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

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As 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. We also assessed parasitism rates in several sampling sites, highlighting the top-down impact of *O. vulgatus* on populations of *P. spumarius*. Based on the geographic occurrences of *O. vulgatus* mined in the literature, we calibrated an ecological niche model to assess its potential distribution in the Holarctic. Our results showed that *O. vulgatus* potential distribution overlaps that of *P. spumarius.* Hence, *O. vulgatus* appears to be a promising biocontrol agent of the meadow spittlebug in Europe and it seems advisable to conduct research on this small parasitoid wasp to assess whether it could contribute to reduce the spread and impact of *X. fastidiosa* in Europe.

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ABSTRACT

As 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. We also assessed parasitism rates in several sampling sites, highlighting the top-down impact of *O. vulgatus* on populations of *P. spumarius*. Based on the geographic occurrences of *O. vulgatus* mined in the literature, we calibrated an

ecological niche model to assess its potential distribution in the Holarctic. Our results showed that *O. vulgatus* potential distribution overlaps that of *P. spumarius.* Hence, *O. vulgatus* appears to be a promising biocontrol agent of the meadow spittlebug in Europe and it seems advisable to conduct research on this small parasitoid wasp to assess whether it could contribute to reduce the spread and impact of *X. fastidiosa* in Europe.

Keywords = biocontrol, Europe, insect vector, meadow spittlebug, *Xylella fastidiosa*, oophagous, parasitoid, Mymaridae

Running headline = *O. vulgatus* to control *P. spumarius* ?

INTRODUCTION

Xylella fastidiosa (Wells, 1987) is a xylem-dwelling insect-borne bacterium, that originates from the Americas and causes a variety of scorch-like diseases in more than 500 species of plants including many cultivated species (Almeida & Nunney 2015; EFSA 2015; EFSA 2018; Sicard et al. 2018). Studies on the economic impact of *X. fastidiosa* are scarce and have primarily focused on the vineyard and wine industry. Yield reduction and management costs to the California grape-growing industry are estimated to more than US$100 million per year (Tumber et al. 2014) and 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; Godefroid et al. 2019). Hence, *X. fastidiosa* represents a serious threat to European agriculture and natural ecosystems.

The spread of *X. fastidiosa* depends on several interacting variables, mainly insect vectors and plant communities as well as landscape, climate features and population dynamics of the bacterium itself. As a consequence, disease management is complex. Reducing its 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 et al. 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 spittlebug *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae) (Cornara et al. 2016; Saponari et al. 2014).

P. spumarius is highly polyphagous and can be locally abundant (Cornara et al. 2018). Species distribution modelling showed that it could occur all over Europe (Cruaud et al. 2018) from sea level to high altitude (*ca* 2000m, (Drosopoulos & Asche 1991)). Therefore, as a vector of *X. fastidiosa*, it represents a new important pest for Europe (Cornara et al. 2018).

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

Currently, information about the natural enemies of the meadow spittlebug are scattered (Cornara et al. 2018). Species of birds, frogs, arachnids and insects (Hymenoptera, Diptera and Coleoptera Carabidae) occasionally feed on *P. spumarius* (Halkka & Kohila 1976; Harper & Whittaker 1976; Henderson et al. 1990; Pagliano & Alma 1997; Phillipson 1960) but predation does not appear to be an important source of mortality. Studies are in progress to test whether the invasive assassin bug *Zelus renardii* (Reduviidae) could be used to control populations of *P. spumarius* in olive orchards (Salerno et al. 2017). However, this insect is generalist (Weirauch et

al. 2012) and introducing reared specimens into the wild may have dramatic unintended effects that need to be evaluated (Van Driesche & Hoddle 2016).

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; Whittaker 1973). A few oophagous species 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). 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*, which is a vector of *X. fastidiosa* both in the larval and adult stages, the insect is killed before it acquires the bacterium from an infected host plant and becomes able to transmit it. The transmission ability of *P. spumarius* populations is therefore reduced early in the season with a potential strong impact on the dynamics of the disease. However, very few is known about the biology and efficiency of these natural enemies *in natura*.

