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UPDATE OF THE ALIEN DISTRIBUTION OF THE JASMINE LACEBUG THROUGH A CITIZEN-SCIENCE APPROACH

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Mori E., Viviano A., Corradetti M., Ancillotto L., Falco C., Mazza G.- Update of the alien distribution of the jasmine lacebug through a citizen-science approach.

Alien lacebugs have been recorded in most Mediterranean countries and, in Italy, in several western coastal regions. In this work, we collected new data on the jasmine lacebug *Corythauma ayyari* through a citizen-science approach. We reported for the first time its presence in three new Italian regions (Lombardy, Piedmont, and Marche), as well as in the hinterland areas of Israel, France, and Spain, up to over 220 km from the nearest coastal area. Through molecular analyses based on the mitochondrial gene of cytochrome oxidase I, we identified three new mitochondrial haplotypes in Italy, suggesting the possibility of recent introductions through plant trading. The increased distribution range towards the European hinterlands by *C. ayyari* needs to be monitored, as these introduced aliens are responsible for severe impacts on ornamental plants. Furthermore, improved controls in port areas should be recommended to prevent new invasions. We strongly recommend using multi-taxon, pan-continental platforms of citizen-science to collect data and keep the distribution of alien species updated.

Key Words: *Corythauma ayyari*; Mediterranean basin; mitochondrial DNA; online citizen-science platforms; Tingidae.

3

INTRODUCTION

The Anthropocene is turning out to be an era characterized by rapid and widespread changes due to the heavy and multifaceted impact of humans on the environment (Lewis and Maslin, 2015). Many scientific studies have raised concerns about the rapid decline and homogenization of global biodiversity in relation to the growing human interaction with ecological dynamics (Eriksen, 2021). One of the main causes of this decline is brought by invasive alien species (Corlett, 2015; Kueffer, 2017). The lack of knowledge of the introduction pathways and the late awareness of the presence of these species when they are already well established and out of control limit the success of management actions (Diagne *et al*., 2021).

Over the last 30 years, it has become increasingly clear that the well-being of the biosphere is closely linked to the wellness of human populations (Adjei *et al*., 2015; Freitas *et al*., 2020; Bernhardt and O'Connor, 2021). The adoption of policies aimed at ensuring long-term usability and responsible trade should be promoted. This can be achieved by providing port authorities with informative brochures and documents to enhance controls over incoming and outgoing goods, as part of adaptive management programs (Vanderhoeven *et al*., 2011; Patoka *et al*., 2018). Managers of the affected areas should follow management recommendations and implement guidelines to address more targeted communication strategies and curtail the incoming and spread of alien species.

Citizen-science represents a useful and effective tool to monitor the distribution of alien species (Mazza *et al*., 2014; Di Febbraro *et al*., 2023; Menchetti *et al*., 2023). Online platforms and citizen-science apps have been shown to act as reliable methods to detect new areas of invasions, particularly multi-taxon ones, e.g., iNaturalist (www.inaturalist.org: e.g., Mori *et al*., 2023; Menchetti *et al*., 2023).

In Europe, the EU Regulation 1143/2014 black-lists only five species of insects, the impacts of which are of conservation concern for European native biodiversity (https://www.specieinvasive.it/specie-di-rilevanza-unionale/specie-di-rilevanza-unionale-2, Accessed on 07.10.2023). In Italy, several other species of insects are listed by the Plant Protection Service ("Servizio Fitosanitario Nazionale", i.e., the Italian public council responsible for protecting plants and plant products from alien harmful organisms) as "quarantine" species, and require addressed monitoring and immediate reporting by researchers and regional councils (Inghilesi *et al*. 2013; Ma-

