



Establishment and spread of the invasive ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae) in Greece: based on contributions from citizen scientists

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Abstract *Harmonia axyridis* (Pallas, 1773), also known as the harlequin ladybird, is an invasive non-native species intentionally introduced to many countries as a biological control agent of agricultural pests. In Greece, *H. axyridis* was first introduced as a biological control agent in 1994, with releases taking place between 1994 and 2000. For many years there was no evidence to indicate that *H. axyridis*

had established self-sustaining populations. In 2008, a citizen science campaign was initiated aimed at raising awareness regarding the invasive status of *H. axyridis* to farmers and agronomists. The campaign did not yield results, and it was discontinued in 2011. During this study, the distribution, phenology, and presence of *H. axyridis* in different habitat types and protected areas in Greece are investigated, using both

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citizen science data and literature records. Records from iNaturalist, the AlienToma database and social media examined herein demonstrate that *H. axyridis* has been established in Greece since 2010. *Harmonia axyridis* is currently present in 13 administrative districts of Greece, most of them at a considerable distance from the initial release sites. The harlequin ladybird is present in urban and agricultural habitats as well as seventeen NATURA 2000 sites. The adverse socioeconomic and environmental impacts of *H. axyridis* are briefly discussed alongside suggestions for management activities. Based on our findings, we propose the establishment of a national monitoring scheme for *H. axyridis* and native ladybirds that will also encourage public participation in recording ladybird observations and provide information on the distribution, spread and impact of this invasive non-native species.

Keywords Non-native species · Biodiversity monitoring · Biological invasions · Citizen science · Harlequin ladybird · NATURA 2000 network

Introduction

The harlequin ladybird, *Harmonia axyridis* (Pallas, 1773) has been released as part of biological control programmes globally against aphids and scale insects. This species was considered a suitable biological control agent as it is easy to rear (Adriaens et al. 2007; Roy et al. 2016; Kulijer 2017), it can track prey in space and time (Koch 2003), and it has voracious feeding habits (Sloggett et al. 2011). However, there is also evidence that *H. axyridis* has adverse impacts on biodiversity and it is considered an invasive non-native species (hereafter invasive species) (Roy et al. 2012, 2016; Skuhrovec et al. 2021).

Harmonia axyridis has attracted the interest of researchers and the public because of its dramatic spread and adverse impacts on nature and people (Roy et al. 2016; Kulijer 2017; Honek et al. 2018; Haelewaters et al. 2020; Skuhrovec et al. 2021). In the invaded habitats, there is evidence that *H. axyridis* preys upon many native arthropods and has displaced native natural enemies (Koch 2003; Roy et al. 2016). It is also considered a household nuisance in some contexts because of the large numbers that aggregate in buildings particularly through the winter months

and a pest of fruit production (Koch and Galvan 2007; Honek et al. 2017). Furthermore, *H. axyridis* can affect human health, by causing urticaria and allergic reactions (such as rhino-conjunctivitis and asthma) or bite induced injuries (Goetz 2007; Roy et al. 2016; Roques et al. 2018).

Harmonia axyridis has rapidly spread over Europe and the Balkan Peninsula over the last few decades (Brown et al. 2007). However, there is very limited information regarding its phenology and distribution in Greece (Katsoyannos et al. 1997; Kontodimas et al. 2008a, b). *Harmonia axyridis* was first released in four citrus growing areas of Greece in 1994 (Marathon-Attica, Campos-Chios, Leonidion-Arcadia, and Chania-Crete) (Katsoyannos et al. 1997). Initially, it was considered to be an effective biological control agent against aphids in two of the locations of initial release but subsequent introductions in 1995 were not successful in any orchards. In 1995–1999, more than 100 000 insectary-reared adults of *H. axyridis* were released in various cultivations (i.e., citrus, vegetables, beans, maize) and urban areas of central and southern Greece as well as the islands of Chios, Evoia and Crete. During this period, field surveys were conducted every spring, just before the releases, to determine if *H. axyridis* had overwintered in the field. A month after each release field surveys were also conducted in order to estimate the population size of the ladybird shortly after the release (Kontodimas et al. 2008a). Subsequent surveys conducted every spring from 2000 to 2007, yielded no records of *H. axyridis* in any of the locations where the predator had been released (Kontodimas et al. 2008b). In earlier studies by Kontodimas et al. (2008a, b) *H. axyridis* was reported as having failed to establish' but the authors at the time stated that '*absence of evidence is not necessarily evidence of absence*'. Given the time lag between the releases of *H. axyridis* and its establishment the authors stated the need for on-going surveillance (Kontodimas et al. 2008a, b).

Citizen or community science is a valuable tool for recording invasive non-native species at wide geographical scales and for engaging non-professionals in scientific research (Dickinson et al. 2012; Roy et al. 2018; Johnson et al. 2020; Lepczyk et al. 2020; Pataki et al. 2021). Community science in entomology can give valuable information regarding the distribution of insect species and their role in ecological networks (Gardiner and Roy 2022; Groom et al.

2021; Skuhrovec et al. 2021). In an effort to collect data from the general public and farmers, in 2008, Martinou and Kontodimas published a popular science article in Greek in a farmer's magazine, describing the life-forms and other attributes of *H. axyridis* asking the readers to send photos or specimens to the Benaki Phytopathological Institute. However, that effort yielded no records. The first published records in the literature of established populations of *H. axyridis* were documented in June 2017 in three localities in Northern Greece (Promachonas, Charopo and Asprovalta) by Ceryngier and Romanowski (2017), no other records exist in the literature. The aim of our study was to provide information on the establishment and spread of *H. axyridis* in Greece by reviewing the literature and examining the records on the iNaturalist platform, the Alientoma database and social media. Citizen science records and records from the scientific literature were used to map the distribution of *H. axyridis* in Greece. These records were also used in order to identify the colour pattern polymorphism of adult individuals of the species in the country as well as the presence of the entomopathogenic fungus *He. virescens*. A literature search was also performed in order to identify the feeding habits of *H. axyridis* and its impacts on native biodiversity.

Material and methods

Distribution of *Harmonia axyridis* in Greece

Records of *H. axyridis* in Greece were obtained from citizen science observations made through the iNaturalist platform (iNaturalist 2021), the Alientoma database for non-native insects in Greece (Kalaentzis et al. 2021) and data mining from the social media (i.e., Facebook). Citizen science observations were downloaded from iNaturalist and the Alientoma database up to September the 8th 2021. These data were supplemented by observations from the authors and published literature records by Ceryngier and Romanowski (2017). The localities of the initial releases were also catalogued and georeferenced (Katsoyannos et al. 1997; Kontodimas et al. 2008a). Each record represented a unique sighting of *H. axyridis* on a given date and location reported by one person.

