Morphological Characterization and Species Distribution Modeling of Honey Bees (*Apis florea and Apis dorsata*) in District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan

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Morphological Characterization and Species Distribution Modeling of Honey Bees (*Apis florea and Apis dorsata*) in District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan

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"In the Name of ALLAH, the most beneficent, the most Merciful"

بسم الله الرحمن الرحيم

Dedication

This thesis is dedicated to my parents' deepest gratitude whose love, & and prayers have always been a source of strength for me.

DECLARATION

I hereby declare that the work presented in the following thesis is my effort and the material contained in the thesis is original work. I have not previously presented any part of this work elsewhere to any other degree.

NABEEL UR REHMAN

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I am highly grateful to **Allah Almighty** *for His countless blessings throughout my life helping me to achieve my goals and giving me the strength in the completion of this M.Phil. dissertation. May Allah guide me on the right path (Ameen). Peace and blessings are upon the* **Holy Prophet Hazrat Muhammad (PBUH),** *the most perfect among all human beings ever born and Who guided his followers to seek knowledge from cradle to grave.*

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Nabeel Ur Rehman

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Abbreviations	Full Name
ABPV	Acute Bee Paralysis Virus
AFB	American foulbrood
AUC	Area Under Curve
BQCV	Black Queen Cell Virus
CBPV	Chronic Bee Paralysis Virus
DWV	Deformed Wing Virus
EFB	European Foulbrood
FR	Frontier Region
ft	feet
KBV	Kashmir Bee Virus
Kg	kilogram
Mya	millions year ago
ROC	Receiver Operating Characteristics
SBV	Sacbrood Virus
SDM	Species Distribution Modeling

ABSTRACT

Honey bees serve as an essential natural source of various products, including honey, royal jelly, wax, pollen, and propolis which are very important medicinally as well as economically. Besides this, their crucial role in achieving sustainable agricultural productivity by effectively pollinating plants and ensuring successful plant reproduction is also astonishing. The biodiversity and preservation of honey bee races depend greatly on morphological characteristics. Studying the morphological characteristics of honey bees holds significant importance in discerning and conserving various subspecies or races, contributing to the preservation of biodiversity. Therefore, this research work was conducted to identify major honey bee species in District Lakki Marwat using morphological characteristics. As honey bees are sensitive to environmental variables and climate changes. Very little information is available about honey bee species distribution, habitat suitability, and variables affecting their distribution in the study area. Therefore, in this study, the distribution pattern and habitat suitability of honey bee species (*Apis florea* and *Apis dorsata*) in District Lakki Marwat associated with various environmental variables were determined using the MaxEnt model. Using recorded data (present-only data) and climatic variables, a potential distribution map was generated using the MaxEnt model. According to the results, mountain regions with sufficient nectar and nutrient resources were found highly suitable habitats for giant honey bees (*A. dorsata*). Although dwarf honey bees (*A. florea*) were recorded from the mountain regions, these species were also recorded from other plain regions throughout the study area. Therefore, it was concluded that the most suitable habitats for *A. florea* were arid regions with hot summers and mild winters like the climate of District Lakki Marwat. Environmental Variables such as temperature seasonality, precipitation seasonality, precipitation of the driest month, precipitation of the wettest month, and the mean temperature had a considerable percent contribution to the model simulation and showed the greatest impacts on contributions on the distribution of honey bee species (*A. florea* and *A. dorsata*). This research work analyzes important environmental factors impacting species distribution and assisting in identifying potential beekeeping areas.

INTRODUCTION

Honey bees are social insects, belonging to the genus *Apis,* order Hymenoptera, and family Apidae. Honey bees are one of the most valuable pollinators in the world. They are responsible for pollinating a variety of crops, such as fruits, seed plants, vegetables, and nuts. They play a crucial role in pollination, helping to fertilize plants and flowers and other varieties of crops, which in turn ensures a healthy and diverse ecosystem. About one-third of all plants consumed by humans are pollinated by bees which are responsible for three-quarters of the world's major food crops (Kevan & Phillips, 2001; Klein *et al.,* 2003).

In addition to giving pollination services, honey bees also produce honey, and royal jelly, which are used as food as well as play an important role in medicines and cosmetics. Other major bee products like bee venom, propolis, pollen, and wax are also very important economically and play a major role in the medicinal and pharmaceutical industries (Michener, 2000).

1.1 Biology of honey bees

Honey bees are little, buzzing insects with characteristic black and yellow stripes on their bodies. Honey bee anatomy comprises the head, thorax, and abdomen. The head has simple and compound eyes, two antennae, mouthparts, including a proboscis, which is a long, slender tongue used to help in nectar collection from flowers. The thorax, which holds three pairs of legs and two pairs of wings, controls movement. The abdomen contains the reproductive organs, digestive system, and honey-storing structures. The distinguished characters like branched, typically plumose, hairs and basitarsi of hind legs that are wider than subsequent tarsal segments differentiate honey bees from the other members of the family. In general, honey bees have a longer proboscis than wasps (Michener, 2000).

Bees developed eusociality from sub-social and solitary behavior. In sub-social insects, a female starts a colony by laying eggs and caring for the young, while in solitary insects, the mother passes away without seeing her young offspring, after gathering enough food for them. Being a social insect, honey bees live in a highly organized colony that contains thousands of individuals and share some of the common characteristics such as caring for brood collectively and cooperatively, division of labor for reproduction and a single colony overlaps several generations (Wilson & Hölldobler, 2005; Srinivasan, 2010).

1.2 History of Honey Bees

Insects were first discovered 350 million years ago, during the Carboniferous Period. Insect fauna changed during the Permian, Mesozoic, Triassic, and Jurassic eras. Many insects exhibiting social behaviors, such as ants and specoid wasps were discovered associated with flowering plants during the Cretaceous period. The current bee fauna has existed for more than 70 million years since the Cretaceous period (Srinivasan, 2010).

During the early Cretaceous period, it is believed that wasp-like ancestors gave rise to bees around 130 million years ago. The earliest bees were solitary and lived in burrows dug into the ground. They ate nectar and pollen from gymnosperm flowers, which were the dominant plants at the time, in their burrows. Approximately 120mya, honey bees evolved with modifications in morphology and behavior to optimize their ability to collect pollen and nectar from flowers (Angiosperms) and ensure the survival of their colony (Gupta, 2014; Sann *et al.,* 2018).

Honey bees and humans coexisted for a long time since the Stone Age. Honey bees' contribution to crop pollination and the production of honey, a sweetener and food source are beneficial to humans (Chirsanova *et al.,* 2021; Kritsky, 2017).

1.3 The Castes

A honey bee colony is a highly organized and complex social structure comprising of a queen, workers, drones, and comprises approximately 50000-60000 individuals and may be up to 100000 (Winston, 1987).

1.3.1 Queen:

Each honey bee colony has a single queen. Its role is to lay eggs and ensure the colony's survival. The queen bee can lay up to 2,000-25000 eggs per day and can live up to several years. The queen is often larger in size compared to the worker bees and has a longer lifespan. The queen also secretes pheromones that influence the behavior of the other bees in the colony, maintaining harmony and order (Winston, 1992). The size of the queen's head and thorax are similar to those of the worker honey bees, but the queen possesses a longer and more robust abdomen in comparison to worker bees (Mortensen *et al*., 2015). The health and longevity of the queen bee are crucial for the survival of the colony. If the queen bee dies, the colony will eventually dwindle and collapse (Amiri *et al.,* 2017). Unsurprisingly, lower, and poor queen bee quality is one of the reasons for increasing colony decline which is continuously reported all over the world (Meixner, 2010). A new queen bee could be effectively produced from worker larvae three days old, however, a queen produced from old larvae exhibits reduced body weight and size. Both queen and workers Both queens and workers develop from fertilized eggs, but due to the quantity of food and nutrition given to young larvae, initiate distinct epigenetically controlled pathways of development (He *et al*., 2017). Adult worker bees primarily feed on pollen and nectars which are stored in a colony as bee bread and honey. In addition to these, the queen bee is also given royal jelly. Royal jelly is the main food, given to growing larvae, which will then develop into queen. The hypopharyngeal and mandibular glands of bees produce royal jelly, which is placed by nurse bees into the queen cell as well as the worker cell. The queen bee received an enormous amount of this food, while worker larvae were fed for upto three days (Wright *et al*., 2018).

1.3.2 Worker

Honey bee workers are typically sterile females who do not lay eggs and perform the majority of tasks within a honey bee colony. These are responsible for collecting nectar and pollen from flowers, building, and repairing the comb, caring for the queen and her offspring, cleaning and maintaining the hive, defending the colony from predators, and processing and storing honey. Tasks performed by each honey bee worker are determined by their age. Young and old honey bee workers have different roles in the colony. Young worker bees, also known as nurse bees, take care of the queen and the brood (larvae and pupae). These bees feed and clean the larvae, maintain the temperature and humidity of the colony, and secrete royal jelly to feed the queen. Old worker bees, on the other hand, have different tasks such as foraging for pollen and nectar, constructing and repairing the comb, and guarding the colony (Winston, 1987).

1.3.3 Drone

Drones are the males of a honey bee colony, distinguished by their larger heads and thorax in contrast to females. Their large eyes meet in the high middle of their head, giving them a "fly-like" appearance. The abdomen of the drone is characterized by thickness and bluntness at the end, in contrast to the pointed abdomen of the females. Their primary role is to mate with the queen of the colony (Mortensen *et al*., 2015).

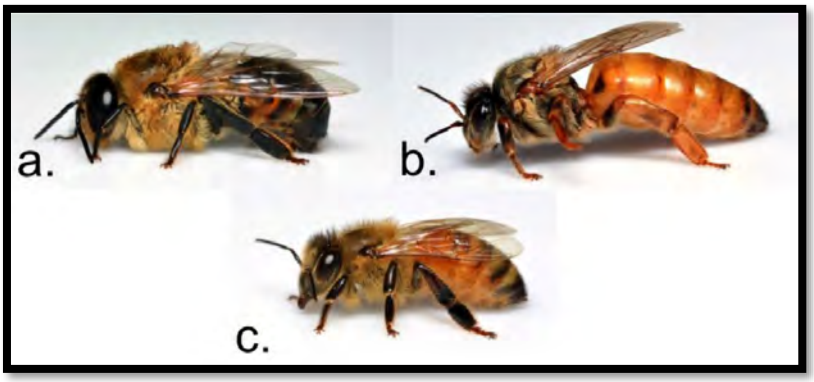

Figure 1.1: Castes of honey bees (a. Drone b. Queen c. Worker)
(Mortensen *et al*., 2015)

1.4 Life Cycle of Honey Bees

Like many other insects, honey bees also develop through complete metamorphosis, and their life cycle is divided into four stages (egg, larva, pupa, and adult). This whole process takes place in a cell of a comb made up of wax (Wang *et al*., 2015).

