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Investigating beetle communities in and around entry points can improve surveillance at national and international scale

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Abstract
Beetles are commonly moved among continents with international trade. Baited traps set up in and around entry points are commonly used to increase chances of early-detection of incoming species and complement visual inspections. A still underestimated benefit of this surveillance approach is the high number and diversity of collected bycatch species. In this study, we exploited a multiyear surveillance program carried out with baited traps at five Spanish ports and their surrounding natural areas to investigate i) the importance of identifying bycatch to more promptly detect nonnative species belonging to non-target groups; ii) patterns of native and nonnative species richness and abundance inside the port areas vs. surrounding natural areas; iii) the occurrence of spillover events between natural areas surrounding ports and the port areas, and iv) whether the native species most commonly introduced into other countries are more abundant in port areas than in surrounding natural areas. A total of 23,538 individuals from 206

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species representing 33 families were collected. The number and taxonomic diversity of the 26 bycatch nonnative beetle species testified that the identification of these unintentionally trapped species can provide additional information on ongoing invasions. Patterns of spillover and native species richness and abundance in port areas vs. surrounding natural areas highlighted a differential ability of different beetle families to colonize port areas. Finally, native species most commonly introduced into other countries were more abundant in port areas than in their surroundings, while the opposite trend occurred for native species that have not been introduced elsewhere. Our study highlighted that the use of traps baited with generic attractants can aid in early-detection of nonnative beetle species, and that the identification of native species can provide useful information on the risk of introduction in other countries.

Keywords
baited traps, Coleoptera, early-detection, insect invasions, nonnative species

Introduction

The constant increase in the volume of goods moved among continents is the primary cause of the impressive number of nonnative insect introductions recorded around the world (Brockerhoff and Liebhold 2017). Beetles (Coleoptera) are the most species-rich group among interceptions at ports of entry worldwide (Nahrung and Carnegie 2021; Turner et al. 2021) as they can be transported via a number of pathways, including wood-packaging materials, logs, processed wood, plants for planting but also as hitchhikers in containers (Wu et al. 2017; Meurisse et al. 2019; Pace et al. 2022; Fenn-Moltu et al. 2023). In addition, beetles include some of the most economically detrimental nonnative insects (Nahrung and Carnegie 2020; Fantle-Lepczyk et al. 2022; Renault et al. 2022). Thus, substantial investments have been undertaken over the last decades to improve pre-border, border and post-border measures aimed at mitigating their arrival and establishment rate (Carnegie et al. 2022; Cuthbert et al. 2022; Haack et al. 2022; Nahrung et al. 2023). This effort has led to the development of a number of innovative tools and strategies that are increasingly exploited to integrate visual inspections at sites where the introduction of nonnative species is more likely (Poland and Rassati 2019; Larson et al. 2020).

The use of traps baited with lures set up in and around entry points is one of the most commonly adopted approaches to early-detect nonnative beetles, especially wood-borers such as bark and ambrosia beetles (Curculionidae: Scolytinae) and longhorn beetles (Cerambycidae) (Poland and Rassati 2019). Examples of surveillance programs based on baited traps are known for a number of developed countries such as Australia (Carnegie et al. 2018, 2022), Austria (Hoch et al. 2020), Canada (Allison et al. 2021), Finland (Melin et al. 2022), France (Fan et al. 2019), Great Britain (Inward et al. 2020), Italy (Rassati et al. 2015a, 2015b), and the USA (Rabaglia et al. 2019), but the same approach can be expected to become increasingly common also in developing economies (Gupta and Sankaran 2021). A still underestimated benefit of trapping protocols developed for the above-mentioned beetle groups is the high number and diversity of bycatch species (Skvarla and Holland 2011; Barringer 2015; DiGirolomo et al. 2021; Thurston et al. 2022; Webster et al. 2022). These unintentionally trapped insects can provide extremely valuable information (Spears and Ramirez 2015) but they are often discarded in surveillance programs because the federal databases only seek presence/absence of target nonnative species.

Bycatch species can be classified into two main categories. The first category includes nonnative or native species belonging to non-target beetle families that are not known to be present in the monitored area. Using trapping protocols developed for longhorn beetles and bark beetles in forested areas of Maine, USA, DiGirolomo et al. (2021) recorded 54 new state records from bycatch species, among which two were new country records. Similarly, using trapping protocols developed for longhorn beetles in a forested area of Canada, Webster et al. (2022) found 300 species new to Prince Edward Island, among which one was a new country record. The second group of bycatch species is represented by native species belonging to the same or different families than the target ones that are already known to occur in the monitored area. These species often represent the majority of trap collections (e.g., Rassati et al. 2015a, 2015b; Fan et al. 2019; Rabaglia et al. 2019; Marchioro et al. 2020, but see Brockerhoff et al. 2006 and Stone et al. 2010) and their records can provide important insights into patterns of beetle abundance, distribution, and diversity in the monitored natural or anthropized areas (Buchholz et al. 2011; Spears and Ramirez 2015; Rassati et al. 2018; Pawson et al. 2020).

