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Research Article

Sexual dimorphism and morphometric analysis of Red Palm Weevil (*Rhynchophorus ferrugineus)* of Khairpur, Sindh, Pakistan

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Abstract

The Red Palm Weevil (*Rhynchophorus ferrugineus*) is a global threat to palm trees. Our study, which delves into the sexual dimorphism and morphometric variations among Red Palm Weevils in the Khairpur district of Sindh, Pakistan, has the potential to influence the development of effective and sustainable management strategies significantly. We collected 210 adult Red Palm Weevils, both males and females, from various palm tree habitats in Khairpur. We measured different body parts using calipers and compared the results between the sexes. The findings revealed clear sexual dimorphism in Red Palm Weevils of Khairpur, with females being larger. These morphometric variations provide valuable insights into the sexual dimorphism and physical characteristics of Red Palm Weevils in Khairpur, Sindh. This information can be directly applied in developing sex-specific control strategies, such as targeted trapping or biological control methods, to mitigate the impact of Red Palm Weevils on palm tree populations in the region. Our research contributes to a comprehensive understanding of the species biology and aids in developing effective and sustainable management strategies for this economically significant pest.

Keywords: Management strategies, Phylogeny, Distribution, Species, Pests, Coleoptera

Introduction

The Red Palm Weevil (*Rhynchophorus ferrugineus*) is a highly destructive pest and a global threat to palm trees. Originally from Southeast Asia, this invasive species has spread to various regions, including the Middle East, North Africa, Europe, and parts of America, causing extensive damage to palm plantations and natural palm populations (Abraham *et al.,* 2013; Milosavljević *et al.,* 2019; El-Sabea *et al.,* 2020). Infestations by the Red Palm Weevil can result in the death of palm trees, leading to economic losses and environmental degradation. This study, therefore, is not just a local investigation but a global effort to understand and manage this pest. It adds to the existing body of research on the biology and management of Red Palm Weevils. Previous studies have investigated various aspects of the weevil's behavior, such as mating behavior and oviposition patterns, to aid in developing effective management strategies (Al-Saghir *et al.,* 2013; El-Shafie *et al.,* 2016). However, studies on the sexual dimorphism and morphometric variations of the weevil are relatively limited.

Several studies have investigated sexual dimorphism and morphometric variations in Red Palm Weevils from different geographical regions. For instance, Jaleel *et al.,* (2017) conducted a study in India and found that females exhibited larger body size and longer rostrum (snout) compared to males. Similarly, Al-Ayedh and Hossain (2014) examined Red Palm Weevils in Saudi Arabia and observed sexual dimorphism in terms of body size, pronotum width, and elytra length, with females being larger than males. The Red Palm Weevil (*R. ferrugineus*) is a notorious pest that seriously threatens palm trees worldwide. It is considered one of the most destructive pests of palm trees, causing extensive damage and economic losses in the date palm industry (Abrol *et al.,* 2014). The weevil primarily infests date palms (*Phoenix dactylifera*), but it can also attack other palm species, including coconut palms and oil palms (Montgomery, 2009). The larvae of the Red Palm Weevil bore into the trunk of palm trees, causing structural weakening and eventually leading to the death of the host plant (Al-Saroj *et al.,* 2017).

The Red Palm Weevil (*R. ferrugineus*) is a highly destructive pest that poses a significant threat to palm trees worldwide. Originally native to Southeast Asia, it has now spread to various regions across the globe (Aziz, 2024), causing extensive damage to palm tree populations and leading to significant economic losses in the palm industry (Borges *et al.,* 2016; Borges *et al.,* 2020). The weevil infests a wide range of palm species, including date palms, coconut palms, and oil palms, and its larvae bore into the trees, feeding on the plant tissues and causing weakening, deformities,

and eventual death of the affected palms (Al-Saffar & Al-Hashimi, 2017; Montesinos-Matías *et al.,* 2019). Understanding the sexual dimorphism and morphometric variations among Red Palm Weevils is essential for elucidating their reproductive biology, behavior, and population dynamics. Sexual dimorphism refers to the differences in physical characteristics between males and females of a species, often associated with their distinct roles in reproduction and resource utilization (Arnqvist & Rowe, 2005; Bandeira *et al.,* 2021). In insects, sexual dimorphism can manifest in various morphological traits, including body size, shape, and coloration, as well as the development of specialized structures for mating and oviposition (Simmons, 2014). The present study provides sexual dimorphism and morphometric variations among Red Palm Weevils. Hopefully, this study will form a baseline for future researchers dealing with Red Palm Weevil fauna.