1.4.1 Eggs

Eggs exhibit a small, poppy seed-like shape and possess a glossy white appearance. A minute opening referred to as a micropyle is situated at the wider end of the egg, which serves as the entry point for sperm The egg typically hatches within 3-4 days, giving rise to small, microscopic larvae (Fisher *et al*., 2014). Honey bees have a unique reproductive system in which fertilized eggs can develop into either workers or queens, depending on their nutrition and care throughout development (Martin *et al*., 2019). If the fertilized egg is laid in a normal cell and fed pollen and nectar, it will develop into a worker bee. However, if the fertilized egg is laid in a specific cell called a "queen cell" and given a particular diet of royal jelly, a protein-rich secretion produced by worker bees, it will develop into a queen bee. Unfertilized eggs emerge and develop into male drone caste (Winston, 1987; Remolina & Hughes, 2008; He *et al*., 2021).

1.4.2 Larvae

Honey bee larvae show a resemblance to small, curved, segmented grubs without legs, displaying a pale whitish color. This larvae phase lasts for approximately nine days. During this stage nurse bees provide honey, pollen, and royal jelly as food to these larvae, however after three days only those larvae that will become queen are exclusively given royal jelly by worker bees while the remaining larvae that will become worker or drone feed on pollen, honey, and water only. Throughout the larval stage, these tiny creatures undergo five molting phases. After this stage, larvae excrete and turn a cocoon all over their body. This next stage is called pupa (Fisher *et al*., 2014).

1.4.3 Pupa

During the pupal phase, there is a significant process of body tissue specialization. The body transforms into its adult form, characterized by three distinct and prominent regions, the head, thorax, and abdomen (Michener, 2000).

Queens develop from the egg stage in 16 days, workers in 21 days, and drones in 24 days (Page *et al*., 2001).

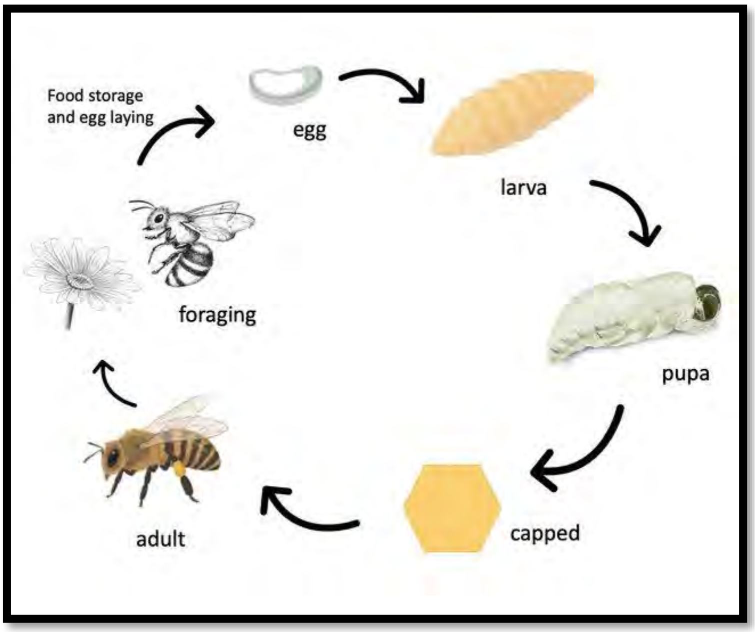

Figure 1.2: Life cycle of honey bee (Chen *et al*., 2021)

1.5 Foraging Behavior of Honey Bees

Foraging behavior is an important factor in effective cross-pollination and varies between pollinator species and even within species. The intensity of cross-pollination is primarily determined by foraging rate, foraging speed, pollinator strength, and pollinator variety (Shivashankara *et al*., 2016). Honey bee pollination services have the potential to increase seed production in sunflowers and potentially benefit other crops that bear fruits and seeds. Depending on the specific cultivar, honey bee pollination may increase fruit set percentage and fruit yields by 10 to 25 percent and 18 to 100 percent respectively (Said *et al*., 2018).

Foraging is done entirely by a particular caste of sterile female workers in honey bee colonies. The flower is never visited by the queen or the drones. A division of honey bee workers performed collective foraging. Some workers search for nectar, while others collect pollen. Some honey bees leave the hive early in the day to search for new foods, while others leave in response to scout or other forager recruiting (Wright *et al*., 2018; Lemanski *et al*., 2019).

1.6 Species

The genus *Apis* contains ten species of honey bees that are well-recognized (Hussain *et al.,* 2018). Honey bees may be classified into three separate groups or lineages, according to phylogenetic studies using mitochondrial (mtDNA) and nuclear DNA markers, giant honey bees (*A. dorsata*, *A. laboriosa*), dwarf honey bees (*A. florea, A. andreniformis*) and cavity-nesting honey bees *A. mellifera, A. cerana, and A. koschevnikovi*) (Arias & Sheppard, 2005). Certain distinctive characteristics are observed in worker honey bees belonging to the genus *Apis*. These are (1) the presence of erect long hairs on compound eyes, (2) a highly convex scutellum, (3) the presence of pollen press on the hind leg, (4) prominent extended marginal (peripheral) and submarginal cells on the forewing, (5) the presence of jugal lobe on the hind wing, (6) absence of hind tibial spurs (7) the presence of bifurcated claws in the female bees (Michener, 2007).

1.6.1 Giant Honey Bees (*A. dorsata* and *A. laboriosa*)

Apis dorsata, often known as the rock honey bee, is a species of honey bee found throughout South and Southeast Asia, including India, Pakistan, Sri Lanka, Nepal, Bangladesh, Bhutan, Myanmar, Thailand, Laos, Vietnam, and the Philippines (Oldroyd & Nanork, 2009). This species has a dark brown or black body with yellow stripes on its abdomen (Wongsiri *et al*., 2012). Workers exhibit a yellow body their thorax is black while the scutellum has a brown coloration pattern. The first abdominal segments are mostly yellow-orange while the last three abdominal segments appear brownish-black. The thorax of the queen is dark brown, while the scutellum appears a lighter brown color. The thorax and scutellum of a drone are dark brown and brown respectively, with a darker hue as compared to a queen (Woyke, 1997). It is known to inhabit mountainous regions, such as the Himalayan highlands, and can be found at altitudes ranging from 1000 to 1700 meters above sea level, and they can even go up to 2000 meters during seasonal migration to find new nesting sites or food sources (Ruttner, 1988; Ruttner, 2013). It often makes its nests high in the air, between 3 and 25 meters above the ground (Gupta, 2014). *A. dorsata* combs are rather enormous, measuring 1.5 to 2.1 meters (4.9 to 6.9 feet) side to side and 0.6 to 1.2 meters (2.0 to 3.9 feet) top to bottom (Haldhar *et al*., 2021). This species is of great economic importance as the honey produced by this species is considerably higher than other species of honey bees. The honey yield from a single colony can range from 50-80 kg (Kishan *et al*., 2017).

1.6.2 Dwarf Honey Bees (*A. florea* and *A. andreniformis*)

The dwarf honey bees, *A. andreniformis,* and *A. florea* are two closely related species of honey bees that have a partially overlapping geographic distribution in southern Asia. They are both subtropical and tropical honey bees that construct a single comb in the open, rather than multiple combs in cavities, which is the characteristic behavior of other more widespread honey bee species that occupy colder climates (Hepbur & Radloff, 2011). *A. florea* workers are typically reddish-brown or rufous in appearance, while *A. andreniformis* workers are predominantly black. This physical characteristic can be used to distinguish the two species of dwarf honey bees in the field (Yan & Bangyu, 1987; Higgs *et al.,* 2009). *A. andreniformis* possesses black hair stripes on the metathoracic tibia and the dorsolateral surface of the hind basitarsus, in contrast to *A. florea*'s white hair. Furthermore, *A. andreniformis* has a blackish pigmentation, whereas *A. florea* has a yellowish pigmentation, giving the appearance that *A. andreniformis* are mostly black bees and *A. florea* are predominantly yellow bees (Smith, 1857; Wongsiri *et al.,* 1997).

Workers of *A. florea* have yellow-colored bodies, which means they have yellow bands on their abdomens. They are predominantly yellow-orange, while the thorax and scutellum regions are black in color. Their queen also exhibits the same coloration pattern, although the light color covers a larger area and extends over more segments of the abdomen. The queen appears lighter in comparison to the worker due to the black apical segment. The thorax, scutellum, and abdomen of the drone honey bee are all black, giving the overall appearance of the drone's body black (Woyke, 1997).

Both species of bee are found throughout Asia. These are often found in warm climates. The species are found in India, Sri Lanka, Pakistan, Oman, Iran, the Indo-China area, Malaysia, the Philippines, Indonesia, Vietnam, and Burma. These species of honey bee are known to be unique for their morphology, defensive mechanism, foraging, and nesting behaviors. They build small and open nests in exposed locations such as tree branches, shrubs, and under-building eaves (Ruttner, 2013; Zewdu *et al.,* 2016; Radjabi *et al.,* 2018).

Figure 1.3: Hive (Nest) of *Apis florea*

Apis florea has been observed to build their nests at different heights ranging from 0.3 to 9.0 meters above the ground. The comb is relatively small, usually less than 1ft long, and contains approximately 14,000 individual cells (Bezabih *et al*., 2014). A healthy colony has around 10,000 bees. The foraging activity of the bees starts early morning; however, it increases and reaches the maximum level from 2:00 PM to 4:00 PM (Rod & Duangphakdee, 2020). The amount of honey produced from a colony of *A. florea* is less than 1 kg per year. This amount is varied and even makes up to 8 kg annually (Thapa, 2003).

1.6.3 Eastern/ Asian Honey Bee (*Apis cerana*)

The Indian or Asian honey bee, *A. cerana* is found all throughout temperate and tropical Asia, from Afghanistan through Korea and Japan, north into the Himalayan foothills and eastern Russia. It is one of the most widely distributed honey bee species in Asia, which is native to several countries in Asia including India, Pakistan, Bhutan, Bangladesh, Myanmar, Indonesia, Nepal, Myanmar, Vietnam, and the Philippines (Ruttner, 1988; Dar *et al*., 2019; Haldhar *et al*., 2021). It has been domesticated in several countries in these regions, including India, Pakistan, Nepal, Thailand, Indonesia, and the Philippines. It is larger in size compared to the dwarf honey bee (*A. florea*), however, it is smaller in size compared to the western honey bee (*A. mellifera*) and giant honey bee (*A. dorsata*) (Kishan *et al*., 2019). *A. cerana* is similar to an *A. mellifera* however there is one key difference in the distal abscissa of the vein-M in the hind wing. The distal abscissa of vein-M in the hind wing of *A. mellifera* is missing however in *A. cerana* the hind wing has a clear distal abscissa of vein-M (Engel, 2012; Rojas-Sandoval, 2022).

