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Nutritional position of managed honey bees during pollination of native plants by the melissopalynology method

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Pollination services are crucial for maintaining ecological stability and ensuring food security for humans. Managed honey bees, which are economically valuable and are experiencing population growth due to the increasing demand for their products, play a significant role in pollination. To produce high-quality honey, beekeepers often choose natural high meadows, characterized by high plant species richness, for their apiaries. This practice, in turn, may contribute to the pollination of native plants, as managed honey bees are likely to forage on diverse floral resources within these meadows In this study, we investigated the nutritional position of managed bees in the pollination of native plants in Iran using the melissopalynology method to determine the extent of their contribution to the pollination of native plants. Ninety-four honey samples were collected from beekeepers located in the natural pastures of two biodiversity hotspots in Iran (Zagros and Alborz). Then, plant pollens were extracted from the honey and photographed by scanning electron microscopy. In the next step, plant species were identified, and their abundance was calculated. The results showed that managed bees visited 54 plant genera, seven of which were non-native plants. Additionally, more plant species and the highest abundance of pollen were observed at altitudes ranging from 1000 to 3000 m. Therefore, beekeepers set up their hives in this altitude range to obtain high-quality honey. In general, in this study, the results of melissopalynological analysis, involving the identification of plant genera and pollen counts, revealed that managed honey bees likely contributed less than 3% to the pollination of native plant species in Iran.

Keywords Pollination ecology, Native pollinator, Zagros, Alborz, Melissopalynology, Pollen

Pollination services play a vital role in natural and agricultural ecosystems, affecting all aspects of biodiversity and maintaining the sustainability of terrestrial ecosystems[1–3]. Pollinating agricultural products provide special services for establishing food security for humans[4–6]. Among the pollinating animals, honeybees play one of the most effective roles in the pollination network[7–10]. However, various factors such as pesticides, human development (including destruction of natural habitats, urban development and road construction), soil pollution and climate change, threaten these insects[11–13].

As one of the most useful insects, honey bees play an important role in pollination and provide food and medicinal services to humans[14–16] Managed honey bees play a prominent role in the pollination of agricultural and garden plants by being placed next to agricultural fields[17,18]. However, these plants cannot pollinate wild bees[19,20]. In some cases, reducing managed bee populations can help increase wild bee populations in fields[21–23]. Due to the economic value of managed honey bees for humans, number of honey bees has increased in recent years to meet human needs[24]. Therefore, currently, managed bees are taken to natural pastures to produce higher quality honey[25].

With more than 8000 natural plant species, Iran has provided suitable pastures for managed bee breeding to produce quality honey[26,27]. Therefore, many beekeepers are legally located in natural pastures[28]. In addition, the Zagros and Alborz mountains are recognized as centers of biodiversity in Iran, and host a significant number of plant species. This high biodiversity is attributed to the region's diverse habitats and climates, as well as a high

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rate of endemism. The presence of diverse natural communities has created suitable conditions for the raising and maintenance of bee colonies managed by beekeepers[26,29].

Previous studies have highlighted the negative effects of managed bees on natural pollinators, especially wild bees[29–34]. Considering that most beekeepers in Iran depend on natural pastures, to the best of our knowledge, no study has been performed to investigate the feeding position of managed bees in the pollination of natural plants.

This study sought to determine the feeding position of managed bees during the pollination of natural plants using the melissopalynology method. The objectives of this study are: (1) What plant genera do managed bees feed on? (2) What is the frequency of each plant genera? (3) The highest number of plant genera carried by honey bees and their abundance are at what altitudes in the Zagros and Alborz mountains? (4) What is the relative contribution of managed honey bees to the pollination of native plant species in the Zagros and Alborz mountains?

