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ANALYSIS OF POLLEN LOADS COLLECTED BY HONEY BEES (*APIS MELLIFERA* L.) FROM LAMBAYEQUE PROVINCE (PERU): BOTANICAL ORIGIN AND PROTEIN CONTENT

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Abstract

In the localities of Illimo, Motupe and Olmos (Phytogeographical province of Lambayeque, Peru in the equatorial dry forest) the availability of flowering along with the botanical origin and protein content of pollen loads harvested by honey bees (*Apis mellifera*), was studied. Based on the melissopalynology 20 pollen load samples were studied and according to qualitative analysis, 14 samples were monofloral and 6 were multifloral. During one apiculture period (September to November) pollen loads were collected every fortnight using pollen traps. Twenty-three principal pollen types were identified belonging to species occurring in the study area. The families Fabaceae and Capparaceae had a significant importance amongst the samples, represented by five and three pollen types, respectively. *Prosopis limensis* and *Acacia macracantha* pollen types occurred most constantly among the samples. A total of 200 g of pollen loads was analyzed. Pollen types such as *Prosopis limensis*, *Capparis scabridae*, *C. avicennifolia* and *Acacia macracantha* are considered the indicators for this geographical origin. The studied pollen loads were also characterized by a high frequency of *Spilanthes leiocarpa*, *Alternanthera peruviana*, *Baccharis glutinosa* and *Parkinsonia aculeata*. *P. limensis* reached the largest amount of pollen grains with 55 812.5 grains/mm^3 (58.9% of frequency) in Olmos and was the dominant pollen ($\geq$ 45%) in the same locality. The highest content of crude protein and amino acids, especially methionine and histidine, also correspond to pollen load samples of Olmos locality. The high representation of native flora distinguishes these pollen loads geographically.

Key words: Honey bees, pollen loads, pollen morphology, protein content, seasonally dry forest.

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INTRODUCTION

One of the most known and studied plant-insect relationship models is between bees and melliferous plants. Due to their harvesting habit, bees are considered to be the most important and efficient plant pollinating insects, and in natural conditions play and important role in maintaining biodiversity of plants [1]. Indeed bees harvest ten to 1000 times more pollen than other insects. Individual bees leave the hive 20 to 30 times a day and the hive's bees may visit 20 to 30 million flowers during a season [2]. Most plants are reproductively dependent upon the activities of the pollinating insects for transportation of pollen from one plant to another, for this reason, this symbiotic relationship ensures reproduction and genetic diversity for the plants, while the insects gain nectar and pollen as food from the plants [3].

In fact, pollen is a source of proteins, free amino acids, lipids, minerals and vitamins which are essential to growth and development of honey bees rather than energy production [4]. In particular, nitrogen is crucial for development of larvae and longevity of adults [5, 6]. It is important to detect which are the main pollen sources of a region and to determine their protein value while pollen is a major component of honey bees' diet. *Apies mellifera* L. transports pollen to the hives in specialized structures of its legs (corbicula), in which pollen, moistened with nectar and saliva, is packaged forming pasty "pellets", called 'pollen loads' [7]. These loads are generally monoespecific due to the high grade of floral constancy of *A. mellifera* [8].

Melissopalynology, the microscopic analysis of pollen from honey sediment and pollen loads, was the first method used for determining the botanical and, in some cases, the geographical origin, therefore, more valuable information about the characterization of honeys and pollen loads can be obtained from melissopalynological studies [9]. This information is helpful for developing an apiary industry and commercial honey production [10]. In addition, apiculture is becoming an increasingly important socio-economic activity in several areas of Peru and particularly in the equatorial dry forest, a biome unique in the world stretching from the Santa Elena peninsula in Ecuador (0^{o}30' South Latitude) to the middle basin of the Chicama river (La Libertad region) in the north of Peru (5^{o}00' South Latitude); however, there are not studies on the potential nectar sources and identification of melliferous plants in this ecosystem**,** consequently, the level of effective use of the vegetation by bees is still to be determined. Characterization of monofloral origin has been recently initiated due to consumer demands. Tsigouri et al. (2004) [11] have emphasized that ''the product name may be supplemented by information referring to floral or vegetable origin, if the product comes wholly or mainly from the indicated source and possesses the organoleptic, physicochemical and microscopic characteristics of the source'' [12, 13]. Generally, blossom honey is considered to be from one source (monofloral) if the pollen frequency of that plant is more than 45% [14].