The worker of *A. cerana* exhibits a yellow coloration pattern. However, in some workers, there is a minimal light coloration pattern present on their abdomen. The first abdominal terga are either completely orange or possess black bands along their posterior margins. The final segment of the abdomen is black. The queen's thorax of *A. cerana* is black while their scutellum is brownish blackish, distinguishing it from their workers. The drone's thorax also exhibits a black coloration pattern. All the abdominal segments and scutellum are also brownish-blackish (Woyke, 1997).

Colonies of *A. cerana* are generally found in cavities such as tree hollows, rock holes, or building walls. *A. cerana* builds several parallel combs that hang from the top of the hive. Each comb has a central mid-rib with six-sided cells attached to the mid-ribs lateral sides. A colony produces 3-5 kg of honey per year on average, although yields can be as high as 10-20 kg (Leven *et al*., 2005).

1.6.4 European Honey Bees (*Apis mellifera*)

Apis mellifera is an extensively domesticated bee species often known as the European honey bee or the Euro-African honey bee. The species is native to Europe, Africa, and the Middle East, but it has been introduced to other regions of the world, including North America, South America, Australia, and portions of Asia (Chapman & Bourke, 2001; Park *et al*., 2014). The body of *A. mellifera* is brown and black with yellow-orange stripes on the abdomen. The bee's body is often covered with light brown or golden-yellow hair. This species often formed multi-comb nests in confined cavities, tree hollows, walls, or shaded areas. On average this species yields 50-60 kg of honey per year (Yadav *et al*., 2017).

1.7 Importance of Honey Bees

Honey bees are an important natural supplier of various items, including honey, wax, and royal jelly. Furthermore, their contribution to the environment is significant, particularly in terms of pollination services, which are critical for food production and agriculture. Beekeepers generally maintain honey bees for the purpose of producing honey and providing pollination services for a profit. Apiculture or beekeeping has the potential to deliver enormous economic benefits to developing countries, particularly rural communities during financial crises (Naz *et al*., 2022).

Bee products have been used for therapeutic purposes since ancient times. Honey, wax, propolis, and other bee products are extensively consumed as food and used in medicinal treatment. The usage of honey to treat diarrhea was even proposed by the respected Muslim Prophet Muhammad (PBUH), with a whole chapter in the Quran called 'Al-Nahl' dedicated to the benefits and significance of bees. Honey is a source of energy and a dependable sweetener for both humans and honey bees (El-Aidy *et al*., 2015). Propolis exhibits excellent pharmacological properties and is utilized as an effective antimicrobial agent to combat various microorganisms including viruses, fungi, molds, and bacteria (Wagh, 2013). Royal Jelly is a very nutritious and well-balanced food source for honey bee larvae. In addition to moisturizing, flying, and stabilizing capabilities, it is known to have antitumor and anti-inflammatory effects, As well as hypotensive and antifatigue action. Furthermore, it has excellent antioxidant and antimicrobial characteristics and can boost immunological function (Nagai & Inoue, 2004). Venom immunotherapy utilizing honey bee venom has been used to treat a variety of conditions including arthritis, multiple sclerosis, back pain, and other diseases (Naz *et al*., 2022).

Pollination is largely recognized as the most important ecosystem service providing control, support, and cultural benefits to the ecosystem. It is essential to produce vegetables, fruits, seed crops, nuts, and cotton as well as for the reproduction of wild plants. Most of the pollinations take place through bees all over the world, i.e., 70% and even up to 85% are also recorded. Honey bees have been recognized as the most effective pollinators for cultivated crops due to their unique body features that allow them to efficiently capture pollen grains (Devkota *et al*., 2016).

1.8 Threats to Honey Bees

In recent times honey bees and their well-being have gained significant attention due to their crucial role in pollination and food production. Honey bees are vulnerable to various environmental threats and diseases, with certain parasites and diseases having been found to contribute significantly to colony mortality and losses (Genersch, 2010).

The parasitic mite *Varroa destructor* affects both brood and adult bees, resulting in varroosis, also known as parasitic mite syndrome. The severity of varroosis relies on both the level of mite infestation in a given colony and the transfer of viral infections from parasite mites to individual bees. Without adequate treatment, varroa-infested colonies in temperate areas usually die within two years. As a result, varroa treatment has become a crucial part of beekeeping methods, as it helps to maintain infection levels under control and prevents colony losses caused by this parasite from reaching the damage threshold (Reams & Rangel, 2022).

Honey bees are threatened significantly by viruses that can affect them at various stages of development and across different castes within their colonies. The presence of viruses is a serious problem for the health and well-being of honey bees. Out of the 18 viruses known to affect honey bees, six are particularly prominent and can cause harmful infections that threaten the health of the entire colony. These viruses include the Deformed wing virus (DWV), Black queen cell virus (BQCV), Sacbrood virus (SBV), Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV), and Chronic bee paralysis virus (CBPV). Studies have shown that viruses that cause infections in honey bees can also be transmitted to other bee species, leading to their decline. In wild bees, viral infections can cause physical deformities, reduced reproductive capability, greater susceptibility to infections, and a higher risk of mortality (Chen & Siede, 2007; Ullah *et al.,* 2021).

In honey bee colonies, only two bacterial infections have been found, both of which are damaging to honey bee larvae but not to adult bees. The first is *Melissococcus plutonius*, which causes European Foulbrood (EFB), and the second is *Paenibacillus larvae*, which causes American Foulbrood (AFB). These are two common bacterial infections that harm honey bees all over the world, causing considerable economic issues and colony losses in the beekeeping business. These diseases typically affect the larval and pupae stages of honey bees, resulting in an unpleasant odor and the progressive decrease of the infected colony, eventually leading to its mortality (Genersch, 2010; Forsgren *et al.,* 2018).

Pesticides have been recognized as one of the major threats to honey bees (Halvorson *et al.,* 2021). Pesticides, including insecticides, herbicides, and fungicides, are frequently used in agriculture to protect crops from pests, diseases, and weeds. However, some pesticides, particularly certain classes of insecticides called neonicotinoids, have been found to have detrimental effects on honey bees and other pollinators (Tudi *et al.,* 2021). The cumulative effect of honey bee exposure to pesticide residue in brood comb can result in a variety of adverse consequences. These include shorter adult bee lifespans, greater brood mortality rates, the increased reproductive potential of *Varroa* mites (owing to delayed development and emergence of adult bees), and increased sensitivity to diseases. These factors lead to a reduction in the general health of honey bee colonies because the afflicted queens and worker bees are unable to satisfy the demands for brood generation and the resources needed to support big colony numbers (Wu *et al.,* 2011).

Honey bees may be affected by climate change in various ways. The physiology and behaviors of honey bees may be directly impacted by climate change. It can change the quality of the floral environment, as well as raise or decrease colony harvesting capability and improvement (Le *et al*., 2008). Assessing potential habitats and studying distribution patterns are the main points in ecological research, which includes studies on the environment, animal protection, and the evaluation of changes at various scales. Species distribution prediction modeling is now one of the most essential tools for creating potential maps. These models illustrate the links between environmental conditions and the existence or absence of species (Parichehreh *et al*., 2022).

1.9 Species Distribution Modeling

Species distribution models (SDMs) are mathematical techniques that integrate measurements of species occurrence or abundance with environmental estimations. It is used to gather ecological and evolutionary insights as well as anticipate distributions across landscapes, which often requires spatial and temporal extrapolation. SDMs are currently widely applied in freshwater, marine, and terrestrial ecosystems (Elith & Leathwick, 2009). Species distribution models offer a wide range of practical uses, including assessing the possible risk posed by pests or invasive species, acquiring knowledge about species biology and biogeography, identifying areas with high concentrations of endangered species, and predicting biodiversity (Beaumont *et al*., 2005). These are frequently utilized in conservation biology, biogeography, and ecology for a variety of objectives (Elith *et al*., 2011).

Modeling a species' geographic distribution is a key topic in ecology. A variety of modeling approaches have been established, the most well-known of which is MaxEnt, a maximum entropy modeling approach (Renner & Warton, 2013). The MaxEnt software program is one of the essential tools for species distribution modeling and ecological niches. This software is popular because of these two factors: 1) MaxEnt consistently overcomes other approaches in terms of predicted accuracy, and 2) the program is extremely simple to use (Phillips *et* al., 2006; Merow *et al*., 2013). This software package is used to find the maximum entropy distribution probability and may be used to estimate the distribution of a target species (Phillips *et* al., 2006).

Most of the previous research studies conducted on distribution modeling have primarily focused on various species, neglecting the exploration of distribution modeling of specific species in Lakki Marwat. Consequently, no prior research has been conducted to explore the distribution patterns of honey bees in the Lakki Marwat region. The purpose of this study was to address this gap and improve our understanding of the actual and potential presence of honey bees in Lakki Marwat. The species distribution models developed as a result of this study will give significant insights into the possible habitats and ranges where honey bees are likely to be found, supporting conservation planning and implementation.

Aim and Objectives

This study aimed at morphological characterization and species distribution modeling of honey bees (*Apis florea* and *Apis dorsata*) in District Lakki Marwat.

Objectives

1. Biodiversity of honey bees based on morphometry in district Lakki Marwat
2. To apply the MaxEnt modeling technique for predicting species distribution and suitable habitats of honey bees (*A. florea* and *A. dorsata*).

MATERIALS AND METHODS

2.1 Study Area

District Lakki Marwat is situated in the South of Khyber Pakhtunkhwa, Pakistan between 32.161° N latitude and 79.191° E longitude. It lies at a height of 200-1000m from sea level. The district covers a total area of 3164 Km^2. Its borderlines connect with districts Bannu and Karak to the north. It touches the boundaries of the district Mianwali of Punjab to its east as well as Frontier Region (FR) Bhattani to its west and district Dera Ismail Khan to the South. The district is surrounded by hills and mountains while the center area is plain. The climate in the district is steppe, with warm and dry summers and mild winters. Summer is the longest season of the year. This season starts in late April and finishes in October. In June, maximum temperatures reported ranged from 42°C to 45°C. Windy storms swept over the area from May to July.

