Ooctonus vulgatus (Hymenoptera, Mymaridae), a potential biocontrol agent to reduce populations of *Philaenus spumarius* (Hemiptera, Aphrophoridae) the main vector of *Xylella fastidiosa* in Europe.

Xavier Mesmin [1, 2], **Marguerite Chartois** [2], **Guenaelle Genson** [2], **Jean-Pierre Rossi** [2], **Astrid Cruaud** [2], **Jean-Yves Rasplus** [Corresp. 2]

[1] AGAP, INRAE, CIRAD, Montpellier SupAgro, Univ Montpellier, San Giuliano, France

[2] CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier, France

Corresponding Author: Jean-Yves Rasplus
Email address: jean-yves.rasplus@inra.fr

As a vector of *Xylella fastidiosa* (Wells, 1987) in Europe, the meadow spittlebug *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera, Aphrophoridae) is a species of major concern. Therefore, tools and agents to control this ubiquitous insect that develops and feeds on hundreds of plant species are wanted. We conducted a field survey of *P. spumarius* eggs in Corsica and provide a first report of *Ooctonus vulgatus* Haliday, 1833 (Hymenoptera, Mymaridae) as a potential biocontrol agent of *P. spumarius* in Europe. To allow species identification, we summarized the main characters distinguishing *O. vulgatus* from other European species of *Ooctonus* and generated *COI* DNA barcodes. Parasitism rates were variable in the four localities included in the survey but could reach 69% (for an average number of eggs that hatched per locality of 109). Based on the geographic occurrences of *O. vulgatus* obtained from the literature, we calibrated an ecological niche model to assess its potential distribution in the Holarctic. Obviously, several questions need to be addressed to determine whether *O. vulgatus* could become an effective biocontrol agent of *P. spumarius* in Europe. So far, *O. vulgatus* has been reared only from *P. spumarius* eggs, but its exact host-range should be evaluated to ensure efficiency and avoid non-target effect. The top-down impact of the parasitoid on vector populations should also be assessed on large data sets. Finally, the feasibility of mass rearing should be tested. We hope this report serves as a starting point to initiate research on this parasitoid wasp to assess whether it could contribute to reduce the spread and impact of *X. fastidiosa* in Europe.

Ooctonus vulgatus (Hymenoptera, Mymaridae), a potential biocontrol agent to reduce populations of *Philaenus spumarius* (Hemiptera, Aphrophoridae) the main vector of *Xylella fastidiosa* in Europe.

Xavier Mesmin[1,2], Marguerite Chartois[2], Guénaëlle Genson[2], Jean-Pierre Rossi[2], Astrid Cruaud[2] & Jean-Yves Rasplus[2£]

[1]AGAP, INRAE, CIRAD, Montpellier SupAgro, Univ Montpellier, San Giuliano, France

[2]CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier, France

[£]corresponding author: jean-yves.rasplus@inrae.fr

ABSTRACT

As a vector of *Xylella fastidiosa* (Wells, 1987) in Europe, the meadow spittlebug *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera, Aphrophoridae) is a species of major concern. Therefore, tools and agents to control this ubiquitous insect that develops and feeds on hundreds of plant species are wanted. We conducted a field survey of *P. spumarius* eggs in Corsica and provide a first report of *Ooctonus vulgatus* Haliday, 1833 (Hymenoptera, Mymaridae) as a potential biocontrol agent of *P. spumarius* in Europe. To allow species identification, we summarized the main characters distinguishing *O. vulgatus* from other European species of *Ooctonus* and generated *COI* DNA barcodes. Parasitism rates were variable in the four localities included in the survey but could reach 69% (for an average number of eggs that hatched per locality of 109). Based on the geographic occurrences of *O. vulgatus* obtained from the literature, we calibrated

an ecological niche model to assess its potential distribution in the Holarctic. Obviously, several questions need to be addressed to determine whether *O. vulgatus* could become an effective biocontrol agent of *P. spumarius* in Europe. So far, *O. vulgatus* has been reared only from *P. spumarius* eggs, but its exact host-range should be evaluated to ensure efficiency and avoid non-target effect. The top-down impact of the parasitoid on vector populations should also be assessed on large data sets. Finally, the feasibility of mass rearing should be tested. We hope this report serves as a starting point to initiate research on this parasitoid wasp to assess whether it could contribute to reduce the spread and impact of *X. fastidiosa* in Europe.

INTRODUCTION

Xylella fastidiosa (Wells, 1987) is a xylem-dwelling insect-borne bacterium that originates from the Americas, infects more than 500 species of plants (EFSA, 2015) and causes a variety of scorch-like diseases in many cultivated species (Almeida & Nunney, 2015; EFSA, 2018; Sicard et al., 2018). Studies on the economic impact of *X. fastidiosa* have primarily focused on the wine and grape industries. Yield reduction and management costs to the California grape industry are estimated at more than US$100 million per year (Tumber, Alston & Fuller, 2014) and a potential introduction of the bacterium in Australia is estimated to cost up to AUD 7.9 billion over 50 years (Hafi et al., 2017).

X. fastidiosa has been recently detected in Europe and is present in Italy (Saponari et al., 2013), France (Denancé et al., 2017), Spain (Olmo et al., 2017), and Portugal (DGAV, 2019). Furthermore, niche modelling has shown that a large part of Europe is climatically suitable for the bacterium (Godefroid et al., 2018, 2019). Hence, *X. fastidiosa* represents a serious threat to European agriculture and natural ecosystems.

The spread of *X. fastidiosa* depends on several interacting factors, mainly insect vectors and plant communities as well as landscape, climate features and population dynamics of the bacterium itself (Krugner et al., 2019). As a consequence, disease management is complex. Reducing bacterium spread requires acting on a set of different biotic and abiotic factors (Almeida et al., 2005) and modelling approaches may help setting up effective strategies (Fierro, Liccardo & Porcelli, 2019). Here we focus on a possible management strategy to control populations of the most common vector of *X. fastidiosa* reported in Europe so far: the meadow

58 spittlebug *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera, Aphrophoridae) (Saponari et al.,
59 2014; Cornara et al., 2016).

60 *P. spumarius* is highly polyphagous (Cornara, Bosco & Fereres, 2018), widely distributed in the
61 Palearctic from sea level to high elevation (about 2000m; e.g. Halkka, Raatikainen & Vilbaste,
62 1975; Lees, Dent & Gait, 1983; Drosopoulos & Asche, 1991; Loukas & Drosopoulos, 1992;
63 Quartau, Borges & André, 1992; Stewart & Lees, 1996; Drosopoulos & Remane, 2000), and was
64 probably introduced to the New World (Whittaker, 1973). Its ability to acquire and transmit *X.*
65 *fastidiosa* was previously demonstrated (Severin, 1950; Saponari et al., 2014; Cornara et al.,
66 2016).

67 So far, a few studies have assessed the impact of different insecticides to reduce juvenile
68 populations of *P. spumarius* in Europe (Dongiovanni et al., 2018; Dáder et al., 2019). However,
69 there is a growing awareness of the need to encourage management practices that safeguard
70 harvests, human health, biodiversity and the environment. Thus, the development of effective
71 biological control programs is desirable. Among biocontrol strategies, augmentative biological
72 control consists in enhancing the effectiveness of naturally occurring natural enemies by the
73 periodic release of specimens (Eilenberg, Hajek & Lomer, 2001; Aubertot & Savary, 2005).
74 Compared to classical biological control it eliminates unintended effects of the introduction of
75 new, non-native, parasitoids or predaceous arthropods (Hoy, 2008). However, as for all biological
76 control programs, augmentative biocontrol requires field investigations to identify potential
77 natural enemies of the target pest.