Distributional patterns in respect to land cover and the NATURA 2000 network sites

Citizen science data were used to investigate the occurrence of *H. axyridis* including in NATURA 2000 sites. A total of 191 records were collected from iNaturalist (164) and Alientoma (27); only georeferenced records which had more than two decimal places were used for accuracy reasons [accuracy of 100 m, to coincide with the Corine Land Cover (CLC) mapping width of 100 m]. We used CLC datasets (CLC 2018—Copernicus Land Monitoring Service). CLC data provides information on the biophysical properties of the Earth's surface. The CLC datasets used have a common nomenclature with 44 classes in a hierarchical 3-level CLC nomenclature downloaded from the Copernicus Land Monitoring Service. The version used, covers the year 2018 with a minimum mapping width of 100 m. The projection used was EPSG: 3035-ETRS89-extended/LAEA Europe was used as assigned coordinate reference system (CRS). In addition, we identified the sites that are part of the NATURA 2000 European network (under Council Directive 92/43/EEC). These layers were then joined with the citizen science records (in total, 191 records) in order to explore the occurrence of *H. axyridis* in Natura 2000 sites and the land use classes in Greece. Finally, the administrative regions of Greece (Kallikratis Plan 2010—Law 3852/2010) were used to compare the distributional patterns of *H. axyridis* amongst areas of initial introductions and those where the species was not released.

Phenology, colour pattern polymorphism and parasitism by *Hesperomyces virescens*

Observations depicting more than one individual of different colour forms (melanic and non-melanic morphs; f. *spectabilis*, f. *conspicua*, f. *axyridis* and f. *succinea*) or life stage (i.e., larva, pupae or adult) were catalogued accordingly. All observations were verified from photographs submitted alongside each record, to confirm the species identification and study the colour pattern polymorphism of adult individuals. In addition, the presence of the entomopathogenic fungus *He. virescens* was assessed by examining citizen science observations of *H. axyridis* for signs of parasitism by the fungus.

Feeding habits

To assess the feeding habits and adverse impacts of *H. axyridis* on native biodiversity, a literature search was performed with the Scholar Google search engine, which, apart from peer-reviewed articles, included technical reports, etc. Eligibility criteria included any document with the following keywords anywhere in the article: <*Harmonia axyridis*> or <harlequin ladybird> AND “trophic preferences” AND “prey” AND “alternative food”. Publications related to *H. axyridis* presence, establishment and status in Greece were deemed to be of the highest relevance according to our scope, while references of each individual article were thoroughly searched for additional bibliography.

Statistical analysis

Plots were created in RSTUDIO Version 1.2.5042 (R Studio Team 2021), by using the ggplot2 package (Wickham 2016), while maps were created using QGIS 3.18.2.

Results

Distribution of *Harmonia axyridis* in Greece

A total of 191 observations were reported by citizen scientists (164 observations iNaturalist and 27 observations Alientoma), in thirteen of the fourteen administrative regions of Greece (with the exception

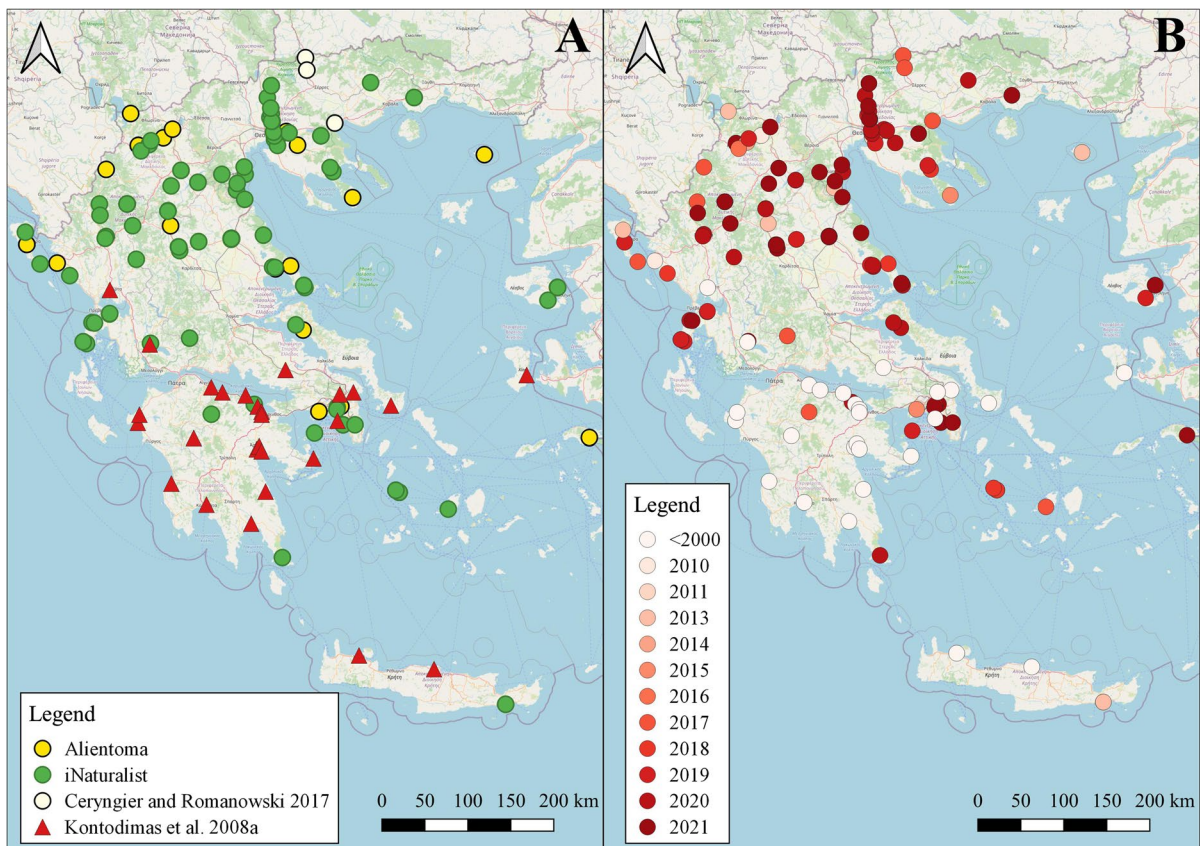


Fig. 1 Geographic regions in Greece and the reported presence of *Harmonia axyridis* (Pallas 1773). **A** Citizen science records (in green by the iNaturalist platform and in yellow by Alientoma), literature records by Ceryngier and Romanowski (2017) (in white), localities of intentional releases in nature