Methods and materials

Study area

This study was carried out in the Zagros and Alborz mountains, which are considered to be hotspots of Iran's biodiversity (Fig. 1). Zagros habitats 3642 plant species, 792 genera and 122 native plant families also, Alborz is habitat 3617 plant species in 876 genera and 146 native plant families, are the habitats of more than 90% of Iran's native plant species[26,27]. This has made the region an important area for maintaining managed bee colonies for honey production in Iran[28].

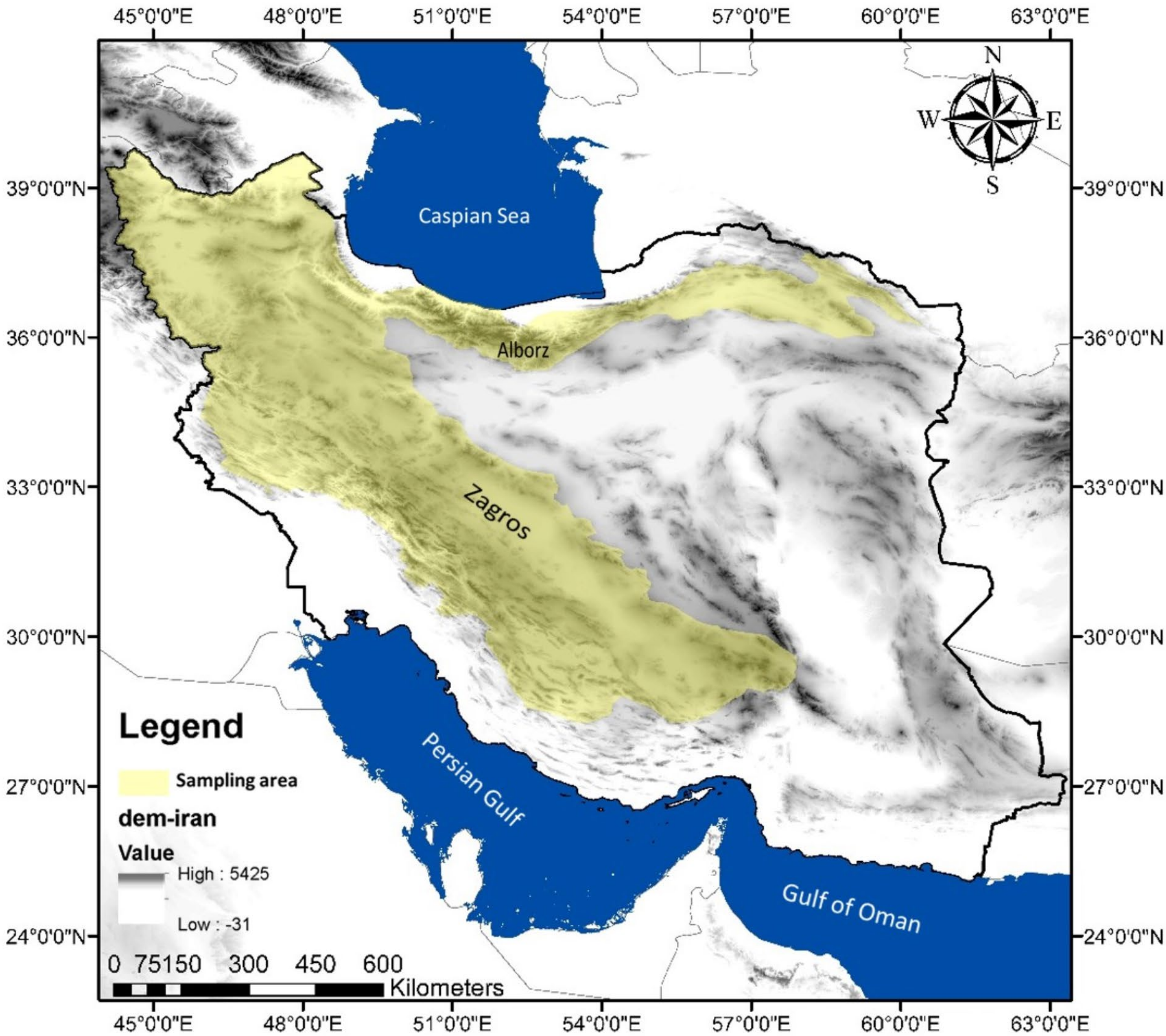

Fig. 1. The sampling locations for the honey bee colonies used in the melissopalynological study. This map was generated using ArcGIS 10.8 software.

