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Hymenoptera wasps associated with the Asian gall wasp of chestnut *(Dryocosmus kuriphilus)* in Calabria, Italy

Vincenzo Palmeri · Pasquale Cascone · Orlando Campolo · Saverio B. Grande · Francesca Laudani · Antonino Malacrinò · Emilio Guerrieri

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Abstract The parasitoid complex of the Asian chestnut gall wasp *Drycosmus kuriphilus* Yasumatsu was studied in Calabria (Italy). A total of 14 different species of parasitoids were collected, of which three are recorded on the Asian gall wasp for the first time. The composition of the parasitoid complex collected in Calabria was compared with that reported from Italy and from Europe. The altitude of the sites of collection seemed to have an effect on the distribution and abundance of the single species of parasitoids.

Keywords *Castanea sativa* · Chalcidoidea · *Eupelmus* · *Mesopolobus* · *Ormyrus* · Parasitoids · *Sycophila* · *Torymus*

The invasive *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), known as the Asian chestnut gall wasp (ACGW), is a major pest of Oriental chestnuts [*Castanea* spp. (Fagaceae)] in its area of origin (China) (Zhang *et al.* 2009). In 2002 it was accidentally introduced into Italy, becoming in a few years a major pest of the European chestnut *Castanea sativa* Miller (Bernardo *et al.* 2013; Brussino *et al.* 2002; Panzavolta *et al.* 2013; Quacchia *et al.* 2013). In summer 2009, *D. kuriphilus* was detected in Calabria, near San Luca village (Reggio Calabria province) (EPPO 2009). Several factors account for the rapid spread of this species throughout Europe: thelytoky; high fertility; low control of infested material circulating in Europe, associated with the lack of a quick diagnostic protocol to assess the presence of the pest in overwintering buds until 2012 (Sartor *et al.* 2012); limited or null control and management of coppice chestnut highly common all over Europe (Abe *et al.* 2007; Bernardo *et al.* 2013). The strong reduction of photosynthetic area due to the formation of galls results in a dramatic reduction of fruit yield (up to 80%) (Battisti *et al.* 2013), tree vigor, wood production and, in the worst cases, in the death of the tree when associated with other biotic stresses (Aebi *et al.* 2006; Kato & Hijii 1997; Payne *et al.* 1983). Chemical control of this pest appears unfeasible and unsustainable for a number of reasons (Bernardo *et al.* 2013). For example, all living instars, except adult, are well protected inside buds and gall tissues; moreover, thelytoky guarantees a reconstitution of pest populations starting from only few adult females escaping the insecticide treatments targeting the adults of the pest, given the impossibility to cover all the galls on the chestnut crown.

The resistance of chestnut trees to *D. kuriphilus* is restricted to a few cultivars of different *Castanea* species. For example, in Japan the cultivars ‘Tzukuba’, ‘Tanzawa’ and ‘Ginyose’ of *Castanea crenata* Siebold

V. Palmeri (✉) · O. Campolo · S. B. Grande · F. Laudani · A. Malacrinò
Dipartimento di Agraria, Mediterranea University of Reggio Calabria, Reggio Calabria, Italy
e-mail: vpalmeri@unirc.it

P. Cascone · E. Guerrieri
Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Portici, NA, Italy

E. Guerrieri (✉)
Department of Life Sciences, The Natural History Museum, London, UK
e-mail: guerrieri@ipp.cnr.it

& Zucc. are reported as resistant to the pest; similarly, in the USA, programs for the genetic improvement of *Castanea dentata* Marshall are in progress (Anagnostakis *et al.* 2009; Bounous & Beccaro 2002; Dini *et al.* 2012; Shimura 1972). The fully resistant cultivar 'Bouche de Bétizac' (*Castanea sativa* Miller x *C. crenata*) avoids the infestation by the gall wasp through a hypersensitive response, killing the larva at the first instar (Dini *et al.* 2012), but there is some concern in exploiting this source of resistance. In particular, both producers and consumers still prefer local cultivars of chestnut, some of which have national and international relevance (*e.g.* 'Marrone di Roccadaspide', 'Marrone di Marradi'). Furthermore, the resistance exhibited by these cultivars was overcome from a ACGW biotype, reducing the viability of this management strategy (Murakami 2010; Panzavolta *et al.* 2012).

Following the experience acquired in Japan (Baker *et al.* 2010) and in the U.S.A. (Rieske 2007), the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), ACGW's natural enemy whose area of origin coincides with that of ACGW, was introduced into several regions of Italy starting in 2005 from Piedmont (Aebi *et al.* 2007; Guerrieri *et al.* 2012; Pugliesi 2012; Quacchia *et al.* 2008).

However, in the last few years it has been reported that the native parasitoid complex could play an important role in the control of invasive pests by an increasing adaptation to the new host starting soon after its introduction (Panzavolta *et al.* 2013; Santi & Maini 2011; Zappalà *et al.* 2012).

The aim of this work was to characterize the composition of the native parasitoid community associated with *D. kuriphilus* in Calabria for the benefit of ongoing and future programs of biological control of the chestnut gall wasp.