78 Currently, information about the natural enemies of the meadow spittlebug are scattered
79 (Cornara et al. 2018). Species of birds, frogs, arachnids, and insects (Hymenoptera, Diptera, and

Coleoptera) occasionally feed on *P. spumarius* (Phillipson, 1960; Halkka & Kohila, 1976; Harper & Whittaker, 1976; Henderson, Hoffman & Jeanne, 1990; Pagliano & Alma, 1997) but predation by native natural enemies does not appear to be an important source of mortality. Studies are in progress to test whether the invasive assassin bug *Zelus renardii* Kolenati, 1857 (Hemiptera, Reduviidae) could be used to control populations of *P. spumarius* in olive orchards (Salerno et al., 2017). However, mass release of this species may be risky for local biodiversity, especially for beneficial arthropods (Ables, 1978). Indeed, it is considered as a generalist predator (Ables, 1978, Cisneros & Rosenheim, 1998; Weirauch, Alvarez & Zhang, 2012, Salerno et al., 2017, but see Cohen & Tang, 1997 who suggest a strong effect of prey body size).

So far, only few parasitoids of *P. spumarius* have been recorded. Adults are attacked by *Verralia aucta* (Fallen, 1817) (Diptera, Pipunculidae) in Europe with relatively high parasitism rates in England: in average 31% in females and 46% in males over four years (Whittaker, 1969, 1973). Parasitism by *V. aucta* has a direct effect on *P. spumarius* population dynamics because it renders the host sterile (Whittaker, 1973). However, this parasitoid does not have an immediate effect on bacterium transmission because adults are only killed after 10-11 weeks of parasitism (Whittaker, 1969), a period during which they are probably still able to spread the bacterium. Contrastingly, an interesting feature of egg parasitoids is that they kill the host in the egg stage, that is, before it can inflict damage to its host plants (Mills, 2010). In the case of *P. spumarius*, the insect is killed before it acquires the bacterium from an infected host plant and becomes able to transmit it. A few egg parasitoids have been recorded in the US: *Ooctonus vulgatus* Haliday, 1833 (Hymenoptera, Mymaridae) and at least two unnamed species of *Centrodora* (Hymenoptera, Aphelinidae) (Weaver & King, 1954). Indeed, the genus *Tumidiscapus*, which is cited as parasitoid

of *P. spumarius* in the US (Weaver & King, 1954), is in fact a synonym of *Centrodora* (Hayat, 1983). However, little is known about the biology and efficacy of egg parasitoids *in natura*.

In this study, a field survey was conducted to identify major egg parasitoids of *P. spumarius* in Corsica. We provide a first report of *Ooctonus vulgatus* in this area. We summarized the main characters separating *O. vulgatus* from other Palearctic species to facilitate identification and generate *COI* DNA barcodes to accurately identify the species. Finally, we reviewed the literature and gathered all available occurrence data (i.e. geographical coordinates) of previously detected populations of *O. vulgatus.* This allowed us to calibrate an ecological niche model linking different climate descriptors to species occurrence data and estimate the potential distribution of the parasitoid in the Holarctic region for comparison with the distribution of *P. spumarius*.

MATERIALS AND METHODS

Sampling and calculation of parasitism rate

Five to ten handfuls of about eight top branches of *Cistus monspeliensis* L. 1753 (cut at 50 cm below the end of the branch) were sampled in four localities (Figure 1). These localities were part of a larger field survey of population dynamics of *P. spumarius* in Corsica. We targeted *C. monspeliensis* to maximize our chances to find eggs of *P. spumarius*. Indeed, we demonstrated in a previous study that, in Corsica, adults of *P. spumarius* seemed to be mainly associated with this species (Cruaud et al., 2018). Sampling was performed between the 12th and the 15th of February 2019. The back of each leaf (about 900 leaves per handful of branches) was inspected in the laboratory for whitish clusters, which were retained and inspected under a binocular

microscope to confirm the presence of eggs of *P. spumarius* (Appendix S1; 109, 148, 167, 187 eggs obtained per site, hence a total of 611 eggs monitored). The morphological identification of *P. spumarius* eggs and first stage nymphs was performed using the descriptions of Weaver and King (1954) (Appendix S1). The pieces of leaf containing the eggs were placed on filter papers in unaerated Petri dishes (i.e. without spur) at room temperature (20.2 ± 1.5 °C), with natural light. Filter papers were kept moist by adding drops of water when necessary. Hatching was monitored every morning from the 18th of February to the 15th of March 2019. Emerging nymphs and parasitoids were killed and stored in 70% Ethanol at 4°C. Parasitism rates were computed in each locality with the following formula: $Parasitism\ rate = \frac{Number\ of\ parasitized\ eggs}{Number\ of\ parasitized\ eggs + Number\ of\ unparasitized\ eggs}$ (Costello & Altieri, 1995).

Morphological identification of the parasitoids

Identification to species was performed using the *Ooctonus* keys by Triapitsyn (2010) and Huber (2012). Specimens were desiccated using HMDS (Heraty & Hawks, 1998) and glued on grey cards. Imaging was performed with a Keyence digital microscope (VHX-5000 Camera color CMOS and the VH-Z100UT lens). Images were then edited in Adobe Photoshop CS6© software.

Molecular identification of the parasitoids

Six individuals were used for molecular identification. Three of them were handled individually (sample codes = XMES00042_0101, XMES00077_0101, XMES00091_0101) and the remaining three were pooled to increase DNA yield (sample code = XMES00041_0189). Total genomic DNA was isolated using the Qiagen DNeasy Blood & Tissue kit without destruction of the specimens.

146 We followed manufacturer's protocol with the following modifications. Samples (whole insects,
147 without dissection or crushing) were incubated overnight in an Eppendorf thermomixer
148 (temperature = 56°C, mixing frequency = 300 rpm). To increase DNA yield, two successive
149 elutions (50 µL each) were performed with heated buffer AE (56°C) and an incubation step of 15
150 minutes followed by centrifugation (6000g for 1 minute at room temperature; see Cruaud et al.
151 2019 for a detailed description of the protocol). Eppendorf microtubes LoBind 1.5ml were used
152 for elution and to store DNA at minus 20°C until PCR amplification. Vouchers were deposited at
153 Centre de Biologie pour la Gestion des Populations (CBGP), Montferrier-sur-Lez, France. The
154 mitochondrial Cytochrome c oxidase I standard barcode fragment (*COI*) was amplified with a
155 cocktail of M13-tailed primers as detailed in Germain et al. (2013). Unpurified PCR products were
156 sent to Eurofins MWG Operon (Ebersberg, Germany) for sequencing using the M13F and M13R
157 primers (Germain et al., 2013; Ivanova et al., 2007). Both strands for each overlapping fragment
158 were assembled in Geneious v11.1.4 (https://www.geneious.com). Geneious was also used to
159 translate consensus sequences to amino acids to detect premature codon stops. All *COI*
160 sequences available on BOLD (Ratnasingham & Hebert, 2007) for *Ooctonus* species were
161 downloaded (last access July 12,2019) and aligned with the newly generated sequences using
162 MAFFT v7.245 (Katoh & Standley, 2013). A maximum likelihood tree was inferred with raxmlHPC-
163 PTHREADS-AVX version 8.2.4 (Stamatakis, 2014). A rapid bootstrap search (100 replicates)
164 followed by a thorough ML search (-m GTRGAMMA) was conducted. Tree visualization and
165 annotation was performed with TreeGraph 2.13 (Stöver & Müller, 2010).
166
167 **Species distribution modelling framework**

Occurrences of *O. vulgatus* were retrieved from the literature and the GBIF database (GBIF.org 2019) (Tables S1&S2). Two hundred and five occurrences were obtained from the literature (Table S1), eight of which were not included in the analysis as no geographic coordinates were available. Forty occurrences were obtained from GBIF (last access: 22 August 2019; Table S2), but were all discarded due to dubious identification or lack of information on sample origin. Therefore, no occurrence obtained from GBIF could be included in the analysis.

