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Native parasitoids associated with *Dryocosmus kuriphilus* in Tuscany, Italy

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Abstract

The invasive *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae) is a major pest of *Castanea* spp. in Asia, the USA and Europe. The aim of this study is to characterize the parasitoid community associated to *D. kuriphilus* in Tuscany and determine their relative occurrence, before the introduction of the exotic parasitoid *Torymus sinensis* Kamijo. Ten hymenopteran parasitoids were collected from *D. kuriphilus* galls. *Mesopolobus fasciiventris* Westwood is reported for the first time as a *D. kuriphilus* associate in Italy. *Torymus flavipes* (Walker) specimens accounted for more than 50% of all collected parasitoids. Facultative hyperparasitoid species were also collected. Furthermore, a higher overall parasitism rate was found on wild chestnut galls (with respect to cultivated ones) and where oak trees were more abundant.

Key words: Asian chestnut gall wasp, cynipid, parasitism, hyperparasitoids, *Mesopolobus fasciiventris*, *Torymus flavipes*.

Introduction

The invasive *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera Cynipidae), the Asian chestnut gall wasp (ACGW), is a major pest of chestnuts, *Castanea* spp. (Fagaceae), in its area of origin (China) (Zhang *et al.*, 2009), as well as in new areas of distribution. Since 1941 it has been reported in neighbouring Countries, first in Japan and later in Korea, where it has caused significant reductions in chestnut yields (Payne *et al.*, 1983; Zhang *et al.*, 2009). The ACGW was recorded in the USA in 1974, first on Chinese chestnuts and, later, also on American chestnuts (Rieske, 2007); in Europe, it was found on the European chestnut *Castanea sativa* Miller in 2002 (Brussino *et al.*, 2002).

Torymus sinensis Kamijo (Hymenoptera Torymidae), ACGW's parasitoid native to China like its host, was introduced into several Countries to control the invasive pest. Following the experience matured in Japan (Moriya *et al.*, 2003) and in the USA (Rieske, 2007), the introduction of this parasitoid has been considered as a sustainable and effective approach for controlling ACGW. Indeed, this parasitoid has been highly successful in reducing the pest population below the economic threshold in Japan (Murakami *et al.*, 2001; Moriya *et al.*, 2003), and may have also affected ACGW populations in the USA (Rieske, 2007). In 2005 *T. sinensis* was introduced in Italy, first in Piedmont (Aebi *et al.*, 2007; Quacchia *et al.*, 2008), and, later, in many other Italian Regions, including Tuscany in 2010.

Some doubts have recently been raised about continuing biological control using *T. sinensis* in Europe (EFSA Panel on Plant Health, 2010; Aebi *et al.*, 2011; Gibbs *et al.*, 2011), because of potential adverse effects. For example, negative interactions might evolve with native parasitoids, leading to their displacement, as reported by Yara *et al.* (2007). Moreover, *T. sinensis* might hybridize with native European species of *Torymus*, as has occurred in Japan with *Torymus beneficus* Yasumatsu et Kamijo (Moriya *et al.*, 1992; Moriya *et al.*, 2003; Yara *et al.*, 2000; Yara *et al.*, 2010). Finally, few studies focusing on the actual host range of *T. sinensis* in the new introduction areas have been carried out (EFSA Panel on Plant Health, 2010; Aebi *et al.*, 2011). Although *T. sinensis* is reported as a generalist parasitoid in China, it seemed to be host specific in Japan (Murakami *et al.*, 1977). However, its host specificity was probably due to the high ACGW population density in the introduction areas, while the same parasitoid could behave differently in ecosystems where several other hosts are largely available (Stone *et al.*, 2002).

Soon after its introduction in Italy, ACGW was used as a suitable host by several native parasitoids that promptly initiated new tritrophic interactions (Speranza *et al.*, 2009; Benvegnù, 2011; Guerrieri *et al.*, 2011; Santi and Maini, 2011; Pollini, 2012; Quacchia *et al.*, 2013). Native parasitoids could be used in biological control to reduce ACGW population density below the economic damage threshold (Aebi *et al.*, 2007), particularly where biotic and abiotic factors make *T. sinensis* ineffective. For example, both Japanese and North American authors have showed how facultative hyperparasitoids negatively affect *T. sinensis* population density (Murakami and Gyoutoku, 1995; Cooper and Rieske, 2007, 2011a).

As highlighted by the European Food Safety Authority (EFSA Panel on Plant Health, 2010), more studies are needed to assess the possible negative effects of ACGW biological control based on the introduction of *T. sinensis*. Similarly, the role and possible use of native parasitoids for ACGW control still need a proper definition (Aebi *et al.*, 2011). The aim of our research was to characterize the complex of native parasitoids associating to the ACGW in areas of Tuscany where *T. sinensis* had not yet been released and to determine their relative abundance. This information is crucial to assess the impact of *T. sinensis* on ACGW native parasitoid communities once the exotic parasitoid colonizes these areas, either naturally or by future introductions.

