacchia *et al*., 2013) but this number fell sharply in 2012 even though the number of galls sampled was much larger (table 1).

Some species were represented by less than 5 specimens in both years, *Eurytoma brunniventris* Ratzeburg, *Eurytoma pistacina* Rondani, *Sycophila biguttata* (Swederus), *Ormyrus pomaceus* (Geoffroy) and *Torymus auratus* (Muller). *Torymus flavipes* (Walker) was the only species present in all years and at all sites (table 1), and it was also recorded from the small samples of just a few galls from Sljeme, Pregrada and Macelj in 2011.

*Eupelmus annulatus* Nees, *Eupelmus urozonus* Dalman, *Mesopolobus tibialis* (Westwood), *Megastigmus dorsalis* (F.) and *Torymus geranii* (Walker) were found more frequently, but not at all sites and not in all years (table 1).

Medvednica was most species-rich (12 species from the total of 15 found in Croatia) while Ozalj had only 2 species.

*E. annulatus*, *E. urozonus*, *T. geranii* and *T. flavipes* all showed female-biased sex ratio (table 2).

No inquiline cynipids (Cynipidae Synergini) were recorded which are an important component of parasitoid communities in oak cynipid galls (Ács *et al*., 2010).

## Discussion and conclusions

This is a first study of parasitoids attacking the invasive cynipid gallwasp *D. kuriphilus* in Croatia. A total of 15 species of parasitoids from five families of Chalcidoidea were found parasitizing *D. kuriphilus* in a relatively short time (2 years research). The parasitoid species found were raised from spring galls only and the community would probably be richer if they were also reared from overwintering galls (Quacchia *et al.*, 2013). All species found have broad host ranges. Except for a few species they are all known to associate only with oak gallwasps and never parasitize other groups of oak insects. In this way they are not real generalists in the broader meaning but are guild specialists on oak cynipid galls (Csóka *et al.*, 2005; Askew *et al.*, 2013). Species we recorded belong to 5 of the 6 chalcid families attacking native oak cynipid gallwasps (Askew *et al.*, 2013). The sites where the samples were taken had naturally growing oak trees with oak cynipid gall fauna and their associate natural enemies. Only Bundek (park) had no oak trees in the vicinity and the less diverse spectrum of species could be attributed to this fact and to the later date of introduction of *D. kuriphilus* (when compared to Medvednica).

This research also has shown that the time lag between the introduction of the new host, *D. kuriphilus*, and the recruitment of native parasitoid community is short what was already showed for other sites in Europe (Aebi *et al*., 2006; Gibbs *et al*., 2011). Research on alien

**Table 1.** Total number of parasitoid species with standardised emergence rates (n.par = number of parasitoids; er = number of emerged adults per 100 galls) from Croatian sampling sites in 2011 and 2012.

| Parasitoids | Medvednica | | | | Bundek | | | | Ozalj | | | | Lovran | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | | 2012 | | 2011 | | 2012 | | 2011 | | 2012 | | 2011 | | 2012 | |
| | n.par | er | n.par | er | n.par | er | n.par | er | n.par | er | n.par | er | n.par | er | n.par | er |
| ***Eupelmus annulatus**** | 5 | 0.179 | 13 | 0.209 | | | | | | | 1 | 0.029 | | | | |
| ***Eupelmus urozonus**** | 73 | 2.627 | 2 | 0.032 | 4 | 0.415 | 21 | 1.008 | | | | | | | 4 | 0.097 |
| ***Eurytoma brunniventris**** | 2 | 0.072 | 2 | 0.032 | | | | | | | | | | | | |
| ***Eurytoma pistacina*** | 1 | 0.036 | | | | | | | | | | | | | | |
| ***Sycophila biguttata*** | 1 | 0.036 | | | | | | | | | | | | | | |
| ***Sycophila variegata*** | 9 | 0.324 | | | | | | | | | | | | | | |
| ***Ormyrus pomaceus*** | 4 | 0.144 | | | | | | | | | | | | | | |
| ***Mesopolobus amaenus*** | | | | | | | | | | | | | | | 6 | 0.145 |
| *Mesopolobus dubius* | 18 | 0.648 | | | | | | | | | | | | | | |
| ***Mesopolobus sericeus*** | 1 | 0.036 | 5 | 0.081 | | | | | | | | | 1 | 0.172 | 5 | 0.121 |
| ***Mesopolobus tibialis*** | 290 | 10.435 | 22 | 0.355 | | | 4 | 0.192 | | | | | | | 3 | 0.073 |
| ***Megastigmus dorsalis**** | 137 | 4.929 | 24 | 0.387 | 10 | 1.036 | | | | | | | | | | |
| ***Torymus auratus*** | | | | | | | | | | | 1 | 0.029 | | | 1 | 0.024 |
| ***Torymus flavipes*** | 106 | 3.814 | 22 | 0.355 | 14 | 1.451 | 2 | 0.096 | 10 | 2.183 | 20 | 0.589 | 3 | 0.517 | 17 | 0.411 |
| ***Torymus geranii*** | 28 | 1.008 | 27 | 0.436 | | | 1 | 0.048 | | | | | | | 2 | 0.048 |
| Total | 675 | | 117 | | 28 | | 28 | | 10 | | 22 | | 4 | | 38 | |
| Total number of galls | 2779 | | 6198 | | 965 | | 2084 | | 458 | | 3398 | | 580 | | 4136 | |