We fitted a correlative model linking different climate descriptors to species occurrences. The Maxent algorithm was chosen to conduct analyses because it does not require absence data (i.e. locations in which we can presume that a species is truly absent) (Phillips et al., 2006). We summarized below the main step of our analysis and details are provided in Appendix S2. The mean temperature and precipitation of the wettest, driest, warmest, and coldest quarters as well as precipitation seasonality were extracted from the Worldclim 2.0 database (Fick & Hijmans, 2017) and used as bioclimatic descriptors (Hijmans et al., 2005). In absence of formal knowledge about climatic factors constraining *O. vulgatus* distribution, we constituted three sets of bioclimatic variables and performed modelling with each of them (Qiao, Soberón & Peterson, 2015; Godefroid et al., 2019). The first set (CLIM1) comprised the mean temperature of the wettest, driest, warmest, and coldest quarters to reflect the impact of temperature constraints on distribution. To highlight the precipitation constraint, we added the precipitation seasonality to CLIM1 and constituted the second set (CLIM2). Finally, we built a third set (CLIM3) by assembling CLIM1 and the precipitation of the wettest, driest, warmest, and coldest quarters to fully account for both extreme temperatures and precipitations in the species distribution models

(SDMs). The Maxent algorithm requires a set of locations where the species has been found (here, a random 70 % of the available occurrences, the other 30 % being used for model validation) and a set of locations where no information about the presence of the species are available (referred to as background points). A total of 10,000 background points were randomly generated in North America and Europe). To render complex response to environmental constraints while reducing model overfitting we first fitted 48 Maxent models using six regularization multiplier (RM) combinations (L, LQ, H, LQH, LQHP, LQHPT with L=linear, Q=quadratic, H=hinge, P=product and T=threshold) and feature class (FC) values (eight values ranging from 0.5 to 4 with increments of 0.5) (Radosavljevic & Anderson, 2014). Optimal FC and RM combinations were determined for each of the three bioclimatic datasets (CLIM1 - CLIM3) using the R language (R core team 2019) and the package ENMeval (Muscarella et al., 2014). Optimal parameters were then used to fit a set of 10 replicate Maxent models using 70% of the dataset. The performance of each model was evaluated using the remaining 30% of occurrences using the area under the receiver–operator curve (AUC, Fielding & Bell, 1997) and the true skill statistics (TSS, Allouche, Tsoar & Kadmon, 2006). Models with AUC < 0.8 were excluded from further analyses (Vicente et al., 2013). Habitat suitability maps (logistic output ranging from 0 to 1) were transformed into binary projections using the threshold that optimized the TSS statistics on the testing data (Guisan, Thuiller & Zimmermann, 2017). Maxent replicate models were fitted and evaluated using the R package biomod2 (Thuiller et al., 2009).

Two different outputs were generated using the set of model prediction. i) Binary predictions were averaged to produce the committee (consensus) averaging (Araújo & New, 2007; Marmion et al., 2009) showing the likelihood of the presence of *O. vulgatus*. This consensus model ranges

212 from 0 (all the models predict absence) to 100% (all the models predict presence) and ii) the
213 median of the logistic outputs (Guisan, Thuiller & Zimmermann, 2017) of the models that depicts
214 the climate suitability across the different models.
215
216 **RESULTS**
217
218 **Parasitism rates.**
219 Out of the 611 eggs monitored, 437 (i.e. 71.5 %) hatched. 277 (63.4%) gave rise to *P. spumarius*
220 nymphs and parasitoids emerged from 160 eggs (36.6%). All parasitoids were identified as *O.*
221 *vulgatus* (Figure 2). No parasitoid emerged from eggs collected in one of the four localities. We
222 observed parasitism rates of 20.5, 48.9 and 69.0% in the three other localities (Figure 1).
223
224 **Guidelines for the identification of *O. vulgatus*.**
225 To help identification, we list below the main features that differentiate *O. vulgatus* from its
226 closest relatives. The genus *Ooctonus* has been recently revised in the Palearctic and Nearctic
227 regions respectively by Triapitsyn (2010) and Huber (2012). *Ooctonus* can be distinguished from
228 other genera of Mymaridae by the following set of characters: tarsi 5-segmented, propodeum
229 with diamond-shaped pattern of carinae (Figure 2F), fore wing venation about one-third the wing
230 length (Figure 2C), with short marginal and stigmal vein, parastigma with hypochaeta next to
231 proximal macrochaeta (Huber, 2012). In the Holarctic region, *O. vulgatus* can be distinguished
232 from other species of *Ooctonus* by the following unique combination of features (Figure 2): vertex
233 without stemmaticum; mesoscutum without median groove; posterior part of scutellum and

234 frenum smooth with weak sculpture laterally; metanotum and propodeum without reticulate
235 sculpture; propodeum without median carina, but with a pentagonal areole formed by
236 dorsolateral carinae; short petiole, 0.9–1.2x as long as metacoxa; forewing at least slightly
237 truncate apically; females funicle with multiporous placoid sensilla (mps) on F7 and F8 only, F5
238 and F6 without mps; single row of six bullae inside the female clava; ovipositor at most 1.4× as
239 long as metatibia and only slightly exerted beyond apex of gaster.
240
241 **Molecular identification of the parasitoid.**
242 Barcode sequences were successfully generated from all samples. All sequences were identical.
243 Phylogenetic analysis confirmed that the most likely identification was *O. vulgatus* (Figure S1).
244
245 **Species distribution modelling.**
246 A total of 200 occurrences (197 obtained from the literature plus the three localities where we
247 sampled *O. vulgatus*) (Figure 3A) were used to model the distribution of *O. vulgatus* in Europe.
248 The optimal Maxent parameters were RM=4 and FC=hinge; RM=4 and FC=hinge and RM=2.5 and
249 FC=hinge for CLIM1, CLIM2, and CLIM3, respectively. With the exception of one model of CLIM2,
250 all models based on these optimal values yielded AUC values >0.8, which indicated that the
251 different bioclimatic data subsets performed well. The consensus model was therefore computed
252 from a set of 29 estimates of climate suitability.
253 Figure 3B shows the median of the climate suitability values for the 29 models considered. Figure
254 3C depicts the proportion of the 29 models indicating that the climate is suitable for *O. vulgatus*.
255 Both Figure 3B and 3C show that the climate is favorable in very large areas covering most of

Western Europe and around the Black Sea. These areas are overlapping with the geographical range of *P. spumarius* (Cruaud et al. 2018).