There has been a limited research on pollen analyses of Peru honeys and pollen loads and more are needed to determine the pollen and plant sources in different regions [15]; due to, for other countries are reported numerous studies. In Argentina, there are several studies on the botanical composition of pollen loads and pollen flora in the central region, where apiculture has a great developed [16, 17], and was recently reported the botanical composition and protein content of pollen collected by *A. mellifera* in the north-west of Santa Cruz (Argentinean Patagonia) [8]. In Spain an analysis was made of the protein content of pollen loads produced by the bees in a hive situated in Viana do Bolo (Ourense, north-west Spain), to establish whether or not the relative quantity of protein in the pollen of each plant species influences the preference made by the bee of the flowers that supply pollen to the hive [18]; likewise, was studied the seasonal variation in vegetation an pollen collected by honey bees in Thessaloniki region, Greece [19], and the seasonal variation of collected pollen loads of honey bees (*Apies mellifera anatoliaca*) in Bursa region, Turkey [20].

The aim of this study was to determine the diversity of plant species used as source of pollen by honey bees (*Apis mellifera*), to identify the morphological characteristics the pollen collected by honey bees and obtained from several beehives at Lambayeque province (Peru) as well as the protein content.

MATERIALS AND METHODS

***Study area*.** The study area is located in the Lambayeque province (Lambayeque region), in the Peruvian north-coast, very next to the Pacific Ocean (79º27' – 80º00' West Longitude and 5º03' – 6º09' South Latitude) (Figure 1). Altitude is 500 m on average. Soil contents sand (10 – 40%), slime (30 – 60%) and clay (10 – 30%). The climate is characterized by a rainy season (December to March) with 100 – 500 mm of precipitation per year and a dry season between April and November; average relative humidity is 69%. Annual temperature is 19.5 and 31.2 ^{o}C. The natural vegetation is composed by the following zones: "El Chaparral" (*Cordia lutea*, *Capparis scabridae*, *Prosopis limensis* and *Capparis avicennifolia*, as predominant species), "El Algarrobal" (*P. limensis*, *C. scabridae*, *C. lutea* and *Acacia macracantha*, as predominant species), "El Zapotal" (*C. scabridae*, as predominant species) and "El Gramadal" (*Spilanthes leiocarpa*, *Ambrosia peruviana* and *Lippia nodiflora*, as predominant species) [21]. In general, the studies area corresponds to the equatorial dry forest [22] or seasonally dry forest, which has been deeply modified by a dense human population whose main activity is agriculture and firewood extraction.

Agriculture activity is based on irrigation. Most of the cultivated area is planted with maize (*Zea mays*), common bean (*Phaseolus vulgaris*), cotton (*Gossypium hirsutum*), rice (*Oryza sativa*) and several fruits as *Citrus* sp. (lemons, oranges, limes and satsumas), passion flower (*Passiflora edulis*), mango (*Mangifera indica*) and avocado (*Persea americana*). Fooder crops, mainly alfalfa (*Medicago* *sativa*) and horticultural plants are cultivated in a lesser degree. Apiculture is complementary to agriculture as bees are used for the pollination of fruit crops.

Reference slides of pollen plants. Plants were identified by Dr. Guillermo E. Delgado and stored in the herbarium HPR at the Universidad Nacional Pedro Ruiz Gallo (UNPRG), Lambayeque (Peru). Anthers were removed from flower buds of fresh plants, acetolyzed with glacial acetic acid, centrifuged, washed once with distilled water, centrifuged again, acetolyzed with anhydrous acetic acid and sulfuric acid [23, 24] mounted in glycerin jelly on glass slides and sealed with paraffin. These slides were included in the reference slide collection at the UNPRG pollen library for later comparison with the pollen types found in the pollen load samples.

Extraction of pollen load samples for microscopic analysis. Twenty pollen load samples were collected from rural areas of Íllimo, Motupe and Olmos, in the Lambayeque province (Table 1). They were mostly composite samples from apiaries although some were from individual colonies (ca. 5 - 100 km apart one from each other) of *Apis mellifera* L., during the period from September to November, 1989. Pollen traps were placed at the hive entrances at 18 h and removed at 18 h on the following day.

After collection, the pollen loads were dehydrated in a drying oven at 40°C until they attained a stable weight, and were then processed with the following methodology: ten grams of well homogenized pollen loads were re-hydrated in warm distilled water (5 ml) for at least two hours and centrifuged for 10 min at 2 500 rpm. The pollen samples were then dehydrated with glacial acetic acid (10 ml) for 60 min and subsequently centrifuged. The decanted sediment was also acetolyzed according to the technique described by Erdtman (1960; 1969) [23, 24] and placed in an aqueous glycerin solution (50%) from one to 24 hours, to re-hydrate and recover the shape of the pollen grains after chemical treatment, and then re-concentrated by centrifugation for mounting on slides. Each of these concentrated acetolyzed pollen sediment samples was mounted in glycerin jelly, and sealed with paraffin, on five slides for analysis and pollen counts. General observations of the slides were first made using an optical light microscope in order to identify and qualitatively analyse the principal pollen types present; in a second step, the first observed 1 500 pollen grains in each sample (~300 grains per slide) were counted in order to determine the percentages of each pollen type [25]. The pollen types present in the pollen load samples were identified, counted, and classified, according to their percentages, as predominant or dominant pollen (D: ≥45%), secondary pollen (S: 16–45%), important minor pollen I: (3–15%) and minor or trace pollen (m: 1–3%) and pollen present (p: <1%) [14]. When one pollen type represented ≥45% of the total number of pollen grains, the sample was classified as a monofloral pollen load [14]. Multifloral pollen load samples were divided into 'oligofloral' when two or more secondary taxa belonged to one botanical family, 'bifloral' when two pollen types had secondary percentages and 'polyfloral' when three or more pollen types were registered with secondary percentages [26].