Material and methods

During present study *R. ferrugineus* were collected from 08 talukas of Khairpur, Sindh, Pakistan. The weevils were captured from infested palm trees using handpicking methods with the help of farmers. A total of 210 adult weevils (105 males and 105 females) were used for this study (Table 1; Figure. 1).

Table 1. Samples of *R. ferrugineus* (Red palm weevil) collected from 08 Talukas of district Khairpur

Sex	Khairpur	Mirwah	Kot diji	Kingri	Sobhodero	Gambat	Faizganj	Nara
Male	17	13	11	12	11	16	8	17
Female	14	12	9	14	13	18	9	16
Total	31	25	20	26	24	34	17	33

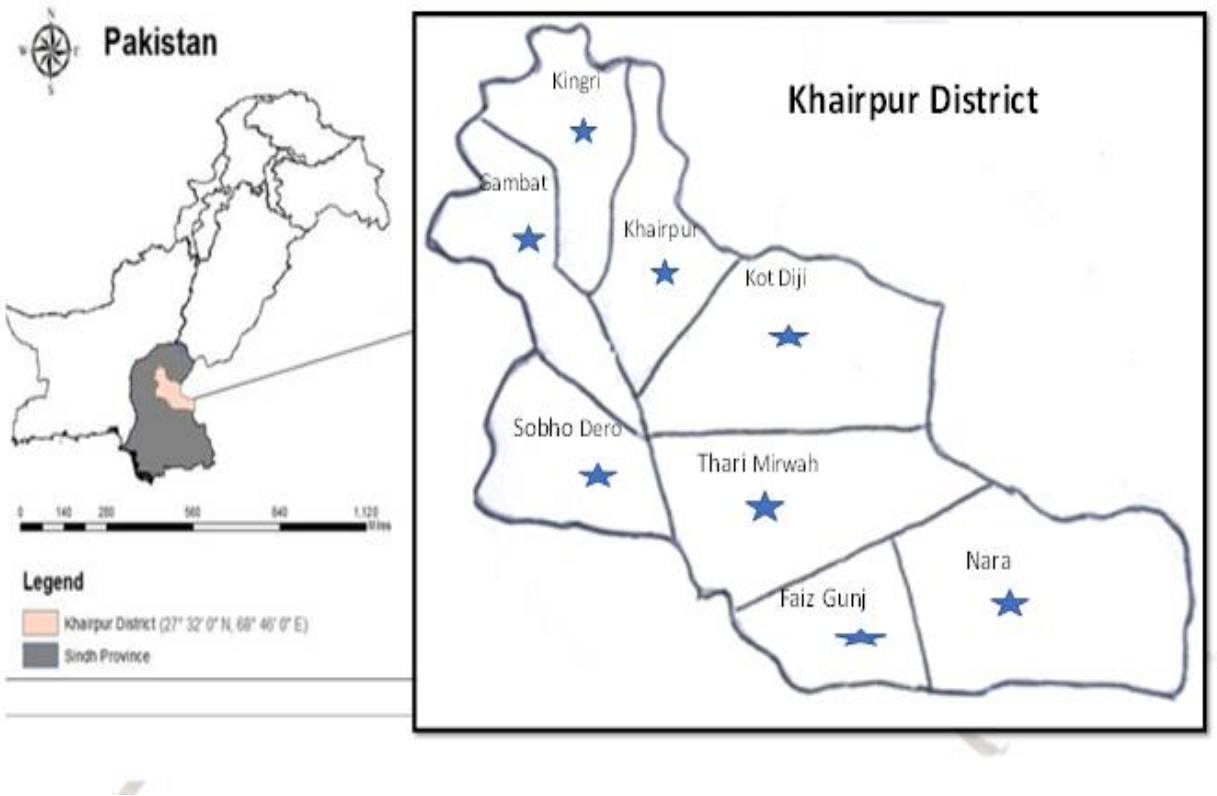

Figure 1. Geographical distribution of *R. ferrugineus* in district Khairpur, Pakistan