DISCUSSION

Ooctonus Haliday, 1833 is a medium-sized genus of Mymaridae containing 37 described species that occur in all zoogeographical regions of the world (Holt et al., 2013) excepted Australasia (Noyes, 2019). *O. vulgatus* has been reared from the eggs of *P. spumarius* and studied only once in North America (Weaver & King, 1954). This species is thus poorly known as confirmed by the limited barcoding record. Indeed, only four barcodes are available in BOLD (two from Virginia United States, one from Ontario Canada, and one from British Columbia Canada). As a likely component of aerial plankton, *O. vulgatus* is expected to be a widespread species distributed in the Holarctic region (ranging from Ireland to the Sakhalin peninsula and from eastern to western coasts of North America, as south as California (Huber, 2012)). The species has been also reported from China (Bai, Jin & Li, 2015) but authors' illustration casts some doubts about specimen identification. There are only a few unquestionable occurrences in the literature for this species (n=197). Here, we provide a first report of *O. vulgatus* in Corsica and assess, for the first time in Europe, its biology as parasitoid of *P. spumarius*. We also confirm its potential large distribution throughout Europe with modelling approaches. More importantly we show that *O. vulgatus* potential distribution in Europe (Figure 3) overlaps that of its host *P. spumarius* (Cruaud et al., 2018), which is not surprising from a biological point of view but is an interesting result in the framework of biological control. This study is preliminary and predictions, especially because they are based on a limited number of occurrences, are indicative only. This study is a

starting point to encourage investigations in other parts of Europe. Sampling efforts should more specifically target areas predicted as suitable for *P. spumarius* but non-suitable for *O. vulgatus* such as eastern areas of Europe.

When studied in North America, observed parasitism rates did not exceed 10% of the sampled eggs of *P. spumarius* (Weaver & King, 1954). Here, we obtained parasitism rates of up to 69 %, but absence of parasitism in one sampled site. While we acknowledge sampling four sites is not enough to have a representative view of *P. spumarius* egg parasitism in Corsica, our results show that parasitism rate can be high though very variable. Further surveys are obviously necessary to better assess the spatial and temporal variability of parasitism rate and understand what is(are) the cause(s) of such variations in Corsica and throughout Europe. Identifying such drivers could open new avenues for conservation biological control against *P. spumarius*, through the implementation of environments favorable to *O. vulgatus* in the vicinity of crops susceptible to *X. fastidiosa*.

The use of mymarids in biological control program has a long history. The most notable instance being the use of *Anaphes nitens* (Girault, 1928) in several countries to successfully control the eucalyptus weevil, *Gonipterus scutellatus* Gyllenhal, 1833 (Coleoptera, Curculionidae), which feeds and reproduces on *Eucalyptus* trees (Doull, 1955). More recently, *Cleruchoides noackae* Lin and Huber, 2007 has been used in South America to control an invasive sap-feeding pest of *Eucalyptus*, *Thaumastocoris peregrinus* Carpintero and Dellapé, 2006 (Hemiptera, Thaumastocoridae) (Martínez, González & Dicke, 2018). Mymarid species were used to control leafhopper vectors of plant pathogens (Hemiptera, Cicadellidae). *Anagrus armatus* (Ashmead, 1887) regulated *Edwardsiana froggatti* (Baker, 1925) (Hemiptera, Cicadellidae), a pest

of apple in New Zealand, with parasitism rates of the eggs reaching 80% (Dumbleton, 1937). More recently, ***Cosmocomoidea*** species were used to target *Homalodisca vitripennis* (Germar, 1821) (Hemiptera, Cicadellidae) a vector of *X. fastidiosa* in California (Irvin & Hoddle, 2010). In all these cases, mymarids helped regulate pest population growth.

However, before any attempts to regulate populations of *P. spumarius* are made, we need to enrich our knowledge on *O. vulgatus*. In particular, the degree of specificity of the *P. spumarius* – *O. vulgatus* interaction needs to be determined to avoid non-target effect of augmentative biocontrol (Van Driesche & Hoddle, 2016). We also need to evaluate our ability to consistently rear *O. vulgatus* in controlled conditions, one of the key obstacles to the use of mymarids in biological control programs (but see Martínez, González & Dicke, 2018). Finally, parasitoids can have complex effects on vector-borne disease by either increasing (Jeger et al., 2011) or decreasing (Martini, Pelz-Stelinski & Stelinski, 2014) pathogen spread. Further research is still needed to better understand the impact of such tri-trophic interactions on plant disease dynamics. While *O. vulgatus* does not directly impact transmission capacity of *P. spumarius*, by killing its host at an early stage of development, it reduces the number of vectors that may acquire the bacterium from an infected host-plant and become able to transmit it.

Again, we consider this study as a starting point to encourage research on this parasitoid wasp to assess whether it could contribute to reduce the spread and impact of *X. fastidiosa* in Europe. Increasing egg parasitism of *P. spumarius* in the fall might significantly reduce population size in the next year and possibly the transmission of the bacterium, without resorting to chemical treatments.

322

323

324

325

326 **REFERENCES**

327 Ables JR. 1978. Feeding Behavior of an Assassin Bug, *Zelus renardii*. *Annals of the Entomological*
328 *Society of America* 71:476–478. DOI: 10.1093/aesa/71.4.476.
329 Allouche O, Tsoar A, and Kadmon R. 2006. Assessing the accuracy of species distribution
330 models: prevalence, kappa and the true skill statistic (TSS). *Journal of applied ecology*
331 43:1223-1232.
332 Almeida RP, Blua MJ, Lopes JRS, and Purcell AH. 2005. Vector transmission of *Xylella fastidiosa*:
333 Applying fundamental knowledge to generate disease management strategies. *Annals of*
334 *the Entomological Society of America* 98:775-786.
335 Almeida RP, and Nunney L. 2015. How do plant diseases caused by *Xylella fastidiosa* emerge ?
336 *Plant Disease* 99:1457-1467.
337 Araújo MB, and New M. 2007. Ensemble forecasting of species distributions. *Trends in ecology*
338 *& evolution* 22:42-47.
339 Aubertot J-N, and Savary S. 2005. Chapitre 4 : Stratégies de protection des cultures, p. 104. In .
340 Expertise Scientifique Collective “Pesticides, Agriculture et Environnement” Available
341 from : http://institutinrafr/Missions/Eclairer-les-decisions/Expertises/Toutes-les-
342 actualites/Pesticides-agriculture-et-environnement.
343 Bai H-F, Jin X-X, and Li C-D. 2015. A taxonomic study of *Ooctonus* (Hymenoptera, Mymaridae)
344 from Heilongjiang, China. *ZooKeys* 479:25–36.
345 Cisneros JJ, Rosenheim JA. 1998. Changes in the Foraging Behavior, Within-Plant Vertical
346 Distribution, and Microhabitat Selection of a Generalist Insect Predator: an Age Analysis.
347 *Environmental Entomology* 27:949–957. DOI: 10.1093/ee/27.4.949.