In this study, we conducted an opportunistic field survey to identify major parasitoids of the eggs of *P. spumarius* in Corsica and provided 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 viable populations of *O.vulgatus.* This allowed us to calibrate ecological niche models 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

Five to ten bunches of branches of *Cistus monspeliensis* L. 1753 were sampled in four localities. This sampling was included in a larger field survey of population dynamics of *P. spumarius* in Corsica (Figure 1). Sampling was performed between the 12th and the 15th of February 2019. The back of each leaf 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). The pieces of leaf containing the eggs were placed on filter papers in Petri dishes 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 larvae and parasitoids were killed and stored in 70% Ethanol at 4°C until identification and molecular analyses.

Morphological identification

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 50 fps and the VH-Z100UT lens). Images were then edited in Adobe Photoshop CS6© software.

126

127 **Molecular identification**

128 DNA was extracted from three individual specimens and a pool of three specimens to increase
129 DNA yield. Total genomic DNA was isolated using the Qiagen DNeasy Blood & Tissue kit without
130 destruction of the specimens. We followed manufacturer's protocol with the following
131 modifications. Samples were incubated overnight in an Eppendorf thermomixer (temperature =
132 56°C, mixing frequency = 300 rpm). To increase DNA yield, two successive elutions (50 µL each)
133 were performed with heated buffer AE (56°C) and an incubation step of 15 minutes followed by
134 centrifugation (6000g for 1 minute at room temperature; (Cruaud et al. 2019). Eppendorf
135 microtubes LoBind 1,5ml were used for elution and to store DNA at -20°C until PCR amplification.
136 Vouchers are deposited at CBGP, Montferrier-sur-Lez, France. The mitochondrial Cytochrome c
137 oxidase I standard barcode fragment (*COI*) was amplified with a cocktail of M13-tailed primers as
138 detailed in Germain et al. (2013). Unpurified PCR products were sent to Eurofins MWG Operon
139 (Ebersberg, Germany) for sequencing using the M13F and M13R primers (Germain et al. 2013;
140 Ivanova et al. 2007). Both strands for each overlapping fragment were assembled in Geneious
141 v11.1.4 (https://www.geneious.com). Geneious was also used to translate consensus sequences
142 to amino acids to detect premature codon stops. All *COI* sequences available on BOLD
143 (Ratnasingham & Hebert 2007) for *Ooctonus* species were downloaded (last access July 12,2019)
144 and aligned with the newly generated sequences using MAFFT v7.245 (Katoh & Standley 2013).
145 A maximum likelihood tree was inferred with raxmlHPC-PTHREADS-AVX version 8.2.4 (Stamatakis
146 2014). A rapid bootstrap search (100 replicates) followed by a thorough ML search (-m

GTRGAMMA) was conducted. Tree visualization and annotation was performed with TreeGraph 2.13 (Stöver & Müller 2010).

Species distribution modelling framework

Occurrences of *O. vulgatus* were mined in the literature and the GBIF database (GBIF.org 2019) (Tables S1&S2). We fitted a correlative model linking different climate descriptors to species occurrences. The Maxent algorithm was chosen to conduct analyses because it dose not require absence data (Phillips et al. 2006). We summarize 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 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 (Godefroid et al. 2019; Qiao et al. 2015). 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 subset of locations where the species has been found (here, a random 70 % of the available occurences, the other 30 % being used for model validation) and a set of locations where no information about the presence of the

