Chalcid parasitoid community associated with the invadingpest Dryocosmus kuriphilus in north-western Italy

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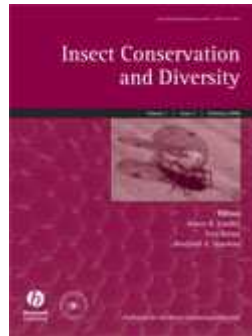A. Quacchia, C. Ferracini, J.A. Nicholls, E. Piazza, M.A. Saladini, F. Tota, G. Melika, A. Alma
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Chalcid parasitoid community associated with the invading pest Dryocosmus kuriphilus in north-western Italy

1 **Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in**

2 **north-western Italy**

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4 Running title: Native parasitoids of *Dryocosmus kuriphilus*

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Abstract

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28 1. Biological invasions of exotic species pose a major threat to native biodiversity. Invaders are

29 known to have direct impacts on native species; however, less well studied are the indirect impacts

30 mediated through the integration of invaders into trophically-linked communities.

31 2. A survey of the chalcid wasp parasitoid community attacking the chestnut gallwasp *Dryocosmus*

32 *kuriphilus* was carried out over a five year period at 26 sites in north-western Italy. More than

33 415,000 galls were collected and more than 10,000 parasitoid specimens emerged. Twenty-seven

34 parasitoid species belonging to six families (Eurytomidae, Pteromalidae, Torymidae, Eupelmidae,

35 Ormyridae, Eulophidae) were identified using morphological and molecular methods; seventeen are

36 new records for the parasitoid community associated with *D. kuriphilus* in Italy. The morpho-

37 species complexes *Megastigmus dorsalis*, *Eupelmus urozonus, E. annulatus* and *Eurytoma pistaciae*

38 were the dominant species, another six morpho-species were encountered regularly but at low

39 frequency, and 13 species were recorded only occasionally. The attack rate of any individual

40 parasitoid species was low, although the more common species appeared to be increasing their use

41 of this novel host.

42 3. Biases observed in the sex ratios of parasitoids emerging from *D. kuriphilus* galls suggest that

43 parasitoid individuals are making life history decisions to take advantage of the high abundance of

44 this host. Overall, these patterns imply that there is ongoing transfer of parasitoids between oak and

45 chestnut galls, and hence a high potential for this invading species to have a major impact on native

46 oak gall communities via indirect competition mediated through shared parasitoids.

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Introduction

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52   The introduction of exotic species is occurring more and more frequently around the world,

53 especially in countries with extensive international exchange of goods and tourists, and poses a

54 widely recognized threat to native biodiversity (Williamson, 1996; Wittenberg & Cock, 2001).

55 Understanding the causes and consequences of biological invasions subsequent to such

56 introductions represents an increasingly important challenge for ecologists and evolutionary

57 biologists. Many invasive species undergo population explosions and spread rapidly since they are

58 released from their normal controls of disease and natural enemies (van Lenteren *et al*., 2006). The

59 resulting ecological impact of invaders can occur at different levels of biological organisation from

60 genetic effects through effects on individuals, populations or communities to effects on ecosystem

61 processes (Parker *et al*., 1999). Expanding populations of exotic species can disrupt ecosystems

62 directly through displacement and extinction of native species (Wilson, 1997), causing both

63 economic and environmental damage. Invasive herbivores may affect populations and communities

64 of native herbivores by competing for the same resource, although mechanisms underlying

65 competition are not always fully understood (Reitz & Trumble, 2002). However, when trophic links

66 are established between invasive species and native communities, invaders can impact indirectly on

67 these communities through perturbed trophic structures (Vitousek *et al*., 1996; Hennemann &

68 Memmot, 2001). Hence understanding the process by which native natural enemies recruitment

69 onto novel invading hosts provides insight into the broader effects of invading species.

70   *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae), native to China, is a key pest

71 of chestnut trees (Fagaceae, *Castanea* spp.) (Payne *et al*., 1983; Moriya *et al*., 1990; Murakami *et*

72 *al*., 1995). This species became established in the mid 20th century in Japan, Korea and the USA. In

73 Europe it was first reported in 2002 from Piedmont in north-west Italy (Brussino *et al*., 2002), but

74 from customs records its introduction can be traced back to 2–3 years beforehand with the

75 importation of nursery material from China (Quacchia *et al*., 2008). Due to the severe impact that

76 the galls induced by this species have on host plant growth and nut production it was added to the

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77 European Plant Protection Organization (EPPO) A2 Action list (EPPO, 2005) in 2003. *Dryocosmus*

78 *kuriphilus* has one generation per year, with parthenogenetic females laying eggs in the buds of

79 *Castanea* spp. during summer which then hatch in 30-40 days. First instar larvae overwinter and

80 grow slowly until the following spring at which point their growth rate increases leading to the

81 induction of galls inside which the gallwasp larvae develop. Native parasitoids that normally attack

82 galls induced by related cynipid gallwasps on oaks (Fagaceae, *Quercus* spp.) have colonised *D.*

83 *kuriphilus* galls everywhere throughout their introduced range (Aebi *et al*., 2006, 2007). Given the

84 high abundance of *D. kuriphilus* galls, the use of this species as a host by oak gall parasitoids may

85 have massive, but to date unstudied, impacts on the oak gall community through the process of

86 apparent competition (Holt, 1977). However, to understand the impacts of this invading species we

87 first need to know basic information on the identity and behaviour of native parasitoids colonising

88 it.