348 Cohen AC, Tang R. 1997. Relative Prey Weight Influences Handling Time and Biomass Extraction
349 in *Sinea confusa* and *Zelus renardii* (Heteroptera: Reduviidae). *Environmental*
350 *Entomology* 26:559–565. DOI: 10.1093/ee/26.3.559.
351 Cornara D, Bosco D, and Fereres A. 2018. *Philaenus spumarius*: when an old acquaintance
352 becomes a new threat to European agriculture. *Journal of Pest Science* 91:957-972.
353 10.1007/s10340-018-0966-0
354 Cornara D, Cavalieri V, Dongiovanni C, Altamura G, Palmisano F, Bosco D, Porcelli F, Almeida
355 RPP, and Saponari M. 2016. Transmission of *Xylella fastidiosa* by naturally infected
356 *Philaenus spumarius* (Hemiptera, Aphrophoridae) to different host plants. *Journal of*
357 *Applied Entomology* 141:80-87.
358 Costello MJ, Altieri MA. 1995. Abundance, growth rate and parasitism of *Brevicoryne brassicae*
359 and *Myzus persicae* (Homoptera: Aphididae) on broccoli grown in living mulches.
360 *Agriculture, Ecosystems & Environment* 52:187–196. DOI: 10.1016/0167-8809(94)00535-
361 M.
362 Cruaud A, Gonzalez A-A, Godefroid M, Nidelet S, Streito J-C, Thuillier J-M, Rossi J-P, Santoni S,
363 and Rasplus J-Y. 2018. Using insects to detect, monitor and predict the distribution of
364 *Xylella fastidiosa*: a case study in Corsica. *Scientific Reports* 8:15628. 10.1038/s41598-
365 018-33957-z
366 Cruaud A, Nidelet S, Arnal P, Weber A, Fusu L, Gumovsky A, Huber J, Polaszek A, and Rasplus J-
367 Y. 2019. Optimised DNA extraction and library preparation for small arthropods:
368 application to target enrichment in chalcid wasps used for biocontrol. *Molecular Ecology*
369 *Resources*. https://doi.org/10.1111/1755-0998.13006
370 Dader B, Vinuela E, Moreno A, Plaza M, Garzo E, Del Estal P, and Fereres A. 2019. Sulfoxaflor
371 and natural Pyrethrin with Piperonyl Butoxide are effective alternatives to
372 Neonicotinoids against juveniles of *Philaenus spumarius*, the european vector of *Xylella*
373 *fastidiosa*. *Insects* 10. 10.3390/insects10080225
374 Denancé N, Legendre B, Briand M, Olivier V, de Boisseson F, Poliakoff F, and Jacques M-A. 2017.
375 Several subspecies and sequence types are associated with the emergence of *Xylella*
376 *fastidiosa* in natural settings in France. *Plant Pathology* 66:1054-1064.

DGAV. 2019. Oficio Circular n.º 2/2019. Primeira deteção de *Xylella fastidiosa* em Portugal. *(available from* https://wwwconfagript/content/uploads/2019/01/Oficio-circular-2_2019_-Xfastidosapdf*)*.

Dongiovanni C, Di Carolo M, Fumarola G, Tauro D, Altamura G, and Cavalieri V. 2018. Evaluation of insecticides for the control of juveniles of *Philaenus spumarius* L., 2015–2017. *Arthropod Management Tests* 43:tsy073.

Doull KM. 1955. The biological control of noxious plants and insects. *Rural Education Bulletin, Lincoln College, New Zealand* 10:98-128.

Drosopoulos S, and Asche M. 1991. Biosystematic studies on the spittlebug genus *Philaenus* with the description of new species. *Zoological Journal of the Linnean Society* 101:169-177.

Drosopoulos S, and Remane R. 2000. Biogeographic studies on the spittlebug *Philaenus signatus* Melichar, 1896 species group (Hemiptera: Aphrophoridae) with the description of two new allopatric species. Ann Soc Entomol Fr 36:269–277

Dumbleton LJ. 1937. Apple leaf-hopper investigations. *New Zealand Journal of Science and Technology* 18:866- 877.

EFSA. 2015. Scientific Opinion on the risk to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction, EFSA Journal 2015;13(1):3989.

EFSA. 2018. Scientific report on the update of the *Xylella* spp. host plant database. *EFSA Journal* 16:5408. https://doi.org/10.2903/j.efsa.2018.5408

Eilenberg J, Hajek A, and Lomer C. 2001. Suggestions for unifying the terminology in biological control. *BioControl* 46:387-400.

Fick SE, and Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International journal of climatology* 37:4302-4315.

Fielding AH, and Bell JF. 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environ Conserv* 24:38-49.

Fierro A, Liccardo A, and Porcelli F. 2019. A lattice model to manage the vector and the infection of the *Xylella fastidiosa* on olive trees. *Scientific Reports* 9:8723.

GBIF.org. 2019. GBIF Home Page. Available from: https://www.gbif.org

Germain JF, Chatot C, Meusnier I, Artige E, Rasplus J-Y, and Cruaud A. 2013. Molecular identification of *Epitrix* potato flea beetles (Coleoptera: Chrysomelidae) in Europe and North America. *Bulletin of Entomological Research* 103:354-362.

Godefroid M, Cruaud A, Streito J-C, Rasplus J-Y, and Rossi J-P. 2018. Climate change and the potential distribution of *Xylella fastidiosa* in Europe. *bioRxiv*. https://doi.org/10.1101/289876

Godefroid M, Cruaud A, Streito J-C, Rasplus J-Y, and Rossi J-P. 2019. *Xylella fastidiosa*: climate suitability of European continent. *Scientific Reports* 9:8844. 10.1038/s41598-019-45365-y

Guisan A, Thuiller W, and Zimmermann NE. 2017. *Habitat suitability and distribution models: with applications in R*: Cambridge University Press.

Hafi A, Randall L, Arthur T, Addai D, Tennant P, and Gomboso J. 2017. Economic impacts of Xylella fastidiosa on the Australian wine grape and wine-making industries. Department of Agriculture and Water Resources ABARES.

Halkka O, Raatikainen M, Vilbaste J. 1975. Clines in the colour polymorphism of *Philaenus spumarius* in Eastern Central Europe. *Heredity* 35:303–309. DOI: 10.1038/hdy.1975.101.

Halkka O, and Kohila T. 1976. Persistence of visual polymorphism, despite a low rate of predation, in *Philaenus spumarius* (L.)(Homoptera, Aphrophoridae). *Annales Zoologici Fennici* 13:185-188.

Harper G, and Whittaker JB. 1976. The role of natural enemies in the colour polymorphism of *Philaenus spumarius* (L.). *Journal of Animal Ecology* 45:91-104.

Hayat M. 1983. The genera of Aphelinidae of the world. *Systematic Entomology* 8:63-102.

Henderson G, Hoffman GD, and Jeanne RL. 1990. Predation on cercopids and material use of the spittle in aphid-tent construction by prairie ants. *Psyche: A Journal of Entomology* 97:43-53.

Heraty JM, and Hawks D. 1998. Hexamethyldisilazane – A chemical alternative for drying insects. *Entomological News,* 109:369–374.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, and Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology: A Journal of the Royal Meteorological Society* 25:1965-1978.

Holt BG, Lessard JP, Borregaard MK, Fritz SA, Araújo MB, Dimitrov D, Fabre PH, Graham GH, Graves GR, Jønsson KA, Nogués-Bravo D, Wang Z, Whittaker RJ, Fjeldså J and Rahbek C. 2013. An update of Wallace's zoogeographic regions of the world. *Science* 339(6115): 74–78.

Hoy MA. 2008. Augmentative biological control. In: Capinera JL, ed. *Encyclopedia of Entomology*: Springer, Dordrecht.

Huber JT. 2012. Revision of the genus *Ooctonus* (Hymenoptera: Mymaridae). *Journal of the entomological Society of Ontario* 143:15-105.

Irvin NA, and Hoddle MS. 2010. Comparative assessments of *Gonatocerus ashmeadi* and the 'new association' parasitoid *Gonatocerus tuberculifemur* (Hymenoptera: Mymaridae) as biological control agents of *Homalodisca vitripennis* (Hemiptera: Cicadellidae). *Biological Control* 55:186-196.

Ivanova NV, Zemlak TS, Hanner RH, and Hebert PDN. 2007. Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes* 7:544-548.

Jeger MJ, Chen Z, Powell G, Hodge S, and van den Bosch F. 2011. Interactions in a host plant-virus–vector–parasitoid system: Modelling the consequences for virus transmission and disease dynamics. *Virus Research* 159:183-193.

Katoh K, and Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30:772-780.

Krugner R, Sisterson MS, Backus EA, Burbank LP, and Redak RA. 2019. Sharpshooters: a review of what moves *Xylella fastidiosa*. Austral Entomology 58:248-267.