Considering by analogy the frequency classes proposed by Louveaux et al. (1978) [14], was established five constancy classes for the pollen types observed in all of the samples analyzed: very constant, present in >75% of the samples; constant, >50% – ≤75%; low constancy, >25% – ≤50%; occasional, ≥5% – ≤25%; and rare, <5%, considering only the simple presence or absence of a pollen type in any of the samples [27].

On the quantitative analysis for determining the number of pollen grains/mm^3, 1 ml of the decanted sediment was resuspended in 4 ml of distilled water, forming a 1:5 dilution; subsequently has took a drop of the sample and placed in the chamber o hemocytometer Neubauer for observation, classification and quantification. Pollen grains were counted under an optical microscope with a 40x objective. We used the modified formula for the white blood cell count (12.5 x p). We took into account the classification proposed by Maurizio (1939) [28], which established five classes according to the pollen grains content in ten grams of honey (Group I: < 20,000; Group II: 20 000 – 100 000; Group III: 100 000 – 500 000; Group IV: 500 000 – 1 000 000; Group V: > 1 000 000.

For nitrogen content determination, 50 mg of pollen loads type (AOAC 1980) [29] was analyzed by the micro-Kjeldahl methods [30], and crude protein was estimated using the factor: 6.25 x N [6]. The pollen samples weighing less than 50 mg were excluded. The analysis of amino acids was performed by using paper chromatography technique determining the RF values (migration of amino acids); the results has expressed in percentage.

The analysis of pollen loads crude protein and amino acids content was made in the Laboratorio de Bromatología y Nutrición of the Universidad Nacional de Trujillo (Peru).

RESULTS

Flowering availability and pollen loads collected by the honey bees. During this study, 20 pollen load samples produced in the province of Lambayeque (Lambayeque, Peru) were analyzed. A total of 52 plant species were found from 22 families and 5 subfamilies (Tables 1 and 4). Twenty-three principal pollen types with percentages ≥1% were recognized in the quantitative analysis, 91% natives and 9% exotic (Table 1).

The quantitative analysis of pollen loads also showed that six of the 20 samples were considered to be monofloral or unifloral, corresponding to Olmos locality with *Prosopis limensis* (n = 6) as the dominant pollen (>45%) and fourteen were considered to be multifloral. In the classification of Ramírez-Arriaga et al. (2011) [26], eight samples of the Íllimo locality and six samples of the Motupe locality were considered bifloral with secondary pollen (16-45%) of *P. limensis* and *Capparis scabridae*, and *P. pallida* and *C. avicennifolia*, respectively; others pollen types, considered as sporadic pollen (<1%), were scarcely visited by honey bees (Tables 2 and 4). In the classification of Novais et al. (2009) [27], *Acacia macracantha*, *Baccharis glutinosa*, *Parkinsonia aculeata* and *Prosopis limensis* are species very constant (>75% of the samples) and *Capparis avicennifolia* and *Cryptocarpus pyriformes* are species constant (>50% – ≤75%) (Table 1).

The most widely represented families were Asteraceae (*Baccharis glutinosa*, *Isocarpha microcephala* and *Spilanthes leiocarpa*) (13%), Fabaceae-Caesalpinioideae (*Caesalpinia glabrata*, *Parkinsonia aculeata* and *Pithecellobium multiflorum*) (13%), Capparaceae (*Capparis avicennifolia*, *C. crotonoides* and *C. scabridae*) (13%), Boraginaceae (*Cordia lutea* and *Heliotropium angiospermum*) (8.7%), Fabaceae-Mimosoideae (*Acacia macracantha* and *Prosopis limensis*) (8.7%) and Nyctaginaceae (*Commicarpus tuberosus* and *Cryptocarpus pyriformis*) (8.7%). The pollen types of *A. macracantha*, *B. glutinosa*, *P. aculeata*, and *P. limensis* were present at all localities sampled; while *Alternanthera peruviana*, *Caesalpinia glabrata*, *Capparis scabridae*, *Isocarpha microcephala*, *Lippia nodiflora* and *Pithecellobium multiflorum*, only occurred in Íllimo locality. *C. crotonoides*, *Citrus limon*, *C. tuberosus*, *H. angiospermum* and *Passiflora edulis* only occurred in Motupe locality, and *Cordia lutea*, *Ipomoea crassifolia*, *Malvastrum tomentosum*, *Salix chilensis* and *Waltheria ovata*, only occurred in Olmos locality (Table 2).