Materials and methods

Study sites

Three chestnut forests were surveyed in Tuscany one year after the first record of ACGW in those sites. Castelpoggio (province of Carrara, Italy; 44°07'04"N, 10°03'59"E; 665 m a.s.l.): ACGW was first recorded in 2008. A survey of natural enemies was carried out in 2009. The site was a wild *C. sativa* forest (coppice and high-forest) mixed with sporadic oaks (mainly *Quercus cerris* L.), with a western exposure. Marradi A (province of Florence, Italy; 44°04'23"N, 11°38'00"E; 478 m a.s.l.): ACGW was first recorded in 2009. A survey of natural enemies was carried out in 2010. The site was a plantation of *C. sativa* cultivar "Marron Buono di Marradi" with a northern exposure, close to annual crop fields and to woodlands of coppice and high-forest wild chestnuts, with sporadic European hornbeams and oaks. Marradi B (province of Florence, Italy; 44°03'35"N, 11°38'54"E; 613 m a.s.l.): ACGW was first recorded in 2009. A survey of natural enemies was carried out in 2010. The site was a plantation of *C. sativa* cultivar "Marron Buono di Marradi" mixed with coppice and high-forest wild chestnuts with a western exposure, surrounded by wide mixed forests dominated by oaks (*Quercus pubescens* Willd. and *Q. cerris*).

T. sinensis was first released in spring 2010 in chestnut forests of Castelpoggio and Marradi. In both areas, 165 specimens were released (Tuscany Regional Committee Resolution n. 646 of 5 July 2010) in sites located at least 1 km from our study sites. Marradi A and Marradi B were also separated from the release sites by mountain ridges.

Gall collection and rearing

Castelpoggio: a total of 150 chestnut trees were selected along three 500-m-long transects at intervals of 10 m. Current-generation galls were collected on 5 and 19 June 2009. On each sampling date, three galls per tree were collected (900 galls in total); galls were picked randomly at a height < 3 m. After collection each gall was dissected in the laboratory to record the number of cells per gall and the content of each cell. Adult parasitoids found in the cells were promptly placed in absolute ethanol and stored at −20 °C.

Marradi: in each site (Marradi A and B) 43 wild chestnut trees and 43 cultivated chestnut trees were selected along two 430-m-long transects at intervals of 10 m. Current-generation galls were collected on 10 and 25 June 2010. On each sampling date, from each site, two galls per tree were collected randomly at a height < 3 m (688 galls in total). Cultivated and wild chestnut galls were stored separately by site. After collection all galls were placed in vials plugged with fine mesh (5 galls/vial) and kept at room temperature (15-25 °C). Vials were checked every other day; when parasitoids emerged they were promptly placed in absolute ethanol and stored at −20 °C. After the end of the emergences of both ACGWs and parasitoids, each gall was dissected to record the number of cells and the content of each cell.

Parasitoid identification

Castelpoggio: Dr. George Melika (Pest Diagnostic Department, Plant Protection & Soil Conservation Directorate of County Vas Tanakajd, Hungary) identified the parasitoid specimens.

Marradi: Parasitoid specimens were identified using available dichotomous keys (Askew, 1961; Graham, 1969; Bouček, 1970; Pujade-Villar, 1993, Graham and Gijswijt, 1998; Askew and Thùroczy, personal communication) and by comparison with type material and museum specimens. DNA was extracted from single specimens by a non-destructive method (Gebiola *et al.*, 2009) and three genes were sequenced (COI, 28S, ITS2) following Gebiola *et al.* (2010). Molecular and morphological data were combined to obtain the final identification. The sex-ratio (expressed as females/males) was calculated for each species.

D. kuriphilus flight activity

On 10 June 2010, four adhesive yellow traps were placed at Marradi B and were replaced weekly until 20 August 2010. Traps were examined in the laboratory to record the number of ACGW captures.

Statistical analyses

The mean numbers of cells per gall between the three collection sites were compared by one-way ANOVA using Bonferroni post-hoc comparison. Two-way ANOVA was used to compare the mean number of cells per gall between cultivated and wild chestnuts from the two Marradi sites. The number of cells per gall was log-transformed to meet requirements of homogeneity and homoscedasticity. Proportions of parasitized cells between collection sites were compared by chi-square test, applying an orthogonal chi-decomposition according to Kimball's (1954) guidelines. Proportions of parasitized cells between cultivated and wild chestnuts from the two Marradi sites were compared by G test (Sokal and Rohlf, 1995).