Loukas M. & Drosopoulos S. (1992) Population genetics of the spittlebug genus *Philaenus* (Homoptera: Cercopidae) in Greece. *Biological Journal of the Linnean Society*, 46, 403-413.

462 Lees DR, Dent CS, Gait PL. 1983. Geographic variation in the colour/ pattern polymorphism of
463 British *Philaenus spumarius* (L) (Homoptera: Aphrophoridae) populations. *Biological*
464 *Journal of the Linnean Society* 19:99–114. DOI: 10.1111/j.1095-8312.1983.tb00779.x.
465 Marmion M, Parviainen M, Luoto M, Heikkinen RK, and Thuiller W. 2009. Evaluation of
466 consensus methods in predictive species distribution modelling. *Diversity and*
467 *Distributions* 15:59-69.
468 Martinez G, González A, and Dicke M. 2018. Rearing and releasing the egg parasitoid
469 *Cleruchoides noackae*, a biological control agent for the *Eucalyptus* bronze bug.
470 *Biological Control* 123:97-104.
471 Martini X, Pelz-Stelinski KS, and Stelinski L. 2014. Plant pathogen-induced volatiles attract
472 parasitoids to increase parasitism of an insect vector. *Frontiers in Ecology and Evolution*
473 doi.org/10.3389/fevo.2014.00008
474 Mills N. 2010. Egg parasitoids in biological control and integrated pest management. In: Consoli
475 FL, Parra JRP, and Zucchi RA, eds. *Egg parasitoids in agroecosystems with emphasis on*
476 *Trichogramma*: Springer, Dordrecht.
477 Muscarella R, Galante PJ, Soley-Guardia M, Boria RA, Kass JM, Uriarte M, Anderson RP, and
478 McPherson J. 2014. ENMeval: An R package for conducting spatially independent
479 evaluations and estimating optimal model complexity forMaxentecological niche
480 models. *Methods in ecology and evolution* 5:1198-1205. 10.1111/2041-210x.12261
481 Noyes JS. 2019. Universal Chalcidoidea Database. Natural History Museum, London. Available
482 from: http://www.nhm.ac.uk/jdsml/research-
483 curation/projects/chalcidoids/indexValidName.dsml (accessed 1 June 2019).
484 Olmo D, Nieto A, Adrover F, Urbano A, Beidas O, Juan A, Marco-Noales E, López MM, Navarro I,
485 Monterde A, Montes-Borrego M, Navas-Cortés JA, and Landa BB. 2017. First detection
486 of *Xylella fastidiosa* infecting cherry (*Prunus avium*) and *Polygala myrtifolia* plants, in
487 Mallorca Island, Spain. *Plant Disease* 101:1820-1820.
488 Pagliano G, and Alma A. 1997. Ricerche etologiche su Gorytini e Alyssonini (Hymenoptera
489 Sphecidae) parassitoidi di Auchenorryncha (Rhynchota Homoptera). *Rivista Piemontese*
490 *di Storia Naturale* 18:173-181.

Phillips SJ, Anderson RP, and Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. *Ecological modelling* 190:231-259.

Phillipson J. 1960. A contribution to the feeding biology of *Mitopus morio* (F) (Phalangida). *Journal of Animal Ecology* 29:35-43.

Qiao H, Soberón J, and Peterson AT. 2015. No silver bullets in correlative ecological niche modelling: insights from testing among many potential algorithms for niche estimation. *Methods in ecology and evolution* 6:1126-1136.

Quartau JA, Borges PAV, André G. 1992. *Philaenus spumarius* (Linnaeus, 1758) new to the Azores (Homoptera, Auchenorrhyncha, Cercopidae). *Boletim da Sociedade Portuguesa de Entomologia* 1:129–136.

R core team. 2019. *R: a language and environment for statistical computing*. Vienna, Austria: R foundation for statistical computing.

Radosavljevic A, Anderson RP. 2014. Making better Maxent models of species distributions: complexity, overfitting and evaluation. *Journal of Biogeography* 41:629–643. DOI: 10.1111/jbi.12227.

Ratnasingham S, and Hebert PDN. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7:355-364.

Salerno M, Russo V, Sefa V, Lamaj F, Basher N, Verrastro V, and Porcelli F. 2017. *Zelus renardii* an assassin bug candidate for *Philaenus spumarius* biocontrol. European conference on Xylella Finding answer to a global problem 13–15 Nov. Palma de Mallorca. p 22-23.

Saponari M, Boscia D, Nigro F, and Martelli GP. 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (Southern Italy). *Journal of Plant Pathology* 95:668.

Saponari M, Loconsole G, Cornara D, Yokomi RK, De Stradis A, Boscia D, Bosco D, Martelli GP, Krugner R, and Porticelli F. 2014. Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy. *Journal of Economic Entomology* 107:1316-1319.

Severin, H.H.P. 1950. Spittle-insect vectors of Pierce's disease virus. Hilgardia 19: 357-382

519 Sicard A, Zeilinger AR, Vanhove M, Schartel TE, Beal DJ, Daugherty MP, and Almeida RPP. 2018.
520 *Xylella fastidiosa*: insights into an emerging plant pathogen. *Annual Review of*
521 *Phytopathology* 56:1-22.
522 Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large
523 phylogenies. *Bioinformatics* 30:1312-1313. 10.1093/bioinformatics/btu033
524 Stewart AJA, Lees DR. 1996. The colour/pattern polymorphism of *Philaenus spumarius* (L.)
525 (Homoptera: Cercopidae) in England and Wales. *Philosophical Transactions of the Royal*
526 *Society of London. Series B: Biological Sciences* 351:69–89. DOI: 10.1098/rstb.1996.0005.
527 Stöver BC, and Müller KF. 2010. TreeGraph 2: Combining and visualizing evidence from different
528 phylogenetic analyses. *BMC Bioinformatics* 11:7.
529 Thuiller W, Lafourcade B, Engler R, and Araújo MB. 2009. BIOMOD–a platform for ensemble
530 forecasting of species distributions. *Ecography* 32:369-373.
531 Triapitsyn SV. 2010. Revision of the Palaearctic species and review of the Oriental species of
532 *Ooctonus* (Hymenoptera: Mymaridae), with notes on extralimital taxa. *Zootaxa* 2381:1-
533 74.
534 Tumber KP, Alston JM, and Fuller KB. 2014. Pierce's disease costs California $104 million per
535 year. *California Agriculture* 68:20-29.
536 Van Driesche R, and Hoddle MS. 2016. Non-target effects of insect biocontrol agents and trends
537 in host specificity since 1985. *CAB Reviews* 11:1-66.
538 Vicente JR, Fernandes RF, Randin CF, Broennimann O, Gonçalves J, Marcos B, Pôças I, Alves P,
539 Guisan A, and Honrado JP. 2013. Will climate change drive alien invasive plants into
540 areas of high protection value? An improved model-based regional assessment to
541 prioritise the management of invasions. *Journal of environmental management*
542 131:185-195.
543 Weaver CR, and King DR. 1954. Meadow spittlebug. *Research bulletin of the Ohio Agricultural*
544 *Experiment Station* 741:1-100.
545 Weirauch C, Alvarez C, and Zhang G. 2012. *Zelus renardii* and *Z. tetracanthus* (Hemiptera:
546 Reduviidae): Biological attributes and the potential for dispersal in two assassin bug
547 species. *Florida Entomologist* 95:641-649.