89   A total of 11 species in five chalcid families (Torymidae, Ormyridae, Eurytomidae,

90 Eupelmidae and Eulophidae) are known to attack *D. kuriphilus* in its native range in China

91 (Murakami *et al*., 1980; Kamijo, 1981; Murakami, 1981; Luo & Huang, 1993). Of these, only

92 *Torymus sinensis* Kamijo, shows high host specificity and a life cycle matching that of its host; the

93 remaining species also attack related cynipid hosts galling oaks. In Japan introduced *D. kuriphilus* is

94 now attacked by a rich parasitoid wasp assemblage of 24 chalcid species from seven families and

95 one braconid species (*Aspilota yasumatsui* Watanabe) (Aebi *et al*., 2006; Abe *et al*., 2007). After its

96 arrival in Korea, *D. kuriphilus* recruited a parasitoid assemblage of 17 chalcid species over a period

97 of only several decades (Ko, 1971; Yasumatsu & Kamijo, 1979; Kamijo, 1981, 1982; Murakami *et*

98 *al*., 1985, 1994, 1995; Ôtake, 1989; Ôtake *et al*., 1982; Kim, 1998). Preliminary surveys of the

99 natural enemies attacking *D. kuriphilus* in Italy were published by Aebi *et al*. (2006, 2007) in which

100 15 species belonging to five chalcid families were identified. Although the communities associated

101 with introduced populations of *D. kuriphilus* are richer than those found in its native Chinese range,

102 the latter is almost certainly understudied. However, the parasitoid communities of *D. kuriphilus* in

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103　both native and introduced ranges show substantial overlap in species composition and consistent

104　biological patterns (Aebi *et al*., 2006). Parasitoid communities based around invading *D. kuriphilus*

105　develop rapidly, involving species shared with local populations of oak gallwasps, typically those

106　with broad host ranges. The novel communities are also diverse, both taxonomically and in terms of

107　the life histories of component species. Although the recruitment of indigenous parasitoids onto

108　invading populations of *D. kuriphilus* has been fast, they have not yet provided effective control of

109　this pest. Given the severity of the damage caused by *D. kuriphilus* and the failure of other methods

110　of control (such as insecticides or selection of resistant cultivars), control by native parasitoids has

111　been augmented by the release of *T. sinensis*, initially into Japan (with rapid effective control;

112　Moriya *et al.*, 1990) and subsequently to both the USA (Cooper & Rieske, 2007; Rieske, 2007) and

113　Italy (Quacchia *et al*., 2008).

114　　　　Here we present a detailed report on the native natural enemies associated with *D. kuriphilus*

115　in the north of Italy where *D. kuriphilus* was first introduced. We provide a qualitative assessment

116　of the species utilising *D. kuriphilus* as a host to examine how the parasitoid community has

117　changed over the past five years since the preliminary surveys of Aebi *et al*. (2006). We also

118　provide quantitative data to examine the rate of parasitoid recruitment onto this novel host, and to

119　examine how the demographics of this novel abundant host may affect life history decisions of the

120　parasitoids attacking it. Finally we discuss the possible future development of the *D. kuriphilus*

121　parasitoid community and its interaction with the parasitoid community attacking hosts on oaks.

Materials and methods

Gall collection and rearing

124　　　　Parasitoids were reared from galls of *D. kuriphilus* collected during the years 2006-2010.

125　Galls were collected from 26 sites within Cuneo province in north-western Italy, within a zone of

126　850 km^2 bounded by 44°38'52'' and 44°12'05'' N and by 7°19'19'' and 7°49'21'' E (Figure 1).

127　This area has extensive infestation by *D. kuriphilus* and encompasses its initial introduction site into

128　Italy, so likely contains the most species-rich parasitoid assemblage available. Sampled chestnut

129 trees were located both in mixed forests and in chestnut orchards; Cuneo province has 40% of its

130 forestry area covered by chestnut and nearly 10% by *Quercus* spp. (IPLA, 2004). Galls were

131 randomly collected by hand from low branches and with the aid of lopping shears from the

132 medium-high canopy. All material was stored in plastic bags, transferred to a forest nursery

133 "Gambarello" at Chiusa Pesio within Cuneo province and separated from any non-gall plant

134 material to avoid contamination by other insects not associated with the galls. Galls were collected

135 twice a year: in winter withered galls formed during the previous spring were sampled, and in

136 summer newly formed galls were collected. The dried winter galls were kept in cardboard boxes

137 provided with extractable skylights while the fresh summer galls were isolated inside Plexiglas and

138 net cubes (40x40x40 cm). In both cases multiple galls (up to 2,000) were kept in every container.