Sample collection

In this study ninety-four honey samples were collected from beekeepers in the Zagros ($n=42$) and Alborz ($n=52$) regions. The bees were collected from natural areas, from May to October 2020–2021. Honey samples, each comprising 300 g of unrefined honey, were individually stored in sterile containers to prevent contamination and maintain sample integrity during the melissopalynological analysis. The honey samples were sourced from beekeepers managing apiaries ranging in size from 70 to 150 beehives. These beekeepers pooled the honey from their respective hives into designated barrels for collection. Consequently, the collected honey samples represented a nearly comprehensive blend of honey from the apiaries of all the participating beekeepers. Location data, including elevation and coordinates, were recorded for each sample. Sampling spanned diverse geographical areas to capture a wide range of vegetation types (On Figshare).

Melissopalynological analyses

The analysis of melissopalynology in this study was based on That of Louveaux et al. (1970). This method was suggested by the International Commission on Bee Botany (ICBB)[35,36].

The Louveaux method[35] was used to extract pollen from honey, which was initially dissolved in 10 g of honey from each sample separately in 20 mL of distilled water on a hot plate at a temperature less than 40 °C. In the next step, the honey wax particles were removed using a cheesecloth filter, and the remaining liquid was poured into 30 mL conical centrifuge tubes. After 3 centrifugation steps (2500–3000 rpm) at room temperature (21–25 °C), pollen was extracted from the honey sample. First, the samples were centrifuged for 10 min (approximately 2500 rpm) and the supernatants were carefully removed to prevent pollen from falling. In the next step, approximately 10 mL of distilled water was added to the remaining sediment, and the samples were subsequently centrifuged for 5 min (2500–3000 rpm). Therefore, the last centrifuge liquid remaining at the end of the tubes was poured into the watch glass and placed on a hot plate at a temperature less than 40 °C so that the water evaporated completely, and the pollen remained in the container. The prepared pollens were subsequently transferred to special bases to be covered with gold. The prepared bases were placed inside a scanning electron microscope (SEM) and, the prepared pollen from the honey sediments was subsequently counted via SEM. Quantitative evaluation of the pollen grains of each honey sample was done based on the Louveaux standard, and to determine the relative abundance, 500 to 1000 pollen grains were counted in each honey sample[35]. Using images via from SEM, pollen grains were identified based on their morphology via international palynology websites and atlases (Fig. 2). This study employed genus-level pollen identification due to the rarity of reliable species-level identification in pollen analysis[36].

Statistical analysis

In this study, Pairwise Spearman Correlations were used to measure the correlation between the number of plant genera identified in honey and their frequency with the number of native plant species of the same genus