169 species are available (referred to as background points). A total of 10,000 background points
170 were randomly generated in North America and Europe). To render complex response to
171 environmental constraints while reducing model overfitting we first fitted 48 Maxent models
172 using 6 regularization multiplier (RM) combinations (L, LQ, H, LQH, LQHP, LQHPT with L=linear,
173 Q=quadratic, H=hinge, P=product and T=threshold) and feature class (FC) values (8 values ranging
174 from 0.5 to 4 with increments of 0.5). Optimal FC and RM combinations were determined for
175 each of the three bioclimatic datasets (CLIM1 - CLIM3) using the R (Team 2019) package ENMeval
176 (Muscarella et al. 2014). Optimal parameters were then used to fit a set of ten replicate Maxent
177 models using 70% of the dataset. The performance of each model was evaluated using the
178 remaining 30% of occurrences using the area under the receiver–operator curve (AUC, Fielding
179 & Bell 1997) and the true skill statistics (TSS, Allouche et al. 2006). Models with AUC < 0.8 were
180 excluded from further analyses (Vicente et al. 2013). Habitat suitability maps (logistic output
181 ranging from 0 to 1) were transformed into binary projections using the threshold that optimized
182 the TSS statistics on the testing data (Guisan et al. 2017). Maxent replicate models were fitted
183 and evaluated using the R package biomod2 (Thuiller et al. 2009).
184 Two different outputs were generated using the set of model prediction. i) Binary predictions
185 were averaged to produce the committee (consensus) averaging (Araújo & New 2007; Marmion
186 et al. 2009) showing the likelihood of the presence of *O. vulgatus*. This consensus model ranges
187 from 0 (all the models predict absence) to 100 (all the models predict presence) and ii) the
188 median of the logistic outputs (Guisan et al. 2017) of the models that depicts the climate
189 suitability across the different models.
190

191 **RESULTS**

192

193 **Sampling, morphological identification and parasitism rates.**

194 All parasitoids reared from the eggs of *P. spumarius* were identified as *O. vulgatus* (Figure 2). No
195 parasitoid emerged from eggs collected in one of the four stations. We observed parasitism rates
196 of 20.5, 48.9 and 69.0% in the three other localities (Figure 1).

197

198 **Guidelines for the identification of *O. vulgatus*.**

199 To help identification, we list below the main features that differentiate *O. vulgatus* from its
200 closest relatives. The genus *Ooctonus* has been recently revised in the Palearctic and Nearctic
201 regions respectively by Triapitsyn (2010) and Huber (2012). *Ooctonus* can easily be distinguished
202 from other genera of Mymaridae by the following set of characters: tarsi 5-segmented,
203 propodeum with diamond-shaped pattern of carinae (Figure 2F), fore wing venation about one-
204 third the wing length (Figure 2C), with short marginal and stigmal vein, parastigma with
205 hypochaeta next to proximal macrochaeta (Huber, 2012). In the Holarctic region, *O. vulgatus* can
206 be discriminated from other species of *Ooctonus* by the following unique combination of features
207 (Figure 2): vertex without stemmaticum; mesoscutum without median groove; posterior part of
208 scutellum and frenum smooth with weak sculpture laterally; metanotum and propodeum
209 without reticulate sculpture; propodeum without median carina, but with a pentagonal areole
210 formed by dorsolateral carinae; short petiole, 0.9–1.2x as long as metacoxa; forewing at least
211 slightly truncate apically; females funicle with multiporous placoid sensilla (mps) on F7 and F8

only, F5 and F6 without mps; single row of six bullae inside the female clava; ovipositor at most 1.4× as long as metatibia and only slightly exerted beyond apex of gaster.

Molecular identification

Barcode sequences were successfully generated from all samples. All sequences were identical. Phylogenetic analysis confirmed that the most likely identification was *O. vulgatus* (Figure S1).

Species distribution modelling

Two hundred and five occurrences were mined in the literature (Table S1), eight of which were not included in the analysis as no geographic coordinates were available. Forty occurrences were mined in GBIF (last access: 22 August 2019; Table S2). Thirty-three occurrences were discarded because no geographic coordinates were available, one occurrence was discarded because specimen identification was dubious and six occurrences were discarded because potential confusion between coordinates of sampling locality and storage locality may have occurred. Therefore, no occurrence mined in GBIF could be included in the analysis. A total of 200 occurrences (197 mined in the literature + the three sampling sites where *O. vulgatus* occurred) (Figure 3A) were used to model the distribution of *O. vulgatus* in Europe.

The optimal Maxent parameters were RM=4 and FC=hinge; RM=4 and FC=hinge and RM=2.5 and FC=hinge for CLIM1, CLIM2 and CLIM3 respectively. With the exception of one model of CLIM2, all models based on these optimal values yielded AUC values >0.8, which indicated that the different bioclimatic data subsets roughly performed similarly well. The consensus model was therefore computed from a set of 29 estimates of climate suitability.