139 All galls were stored outdoors at ambient conditions.

Parasitoid collection and identification

141 Rearing boxes were checked once per week until the emergence of the first parasitoid wasp,

142 after which parasitoids were collected daily and their date of emergence recorded. Gall-inducing

143 and parasitoid wasps were removed using an entomological pooter, then stored in 99% ethanol. All

144 the parasitoids were initially identified using morphological characters. Voucher specimens of

145 parasitoids were deposited at the University of Turin, DIVAPRA Entomology section. Some of the

146 parasitoid taxa encountered contain morphologically-cryptic species distinguishable only through

147 the use of molecular markers; these groups include *Megastigmus dorsalis* (Fabricius), *Eurytoma*

148 *brunniventris* Ratzeburg and the *Eupelmus urozonus* Dalman/*E. annulatus* Nees complex

149 (Kaartinen *et al*., 2010; Nicholls *et al*., 2010). Between 7 and 43 individuals per morpho-species

150 complex were sequenced to determine whether multiple species were present. The sample for each

151 morpho-species was derived evenly from the winter and summer collections of their hosts. Samples

152 were sequenced either for the cytochrome *b* gene (*Megastigmus*, *Eurytoma*) following methods in

153 Nicholls *et al*. (2010) or the cytochrome oxidase I gene (*Eupelmus*) following Kaartinen *et al*.

154 (2010). These molecular methods were only used for a qualitative assessment of the presence of

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155 cryptic species to provide a comprehensive picture of the species diversity attacking *D. kuriphilus*.

156 As resources were not available for molecular identification of every parasitoid specimen all the

157 quantitative analyses of abundance described below were performed at the level of morpho-species

158 rather than genetic species.

Abundance patterns in the parasitoid community

160 The number of emerged adults of each parasitoid species was counted and a standardised

161 emergence rate was calculated by dividing the number of emerged adults by the number of galls

162 sampled in that collecting period. Since *D. kuriphilus* galls are multilocular, multiple parasitoids of

163 the same species could emerge from the same gall; our rearing method meant we were unable to

164 determine if this was the case. Hence our emergence rate is not a measure of the exact rate of

165 parasitism, but does provide a method of determining relative parasitoid abundance per unit of

166 sampling effort. Changes over time in the emergence rate of each species in each season were tested

167 for using a logistic regression implemented in SPSS version 17.0. Dates of emergence of the 4 most

168 common parasitoid morpho-species from galls collected in 2009 were plotted to assess whether

169 emergence (and hence parasitism of new hosts) coincided with the development of the next

170 generation of host galls. The secondary sex ratios (i.e. the sex ratio of emerging adults) for each of

171 these 4 species in each season were tested against the null hypothesis of a 50:50 sex ratio, using a χ^2

172 goodness-of-fit test to examine whether they showed a similar bias to that seen in parasitoids

173 attacking other invading cynipid hosts.

Results

Parasitoid species emerging from galls

176 In total 415,224 galls were collected (371,855 withered winter galls and 43,369 newly formed

177 spring galls; Table 1). A total of 10,077 native parasitoid specimens from the superfamily

178 Chalcidoidea emerged across five years. Twenty-three species in six chalcid families (Eurytomidae,

179 Pteromalidae, Torymidae, Eupelmidae, Ormyridae, Eulophidae) were identified using

180 morphological characters (Table 2). The molecular analyses revealed the presence of cryptic species

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181   within all of the morpho-species complexes tested, thus adding a further 4 species to the list of

182   parasitoids attacking *D. kuriphilus*. Both cryptic species recognised by Nicholls *et al.* (2010) were

183   found within the *M. dorsalis* morpho-species, although one was sampled at much higher frequency

184   (all but one of the 43 individuals screened). The presence of *E. brunniventris* was confirmed, but

185   some individuals provisionally identified as this species were in fact *Eurytoma adleriae* Zerova.

186   Individuals assigned to the morpho-species *Eupelmus urozonus* were found to be either *E. urozonus*

187   or *E. fulvipes* Förster, and individuals identified morphologically as *Eupelmus annulatus* were either

188   *E. annulatus* or *E. spongipartus* Förster.

189   Some parasitoid species were found to attack *D. kuriphilus* galls only occasionally, with

190   fewer than ten specimens in total recorded sporadically across the survey period. This was the case

191   for *Aulogymnus arsames* (Walker), *Baryscapus* sp., *Pediobius chilaspidis* Bouček, *P. saulius*

192   (Walker), *Pediobius* sp., *Eupelmus splendens* Giraud, *Ormyrus pomaceus* (Geoffroy), *O. nitidulus*

193   (Fabricius), *Cecidostiba* sp., *Mesopolobus amaenus* (Walker), *M. tarsatus* (Nees), *M. tibialis*

194   (Westwood) and *Torymus flavipes* (Walker). Other species were found more consistently with

195   records from the majority of years, but were only present at low frequency. This set of species

196   included *Sycophila variegata* (Curtis), *S. biguttata* (Swederus), *E. brunniventris*, *Mesopolobus*

197   *sericeus* (Förster), *Aulogymnus* sp. and *Aprostocetus* sp. (Table S1). Some of these species appeared

198   to be increasing in abundance over time, and often were absent in the early years of sampling, so

199   our data may reflect the initial colonisation of *D. kuriphilus* by these species.