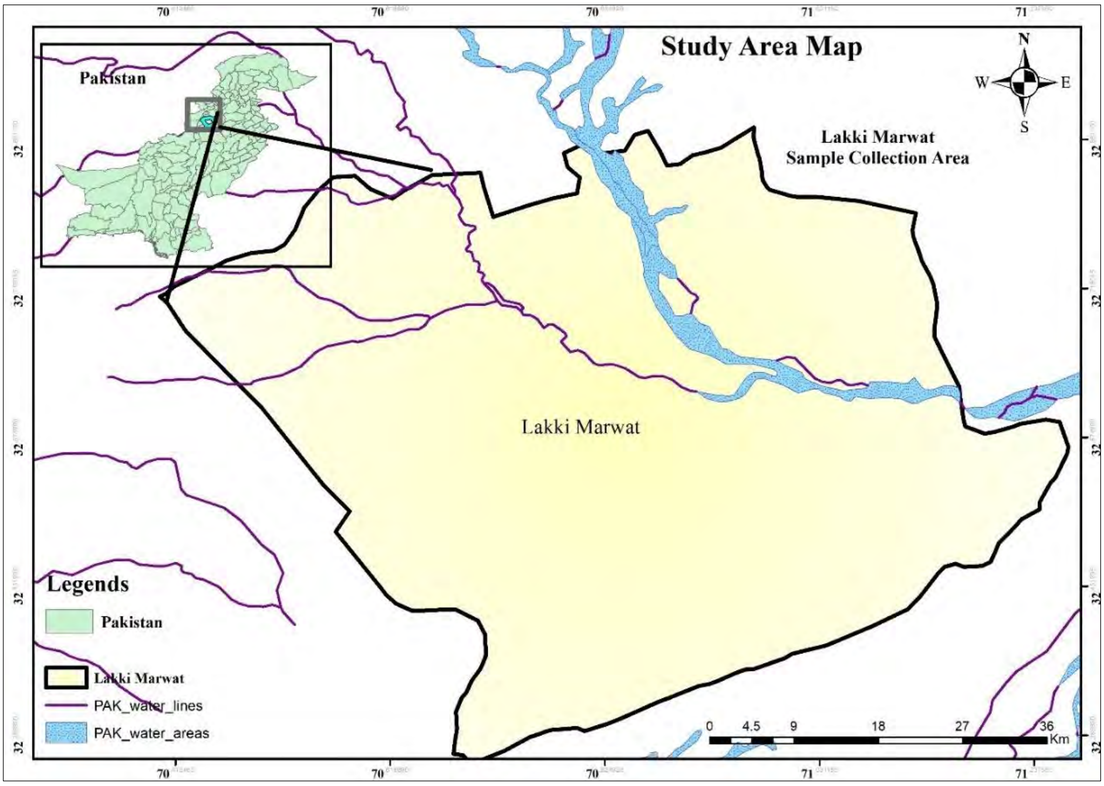

Figure 2.1: Study Area Map

2.2 Collection of Samples (Honey Bees)

Samples of honey bees were collected randomly from different regions in District Lakki Marwat with the help of a sweeping net. These regions were mountains and desert plain areas. Honey bees were captured and preserved individually in little plastic or glass containers with 70% ethanol (Figure2.4) labeled accordingly. The stored samples were taken to the Parasitology and Entomology lab, Quaid-I-Azam University, Islamabad for additional morphometric analysis to identify the species of honey bees collected from hives at different sites in the study area.

Figure 2.2: Collecting Samples (Honey bees)

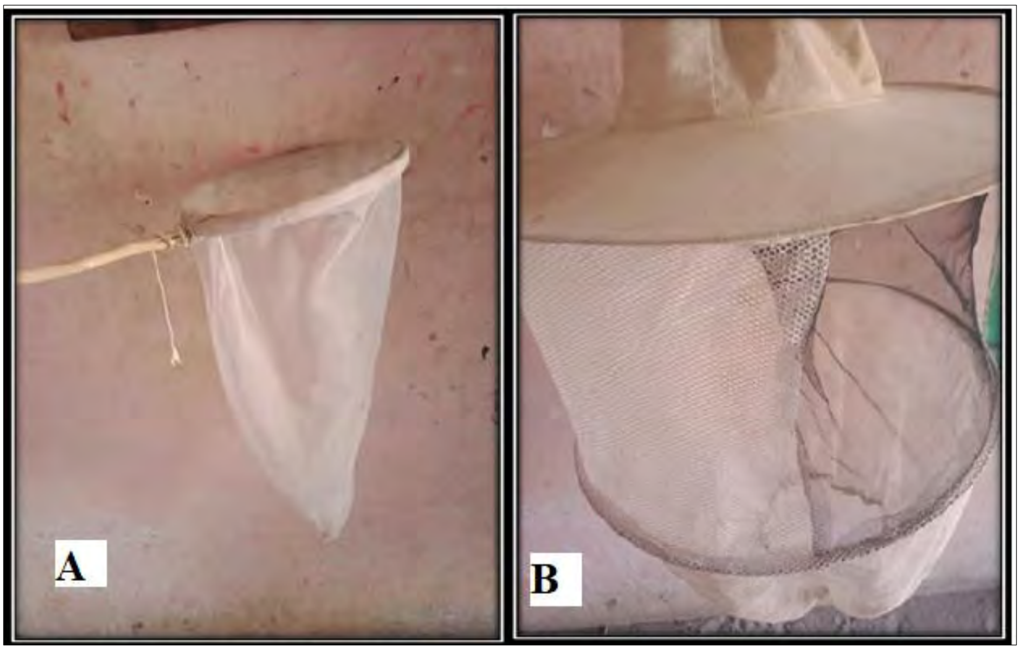

Figure 2.3: A. Sweeping net B. Cap

Figure 2.4: Preserved honey bee samples

2.3 Morphological Identification

The ethanol-preserved honey bees were rinsed with distilled water, kept in a Petri dish, and put in an incubator for 5 to 10 min at 37o C to dry. Honey bees were identified morphologically up to species level according to standard characters and Asian honey bees' manual techniques [Ref. 1] and Ruttner key (Ruttner, 1988 and

Ruttner, 2013) for identification under the stereomicroscope (OPTIKA, SN 355093). Different body parts e.g., body length, forewing length and width, hindwing length, hindwing width, hindleg length, foreleg length, and antennal length were measured according to the (Ruttner, 1988; Ruttner, 2013). The external morphology of the above body parts of honey bees was measured with a calibrated ocular microscope and scale.

2.4 Data Analysis

In terms of relative abundance, collected honey bees were analyzed using the given formula.

$$Relstaive\ Abundance = \frac{n}{L} \times 100$$

Where n= number of each species specimens

L = Total number of samples

2.5 Modeling Procedure

2.5.1 Selection of Present Data and Environmental Variables

A total of 30 presence points of the honey bees (*Apis florea and Apis dorsata*) in the study area were recorded for the MaxEnt analysis. The presence-only data were screened in ArcGIS 10.8 (SDM toolbox). Data on the environmental variables were downloaded from Worldclim (https://www.worldclim.org/). Initially, 19 variables were selected. All the variables were run through the SDM toolbox to check for correlation among the variables. A pairwise correlation matrix with a threshold of 0.7 was carried out for this purpose. Variables with high impact on the distribution of the species were retained and others were excluded if the correlation was equal to 0.7 or higher. Finally, only 9 bioclimatic variables (Table 2.1) were selected and processed for honey bee species distribution modeling across the entire study area.

Table 2.1: List of environmental variables used in modeling potential habitats for honey bees in Lakki Marwat

Environmental (Bioclimatic) Variables	Code	Unit
Annual temperature means	Bio_1	°C
Isothermality	Bio_3	°C
Temperature Seasonality	Bio_4	°C
Mean Temperature of Warmest Quarter)	Bio_10	°C
Precipitation of Driest Month	Bio_14	mm
Precipitation Seasonality	Bio_15	mm
Precipitation of Wettest Quarter	Bio_16	mm
Precipitation of Driest Quarter	Bio_17	mm
Precipitation of Warmest Quarter	Bio_18	mm

2.6 MaxEnt Modeling Distribution

MaxEnt software (Version 3.4.4), developed by (Phillips 2006; and Phillips *et al.,* 2008), was used to model the honey bee habitat's size and appropriateness in the district of Lakki Marwat. This software is designed to model climatically favorable habitats for species by integrating occurrences with climatic data. Present data background data were used for habitat suitability and species response. The species occurrence points and bioclimatic variables (Table 2.1) were utilized as model inputs.

Ten MaxEnt model replicate runs were done on solely current data (data collected in the field), with a set number of background points chosen at random for each run. After collecting all of the information (presence data and environmental layers), the MaxEnt model running was started. The output of the MaxEnt model was then analyzed with ArcGIS version 10.5 software. The distribution of species was examined.

2.7 Model Simulation and Evaluation

The presence data of honey bees collected during the field surveys were formatted as a CSV file. The environmental variables were adjusted according to the specific requirements of MaxEnt software (version 3.4.4). A random seed option was utilized while maintaining a 25% random test percentage. Ten replicates were run using the subset of data.

The default settings were retained, including generating 10,000 background points randomly, performing 500 maximum iterations with a convergence threshold set at 0.00001, and a regularization multiplier of 1. A jackknife estimator was used to estimate the significance and contribution of each variable.

The MaxEnt model's performance was measured using receiver operating characteristic (ROC) values, with rejection indicated by a ROC value of 0.5-0.6, poor performance indicated by 0.6-0.7, average performance indicated by 0.7-0.8, good performance indicated by 0.8-0.9, and excellent performance indicated by a ROC value of 0.9-1.0. The obtained output was used to categorize the suitable habitat range of honey bees.

2.8 Ethical Approval

The Bio-Ethical Committee (BEC) of Quaid-i-Azam University Islamabad granted proper approval for the research study under reference number BEC-FBS-QAU2023-471.

RESULTS

Tables 3.1 and 3.3 illustrate the morphometric measurements of morphological traits of *Apis florea* and *Apis dorsata* respectively, recorded in the Lakki Marwat, while tables 3.2 and 3.4 shows comparative analysis of the current study morphometric measurements of *A. florea* and *A. dorsata* respectively with previous study.

3.1 Morphology and measurements of different body parts of *Apis florea* in millimeters (mm)

The full body length varies from 9.43-10.29 having a mean value of 9.86±0.21. The length of a head was 2.4459-2.5613mm with a mean value of 2.50±0.02**.** Length of antenna varied from 2.97-2.99 with mean value 2.98±0.006. Different segments of hind leg that is coxa, trochanter, femur, tibia, metatarsus, and tarsus have length in the range of 0.65-0.76, 0.40-0.43, 1.78-1.84, 2.00-2.10, 1.35-1.41, and 1.18-1.22 respectively having mean value of 0.70±0.02, 0.41±0.006, 1.81±0.01, 2.05±0.02, 1.38±0.01, and 1.20±0.007 respectively. Similarly, length of front leg varied between 5.10-5.24 having mean value of 5.17±0.03. The length and width of the forewing and hind wings also has an important role in morphometry of honey bees. The length and width of the fore wings of *A. florea* varied between 6.87-6.95 and 2.02-2.06, having a mean value of 6.91±0.01 and 2.04±0.008 respectively (Table 3.1).

