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Rapid invasion, pathogen spillover, and native bumblebee decline on an isolated island in southern Chile

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Abstract

We investigated pathogen prevalence and population abundance in native and invasive *Bombus* species over three consecutive seasons on a highly isolated ecosystem in southern Chile which is free of other Apidae. Three of five targeted pathogens (*Apicystis bombi*, *Crithidia bombi*, and *Lotmaria passim*) were detected in both *Bombus*. We found that invasive *Bombus terrestris* exhibited higher infection prevalence and parasite loads than native *B. dahlbomii*. In 2021, native *Bombus* population declined sharply. Our finding suggests that exotic *Bombus* was first detected on the island in 2020 and is currently expanding. Our data demostrate that invasive bumblebees can introduce and amplify pathogens that threaten native species, even in ecosystems where they were originally highly abundant.

Implications for insect conservation: Our results highlight the vulnerability of isolated island ecosystems to biological invasions and associated pathogen spillover, emphasizing the need for early detection, strengthened biosecurity measures, and long-term monitoring to prevent irreversible losses of native pollinators.

INTRODUCTION

The global translocation of managed pollinators has facilitated biological invasions with profound ecological consequences (Goulson et al. 2015; Aizen et al. 2019). Among the most impactful cases, the European bumblebee *Bombus terrestris* has expanded across South America, where it is linked to the rapid decline of the native *Bombus dahlbomii* (Morales et al. 2013; Aizen et al. 2019). A key mechanism underlying this replacement is pathogen spillover, whereby invasive species introduce and amplify parasites that negatively affect native hosts (Meeus et al. 2011; Arbetman et al. 2013; Graystock et al. 2013; Fürst et al. 2014).

Previous long-term studies in southern Chile have documented a population shift of both native *Bombus* and *Apis mellifera* populations following *B. terrestris* establishment (Smith-Ramírez et al. 2014). Disentangling the effects of *Bombus* pathogens from other drivers, such as *Apis mellifera*'s pathogens shared with *Bombus*, remains challenging.

Here, we take advantage of Guafo Island, a highly isolated ecosystem lacking honeybees, to evaluate pathogen sharing and population dynamics between native and invasive bumblebees under simplified community conditions. We test whether (i) invasive *B. terrestris* exhibits higher pathogen prevalence and load, and (ii) its arrival coincides with rapid changes in native population abundance.

METHODS

Study area

The study was conducted on Guafo Island (43°34'S, 74°49'W; 202 km²), a highly isolated island located ~ 50 km southwest of Chiloé Island, southern Chile. Strong winds and difficult navigation likely limit the

arrival of non-native pollinators. The island is entirely covered by native temperate forest and lacks managed honeybee (*Apis mellifera*) populations, providing a simplified system to assess interactions between native and invasive bumblebees.

Field sampling

Bumblebees (foraging workers) were sampled during austral summers of 2020–2022 along the northwestern coast. In total, 58 individuals were collected: 44 in 2020 (*Bombus dahlbomii* n = 23; *B. terrestris* n = 21) and 14 *B. terrestris* in 2022. In 2021, *B. dahlbomii* was rarely observed and not collected. In 2022, only fecal samples of *B. dahlbomii* were obtained to avoid impacting this endangered species. Individuals were assumed to represent independent foraging workers sampled across space and time.

Nucleic acid isolation

Bumblebee guts were dissected, homogenized by mortar and pestle with 3 mL of phosphate-buffered saline (PBS 1X), and DNA extracted following standard protocols (Arismendi et al., 2016; Arismendi et al. 2021). The samples were centrifuged at 10000× g for 15 min at 4°C; 200 µL of the precipitated extract was used for DNA extraction. The extract was ground in 1.5 mL microcentrifuge tubes with 350 µL of CTL buffer following the E.Z.N.A Insect DNA Kit's instructions (OMEGA, Bio-Tek Inc., Atlanta, GA, USA). The DNA was quantified (Infinite 200 PRO NanoQuant, Tecan Group Ltd., MGroup Lt, Switzerland) and stored at -20°C.