Results

The mean number of cells per gall was significantly different among the three study sites (one-way ANOVA, df = 2, F = 21.18, $P < 0.001$). The value was higher at Castelpoggio (4.12 ± 0.11 SE) than at either Marradi A (2.91 ± 0.10 SE; Bonferroni test, $P < 0.001$) or Marradi B (3.14 ± 0.10 SE; Bonferroni test, $P < 0.001$). In contrast, no differences were recorded between the two sites at Marradi (Bonferroni test, P = 0.396). A higher mean number of cells was recorded on cultivated chestnuts (3.29 ± 0.11 SE) in respect to wild chestnuts (2.99 ± 0.10 SE) from both sites at Marradi (two-way ANOVA, df = 1, F = 4.31, P = 0.039).

Living ACGW larvae were found in 93.14% of the cells at Castelpoggio. The total ACGW mortality within the galls was 6.86%; empty cells were 6.35% and overall parasitism rate (parasitized cells / total number of cells × 100) was 0.51% (table 1). Nineteen parasitoid specimens were recorded, 11 of which were adults belonging to six species of three Hymenoptera families (Torymidae, Pteromalidae and Eupelmidae) (table 1). Parasitoid identification was not possible for the preimaginal stages (eight specimens).

From galls collected at Marradi A and B in 2010, a total of 977 ACGW adults and 59 adult parasitoids emerged. ACGW adults emerged from 45.25% of the cells counted during gall dissection, whereas dead ACGW adults were recorded in 43.26% of the cells. Dead ACGW adults inside the cells were not considered in the mortality assessment because laboratory conditions may have affected galls, causing a higher adult mortality during emergence (Ôtake, 1982). Total ACGW mortality was 11.49%; empty cells were 8.11% and overall parasitism rate was 3.38%. The parasitism rate at Marradi was calculated by taking into account the number of cells containing parasitoid exuviae, easily distinguishable from those of ACGW.

The total parasitism rate recorded at Marradi B (5.74%) was significantly higher than those at Marradi A (0.60%) and Castelpoggio (0.51%) (table 1).

Fifty-nine parasitoids emerged from Marradi A and B galls, for a total of six parasitoid species belonging to five different Hymenoptera families (Torymidae, Eurytomidae, Pteromalidae, Eupelmidae and Ormyridae) (table 1). Differences emerged between the two sites: at Marradi A, only two parasitoid species were collected reflecting a low number of community associates; conversely, at Marradi B, five parasitoid species were collected (table 1). *Torymus flavipes* (Walker) was the only species collected in all three study sites (table 1). *T. flavipes* sex ratio at Marradi (A and B together) was 5.67. *Eupelmus urozonus* Dalman was collected in two out of three sites; its occurrence was higher at Marradi B than at Castelpoggio (table 1). Its sex ratio was 2. All other identified species were collected each from one site only. A single male of the genus *Ormyrus* was collected at Marradi A (table 1). Adult occurrence of *T. flavipes* at Marradi B was the highest recorded in all study sites, followed by that of *Mesopolobus tibialis* (Westwood), also at Marradi B. Among the parasitoids recorded at Castelpoggio, *Mesopolobus tarsatus* (Nees) was the most abundant (table 1).

In the laboratory, ACGW adult emergence started on 28 June 2010 and lasted until the end of July (figure 1), virtually overlapping with field emergences. This result is in line with what observed in other Regions of Italy (Bernardo *et al.*, 2013). Indeed, most of ACGW emergences were recorded from 7 to 22 July 2010, both in laboratory and field conditions (96.59% and 94.50% respectively). Adult parasitoids emerged in the laboratory from 21 June to 14 July 2010, a week before ACGW (figure 1). *Ormyrus* sp., *M. tibialis* and *T. flavipes* were the first parasitoids to emerge in the laboratory. *T. flavipes* emergence reached its peak on 28 June 2010 and lasted until 14 July (figure 1). The first Eupelmidae emerged on 30 June 2010, and more specifically *E. urozonus* emergence occurred until 12 July.

Finally, a significantly greater number of parasitoids were recorded in galls collected at Marradi on wild chestnuts than on cultivated ones (table 2). Here differences emerged between the two Marradi sites, in that parasitoids at Marradi A were lower than those recorded at Marradi B both considering wild chestnuts or cultivated chestnuts (table 2).

Discussion

Our study extends our knowledge about the parasitoid community associated to *D. kuriphilus* in Tuscany before

Table 1. Native parasitoids from *D. kuriphilus* galls collected in Tuscany (Italy) at Castelpoggio (Carrara) and Marradi (A and B) (Florence). n = total number of cells examined.

