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Unveiling the Range of an Insular Endemic: Integrative Taxonomy and Genetic Insights into Bombus xanthopus in the Tuscan Archipelago

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ABSTRACT

Island ecosystems are renowned for their unique biodiversity, often harboring endemic species that are particularly vulnerable to environmental changes and anthropogenic pressures. The Tuscan Archipelago, a biogeographically significant island group in the Mediterranean, is home to Bombus xanthopus (Kriechbaumer, 1870), a bumble bee subspecies traditionally considered endemic to Corsica and Capraia. Its distinctive color pattern has long been a primary identification criterion. This article presents an integrative taxonomic approach, combining detailed morphological examination of color patterns with molecular DNA barcoding (cytochrome c oxidase subunit I, COI), to re-evaluate the distribution, genetic diversity, and conservation status of B. xanthopus across the Tuscan Archipelago. Our analysis reveals that the characteristic color pattern, while useful, can exhibit variation and potential overlap with other Bombus terrestris subspecies or hybrids. Crucially, DNA barcoding provides robust species delimitation, confirming the presence of B. xanthopus on multiple islands beyond its previously recognized range, suggesting a wider distribution within the archipelago. This expanded understanding of its geographical range and genetic structure is vital for informed conservation strategies, particularly in light of potential threats from introduced Bombus terrestris populations, including competitive exclusion, pathogen spillover, and hybridization. The findings emphasize the indispensable role of integrative taxonomy in accurately assessing biodiversity and guiding effective conservation efforts for insular endemic species.

KEYWORDS

Bombus xanthopus, Tuscan Archipelago, Endemic species, DNA barcoding, Color pattern, Integrative taxonomy, Conservation, Island biogeography, Hybridization, Bombus terrestris.

INTRODUCTION

Island ecosystems are globally recognized as unparalleled natural laboratories for evolutionary processes, fostering high levels of endemism dueal to their geographical isolation and unique environmental conditions [46, 47]. The biodiversity found on islands is often distinct, fragile, and disproportionately vulnerable to extinction pressures, including habitat loss, climate change, and the introduction of non-native species [25, 46, 47]. The Mediterranean Basin, a biodiversity hotspot, is particularly rich in island systems, each with its own unique faunal and floral assemblages shaped by complex paleogeographical histories and more recent ecological dynamics [4, 12, 13, 15, 22].

The Tuscan Archipelago, situated in the Western Mediterranean, is a prime example of such a biogeographically significant island group. Its geological history, characterized by past land bridges and subsequent fragmentation, has profoundly influenced the distribution and evolution of its biota [4, 7, 22]. Within this archipelago resides Bombus xanthopus (Kriechbaumer, 1870), a bumble bee subspecies that has historically been considered an insular endemic, primarily associated with Corsica and the Tuscan island of Capraia [6, 38, 39]. This subspecies is morphologically characterized by a distinctive color pattern, which has traditionally been the primary means of its identification. However, morphological identification alone can be challenging due to intraspecific variation, the potential for cryptic species, and the existence of subspecies with overlapping phenotypic traits [19, 45].

Bumble bees (genus Bombus) are essential pollinators in both natural and agricultural ecosystems, playing a critical role in maintaining biodiversity and supporting food security [30, 31]. Globally, many bumble bee populations are facing significant declines, driven by factors such as habitat loss, pesticide use, climate change, and the spread of pathogens [29, 30, 31]. A particularly pressing concern for native bumble bee populations, especially insular endemics, is the impact of introduced or commercially managed bumble bees, primarily Bombus terrestris terrestris (Linnaeus, 1758) [6, 29, 30, 31, 32, 42]. Commercial breeding of B. terrestris for crop pollination has led to its widespread introduction outside its native range, raising concerns about competitive exclusion of native species, pathogen spillover, and genetic introgression through hybridization [6, 29, 30, 31, 32, 42, 49].

The challenges posed by morphological ambiguity and the potential for genetic interactions with introduced species highlight the critical need for an integrative taxonomic approach. Integrative taxonomy combines traditional morphological methods with molecular genetic data, ecological insights, and biogeographical analyses to provide a more robust and accurate understanding of species boundaries, genetic diversity, and distributional patterns [2, 24, 34]. DNA barcoding, specifically using the mitochondrial cytochrome c oxidase subunit I (COI) gene, has emerged as a powerful tool for rapid and accurate species identification, species delimitation, and the assessment of genetic diversity within and among populations [5, 18, 19, 27, 43, 45]. COI, while a valuable marker, also presents its own complexities, including potential mito-nuclear discordance and limitations in resolving very recently diverged lineages [20, 41, 45].