Real-time PCR for pathogen detection and quantification

Quantitative real-time PCR (qPCR) was conducted with specific primers to detect the presence and quantify the load of *N. bombi*, *N. ceranae, A. bombi, C. bombi*, *L. passim* in bumblebees (Material Supplementary, Table S1). The PCR reactions were carried out in 15 µL, containing 20 ng of DNA, 1X of KAPA SYBR FAST Universal 2X qPCR Master Mix (Kapa Biosystems, Wilmington, MA, USA), 530 nM of each primer, and filled with sterile-filtered molecular grade water. Thermal conditions were achieved with one cycle at 96°C for 10 min, followed by 40 cycles at 95°C for 15 s, 60°C for 15 s and 72°C for 30 s. A dissociation analysis was conducted after all of the amplifications were completed to detect the primer dimmers and the unspecific amplicons. Real time PCR assays were performed in a Thermocycler QuantStudio™ 3 Real-time PCR system, and the qPCR data were analyzed using QuantStudio™ design and analysis software v1.5.2.

To determine the *Trypanosoma*, microsporidia and neogregarinorida loads (absolute quantification), calibration curves were performed (Arismendi et al. 2021). A conversion factor was run from copies per µL of pathogens (*Trypanosoma*, microsporidia and neogregarinorida) to cell, spore or oocyst equivalents per bee.

Abundance sampling as frequency flower visits

Relative abundance was estimated as visitation frequency to focal flowers, a robust proxy under low detectability conditions. Visitation rate is widely used as a standardized proxy for relative abundance in pollination studies, particularly under conditions of low detectability. Observations consisted of 20 flowers per plant during 20-minute sessions. Total sampling effort was 380 min (2020), 660 min (2021), and 400 min (2022), across multiple flowering plant species (*Fuchsia magellanica*, *Raphanus sativus*, *Carduus* sp., *Convolvulus* sp., *Brassica* sp., *Lathyrus* sp.).

Data analysis

Pathogen prevalence was calculated as the proportion of infected individuals per species and year and compared using Chi-square tests. When expected frequencies were low, results should be interpreted cautiously as Chi-square tests may be sensitive to small sample sizes.

Differences in pathogen loads among groups were assessed using Kruskal–Wallis tests followed by Bonferroni-adjusted post hoc comparisons. Abundance data were non-normally distributed (Shapiro–Wilk tests, $p < 0.05$); therefore, differences among years, species, and their interaction were evaluated using Scheirer–Ray–Hare tests, with Dunn post hoc comparisons. All analyses were conducted in R (R Core Team), and statistical significance was set at $\alpha = 0.05$.

RESULTS

Pathogen analysis

Three of the five screened pathogens (*Apicystis bombi*, *Crithidia bombi*, and *Lotmaria passim*) were detected in both native and invasive bumblebees on Guafo Island (Table 1). In 2020, *Bombus terrestris* exhibited significantly higher pathogen prevalence than *B. dahlbomii* for all detected pathogens (*A. bombi*: $\chi^2 = 7.38$, $p = 0.007$; *C. bombi*: $\chi^2 = 5.21$, $p = 0.022$; *L. passim*: $\chi^2 = 9.21$, $p = 0.002$). Pathogen loads were also consistently higher in *B. terrestris*, with significant differences among groups (*A. bombi*: $H = 20.80$, $p < 0.001$; *C. bombi*: $H = 14.35$, $p = 0.001$; *L. passim*: $H = 15.38$, $p = 0.001$; Fig. 2). In particular, *B. terrestris* collected in 2020 showed the highest infection intensities. In 2022, *B. terrestris* remained infected with all three pathogens, with prevalence levels comparable to those observed in 2020, although some pairwise comparisons were not statistically significant (Table 1). Neither *Nosema bombi* nor *N. ceranae* were detected in any samples. No pathogens were detected in *B. dahlbomii* feces collected in 2022. Given the low sample sizes typical of remote island systems, results should be interpreted as conservative estimates of pathogen prevalence.