In this study, we exploited a multi-year surveillance program carried out at five Spanish ports and their surrounding natural areas aimed at early-detection of nonnative bark and ambrosia beetles and longhorn beetles to investigate a number of mechanisms that can improve surveillance strategies at national and international scale. First of all, we assessed the importance of identifying bycatch species that can be trapped in the context of such surveillance activities to improve the chances of detecting nonnative species belonging to non-target groups. Second, we compared patterns of native and nonnative species richness and abundance inside the port areas vs. surrounding natural areas to understand whether the latter changes depending on the beetle family. Third, we used native species records of both target and non-target families to investigate the occurrence of spillover (i.e., the movement of organisms from one distinct habitat type to another) events between natural areas surrounding ports and the port areas. Fourth, we tested the hypothesis that the native species most commonly introduced into other countries are more abundant in port areas than in surrounding natural areas, while the opposite trend occurs for native species that have not been introduced into other countries.

Methods

Study sites

The trapping survey was carried out from 2017 to 2021 in five coastal towns located along the Spanish coast, namely Alicante, Castellon de la Plana, Gandia, Sagunto, and Valencia (Suppl. material 1 and Suppl. material 3: fig. S1A, B). These cities were selected as they host the only state ports of the Valencia region that import/export commodities from/to foreign countries. In each city, traps were set up both within the port area and in a natural area surrounding the port area (mean distance: 2.8 ± 1.3 km). With the term “natural areas” we refer to remnant vegetation areas within or surrounded by urban areas. In Alicante, Castellon de la Plana and Gandia, a pair of traps was used (1 inside and 1 outside the port) (Suppl. material 3: fig. S1C); in Sagunto and Valencia, instead, the trap located outside the port was coupled with 2 and 3 traps inside the port area, respectively, as these ports are larger and trade more commodities than the others. Traps were active at all sites in 2019, 2020, and 2021; traps were set up also in Alicante in 2017, and Alicante and Castellon de la Plana in 2017 and 2018. In Alicante and Valencia, the ports were mostly surrounded by urban areas, while the landscape around the port of Castellon de la Plana, Gandia and Sagunto was more heterogeneous with a mosaic of urban areas, crop fields and forested areas. In all cases, conifers were dominant over broadleaf trees. The latter were mostly restricted to some parks and private gardens.

Trapping design, lures and species identification

The trapping network was meant to target nonnative bark and ambrosia beetles and longhorn beetles. For this reason, black crossvane traps (Crosstrap, Econex, Spain) were used. This trap type is composed by four 19 × 100 cm flexible and sliding coated panels above a funnel measuring 48 cm square with an opening of about 40 cm deep attached to a screw cap collecting jar (9.5 cm diameter × 21 cm deep) (Suppl. material 3: fig. S1D). This trap was found to efficiently collect both longhorn beetles and bark and ambrosia beetles (Alvarez et al. 2015; Faccoli et al. 2020). Traps were baited with (-)-alpha-pinene (release rate of 300 mg/day at 25 °C), ethanol (2000 mg/day at 25 °C), and a blend of ipsenol (95.24%), ipsdienol (4.75%), and (s)-(+)-cis-verbenol (0.02%) (release rate of 3.71 mg/day at 25 °C). These volatiles were selected as they are known to attract a wide range of conifer-associated (alpha-pinene, ipsenol, ipsdienol, and verbenol) and broadleaf-associated (ethanol) bark and ambrosia beetles and longhorn beetles (Miller et al. 2005, 2011; Miller 2006; Miller and Rabaglia 2009; Ranger et al. 2021) and because they were previously used together or separately in surveillance programs (Brockerhoff et al. 2006; Rassati et al. 2014, 2015a, 2015b; Rabaglia et al. 2019). All lures were purchased from Econex, Spain. The tops of the traps were hung about 2 m off the ground, using suitable supports such as building structures, wire fences, and metal girders in port areas and tree branches in surrounding natural areas. All traps were in relatively open areas where insects could approach from several directions. Trap collecting cups were half-filled with 50% solution of ethylene glycol to kill and preserve captured beetles, and the solution was replaced at each trap check. Traps were emptied once per month from March to September of each year (total of 6 trap checks). Lures were replaced monthly.

Bark and ambrosia beetles, longhorn beetles and all the other bycatch beetle species were identified to species or at least genus level. All beetles that were identified at species level were classified as native or nonnative using available literature (Löbl and Smetana 2007, 2008, 2010; Beenen and Roques 2010; Denux and Zagatti 2010; Roy and Migeon 2010; Löbl and Löbl 2015, 2016, 2018; Alonso-Zarazaga et al. 2017).