200   Finally, four species were recorded every year at high frequency: *M. dorsalis*, *E. urozonus*, *E.*

201   *annulatus* and *Eurytoma pistaciae* Rondani (Table S1). The most frequently collected species was

202   *M. dorsalis*. Overall, this morpho-species accounted for 33.3% of all parasitoid emergences during

203   the study, followed by *E. pistaciae*, *E. annulatus* and *E. urozonus* with 30.1%, 14.1%, and 14.0%,

204   respectively. Three of these species, *M. dorsalis, E. annulatus* and *E. pistaciae*, showed significant

205   increases in emergence rate from overwintering galls over the sampling period (R^2= 0.938, *P*=

206   0.007; R^2 =0.930, *P*= 0.008; R^2 =0.790, *P*= 0.044 respectively). Neither overwintering *E. urozonus*

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207 nor any species emerging from galls collected in summer showed significant increases in

208 emergence rate over time (Figure 2). All four species showed an emergence pattern consisting of

209 two generations per year (Figure 3). The first generation overwintered in *D. kuriphilus* galls and

210 emerged from the end of April until early June. The second generation resulted from attack of

211 developing galls, with emergence beginning at the end of June and continuing until the end of July

212 or early August (Figure 3). Of these four common parasitoid morphospecies, *M. dorsalis,*

213 *Eupelmus urozonus* and *E. annulatus* showed a general pattern of strongly male-biased sex ratios in

214 their overwintering generations and weakly female-biased ratios in the generations developing

215 within freshly growing *D. kuriphilus* galls (Table 3). The fourth species, *E. pistaciae*, typically

216 showed no bias in sex ratios with the exception of a male bias in the overwintering 2007 generation

217 and a female bias in the summer 2010 generation.

Discussion

219 A total of 27 species in six chalcid families (Eurytomidae, Pteromalidae, Torymidae,

220 Eupelmidae, Ormyridae, Eulophidae) were found to be associated with *D. kuriphilus* galls during

221 the five year period 2006-2010. Seventeen of these species were not recorded by Aebi *et al*. (2006)

222 so represent recent additions to the parasitoid community attacking this pest in Italy, although four

223 of them are cryptic species revealed by molecular analyses so may have been present but simply

224 unrecognised in earlier surveys (for example see Aebi *et al.*, 2007). Aebi *et al*. (2006) recorded five

225 further species associated with *D. kuriphilus* during the early years of the pest's establishment that

226 were not recorded in this study (*Torymus scutellaris* (Walker), *T. auratus* (Müller), *Sycophila*

227 *iracemae* Nieves-Aldrey*, Mesopolobus mediterraneus* (Mayr) and *Baryscapus pallidae* Graham;

228 Table 2), bringing the total number of parasitoid species known to attack *D. kuriphilus* in Italy to

229 32. Many of these species appear to parasitise *D. kuriphilus* only occasionally and therefore may be

230 at a very early stage in their recruitment onto this novel host. However, four species (*M. dorsalis*, *E.*

231 *urozonus*, *E. annulatus* and *E. pistaciae*) are more abundant and appear to have incorporated *D.*

232 *kuriphilus* into their regular host range.

233   In its native distribution *D. kuriphilus* populations are kept at low densities by natural

234   enemies; in contrast in regions where it has invaded (Japan, South Korea and the USA) the attack

235   rates of indigenous parasitoid species have remained low many years after the arrival of the pest

236   (typically less than 2%) (Murakami *et al*., 1995; Ito & Hijii, 2000; Stone *et al*., 2002; Aebi *et al*.,

237   2007). This study confirms the same pattern in Italy more than 10 years after *D. kuriphilus* first

238   invaded, despite the species richness of the associated parasitoid community. The emergence rate

239   across all indigenous parasitoids from the summer galls was typically 2-3 individuals per 100 galls,

240   and although the emergence rate from winter galls increased over the study period it still remained

241   relatively low (6 individuals per 100 galls). In addition, since *D. kuriphilus* galls are multilocular,

242   any individual female parasitoid may lay multiple eggs within a single gall so the overall rate of

243   parasitism may well be even lower than our emergence data suggest. As a result, it appears unlikely

244   that current activities of indigenous parasitoids will be able to bring about control of this serious

245   pest. Given the economic and ecological damage caused by *D. kuriphilus* and considering the lack

246   of alternative effective control strategies (EFSA, 2010), this ineffective control by indigenous

247   natural enemies highlights the need for biological control using the exotic parasitoid *T. sinensis*

248   (Quacchia *et al*., 2008; Gibbs *et al*., 2011).

249   The degree to which native parasitoids may help impose some control over *D. kuriphilus* also

250   depends on how well integrated this pest becomes into the oak gall community. The data presented

251   here indicate that although some recruitment of oak gall parasitoids has occurred, there is still a

252   mismatch between the phenology of gall development by the univoltine *D. kuriphilus* and the

253   emergence times of native natural enemies. This pattern is also found for parasitoids that are shared

254   with oak galls in the pest's native China (Murakami *et al*., 1980). The presence of this phenological

255   mismatch implies that parasitoid exchange between chestnut galls and oak galls is an ongoing

256   process, with occasional use of chestnut galls by a community that has evolved around the use of

257   bivoltine oak galls. However, the extremely high abundance of *D. kuriphilus* galls in its invaded

258   European range represents a massive unexploited resource available for any lineages within

259 parasitoid species that have a phenology more suited to that of *D. kuriphilus*. This could provide

260 these lineages with a selective advantage, potentially leading to the development of genetically

261 discrete host-associated ecotypes, one attacking oak galls and the other cycling on chestnut galls.

262 Such a scenario implies that over time the communities centred around the two resources of oak

263 galls and chestnut galls remain relatively distinct, with little exchange of individuals between them.

264 However, genetic data are required to establish whether this may be a possibility or whether

265 parasitoids consider oak- and chestnut-galling cynipids to be ecologically equivalent hosts within a

266 single community.