Parasitoid species	Family	Parasitoids / cells (%) Castelpoggio n = 3715	Marradi A n = 992	Marradi B n = 1167
Torymus flavipes (Walker)	Torymidae	0.05	0.30	3.17
Torymus auratus (Muller)	Torymidae	0.05	-	-
Megastigmus dorsalis (F.)	Torymidae	0.03	-	-
Eurytoma brunniventris Ratzeburg	Eurytomidae	-	-	0.26
Mesopolobus tarsatus (Nees)	Pteromalidae	0.08	-	-
Mesopolobus tibialis (Westwood)	Pteromalidae	-	-	0.69
Mesopolobus fasciiventris Westwood	Pteromalidae	0.03	-	-
Eupelmus urozonus Dalman	Eupelmidae	0.05	-	0.51
Eupelmus annulatus (Nees)	Eupelmidae	-	-	0.09
Ormyrus spp.	Ormyridae	-	0.20	-
Undetermined		0.22	0.10	1.03
Total parasitism rates		0.51	0.60	5.74

χ^2 and Kimball's tests results Comparisons of total parasitism rates	χ^2	df	P
Marradi A vs Marradi B	71.758	1	< 0.001
Castelpoggio vs (Marradi A + B)	72.941	1	< 0.001
Total	164.699	2	< 0.001

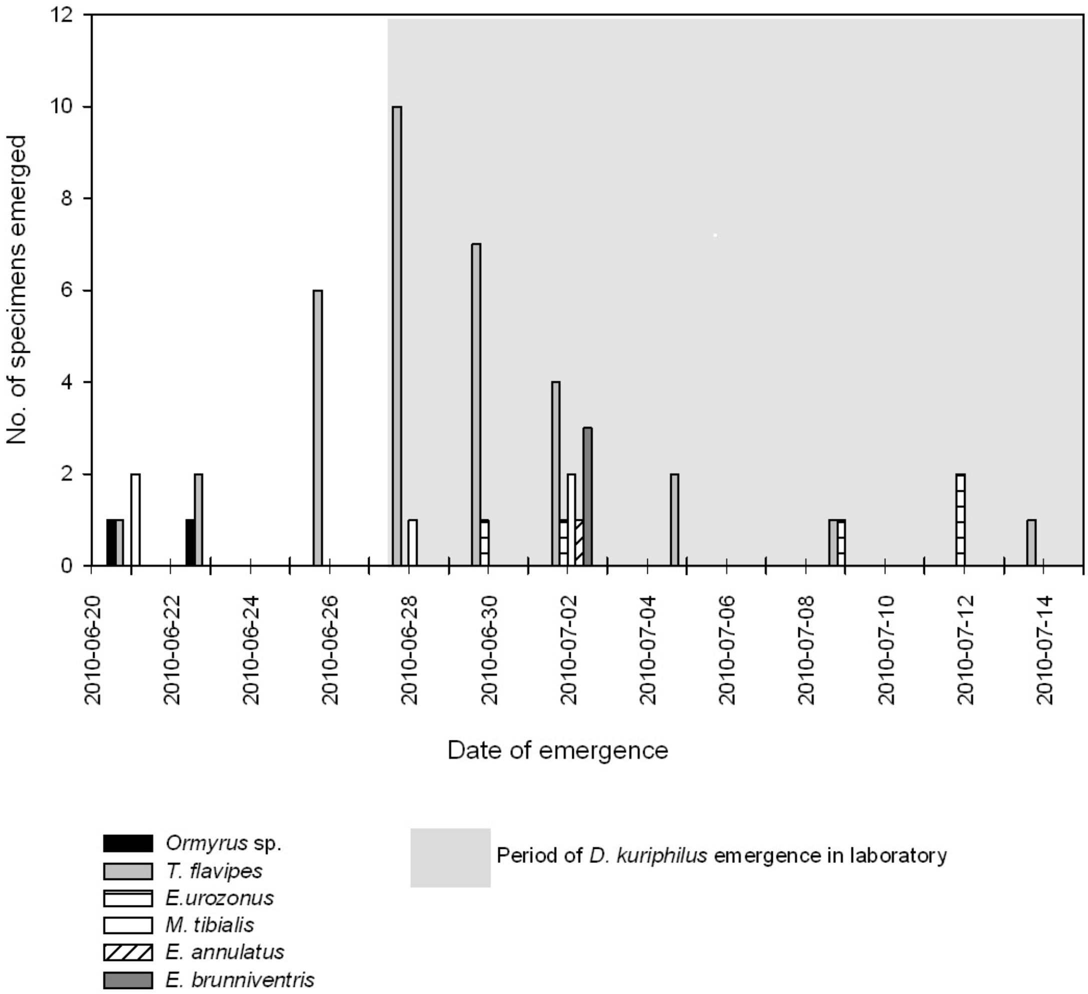

Figure 1. Number of *D. kuriphilus* native parasitoids emerged in the laboratory from galls collected in Tuscany (Italy) at Marradi A and B (Florence).

Table 2. Overall parasitism rates of *D. kuriphilus* by native parasitoids in cultivated and wild chestnut galls collected in Tuscany (Italy) at Marradi A and B (Florence).