The quantitative analysis of pollen loads showed to *Prosopis limensis* as the species with more number of pollen grains/mm^3 with 45 412.5 grains/mm^3 (42.3% of frequency) for Íllimo locality, 16 450 (38.9%) for Motupe locality, and 55 812.5 (58.9%) for Olmos locality. In Íllimo locality, *P. limensis* was followed by *Capparis scabridae* with 21 237.5 (19.8%), in Motupe locality by *Capparis avicennifolia* with 9 875 (23.4%) and in Olmos locality by *Acacia macracantha* with 17 650 (18.6%) (Table 3). *Prosopis limensis* was exceptionally abundant in both Olmos and Íllimo localities, and this unusual availability of pollen was intensely utilized by honey bees. The other pollen types were less than 10 000 grains/mm^3. The Maurizio´s 1939 [28] classification of pollen loads revealed low pollen amounts in samples of both Motupe and Olmos localities (Group II) and moderate pollen amounts in samples of Íllimo locality (Group III).

General description of the pollen loads. Pollen grains of the Fabacaeae-Mimosoideae (*Acacia macracantha* and *Pithecellobium multiflorum*) are polyads with outline circular or elliptic, respectively, with exception of *Prosopis limensis*; in the Fabaceae-Caesalpinoideae (*Caesalpinia glabrata* and *Parkinsonia aculeata*) the pollen grains are monads. In the rest of species, the pollen grains are monad, spheroidal, subprolate or prolate; isopolar or apolar; radiosymmetric; psilate, reticulate, foveolate, echinate, echinolophate or microechinate; and inaperturate, periporate, tricolpate, tricolporate or stephanocolporate (Table 5).

Protein and aminoacids content of collected pollen loads. Protein content of most collected pollen was variable. Optimal levels [>20% of protein, according to Shaw (1999)] [31] were noted on pollen loads of the localities of Olmos (21.63%) and Íllimo (20.24%), while lightly low levels of protein were found in the pollen loads of the locality of Motupe (19.75%) (Table 6). Optimal levels of amino acids, especially methionine and histidine, were also noted on pollen loads of the localities of Olmos and Motupe, while significative low levels of amino acids were found in the pollen loads of the locality of Motupe (Table 7).

DISCUSSION

In the equatorial dry forest of the Lambayeque province (Lambayeque, Peru), that includes the localities studies of Íllimo, Motupe and Olmos, pollen and nectar sources mostly come from native species; only *Passiflora edulis* (amazonic species) and *Citrus limon* (asiatic species) were the unique exotic species. Flower availability is brief as generally occurs in semi-arid [32], and arid ecosystems. It begins toward the end of winter (august) and continues through spring (september to december) until early summer (january) [33].

Native polliniferous taxa were the dominant pollen with 21 species (91%) and also produced monofloral pollen loads (70% in both Íllimo and Motupe localities) and multifloral pollen loads (30% in Olmos locality). Twenty-three principal pollen types (belonging12 families and 21 genera) were classified from dominant pollen to trace pollen and twenty-nine pollen types (belonging 17 families and 29 genera) were classified as sporadic pollen; this floristic diversity recorded in the study area [34, 35] indicates the high plant diversity of the region, reflected in the pollen harvested by *A. mellifera*, and reveals a wide potential botanical spectrum for apicultural activities.

Llatas (2011) [36] reported that the families Asteraceae, Fabaceae, Capparaceae, Malvaceae and Nyctaginaceae are represented by numerous species in the study area. These same families are represented by 15 principal pollen types in the present study, constituting 71.4% of the 21 pollen types found. This broad sampling of the local flora by *A. mellifera* is similar to that seen in other studies in several countries [37, 19, 20, 27]. Likewise, of the 15 woody species of Fabaceae (subfamilies Caesalpinioideae and Mimosoideae) cited by Llatas (2011) [36] for the studied region, five principal pollen types were found in our analysis. This wide range of pollen types confirms the importance of this family in maintaining *A. mellifera* and other bees in the equatorial dry forest (especially in the Íllimo, Motupe and Olmos localities). On the other hand, *Prosopis* was recognized as a relevant source of nectar and pollen for honey bees by Genise et al. (1990) [38] and by Andrada & Tellería (2005) [16], respectively, but from our results it is evident that the pollen of *P. limensis* is also intensely utilized by honey bees. However, within the great diversity of pollen sources, the plants intensely utilized by honey bees were few. This foraging pattern was also observed in other studies, performed in different ecosystems [39].

In summary, pollen of native plants of the Lambayeque province is intensely utilized by honey bees and, in general, is a nutritional food for them because it combines high protein content and lipids. Beekeepers should take these results into account in apiculture exploitation of the others regions as the Caldén district (Argentina) [16], in the Mediterranean region of Anatolia (Turkey) [9], in the Maharashtra state (India) [10] and in other regions especially in the equatorial dry forest of the Ecuador and Peru. Consequently, it is necessary to increase the ecological studies in order to make decisions regarding sustainable resource management of this region.