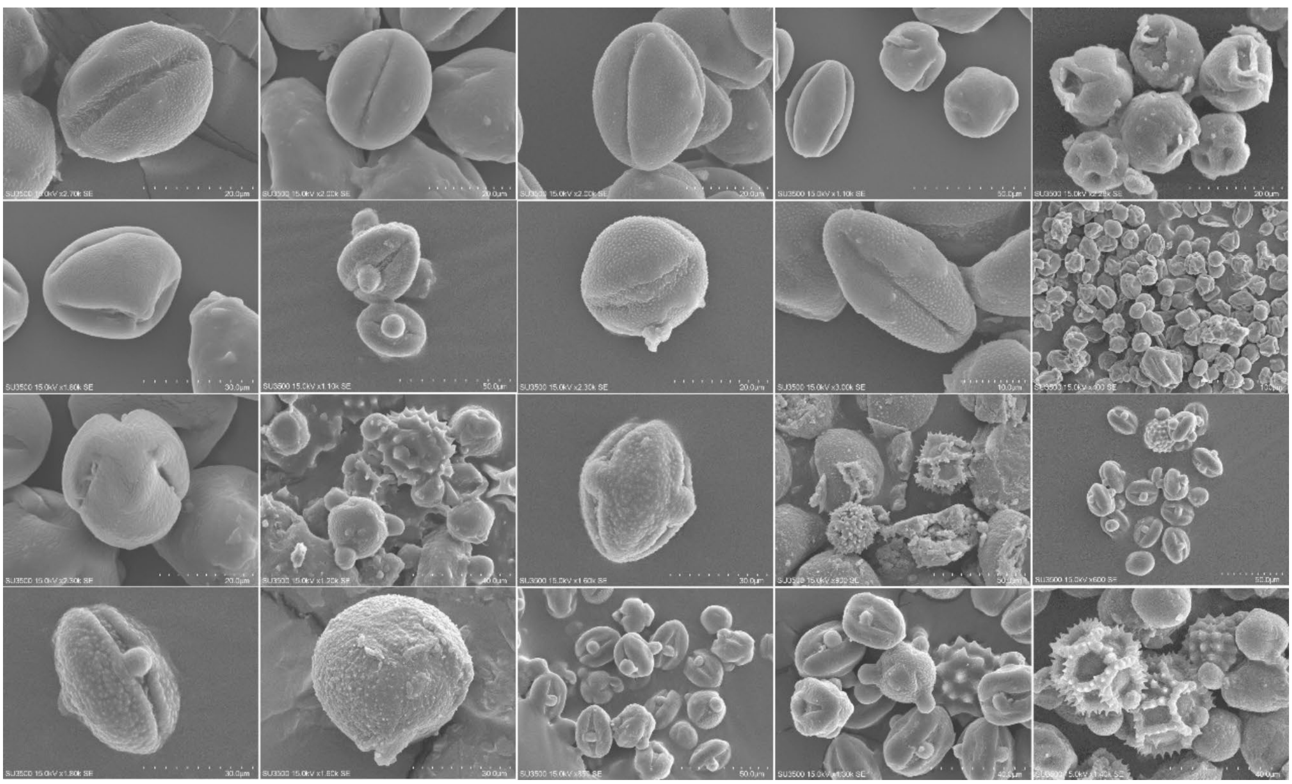

Fig. 2. Some of the pollen extracted in this study.

in Iran. Additionally, to determine the height interval where the highest plant species and the highest pollen abundance were observed, a regression was performed by Fitted Line Plot-Minitab 19. The number of identified plant species, and their abundance were considered as dependent variables. In both regressions, the location height of each sample was considered an independent variable.

Results

The identification of 42 plant families and 54 plant genera was the result of melissopalynology in this study. Additionally, analysis of the pollen samples revealed that Asteraceae (38%), Fabaceae (13.5%), Rosaceae (6%), Apocynaceae and Apiaceae (5%) were the most dominant plant families, on the other hand, *Centaurea* 9.12%, *Senecio* 6.3%, *Achillea*, 6%, *Rosa* 5.9%, *Cirsium*4.2%, *Atriplex* 4.1%, *Torilis* 4.1%, and *Robinia* 4% were the most abundant genera among the identified plant genera (Fig. 3).

Out of the 54 plant genera identified in this study, seven non-native genera were identified, which showed that 13% of the visits by managed honey bees were to non-native plants (Fig. 4).

The results of Pairwise Spearman Correlations showed that the frequency of identified plant genera was significantly correlated (P-value 0.001, correlation 0.478) with the number of plant species of those genera. That is, the frequency of identified pollens of plant genera depends on the number of plant species of that genus in Iran.