We used the term nonnative to define species not known to be native to Spain or not of Western Palearctic origin. Subsequently, Spanish native species were further divided into two groups, i.e., species that were not and species that were introduced outside Spain (again considering both other continents and other areas outside their native distributional range). Information regarding introduction outside Spain was recovered primarily from the scientific literature cited above (e.g., Catalogue of Palaearctic Coleoptera book series) and on-line resources.

Data analysis

Generalized linear mixed models with a Gaussian distribution were used for all analyses. The occurrence of differences in species richness and abundance of target and non-target beetle families in port areas vs. surrounding natural areas was investigated separately for native and nonnative species within each family but only when they were represented by at least 50 individuals and 3 species. The model included the mean number of species (i.e., species richness) or the mean number of individuals (i.e., abundance) caught per year and site as continuous response variable, the habitat type (port area vs. surrounding natural area) as categorical explanatory variable, and the year and site as crossed random factors. For ports where more than one trap was present both species richness and abundance were averaged by the number of traps. Abundance was ln-transformed to improve linearity.

The occurrence of spillover events of native species between natural areas surrounding ports and port areas was investigated only for families represented by at least 50 individuals and 3 species, running separate analyses for each family. The model included the abundance of native species collected in the port area as a continuous response variable and the abundance of native species collected in the surrounding natural area as continuous explanatory variable. Abundance of each native species was obtained by pooling the number of individuals caught in the port area or surrounding natural area during a given year at a given site. The insect species, year and site were included in the models as crossed random factors. For ports where more than one trap was present pooled abundance values were averaged by the number of traps. Abundance was ln-transformed to improve linearity.

The relationship between occurrence at port areas vs. surrounding natural areas and likelihood of introduction into other countries was tested using native species abundance as a continuous response variable, and their status (introduced vs. not-introduced in other countries), habitat type (port area vs. surrounding natural area), and the interaction between the latter two variables as categorical explanatory variables. For each native species and habitat type, the abundance was obtained averaging the number of individuals by year and site. For ports where more than one trap was present abundance values were also averaged by the number of traps. The insect species was included in the model as random factor. Pairwise comparisons between port areas and surrounding areas for introduced vs. not-introduced species were run using Tukey correction of p-values.

All the analyses were performed in R software version 4.1.1 (R Core Team 2021). Models were fitted using the 'glmmTMB' package (Brooks et al. 2022) and validated using the 'DHARMa' package (Hartig 2022). In the Results section the omnibus chi-square test is reported.

Results

General results

A total of 23,538 individuals from 206 species representing 33 families were collected (Suppl. material 2). The family Curculionidae was the most species rich (42 species), followed by Coccinellidae (17 species) and Nitidulidae (16 species). Sixteen families were represented only by one or two species each. Curculionidae was also the most abundant family (18,154 individuals), followed by Tenebrionidae (3,475 individuals). Among the other beetle families, five were represented by 100 to 500 individuals (i.e., Bostrichidae, Laemophloeidae, Zopheridae, Cerambycidae and Dermestidae, in decreasing order) and eighteen families were represented by 10 or less individuals (Suppl. material 2).

Among the trapped species, eight were nonnative beetles representing the main target of the surveillance program, seven Scolytinae beetles (i.e., *Coccotrypes dactyliperda* (Fabricius), *Dactylotripes longicollis* (Wollaston), *Gnathotrichus materiarius* (Fitch), *Hypothenemus eruditus* Westwood, *Ips calligraphus* (Germar), *Xyleborus bispinatus* Eichhoff, *Xylosandrus germanus* (Blandford)) and one longhorn beetle (i.e., *Xylotrechus stebbingi* Gahan) (Suppl. material 2). Most of these eight nonnative target species are already widely established in Europe, whereas the bark beetle *I. calligraphus* and the ambrosia beetle *X. bispinatus* represent first records for Europe and Spain, respectively. Among bycatches, 38 were native bark and ambrosia beetles or native longhorn beetles (24 and 14 species, respectively), whereas the remaining bycatch 167 species were native (141) or nonnative species (26) belonging to other beetle families. The most abundant species were the native bark beetles *Hylurgus micklitzii* (Wachtl) and *Orthotomicus erosus* (Wollaston) (10,096 and 6,219 individuals, respectively), followed by the nonnative darkling beetle *Tribolium castaneum* (Herbst) (1,821 individuals). By contrast, 159 species were represented by 10 or less individuals.