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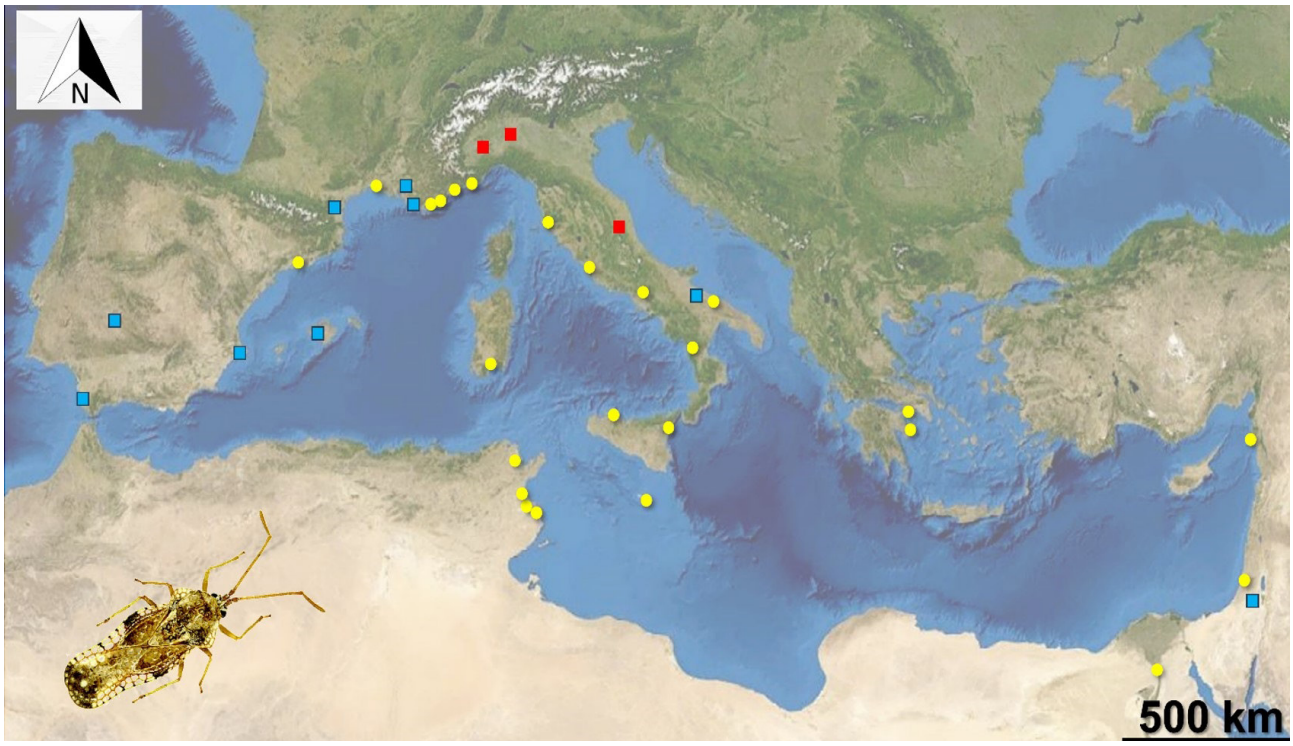

Fig. I - The current distribution of the jasmine lacebug in the Mediterranean countries. Yellow dots refer to occurrences before 2020 (summarised by Mazza *et al*., 2020), blue squares refer to new areas, red ones to new areas and genotyped specimens.