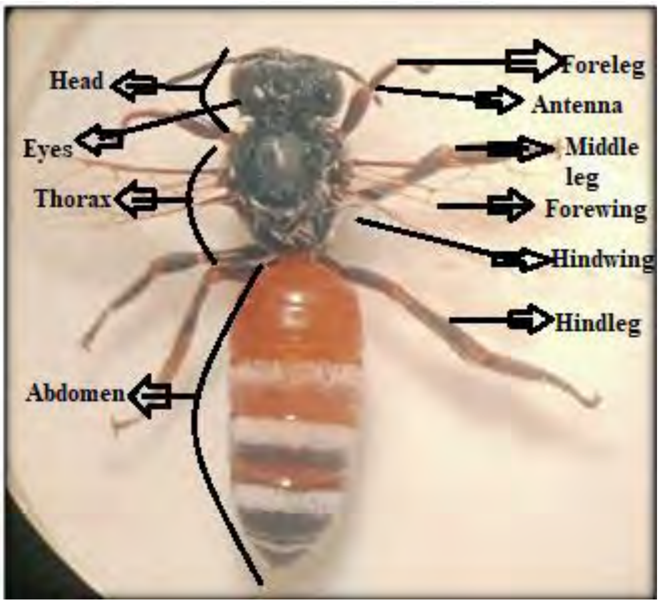

Figure 3.1: Body parts of *Apis florea*

.**Table 3.1: Morphometric measurements of *A. florea* in millimeters (mm) N =55**

Parameters	Mean	StDev	SE Mean	95% CI for µ
Body Length	9.86	1.60	0.20	(9.43-10.29)
Forewing Length	6.92	0.14	0.02	(6.87-6.95)
Forewing Width	2.04	0.06	0.00	(2.02-2.06)
Hindwing Length	4.84	0.25	0.03	(4.77-4.91)
Hindwing Width	1.12	0.08	0.01	(1.10-1.14)
Fore Leg Length	5.20	0.25	0.03	(5.10-5.24)
Midleg length	5.70	0.38	0.05	(5.62-5.83)
Hind Leg Length	7.65	0.30	0.04	(7.58-7.75)
Coxa	0.70	0.21	0.02	(0.65-0.76)
Trochanter	0.40	0.04	0.006	(0.40-0.43)
Femur Length	1.80	0.09	0.01	(1.78-1.84)
Tibia Length	2.05	0.18	0.02	(2.00-2.10)
Meta Tarsus Length	1.40	0.11	0.01	(1.35-1.41)
Tarsus Length	1.20	0.05	0.007	(1.18-1.22)
Antennal Length	3.00	0.05	0.006	(2.97-2.99)
Head Length	2.50	0.20	0.03	(2.44-2.56)
Head Width	2.40	0.17	0.023	(2.34-2.43)
Abdominal Length	4.61	0.40	0.05	(4.50-4.72)
Proboscis Length	2.60	0.25	0.03	(2.53, 2.67)

µ: mean of Body Length, Forewing Length, Forewing Width, Hindwing Length, Hindwing Width, Fore Leg Length, Midleg length, Hind Leg Length, Coxa, Trochanter, Femur Length, Tibia, Length, Meta Tarsus Length, Tarsus Length, Antennal Length, Head Length, Head Width, Abdominal Length, Proboscis Length

Table 3.2: Comparative analysis of current study morphometric measurements in millimeters (mm) of *A. florea* with the previous report (p<0.05)

Parameters	Mean (Present Study)	Makkar *et al*., (2020) (Previous study)
Body Length	9.86	8.66
Forewing Length	6.92	6.27
Forewing Width	2.04	2.57
Hindwing Length	4.84	4.53
Hindwing Width	1.12	1.65
Head Length	2.50	1.25
Head Width	2.38	2.62
Abdominal Length	4.61	3.77

3.2 Morphology and measurements of different body parts of *Apis dorsata* in millimeters (mm)

The full body length varies from 916.96-18.17 having a mean value of 17.57±0.29. The length of a head was 3.81-4.02 with a mean value of 3.91±0.04**.** The length of antenna varied from 3.99-4.46 with mean value 4.22±0.11. Different parts of hind leg that is coxa, trochanter, femur, tibia, metatarsus, and tarsus have length in the range of 0.84-0.94, 0.57-0.79, 2.02-2.54, 2.53-3.24, 1.72-2.48, and 1.41-1.82 respectively having mean value of 0.89±0.02, 0.68±0.05, 2.28±0.12, 2.88±0.17, 2.10±0.18, and 1.61±0.09 respectively. Similarly, length of foreleg varied between 8.12-10.88 having mean value of 9.50±0.66. The length and width of the forewings of *A. dorsata* varied between 12.45-13.58 and 3.78-4.11, having a mean value of 13.01±0.26 and 3.95±0.07 respectively. (Table 3.3).

Table 3.3: Morphometric measurements of *A. dorsata* in millimeters (mm) N= 21

Parameters	Mean	StDev	SE Mean	95% CI for µ
Body Length	17.57	1.33	0.29	(16.96-18.17)
Forewing Length	13.01	1.23	0.26	(12.45-13.58)
Forewing Width	3.95	0.36	0.07	(3.784.11)
Hindwing Length	8.44	0.51	0.11	(8.21-8.68)
Hindwing Width	2.33	0.21	0.04	(2.23-2.43)
Fore Leg Length	9.50	3.04	0.66	(8.12-10.88)
Midleg length	1.00	0.00	0.00	(1.00-1.00)
Hind Leg Length	13.48	0.82	0.18	(13.10-13.86)
(i) Coxa	0.89	0.10	0.02	(0.84- 0.94)
(ii) Trochanter	0.68	0.24	0.05	(0.57- 0.79)
(iii) Femur Length	2.28	0.56	0.12	(2.02- 2.54)
(iv) Tibia Length	2.88	0.77	0.17	(2.53-3.24)
(v) Meta Tarsus Length	2.10	0.84	0.18	(1.72- 2.48)
(vi) Tarsus Length	1.61	0.44	0.09	(1.41-1.82)
Antennal Length	4.22	0.52	0.11	(3.99-4.46)
Head Length	3.91	0.22	0.04	(3.81-4.02)
Head Width	3.88	0.23	0.05	(3.77-3.98)
Abdominal Length	9.56	0.81	0.17	(9.19-9.93)
Proboscis Length	3.75	0.24	0.05	(3.64-3.86)

µ: mean of Body Length, Forewing Length, Forewing Width, Hindwing Length, Hindwing Width, Fore Leg Length, Midleg length, Hind Leg Length, Coxa, Trochanter, Femur Length, Tibia, Length, Meta Tarsus Length, Tarsus Length, Antennal Length, Head Length, Head Width, Abdominal Length, Proboscis Length

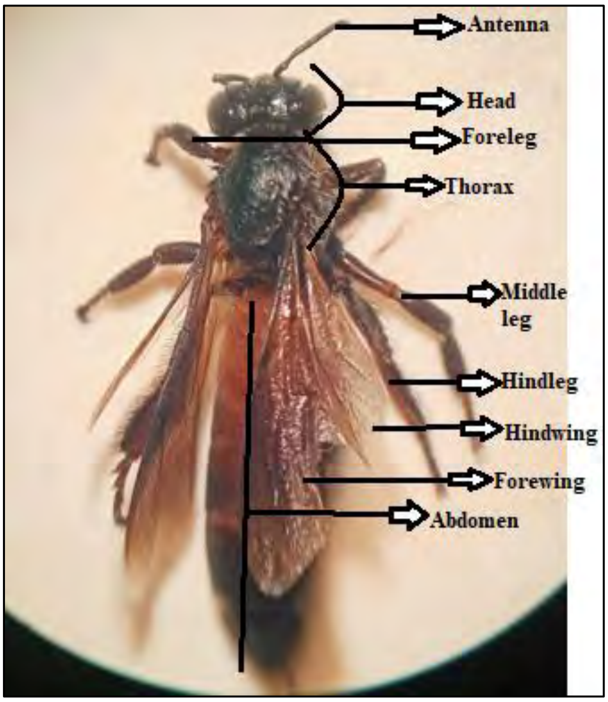

Figure 3.2: Body parts of *Apis dorsata*

Table 3.4: Comparative analysis of current study morphometric measurements in millimeters (mm) of *A. dorsata* with the previous report ($p<0.05$)

Parameters	Mean (Present Study)	Makkar *et al*., (2020) (Previous study)
Body Length	17.57	17.97
Forewing Length	13.01	12.5
Forewing Width	3.95	4.43
Hindwing Length	8.44	8.60
Hindwing Width	2.33	2.61
Head Length	3.91	3.39
Head Width	3.88	4.34
Abdominal Length	9.56	9.65

3.3 Relative Abundance

Among the 500 collected honey bee samples, 440 belong to the species *Apis florea,* while 60 samples correspond to *Apis dorsata*. Calculating the relative abundance, *Apis florea* constitutes 88% of the total samples, while *Apis dorsata* accounts for 12%. This suggests that *Apis florea* is the dominant species in study areal. The higher prevalence of *Apis florea* might indicate its adaptability to the local environment or ecological factors favoring its proliferation.

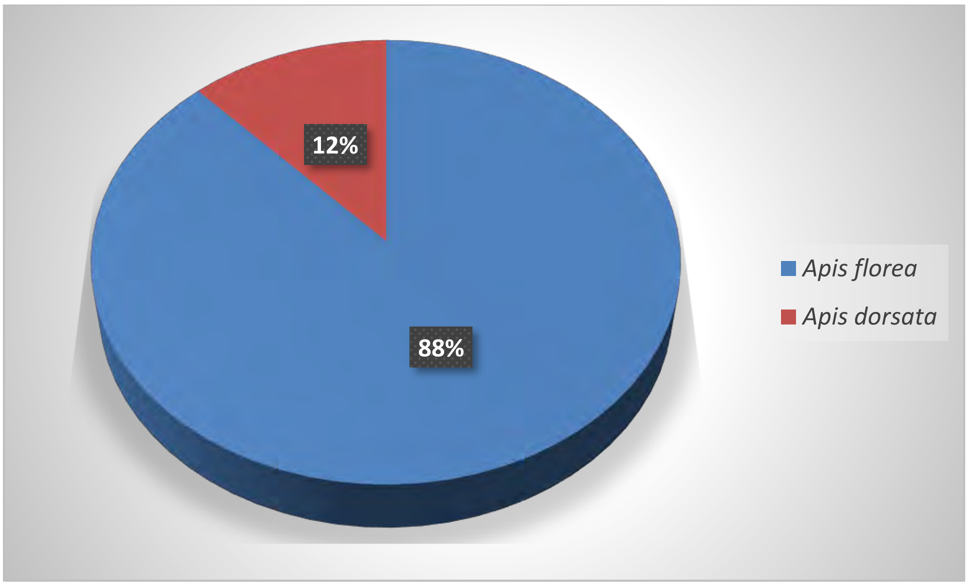

Figure 3.3: Relative Abundance of *Apis florea and Apis dorsata*

3.4 MaxEnt Results for *Apis florea*

3.4.1 Average Sensitivity vs Specificity for *Apis florea*

The present research achieved a reliable and significant model using Area under Curve (AUC) value. The receiver operating characteristics (ROC) outcomes revealed an average AUC value is 0.970, suggesting that the predictions derived from MaxEnt were excellent.