	Parasitism rates (%)		
	Marradi A	Marradi B	Total
Cultivated chestnut galls (CCG)	0.32	3.97	2.12
Wild chestnut galls (WCG)	1.08	7.65	5.04

G test results Comparisons of parasitism rates	G	df	P
CCG versus WCG	13.747	1	< 0.001
CCG: Marradi A versus Marradi B	22.973	1	< 0.001
WCG: Marradi A versus Marradi B	24.542	1	< 0.001
Total	61.262	3	< 0.001

the introduction of the exotic parasitoid *T. sinensis*. Indeed, the parasitoid had not yet been introduced at Castelpoggio and it was released at the time of our samplings (2010) in chestnut forests located at least 1 km from our study sites at Marradi. It is unlikely that the parasitoid had already reached Marradi sites at the time of gall sampling. Indeed, the natural spread of *T. sinensis* is very low during the first three years after release (less than 1 km / year) (Moriya *et al.*, 2003). Furthermore, mountain ridges separated release sites from our study sites at Marradi, and a very low number of specimens were released. Our observations may help in assessing the interactions among native parasitoids of ACGW and *T. sinensis,* when the latter will reach our study sites.

We were able to identify most of the parasitoid-abandoned cells. Ôtake *et al.* (1982) stated that parasitoid- and ACGW-abandoned cells were indistinguishable.

These contrasting results could be at least partially due to the different species composition of the parasitoid complex in Japan. However, in a very few cases it was not possible to distinguish parasitoid-abandoned cells from ACGW-abandoned ones. Indeed, in a few cases no exuvial remains were observed inside parasitoid abandoned cells, as assessed by dissecting the galls.

Ten hymenopteran parasitoid species were collected from ACGW galls, nine of which had been already recorded associating to ACGW in Italy (Speranza *et al.*, 2009; Santi and Maini, 2011; Pollini, 2012; Quacchia *et al.*, 2013), whereas *Mesopolobus fasciiventris* Westwood is reported from the first time as an ACGW associate in Italy. More than 50% of the adult parasitoids collected were identified as *T. flavipes*. This is a common species attacking several oak gall wasps (Noyes, 2012). The second-most-abundant species was *M. tibialis*, known to develop on numerous gall wasps on different plant species (Noyes, 2012), followed by *E. urozonus*, both from Castelpoggio and Marradi galls. *E. urozonus* can develop either as a primary parasitoid or as a hyperparasitoid (Murakami *et al.*, 1995) and has a wide host range (Yasumatsu and Kamijo, 1979). The occurrence of other species was sporadic, including that of *M. fasciiventris,* frequently reported as a parasitoid of oak gall wasps (Askew, 1961). Within just one year from the first ACGW record, in each of the three study sites, up to six native parasitoid species generally associated with oak gall wasps had already adapted to this new host on chestnut trees. The recruitment of several parasitoid species in such a short period of time is in agreement with other European studies, which showed a similar time lag between the introduction of *D. kuriphilus* and the development of a native parasitoid community (Aebi *et al*., 2006; Matošević and Melika, 2013).

The higher overall parasitism rate we recorded at Marradi B could be related to the larger presence of oak trees in this site. Except *Eupelmus* species, all parasitoids recorded on ACGW are known to associate mainly with oak gall wasps (Aebi *et al.*, 2006). The parasitism rate observed at Marradi B was not high, but encouraging. Whereas in some areas of Italy the native parasitoid emergence rate is typically 2-3 specimens per 100 galls (Quacchia *et al.*, 2013), in Marradi B the rate was 16 specimens per 100 galls. However, further surveys are needed at the same study site to verify if native parasitoids will be able to control ACGW population density, because high annual fluctuations in parasitism by native parasitoids have been observed in Japan (Ôtake *et al.*, 1982; Ôtake, 1989).

The overall parasitism rate on wild chestnut galls was higher than on cultivated chestnut galls. This could be due to the higher number of oaks present near the wild chestnuts in both Marradi A and Marradi B. However, it could be also due to the lower gall size recorded on wild chestnuts, in agreement with other authors (Ôtake *et al.*, 1982; Cooper and Rieske, 2010; 2011a; Bernardo *et al.*, 2013) who stated that parasitism is negatively correlated with the gall size. However, multiple-year studies are needed because the gall size can vary annually at the same site (Panzavolta *et al.*, 2012; Bernardo *et al.*, 2013). Furthermore, during the gall samplings, differences in tree phenology were observed, inasmuch as budburst in wild and in cultivated chestnut trees occurs in different times and this may have affected gall parasitism, as suggested by Cooper and Rieske (2011b) for Chinese and American chestnuts. The infection by parasitic fungi could be a limiting factor on AGCW population and its parasitoid complex (Addario and Turchetti, 2011).