Of the twenty-three principal pollen types studied, the most important were the woody species *Prosopis limensis*, *Acacia macracantha*, *Capparis scabridae* and *C. avicennifolia*, and herbaceous species *Spilanthes leiocarpa*. The general morphology of *P. limensis* was similar that of many species of *Prosopis*: monad, isopolar, psilate, tricolporate and radially symmetric [40], and especially to the description given by Delgado (1984) [41] for *P. pallida*, now known as *P. limensis*, which was collected in the Lambayeque region. The pollen of *Acacia* is one of the more extensively studied with regard to its morphological aspects [42]. In the subgenus *Acacia*, the exine surface is not always smooth, and the frequent presence of a supramicrorugulate exine is demonstrated using SEM; polyads are acalymmate, with 8 or 16 pollen grains, radially symmetrical, 20-51 μm in greatest diameter; or with 32 pollen grains, asymmetrical, elliptical in outline, 60-68 μm in greatest diameter, and exine 1-3 μm thick [43]. In our study the pollen of *A. macracantha* are polyads with 16 pollen grains, psilate to reticulate, and exine >2,0 μm thick, characteristic very similar with the reported by Caccavari & Dome (2000) [43] and Ventura & Huamán (2008) [44]. Pollen grains of various species of *Capparis* as observed under light microscope (LM) showed the following pollinic characteristics: prolate, subprolate and prolate spheroidal; tricolporate (3-zonocolporate) and surface reticulate, aerolate,

microreticulate and spinulate [45, 46]. In Peru there are no studies on pollen morphology about *Capparis* genus; however, in the present work the studied species were described as monad, prolate, tricolporate and reticulate. In the case of *Spilantes leiocarpa*, the pollinic model is similar to that described in numerous Asteraceae species: monad; isopolar; radiosymmetrical; trizonocolporate and fossaperturate; oblate-spheroidal, spheroidal, suboblate, prolate-spheroidal and subprolate, and echinolophate [47, 48].
Honey bees collected pollen widely different protein content; but has, in general, high levels of protein (>20%), enough to satisfy the nutritional requirements of honey bees [31]. Andrada & Tellería (2005) [16] showed that within this group, pollen from Brassicaceae and Rhamnaceae have the major protein content, followed by Fabaceae, including *Prosopis* sp.; likewise, different protein content were found in Asteraceae with some species that have low protein content and others species have high protein content. In our study eight species, corresponding to Fabacaeae and Asteraceae families, were analyzed.

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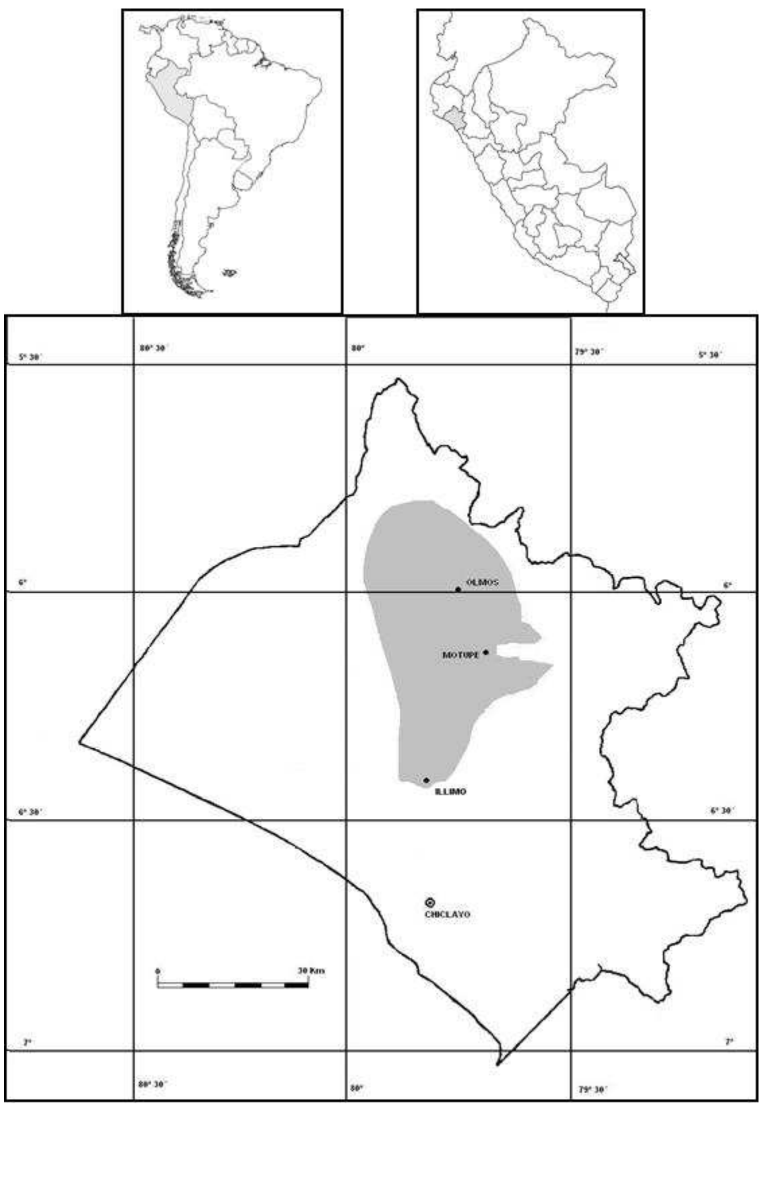