Morphometric measurements

Morphometric measurements were taken using a caliper in the Entomology Laboratory of the Department of Zoology Shah Abdul University Khairpur, Sindh. The various body parts of weevils were examined using a binocular stereo microscope and a set of calipers to do a morphometric examination of the insects. The following dimensions included: body length (l), abdomen length (al), abdomen width (aw), pronotum width (pw), pronotum length (pl), head size (hs), and length from the tip of the rostrum to antennal insertion (ta) (Mizzi *et al.,* 2009; Tambe *et al.,*2013).

Phylogeny

The DNA of *Rhynchophorus ferrugineus* was extracted through standard molecular technique. The extracted DNA sample was sent to Macrogen Korea for sequencing. The sequence was blasted in NCBI. Using the Tamura-Nei model, the MEGA X software tool, version 10.0.5, created a Neighbor-Joining phylogenetic tree. All nucleotide data were analyzed, and numerous sequences

were aligned using BioEdit version 7.2.6. The sequence was submitted in NCBI with accession number OQ452923.1.

Results

Rhynchophorus ferrugineus

Size: In general, female red palm weevils are larger than males. Adult females can reach a length of about 25-40 millimeters, whereas adult males are typically smaller, measuring around 20-30 millimeters (Table 2 & 3, Fig. 2).

Rostrum (snout): The rostrum, or snout, of male red palm weevils tends to be longer and more curved than that of females. The extended rostrum in males is used during courtship and mating.

Antennae: The antennae of male and female red palm weevils differ in structure. The male's antennae are longer and thicker than the female's. These antennae play a role in sensory perception and locating potential mates (Tables 2 & 3).

Abdomen: The abdominal segments of female red palm weevils are typically broader and more rounded than males. This difference is more noticeable in mature adults.

Ovipositor: Only female red palm weevils possess an ovipositor, a specialized structure for laying eggs. The ovipositor is located at the end of the female's abdomen and is used to insert eggs into palm tree trunks or other suitable sites for reproduction.

Pronotum: The pronotum, which is the upper part of the thorax, is more curved and has a distinctive hump in males compared to the flatter pronotum of females (Table. 3).