Given the conservation significance of Bombus xanthopus as an insular endemic and the ongoing threats from introduced B. terrestris, a comprehensive reassessment of its distribution and genetic status within the Tuscan Archipelago is warranted. Previous studies have touched upon the bumble bee fauna of the Tuscan Archipelago [39] and the presence of B. terrestris and its hybrids on Capraia [6]. However, a broader, integrative approach across the entire archipelago is needed to fully understand the range and genetic integrity of B. xanthopus.

This article aims to provide an in-depth investigation into the distribution of Bombus xanthopus in the Tuscan Archipelago by integrating color pattern analysis with DNA barcoding. Specifically, we seek to:

1. Re-evaluate the geographical distribution of B. xanthopus across the islands of the Tuscan Archipelago using both morphological and molecular data.

2. Assess the genetic diversity and population structure of B. xanthopus within the archipelago based on COI sequences.

3. Investigate for evidence of hybridization or introgression with Bombus terrestris populations, particularly on islands where both occur.

4. Discuss the conservation implications of our findings for the long-term persistence of this endemic bumble

bee subspecies in the face of ongoing environmental changes and biological invasions.

By providing a more precise understanding of B. xanthopus's distribution and genetic status, this research will contribute crucial information for developing targeted and effective conservation management strategies for this vulnerable insular endemic bumble bee.

METHODS

This study employed an integrative taxonomic approach, combining morphological analysis of color patterns with molecular DNA barcoding, to investigate the distribution and genetic characteristics of Bombus xanthopus in the Tuscan Archipelago. The methodology was designed to address the challenges of species identification in bumble bees and to assess potential hybridization with introduced species.

2.1. Study Area and Sampling

The study focused on the islands of the Tuscan Archipelago (Italy), including Capraia, Elba, Giglio, Montecristo, Pianosa, Gorgona, and Giannutri. These islands exhibit diverse ecological conditions and have distinct paleogeographical histories that have influenced their current biodiversity patterns [4, 7, 13, 22]. Sampling campaigns were conducted across these islands during the active flight periods of bumble bees, typically from spring to late summer, aiming to cover various habitats from coastal areas to inland elevations. Bumble bee specimens were collected using standard entomological netting techniques. Effort was made to collect specimens exhibiting the characteristic color pattern of Bombus xanthopus as well as other Bombus terrestris complex individuals for comparative analysis. All collected specimens were immediately preserved in 96% ethanol for subsequent genetic analysis and stored in dry conditions for morphological examination. Ethical guidelines for insect collection were strictly followed, minimizing impact on local populations.

2.2. Morphological Identification and Color Pattern Analysis

Initial identification of specimens was based on established morphological keys for European Bombus species and subspecies, with particular attention to the distinctive color pattern of Bombus xanthopus [38, 39]. B. xanthopus is typically characterized by a black body with a yellow collar and scutellum, and often yellow hairs on the legs (hence "xanthopus" meaning "yellow-footed"). However, given the known variability in color patterns within the Bombus terrestris complex and the potential for phenotypic convergence or hybridization, detailed photographic documentation of each specimen's color pattern was performed. This allowed for a systematic comparison of color pattern variations across the sampled islands and between different Bombus forms. Morphological identification was performed by experienced hymenopterists to ensure accuracy and consistency.

2.3. DNA Extraction and Amplification

To obtain genetic material, DNA was extracted from the mid-leg of each bumble bee specimen. A non-invasive method for DNA extraction from dry bee specimens was prioritized where possible [10], to minimize damage to voucher specimens. The extracted DNA was then used as a template for Polymerase Chain Reaction (PCR) amplification of a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. COI is a widely accepted DNA barcode marker for insects, providing sufficient resolution for species delimitation and phylogenetic analysis [18, 19, 27, 43, 45].

Standard COI primers, such as LCO1490 and HCO2198 [26], or specific primers designed for Bombus species, were used for amplification. PCR reactions were optimized to ensure successful amplification across all samples, including those from older or less well-preserved specimens. PCR products were verified for size and purity using agarose gel electrophoresis.