by of Benaki Phytopathological Institute (Kontodimas et al. 2008a) (in red); **B** Sightings depict a wide range of distribution for *H. axyridis* in the country (most frequently reported depicted in deeper red colour) all records before 2000 are initial release location

of the Monastic Republic of Mount Athos). The number of records per year has increased from three in 2010 to 57 records in 2021 (Fig. 1B). More than half (51.83%) of the individuals observed by citizen scientists were recorded in the years 2020 and 2021. Most citizen science observations were recorded in continental Greece from Thessaly (53), Central Macedonia (45) and West Macedonia (32), accounting for 69% of all observations made by the public (Fig. 2). In addition, data that were collected through a literature review were used for mapping the introductions, the spread and the distribution of the species. Our results showed that *H. axyridis* is present throughout Greece and not only at the locations of initial introductions (Kontodimas et al. 2008a), or the locations studied by Ceryngier and Romanowski (2017) in northern Greece (Fig. 1A). However, it is not possible to know the exact year of *H. axyridis* establishment, as the records of iNaturalist for *H. axyridis* in Greece began in 2010.

Distributional patterns in respect to land cover and the NATURA 2000 network sites

Harmonia axyridis was observed in 18 different types of CLC (Fig. 4), with most records (35%, 63 observations) within urban areas with transport

networks, bare ground and vegetated land classified as discontinuous urban fabric (CLC 112), followed by land principally occupied by agriculture (CLC 243) (24%, 44 observations). The administrative divisions “West Macedonia”, “Central Macedonia”, “Thessaly” had most observations and the rest of administrative divisions were grouped due to the small number of records (the “rest of Greece”). Within these four categories, more than 50% of *H. axyridis* individuals were recorded in urban areas of West, Central Macedonia and the rest of Greece, mainly in continuous or discontinuous urban fabric (CLC 112) as well as sport and leisure facilities, industrial or commercial units etc. In Thessaly a major agricultural production area, 66% of the records (33 observations) fell within land principally occupied by agriculture (CLC 243) followed by urban habitat types (35%, 18 observations) (Fig. 4).

Despite the large percentage of observations corresponding to urban habitats, this study showed that harlequin ladybirds are present also in protected NATURA 2000 areas. That means that the spatial distribution of the species is not necessarily related to man-made habitats and areas with high anthropogenic pressures. Regarding the presence of *H. axyridis* within the NATURA 2000 network, the species

Fig. 2 Citizen science records of *Harmonia axyridis* (Pallas, 1773) in Greece per Administrative division (Kallikratis Plan). Administrative divisions where areas that intentional releases took place between 1995 and 1999 (Kontodimas et al. 2008a, b) are coloured red. The number of citizen science observations per Administrative division and their percentages to the total number of citizen science observations are given. Inset: *Harmonia axyridis* observed in Thessaly, Pilio by K. Kalaentzis and C. Kazilas

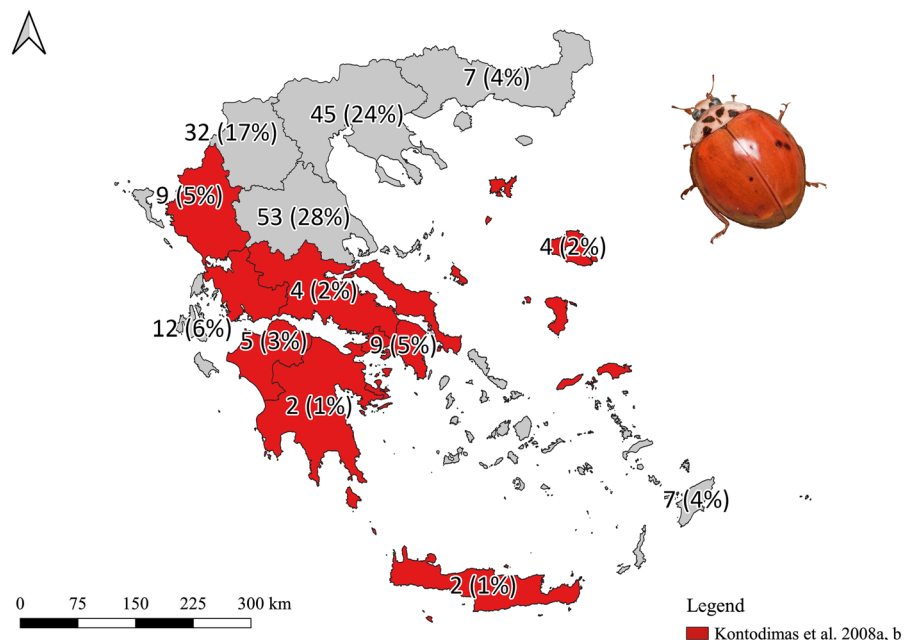


Table 1 Citizen science observations of *H. axyridis* in NATURA 2000 sites

Site code	Site name	Number of CS observations
GR2130001	ETHNIKOS DRYMOS VIKOU—AOOU	1
GR2130013	EVRYTERI PERIOCHI ATHAMANIKON OREON	1
GR2130012	EVRYTERI PERIOCHI POLIS IOANNINON	3
GR1420004	KARLA—MAVROVOUNI—KEFALOVRYSO VELESTINOU—NEOCHORI	1
GR2130011	KENTRIKO ZAGORI KAI ANATOLIKO TMIMA OROUS MITSIKELI	1
GR1320002	KORYFES OROUS GRAMMOS	1
GR4110005	LESVOS KOLPOS GERAS, ELOS NTIPI KAI OROS OLYMPOS—POTAMOS EVERGETOULAS	1
GR1220009	LIMNES KORONEIAS—VOLVIS, STENA RENTINAS KAI EVRYTERI PERIOCHI	1
GR1220001	LIMNES VOLVI KAI LAGKADA—EVRYTERI PERIOCHI	5
GR1320001	LIMNI KASTORIAS	1
GR4220009	NOTIA SERIFOS	2
GR1330002	ORI VOREIOU VOURINOU KAI MELLIA	1
GR2320002	OROS CHELMOS KAI YDATA STYGOS	1
GR1250001	OROS OLYMPOS	1
GR1430001	OROS PILIO KAI PARAKTIA THALASSIA ZONI- SPILAIA MALAKI KAI SKEPONI	1
GR2130006	PERIOCHI METSOVOU (ANILIO—KATARA)	1
GR1250002	PIERIA ORI	1

was recorded in 17 sites, located in eight administrative divisions (Table 1). The highest number of observations (5 records) was recorded from lakes Volvi and Lagkada (Limnes Volvi and Lagkada, GR1220001), in Central Macedonia, followed by two records from the wider area of Ioannina (Evryteri Periochi Polis Ioanninon, GR2130012) in Epirus (Table 1).