Figure 3B shows the median of the climate suitability values for the 29 models considered. For each pixel of the map, we computed the median of the 29 estimates of climate suitability values. Figure 3C depicts the proportion of the 29 models indicating that the climate is suitable for *O. vulgatus*. 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 which occur in all biogeographic regions of the world excepted Australia (Noyes 2019). Among them, *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 poor 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, the tiny *O. vulgatus* is supposed to be a widespread species distributed in the Holarctic region 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 cited from China (Bai et al. 2015) but the illustration proposed casts some doubts about the identification of this specimen. However, there is only a few unquestionable occurrences in the literature for this species (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

256 that *O. vulgatus* potential distribution in Europe (Figure 3) overlaps that of its host *P. spumarius*
257 (Cruaud et al. 2018), which is not surprising from a biological point of view but is an interesting
258 result in the framework of biological control. This study is preliminary and predictions, especially
259 because they are based on a limited number of occurrences, are indicative only. It is noteworthy
260 that this lack of occurrences is the common condition for most organisms and that species
261 distribution models were developed to overcome this lack of information. This study is a starting
262 point to encourage investigations in other parts of Europe. Sampling efforts should more
263 specifically target areas predicted as suitable for *P. spumarius* but non-suitable for *O. vulgatus*
264 such as eastern areas of Europe.

265 When studied in North America, observed parasitism rates did not exceed 10% of the
266 sampled eggs of *P. spumarius* (Weaver & King 1954). Here, we obtained parasitism rates of up to
267 69 %, but absence of parasitism in one sample site. Parasitism rates can thus be high but are also
268 highly variable. Further surveys are obviously necessary to estimate what could be the drivers of
269 parasitism rates in Corsica and throughout Europe. Identifying such drivers could open new
270 avenues for conservation biological control against *P. spumarius*, through the implementation of
271 environments favorable to *O. vulgatus* in the vicinity of crops susceptible to Xf.

272 The use of mymarids in biological control program has a long history. The most notable
273 instance being the use of *Anaphes nitens* (Girault, 1928) in several countries to successfully
274 control the eucalyptus weevil *Gonipterus scutellatus* Gyllenhal, 1833, which feeds and breeds on
275 *Eucalyptus* trees (Doull 1955). More recently, *Cleruchoides noackae* Lin and Huber, 2007 has been
276 used in South America to control an invasive sap-feeding pest of *Eucalyptus*, *Thaumastocoris*
277 *peregrinus* Carpintero and Dellapé, 2006 (Hemiptera: Thaumastocoridae) (Martinez et al. 2018).

Mymarid species were used to control leafhoppers or sharpshooters vectors of plant pathogens (Hemiptera: Cicadellidae). *Anagrus armatus* (Ashmead, 1887) regulated *Edwardsiana froggatti* (Baker, 1925), a pest of apple in New Zealand, with parasitism rates of the eggs reaching 80% (Dumbleton 1937). More recently, *Gonatocerus* species were used to target *Homalodisca vitripennis* (Germar, 1821) an efficient vector of *Xylella* in California (Irvin & Hoddle 2010). In all these cases, mymarids help regulate pest population growth without the need for insecticides. However, before any attempts to regulate populations of *P. spumarius* are made, we need to enrich our biological knowledge on the species, its specificity and its ecology. In particular, the degree of specificity of the *P. spumarius – O. vulgatus* interaction needs to be addressed 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 as biological control (but see Martinez et al., 2018).

Again, we consider this study as a starting point to encourage research on this small 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 disease, without resorting to chemical treatments.

ACKNOWLEDGEMENTS

This work was funded by the Collectivité Territoriale de Corse and the European Union Horizon 2020 research and innovation program under Grant Agreement No. 727987 XF-ACTORS. XM thanks Laurent Julhia for providing sensors to measure room temperature.

AUTHOR's CONTRIBUTIONS

XM: field survey, rearing experiments, writing of the original draft; MC: occurrence mining, species distribution modelling, writing of the original draft; GG: lab work; JPR: species distribution modelling, writing of the original draft; AC: funding acquisition, project administration, supervision, phylogenetic analysis, writing of the original draft; JYR: conceptualization of the study, funding acquisition, project administration, supervision, identification of the parasitoid, review of current knowledge on the parasitoid, occurrence mining, writing of the original draft. All authors commented on the MS.