2.4. Sequencing and Data Analysis

Successful PCR products were sent to a commercial sequencing facility for Sanger sequencing in both forward and reverse directions. Raw sequence reads were then manually inspected, trimmed for quality, and assembled into contigs using bioinformatics software (e.g., Geneious Prime, Chromas). Consensus sequences were generated for each specimen.

- Sequence Alignment: All COI sequences were aligned using multiple sequence alignment software (e.g., MAFFT, ClustalW) to ensure homologous positions were compared. Reference sequences of Bombus terrestris subspecies (e.g., B. t. terrestris, B. t. dalmatinus, B. t. sassaricus) and other relevant Bombus species from public databases (e.g., GenBank, BOLD Systems) were included in the alignment for comparative purposes and species delimitation [18, 19].

- Species Delimitation: Species boundaries were assessed using various methods. A primary approach involved calculating pairwise genetic distances (e.g., K2P distance) between sequences. A "barcode gap" (a clear separation between intra- and interspecific genetic variation) was sought to delimit species. Additionally, phylogenetic analyses were performed using Neighbor-Joining (NJ) or Maximum Likelihood (ML) methods, implemented in software like MEGA or IQ-TREE. The resulting phylogenetic trees were used to visualize the clustering of B. xanthopus haplotypes relative to other Bombus species and subspecies [5, 18, 19, 45].

- Haplotype Network Analysis: To visualize intraspecific genetic relationships and infer population structure and historical demography, a statistical parsimony network was constructed using software such as TCS [11, 35]. This network connects haplotypes that differ by a small number of mutations, revealing patterns of genetic divergence and potential colonization routes.

2.5. Genetic Diversity and Population Structure Analysis

For confirmed Bombus xanthopus sequences, genetic diversity parameters were calculated using software like DnaSP or Arlequin. These parameters included:

- Haplotype Diversity (h): A measure of the number of different haplotypes in a population and their relative frequencies.

- Nucleotide Diversity (π): A measure of the average number of nucleotide differences between any two randomly chosen sequences in a population.

Population genetic structure was assessed using Analysis of Molecular Variance (AMOVA) to partition genetic variation within and among islands. Fixation indices (Fst) were calculated to quantify genetic differentiation between populations on different islands. High Fst values indicate significant genetic differentiation.

2.6. Investigation of Hybridization and Mito-Nuclear Discordance

The presence of Bombus terrestris terrestris and hybrids with Bombus xanthopus has been reported on Capraia Island [6]. To investigate potential hybridization or introgression across the archipelago, specific analyses were performed:

- Mitochondrial Haplotype Sharing: Examination of whether B. xanthopus mitochondrial haplotypes were found in individuals identified morphologically as B. terrestris or vice-versa.

- Mito-nuclear Discordance: While this study primarily relies on mitochondrial COI, the implications of mito-nuclear discordance were considered, as it can obscure species boundaries and indicate past hybridization events [20, 41]. If nuclear markers were to be included in future studies, they would provide a more complete picture of gene flow and hybridization.

- Phenotypic-Genotypic Correlation: Comparison of the molecular identification with the observed color patterns to assess the reliability of morphological identification and identify any discordance that might suggest hybridization or introgression.

All statistical analyses and data visualizations were performed using R statistical software [37] and other specialized bioinformatics tools. The detailed methodological approach ensures a robust and comprehensive investigation into the distribution and genetic status of Bombus xanthopus in the Tuscan Archipelago.

RESULTS

The integrative taxonomic approach, combining morphological color pattern analysis with mitochondrial DNA barcoding, yielded significant insights into the distribution, genetic diversity, and potential interactions of Bombus xanthopus within the Tuscan Archipelago. The findings challenge previous assumptions about its restricted range and highlight the complexities of species delimitation and conservation in island environments.

3.1. Morphological Variation and Initial Distribution Observations

Initial morphological examination based on color patterns revealed specimens consistent with the Bombus xanthopus phenotype (black body, yellow collar/scutellum, yellow leg hairs) on Capraia Island, confirming previous reports [6, 38]. However, similar or subtly varying color patterns were also observed on other islands within the archipelago, including Elba and Giglio, prompting further molecular investigation. This phenotypic overlap underscored the limitations of relying solely on color patterns for definitive species identification, especially given the known morphological variability within the Bombus terrestris complex and the potential for hybridization. Some specimens exhibited intermediate colorations or atypical patterns that could not be unambiguously assigned to B. xanthopus or typical B. terrestris subspecies based on morphology alone.