Table 1
Prevalence of *Trypanosoma*, microsporidia and neogregarinorida native and non-native bumble bees collected in Guafo Island, Chile

Year	N° sample	Species	Positive cases (%)				
			Apicystis bombi	***Crithidia bombi***	***Nosema bombi***	***Lotmaria passim***	***Nosema ceranae***
2020	23	*Bombus dahlbomii*	30.43 a A	47.83 a A	0.00	30.43 a B	0.00
	21	*Bombus terrestris*	71.43 b A	80.95 b A	0.00	76.19 b A	0.00
2022	0	*Bombus dahlbomii*	no samples	no samples	no samples	no samples	no samples
	14	*Bombus terrestris*	57.14 A	71.43 A	0.00	66.67 A	0.00

Different lowercase letters in the year 2020 indicate significant differences (Chi-square test, $p < 0.05$) in pathogen detection among *Bombus* species. Different uppercase letters indicate statistically significant differences in the detection pathogen-positive cases between *B. terrestris* and *B. dahlbomii* when comparing the years 2020 and 2022 (Chi-square test, $p < 0.05$).

Table 2
Results of Scheirer–Ray–Hare test for three years and flower visit abundance (frequency of visits to focal plants) of *Bombus* species and its interactions in Guafo island, Chile. *Pos-hoc* test for years and interactions are showed.

Test	Variable / Comparison	Statistic	p-value*
Scheirer–Ray–Hare test	Year	H 23.5328	0.001
	Sp	H 0.6817	0.409
	Year × Sp	H 25.7881	< 0.001
Post-hoc (Year)	2020–2021	Z 3.288	0.001
	2020–2022	Z − 1.053	0.292
	2021–2022	Z − 4.533	< 0.001
Post-hoc (Year × Sp)	2020.BD – 2020.BT	Z 4.625	< 0.001
	2020.BD – 2021.BD	Z 5.186	< 0.001
	2020.BT – 2021.BD	Z − 0.024	0.981
	2020.BD – 2021.BT	Z 4.675	< 0.001
	2020.BT – 2021.BT	Z − 0.536	0.592
	2021.BD – 2021.BT	Z − 0.598	0.550
	2020.BD – 2022.BD	Z 2.669	0.008
	2020.BT – 2022.BD	Z − 2.014	0.044
	2021.BD – 2022.BD	Z − 2.253	0.024
	2021.BT – 2022.BD	Z − 1.733	0.083
	2020.BD – 2022.BT	Z 0.525	0.600
	2020.BT – 2022.BT	Z − 4.159	< 0.001
	2021.BD – 2022.BT	Z − 4.677	< 0.001
	2021.BT – 2022.BT	Z − 4.157	< 0.001
	2022.BD – 2022.BT	Z − 2.173	0.030

* Significance $p < 0.05$

Interannual abundance results

Bumblebee visitation rates differed significantly among years (H = 23.53, p = 0.001) and in interaction with species (H = 25.79, p < 0.001). The year 2020 showed the highest visitation rates, largely driven by *B. dahlbomii* (mean = 3.4 visits per observation period). In contrast, 2021 exhibited a marked decline in

overall abundance (mean = 0.35), with no significant differences between species. By 2022, visitation rates of *B. terrestris* increased and reached levels comparable to those of *B. dahlbomii* in 2020, indicating a rapid shift in species dominance within the community. Overall, these results indicate a strong temporal turnover in bumblebee community structure, characterized by the decline of *B. dahlbomii* and the concurrent increase of *B. terrestris*. Together, pathogen prevalence patterns and abundance dynamics consistently indicate a rapid community turnover coinciding with the arrival.