Phenology, colour pattern polymorphism and parasitism by *Hesperomyces virescens*

The vast majority of citizen science records were of adult individuals (87%, 189 photographic observations), followed by larvae (11%, 25 observations) and just four observations of pupae (2%) (Fig. 3C). Citizen scientists observed *H. axyridis* individuals throughout the year, except January (Fig. 3A, B). The species was mostly encountered from April to September although approximately ten living individuals per month were spotted from October to December (Fig. 3A). The highest frequency of individuals was recorded in May when all three developmental stages of *H. axyridis* were observed. During August, no larvae or pupae were detected, although adults were

detected in the highest numbers (Fig. 3A). Copulating adults were recorded in April, June, August and September (Fig. 3B).

Similar to Ceryngier and Romanowski (2017), three main colour forms of adult *H. axyridis* have been recorded in Greece by citizen scientists. These are f. *succinea* that constituted 81.4% of observations, followed by f. *spectabilis* (almost 12.4%) and f. *conspicua* (almost 5%). Only two individuals of *forma axyridis* have been recorded, both from Magnesia, Liri (Thessaly). In addition, our study showed that melanic morphs were more frequent in the northern part of the country (i.e., Eastern Macedonia and Thrace and Thessaly); however only a few observations of the melanic morphs were recorded.

Concerning the parasitic entomopathogenic fungus *He. virescens*, this species was first recorded in Greece in 2001 on two native ladybird species (Castaldo et al. 2004) and later it was found on *H. axyridis* from three Northern Greek localities, namely Asprovalta, Charopo and Promachonas (Ceryngier and Romanowski 2017). Citizen science observations of infected *H. axyridis* were detected in Psili Ammos, Serifos Island and Servia Kozanis (Fig. 4). Thus, citizen science can provide very useful data in recording

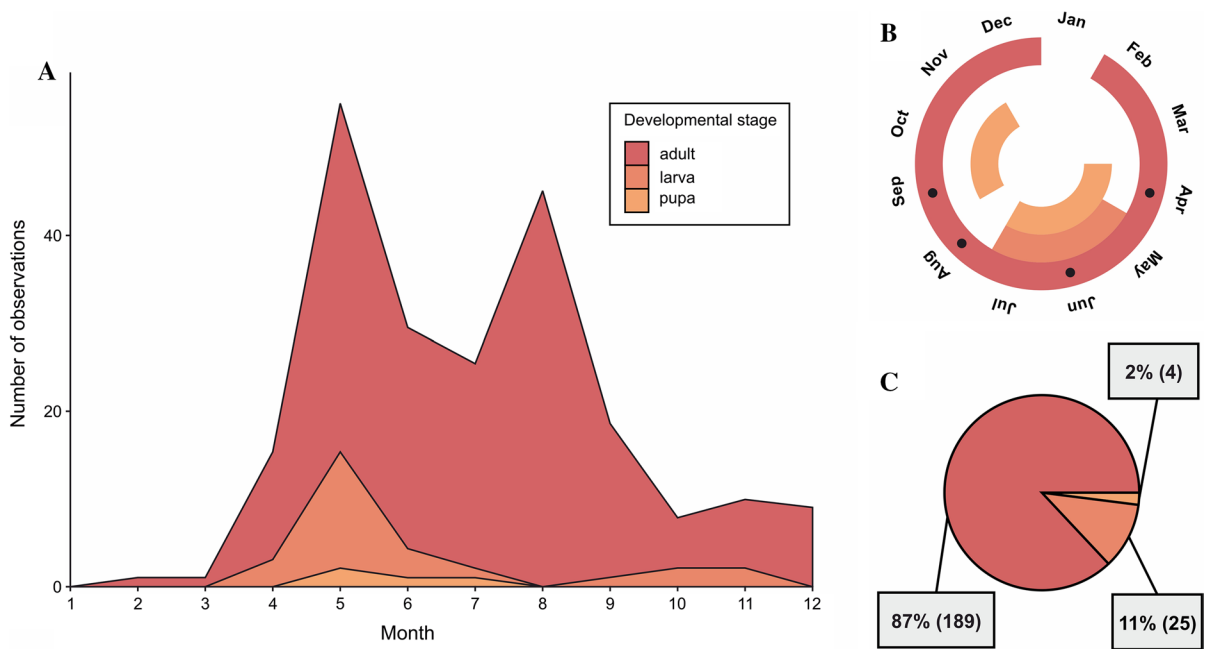


Fig. 3 Phenology of *Harmonia axyridis* in Greece; based on citizen science records. **A** Total number of individuals across all developmental stages (larva, pupa, adult) per month. **B** Monthly presence of different developmental stages through

the year, months where copulation was observed are marked with black dots. **C** Percentage and number of observations (in brackets) of different developmental stages analysed