The results of the regression showed that the highest number of plant species and the highest abundance of pollen were detected in the samples (On Figshare) that were collected at an altitude of 2500 –500 m (Fig. 5).

Also, our findings showed that beekeepers have the majority of settlements in exactly this altitude range (500–2500 m) (Fig. 6).

Discussion

This study was conducted to determine the nutritional position of managed honey bees during the pollination of native plants in Iran. The results of this study showed that managed bees visit and feed on 54 plant genera. 87% of the plants were native plant species. Asteraceae and Fabaceae plant families as the most abundant plant families and the *Robinia* genus (Non-native) is among the most abundant plant genera visited by managed bees. Additionally, our results showed that the highest abundance of pollen and the highest number of plant species were detected at an altitude of 500–2500 m. In general, our results showed that despite the presence of beekeepers in natural pastures, managed bees have a very small contribution to the pollination of native plants.

Asteraceae and Fabaceae are known as the largest plant families in Iran in terms of the number of species[37–40] and the highest number of visits by managed bees has been made to these two families, and the[41–43] studies have reached similar results in their studies. Approximately 1/7 of all managed honey bee visits involved nonnative plants, and the results of these visits[44–46] support our findings. Our results showed that the abundance of pollen and the number of plants visited by managed honey bees are directly related to the number and abundance of plant species in their breeding place, which is greatest at altitudes of 500–2500 m. More than 77% of the studied beekeepers were located in high pastures (1000–3000 m), and Iran's natural honey bees are also present at the same altitude and within the same habitat range[47]. It can be said that managed bees are raised in pastures with the highest plant diversity[26,27] studies have shown that the heights of Zagros and Alborz are the main habitats of Iran's native plants[48,49] studies show that the feeding of bees is influenced by the plants where they are housed.

Zagros and Alborz together constitute the habitat of 1668 native plant genera[26,27,50]. However, managed bees visited only 47 native plant genera. It can be said that managed bees probably participate in the pollination of only 2.8% of native plants and, that more than 97% of native plant species depend on natural pollinators. The results of[29,34,51,52] studies confirm our findings.

The presence of managed bees in the highland pastures of Iran, which has the highest plant diversity, not only contributes little to the pollination of native plants, but also poses a significant threat to natural pollinators, especially natural bees. Studies have shown that managed bee colonies at high density can threaten the natural bee population by affecting food sources[5,25,29–31,34,52–54].

Our results showed that managed bees are established in habitats rich in native plant diversity and make a very small contribution to the pollination of native plants. Iran ranks third in honey production and ranks second in the world in the number of managed bee colonies[55]. Therefore, managed bees, especially native bees, are present in the habitats of natural pollinators[47], and are considered a threat to their populations. Since domestic livestock grazing in addition to beekeeping puts additional pressure on natural habitats, studies based on pollination ecology, identification of threat factors[56] and effects of beekeeping and domestic livestock grazing on native pollinators via a conservation approach are highly recommended.