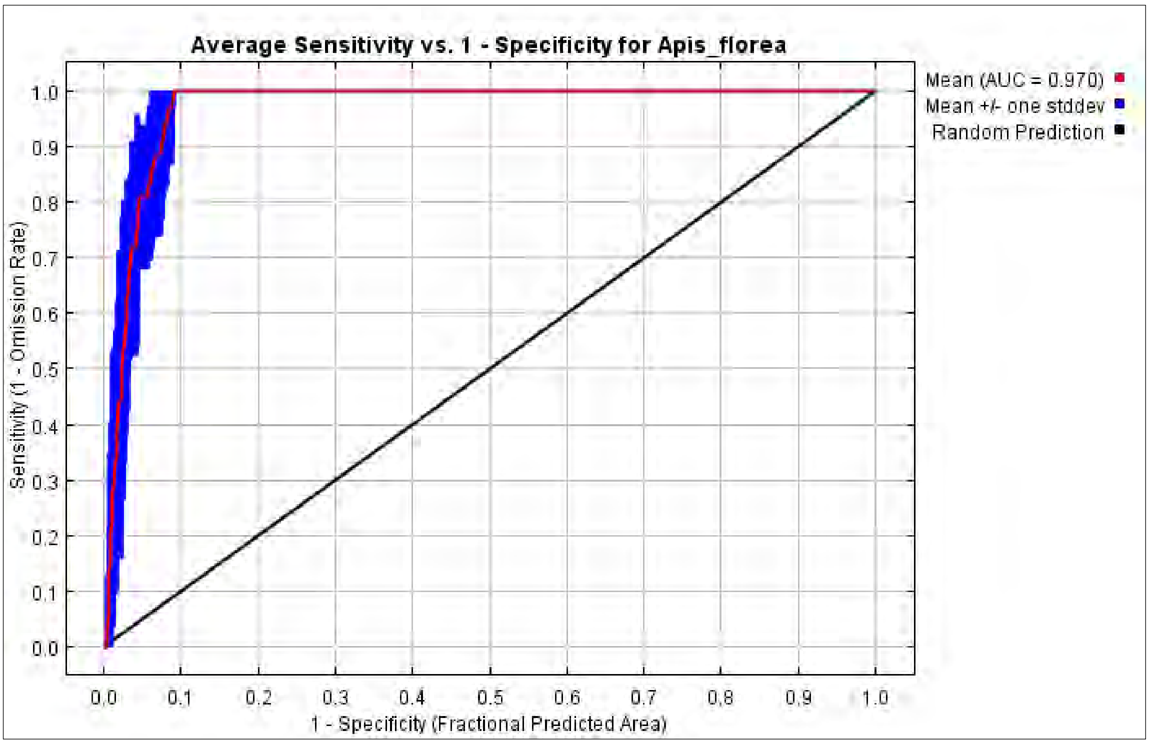

Figure 3.4: Results of Area Under Curve (AUC) Analysis of *Apis florea*

3.4.2 Important Variables Determining Habitat Suitability

Table 3.5 shows approximate environmental variables' contributions to the MaxEnt model. The contribution of each variable in predicting the habitat suitability of *A. florea* was determined by the MaxEnt model (Table 3.5). The results showed that the temperature seasonality contributed highly to the selection of the habitat of *A. florea*

Other variables that contribute to *A. florea*' habitat suitability are precipitation seasonality, precipitation of the wettest quarter, mean temperature of the warmest quarter.

Table 3.5: Relative contributions of the environmental variables to the MaxEnt model of *Apis florea*

Variable	Percent contribution	Permutation importance
wc2.1_30s_bio_4	30.7	14.9
wc2.1_30s_bio_15	22.8	2.2
wc2.1_30s_bio_16	16.5	9
wc2.1_30s_bio_10	16.3	2
wc2.1_30s_bio_18	5.7	32.6
wc2.1_30s_bio_17	3.4	32.1
wc2.1_30s_bio_14	1.9	5.4
wc2.1_30s_bio_3	1.9	0.3
wc2.1_30s_elev	0.6	1.5

3.4.3 Response curves

The MaxEnt prediction is impacted by each environmental variable, as seen by these curves (Figure 3.5). The curves show how the predicted probability of presence changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. The curves show the mean response of the 10 replicates MaxEnt runs (red) and the mean +/- one standard deviation (blue, two shades for categorical variables).

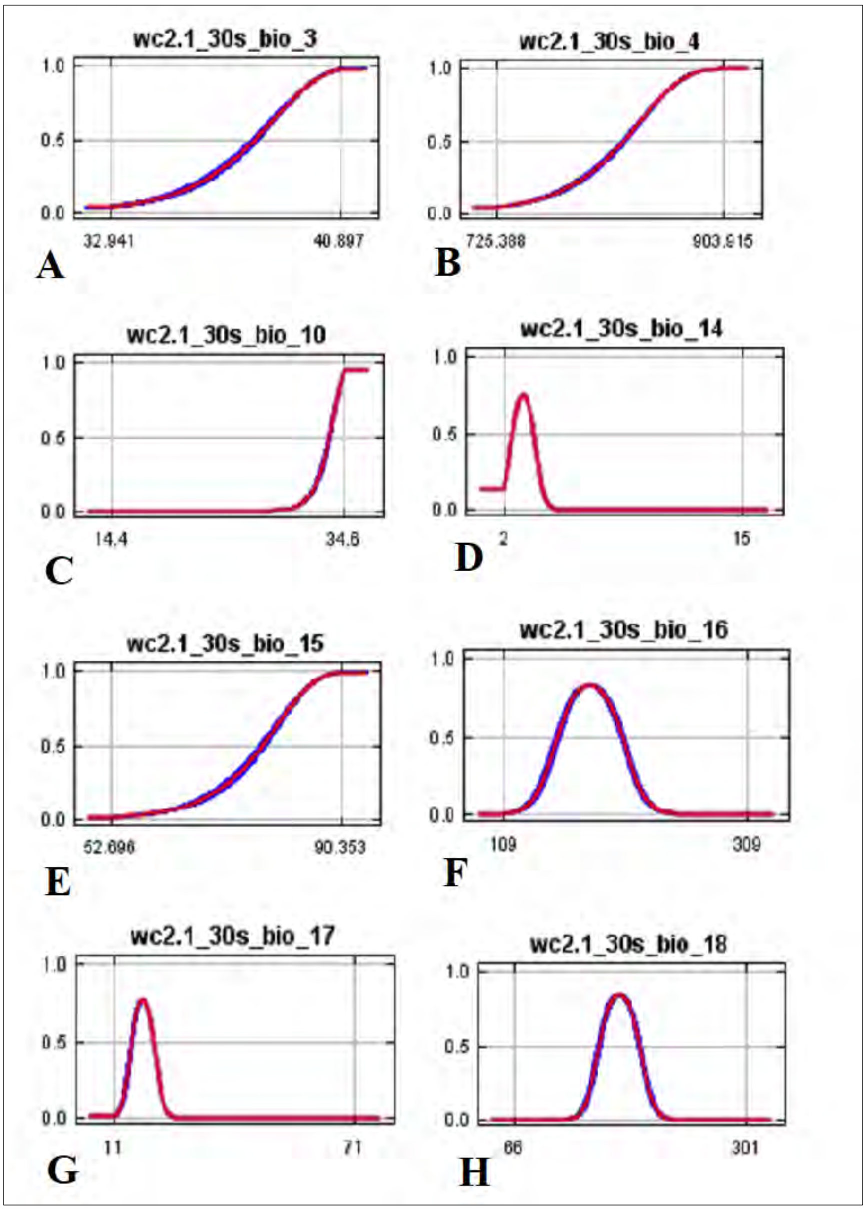

Figure 3.5: Response curve of environmental variables to MaxEnt prediction of *Apis florea* (A. Isothermality, (B) Temperature Seasonality (C) Mean Temperature of Warmest Quarter (D) Precipitation od Driest Month (E) Precipitation Seasonality (F) Precipitation of Wettest Quarter (G) Precipitation of Driest Quarter (H) Precipitation of Warmest Quarter)

3.4.4 Jackknife test

Figure 3.6 displays the MaxEnt model for honey bees' relative predictive value of jackknife regularized test gain, which is based on several environmental variables.

The jackknife test results revealed that temperature seasonality (bio_10), when used in isolation, has the highest gain among environmental variables followed by precipitation of driest quarter (bio_17), precipitation of warmest quarter (bio_18).

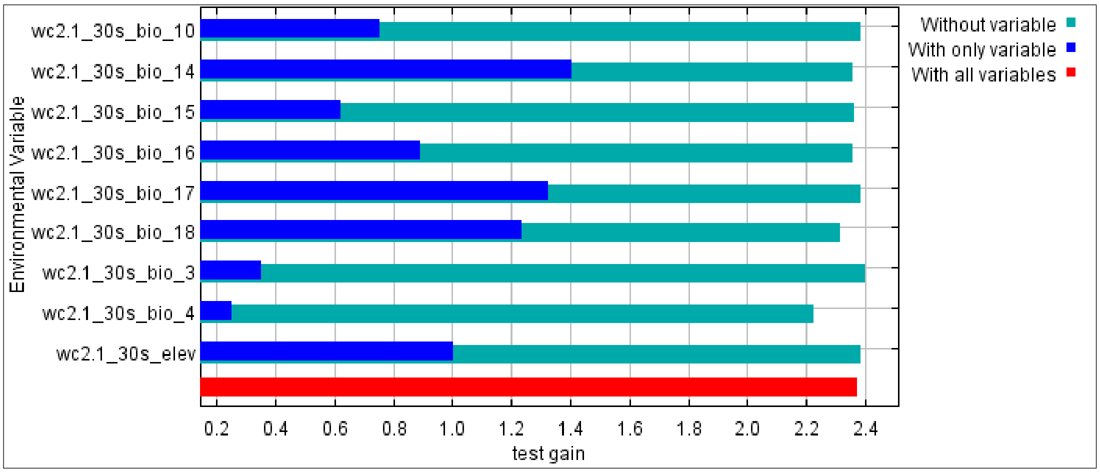

Figure 3.6: Jackknife test gain for *Apis florea*

3.4.5 Suitable Area

Five classes are categorized based on the threshold predicted habitat suitability map of honey bees: Very highly suitable, highly suitable, moderately suitable, least suitable, and unsuitable (0.00-0.08).