In our study, the presence of native insects reported as facultative hyperparasitoids, such as *Eurytoma brunniventris* Ratzeburg, *E. urozonus* and *Eupelmus annulatus* (Nees) (Murakami and Gyoutoku, 1995; Murakami *et al.*, 1995), was recorded. Specifically, *E. urozonus* and *E. brunniventris* had an important role in the delay in the population increment of the introduced *T. sinensis* in some areas of Japan (Murakami and Gyoutoku, 1995). Therefore, investigating the presence and density of these hyperparasitoids could help in predicting the results of biological control with *T. sinensis* (Murakami *et al.*, 1994).

Among the species collected, *T. flavipes* was not only the most abundant but also the only one collected in all three study sites. This species has recently been recorded, from the same host, in the nearby Emilia-Romagna region (Santi and Maini, 2011); moreover, it is apparently the dominant species in other regions (personal observations by Guerrieri E.). In our laboratory rearing (2010) *T. flavipes* emerged from galls about one week before ACGW adults. This result is concordant with that of Santi and Maini (2011), though they recorded insect emergences in the field. Laboratory conditions seemed not to affect ACGW emergence period which virtually overlapped with that recorded in the field. It is possible to speculate that the same could apply to parasitoid emergence as well. The role of *T. flavipes* in ACGW control needs to be assessed carefully. Further research should focus on trophic relationships of *T. flavipes* both with oak gall wasps and ACGW.

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References

ADDARIO E., TURCHETTI T., 2011.- Parasitic fungi on *Dryocosmus kuriphilus* in *Castanea sativa* necrotic galls.- *Bulletin of Insectology*, 64: 269-273.

AEBI A., SCHÖNROGGE K., MELIKA G., ALMA A., BOSIO G., QUACCHIA A., PICCIAU L., ABE Y., MORIYA S., YARA K., SELJAK G., STONE G. N. 2006.- Parasitoid recruitment to the globally invasive chestnut gall wasp *Dryocosmus kuriphilus*, pp. 103-121. In: *Galling arthropods and their associates. Ecology and evolution* (OZAKI K., YUKAWA J., OHGUSHI T., PRICE P. W., Eds).- Springer-Verlag, Tokyo, Japan.

AEBI A., SCHÖNROGGE K., MELIKA G., QUACCHIA A., ALMA A., STONE G. N., 2007.- Native and introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*.- *Bulletin OEPP/EPPO Bulletin*, 37: 166-171.

AEBI A., SCHÖENENBERGER N., BIGLER F., 2011.- Evaluating the use of *Torymus sinensis* against the chestnut gall wasp *Dryocosmus kuriphilus* in the Canton Ticino, Switzerland.- Agroscope, Zürich, Switzerland.

ASKEW R. R., 1961.- A study of the biology of species of the genus *Mesopolobus* Westwood (Hymenoptera: Pteromalidae) associated with cynipid galls on oak.- *Transactions of the Royal Entomological Society of London*, 113: 155-173.

BENVEGNÙ I., 2011.- Indagini sul cinipide del castagno (*Dryocosmus kuriphilus*) nell'Italia nord-orientale. 55 pp., *M.sc. Thesis*, University of Padua, Department of Environmental Agronomy and Crop Production.

BERNARDO U., IODICE L., SASSO R., TUTORE V. A., CASCONE P., GUERRIERI E., 2013.- Biology and monitoring of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) on *Castanea sativa* in Campania (Southern Italy).- *Agricultural and Forest Entomology*, 15: 65-76.

BOUČEK Z., 1970.- On some British *Megastigmus* (Hym. Torymidae), with a revised key to the west European species.- *Entomologist's Gazette*, 21: 265-275.

BRUSSINO G., BOSIO G., BAUDINO M., GIORDANO R., RAMELLO F., MELIKA G., 2002.- Pericoloso insetto esotico per il castagno europeo.- *Informatore Agrario*, 58 (37): 59-61.

COOPER W. R., RIESKE L. K., 2007.- Community associates of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Eastern North America.- *Annals of the Entomological Society of America*, 100: 236-244.

COOPER W. R., RIESKE L. K., 2010.- Gall structure affects ecological associations of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae).- *Environmental Entomology*, 39: 787-797.

COOPER W. R., RIESKE L. K., 2011a.- A native and an introduced parasitoid utilize an exotic gall-maker host.- *BioControl*, 56: 725-734.

COOPER W. R., RIESKE L. K., 2011b.- Chestnut species and jasmonic acid treatment influence development and community interactions of galls produced by the Asian chestnut gall wasp, *Dryocosmus kuriphilus*.- *Journal of Insect Science*, 11: 1-14.

EFSA PANEL ON PLANT HEALTH (PLH), 2010.- Risk assessment of the oriental chestnut gall wasp, *Dryocosmus kuriphilus* for the EU territory and identification and evaluation of risk management options.- *EFSA Journal*, 8 (6): 1619. doi:10.2903/j.efsa.2010.1619.