DATA ACCESSIBILITY

COI sequences were deposited on NCBI (Accession ID#XXXX).

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494

495 **FIGURE CAPTIONS**

496

497 **Figure 1. Parasitism rate of *Philaenus spumarius* eggs in the four sites sampled in Corsica.**

498 Size of the pie chart is proportional to the total number of eggs that hatched from each locality

499 (n). Slices indicate the relative proportion of *O. vulgatus* (dark grey) and larvae of *P. spumarius*

500 (light grey) that emerged from the pool of eggs. The map was built with the R package maps,

501 using data from UNESCO (1987) through UNEP/GRID-Geneva.

502

503 **Figure 2. Morphology of *Ooctonus vulgatus Haliday*, 1833**. A. ♂ Antenna. B. ♀ Antenna. C.

504 habitus. D. Head front view. E. Mesosoma lateral view. F. ♂ Propodeum. G. Mesosoma dorsal

505 view. All scales = 100 µm except habitus.

506

507 **Figure 3. Geographical distribution of *O. vulgatus.***

508 A. Distribution of *O. vulgatus* occurrences collected from the literature and GBIF.org. B Consensus

509 model of climate suitability estimated by Maxent: median of model outputs. C Consensus model

510 of climate suitability estimated by Maxent: proportion of models predicting *O. vulgatus* presence

511 in Europe.

Figure 1

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

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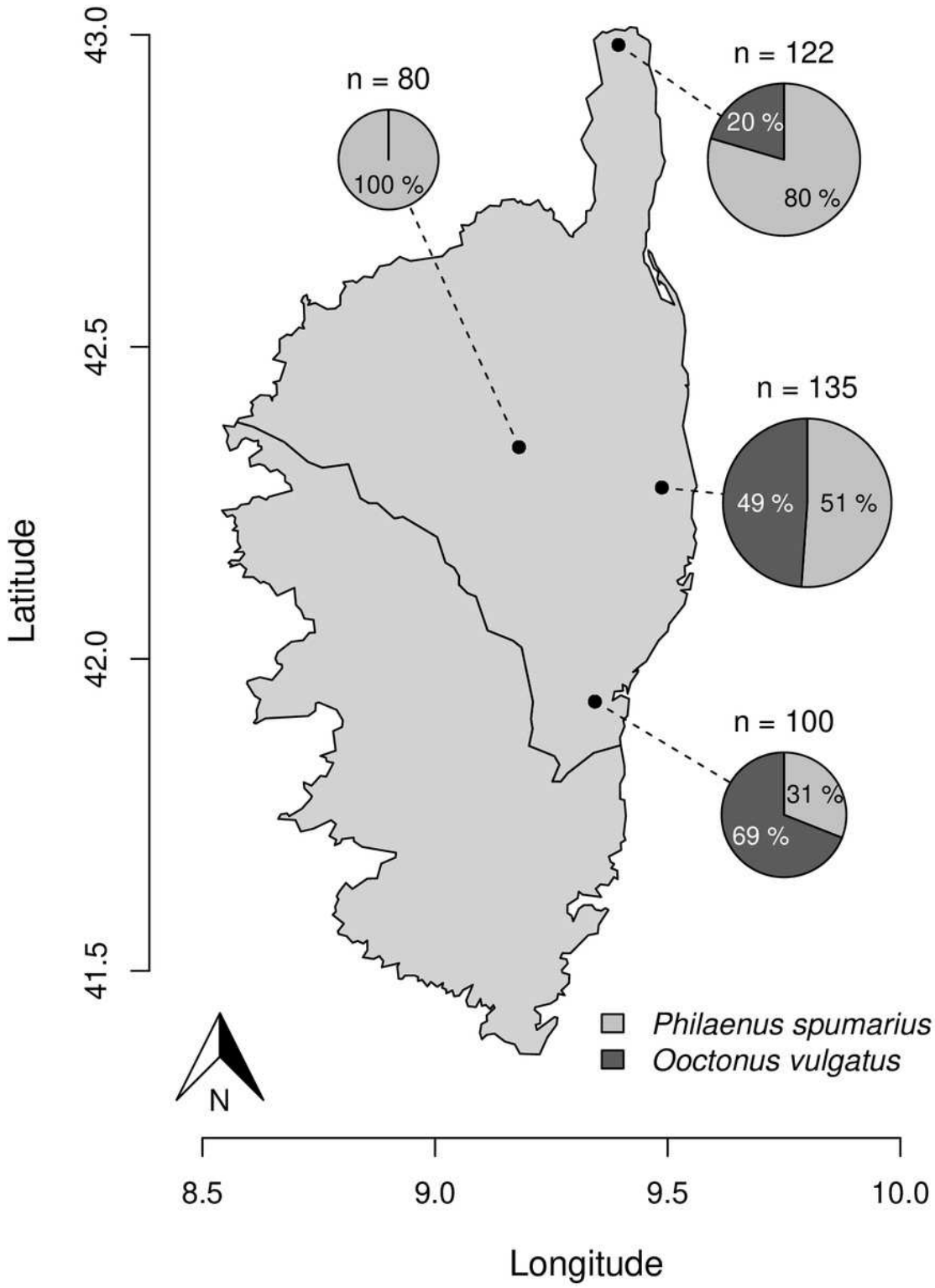