The predicted potential distribution of *A. florea* in the below map (Figure 3.7) shows that the red color 0.7 to 1.00 is a very high predicted suitable habitat, while 0.53 to 0.69 is a highly suitable habitat, 0.36 to 0.59 is moderately suitable, 0.18 to 0.35 less suitable, and 0-17 is poor habitat.

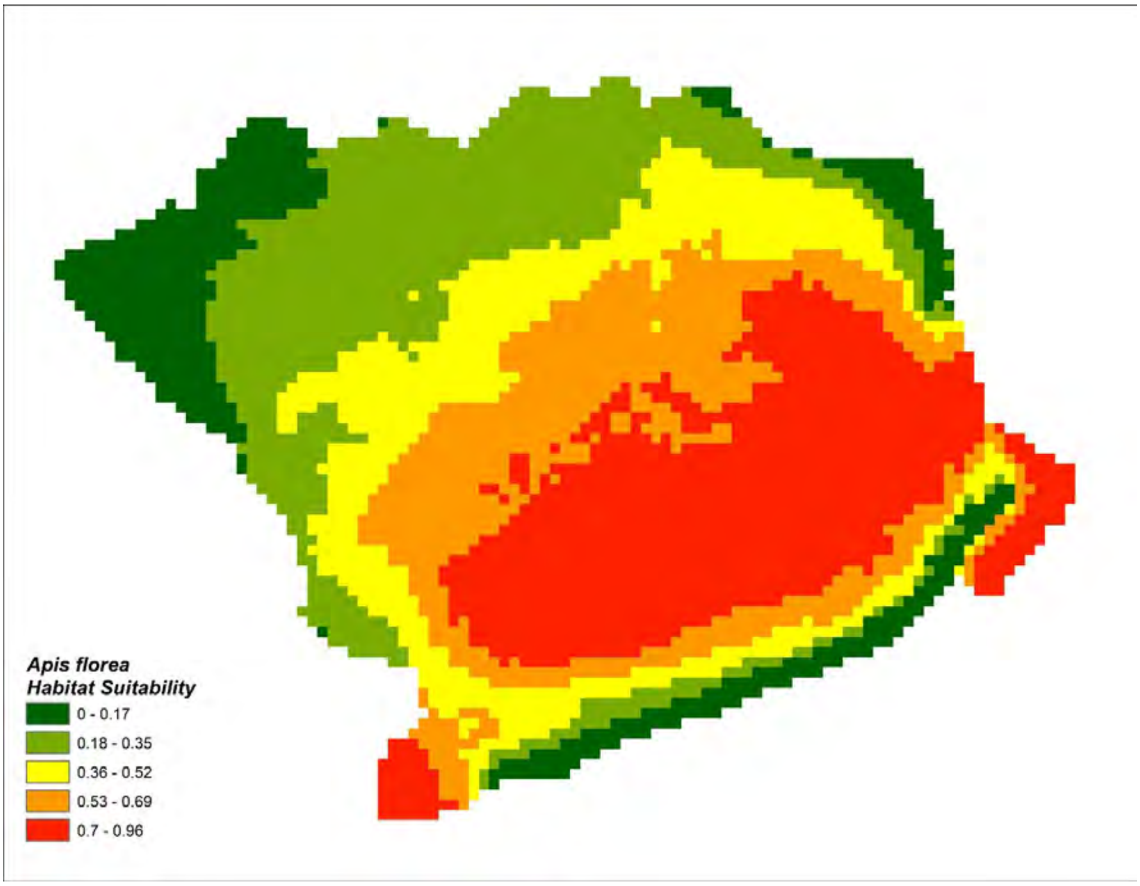

Figure 3.7: Potential Habitat of *Apis florea* using MaxEnt software

Green color (0-0.17) = Poor habitat

Light green color (0.18-0.35) = Less suitable habitat

Yellow color (0.36-0.52) = Moderate habitat

Orange color (0.53-0.69) = Highly suitable habitat

Red color (0.7-0.96) = Highly predicted suitable habitat

3.5 MaxEnt Result for *Apis dorsata*

3.5.1 Average Sensitivity vs Specificity

The Area Under the Curve (AUC) of the Receiving Operating Characteristics (ROC) curve serves as a measure for evaluating the predictive performance of the constructed model. An average AUC value was 0.832, suggesting that the prediction achieved from the MaxEnt model was good.

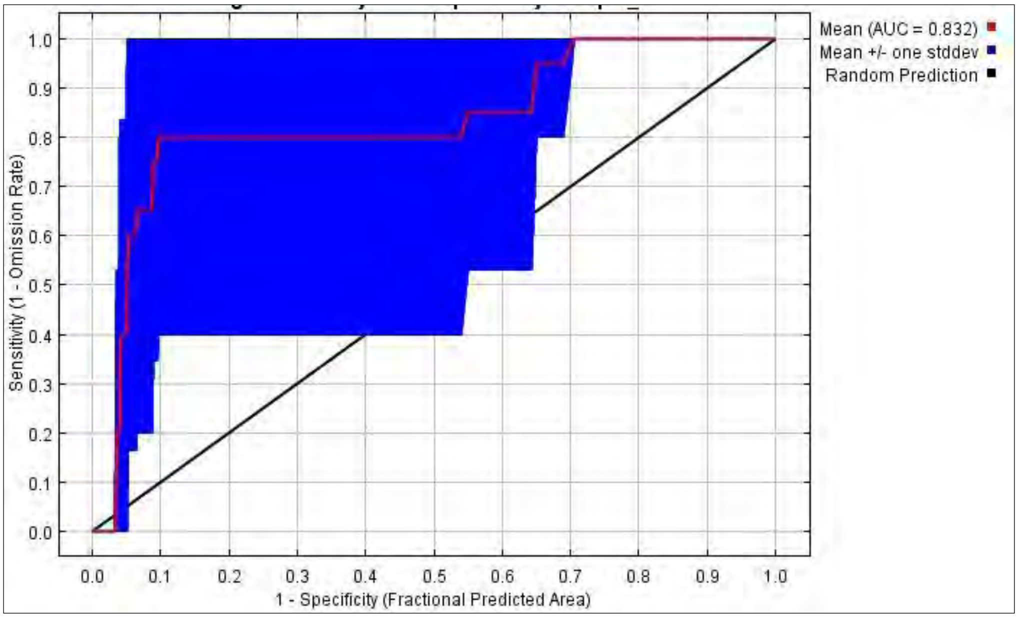

Figure 3.8: Area Under Curve (AUC) Analysis of *Apis dorsata*

3.5.2 Analysis of Variable Contributions

The table 3.6 below the contributions of each environmental variable to the MaxEnt model in predicting the habitat suitability of giant honey bees (*A. dorsata)*. The results showed that the precipitation seasonality contributed highly (77.2%) to the selection of the habitat of *A. dorsata* honey bee species.

Other variables that contribute to *A. dorsata's* habitat suitability are precipitation seasonality, precipitation of the driest month, mean temperature of the coldest month, etc.

Table 3.6: Relative contributions of the environmental variables to the MaxEnt model of *Apis dorsata*

Variable	Percent contribution	Permutation importance
wc2.1_30s_bio_15	77.2	55
wc2.1_30s_bio_14	9.1	16.5
wc2.1_30s_bio_6	7.8	9.8
wc2.1_30s_bio_8	2.2	12.1
wc2.1_30s_bio_17	1.3	0.7
wc2.1_30s_elev	1.2	2.2
wc2.1_30s_bio_12	0.7	3.7
wc2.1_30s_bio_19	0.6	0.0

3.5.3 Response Curve

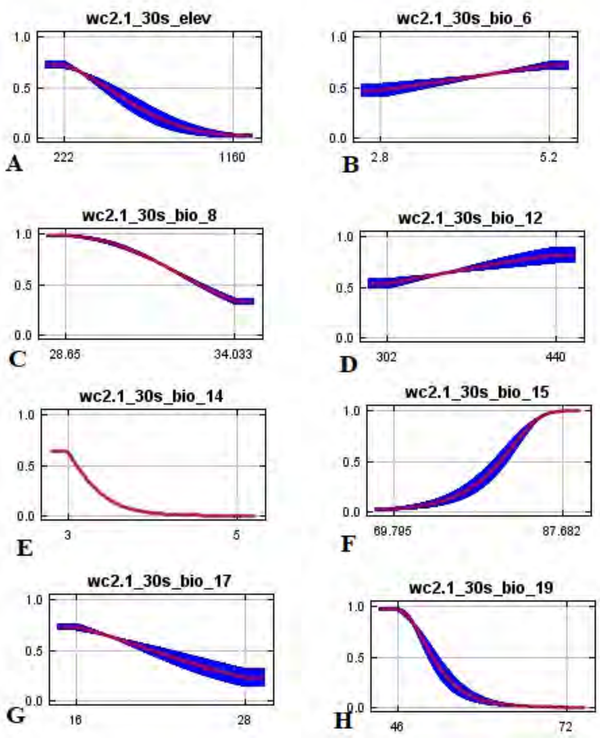

Figure 3.9: Response curve of environmental variables to MaxEnt prediction of *Apis dorsata* (A. elevation, (B) Mean Temperature of the Coldest Month (C) Mean Temperature of the Wettest Month (D) Annual Precipitation (E) Precipitation of the Driest Month (F) Precipitation Seasonality (G) Precipitation of the Driest Quarter (H) Precipitation of Coldest Quarter)

3.5.4 Habitat Suitability Area of *Apis dorsata*

The predicted potential distribution of *A. dorsata* in the below map (Figure 3.10) indicated that most of the green color 0-0.013 is a very unsuitable habitat, while light green color 0.14-0.31 is less suitable, yellow color 0.32-0.52 moderately suitable, but on other hands orange color, 0.53-0.76 is highly suitable habitat and red color 0.77-1 is highly suitable.