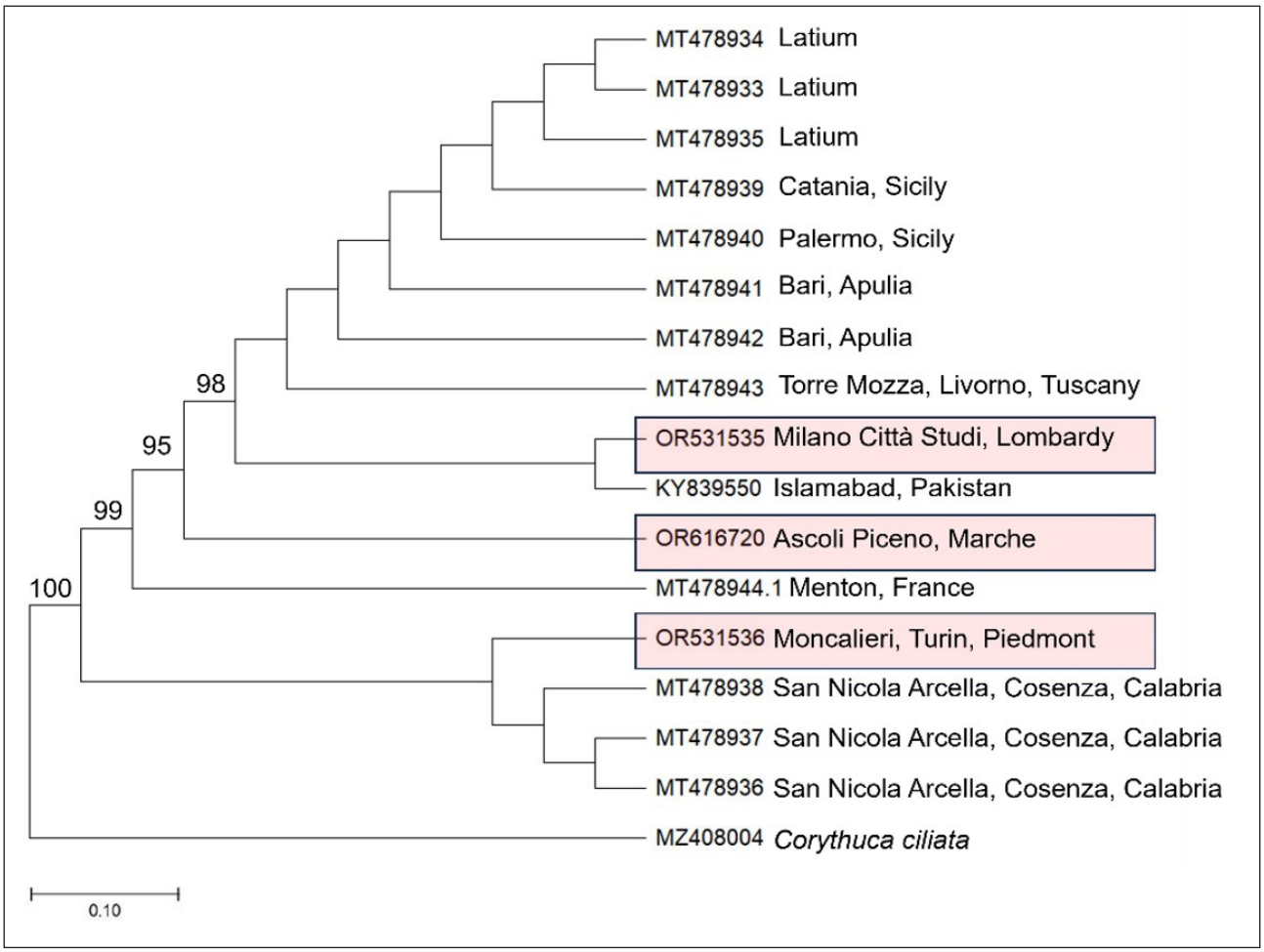

Fig. II - ML phylogenetic tree derived from the alignment of COXI sequences (638 bp) amplified from selected specimens (red boxes) and NCBI retrieved samples of *Corythauma ayyari*. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

rianelli *et al*. 2019). Besides all those black-listed species, a huge number of alien species of insects is reported for Italy (Inghilesi *et al*., 2013; Molfini *et al*., 2020) and many of those are known to affect ornamental and/or cultivated plant species (Mazza *et al.,* 2018; Farina *et al*., 2020; Stroiński *et al.,* 2022; Mori *et al*., 2023). The recent Italian Legislative Decree 19/2021 ("Rules for the protection of plants from harmful organisms"), which entered into force on 13 March 2021, imposes that any previously unrecorded species in each Italian region should be rapidly signaled to the regional Plant Protection Service before any publication (both scientific and newspaper articles). This Legislative Decree also highlights the importance of keeping updated on the distribution of harmful taxa.

Table 1 - New areas of the presence of the invasive jasmine lacebug in Mediterranean countries. Accession numbers refer to molecularly analysed specimens (see Fig. II).

Date	Country	Locality	Region	Source	Latitude	Longitude	Accession number
26/7/2020	Italy	Trani	Apulia	Facebook	41.273357°N	16.422636°E	NA
18/6/2021	Italy	Moncalieri (Torino)	Piedmont	Facebook	45.001488°N	7.688634°E	OR531536
27/6/2021	Italy	Milano - Città Studi	Lombardy	iNaturalist	45.480078°N	9.222264°E	OR531535
31/7/2023	Italy	Ascoli Piceno	Marche	iNaturalist	42.854195°N	13.597613°E	OR616720
13/9/2020	France	Avignone	Vaucluse	iNaturalist	43.967247°N	4.899333°E	NA
15/6/2022	France	Marseille	Provence-Alpes-Côte d'Azur	iNaturalist	43.296625°N	5.385980°E	NA
01/8/2022	France	Perpignan	Pyrénées-Orientales	iNaturalist	42.672163°N	2.992420°E	NA
09/9/2021	Spain	Palma de Mallorca	Balearic Islands	iNaturalist	39.566744°N	2.651096°E	NA
01/11/2022	Spain	Denia	Comunidad Valenciana	iNaturalist	38.890993°N	0.146938°E	NA
29/8/2022	Spain	Villanueva de la Serena	Extremadura	iNaturalist	38.973007°N	5.799356°W	NA
30/8/2022	Spain	Villanueva de la Serena	Extremadura	iNaturalist	38.968970°N	5.799727°W	NA
16/9/2023	Spain	Jerez	Andalusia	iNaturalist	36.662833°N	6.119104°W	NA
10/9/2022	Israel	Jerusalem	NA	iNaturalist	31.778291°N	35.181786°E	NA

The jasmine lacebug *Corythauma ayyari* is an invasive insect (Hemiptera: Tingidae) native to Southern Asia from Pakistan to Indonesia, but also recorded as an alien species in several countries of the Mediterranean basin (Mazza *et al*., 2020). The unmistakable aspect of this species, showing a subspherical pronotal hood and the extensive damage it may provoke to jasmine species, makes it a suitable species for citizen-science survey (Mazza *et al*., 2020).