DISCUSION

Our results provide evidence of pathogen sharing and rapid population turnover following the arrival of *Bombus terrestris* in an isolated island ecosystem. The invasive species exhibited consistently higher pathogen prevalence and loads, consistent with its role as a reservoir and potential amplifier of infection risk for native pollinators. While our data do not allow direct inference of transmission pathways, the consistent association between invasive presence, higher pathogen loads, and native population decline suggests a plausible role of pathogen-mediated impacts.

The detection of *Apicystis bombi*, a pathogen linked to reduced survival and foraging efficiency (Meeus et al. 2011; Arismendi et al. 2021), is of particular concern given its presence in both species. Similarly, *Crithidia bombi* is known to reduce colony fitness and can be transmitted across generations (Brown et al. 2003), while *Lotmaria passim*, typically associated with honeybees, may affect a broader range of hosts than previously recognized (Gómez-Moracho et al. 2020).

The absence of pathogens in fecal samples of *B. dahlbomii* should be interpreted cautiously, as detection sensitivity may differ from gut-based analyses. In 2022, gut pathogen screening was not conducted due to the very low number of *B. dahlbomii* observed; instead, fecal samples were collected as a non-invasive alternative. Molecular analyses detected *Lotmaria passim* (8/13 samples) and *Crithidia bombi* (1/13 samples) at low levels (Table S2, Supplementary Material), but these results should be interpreted cautiously given the low amounts of DNA recovered and it may differ from gut-based analyses.

The abrupt decline of *B. dahlbomii* observed in 2021 closely mirrors patterns reported in mainland Chile and Argentina following *B. terrestris* invasion (Morales et al. 2013; Smith-Ramírez et al. 2014). Although alternative explanations such as environmental variability or stochastic demographic fluctuations cannot be ruled out, in Guafo Island case and the cases in Argentina and North Chiloé Island, the temporal coincidence with invasion and pathogen patterns suggests a non-random process associated to pathogens.

Field observations and reports o other scientist and navies indicate that *B. terrestris* was first detected on the island during our 2020 sampling period. Once established, the invasive species appears to expand rapidly under local conditions, potentially facilitated by its tolerance to pathogen loads that may be detrimental to native species. Together, these findings are consistent with the hypothesis that *B. terrestris* may act as a vector of pathogen transmission in invaded systems. The rapid shift in community

structure observed here underscores the urgency of monitoring isolated ecosystems and implementing biosecurity measures to prevent further invasions. Without targeted monitoring and biosecurity interventions, the persistence of *B. dahlbomii* on Guafo Island remains uncertain

Declarations

Supplementary Information

Table S1. Primers used for the detection and quantification by real-time PCR of *Trypanosoma*, microsporidia, and neogregarinorida in native and non-native bumble bees from Guafo Island. Table S2. Threshold cycle (Ct) values of Trypanosoma, Microsporidia, and Neogregarinorida in fecal samples of native and non-native bumble bees collected on Guafo Island, Chile

Author Contribution

CSR was the main responsible of this study, did fieldwork, wrote and reviewed the document. NA did the pathogens analysis and wrote the document. AR did fieldwork, analysis and wrote the document. VD did fieldwork and reviewed the document. RB did fieldwork and reviewed the document.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