Patterns of species richness and abundance in port areas vs. surrounding natural areas

Significant differences in native species richness and abundance between port areas and surrounding natural areas were found for three out of the five analyzed beetle families (Fig. 1). In particular, both species richness and abundance were significantly higher in natural areas surrounding ports than in the port areas for Curculionidae (species richness: $\chi^2_1 = 7.18$, $p = 0.007$, Fig. 1E; abundance: $\chi^2_1 = 80.16$, $p < 0.001$, Fig. 1F), Tenebrionidae (species richness: $\chi^2_1 = 5.17$, $p = 0.023$, Fig. 1I; abundance: $\chi^2_1 = 17.25$, $p < 0.001$, Fig. 1J),

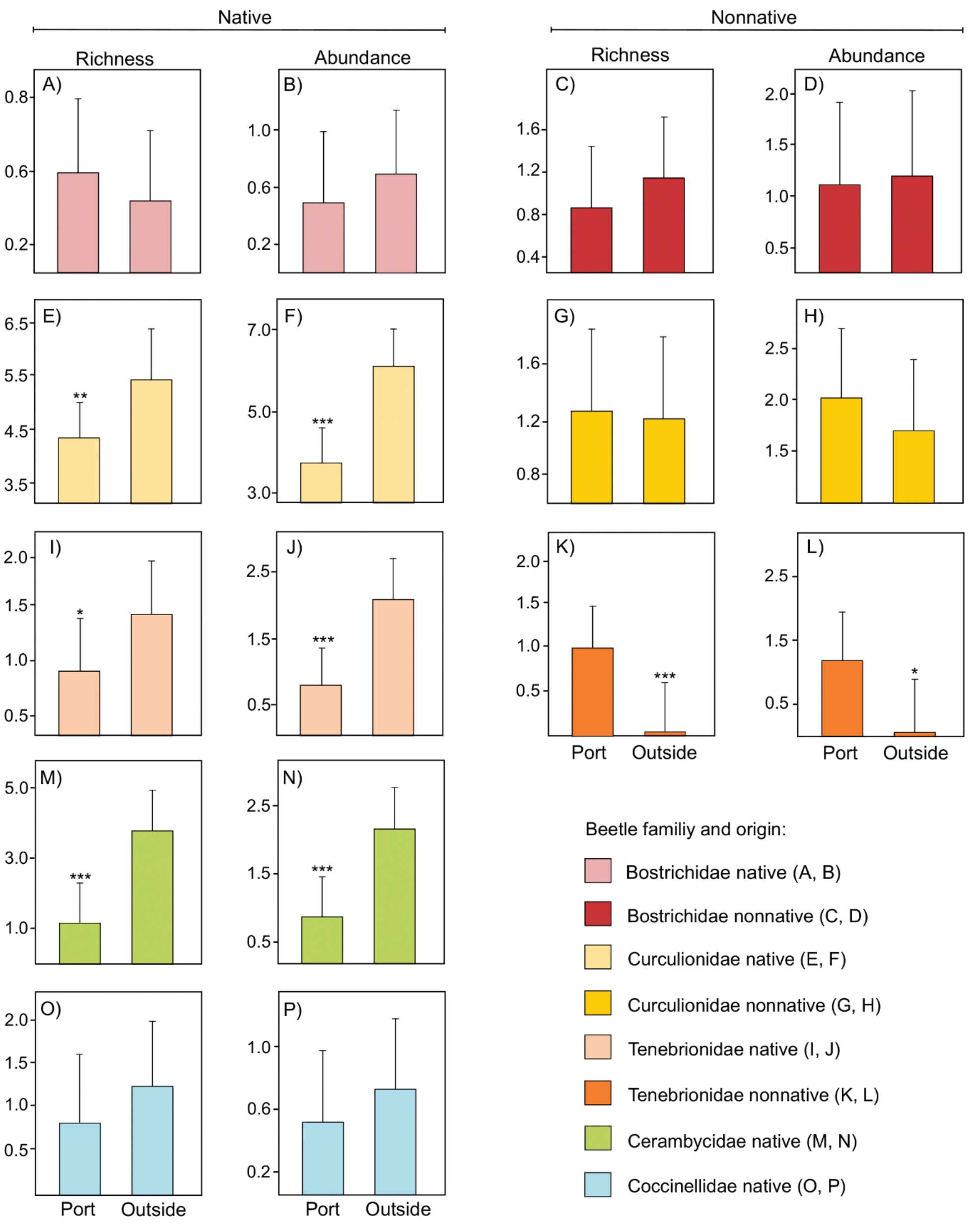

Figure 1. Mean number of species (i.e., species richness) and individuals (i.e., abundance) of native and nonnative beetle species collected in port areas ("Port") vs. surrounding natural areas ("Outside"). Trends are shown separately for the different beetle families. Abundance is log-transformed according to data transformation used in statistical analysis. P-values: * = 0.01 - 0.05; ** = 0.01 - 0.001; *** = < 0.001.

and Cerambycidae (species richness: $\chi^2_1 = 37.72$, $p < 0.001$, Fig. 1M; abundance: $\chi^2_1 = 33.58$, $p < 0.001$, Fig. 1N), but did not differ between the two habitats for Bostrichidae (species richness: $\chi^2_1 = 0.78$, $p = 0.377$, Fig. 1A; abundance: $\chi^2_1 = 1.05$, $p = 0.306$, Fig. 1B) and Coccinellidae (species richness: $\chi^2_1 = 1.05$, $p = 0.307$, Fig. 1O; abundance: $\chi^2_1 = 0.91$, $p = 0.340$, Fig. 1P). Considering beetle species composition, four families were composed by species found only in the port areas, seven by species found only in surrounding natural areas, and four by species shared between the two habitats (Table 1).