This lacebug has been reported on a plethora of ornamental plant species belonging to several families (Acanthaceae, Apocynaceae, Lamiaceae, Malvaceae, Musaceae, Oleaceae, Verbenaceae, and Zingiberaceae), therefore being regarded as a pest for the human economy. Control of this species requires plant destruction (Mazza *et al*., 2020) or, as in this case, ozone treatments (M. Iemma, unpublished). As to Italy, the jasmine lacebug has been confirmed for several regions (Apulia, Calabria, Campania, Latium, Tuscany, Liguria, Sicily, and Sardinia), whereas direct research in other ones (Lombardy, Marche, Abruzzi, and Basilicata) did not provide any positive result (EM, unpublished data). All regions of recorded presence of *C. ayyari* in Italy are located on the coastline, thus suggesting the importance of port areas for the diffusion of this alien insect, i.e., through the trade of potted plants. This pest is not included in any list of European and Mediterranean Plant Protection Organization (EPPO) and the pest status in Italy is officially declared as "transient" (https://gd.eppo.int/taxon/COTMAY), but as already reported for the palm borer *Paysandisia archon*, the distribution and the status need to be updated (Mori *et al.*, 2023). Thus, after three years since the previous report, the aim of this work was to update the known distribution of this species in the Mediterranean basin. Furthermore, we assessed the potential origin of the new Italian populations by means of genetic analyses.

Fig. III - Jasmine plants *Jasminum officinale* L. affected by *Corythauma ayyari* in Ascoli Piceno (photo by Marida Corradetti).

MATERIALS AND METHODS

In September 2023, we conducted a media campaign on social networks (Facebook, Instagram, Twitter, and TikTok), and on iNaturalist to collect as much data as possible on the occurrence of *C. ayyari* in the Mediterranean countries. We only considered occurrences from 2020 to 2023 which were authenticated through the use of photographic evidence. Every photograph was examined and validated, following the main characteristics of adults of this species (i.e., small, rectangular, dorsoventrally flattened and cream coloured with brown patches, pronotum with a spherical dome-shaped hood, transparent wings, like texture covering the insect body). Subsequently, authors of each record were contacted, requesting precise dates and coordinates. Then, a dataset was compiled including an unequivocal code for each record, photos of each individual, locality, coordinates, and dates.

Biological samples of this species were collected by the authors and, when possible, by asking observers to do the same. The samples were preserved in absolute ethanol for molecular analyses. In these cases, DNA was extracted using the Qiagen Blood and Tissue kit (©Qiagen, Inc, Tokyo, Japan). A portion (638 bp) of the mitochondrial DNA cytochrome oxidase I gene (hereafter, COXI) was amplified by using universal primers for COXI: HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO 1490 (5'- GGTCAACAAATCATAAAGATATTGG-3') (Folmer *et al*., 1994). PCRs were conducted on an Eppendorf MasterCycler X50 thermal cycler in 25 µl mix including 100ng of each DNA sample, buffer 10 ×, 12 mM MgCl2, 200 µM dNTPs, 0.2 µM of each primer, and one unit of Taq polymerase (©Life Technologies, Waltham, Massachusetts, USA). PCR conditions included initial denaturing at 94°C for 5 min, followed by 35 cycles of 94 °C for 45″, annealing at 49°C for 30″, extending at 72 °C for 1 min, and a final extension to 72 °C for 10 min. PCR products were run by electrophoresis on 2.0% agarose gel, containing 0.5 mg/ml of SYBR gel staining. Successful amplifications were then purified (ExoSAP-IT PCR clean-up Kit, ©Applied Biosystems, Foster City, California, USA) and sequenced through the chain termination method at the BMR Genomics (https://www.bmr-genomics.it/, Padua, Italy). Sequences were analyzed with the Mega XI software (Tamura *et al*., 2021). The software JModelTEST 304 (Posada and Crandall, 1998) was used to test the most accurate model of substitution using the Bayesian information criterion (BIC) and Akaike's Information Criterion (AIC), corrected for the heterogeneity between sites (gamma [G]). Lacebug sequences obtained in this study were aligned with previously published COXI sequences of similar species available on GenBank (http://www.ncbi.nlm.nih.gov). The Tamura-Nei 93 nucleotide substitution model was selected. Phylogenetic reconstructions were conducted by applying the maximum likelihood method, through the software SeaView (Gouy *et al*., 2010). We selected optimized choices, and we obtained the tree-searching operations by Nearest-Neighbour Interchange (NNI) and Subtree Pruning–Regrafting (SPR). A sequence of plane-tree lace tingid *Corythucha ciliata* was included as an outgroup.