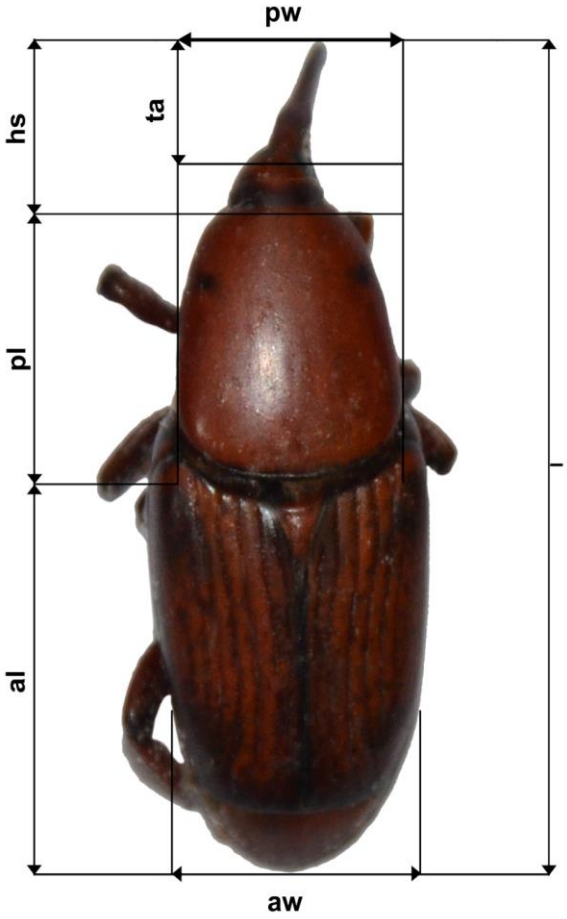

Figure 2. Schematic representation of the measures taken on the different body parts of the Red Palm Weevil (*Rhynchophorus ferrugineus)* (l): whole body length; (al): abdomen length; (aw): abdomen width; (pl): pronotum length; (pw): pronotum width;(hs): head size; (ta): length from the tip of rostrum to the antennal insertion.

Table 2. Morphometric measurements of *R. ferrugineus* (Male)

Male (n=15)	**Morphometric Parameters (mm)**						
	L	**Al**	**Aw**	**Pl**	**Pw**	**Hs**	**Ta**
Min	23.10	10.12	7.71	7.45	6.18	5.85	4.57
Max	36.90	15.34	12.68	13.18	10.84	9.72	6.80
Average	30	12.73	10.19	10.32	8.51	7.79	5.69

Table 3. Morphometric measurements of *R. ferrugineus* (Female)

Female (n=15)	Morphometric Parameters (mm)						
	L	**Al**	**Aw**	**Pl**	**Pw**	**Hs**	**Ta**
Min	24.10	10.10	8.35	8.11	6.69	7.12	5.34
Max	37.94	16.34	11.83	12.54	10.68	9.65	8.01
Average	31.02	13.22	10.09	10.33	8.69	8.39	6.69

Life cycle *Rhynchophorus ferrugineus*

The females use their long beak, a rostrum, to cut a hole into the palm tissue to lay their eggs. After that, eggs are placed in this crater. Eggs can be laid in wounds, fissures, and crevices in the trunk anywhere from the collar region at the roots all the way up to the base of frond petioles and axils near the crown of the palm tree. These areas can be found on the trunk from the roots up to the crown of the palm tree. Females can deposit anything from 58 to 531 eggs, which can take anywhere from one to six days to hatch. After emerging from their eggs, the larvae feed on the palm tissue surrounding them and eventually burrow their way into the heart of the palm. The tunnels the larvae create while feeding become filled with frass (excrement and chewed fibers that emit a pungent odor) and sap from the plants on which they feed. Before reaching the pupal stage, larvae may go through anywhere from three to seven instars or stages, each of which can last anywhere from one to two months. The cocoons in which the larvae develop into adulthood can be found in the palm tree's trunk or in hidden locations at the base of the palm fronds. The time spent in the pupal stage might range from 11 to 45 days. It takes to mature from an egg to an adult, ranging from 45 to 139 days. Mature Red Palm Weevils emerge from their cocoons and can begin laying eggs approximately eight to ten weeks after emerging. Mature weevils have a lifespan of approximately two to three months, during which time they consume palms, mate multiple times, and lay eggs before eventually passing away. There is a small imbalance in favor of females in the gender ratio (1 male to about 1.2 females).

***R. ferrugineus* global distribution**

This species is distributed in China, Bangladesh, Cambodia, India, Japan, Pakistan, Philippines, Laos, Sri Lanka, Taiwan, and Vietnam, are thought to be this species' native habitats; data from Indonesia, Malaysia, Burma, Singapore, and Thailand primarily or completely refer to *R.*

vulneratus, R. ferrugineus has now been reported and confirmed from Albania, Algeria, Aruba, Bahrain, Bosnia and Herzegovina, Croatia, Curaçao, Cyprus, Egypt, France, Greece, Israel, Italy (incl. Sicily and Sardinia), Jordan, Kuwait, Libya, Malta, Monaco, Montenegro, Morocco, Oman, Palestine, Portugal (incl. Madeira), Qatar, Saudi Arabia, Slovenia, Spain (incl. the Balearic and Canary islands), Syria, Tunisia, Turkey, and United Arab Emirates. Reports from Australia, Papua New Guinea, Samoa, the Solomon Islands, and Vanuatu have not been verified but are most likely samples of the nearby native species *R. bilineatus*.