For nonnative species, analyses were carried out only for three families, among which significant differences between the two habitats were observed for Tenebrionidae (species richness: $\chi^2_1 = 15.98$, $p < 0.001$, Fig. 1K; abundance: $\chi^2_1 = 9.35$, $p = 0.002$, Fig. 1L) but not for Bostrichidae (species richness: $\chi^2_1 = 1.34$, $p = 0.248$,

Table 1. Number of native and nonnative species for each beetle family collected exclusively in the port areas, exclusively in the surrounding natural areas, or shared between the two habitats.

	Native						Nonnative					
	Exclusive to port areas		Exclusive to surrounding areas		Shared		Exclusive to port areas		Exclusive to surrounding areas		Shared	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Anamorphidae	–	–	1	100	–	–	–	–	–	–	–	–
Anthicidae	5	100	–	–	–	–	–	–	–	–	–	–
Anthribidae	–	–	1	100	–	–	1	100	–	–	–	–
Bostrichidae	2	40	1	20	2	40	–	–	–	–	3	100
Buprestidae	–	–	3	100	–	–	–	–	–	–	–	–
Cantharidae	–	–	1	100	–	–	–	–	–	–	–	–
Carabidae	6	60	4	40	–	–	–	–	–	–	–	–
Cerambycidae	1	7.1	8	57.2	5	35.7	–	–	–	–	1	100
Chrysomelidae	5	62.5	1	12.5	2	25	1	100	–	–	–	–
Cryptophagidae	–	–	1	33.3	2	66.7	–	–	–	–	–	–
Cleridae	–	–	2	66.7	1	33.3	1	100	–	–	–	–
Coccinellidae	5	33.3	6	40	4	26.7	1	50	–	–	1	50
Curculionidae	12	37.5	9	28.1	11	34.4	4	40	4	40	2	20
Dasytidae	–	–	1	100	–	–	–	–	–	–	–	–
Dermestidae	7	70	1	10	2	20	1	100	–	–	–	–
Elateridae	3	42.9	3	42.9	1	14.2	–	–	–	–	–	–
Hydrophilidae	1	100	–	–	–	–	–	–	–	–	–	–
Hybosoridae	1	100	–	–	–	–	–	–	–	–	–	–
Histeridae	–	–	2	50	2	50	–	–	–	–	–	–
Laemophloeidae	1	50	–	–	1	50	–	–	–	–	–	–
Lampyridae	–	–	–	–	1	100	–	–	–	–	–	–
Latridiidae	–	–	1	50	1	50	–	–	–	–	–	–
Malachiidae	–	–	2	100	–	–	–	–	–	–	–	–
Monotomidae	–	–	–	–	1	100	–	–	–	–	–	–
Mycetophagidae	1	50	–	–	1	50	–	–	–	–	1	100
Nitidulidae	2	28.7	4	57	1	14.3	7	78	–	–	2	22
Oedemeridae	–	–	1	100	–	–	–	–	–	–	–	–
Ptinidae	1	20	3	60	1	20	–	–	–	–	1	100
Scarabaeidae	2	33.3	2	33.3	2	33.3	–	–	–	–	–	–
Silvanidae	1	100	–	–	–	–	1	100	–	–	–	–
Tenebrionidae	1	12.5	2	25	5	62.5	4	80	–	–	1	20
Trogossitidae	–	–	–	–	1	100	–	–	–	–	–	–
Zopheridae	–	–	–	–	1	100	–	–	–	–	–	–

Fig. 1C; abundance: χ^2_1 = 0.12, p = 0.724, Fig. 1D) and Curculionidae (species richness: χ^2_1 = 0.03, p = 0.853, Fig. 1G; abundance: χ^2_1 = 0.86, p = 0.358, Fig. 1H). For Tenebrionidae, contrary to what was found for native species, species richness and abundance were significantly higher in port areas than in the surrounding natural areas (Fig. 1K, L). Considering beetle species composition, five families were composed by nonnative species found only in the port areas, and four by nonnative species shared between the two habitats (Table 1). No beetle family was characterized by nonnative species recorded exclusively in the surrounding natural areas.

Spillover of native beetle species from natural areas surrounding ports to the port areas

The number of native beetle individuals collected inside port areas was significantly affected by the number of individuals of the same native species collected in the surrounding natural areas for Bostrichidae (χ^2_1 = 4.30, p = 0.038, Fig. 2A), Curculionidae (χ^2_1 = 17.70, p < 0.001, Fig. 2B), Cerambycidae (χ^2_1 = 5.15, p = 0.023, Fig. 2D) and Coccinellidae (χ^2_1 = 17.77, p < 0.001, Fig. 2E) but not for Tenebrionidae (χ^2_1 = 0.01, p = 0.941, Fig. 2C) (Fig. 2). Nonetheless, the trend was different depending on the beetle family. For Curculionidae, the abundance of native species in the port areas increased with increasing abundance of the same species in the surrounding natural areas (Fig. 2B), whereas for Bostrichidae, Cerambycidae and Coccinellidae abundance in port areas decreased with increasing abundance in the surrounding natural areas (Fig. 2A, D, E).