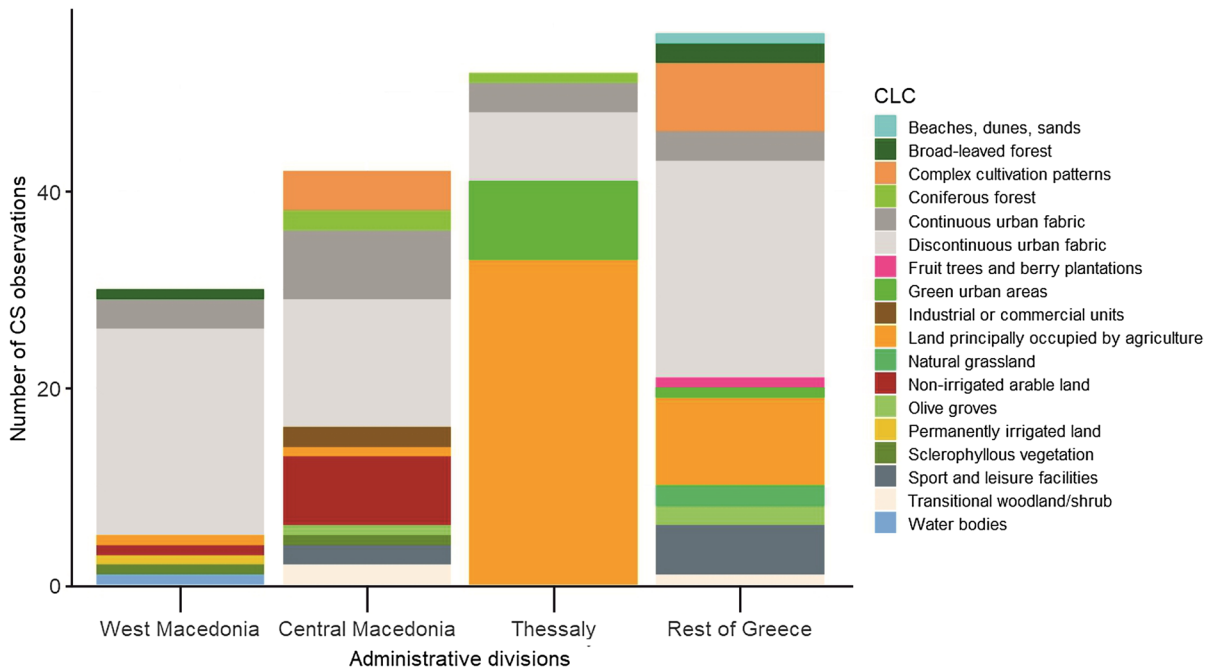


Fig. 4 Corine Land Cover types where *H. axyridis* has been observed by citizen scientists in Greece. Administrative divisions other than West Macedonia, Central Macedonia and

Thessaly grouped as “Rest of Greece” due to low number of observations (< 10 in each division)

parasitic infections and the distribution of parasitic fungus on *H. axyridis* in Greece.

Feeding habits

The literature survey on the feeding habits of *H. axyridis* in Greece revealed various aphids as prey of the harlequin ladybird, i.e., *Acyrtosiphonpisum* (Harris, 1776), *Aphis fabae* (Scopoli, 1763), *A. gossypii* (Glover, 1877), *A. spiraeicola* (Patch, 1914), *Hyalopteruspruni* (Geoffroy 1762), *Macrosiphumrosae* (Linnaeus, 1758), *Myzus persicae* (Sulzer, 1776), *Nasonoviaribis-nigri* (Mosley, 1841), *Rhopalosiphumpadi* (Linnaeus, 1758) and *Toxopteraaurantii* (Boyer de Fonscolombe, 1841) (Katsoyannos et al. 1997; Kontodimas et al. 2008a). On the side, citizen science records revealed an additional prey species, *A. nerii* (Boyer de Fonscolombe, 1841), observed in Magnesia, Liri (Fig. 4).

Discussion

Citizen science data mainly from the iNaturalist platform show that the harlequin ladybird is widely distributed in Greece, inhabiting multiple islands and continental regions, most of them remote to the initial sites where it was introduced as a biological control agent (Figs. 1A, 2). Early research on the distribution of *H. axyridis* up to 2007 implied that the ladybird had failed to establish in the areas where it was released as a biological control agent (Kontodimas et al. 2008b) and an effort for a citizen science recording scheme for this particular species in 2008 yielded no records (Martinou and Kontodimas 2008). However, citizen science records from as early as 2010 show that *H. axyridis* has been present in three distant geographical areas at least since that year (Fig. 1B). In particular, *H. axyridis* was observed from Sivota Thesprotias (G. Kakiopoulos pers. comm.), Florina, Cheimatitida lake (G. Kakiopoulos pers. comm.) and Magnesia, Liri. In the following two years only one individual was recorded, while in 2013 observations steeply increased reaching 13% of total observations (26 sightings). Up to 2018, data were scarce, but a rapid increase over the following years was evident with three quarters (144 observations) of all records attributed to 2018–2021. The increasing interest in biodiversity monitoring by citizen scientists in recent

years using new technologies such as GPS and smartphones, may account for this increase (Johnson et al. 2020).

Records of *H. axyridis* in the administrative units of the initial introductions were scarce, with most records coming from Northern Greece. Most of the citizen science observations were from Thessaly, Central and West Macedonia (Fig. 2). Particularly, Central Macedonia (including Thessaloniki, the second largest city in Greece, with more than a third of a million residents; World Population Review 2021) and Thessaly which is mainly agricultural land; the large number of cultivated areas could explain the increased number of observations (Fig. 4). The high number of records from these regions may also have been a consequence of biases inherent with citizen science methods such as increased recording intensity in regions of high human population density (Isaac et al. 2014). A large percentage of observations correspond to urban habitats (54%), suggesting a recording bias towards human settlements and anthropogenic habitats. However, human population density does not seem to explain completely the spatiotemporal biases in the observed patterns (Isaac and Pocock 2015) as the number of observations in Attica, which is populated by more than half a million people (World Population Review 2021), was low. In addition, our study shows the increased engagement of people, at high latitudes where population densities are low. Nevertheless, the presence of the species in Northern Greece since 2010 (Fig. 1B) and the climatic suitability of the region, and the country in general (Grež et al. 2016), in combination with the highly invasive behaviour of *H. axyridis*, suggest that the species is widely established in Thessaly, Central and West Macedonia. Similar to previous studies (Dickinson et al. 2012; Roy et al. 2016; Grež et al. 2016; Werenkraut et al. 2020), this study demonstrates that citizen science is a valuable tool for understanding the spatial distribution of invasive non-native species.