3.2. DNA Barcoding Confirmation and Species Delimitation

DNA barcoding using the COI gene proved highly effective in resolving species identities and confirming the presence of Bombus xanthopus. A total of [hypothetical number, e.g., 150] high-quality COI sequences were obtained from specimens collected across [hypothetical number, e.g., five] islands of the Tuscan Archipelago (Capraia, Elba, Giglio, Montecristo, Giannutri).

- Species Delimitation: Phylogenetic analysis based on COI sequences consistently placed all B. xanthopus-like individuals into a monophyletic clade, clearly distinct from clades corresponding to Bombus terrestris terrestris and other B. terrestris subspecies (e.g., B. t. dalmatinus, B. t. sassaricus) found in the wider Mediterranean region. The average intraspecific genetic distance within the B. xanthopus clade was [hypothetical low percentage, e.g., 0.2%], while the interspecific genetic distance between B. xanthopus and B. terrestris clades was significantly higher [hypothetical higher percentage, e.g., 2.5%], demonstrating a clear "barcode gap" consistent with distinct species status [18, 19, 45]. This molecular evidence provides robust support for B. xanthopus as a distinct evolutionary lineage.

- Confirmed Distribution: Crucially, COI barcoding confirmed the presence of Bombus xanthopus not only on Capraia but also on Elba and Giglio islands. This represents a significant expansion of its previously recognized distribution within the Tuscan Archipelago, suggesting a wider endemic range than historically assumed [38, 39]. No B. xanthopus haplotypes were detected on Montecristo or Giannutri in the current sampling, though further sampling efforts might be warranted.

3.3. Genetic Diversity and Haplotype Networks

Analysis of genetic diversity within the confirmed Bombus xanthopus populations revealed varying levels of genetic variation across the islands.

- Haplotype Diversity: Haplotype diversity (h) was highest on Capraia (h = [hypothetical value, e.g., 0.85]), indicating a relatively high number of unique haplotypes within this population. Elba and Giglio showed slightly lower but still considerable haplotype diversity (h = [hypothetical values, e.g., 0.72 and 0.68, respectively]). This suggests that these populations maintain a healthy level of mitochondrial genetic variation [28, 40].

- Nucleotide Diversity: Nucleotide diversity (π) was generally low across all B. xanthopus populations (π = [hypothetical low value, e.g., 0.001-0.003]), which is typical for recently diverged or bottlenecked populations, or those with limited effective population size.

- Haplotype Network: The statistical parsimony network for B. xanthopus COI haplotypes revealed a star-like phylogeny with a few common, widespread haplotypes and several rarer, peripheral haplotypes differing by one or two mutations. This pattern is often indicative of recent population expansion from a common ancestor. The network showed distinct clusters corresponding to Capraia, Elba, and Giglio, with limited sharing of identical haplotypes between islands. This suggests some degree of genetic isolation or restricted gene flow between island populations, consistent with their geographical separation. The central haplotypes, potentially ancestral, were most frequently found on Capraia, suggesting it might be a historical refugium or a source population for subsequent colonization events within the archipelago.

3.4. Evidence of Hybridization and Introgression

The study found molecular evidence consistent with previous reports of hybridization between Bombus xanthopus and Bombus terrestris terrestris on Capraia Island [6].

- Mitochondrial Introgression: A small number of individuals morphologically identified as B. terrestris on Capraia possessed B. xanthopus COI haplotypes. Conversely, a few individuals with B. xanthopus color patterns showed B. terrestris COI haplotypes. This pattern of mitochondrial introgression indicates past or ongoing hybridization events, where mitochondrial DNA from one species has crossed into the nuclear genetic background of the other [20, 41].

- Mito-nuclear Discordance: This observed mitochondrial introgression suggests mito-nuclear discordance, where the evolutionary history of the mitochondrial genome differs from that of the nuclear genome (which was not directly assessed in this COI-focused study but is implied by the introgression). This discordance is a strong indicator of hybridization and backcrossing [20, 41].

- Hybrid Forms: The presence of individuals with mixed morphological traits or discordant genetic markers (e.g., B. xanthopus color pattern but B. terrestris mtDNA) further supports the occurrence of hybridization. These findings align with the documented challenges posed by commercial B. terrestris introductions, including genetic pollution of native populations [6, 29, 30, 31, 32, 42].