Likelihood of native species being introduced into other countries

The number of collected native beetle individuals was significantly affected by the interaction between habitat and status (χ^2_3 = 29.86, p < 0.001). In particular, the abundance of native species that have never been introduced into other countries was significantly higher in natural areas surrounding ports than in the port areas (p < 0.001, Fig. 3A), whereas the opposite trend was found for species that have been introduced at least once into another country (p = 0.026, Fig. 3B).

Discussion

New nonnative beetle species are moved outside their native range on a yearly basis (Brockeroff and Liebhold 2017) and their introduction and establishment rates are expected to increase in the next years due to climate change (Pureswaran et al. 2022). Traps set up in and around entry points are commonly used to increase chances of early-detection of incoming species and complement visual inspections (Poland and Rassati 2019). Exploiting trapping data from a multiyear surveillance programs carried out in Spain, we showed that the most commonly adopted trapping protocols for bark and ambrosia beetles and longhorn beetles allowed the collection of a substantial

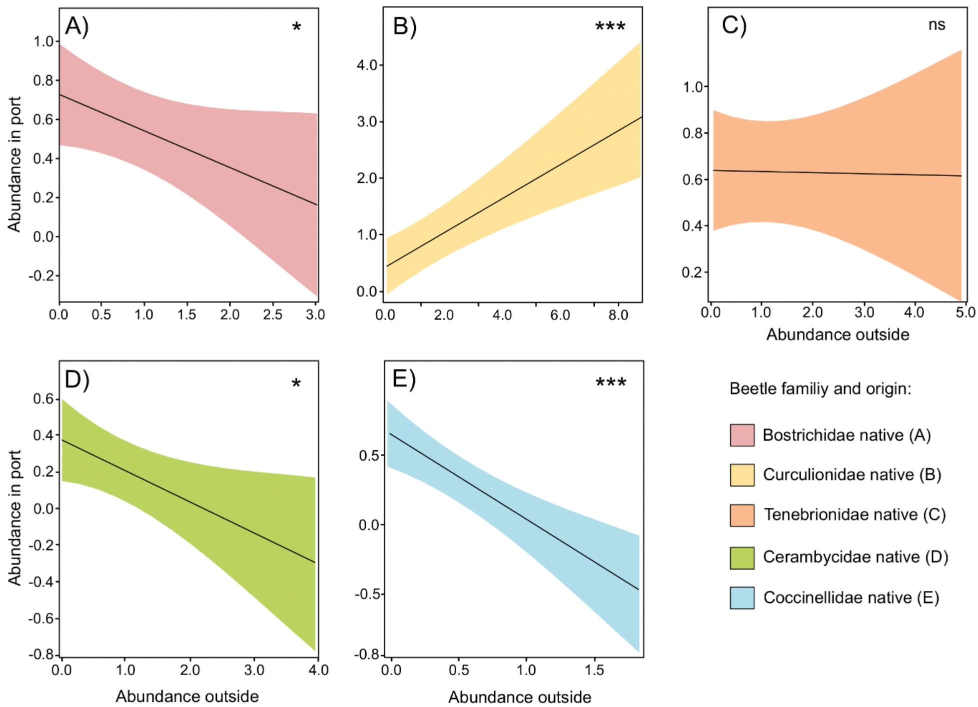

Figure 2. Relation between the number of individuals of native beetle species collected in port areas and the number of individuals of the same native beetle species collected in the surrounding natural areas shown separately for the different beetle families. P-values: * = 0.01 - 0.05; ** = 0.01 - 0.001; *** = < 0.001; ns = not significant (> 0.05).

number of non-target species, both native and nonnative. In addition, we highlighted that the study of beetle communities living in and around entry points could provide important insights into the likelihood of certain beetle species or families to colonize port areas and subsequently be potentially introduced into other countries with trade.