548 Whittaker JB. 1969. The biology of Pipunculidae (Diptera) parasitising some British Cercopidae
549 (Homoptera). *Physiological Entomology* 44:17-24.
550 Whittaker JB. 1973. Density regulation in a population of *Philaenus spumarius* (L.) (Homoptera:
551 Cercopidae). *Journal of Animal Ecology* 42:163-172.
552
553 **FIGURE CAPTIONS**
554
555 **Figure 1. Parasitism rate of *Philaenus spumarius* eggs in the four sites sampled in Corsica.**
556 Size of the pie chart is proportional to the total number of eggs that hatched from each locality
557 (n). Slices indicate the relative proportion of *O. vulgatus* (dark grey) and nymphs of *P. spumarius*
558 (light grey) that emerged from the pool of eggs. GPS coordinates of sampling localities:
559 42.984205 °N, 9.395287 °E (Ersa); 42.338849 °N, 9.180636 °E (Tralonca); 42.274756 °N,
560 9.487185 °E (Canale-di-Verde); 41.931726 °N, 9.343731 °E (Ventiseri). The map was built with the
561 R package maps, using data from UNESCO (1987) through UNEP/GRID-Geneva.
562
563 **Figure 2**. **Morphology of *Ooctonus vulgatus Haliday*, 1833**. A. ♂ Antenna. B. ♀ Antenna. C.
564 habitus. D. Head front view. E. Mesosoma lateral view. F. ♂ Propodeum. G. Mesosoma dorsal
565 view. All scales = 100 µm except habitus. Photo credit: Jean-Yves Rasplus INRA.
566
567 **Figure 3. Geographical distribution of *O. vulgatus.***
568 A. Distribution of *O. vulgatus* occurrences collected from the literature. B Consensus model of
569 climate suitability estimated by Maxent: median of model outputs. C Consensus model of climate
570 suitability estimated by Maxent: proportion of models predicting *O. vulgatus* presence in Europe.

Figure 1

Parasitism rate of *Philaenus spumarius* eggs in the four sites sampled in Corsica.

Size of the pie chart is proportional to the total number of eggs that hatched from each locality (n). Slices indicate the relative proportion of *O. vulgatus* (dark grey) and nymphs of *P. spumarius* (light grey) that emerged from the pool of eggs. GPS coordinates of sampling localities: 42.984205°N, 9.395287°E (Ersa); 42.338849°N, 9.180636°E (Tralonca); 42.274756°N, 9.487185°E (Canale-di-Verde); 41.931726°N, 9.343731°E (Ventiseri). The map was built with the R package maps, using data from UNESCO (1987) through UNEP/GRID-Geneva.

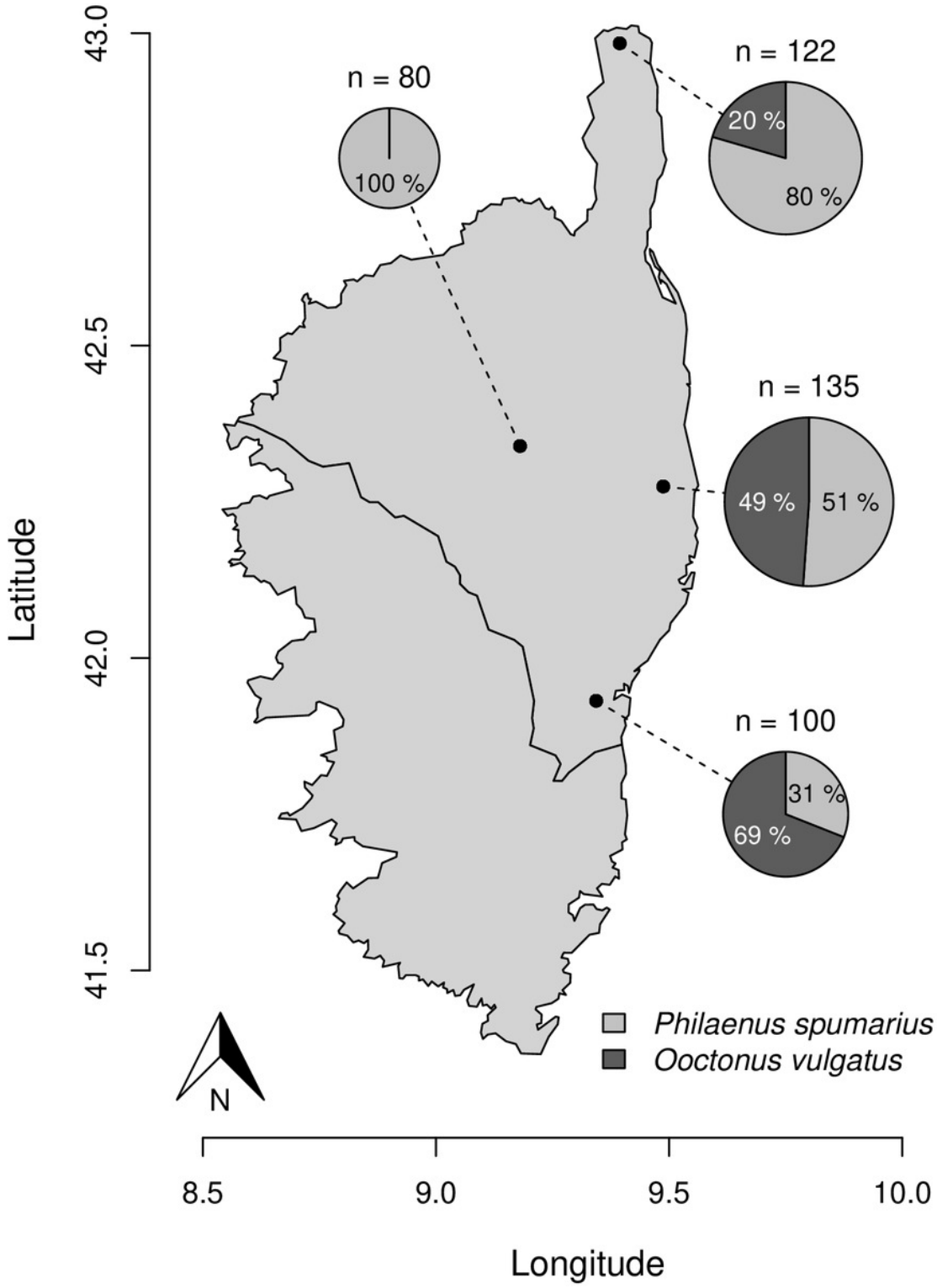

n = 80
100 %
n = 122
20 %
80 %
n = 135
49 %
51 %
n = 100
31 %
69 %
Philaenus spumarius
Ooctonus vulgatus
N
43.0
42.5
42.0
41.5
Latitude
8.5
9.0
9.5
10.0
Longitude

Figure 2

Morphology of *Ooctonus vulgatus Haliday*, 1833.

A. ♂ Antenna. B. ♀ Antenna. C. habitus. D. Head front view. E. Mesosoma lateral view. F. ♂ Propodeum. G. Mesosoma dorsal view. All scales = 100 µm except habitus. Photo credit: Jean-Yves Rasplus INRA.