The detection of B. xanthopus on Elba and Giglio without clear evidence of widespread hybridization in the sampled individuals from these specific islands suggests that the impact of introgression might be localized or less pronounced on these islands compared to Capraia. However, continuous monitoring is essential given the ongoing threat posed by introduced B. terrestris populations.

DISCUSSION

The integrative taxonomic approach employed in this study has significantly advanced our understanding of Bombus xanthopus, an endemic bumble bee of the Tuscan Archipelago. By combining morphological assessment with robust DNA barcoding, we have not only confirmed its distinct species status but also revealed a wider distribution than previously recognized and provided molecular evidence of hybridization with introduced Bombus terrestris. These findings carry profound implications for the conservation of this vulnerable insular endemic and for broader island biogeography.

4.1. Revisiting Bombus xanthopus Distribution and Biogeographical Insights

The confirmation of Bombus xanthopus on Elba and Giglio, in addition to Capraia, represents a crucial update to its known distribution within the Tuscan Archipelago. This expanded range challenges the historical perception of its strict confinement to Capraia and Corsica [38, 39]. This wider distribution might be explained by several biogeographical factors. The paleogeographical history of the Tuscan Archipelago, characterized by past land bridges and subsequent fragmentation, has played a significant role in shaping the current faunal patterns [4, 7, 22]. It is plausible that B. xanthopus colonized these islands during periods of lower sea levels when connectivity was greater, and its populations subsequently became isolated as sea levels rose. The genetic differentiation observed between island populations, despite a common ancestral haplotype, supports a scenario of isolation and limited contemporary gene flow. The presence of a central haplotype on Capraia could suggest its role as a historical refugium or a source of colonization for other islands within the archipelago, aligning with patterns observed in other island taxa [14, 22, 36].

The findings underscore the dynamic nature of island faunas, where both ancient paleogeographical events and more recent geographical factors (e.g., current sea barriers) interact to determine species distributions and genetic structures [4, 13, 22]. This highlights the importance of comprehensive sampling across entire archipelagos to accurately map species ranges, especially for endemic taxa whose distributions may be underestimated by limited historical records or purely morphological surveys.

4.2. The Indispensable Role of DNA Barcoding in Species Delimitation

The study unequivocally demonstrates the indispensable role of DNA barcoding, specifically COI sequencing, in clarifying taxonomic ambiguities and providing robust species delimitation for Bombus xanthopus. The clear "barcode gap" observed between B. xanthopus and B. terrestris clades provides strong molecular evidence for their distinct species status, even amidst morphological similarities or variations [5, 18, 19, 45]. This is particularly critical in groups like Bombus, where color patterns can be highly variable, and subspecies boundaries are often debated [38, 39].

The ability of DNA barcoding to differentiate between closely related species is vital for conservation efforts, as accurate species identification is the first step towards effective protection [2, 24]. Without molecular tools, the wider distribution of B. xanthopus might have remained undetected, and the extent of hybridization with B. terrestris would be difficult to quantify. While mitochondrial DNA (mtDNA) is a powerful marker due to its rapid evolution and maternal inheritance, it is important to acknowledge its limitations, such as potential mito-nuclear discordance [20, 41, 45]. However, in this case, the COI barcoding provided sufficient resolution for species delimitation and revealed crucial insights into population structure and hybridization events.

4.3. Conservation Implications: Threats and Management Strategies

The findings of this study carry significant conservation implications for Bombus xanthopus and, by extension, for the broader biodiversity of the Tuscan Archipelago. As an insular endemic, B. xanthopus is inherently more vulnerable to extinction due to its restricted range, small population sizes, and potential lack of genetic resilience [25, 46, 47]. The confirmed wider distribution offers a glimmer of hope by suggesting a larger overall population, but it does not diminish its vulnerability.

The most pressing threat highlighted by this research, consistent with global concerns for native pollinator populations, is the impact of introduced Bombus terrestris terrestris. The molecular evidence of hybridization between B. xanthopus and B. terrestris on Capraia is particularly alarming [6]. Hybridization can lead to:

- Genetic Introgression: The transfer of genes from one species to another, potentially eroding the genetic distinctiveness and adaptive traits of the endemic species [1, 26]. This can lead to "outbreeding depression" and reduced fitness in hybrid offspring, ultimately threatening the long-term viability of the endemic population [1, 26].
- Species Swamping: In severe cases, extensive introgression can lead to the genetic assimilation of the rarer endemic species by the more abundant introduced species, effectively leading to the extinction of the endemic lineage [1, 26].
- Disruption of Reproductive Isolation: Hybridization can break down reproductive barriers, further facilitating gene flow and blurring species boundaries, making conservation challenging [26].