267 Our data provide some preliminary suggestions that *D. kuriphilus* has been integrated into the

268 oak gall community to a sufficient extent that it has impacted upon the behaviour of parasitoids.

269 The observed biases in sex ratios for some parasitoid species suggest that female parasitoids may be

270 altering their laying behaviour in response to the presence of this novel host. However, this remains

271 untested since we did not assess the sex ratios of the corresponding species emerging from native

272 oaks galls, so the observed biases may actually reflect a population-level bias apparent across all

273 hosts. Nevertheless, given its novelty, native parasitoids may view *D. kuriphilus* as a resource of

274 unpredictable or lower quality, and therefore individuals may alter their laying behaviour to take

275 advantage of this novel yet abundant host whilst minimising the risk to overall fitness (Charnov *et*

276 *al*., 1981). In this case the male-biased sex ratio we observed emerging from overwintering galls

277 may be due to the high relative abundance of chestnut galls in infested areas that act as a sink for

278 excess male eggs that otherwise would not be laid, a phenomenon associated with other invading

279 gallwasp species at high abundance (Hails & Crawley, 1991; Schönrogge *et al*., 2000).

280 Such integration of the invading *D. kuriphilus* into the native oak gall community has broader

281 implications for the evolution of this community. All the parasitoids attacking *D. kuriphilus* have

282 very broad host ranges, a general pattern also seen during the recruitment of parasitoids onto other

283 invading gallwasps (Stone *et al*., 1995). Our data confirm the presence of some generalist parasitoid

284 species that were predicted to recruit onto chestnut galls by Aebi *et al.* in 2006, and we could

285   reasonably expect further generalist species to start attacking *D. kuriphilus* in the near future. In

286   addition, although there is limited evidence for the recruitment of cynipid inquilines onto *D.*

287   *kuriphilus* galls (Aebi *et al*., 2006), if these important constituents of the oak gall community were

288   to start utilising chestnut galls we could also expect them to bring along greater parasitoid diversity

289   (Schönrogge *et al*., 1996) and hence tighten further the trophic links between oak and chestnut gall

290   communities. Given the very large population sizes of *D. kuriphilus* that can act as a significant

291   breeding ground for parasitoids, the presence of these trophic links means there is the potential for

292   severe negative impacts on native oak gall cynipids through the process of apparent competition

293   mediated through shared parasitoid species (Holt, 1977). The larger population sizes that may result

294   from increased use of the abundant *D. kuriphilus* could in turn lead to increased levels of parasitism

295   imposed upon native oak gallers, potentially driving their populations locally extinct (Hassel, 2000)

296   or inducing severe perturbations in the functioning of native communities (Henneman & Memmott,

297   2001). Such effects may be particularly likely to occur in the oak-chestnut galler community, given

298   that the parasitoid species most frequently attacking *D. kuriphilus* have broad host ranges within

299   native communities (see for example Askew, 1966). In addition, at least one of the commonly

300   encountered species, *E. urozonus*, is known also to attack leaf miners (Askew & Nieves-Aldrey,

301   2000), so impacts of increased parasitoid attack could extend into the wider community of insects

302   on both chestnuts and oaks.

303   One further complication is that one of the commonest parasitoids attacking *D. kuriphilus* is

304   *E. urozonus*, a species that can act as a hyperparasitoid and therefore can impose mortality upon

305   multiple trophic levels within a community. A similar phenomenon has recently been observed for

306   the parasitoid *Ormyrus labotus* Walker in the USA (Cooper & Rieske, 2011); in addition to

307   parasitising *D. kuriphilus*, this species also attacks the introduced biological control agent *T.*

308   *sinensis*. Hence it is imperative that monitoring of the community associated with *D. kuriphilus*

309   continues and that action is taken to minimise the impact this invader has on the wider ecosystem.

310   Furthermore, the potential for apparent competition can be examined in more detail using genetic

311   data to assess levels of gene flow among parasitoid populations attacking native oak galls and those

312   attacking chestnut galls. This would allow rates of parasitoid exchange between the different hosts

313   to be assessed, and therefore the degree to which parasitoids from chestnut galls could impact upon

314   native communities.