GEBIOLA M., BERNARDO U., MONTI M. M., NAVONE P., VIGGIANI G., 2009.- *Pnigalio agraules* (Walker) and *Pnigalio mediterraneus* Ferrière and Delucchi (Hymenoptera: Eulophidae): two closely related valid species.- *Journal of Natural History*, 43: 2465-2480.

GEBIOLA M., BERNARDO U., BURKS R. A., 2010.- A reevaluation of the generic limits of *Pnigalio* Schrank (Hymenoptera: Eulophidae) based on molecular and morphological evidence.- *Zootaxa*, 2484: 35-44.

GIBBS M., SCHÖNROGGE K., ALMA A., MELIKA G., QUACCHIA A., STONE G. N., AEBI A., 2011.- *Torymus sinensis*: a viable management option for the biological control of *Dryocosmus kuriphilus* in Europe?- *BioControl*, 56: 527-538.

GRAHAM M. W. R., 1969.- The Pteromalidae of north-western Europe (Hymenoptera: Chalcidoidea).- *Bulletin of the British Museum (Natural History) Entomology Supplement*, 16: 1-908.

GRAHAM M. W. R., GIJSWIJT M. J, 1978.- Revision of the European species of *Torymus* Dalman (s. lat.) (Hymenoptera: Torymidae).- *Zoologische Verhandelingen (Leiden)* 317: 1-202.

GUERRIERI E., BERNARDO U., IODICE L., GEBIOLA M., 2011.- Identificazione morfo-bio-molecolare ed interazioni trofiche degli antagonisti autoctoni di *Dryocosmus kuriphilus* Yasumatsu in Campania: metodologia e risultati preliminari.- *Atti dell'Accademia Nazionale Italiana di Entomologia*, 58: 115-120.

KIMBALL A. W., 1954.- Short-cut formulae for the exact partition of chisquare in contingency tables.- *Biometrics*, 10: 452-458.

MATOŠEVIĆ D., MELIKA G., 2013.- Recruitment of native parasitoids to a new invasive host: first results of *Dryocosmus kuriphilus* parasitoid assemblage in Croatia.- *Bulletin of Insectology*, 66 (2): 231-238.

MORIYA S., INOUE K., SHIGA M., MABUCHI M., 1992.- Interspecific relationship between an introduced parasitoid, *Torymus sinensis* Kamijo, as a biological control agent of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, and an endemic parasitoid, *T. beneficus* Yasumatsu et Kamijo.- *Acta Phytopathologica et Entomologica Hungarica*, 27: 479-483.

MORIYA S., SHIGA M., ADACHI I., 2003.- Classical biological control of the chestnut gall wasp in Japan, pp. 407-415. In: *Proceedings of the 1st international symposium on biological control of arthropods* (VAN DRIESCHE R. G., Ed.), Honolulu, 14-18 January 2002. United States Department of Agriculture, Forest Service, Washington, USA.

MURAKAMI Y., UMEYA K., OHO N., 1977.- A preliminary introduction and release of a parasitoid (Chalcidoidea, Torymidae) of the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Cynipidae) from China.- *Japanese Journal of Applied Entomology and Zoology*, 21: 197-203.

MURAKAMI Y., GYOUTOKU Y., 1995.- A delayed increase in the population of an imported parasitoid, *Torymus (Syntomaspis) sinensis* (Hymenoptera: Torymidae) in Kumamoto, Southwestern Japan.- *Applied Entomology and Zoology*, 30: 215-224.

MURAKAMI Y., HIRAMATSU T., MAEDA M., 1994.- Parasitoids complexes of the chestnut gall wasp (Hymenoptera: Cynipidae) in two localities before introduction of *Torymus (Syntomaspis) sinensis* (Hymenoptera: Torymidae), with special reference to prediction of results after release of the parasitoid.- *Japanese Journal of Applied Entomology and Zoology*, 38: 29-41.

MURAKAMI Y., OHKUBO N., MORIYA S., GYOUTOKU Y., KIM C. H., Kim J. K., 1995.- Parasitoids of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in South Korea with particular reference to ecologically different types of *Torymus (Syntomaspis) sinensis* (Hymenoptera: Torymidae).- *Applied Entomology and Zoology*, 30: 277-284.

MURAKAMI Y., TODA S., GYOUTOKU Y., 2001.- Colonization of imported *Torymus* (*Syntomaspis*) *sinensis* Kamijo (Hymenoptera: Torymidae) parasitic on the chestnut gall wasp (*Dryocosmus kuriphilus)* (Hymenoptera: Cynipidae). 7. Success in the eighteenth year after release in Kumamoto (Japan).- *Kyushu Plant Protection Research*, 47: 132-134.