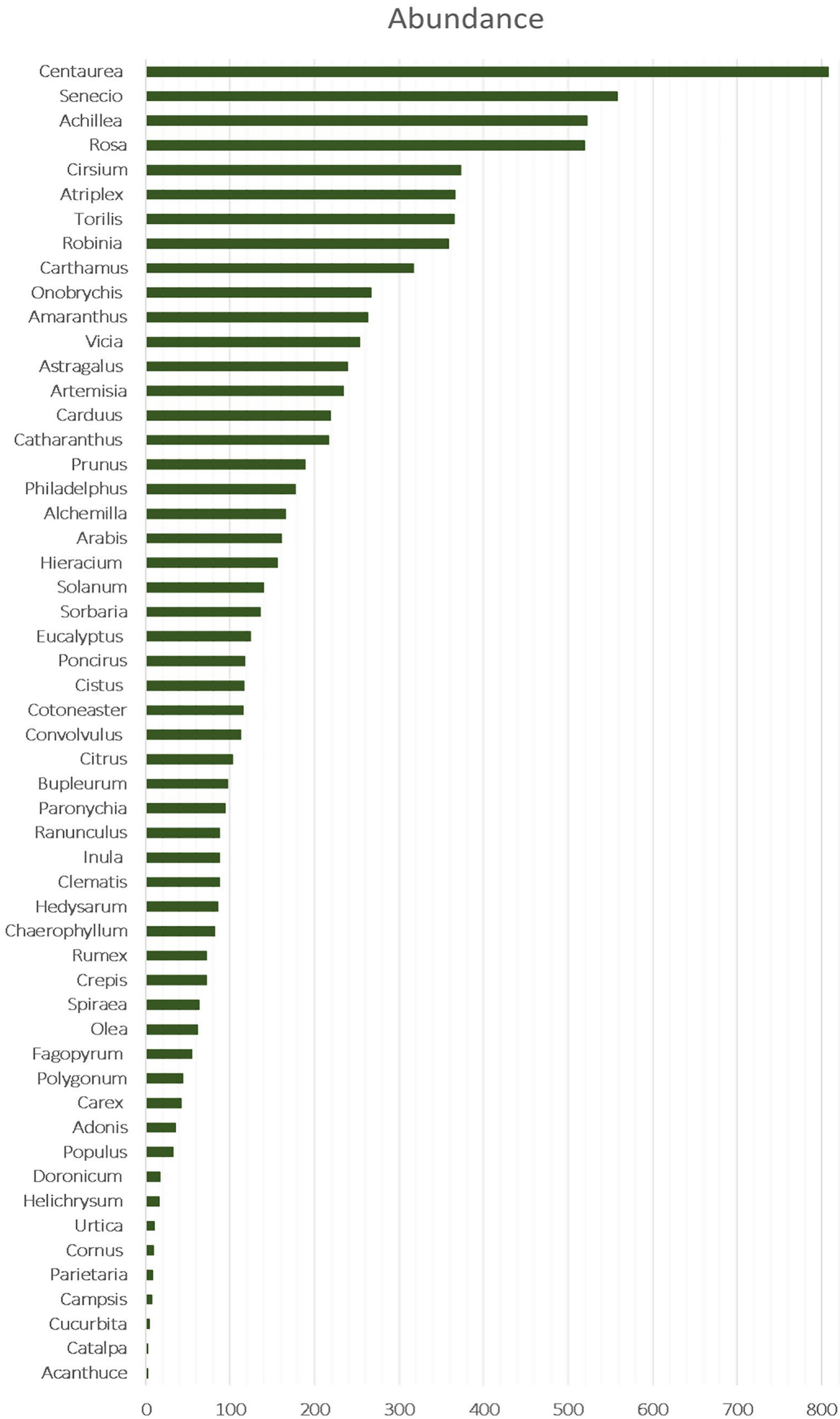

Fig. 3. Melissopalynological analysis of honey samples, illustrating the diversity of the plant genera represented and their relative abundance. The bars represent the percentage of pollen grains of each genus identified.