Distribution of *Rhynchophorus ferrugineus* in district Khairpur

This species is widely distributed in various talukas in the Khairpur district. The highest population of this species was observed in the taluka Gambat, followed by Nara and Khairpur. The lowest population of *R. ferrugineus* was observed in taluka Faiz Ganj, followed by Kot Deji and Kingri. In addition to this, a medium population of *R. ferrugineus* was observed in taluka Mir Wah and Sobho dero (See Table 1 & Fig. 1).

Construction of phylogenetic tree of *R. ferrugineus* on the basis based on Cytochrome oxidase I (*COI)* gene

The nucleotide sequence of the Cytochrome oxidase I (*COI)* gene was taken from *R. ferrugineus* and was subjected to BLAST analysis, and relevant available sequences in the Gene Bank were used for the alignment by BioEdit software and a phylogenetic tree was constructed in the MEGA6 to indicate the affinities of *R. ferrugineus* with already reported species in NCBI (National Center for Biotechnology Information). The present study showed close affinities with *R. ferrugineus* species reported from the Middle East with accession number (GU581539.1) shown in Figure 3.

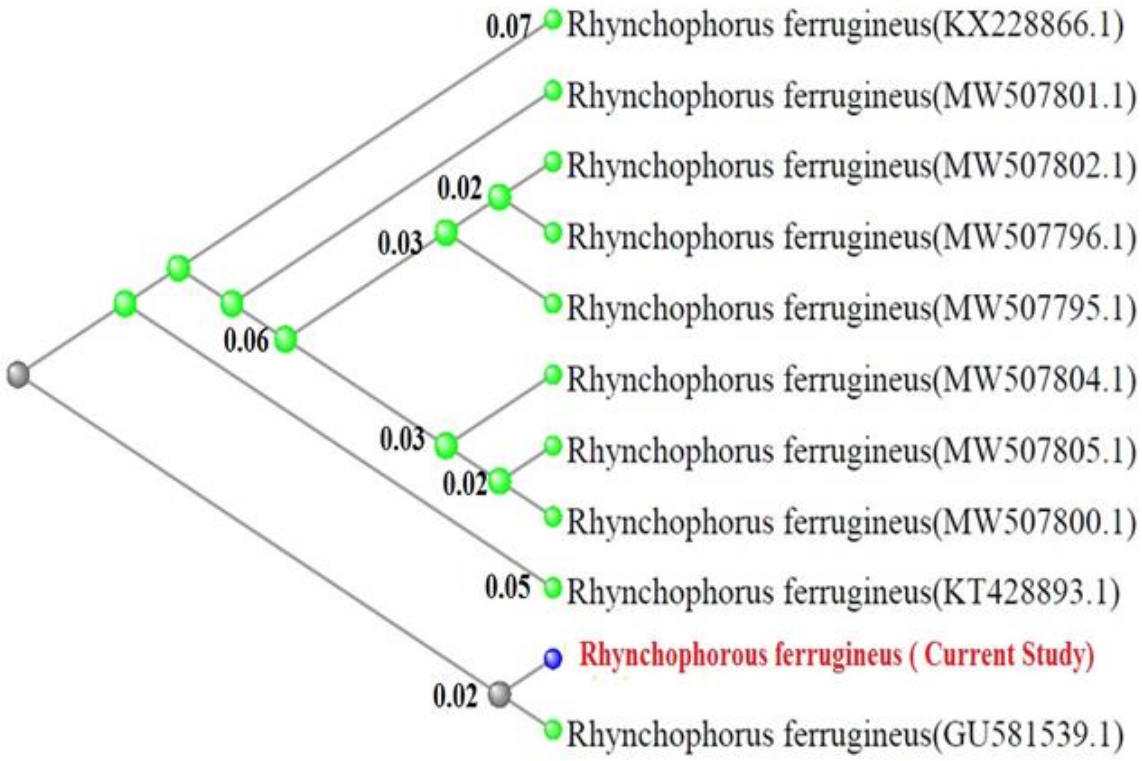

Figure 3. Phylogenetic tree of *R. ferrugineus*

Discussion

The morphometric analysis revealed clear sexual dimorphism in various aspects of the Red Palm Weevils' body structure. Overall, males were found to be significantly smaller in size compared to females. This difference in size is a common phenomenon observed in many insect species and can be attributed to sexual selection and differences in reproductive roles. Mizzi *et al.*, (2009) studied the morphometrics and sexual dimorphism in Red Palm Weevil, *R. ferrugineus* (Olivier, 1790) in Malta. The present study found that the most prominent morphological difference between male and female weevils was observed in the rostrum length. The rostrum, or snout-like projection, was significantly longer in males compared to females. This finding is consistent with previous research on *R. ferrugineus* and other related weevil species. The elongated rostrum in males likely plays a role in intrasexual competition, mate recognition, or in accessing plant tissues during feeding and oviposition. Similarly, Tambe *et al.*, (2013) studied the sexual size differences and color polymorphism of *R. phoenicis* in the Southwest region of Cameroon.

Additionally, the present study examined the pronotum width, elytra length, and body weight as indicators of sexual dimorphism. Although there were slight differences in these parameters

between males and females, they were not statistically significant. This suggests that while rostrum length is a robust indicator of sexual dimorphism, other morphological characteristics may not show significant differences between the sexes in this particular population. Understanding sexual dimorphism in Red Palm Weevils is essential for effective pest management strategies. The differences in body size and rostrum length between males and females can impact their