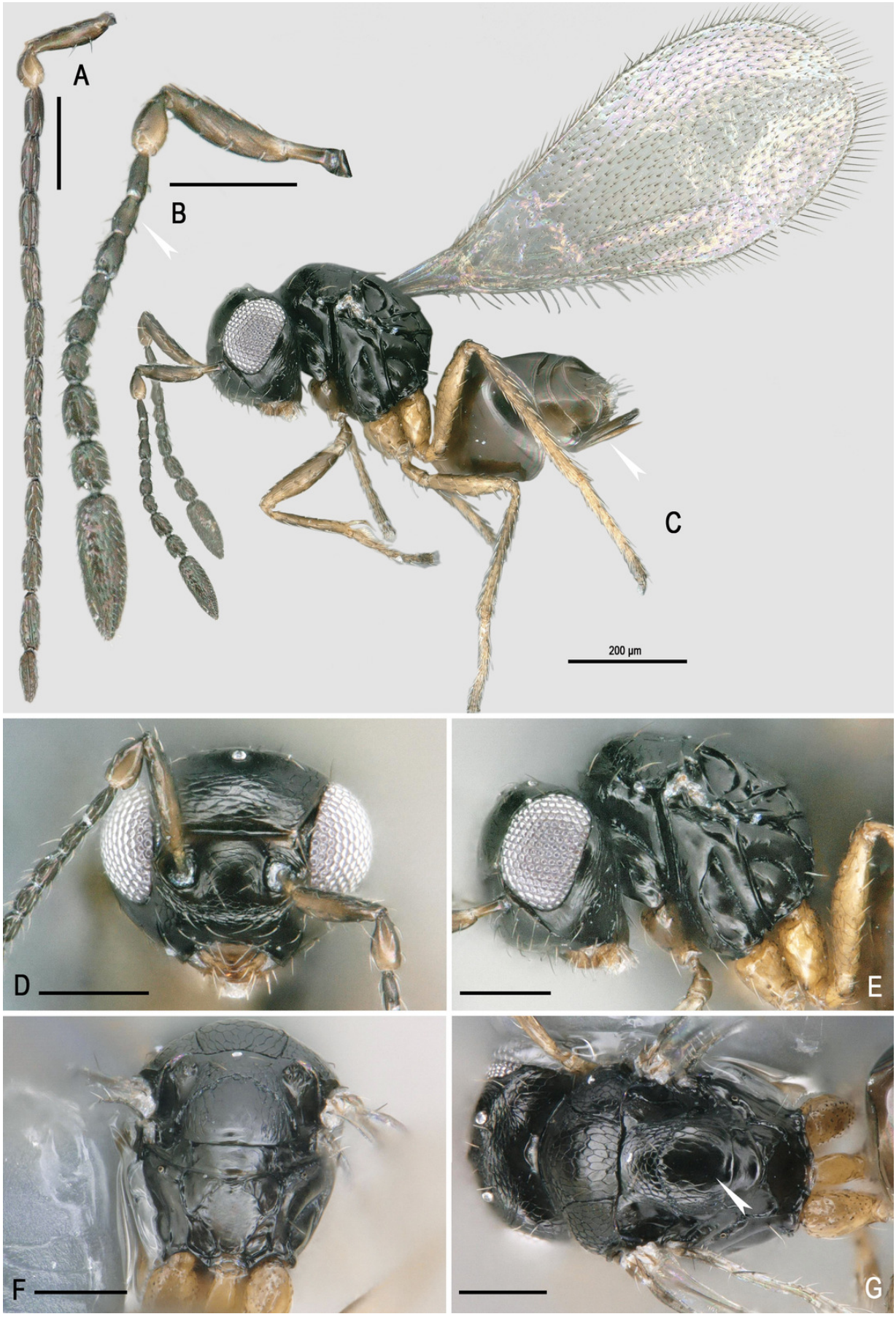
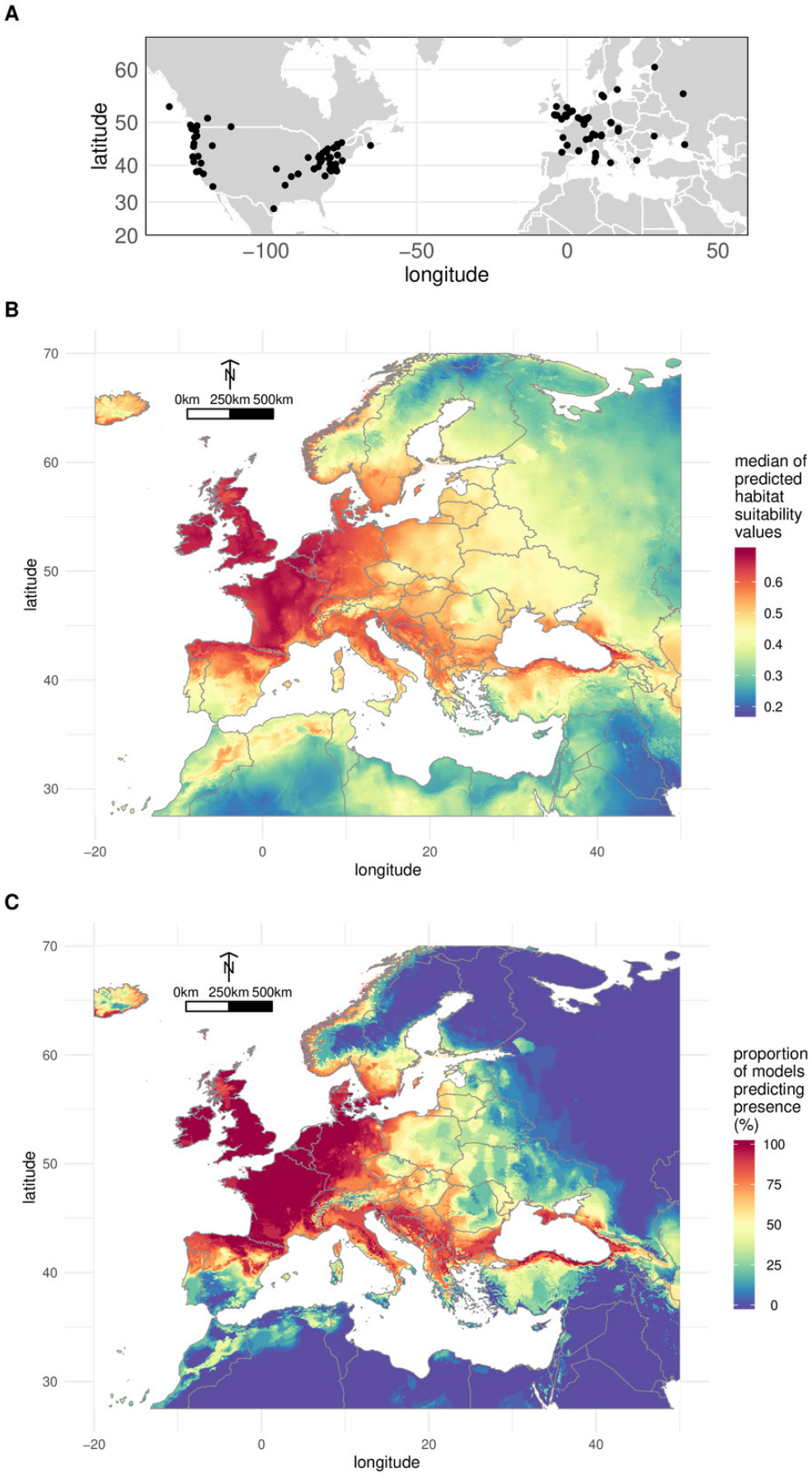

A
B
C
200 µm
D
E
F
G

Figure 3

Geographical distribution of *O. vulgatus.*

A. Distribution of *O. vulgatus* occurrences collected from the literature. B Consensus model of climate suitability estimated by Maxent: median of model outputs. C. Consensus model of climate suitability estimated by Maxent: proportion of models predicting *O. vulgatus* presence in Europe.


A
latitude
longitude
60
50
40
30
20
−100
−50
0
50
B
0km 250km 500km
median of predicted habitat suitability values
0.6
0.5
0.4
0.3
0.2
latitude
longitude
70
60
50
40
30
−20
0
20
40
C
0km 250km 500km
proportion of models predicting presence (%)
100
75
50
25
0
latitude
longitude