Figure 2

Morphology of *Ooctonus vulgatus Haliday*, 1833

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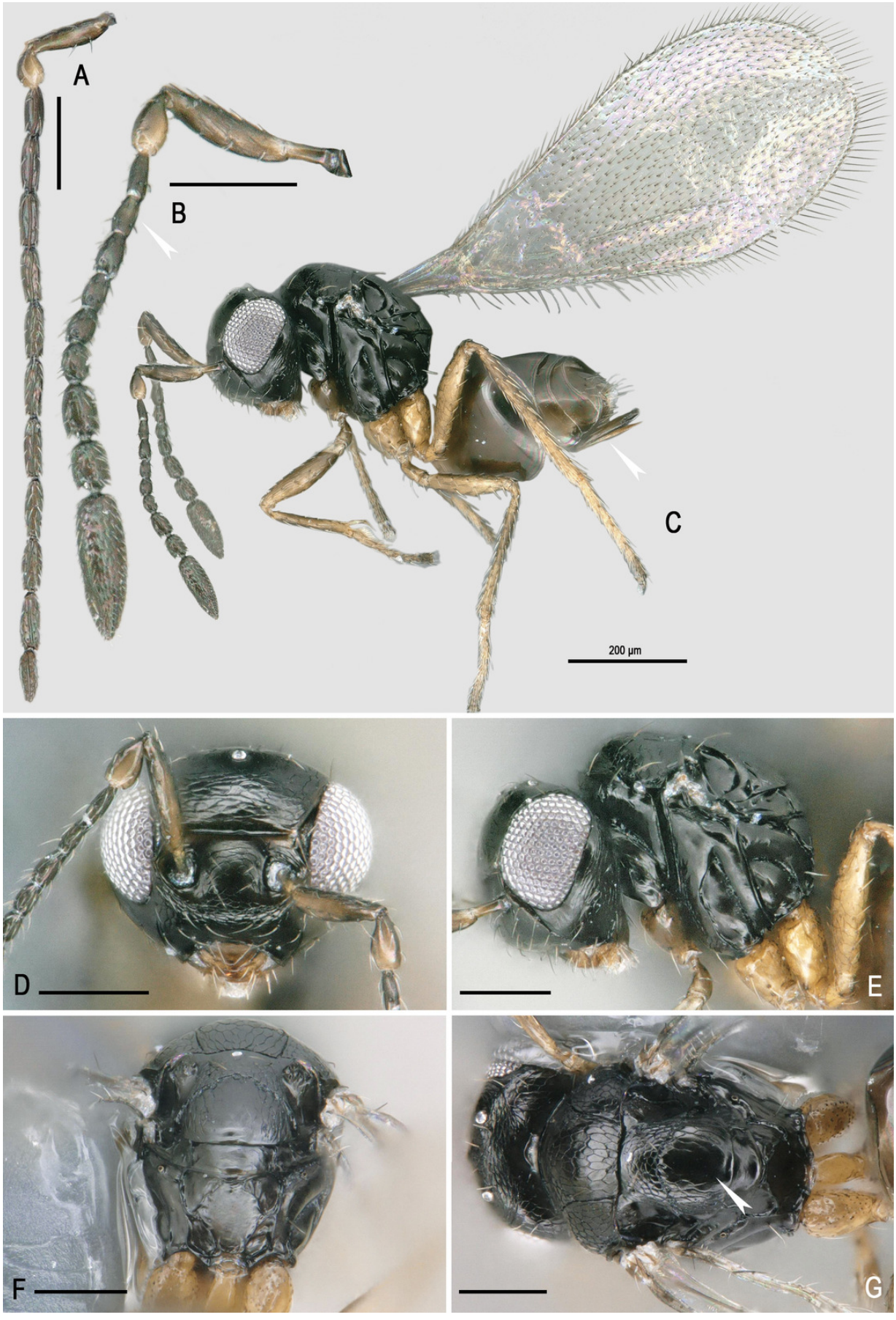