behavior, reproductive success, and response to control measures. For instance, knowledge of sexual dimorphism can aid in the development of targeted trapping methods or pheromone-based attractants, taking into account the specific behavioral and physiological characteristics of each sex. Rugman-Jones *et al.,* (2013) investigated the specific status and invasion history of this serious economic pest and to find potential common entry points, mitochondrial cytochrome oxidase subunit I (COI) gene DNA sequences from RPW populations throughout their native and invaded ranges were compared. Analyses of COI haplotype data provide conclusive support, which is supported by sequences of additional nuclear gene regions. Only the northern and western regions of continental southeast Asia, Sri Lanka, and the Philippines are home to the real *R. ferrugineus,* which is also the cause of nearly all invasive populations globally. The second species, which was previously known as *R. ferrugineus* but was later synonymized under the name *R. vulneratus* (Panzer), has a more southern range across Indonesia and is only responsible for one invasive population, that in California, United States. During the present study nucleotide sequence of Cytochrome oxidae I (*COI)* gene was taken from *Rhynchophorus ferrugineus* and were subjected to BLAST analysis and relevant available sequences in the Gene Bank were used for the alignment by BioEdit software and phylogenetic tree were constructed in the MEGA6 to indicate the affinities of *Rhynchophorus ferrugineus* with already reported species in NCBI (National Center for Biotechnology Information). Present study showed close affinities with *Rhynchophorus ferrugineus* species reported from the Middle East with accession number (GU581539.1). More recently, Suhriani *et al.,*(2023) carried out morphometric and molecular studies on grain weevils *Sitophilus* (Curculionidae) from different ecological zones of Khairpur Sindh, Pakistan. The observed sexual dimorphism in Red Palm Weevils of Khairpur, Sindh, Pakistan, aligns with previous studies conducted on this species. The phylogeny also gives the first molecular report from the study area.

Conclusion

This study investigated the sexual dimorphism and morphometry of Red Palm Weevils in Khairpur, Sindh, Pakistan. The results revealed significant physical differences between male and female weevils, with males exhibiting larger body size and distinct morphometric features. These differences are likely influenced by sexual selection and reproductive strategies. Understanding sexual dimorphism and morphometry can have practical implications for pest management strategies, such as sex-specific control measures. Further research is warranted to explore the underlying mechanisms driving sexual dimorphism and morphometry in Red Palm Weevils. The phylogeny also gives the first molecular report from the study area. Overall, the findings contribute to efforts to mitigate the impact of this destructive pest on palm plantations.

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