Species in bold indicate species found in Italy and with an asterisk the most abundant parasitoid species in north-western Italy (Quacchia *et al.*, 2013; Santi and Maini, 2012).

**Table 2.** Number of males and females for parasitoids reared from *D. kuriphilus* galls from Croatian sampling sites in 2011 and 2012

| Parasitoid species | Family | Males | Females | Total | $\chi^2$ |
|---|---|---|---|---|---|
| *Eupelmus annulatus* | Eupelmidae | 3 | 16 | 19 | 8.895* |
| *Eupelmus urozonus* | Eupelmidae | 35 | 69 | 104 | 11.115** |
| *Eurytoma brunniventris* | Eurytomidae | 3 | 1 | 4 | NS |
| *Eurytoma pistacina* | Eurytomidae | 0 | 1 | 1 | NS |
| *Sycophila biguttata* | Eurytomidae | 1 | 0 | 1 | NS |
| *Sycophila variegata* | Eurytomidae | 0 | 5 | 5 | NS |
| *Ormyrus pomaceus* | Ormyridae | 2 | 2 | 4 | NS |
| *Mesopolobus amaenus* | Pteromalidae | 0 | 6 | 6 | 6.000* |
| *Mesopolobus dubius* | Pteromalidae | 3 | 10 | 13 | NS |
| *Mesopolobus sericeus* | Pteromalidae | 5 | 7 | 12 | NS |
| *Mesopolobus tibialis* | Pteromalidae | 90 | 229 | 319 | 60.567*** |
| *Megastigmus dorsalis* | Torymidae | 61 | 110 | 171 | 14.041*** |
| *Torymus auratus* | Torymidae | 0 | 2 | 2 | NS |
| *Torymus flavipes* | Torymidae | 63 | 131 | 194 | 23.835*** |
| *Torymus geranii* | Torymidae | 3 | 55 | 58 | 46.621*** |

The observed sex ratios were tested against expected 50:50 ratio (all df = 1; *P < 0.05; **P < 0.001; ***P < 0.001; NS = not significant).

gallwasps in the United Kingdom showed that a rapid recruitment of native parasitoids by new hosts occurs in the first years and later this process slows down, with fewer and new parasitoid species shifting to the new host (Schönrogge *et al.*, 1995; 1996a; 1996b; 2011). The "Geographical-spread hypothesis" predicts that the number of parasitoids which attack an invasive species will increase as the invader increases its geographical range, accumulating parasitoids during expansion (Cornell and Hawkins, 1993; Hawkins, 2005). It can be expected that *D. kuriphilus* will have richer parasitoid complex from year to year especially on the sites that have been first invaded but later it will reach the saturation level and no or few new parasitoid species will shift to the new host. The extent to which the "Geographic Spread hypothesis" is true depends on the regional parasitoid pool. If the invader spreads across a region with a largely homogeneous parasitoid pool (which is true for much of the Western Palaearctic), then after initial recruitment the pest will largely meet new populations of the same parasitoid species (Bailey *et al.*, 2009).

It is estimated that *D. kuriphilus* was introduced to Medvednica in 2007 or 2008 (Matošević *et al.*, 2010), the first galls were found in Ozalj, Lovran and Bundek in 2010 and in two years the parasitoids have started to detect and exploit the new host. The novel gall of *D. kuriphilus* and its development on a novel plant host (it is the only cynipid to develop on *Castanea*) are not significant barriers to its detection and exploitation by some parasitoids usually resident on oak galls (Aebi *et al.*, 2006). This is despite the fact that host oak taxon is the most important factor structuring the communities of parasitoids associated with oak cynipids (Bailey *et al.*, 2009).

The number of parasitoid species per site reflects the date of host arrival. The parasitoids were found at all sites, but Medvednica as the site of first introduction and hence longest host availability, has the most parasitoid species. Bundek parkland is without oak trees and only four parasitoid species have been found. In Ozalj and Lovran the differences between 2011 and 2012 can be seen, the sites have been invaded in 2009 and gradual increase in number of parasitoid species occurred (table 1).