Acknowledgements

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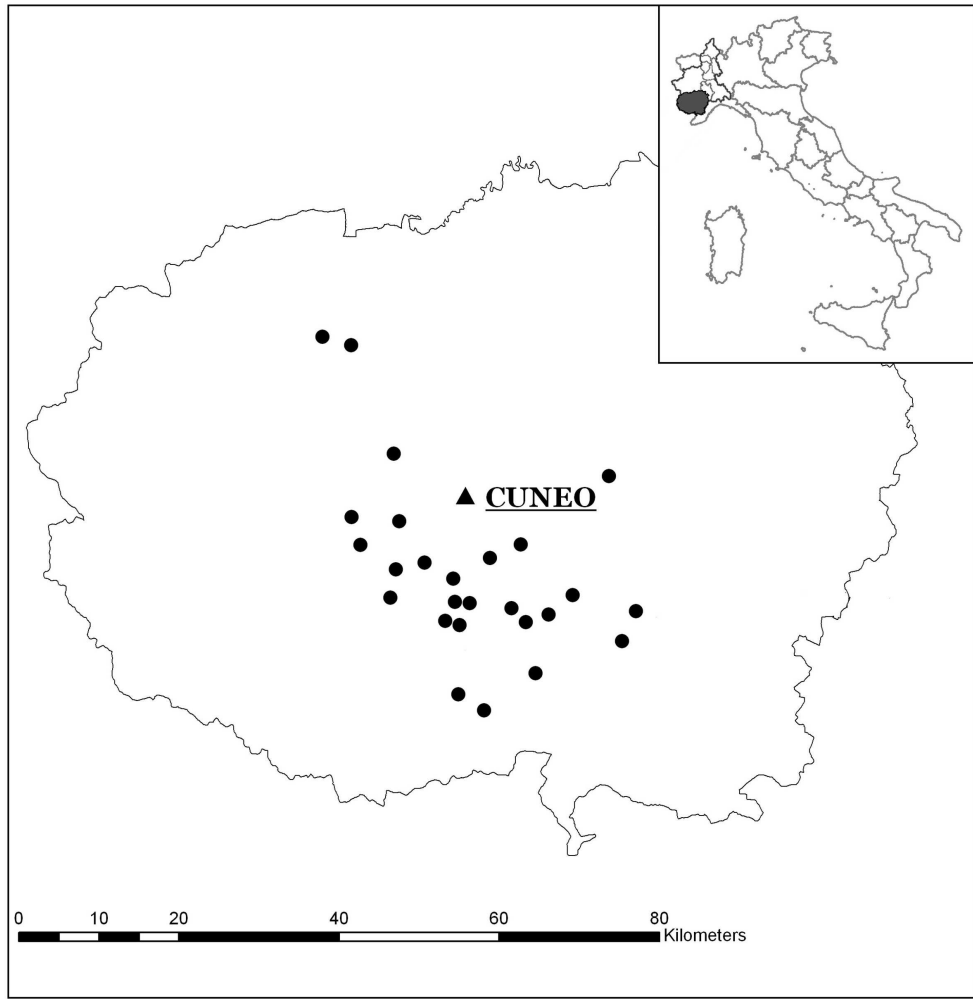
Location of the 26 sampling sites in Cuneo province, with inset showing the location of Cuneo province within Italy.
204x209mm (300 x 300 DPI)

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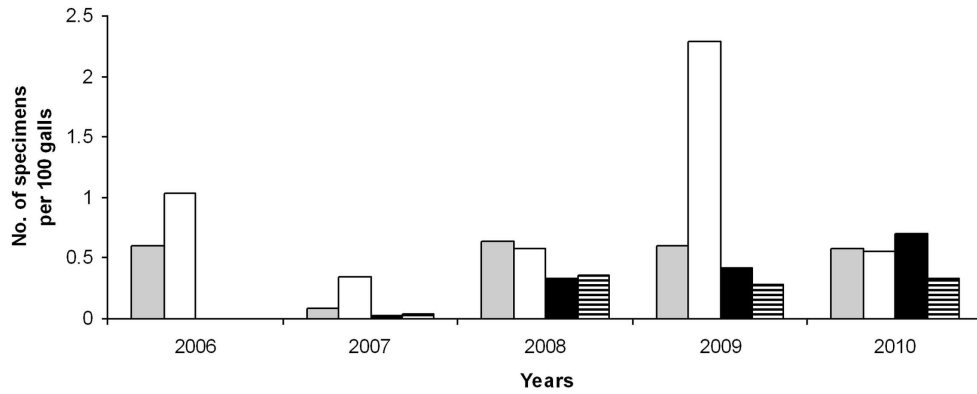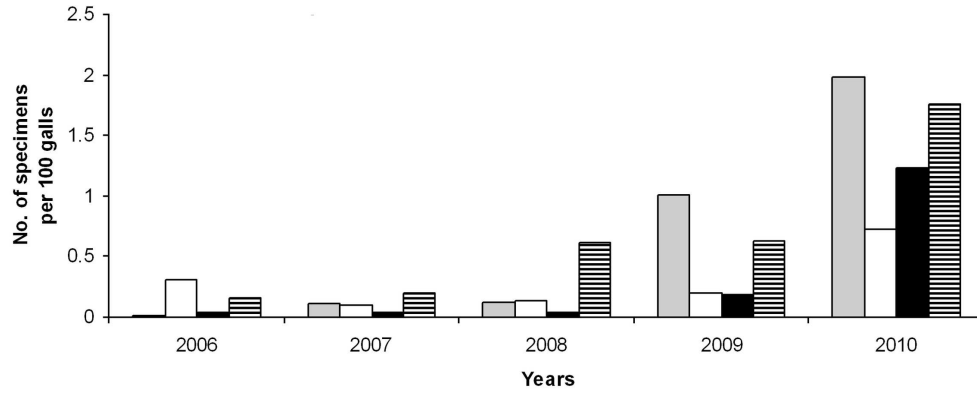
Emergence rates of the four most common parasitoid species attacking Dryocosmus kuriphilus galls in Cuneo province, Italy, over the years 2006-2010. The upper graph shows winter emergence, the lower one shows summer emergence. Shading of bars indicates species: grey Megastigmus dorsalis, white Eupelmus urozonus, black Eupelmus annulatus, stripes Eurytoma pistaciae.
196x183mm (300 x 300 DPI)
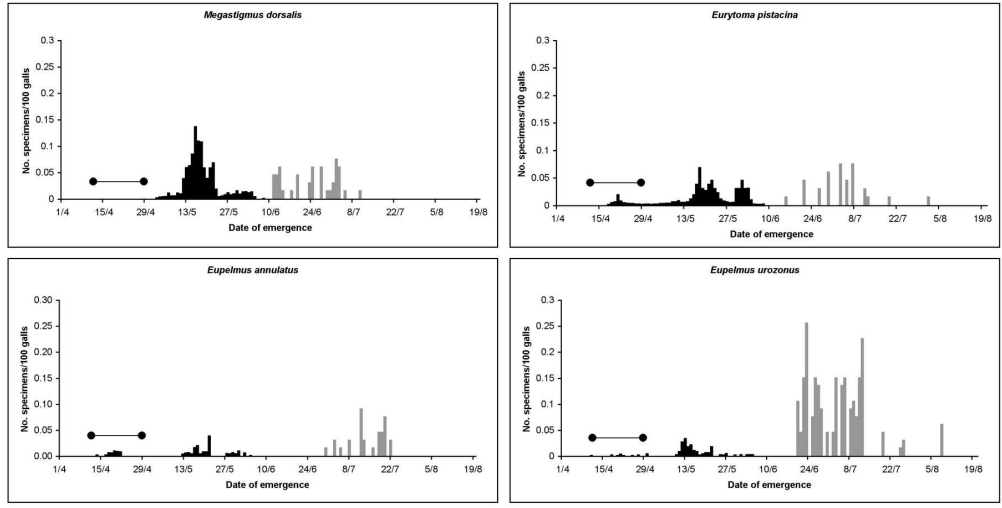
Seasonal patterns of emergence of the four most common parasitoid species attacking Dryocosmus kuriphilus galls in Cuneo province, Italy, in 2009. Black bars show emergence from withered galls collected in winter 2008-2009, grey bars show emergence from newly formed galls collected in summer 2009; the line indicates the growth period of D. kuriphilus galls.