Plant material was collected in three sites, characterized by different altitudes and age of the plants. The first site, located in Scilla (RC) (38° 14' 36.2"N; 15° 43' 7.8"E, 270 m a.s.l.), was a 15-year-old chestnut coppice; the second site, located in Melia (RC) (38° 12' 49.9"N; 15° 46' 23.1"E, 600 m a.s.l.), was an 18-year-old coppice; the third site, located in Sant'Angelo (RC) (38° 13' 9.9"N; 15° 45' 58.9"E, 1000 m a.s.l.), was a 13-year-old chestnut coppice characterized by plants of *Quercus* spp. growing near and inside it. At each site 72 galls of *D. kuriphilus* were collected weekly from June to September 2011. The collected galls were kept in aerated boxes placed in a climate-controlled chamber at 25±1°C and 75±5% r.h. Emerging parasitoids were isolated in small vials and identified to species level by comparing them with material authoritatively identified and stored at the Natural History Museum of London (UK). The average number of gall chambers was calculated by dissecting the galls at the end of the collection. The percentage of parasitized galls was calculated with the following ratio:

$$parasitized\ \ galls(\%) = \frac{Pg}{Tg} \times 100$$

where: *Pg* is the number of galls from which at least one parasitoid emerged and *Tg* is the total number of collected galls.

Data about the number of gall chambers among the different altitudes were subjected to one-way ANOVA using R statistical software version 2.3.0 (R Development Core Team 2008).

A total of 14 parasitoid species were identified. The following species were already reported as parasitoids of *D. kuriphilus*: *Eupelmus urozonus* Dalman, *E. annulatus* Nees (Hym., Eupelmidae), *Megastigmus dorsalis* (Fabricius) (Hym., Torymidae), *M. fasciiventris* Westwood, *M. sericeus* (Forster), *M. tarsatus* Nees, *M. tibialis* (Westwood) (Hym., Pteromalidae), *Mesopolobus amaenus* (Walker) (Hym., Pteromalidae), *Ormyrus nitidulus* Fabricius (Hym., Ormyridae), *O. pomaceus* Geoffroy (Hym., Ormyridae), *Torymus auratus* Müller (Hym., Torymidae). The following species are reported for the first time as parasitoids of *D. kuriphilus*: *Eupelmus vesicularis* (Retzius) (Hym., Eupelmidae), *Sycophila binotata* Fonscolombe, *S. flavicollis* Walker (Hym., Eurytomidae).

Among new associations with the chestnut gall wasp, *E. vesicularis* is also known to attack other Cynipidae (gen. *Synergus*), Eulophidae, Eurytomidae, Pteromalidae and Torymidae. For this reason we cannot exclude a possible role of this species as a hyperparasitoid (Askew *et al.* 2013; Noyes 2012).

The number of chambers in the galls ranged from one to four, with a mean value of 2.83±0.09, without statistical differences among the sites ($F_{2,\ 117} = 1.835$; $P = 0.164$). Overall, 7.93% of collected galls were parasitized. The mean rate of parasitized galls ranged from 0.79% in Scilla, to 9.92% in Melia, and to 13.09% in Sant'Angelo, suggesting an increase of the level of parasitism with altitude. Differences in the composition of the parasitoid complexes were observed among sites, suggesting an increase of diversity with altitude: in

Scilla, only specimens belonging to *Eupelmus* genus emerged from the galls; in Melia, the parasitoid complex was composed of *Sycophila* (41%), *Mesopolobus* (32%), *Eupelmus* (15%) and *Ormyrus* (11%); the highest diversity of the parasitoid complex was found in Sant'Angelo, as composed of *Mesopolobus* (48%), *Sycophila* (31%), *Megastigmus* (13%), *Eupelmus* (4%), *Ormyrus* (2%) and *Torymus* (2%).

The parasitization was not constant during the entire sampling period: in Scilla (from 0 to 2.77% of parasitized galls) the wasps emerged only in mid-June and mid-September, whereas in the two other locations, Melia (2.77–22.22%) and Sant'Angelo (8.33–19.45%), the parasitoid emergence was observed throughout the entire period of sampling, with a maximum value recorded in mid-July.

Approximately 37% and 27% of the reared parasitoids belonged to the genera *Mesopolobus* (of which *M. amaenus* 47.37%, *M. fasciiventris* 13.16%, *M. sericeus* 2.63%, *M. tarsatus* 13.16%, *M. tibialis* 23.68%) and *Sycophila* (*S. binotata* 80.77%, *S. flavicollis* 19.23%), respectively, while each of the genera *Eupelmus* (*E. annulatus* 28.57%, *E. urozonus* 57.14%, *E. vesicularis* 14.29%) and *Ormyrus* (*O. nitidulus* 60%, *O. pomaceus* 40%), as well as the species *M. dorsalis* and *T. auratus,* represented approximately 9% of the total. It is worthwhile noticing the absence of *Torymus flavipes* (Walker) (Torymidae) in the parasitoid complex collected in Calabria. In fact, this parasitoid species has been collected in different parts of Italy and Europe – representing in some cases the dominant species (Matoševič & Melika 2013; Panzavolta *et al.* 2013; Quacchia *et al*. 2013; Santi & Maini 2011). A possible reason for this could be the date of the first collection of galls (June). However, in other parts of Italy where *T. flavipes* has been reared from *D. kuriphilus*, the emergence of the parasitoid lasted until the end of June (Panzavolta *et al.* 2013; Santi & Maini 2011).

The complex of parasitoids identified in Calabria partially overlaps with those found in northern Italy and central Italy (Panzavolta *et al*. 2013; Quacchia *et al.* 2013), and in other countries where *D. kuriphilus* is present (Aebi *et al.* 2006).

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