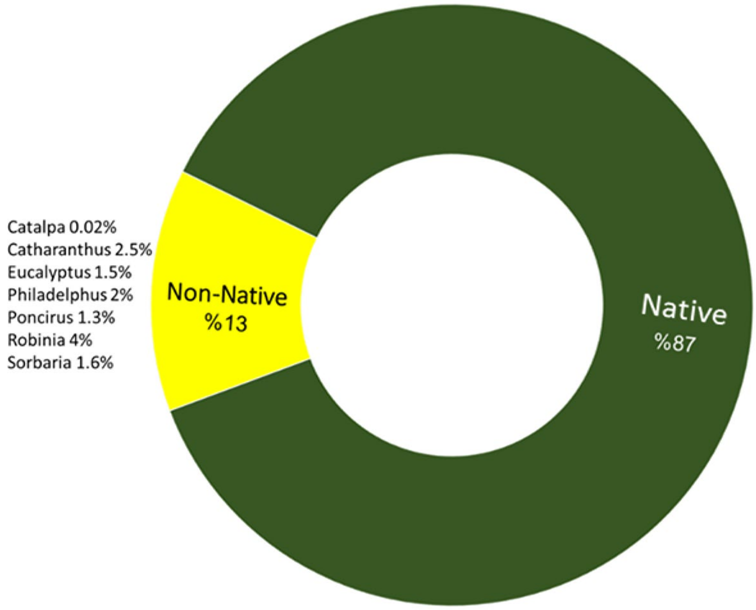

Fig. 4. The percentage abundance of native and non-native plant genera (information obtained from melissopalynology analysis) visited by managed honey bees.

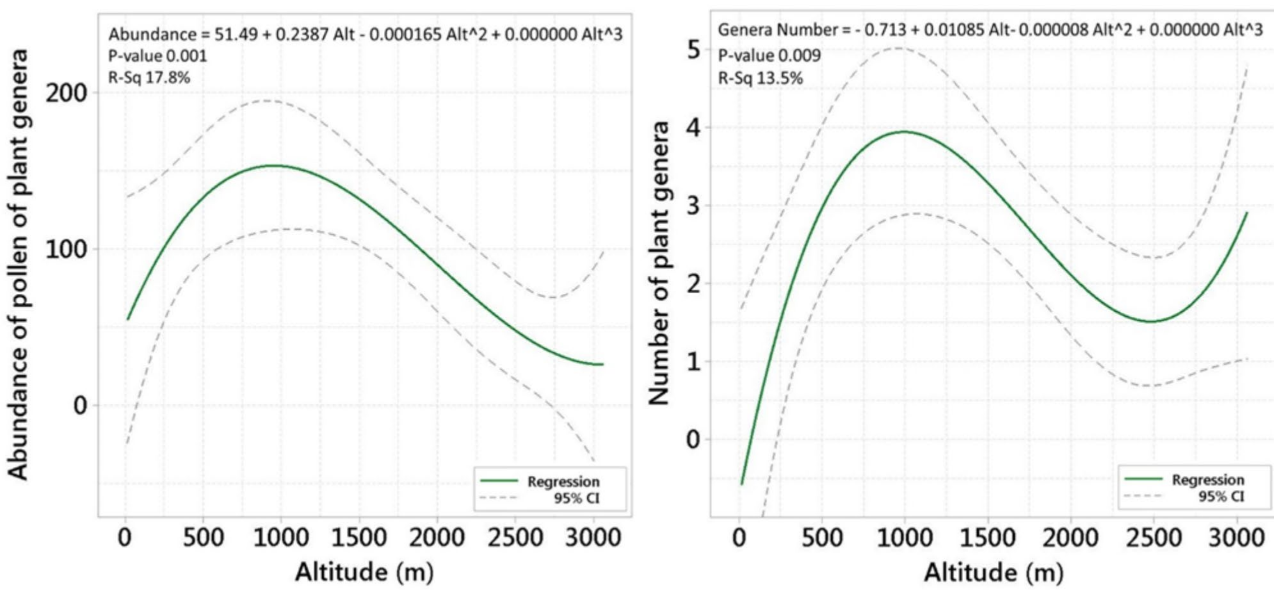

Fig. 5. Cubic regression analysis of the relationship between the number of identified plant genera and the abundance of pollen and the altitude of the sampling site.