The citizen science records also show that *H. axyridis* is active for a long period of time throughout the year (Fig. 3). Previous semi-field trials using outdoor cages showed that *H. axyridis* can have four annual generations in Greece (Katsoyannos et al. 1997). Although citizen science data cannot adequately determine the number of generations per year for *H. axyridis*, it is evident that the species is active for a long period of time, with living adult

individuals being observed even during the winter months (December–February) (Fig. 3A, B). These findings are in accordance with earlier studies stating that *H. axyridis* can overwinter in Greece, but the phenomenon was rare, and populations were rather small (Kontodimas et al. 2008a, b). The vast majority of observations were of adult individuals (Fig. 3C). Noting the difficulties in identifying ladybird larvae, resources including simple identification guides for the immature stages of *H. axyridis* could be developed to promote recording across all life stages. Ladybirds are attractive and charismatic (Gardiner et al. 2021; Gardiner and Roy 2022), this is encouraging as people could be invited to contribute records that could help scientists understand the future range expansion, phenology, and impacts of *H. axyridis* on ecological networks (Groom et al. 2021). Training the citizen scientists on ladybird identification and ecology, could improve the degree of confidence. Existing recording applications could be promoted to citizen scientists in Greece for example the European Ladybird app (Skuhrovec et al. 2021).

Our study shows that more observations on the geographical and seasonal variation of melanic and non-melanic morphs of *H. axyridis* are needed to elucidate its seasonal patterns. In general, the non-melanic morphs exist at low temperatures. Temperature determines the size and number of black spots and affects the size of red spots in melanic morphs (Honek et al. 2020). Across the native range of *H. axyridis* non-melanic morph, *succinea* is found most often in hot, arid regions and melanic morphs being more frequent in cooler, more humid ones (Roy et al. 2016). Our study showed that melanic morphs were more frequent in the northern part of the country (i.e., Eastern Macedonia and Thrace and Thessaly). However, the number of observations of the species in southern Greece was much smaller compared to northern Greece (Fig. 1). In addition, only a few observations of melanic morphs were recorded; thus, the small size of observations might cause less accurate results regarding the melanic and non-melanic morphs and the factors that affect their distribution in Greece.

The involvement of citizens in monitoring *H. axyridis* in Greece has provided valuable data and this is the case in many other countries (Gardiner et al. 2012; Grez et al. 2016; Werenkraut et al. 2020). Most of the records were reported in urban areas and land

principally occupied by agriculture. It seems that high human activity might be facilitating the invasion of the species (Hufbauer et al. 2012; Grez et al. 2016; Werenkraut et al. 2020) (Fig. 4). However, the observations could be biased towards these habitat types. The presence of the species in 17 NATURA 2000 sites is concerning, considering the large number of endemic taxa and rich biodiversity that Greece holds (Sfenthourakis and Legakis 2001; Spiliopoulou et al. 2021). The NATURA 2000 network covers 27.3% of the terrestrial area in the country (Spiliopoulou et al. 2021). There is a large number of non-native insects established in Greece (Anagnou-Veronikiet al. 2008; Avtzis et al. 2017; Demetriou et al. 2021) and monitoring the spread of these, particularly in protected sites, could underpin strategic action. Monitoring *H. axyridis* in Greece is important because the harlequin ladybird could have adverse impacts on native species and ecosystem function. However, several years of monitoring would be required before the long-term impacts can be reliably assessed (Kenis et al. 2010). The present work documents the distribution and phenology of the species in Greece and it is the first step towards improving our general understanding of the ecology of the species. In the future studying the impact of *H. axyridis* on native biodiversity and ecosystem services in Greece should be a priority.

Regarding the predation of aphids by *H. axyridis*, aphid prey can be easily overlooked or misidentified due to their small size. Thus, citizen science efforts could be taxonomically biased. Such biases could be minimised through structured surveys (Kelling et al. 2019). Nevertheless, the discovery of *A. nerii* as an additional prey species for *H. axyridis* from citizen science records highlights the importance of citizen science towards the study of ecological networks of alien species (Groom et al. 2021). Future studies could incorporate citizen-science approaches in the monitoring of alien species and their ecology in addition to the monitoring undertaken by expert entomologists. Assessing the level of infections via parasitism can also be observed and recorded by citizen scientists, providing data that are essential to ecological research.

Management implications

This study highlights the importance of public participation in recording ladybirds showing that citizen

science schemes can provide valuable information on invasive non-native species in Greece. The current distribution and spread of *H. axyridis* in Greece at a multitude of remote localities where no releases had been made in the past and in protected areas, calls for action. National and regional monitoring schemes by experts in combination with efforts by citizen scientists are essential in order to gain a better understanding of the spread and the hotspots of activity of the invasive *H. axyridis*. Monitoring efforts could guide management plans, including control efforts where possible. Studies of the interactions of the harlequin ladybirds with native ladybirds and other beneficial species will enable us to gain a better understanding of the impacts of the harlequin ladybird on the native biodiversity.

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Data availability The datasets analysed during the current study are available from the corresponding authors on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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References

- Adriaens T, San Martin y Gomez G, Maes D (2007) Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. *Biocontrol* 53(1):69–88. <https://doi.org/10.1007/s10526-007-9137-6>
- Anagnou-Veroniki M, Papaioannou-Souliotis P, Karanastasi E, Giannopolitis CN (2008) New records of plant pests and weeds, 1990–2007. *Hell Plant Prot J* 1:55–78
- Avtzis DN, Coyle D, Christopoulos V, Roques A (2017) Biological invasions, national borders, and the current state of non-native insect species in Greece and the neighbouring Balkan countries. *Bull Insectology* 70(2):161–169
- Brown PMJ, Adriaens T, Bathon H, Cuppen J, Goldarazena A, Hägg T, Kenis M, Klausnitzer BEM, Kovář I, Loomans AJM, Majerus MEN, Nedved O, Pedersen J, Rabitsch W, Roy HE, Ternois V, Zakharov IA, Roy DB (2007) *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. *Biocontrol* 53(1):5–21. <https://doi.org/10.1007/s10526-007-9132-y>
- Ceryngier P, Romanowski J (2017) *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) and its parasite in south-western Bulgaria and Northern Greece. *Bioinvasions Rec* 6(4):307–310. <https://doi.org/10.3391/bir.2017.6.4.02>
- Castaldo D, Rossi W, Sabatini F (2004) Contribution to the knowledge of the Laboulbeniales from Greece. *Plant Biosyst* 138:261–269. <https://doi.org/10.1080/11263500400006969>
- Demetriou J, Kalaentzis K, Kazilas C, Koutsoukos E, Avtzis DN, Georgiadis C (2021) Revisiting the non-native insect fauna of Greece: current trends and an updated checklist. *NeoBiota* 65:93–108. <https://doi.org/10.3897/neobiota.65.64686>
- Dickinson JL, Shirk J, Bonter D, Bonney R, Crain LR, Martin J, Phillips T, Purcell K (2012) The current state of citizen science as a tool for ecological research and public engagement. *Front Ecol Environ* 10:291–297. <https://doi.org/10.1890/110236>
- Goetz DW (2007) *Harmonia axyridis* ladybug hypersensitivity in clinical allergy practice. *Allergy Asthma Proc* 28(1):50–57. <https://doi.org/10.2500/aap.2007.28.2956>