152x78mm (300 x 300 DPI)

Table 1. Number of *Dryocosmus kuriphilus* galls collected from each year and site in Cuneo

Province, Italy.

Place	2006 winter	2006 summer	2007 winter	2007 summer	2008 winter	2008 summer	2009 winter	2009 summer	2010 winter	2010 summer	Total galls collected
Robilante	3,000		12,000	1,200	13,200	1,000	33,080		14,300	250	78,030
Peveragno	3,000		12,000	892			22,400		7,850		46,142
Boves	3,000		12,000	100			10,000		10,600		35,700
Chiusa Pesio			1,425	4,400	4,600	2,500				200	13,125
Boves (Mellana)		1,300	28,000		29,000		19,900	20	12,100		90,320
S. Bartolomeo		1,200									1,200
Cuneo (via Ripa)		700			6,400		24,800	1,800	10,000		43,700
Boves (Cerati)		757									757
Busca				1,430							1,430
Montefallonio				5,000		200					5,200
Fontanelle				100							100
Pianfei				7,870				50			7,920
Valgrana				2,000							2,000
Bernezzo				1,100							1,100
Cuneo (Tetti Pesio)					26,000						26,000
Vasco						500		800			1,300
Vernante										600	600
Caraglio							3,000		6,700		9,700
Cervasca						300	15,200	700	5,000		21,200
Gambara							3,000		9,000		12,000
Roccasparvera							1,000				1,000
Montemale								2,100			2,100
Rio Secco								1,200			1,200
Martiniana Po									10,300		10,300
Sanfront										3,000	3,000
Limone										100	100
Total winter	9,000		65,425		79,200		132,380		85,850		371,855
Total summer		3,957		24,092		4,500		6,670		4,150	43,369
											415,224

Table 2. Parasitoid species attacking the chestnut gallwasp *Dryocosmus kuriphilus* in north-western Italy. Species names in bold indicate cryptic species pairs which molecular data resolve; not all individuals collected each year from these species pairs were sequenced, so the presence of the first mentioned species indicates presence of the corresponding morpho-species. Data for the years 2002-2005 are from Aebi *et al*., 2006.

Species	Family	2002-2005	2006	2007	2008	2009	2010
Aprostocetus sp.	Eulophidae		X	X	X	X	X
Aulogymnus arsames	Eulophidae					X	
Aulogymnus sp.	Eulophidae			X	X	X	X
Baryscapus pallidae	Eulophidae	X					
Baryscapus sp.	Eulophidae						X
Pediobius chilaspidis	Eulophidae			X			
Pediobius saulius	Eulophidae					X	X
Pediobius sp.	Eulophidae			X	X		X
Eupelmus annulatus*	Eupelmidae		X	X	X	X	X
Eupelmus spongipartus*	Eupelmidae					X	
Eupelmus splendens	Eupelmidae			X		X	
Eupelmus urozonus	Eupelmidae	X	X	X	X	X	X
Eupelmus fulvipes	Eupelmidae					X	
Eurytoma brunniventris	Eurytomidae	X		X	X	X	X
Eurytoma adleriae	Eurytomidae						X
Eurytoma pistaciae	Eurytomidae	X	X	X	X	X	X
Sycophila variegata	Eurytomidae	X	X	X	X	X	X
Sycophila biguttata	Eurytomidae	X		X		X	X
Sycophila iracemae	Eurytomidae	X					
Ormyrus nitidulus	Ormyridae					X	
Ormyrus pomaceus	Ormyridae	X				X	X
Cecidostiba sp.	Pteromalidae				X		X
Mesopolobus amaenus	Pteromalidae				X		
Mesopolobus mediterraneus	Pteromalidae	X					
Mesopolobus sericeus	Pteromalidae	X		X	X	X	X
Mesopolobus tarsatus	Pteromalidae	X				X	
Mesopolobus tibialis	Pteromalidae				X		
Megastigmus dorsalis (sp1)	Torymidae	X	X	X	X	X	X
Megastigmus dorsalis (sp2)	Torymidae					X	
Torymus auratus	Torymidae	X					
Torymus flavipes	Torymidae	X		X			
Torymus scutellaris	Torymidae	X					