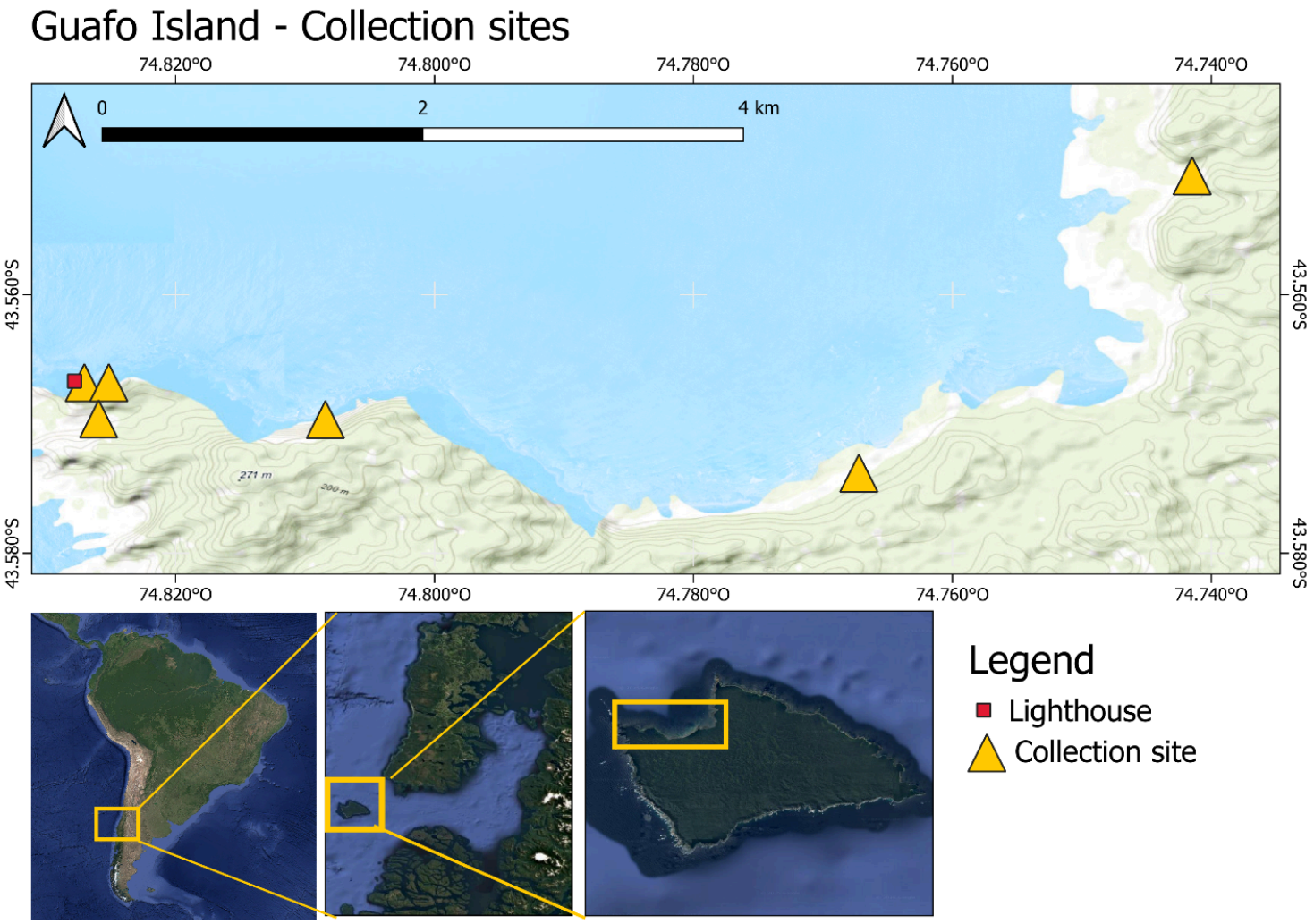


Figure 1

Map of Guafo Island, Chile, indicating the collection sites.