Figure 1. Geographical location of the study area, the Lambayeque province, Peru.

Table 1. Principal pollen types identified in 20 pollen loads collected by honey bees in Lambayeque province.

Family	Species	Popular name	Locality (samples)		
			Íllimo (8)	**Motupe (6)**	**Olmos (6)**
Amaranthaceae	*Alternanthera peruviana* (Moquin) Suessenguth	Paja blanca	X		
Asteraceae	*Baccharis glutinosa* Pers.	Chilco hembra	X	X	X
	Isocarpha microcephala (DC.) Blake	Isocarpa	X		
	Spilanthes leiocarpa DC.	Turre macho	X	X	
Boraginaceae	*Cordia lutea* Lam.	Overo			X
	Heliotropium angiospermum Murray	Hierba de alacrán		X	
Capparaceae	*Capparis avicennifolia* H.B.K.	Vichayo		X	X
	C. crotonoides H.B.K.	Yunto		X	
	C. scabridae H.B.K.	Sapote	X		
Convolvulaceae	*Ipomoea crassifolia* Cav.	Bejuco			X
Fabaceae-Caesalpinoideae	*Caesalpinia glabrata* Kunth	Charán	X		
	Parkinsonia aculeata L.	Azote de cristo	X	X	X
	Pithecellobium multiflorum (H.B.K.) Benth.	Angolo	X		
Fabaceae-Mimosoideae	*Acacia macracantha* H. & B. ex Wild.	Faique	X	X	X
	Prosopis limensis Benth. in Hook.	Algarrobo	X	X	X
Malvaceae-Malvoideae	*Malvastrum tomentosum* (Linnaeus) S.R. Hill	Raja mano			X
Malvaceae-Sterculoideae	*Waltheria ovata Cav.*	Membrillejo			X
Nyctaginaceae	*Commicarpus tuberosus* (Lam.) Standl.	Hierba de la purgación		X	
	Cryptocarpus pyriformis H.B.K.	Chope	X		X
Passifloraceae	**Passiflora edulis* Sims	Maracuyá		X	
Rutaceae	**Citrus limon* (L.) Burm.	Limonero		X	
Salicaceae	*Salix chilensis* Molina	Sauce			X
Verbenaceae	*Lippia nodiflora* (L.) Mich.	Turre hembra	X		

*Exotic species

Table 2. Samples of pollen loads (PL) collected by honey bees in Lambayeque province.

Samples	Location	Botanical origin
PL01	Cruz Verde (Íllimo)	Blossom (monofloral)
PL02	Huaca Bandera (Íllimo)	Blossom (monofloral)
PL03	Jayanca (Íllimo)	Blossom (monofloral)
PL04	La Leche (Íllimo)	Blossom (monofloral)
PL05	La Merced (Íllimo)	Blossom (monofloral)
PL06	La Salina (Íllimo)	Blossom (monofloral)
PL07	Puente Machuca (Íllimo)	Blossom (monofloral)
PL08	Sapamé (Íllimo)	Blossom (monofloral)
PL09	Anchovira (Motupe)	Blossom (monofloral)
PL10	Cholocal (Motupe)	Blossom (monofloral)
PL11	La Viña (Motupe)	Blossom (monofloral)
PL12	Motupe (Motupe)	Blossom (monofloral)
PL13	Prada (Motupe)	Blossom (monofloral)
PL14	Salitral (Motupe)	Blossom (monofloral)
PL15	Corral de Arena (Olmos)	Blossom (multifloral)
PL16	El Porvenir (Olmos)	Blossom (multifloral)
PL17	El Pueblito (Olmos)	Blossom (multifloral)
PL18	El Puente (Olmos)	Blossom (multifloral)
PL19	Insculás (Olmos)	Blossom (multifloral)
PL20	La Noria (Olmos)	Blossom (multifloral)

Table 3. Quantitative analysis in 20 pollen loads collected by honey bees in Lambayeque province.