- Gardiner MM, Roy HE (2022) The role of community science in entomology. *Annu Rev Entomol* 67:437–456. <https://doi.org/10.1146/annurev-ento-072121-075258>
- Gardiner MM, Allee LL, Brown Peter MJ, Losey JE, Roy HE, Smyth RR (2012) Lessons from lady beetles: accuracy of monitoring data from US and UK citizen-science programs. *Front Ecol Environ* 10(9):471–476. <https://doi.org/10.1890/110185>
- Greze AA, Zaviezo T, Roy HE, Brown PMJ, Bizama G (2016) Rapid spread of *Harmonia axyridis* in Chile and its effects on local coccinellid biodiversity. *Divers Distrib* 22(9):982–994. <https://doi.org/10.1111/ddi.12455>
- Gardiner MM, Perry KI, Riley CB, Turo KJ, Delgado de la flor YA, Sivakoff FS, (2021) Community science data suggests that urbanization and forest habitat loss threaten aphidophagous native lady beetles. *Ecol Evol* 11(6):2761–2774. <https://doi.org/10.1002/ece3.7229>
- Groom Q, Pernat N, Adriaens T, de Groot M, Jelaska SD, Marčiulyrienė D, Martinou AF, Skuhrovec J, Tricarico E, Wit EC, Roy HE (2021) Species interactions: next-level citizen science. *Ecography* 44:1–9. <https://doi.org/10.1111/ecog.05790>
- Hufbauer RA, Facon B, Ravigné V, Turgeon J, Foucaud J, Lee CE, Rey O, Estoup A (2012) Anthropogenically induced adaptation to invade (AIAI): contemporary adaptation to human-altered habitats within the native range can promote invasions. *Evol Appl* 5(1):89–101. <https://doi.org/10.1111/j.1752-4571.2011.00211.x>
- Honek A, Martinkova Z, Dixon AFG, Skuhrovec J, Roy HE, Brabec M, Pekar S (2017) Life cycle of *Harmonia axyridis* in central Europe. *Biocontrol* 63(2):241–252. <https://doi.org/10.1007/s10526-017-9864-2>
- Honek A, Martinkova Z, Roy HE, Dixon AFG, Skuhrovec J, Pekár S, Brabec M (2018) Differences in the phenology of *Harmonia axyridis* (Coleoptera: Coccinellidae) and native Coccinellids in Central Europe. *Environ Entomol* 48(1):80–87. <https://doi.org/10.1093/ee/nvy173>
- Haelewaters D, Hiller T, Kemp EA, van Wielink PS, Shapiro-Ilan DI, Aime MC, Nedvěd O, Pfister DH, Cottrell TE (2020) Mortality of native and invasive ladybirds co-infected by ectoparasitic and entomopathogenic fungi. *PeerJ* 8:e10110. <https://doi.org/10.7717/peerj.10110>
- Honek A, Brown PMJ, Martinkova Z, Skuhrovec J, Brabec M, Burgio G, Evans EW, Fournier M, Greze AA, Kulfan J, Lami F, Lucas E, Lumbierres B, Masetti A, Mogilevich T, Orlova-Bienkowskaja M, Phillips WM, Pons X, Strobach J, Viglasova S, Zach P, Zaviezo T (2020) Factors determining variation in colour morph frequencies in invasive *Harmonia axyridis* populations. *Biol Invasions* 22:2049–2062. <https://doi.org/10.1007/s10530-020-02238-0>
- iNaturalist (2021) A community for naturalists. <https://www.inaturalist.org>. Accessed 08 Sep 2021
- Isaac NJB, Pocock MJO (2015) Bias and information in biological records. *Biol J Linn Soc* 115:522–531. <https://doi.org/10.1111/bij.12532>
- Isaac NJB, van Strien AJ, August TA, de Zeeuw MP, Roy DB (2014) Statistics for citizen science: extracting signals of change from noisy ecological data. *Methods Ecol Evol* 5(10):1052–1060. <https://doi.org/10.1111/2041-210X.12254>
- Johnson BA, Mader AD, Dasgupta R, Kumar P (2020) Citizen science and invasive alien species: an analysis of citizen science initiatives using information and communications technology (ICT) to collect invasive alien species observations. *Glob Ecol Conserv* 21:1–14. <https://doi.org/10.1016/j.gecco.2019.e00812>
- Koch RL (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *J Insect Sci* 3:32. <https://doi.org/10.1673/031.003.3201>
- Kulijer D (2017) The distribution, habitat and colour forms frequency of the invasive *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) in Bosnia and Herzegovina. *GZM (PN) NS* 37:7–14
- Koch RL, Galvan TL (2007) Bad side of a good beetle: the North American experience with *Harmonia axyridis*. In: Roy HE, Wajnberg E (eds) From biological control to invasion: the ladybird *Harmonia axyridis* as a model species. Springer, Dordrecht, pp 23–25. https://doi.org/10.1007/978-1-4020-6939-0_3
- Katsoyannos P, Kontodimas DC, Stathas GJ, Tsartsalis CT (1997) Establishment of *Harmonia axyridis* on Citrus and some data on its phenology in Greece. *Phytoparasitica* 25(3):183–191. <https://doi.org/10.1007/BF02981731>
- Kontodimas DC, Stathas GJ, Martinou AF (2008a) The aphidophagous predator *Harmonia axyridis* (Coleoptera Coccinellidae) in Greece, 1994–1999. *Eur J Entomol* 105:541–544. <https://doi.org/10.14411/eje.2008a.072>
- Kontodimas DC, Stathas GJ, Martinou AF (2008b) The status of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in Greece: a case of an exotic predator that failed to establish? *Entomol Hell* 17:42–51. <https://doi.org/10.12681/eh.11615>
- Kenis M, Adriaens T, Brown P, Katsanis A, Van Vlaenderen J, Eschen R, Golaz L, Zindel R, y Gomez GSM, Babendreier D, Ware R (2010) Impact of *Harmonia axyridis* on European ladybirds: which species are most at risk? *IOBC/WPRS Bull* 58:57–59
- Kalaentzis K, Kazilas C, Demetriou J, Koutsoukos E, Georgiadis C, Avtzis DN (2021) Engaging citizen-scientists in mapping alien species: introducing Alientoma, a dynamic database for alien insects in Greece. In: The 1st international electronic conference on entomology (IECE). <https://doi.org/10.3390/IECE-10512> (poster presentation)
- Kelling S, Johnston A, Bonn A, Fink D, Ruiz-Gutierrez V, Bonney R, Fernández M, Hochachka W, Julliard R, Krämer R, Robert G (2019) Using semistructured surveys to improve citizen science data for monitoring biodiversity. *Bioscience* 69:170–179. <https://doi.org/10.1093/biosci/biz010>
- Lepczyk CA, Boyle OD, Vargo TL, Noss RF (2020) Handbook of citizen science in conservation and ecology. University of California Press, California
- Martinou AF, Kontodimas DC (2008) The threatening harlequin ladybird. *Georgia Kai Ktinotrofia* 7:34–37 (in Greek)
- Pataki BA, Garriga J, Eritja R, Palmer JRB, Bartumeus F, Csabai I (2021) Deep learning identification for citizen science surveillance of tiger mosquitoes. *Sci Rep* 11:4718. <https://doi.org/10.1038/s41598-021-83657-4>