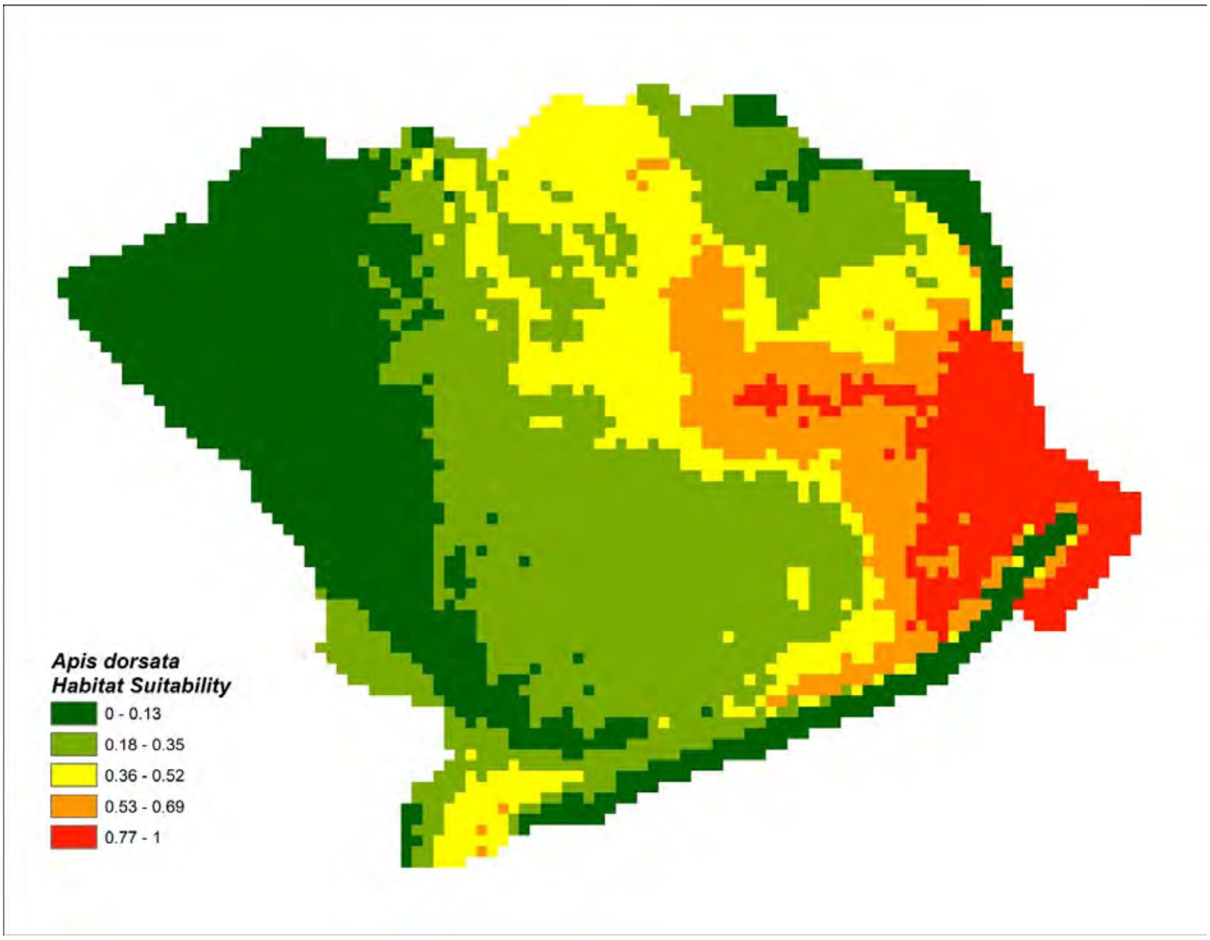

Figure 3.10: Potential Habitat of *Apis dorsata* using MaxEnt software

Green color (0-0.13) = Poor habitat

Light green color (0.18-0.35) = Less suitable habitat

Yellow color (0.36-0.52) = Moderate habitat

Orange color (0.53-0.69) = Highly suitable habitat

Red color (0.7-1) = Highly predicted suitable habitat

DISCUSSION

Species identification is a challenging task, and approximately 0.01% of the estimated 15 million species were identified successfully by only a few taxonomists. Even the use of morphological characteristics for the identification of species still required a team of taxonomists to identify species globally (Mustafa *et al*., 2022). This research work was conducted to identify and classify major honey bee species in the study area. Different body characteristics such as body color, the color of bands on the abdomen, the color of hairs present on the abdomen or legs, and wing venations.

The morphological characterization of honey bee species holds pivotal importance in ecological research, aiding in species identification, and taxonomic classification. An essential component of proper specie identification and categorization is consistency in physical characteristics. In the current research work, two honey bee species were identified: *Apis florea* known as dwarf honey bee and other is *Apis dorsata, a* giant honey bee. The morphological analysis of the honey bee species in the current research work aligns with the findings of the previous studies. These studies have consistently identified specific morphological traits that are used to differentiate honey bee species, such as body size, wing length, color pattern, etc. A study conducted by Mustafa *et al*., (2022) and Khan *et al*., (2014) in a neighboring province i.e., Punjab also reported similar morphological traits for honey bee species. This uniformity supports the reliability of these morphological traits for accurate species identification. Ruttner, (1988) and Hepburn *et al*., (1999) have also emphasized certain important morphological traits for the identification and classification of honey bee species.

The presence of these two recorded species from respective localities is in accordance with the study conducted by Mustafa *et al*., (2022). They recorded these two species from different locations in six districts of Punjab. The reasons might be the temperature, humidity, and altitude of the areas which are not different as much. But the only difference between these two studies is that they also recorded *Apis mellifera* honey bee species which is not recorded in the current study. It might be due to the difference in the number of locations. Mustafa *et al*., (2022) collected the samples from six districts while in the present study, samples were collected from only one district.

In addition to *A. mellifera* and *A. dorsata*, Khan *et al*., (2014) also reported *Apis cerana* in their study and they stated that this species is found at an altitude of higher than 2000 meters, which is in accordance with a study conducted by Rahman (1940) who also mentioned an altitude of 3333 meters from sea level for *A. cerana.* Our results might be in accordance with Khan *et al*., (2014) because they did not record any *A. florea* honey bee specie at such a high altitude of 2000 meters, because *A. florea* are mostly found at an altitude of 600 meters, which is within range for the species in current study. The reason that *A. cerana* was not recorded in the current study might be the difference between the altitudes of the study areas. The current study area, district Lakki Marwat, is located at an altitude of 200-1000 meters from sea level which is lower than that of previous studies by Rahman (1940); and Khan *et al*., (2014). The findings about *A. cerana* in the present research work are in accordance with these previous studies because no *A. cerana* species was recorded in the current study. Similarly, the climate of Lakki Marwat is also warm while *A. cerana* are found in cold areas like the Himalayan mountains, Murree (Koeniger 1976; Khan *et al*., (2014)). The other possible factors might be the loss of habitats, plains, and desert areas of Lakki Marwat.

Nasreen & Rafiq, (1990) reported that wild colonies of *A. dorsata* are mainly found throughout foothills, small hills, and plains at an altitude of <1200 meters height, and *A. florea* is located at an altitude of 600 meters, which is in accordance with the current research work.

This study also depicted the distribution of honey bees in the district Lakki Marwat using the MaxEnt modeling technique. Several studies have been conducted to predict the suitable habitat and distribution of honey bees in many countries. This might be the first study to use the MaxEnt model to predict habitat suitability and distribution modeling in the southern regions of Khyber Pakhtunkhwa, Pakistan. The accuracy obtained of ROC-AUC *A. florea* and *A. dorsata* was 0.97 and 0.83 respectively, which is an excellent model because AUC was more than 0.90 as advised by (Araujo *et al*., 2005; Giovanelli *et al*., 2010). The finding of the current research work regarding AUC value is in accordance with the study conducted by Parichehreh *et al*., (2022). They also found an AUC value of more than 0.90.

The most significant climatic factor impacting insect range and the biological life cycle is temperature as studied by Bale *et al*., (2022). In this study, temperature seasonality was the most important variable in determining suitable habitat for *A. florea* (Table 3.5). The distribution model map for *A. florea* in the study, conducted by Parichehreh *et al*., (2022) found that regions having dry climates with warm and hot summers could potentially considered suitable habitats for *A. florea.* The climate of the current study area (District Lakki Marwat) is also dry with warm and hot summers; therefore, the distribution model predicted an excellent model (Figure 3.7). This is similar to the fact that *A. florea* is a dry-tolerant species, found all around the world such as in Bangladesh, India, Pakistan, Oman, Saudi Arabia, Muscat, Vietnam, Sumatra, Borneo, China, Indonesia, Sudan, and the Philippines, where the climate ranges from desert and semi-arid regions to subtropical and tropical (Hepburn & Radloff, 2011).

Environmental variables that are related to temperature are the major factors that determine the distribution of both honey bee species (Ghassemi *et* al., 2022). The giant honey bee (*A. dorsata*) is a seasonally migrated species that migrates with changes in temperature (Woyke *et al*., 2012).

In the current research work, *A. dorsata* species were mostly recorded and collected from mountain regions of the study area. Analysis of the MaxEnt habitat modeling in a map (Figure 3.10) also showed that mountain regions are highly suitable habitats for *A. dorsata* as shown by the red color. Similar results were obtained in a study conducted by those who reported that the potential suitability of *A. dorsata* was recorded in hilly areas (dry habitat) (Basrowi *et al*., 2022).

This research work presents the first attempt to model the distribution of honey bee species, *A. florea,* and *A. dorsata.* The findings suggest that various environmental variables impact the habitat suitability of honey bee species. Among these temperature and precipitation seasonality and precipitation of wettest quarter exert great influence habitat suitability of these honey bee species.

Conclusion

Morphometric characteristics are one of the suitable approaches for distinguishing honey bee species. Using MaxEnt software showed that giant honey bee species (*A. dorsata*) prefer mountain regions The results showed that the AUC of *A. florea* and *A. dorsata* were 0.970 and 0.832 respectively which revealed an excellent model. It is concluded that both honey bee species (*A. florea* and *A. dorsata*) were distributed irregularly. MaxEnt modeling showed that highly suitable habitats for *A. florea* and *A. dorsata*. This study includes important environmental factors that affect species distribution as well, which may assist in identifying potential beekeeping regions.

Recommendations

In the research area, honey bee species need management and conservation strategies that should be efficient, scientific, and long-term. Additionally, both species must be conserved and protected through the creation and implementation of laws and policies. Therefore, the findings of this research work can be implanted in the conservation program of honey bee species, including identifying the locations, which are already occupied by honey bees.

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the distribution pattern and habitat suitability of honey bee species (Apis florea and Apis dorsata) in District Lakki Marwat associated with various environmental variables were determined using the

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