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Figure 3

Geographical distribution of *O. vulgatus*

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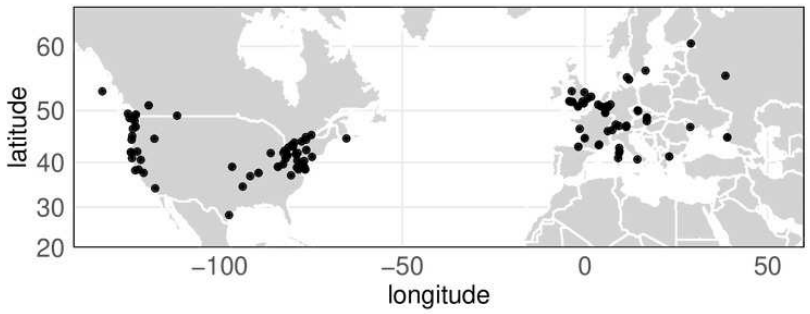
A



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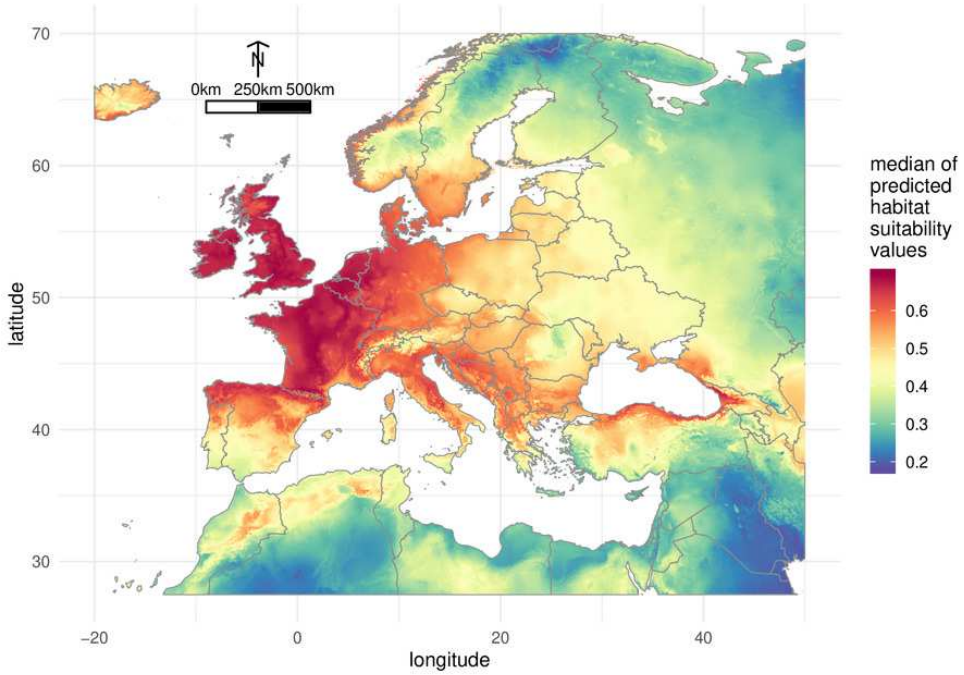

C