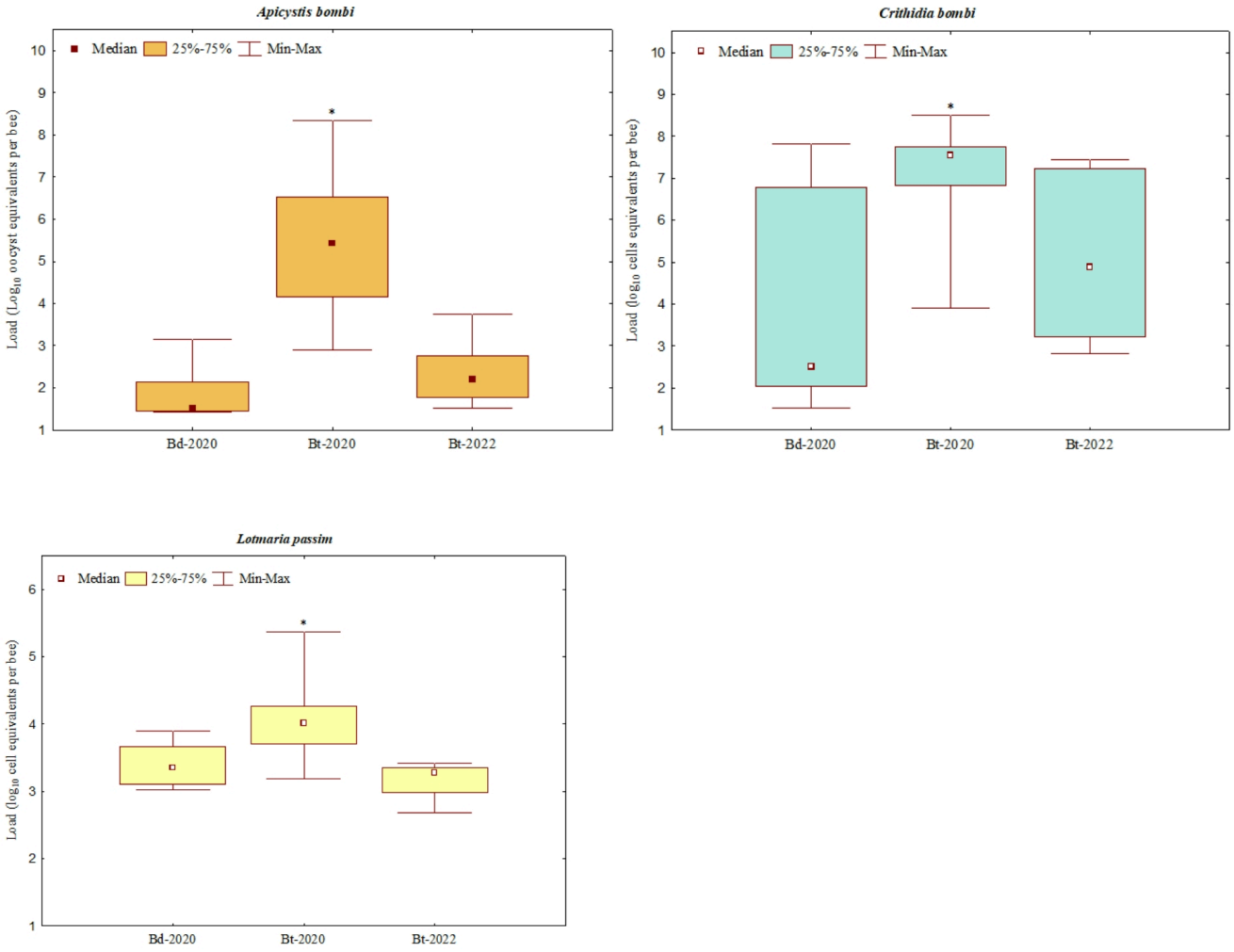


Figure 2

Apicistys bombi, *Crithidia bombi* and *Lotmaria passim* loads quantified in *Bombus dahlbomii*(Bd) and *B. terrestris* (Bt) collected in 2020 and 2022. Asterisks on the box plot indicate significant differences ($p < 0.05$) of pathogen loads among *Bombus* species according to the Kruskal-Wallis H test.