**Bouček's (1970) consideration of *Eupelmus annulatus* and *E. spongipartus* as synonyms appears to be incorrect and the names do represent two distinct species (Gibson, 2011).*

Table 3. Number of females and males of the four most abundant parasitoid species emerging from

Dryocosmus kuriphilus galls in north-western Italy in the period 2006-2010. The observed sex

ratios were tested against an expected 50:50 ratio (all d.f. = 1, *P<0.05, **P<0.01, ***P<0.001;

NS=not significant).

2006		winter			summer	
	F	M	$\chi2$	F	M	$\chi2$
Megastigmus dorsalis	0	1	NS	22	2	16.67***
Eupelmus urozonus	12	16	NS	38	3	29.88***
Eupelmus annulatus	3	0	NS	-	-	-
Eurytoma pistaciae	6	8	NS	-	-	-

2007		winter			summer	
	F	M	$\chi2$	F	M	$\chi2$
Megastigmus dorsalis	14	60	28.59***	8	12	NS
Eupelmus urozonus	20	45	9.62**	46	38	NS
Eupelmus annulatus	18	3	10.71**	4	2	NS
Eurytoma pistaciae	32	93	29.77***	2	8	NS

2008		winter			summer	
	F	M	$\chi2$	F	M	$\chi2$
Megastigmus dorsalis	11	86	57.99***	20	9	4.17*
Eupelmus urozonus	29	82	25.31***	15	11	NS
Eupelmus annulatus	10	19	NS	9	6	NS
Eurytoma pistaciae	241	245	NS	11	5	NS

2009		winter			summer	
	F	M	$\chi2$	F	M	$\chi2$
Megastigmus dorsalis	227	1115	587.59***	28	12	6.40*
Eupelmus urozonus	78	177	38.44***	96	57	9.94**
Eupelmus annulatus	77	165	32.00***	25	3	17.29***
Eurytoma pistaciae	391	446	NS	13	6	NS

2010		winter			summer	
	F	M	$\chi2$	F	M	$\chi2$
Megastigmus dorsalis	401	1300	475.13***	15	9	NS
Eupelmus urozonus	446	180	113.03***	17	6	5.26*
Eupelmus annulatus	400	652	60.37***	21	8	5.83*
Eurytoma pistaciae	757	754	NS	12	2	7.14**

Table S1. Emergence data for the 10 parasitoid species found to attack *Dryocosmus kuriphilus* galls regularly in north-western Italy during the period 2006-2010. Both the total number of specimens (ns) and the standardised emergence rate (er; number of adults emerging per 100 galls) are presented.

	2006 winter		2006 summer		2007 winter		2007 summer		2008 winter		2008 summer		2009 winter		2009 summer		2010 winter		2010 summer	
	ns	er	ns	er	ns	er	ns	er	ns	er	ns	er	ns	er	ns	er	ns	er	ns	er
Megastigmus dorsalis	1	0.01	24	0.61	74	0.11	20	0.08	97	0.12	29	0.64	1342	1.01	40	0.60	1701	1.98	24	0.58
Eurytoma pistaciae	14	0.16	-	-	125	0.19	10	0.04	486	0.61	16	0.36	837	0.63	19	0.28	1511	1.76	14	0.34
Eupelmus annulatus	3	0.03	-	-	21	0.03	6	0.02	29	0.04	15	0.33	242	0.18	28	0.42	1052	1.23	29	0.70
Eupelmus urozonus	28	0.31	41	1.04	65	0.10	84	0.35	111	0.14	26	0.58	255	0.19	153	2.29	626	0.73	23	0.55
Sycophila variegata	-	-	6	0.15	1	0.00	-	-	4	0.01	3	0.07	18	0.01	2	0.03	74	0.09	5	0.12
Sycophila biguttata	-	-	3	0.08	-	-	6	0.02	-	-	2	0.04	3	0.00	1	0.01	33	0.04	-	-
Eurytoma brunniventris	-	-	-	-	-	-	50	0.21	1	0.00	9	0.20	10	0.01	-	-	1	0.00	-	-
Mesopolobus sericeus	-	-	-	-	2	0.00	-	-	19	0.02	-	-	10	0.01	-	-	161	0.19	15	0.36
Aulogymnus sp.	-	-	-	-	31	0.05	-	-	21	0.03	-	-	26	0.02	-	-	16	0.02	-	-
Aprostocetus sp.	-	-	23	0.58	47	0.07	-	-	1	0.00	-	-	5	0.00	-	-	16	0.02	-	-
total	92	0.51	97	2.45	366	0.56	176	0.73	769	0.97	100	2.22	2748	2.08	243	3.64	5191	6.05	110	2.65