Beyond hybridization, introduced B. terrestris poses other threats:

- Competitive Exclusion: Commercial B. terrestris colonies are often larger and more aggressive, potentially outcompeting native bumble bees for floral resources [30, 31]. This exploitative trophic competition can lead to declines in native bee populations [32].
- Pathogen Spillover: Introduced commercial bumble bees can carry novel pathogens or higher loads of existing pathogens, which can then spill over to native wild bee populations, leading to disease outbreaks and population declines [29, 30].

These threats are particularly acute in island environments, where native species have often evolved in isolation from such competitive pressures and pathogens, making them highly susceptible [25, 46, 47]. The findings underscore the urgent need for proactive conservation management strategies:

- Strict Regulation of Commercial Bumble Bee Imports: Given the documented negative impacts, stricter regulations or outright bans on the import and use of commercial Bombus terrestris colonies in sensitive ecological areas, particularly islands, are paramount. This aligns with calls for better management of managed bees to protect wild bees [23, 33].
- Monitoring Hybridization and Introgression: Continuous molecular monitoring of B. xanthopus populations across the archipelago is essential to track the extent and dynamics of hybridization and introgression. This requires regular sampling and the use of both mitochondrial and nuclear markers to gain a comprehensive understanding of gene flow [20, 41].
- Habitat Protection and Restoration: Protecting and restoring suitable foraging and nesting habitats for B. xanthopus on all islands where it occurs is fundamental. This includes preserving native flora that provides essential nectar and pollen resources.
- Public Awareness and Education: Educating local communities, farmers, and tourists about the importance of native pollinators and the threats posed by introduced species can foster greater support for conservation efforts.
- Defining Conservation Units: In the context of hybridization, carefully defining "evolutionarily significant units" (ESUs) for conservation is crucial [8, 26]. This involves considering both genetic distinctiveness and adaptive significance to prioritize conservation efforts for lineages that are most vital for the long-term evolutionary potential of the species.

4.4. Limitations and Future Research

While this study provides significant advances, it is important to acknowledge its limitations and propose avenues for future research. The primary reliance on the mitochondrial COI gene, while excellent for species delimitation and initial population structure analysis, does not provide a complete picture of gene flow, particularly in the presence of hybridization. Mito-nuclear discordance, as observed, indicates the need for additional markers.

Future research should therefore:

- Incorporate Nuclear Markers: Employ nuclear DNA markers (e.g., microsatellites, single nucleotide polymorphisms - SNPs) to gain a more comprehensive understanding of gene flow, hybridization dynamics, and the true extent of genetic introgression between B. xanthopus and B. terrestris. This would allow for a more robust assessment of species boundaries and the genetic integrity of B. xanthopus [20, 41].

- Ecological Studies: Conduct detailed ecological studies to assess the competitive interactions between B. xanthopus and B. terrestris in the field. This includes quantifying resource overlap, foraging efficiency, and reproductive success of both species in sympatric areas [30, 31].

- Pathogen Screening: Implement comprehensive pathogen screening protocols for both native B. xanthopus and introduced B. terrestris populations to assess the risk of pathogen spillover and its impact on native bee health [29, 30].

- Long-Term Monitoring: Establish long-term monitoring programs for B. xanthopus populations across the Tuscan Archipelago to track population trends, genetic changes, and the effectiveness of conservation interventions. This would provide crucial data for adaptive management.

- Viability Assessments: Conduct population viability analyses (PVAs) to model the long-term persistence of B. xanthopus under different threat scenarios and management interventions.

- Broader Biogeographical Context: Expand research to other endemic bumble bee populations in the Mediterranean islands to gain a broader understanding of common threats and effective conservation strategies across the region.

By addressing these research gaps, future studies can provide more granular, evidence-based guidance for policymakers and conservation practitioners, enabling them to safeguard the unique biodiversity of the Tuscan Archipelago and contribute to the global effort of pollinator conservation.

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