Species	Íllimo		Motupe		Olmos	
	N° pollen grains/mm^3	*Freq. (%)	N° pollen grains/mm^3	Freq. (%)	N° pollen grains/mm^3	Freq. (%)
Acacia macracantha	7 887.5	7.35	1 987.5	4.70	17 650.0	18.64
Alternanthera peruviana	8 000.0	7.46				
Baccharis glutinosa	1 100.0	1.02	5 875.0	14.16	4 587.5	4.84
Caesalpinia glabrata	4 700.0	4.40				
Capparis avicennifolia			9 875.0	23.36	2 100.0	2.22
C. crotonoides			587.5	1.39	1 425.0	1.51
C. scabridae	21 237.5	19.80				
Citrus limon			700.0	1.66		
Commicarpus tuberosus			1 287.5	3.05		
Cordia lutea					600.0	0.63
Cryptocarpus pyriformis	1 987.5	1.85			3 375.0	3.56
Heliotropium angiospermum			587.5	1.39		
Ipomoea crassifolia					687.0	0.73
Isocarpha microcephala	1 087.5	1.01				
Lippia nodiflora	1 312.5	1.22				
Malvastrum tomentosum					1 125.0	1.20
Parkinsonia aculeata	4 262.5	3.97	1 262.5	2.99	5 900.0	6.23
Passiflora edulis			1 112.5	2.63		
Pithecellobium multiflorum	1 037.0	0.97				
Prosopis limensis	45 412.5	42.33	16 450.0	38.91	55 812.5	58.92
Salix chilensis					1 050.0	1.11
Spilanthes leiocarpa	9 250.0	8.62	2 437.5	5.76		
Waltheria ovata					400.0	0.42

*Freq., frequency

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Table 4. Pollen species and their percentages in the 20 pollen loads collected by honey bees in Lambayeque province.

Locality/ Samples (PL)	Pollen dominant (>45%)	Secondary pollen (16-45%)	Important minor pollen (3-15%)	Trace pollen (1-3%)
Íllimo (average of 8 samples)				
		Prosopis limensis	*Spilanthes leiocarpa*	*Cryptocarpus pyriformis*
		Capparis scabridae	*Alternanthera peruviana*	*Lippia nodiflora*
			Acacia macracantha	*Baccharis glutinosa*
			Caesalpinia glabrata	*Isocarpha microcephala*
			Parkinsonia aculeata	*Pithecellobium multiflorum*
Motupe (average of 6 samples)				
		Prosopis limensis	*Baccharis glutinosa*	*Parkinsonia aculeata*
		Capparis avicennifolia	*Spilanthes leiocarpa*	*Capparis crotonoides*
			Acacia macracantha	**Citrus limon*
			Commicarpus tuberosus	**Passiflora edulis*
				Heliotropium angiospermum
Olmos (average of 6 samples)				
	Prosopis limensis	*Acacia macracantha*	*Parkinsonia aculeata*	*Capparis avicennifolia*
			Baccharis glutinosa	*Capparis crotonoides*
			Cryptocarpus pyriformis	*Malvastrum tomentosum*
				Salix chilensis
				Ipomoea crassifolia
				Cordia lutea
				Waltheria ovata

*Exotic species

Sporadic pollen (< 1%): *Amaranthus spinosus* (Amaranthaceae), *Sarcostemma clausum* (Asclepiadaceae), *Ambrosia peruviana*, *Conyza bonariensis*, *Helianthus annus* and *Tessaria integrifolia* (Asteraceae), *Apodanthera biflora*, *Cucumis dipsaceus* and *Cucurbita moschata* (Cucurbitaceae), *Ricinus communis* and *Chamaesyce hypericifolia* (Euphorbiaceae), *Inga feullei* (Fabaceae-Mimosoideae), *Cajanus indicus*, *Lablad niger* and *Medicago sativa* (Fabaceae-Papilionoideae), *Persea americana* (Lauraceae), *Psittacanthus linearis* (Loranthaceae), *Allium cepa* (Liliaceae), *Gossypium hirsutum* and *Sidastrum paniculatum* (Malvaceae-Malvoideae), *Argemone subfusiformis* (Papaveraceae), *Passiflora foetida* (Passifloraceae), *Sorghum vulgare* and *Zea mays* (Poaceae), *Galvesia fruticosa* (Scrophulariaceae), *Lycopersicon pimpinellifolium*, *Exodeconus prostratus* and *Nicotiana plumbaginifolia* (Solanaceae) and *Lippia alba* (Verbenaceae).

Table 5. Variation in pollen characters of the Peruvian species collected in Lambayeque province.