NOYES J. S., 2012.- Universal Chalcidoidea database. [online] URL: http://www.nhm.ac.uk/chalcidoids

ÔTAKE A., 1982.- Weighing of galls as a means of studying the ecology of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) and its parasitoids.- *Applied Entomology and Zoology*, 17: 148-150.

ÔTAKE A., 1989.- Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae): analyses of records on cell contents inside galls and on emergence of wasps and parasitoids outside galls.- *Applied Entomology and Zoology*, 24: 193-201.

ÔTAKE A., SHIGA M., MORIYA S., 1982.- A study on parasitism of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) by parasitoids indigenous to Japan.- *Bulletin of the Fruit Tree Research Station A*, 9: 177-192.

PANZAVOLTA T., BRACALINI M., CROCI F., CAMPANI C., BARTOLETTI T., MINIATI G., BENEDETTELLI S., TIBERI R., 2012.- Asian chestnut gall wasp in Tuscany: gall characteristics, egg distribution and chestnut cultivar susceptibility.- *Agricultural and Forest Entomology*, 14: 139-145.

PAYNE J. A., JAYNES R. A., KAYS S. J., 1983.- Chinese chestnut production in the United States: practice, problems, and possible solutions.- *Economic Botany*, 37: 187-200.

POLLINI A., 2012.- Situazione degli antagonisti indigeni di *Dryocosmus kuriphilus* in castagneti dell'Emilia-Romagna, pp. 76-78. In: *Atti del convegno stato dell'arte nella difesa delle avversità del castagno* (Castanea sativa *Mill.)*, (BELLINI E., Ed.), Marradi (Florence), 9 September 2011. Centro di Studio e Documentazione sul Castagno, Marradi (Florence), Italy.

PUJADE VILLAR J., 1993.- Especies de Mesopolobus (Hym., Pteromalidae) asociadas a agallas de Cynipini (Hym., Cynipidae) del nordeste ibérico y notas sobre la validez de *M. lichtensteini* (Mayr, 1903).- *Eos*, 69: 63-73.

QUACCHIA A., MORIYA S., BOSIO G., SCAPIN I., 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: 829-839.

QUACCHIA A., FERRACINI C., NICHOLLS J. A., PIAZZA E., SALADINI M. A., TOTA F., MELIKA G., ALMA A., 2013.- Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in north-western Italy.- *Insect Conservation and Diversity*, 6: 114-123.

RIESKE L. K., 2007.- Success of an exotic gallmaker, *Dryocosmus kuriphilus*, on chestnut in the USA: a historical account.- *Bulletin OEPP/EPPO Bulletin*, 37: 172-174.

SANTI F., MAINI S., 2011.- New association between *Dryocosmus kuriphilus* and *Torymus flavipes* in chestnut trees in the Bologna area (Italy): first results.- *Bulletin of Insectology*, 64: 275-278.

SOKAL R. R., ROHLF F. J., 1995.- *Biometry: the principles and practice of statistics in biological research* (3rd edition).- W.H. Freeman and Company, New York, USA.

SPERANZA S., STACCHIOTTI M., PAPARATTI B., 2009.- Endemic parasitoids of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cinipidae) in Central Italy.- *Acta Horticulturae*, 844: 421-424.

STONE G. N., SCHÖNROGGE K., ATKINSON R. J., BELLIDO D., PUJADE-VILLAR J., 2002.- The population biology of oak gall wasps (Hymenoptera: Cynipidae).- *Annual Review of Entomology*, 47: 633-668.

YARA K., YANO E., SASAWAKI T., SHIGA M., 2000.- Detection of hybrids between introduced *Torymus sinensis* and native *T. beneficus* (Hymenoptera: Torymidae) in central Japan, using malic enzyme.- *Applied Entomology and Zoology*, 35: 201-206.

YARA K., SASAWAKI T., KUNIMI Y., 2007.- Displacement of *Torymus beneficus* (Hymenoptera: Torymidae) by *T. sinensis*, an indigenous and introduced parasitoid of the chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Japanese chestnut fields: possible involvement in hybridization.- *Biological Control*, 42: 148-154.

YARA K., SASAWAKI T., KUNIMI Y., 2010.- Hybridization between introduced *Torymus sinensis* (Hymenoptera: Torymidae) and indigenous *T. beneficus* (late-spring strain), parasitoids of the Asian chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae).- *Biological Control*, 54, 14-18.

YASUMATSU K., KAMIJO K., 1979.- Chalcidoid parasites of *Dryocosmus kuriphilus* Yasumatsu (Cynipidae) in Japan, with descriptions of five new species (Hymenoptera).- *Esakia*, 14: 93-111.

ZHANG Z. Y., TARCALI G., RADÓCZ L., FENG Y. Q., SHEN Y. Y., 2009.- Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu in China and in Hungary.- *Journal of Agricultural Sciences*, 38: 123-128.

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