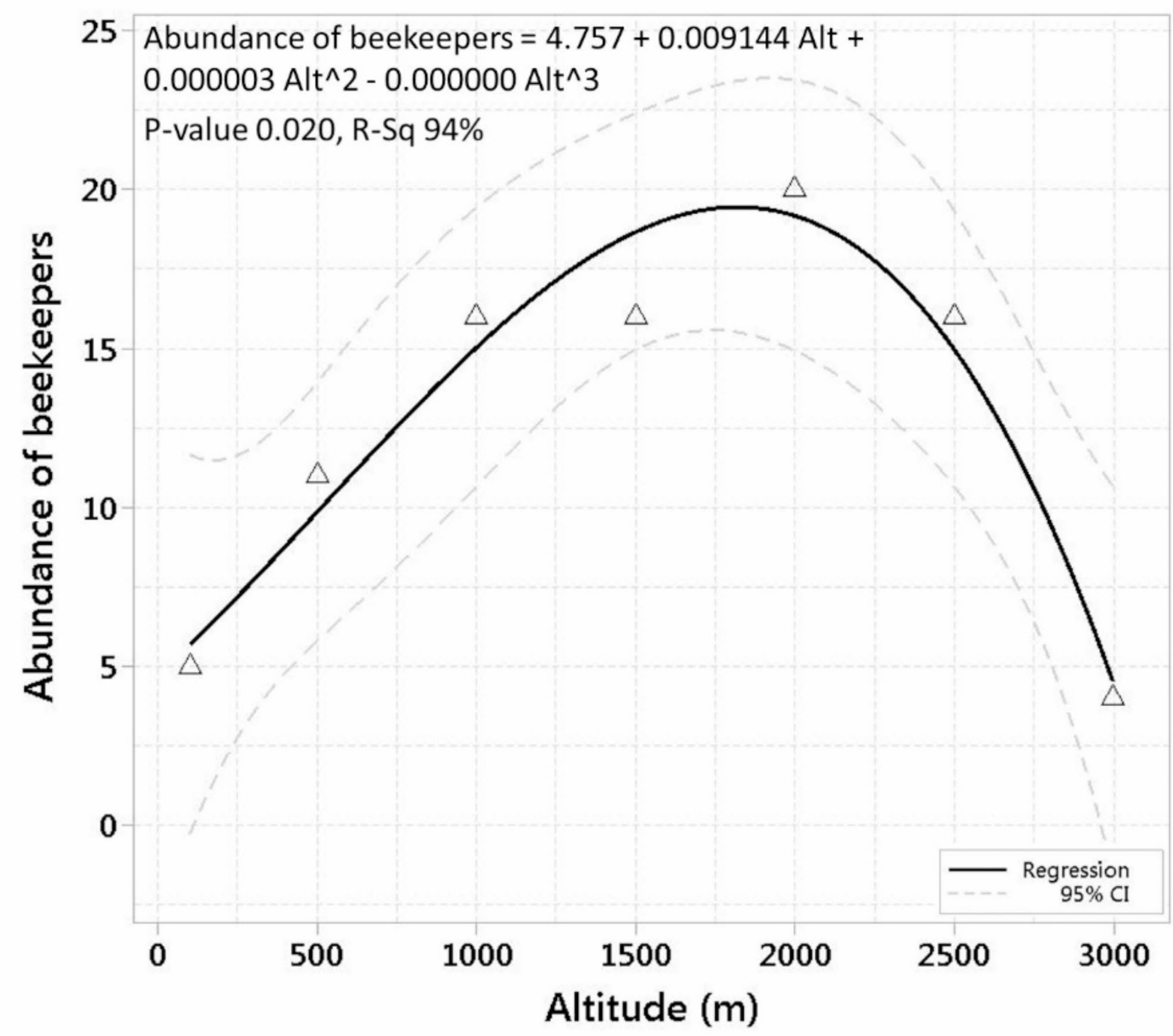

Fig. 6. The relationship between the altitude range of the habitat and the number of beekeepers.

Data availability

https://figshare.com/s/7ff7885ada4a6d002983.

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Author contributions

Z.S. and F.S. : Filed work; Writing - original draft; Writing - review & editing; Supervision; Project administration; Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Investigation;

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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