Species	Polar length (P)/ Equatorial diameter (D) (µm)	P/E	Shape and symmetry	Exine (Sculpture and thickness/µm)	Aperture
Acacia macracantha	34-36	-	Polyad; circular in ouline; 16 grains in number	Psilate to reticulate; > 2,0	Inaperturate
Alternanthera peruviana	14-16	-	Spheroidal; monad, apolar, radiosym-metric	Foveolate; 1,0	Periporate (10 - 12 pores)
Baccharis glutinosa	20-24/15-17	1.37	Prolate; monad, isopolar, radiosym-metric	Echinate; 1,5	Tricolporate; colpi lengthy, moderately broad
Caesalpinia glabrata	40-42/36-38	1.10	Prolate-spheroidal; monad, isopolar, radiosym-metric	Reticulate; 2,5	Tricolporate; colpi lengthy (38 x 5 µm)
Capparis avicennifolia	22-27/15-18	1.48	Prolate; monad, isopolar, radiosym-metric	Reticulate; 1,5 - 2,0	Tricolporate; colpi lengthy, moderately broad
C. crotonoides	24-25/15-16	1.58	Prolate; monad, isopolar, radiosym-metric	Reticulate; 1,5	Tricolporate; colpi lengthy, moderately broad
C. scabridae	24-31/15-18	1.66	Prolate; monad, isopolar, radiosym-metric	Reticulate; 1,5	Tricolporate; colpi lengthy, moderately broad
Citrus limon	24-27/11-18	1.75	Prolate; monad, isopolar, radiosym-metric	Foveolate to reticulate; 1,5 - 2,0	Tricolporate
Commicarpus tuberosus	70-75	-	Spheroidal; monad, apolar, radiosym-metric	Echinate; ± 6,0	Periporate; pores circular, ± 65-70, Ø 3,5 µm
Cordia lutea	40-50/31-35	1.36	Subprolate to prolate; monad, apolar, radiosym-metric	Micro-reticulate; 1,0 - 1,5	Tricolporate; colpi lengthy, moderately broad
Cryptocarpus pyriformis	15-27/11-20	1.35	Prolate;	Micro-echinate;	Tricolpate

			monad, isopolar, radiosym-metric	± 1,0	
Heliotropium angiospermum	20-25/14-19	1.36	Prolate; monad, isopolar, radiosym-metric	Psilate; 2,5	Tricolporate; colpi lengthy, moderately broad
Ipomoea crassifolia	75-80	-	Spheroidal; monad, apolar, radiosym-metric	Echinate (echina 5 µm); 3,0 – 3,5	Periporate (160 - 165 pores)
Isocarpha microcephala	11-15	-	Spheroidal; monad, apolar, radiosym-metric	Echinate; 1,0	?
L. nodiflora	20-25/16-20	1.25	Subprolate to prolate; monad, isopolar, radiosym-metric	Psilate; 1,0 -1,5	Tricolporate; colpi lengthy; pores circular, Ø 2,0 µm
Malvastrum tomentosum	16-20	-	Spheroidal; monad, apolar, radiosym-metric	Echinate (echina > 5 µm); > 2,0	Periporate (12 - 24 pores)
Parkinsonia aculeata	20-27/18-20	1.23	Subprolate; monad, isopolar, radiosym-metric	Reticulate; 1,5	Tricolporate colpi lengthy (28 x 2 µm)
Passiflora edulis	47-67/43-58	1.12	Prolate spheroidal; monad, isopolar, radiosym-metric	Reticulate; 1,5	?
Pithecellobium multiflorum	80-70	-	Polyad; ellipsoidal in ouline; 20 grains in number	Reticulate; 2,0	Inaperturate?
Prosopis limensis	25-31/15-20	1.60	Prolate; monad, isopolar, radiosym-metric	Psilate; 1,5	Tricolporate; colpi lengthy; pores circular, Ø 4,5 µm
Salix chilensis	15-20/9-15	1.45	Prolate; monad, isopolar, radiosym-metric	Reticulate; 1,2 – 1,5	Tricolporate; colpi lengthy
Spilanthes leiocarpa	15-22/13-18	1.19	Subprolate; monad, isopolar, radiosym-metric	Echinolophate (echina 5 µm); 1 - 1,5	Tricolporate; colpi lengthy
Waltheria ovata	45-55/37-45	1.21	Subprolate; monad,	Micro-echinate; 1,0	Stephano-colporate

		isopolar, radiosym-metric	

Table 6. Moisture, ash and crude protein content of pollen loads collected by honey bees in Lambayeque province.

Locality	Moisture (%)	Ash (%)	Crude protein (%)
Íllimo	21.64	4.72	20.24
Motupe	19.13	4.28	19.75
Olmos	21.53	5.07	21.63
Mean	20.76	4.69	20.54

Table 7. Amino acids content of pollen loads collected by honey bees in Lambayeque province.

Amino acids (%)	Locality			Mean
	Íllimo	Motupe	Olmos	
Phenylalanine	6.69	6.92	7.00	6.87
Histidine	5.23	5.61	5.69	5.51
Isoleucine	6.23	6.77	6.77	6.54
Leucine	6.69	6.85	6.92	6.82
Lysine	3.69	3.69	3.85	3.74
Methionine	6.31	6.69	6.77	6.57
Threonine	4.15	4.38	4.46	4.33
Valine	6.07	6.15	6.23	6.15
Mean	5.63	5.87	5.96	

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