The first findings of *I. calligraphus* and *X. bispinatus* in Europe (Mas and Johnson 2023) and Spain (Gallego et al. 2022), respectively, confirmed that baited traps used at entry points are a valuable complementary tool to visual inspections routinely carried out by phytosanitary inspectors (Rassati et al. 2015a; Fan et al. 2019; Rabaglia et al. 2019). The number and taxonomic diversity of the 26 bycatch nonnative beetles also testified that the identification of these unintentionally trapped species can provide important additional information on the ongoing invasions. This is valid not only for other wood-boring or forest-related beetles (e.g., Bostrichidae, Nitidulidae, Tenebrionidae) and their associates (e.g., Cleridae, Zopheridae), whose attraction to tree volatiles (Miller 2006, 2020; Jurc et al. 2012; Miller et al. 2015; Miller 2023) and/or bark beetle pheromones is well known (Miller and Asaro 2005; Allison et al. 2013; Miller et al. 2015), but also for other beetles, such as Coccinellidae, Dermestidae or Elateridae, which can be caught more or less accidentally (Olivier-Espejel et al. 2016). Invasions by the latter beetles are often overlooked (Ruzzier et al. 2020, 2021a, Nahrung and

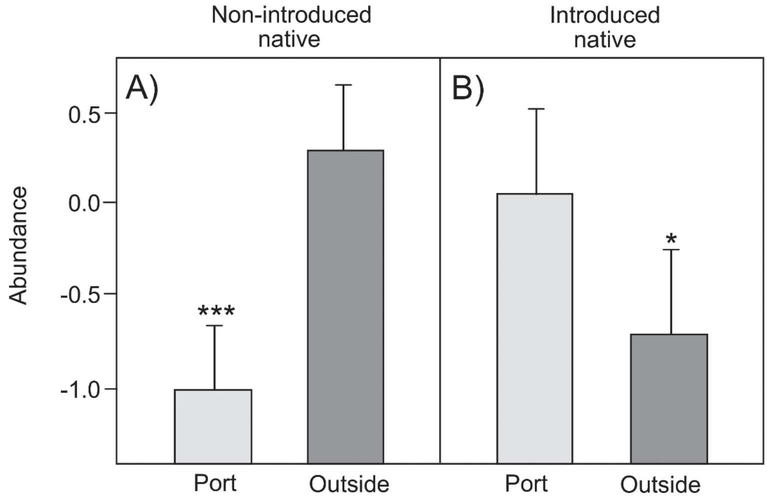

Figure 3. Abundance of beetle species collected at port areas ("Port") and their surrounding natural areas ("Outside") for native species that have not been introduced into other countries (**A**) and native species that have been introduced at least into one country outside the native range (**B**). P-values: * = 0.01 - 0.05; ** = 0.01 - 0.001; *** = < 0.001.

Carnegie 2022), just like their potential economic or ecologic impacts in the invaded areas, and baited traps, even if not specifically designed for this aim, can help to increase chances of their early detection (Ruzzier et al. 2021b).

We also found that patterns of native species richness and abundance inside port areas vs. surrounding natural areas changed depending on the beetle family and between native vs. nonnative species. The number of species and individuals of native Curculionidae, Cerambycidae and Tenebrionidae, for example, was found to be higher in natural areas surrounding ports than in the port areas as already reported in previous studies (Rassati et al. 2014, 2015a; Hoch et al. 2020); this trend is likely linked to a higher availability of host trees or preys in natural areas than in the highly anthropized ports. Instead, the opposite or different trends observed for native Coccinellidae, native and nonnative Bostrichidae, and nonnative Curculionidae and Tenebrionidae, along with records of both native and nonnative beetles collected exclusively in port areas, highlighted that the latter habitat hosts a number of beetle species that successfully exploit woody materials or traded goods as feeding and reproductive substrates and that tend to not spread in the nearby natural areas (Cogburn 1973; Fenn-Moltu et al. 2023). These native and nonnative species can have emerged in the port areas from woody materials or goods either imported from other ports via maritime trade (Rassati et al. 2018; Meurisse et al. 2019) or originated from factories, sawmills or production sites (Meurisse et al. 2021); nonetheless, the low number of traps that we deployed does not allow us to exclude that we under sampled the beetle community in natural areas surrounding ports and that some species that we found exclusively in the port areas were instead also present in natural areas surrounding them. Irrespective of the origin, their records are important not only because they can be used to identify when and where export phytosanitary risks are greatest

(Pawson et al. 2020), but also because they could represent adventive populations originating from geographically distant areas of the native range which can potentially behave differently or have a higher fitness than the resident population (Nelufule et al. 2022).