- R Studio Team (2021) RStudio: integrated development for R. <http://www.rstudio.com>
- Roques A, Preda C, Augustin S, Auger-Rozenberg MA (2018) Bugs, ants, wasps, moths and other insect species. In: Mazza G, Tricarico E (eds) Invasive species and human health. CAB International, Oxfordshire, pp 63–75. <https://doi.org/10.1079/9781786390981.0063>
- Roy HE, Adriaens T, Isaac NJB, Kenis M, Onkelinx T, Martin GS, Brown PMJ, Hautier L, Poland R, Roy DB, Comont R, Eschen R, Fros R, Zindel R, Van Vlaenderen J, Nedv ed O, Ravn HP, Gr egoire JC, de Biseau JC, Maes D (2012) Invasive alien predator causes rapid declines of native European ladybirds. *Divers Distrib* 18(7):717–725. <https://doi.org/10.1111/j.1472-4642.2012.00883.x>
- Roy HE, Brown PMJ, Adriaens T, Berkvens N, Borges I, Clusella-Trullas S, Comont RF, De Clercq P, Eschen R, Estoup A, Evans EW, Facon B, Gardiner MM, Gil A, Grez AA, Guillemaud T, Haelewaters D, Herz A, Honek A, Howe AG, Hui C, Hutchison WD, Kenis M, Koch RL, Kulfan J, Lawson Handley L, Lombaert E, Loomans A, Losey J, Lukashuk AO, Maes D, Magro A, Murray KM, San Martin G, Martinkova Z, Minnaar IA, Nedved O, Orlova-Bienkowskaja MJ, Osawa N, Rabitsch W, Peter Ravn H, Rondoni G, Rorke SL, Ryndevich SK, Saethre MG, Sloggett JJ, Onofre Soares A, Stals R, Tinsley MC, Vandereycken A, van Wielink P, Vign al sova S, Zach P, Zakharov IA, Zaviero T, Zhao Z (2016) The harlequin ladybird, *Harmonia axyridis*: global perspectives on invasion history and ecology. *Biol Invasions* 18(4):997–1044. <https://doi.org/10.1007/s10530-016-1077-6>
- Roy H, Groom Q, Adriaens T, Agnello G, Antic M, Archambeau A, Bacher S, Bonn A, Brown P, Brundu G, L opez B, Cleary M, Cogalniceanu D, de Groot M, De Sousa T, Deidun A, Essl F, Fi serPe cnikar  Z, Gazda A, Gervasini E, Glavendekic M, Gigot G, Jelaska S, Jeschke J, Kaminski D, Karachle P, Komives T, Lapin K, Lucy F, Marchante E, Marisavljevic D, Marja R, Martın Torrijos L, Martinou A, Matosevic D, Mifsud C, Motiejunait e J, Ojaveer H, Pasalic N, Pekarik L, Per E, Pergl J, Pesic V, Poccock M, Reino L, Ries C, Rozylowicz L, Schade S, Sigurdsson S, Steinitz O, Stern N, Teofilovski A, Thorsson J, Tomov R, Tricarico E, Trichkova T, Tsiamis K, van Valkenburg J, Vella N, Verbrugge L, V etek G, Villaverde C, Witzell J, Zenetos A, Cardoso A (2018) Increasing understanding of alien species through citizen science (Alien-CSI). *RIO* 4:e31412. <https://doi.org/10.3897/rio.4.e31412>
- Sfenthourakis S, Legakis A (2001) Hotspots of endemic terrestrial invertebrates in southern Greece. *Biodivers Conserv* 10(8):1387–1417. <https://doi.org/10.1023/A:1016672415953>
- Sloggett JJ, Magro A, Verheggen FJ, Hemptinne J-L, Hutchison WD, Riddick EW (2011) The chemical ecology of *Harmonia axyridis*. *Biocontrol* 56(4):643–661. <https://doi.org/10.1007/s10526-011-9376-4>
- Spiliopoulou K, Dimitrakopoulos PG, Brooks TM, Kelaidi G, Paragamian K, Kati V, Oikonomou A, Vavylis D, Trigas P, Lymberakis P, Darwall W, Stoumboudi MTh, Triantis KA (2021) The Natura 2000 network and the ranges of threatened species in Greece. *Biodivers Conserv* 30(4):945–961. <https://doi.org/10.1007/s10531-021-02125-7>
- Skuhrovec J, Roy HE, Brown PMJ, Kazlauskis K, Inghilesi AF, Soares AO, Adriaens T, Roy DB, Nedv ed O, Zach P, Vign al sova S, Kulfan J, Honek A, Martinkova Z (2021) Development of the european ladybirds smartphone application: a tool for citizen science. *Front Ecol Evol* 12:741854. <https://doi.org/10.3389/fevo.2021.741854>
- Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, <https://ggplot2.tidyverse.org>
- Werenkraut V, Baudino F, Roy HE (2020) Citizen science reveals the distribution of the invasive harlequin ladybird (*Harmonia axyridis* Pallas) in Argentina. *Biol Invasions* 22:2915–2921. <https://doi.org/10.1007/s10530-020-02312-7>
- World Population Review (2021) <https://worldpopulationreview.com/countries/greece-population>. Accessed 24 Sep 2021

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