RESULTS

We obtained 23 new occurrences of *C. ayyari* with respect to the previous review (Mazza *et al*., 2020). Ten of these records confirmed previous occurrences (six in Tel Aviv, two in Rome, one in Barcelona, and one in Monaco, The Principality); the other 13 referred to new areas of occurrence (Table 1).

Only two records came from a social network (Facebook), whereas all the others were uploaded on iNaturalist platform. In particular, the species expanded its range in the Israel hinterland, France, and Spain, with first records from Balearic Islands and from the Extremadura region (Fig. I; Table 1).

As for Italy, we obtained the first occurrence reports for Marche, where the species was searched but not recorded in 2019, Lombardy, and Piedmont (Figs I and II). Individuals from the first two regions clustered with *C. ayyari* from most of the native and invaded ranges, whereas the one from Piedmont was included within the clade including specimens from Calabria, i.e., the sister group of the one including sampling from most of Italy and Southern France (Fig. II).

DISCUSSION

Our work showed a remarkable range expansion of the jasmine lacebug in Mediterranean countries since its first observations (Mazza *et al*., 2020). Particularly, we recorded the presence of this species also in areas where it was not detected in our previous local surveys, thus suggesting that the invasion may have followed the purchase of the host plant by the garden owner, i.e., the host plant might have been bought before the insect attack.

In particular, three new Italian regions and several new areas of Southern France and Spain (i.e., coastal area of Southern regions, Balearic Islands and Extremadura) were found to be colonized by this alien insect. As to Italy, both Marche and Lombardy regions were explored in 2019, but no sign of *C. ayyari* was detected on local potted jasmine plants. Afterward, plants of jasmine in both regions were found infested by *C. ayyari*, suggesting that a further trade of host plants after 2019 may have brought individuals of this alien species to other parts of the study area. However, we did not detect new genetic haplotypes, therefore suggesting that no new introduction event from the native country of *C. ayyari* may have occurred in the last year. In other words, all new genetically-analysed specimens clustered with previously analysed ones.

Port areas constitute a typically perturbed anthropic environment, widespread and highly functionally interconnected on a global scale. Therefore, such areas have a huge potential to represent hubs for the diffusion of aquatic and terrestrial alien species, including species affecting ornamental plants and native biodiversity (Pennacchio *et al*., 2017; Binazzi *et al*., 2019; Mori *et al*., 2022). Comparing the distribution of this species in the invaded range between this work and previous reviews (Rietschel, 2015; Mazza *et al*., 2020), we may suggest that the stabilization process of the jasmine lacebug may have started from port or circum-port areas, and then moved through secondary trade movements towards the hinterland. Therefore, the first record of this alien insect in Extremadura, i.e., in the central-western part of the Iberian Peninsula, at over 220 km from the nearest coastal area, may suggest that national trade from coastlines may have helped this species to reach previously unoccupied areas.

Evidence indicates that the jasmine lacebug brings host plants to death, therefore representing a clear threat to public and private gardens (Streito *et al*., 2010; Novoselsky and Freidberg, 2013), also given the wide range of plants it may affect (Fig. III), thus indicating this lacebug as a suitable species for citizen-science records. The use of multi-taxon, open-access citizen-science platforms should be promoted, as it is one of the main methods for early detection of alien species and it may lead to outline invasion risk maps, by indicating areas where to exercise the greatest research effort to limit the impacts by alien species through prompt actions. (Di Febbraro *et al*., 2019, 2023; Menchetti *et al*., 2023).

Increasing controls in port areas, and a good communication on the impacts provided also by alien species not included in blacklists or in quarantine lists, such as the jasmine lacebug, should be required over incoming and outgoing goods, as part of adaptive management programs, to minimize (Vanderhoeven *et al*., 2011; Bacon *et al*., 2012; Patoka *et al*., 2018). Managers of the affected areas should follow management recommendations and implement guidelines to address more targeted communication strategies and curtail the incoming and spread of alien species.

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