For the potential spillover of native species between the two habitats, we found that abundance inside port areas was positively affected by the abundance in the surrounding natural areas only for Curculionidae; on the contrary, a negative relation between the two variables was found for most of the other families tested. The constant movement of native species from areas surrounding ports to the port areas was already observed in Curculionidae, especially for bark and ambrosia beetles, for which abundance of native species in ports was found to increase with increasing amount of forest cover in the surrounding areas (Rassati et al. 2018). The opposite pattern observed for Bostrichidae, Cerambycidae and Coccinellidae, i.e., decreasing abundance inside the port with increasing abundance in the surrounding areas, was somewhat unexpected. A possible explanation is that most of these taxa present species with a modest dispersal capability at the adult stage, and that in general tend to remain in closer proximity to their food source or reproductive substrate. In addition, ports, being artificial ecosystems, do not promote colonization by those species that are sensitive to disturbance and that require specific ecological conditions (i.e., specialists). Finally, we cannot exclude that the low number of traps deployed might have led us to underestimate the species abundance in one of the two habitats or both, and might have affected the spillover trends that we observed. In general, a frequent occurrence of spillover events from areas surrounding ports to port areas may increase the likelihood of certain beetle species being introduced into other countries with export, mechanisms that would contribute to explain why Curculionidae are one of the most commonly intercepted beetle family at points of entry worldwide (Nahrung and Carnegie 2021; Turner et al. 2021). However, the relatively low number of Bostrichidae species and individuals collected in this study compared to the high number of Bostrichidae interceptions at entry points in other countries (Nahrung and Carnegie 2021; Turner et al. 2021) highlights that also other mechanisms can determine the risk of introduction outside the native range. The ecological and biological characteristics of beetle species, such as polyphagy (Nahrung and Carnegie 2020), and the ability of colonizing timber-in-service, wood-packaging materials or round wood present in the port area (Meurisse et al. 2019; Horwood et al. 2022) are two important examples.

Finally, we found that native species introduced into other countries were more abundant in the port areas than in the surrounding natural areas, while the opposite trend occurred for native species that have not been introduced elsewhere. Higher catches in port areas than in surrounding areas of native species which invaded other countries can be due to two not mutually exclusive mechanisms. The first one is that these species mostly live in port areas and thus have higher chances to colonize woody materials or goods ready for exportation or randomly enter containers as hitchhikers, and then to be introduced in recipient countries (Meurisse et al. 2019); the second one is that they are species commonly moved via international and domestic trade which arrived into the monitored ports inside imported commodities or containers and were then intercepted mostly by baited traps set up in port areas. Irrespective of the mechanism, some of the most abundant beetle species we collected in port areas, such as *T. castaneum* and *Rhyzo-*

pertha dominica Fabricius, are also the most intercepted Tenebrionidae and Bostrichidae at ports of entry worldwide (Turner et al. 2021). A similar pattern was found in a previous study, i.e., the most commonly intercepted longhorn beetle and bark and ambrosia beetle in the United States during 1985–2000 on exports from Italy (Haack 2006) corresponded to the second most commonly collected Cerambycidae and the most commonly collected Scolytinae in Italian ports (Rassati et al. 2018). This suggests that the abundance of beetle species in port areas or other shipping points can be potentially considered as a proxy for their likelihood of being introduced into other countries.

Conclusions

When strategies aimed at preventing arrival of nonnative species fail, the first opportunity to prevent permanent establishment of an invading species stems from effective surveillance (Liebhold and Tobin 2008; Nahrung et al. 2023). Our study highlighted that the use of traps baited with generic attractants can be considered not only an efficient strategy to monitor and potentially intercept incoming beetle species of both target and non-target beetle families, but also suggested that the identification of native species trapped in the port areas along with nonnative ones might prove useful to estimate the risk of introduction into other countries. The establishment of a permanent national trapping network in and around entry points and the subsequent exchange of trapping records among countries would represent a key feature of a collaborative global biosecurity program, allowing biosecurity agencies to better identify risks, and invasion scientists to better understand drivers of new invasions (Hulme 2021). Such a trapping network should consider the use of more than a single trap in and around entry points. A higher number of traps would allow to more accurately describe the community of beetles living in port areas and natural areas surrounding them, and thus to better investigate differences and spillover events occurring between these two habitats. However, deploying more traps would lead to increasing costs and efforts needed for inspecting and managing them, as well for identification of trapped specimens. The recent developments in terms of optimization techniques for survey planning (Koch et al. 2020) and technological advances on automatic insect identification (Wührl et al. 2022) might help to overcome these issues.

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Supplementary material 1

Number, position (port area vs. surrounding area), city, and geographic coordinates for each of the 14 traps used during this study

Authors: Hugo Mas, Giacomo Santoiemma, José Luis Lencina, Diego Gallego, Eduardo Pérez-Laorga, Enrico Ruzzier, Davide Rassati

Data type: table (docx. file)

Link: https://doi.org/10.3897/neobiota.85.103904.suppl1

Supplementary material 2

List of beetle species trapped from 2017 to 2021

Authors: Hugo Mas, Giacomo Santoiemma, José Luis Lencina, Diego Gallego, Eduardo Pérez-Laorga, Enrico Ruzzier, Davide Rassati

Data type: table (docx. file)

Explanation note: List of beetle species trapped from 2017 to 2021 at the five Spanish ports and their surrounding natural areas divided by family. For each species the abundance in the port areas and surrounding areas, the cities where it was found, and whether it was introduced or not outside its native range is reported.

Link: https://doi.org/10.3897/neobiota.85.103904.suppl2

Supplementary material 3

Area of Spain and position where baited cross-traps were set up both inside and outside the selected ports of entry

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Data type: figure (docx. file)

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