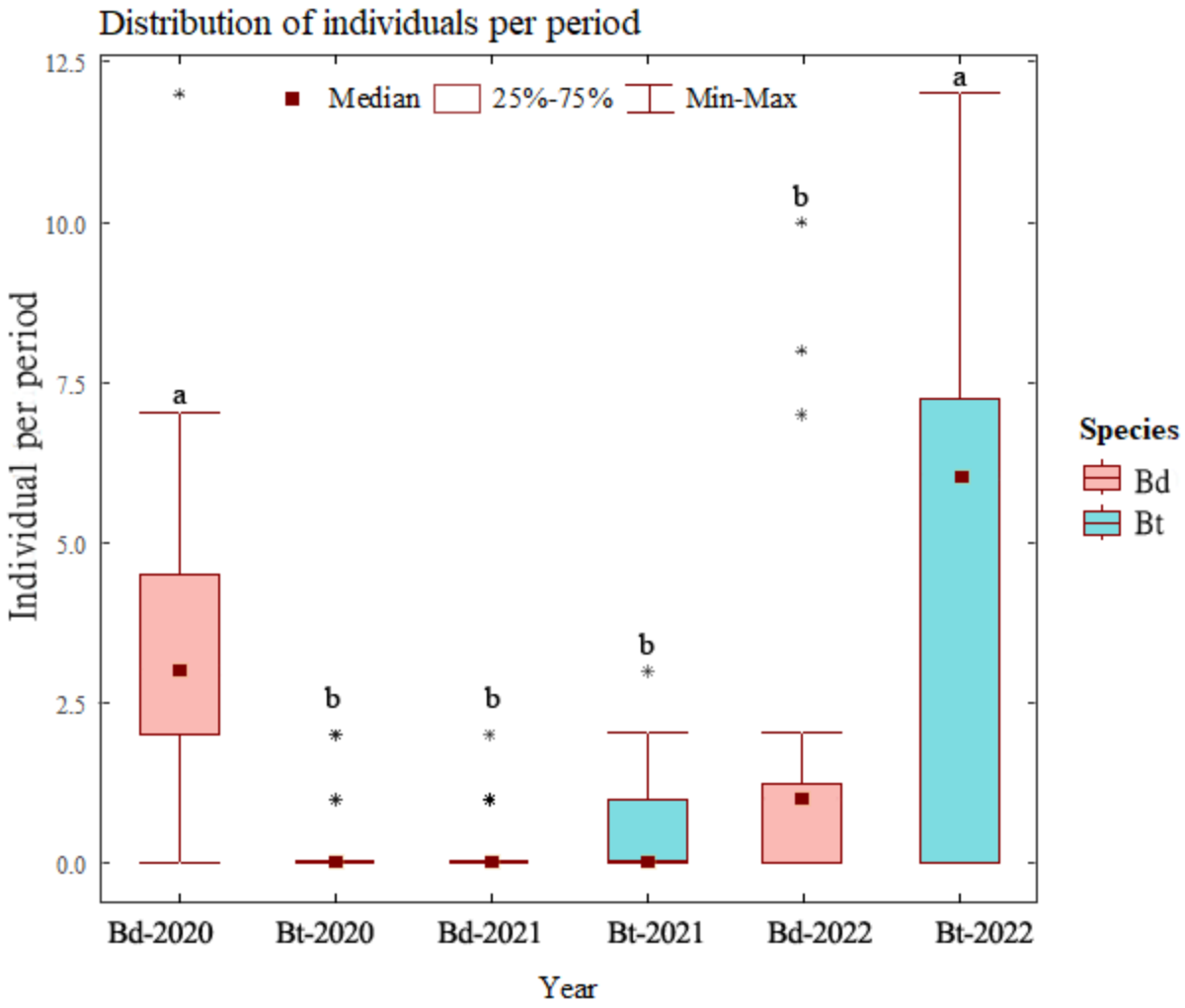


Figure 3

Distribution of individuals per period for *Bombus dahlbomii* (Bd) and *B. terrestris* (Bt) collected in 2020, 2021 and 2022. Letters show significant differences according to Scheirer–Ray–Hare (SRH).

Supplementary Files

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