*T. flavipes* was found at all sites and in both years (and in one small sample from Sljeme in 2010 and Pregrada and Macelj in 2011) (table 1) and it was in between the first species to exploit the new host in Croatia. Quacchia *et al.* (2013) found *T. flavipes* in Italy only occasionally and in small numbers while Santi and Maini (2011) found this species associating with *D. kuriphilus* in the Bologna area of Italy and they discuss its ability to find the host earlier than other parasitoid species, finding it a truly promising natural enemy of *D. kuriphilus* for natural biological control. Panzavolta *et al.* (2013) found *T. flavipes* as most abundant species on all researched sites in Tuscany, Italy. *T. geranii* was found in Medvednica in all years and in Bundek and Lovran two years after introduction of its host (table 1). The species was recorded in Italy for the first time in the Bologna area in 2010 and 2011 (Santi and Maini, 2012). *M. tibialis* was abundant on Medvednica and found also in Bundek and Lovran but in Italy it was recorded as only an occasional species in low numbers (Quacchia *et al.*, 2013). *E. annulatus*, *E. urozonus* and *Mesopolobus sericeus* (Forster) were present at the Croatian sites and they were the most abundant species in Italy (Quacchia *et al.*, 2013). *Mesopolobus dubius* (Walker) has not yet been recorded from the parasitoid complex of *D. kuriphilus* in Europe (Aebi *et al.*, 2007, Quacchia *et al.*, 2013). It has to be noted that the sampling period and number of galls in Croatia were lower when compared to the Italian research (Quacchia *et al.*, 2013) so influencing the comparability of our results and perhaps contributing to the lower number of species recorded in Croatia.

The emergence rates (er) from spring galls were similar to those found in Italy (Quacchia *et al.*, 2013), except for *M. tibialis*, *M. dorsalis* and *T. flavipes* which were quite abundant in 2011 at Medvednica (table 1).

This abundance fell sharply in 2012 also. However, the emergence rate for all sites is relatively low. The addition of new species in 2012 on the sites that were most recently colonised can be noticed (Bundek, Ozalj and Lovran) (table 1).

The sex ratios for the most abundant parasitoid species appeared to be female-biased (table 2), while the Italian data, on the contrary, showed male-biased sex ratio (Quacchia *et al.*, 2013). Our data set is small and thus it may be too early to speculate on whether this difference is significant, and, if so, due to local mate competition (LMC), complementary sex determination, host quality, inequalities in competitive ability or other reasons (e.g., Charnov *et al.*, 1981; Hardy, 1994; Wogin *et al.*, 2012).

The rapid recruitment of oak gall cynipid parasitoids to *D. kuriphilus* may suggest there is a potential of biological control using native parasitoids as other control measures are practically of no effect (EFSA, 2010). However, while recruitment of parasitoids to this novel host was quite quick there is a question of actual influence of the parasitoids on decreasing the infestation rate of *D. kuriphilus.* There is a mismatch between the phenology of gall development of *D. kuriphilus* and emergence times of native natural enemies (Quacchia *et al.*, 2013). *T. flavipes* has shown better timing for parasitizing the novel host and could be a good potential in biological control of *D. kuriphilus* (Santi and Maini, 2011). Our results show that two native species of *Torymus* (*T. flavipes* and *T. geranii*) and *M. dorsalis* have been reared from *D. kuriphilus* galls which could provide a good possibility for native biological control but further research in matching of host and parasitoid phenology and percentages of parasitism is needed. Native *Torymus* species and *M. dorsalis* have very fine phenological and morphological differences (Schönrogge *et al.*, 2006), compared to other native polyphagous parasitoids, which could make them more efficient in *D. kuriphilus* control. Recent studies revealed the presence of cryptic species within *Eupelmus*, *Torymus*, *M. dorsalis* and other parasitoid species (Nicholls *et al.*, 2010a; 2010b; Quacchia *et al.*, 2013) which impossible to distinguish morphologically and thus the genetic analyses is in need to decide which species might be the most appropriate for the biological control purposes.

If an invasion is successful, a new assemblage around an invader will be generated and the food-web complexity of the entire local community will be increased (Cornell and Hawkins, 1993). High abundance of a new host can also have negative impacts through increased levels of parasitism on other native communities that share the same parasitoid species (in this case oak cynipids, leaf miners) via apparent competition (Hawkins, 2005). *E. urozonus* was found as a parasitoid of the native oak leafminer community in nearby locations (Matošević and Melika, 2012), so high abundance of parasitoids could lead to higher levels of parasitism in native oak gall and leafminer communities, negatively influencing their population densities.

In conclusion, the present study has shown that the invasive host *D. kuriphilus* has recruited native parasitoid species that could have the potential in influencing its population densities. The recruitment has occurred quite rapidly and the sites that have been first invaded have a richer parasitoid species spectrum than recently invaded sites. *T. flavipes* and *M. dorsalis* were the most abundant species that have shifted onto the new host, regardless of site tree species composition.

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