



Available on <http://www.pjbt.org>
 Pakistan Journal of Biotechnology
 (PJB)
 (P-ISSN: 1812-1837 and E-ISSN: 2312-7791)



STUDIES ON THE MORPHOLOGY AND MOLECULAR CHARACTERIZATION OF STORED GRAIN WEEVILS *SITOPHILUS* (CURCULIONIDAE: COLEOPTERA) FROM KHAIRPUR, SINDH-PAKISTAN.

Shahnour Suhriani¹, Waheed Ali Panhwar^{1*} and Abdul Manan Shaikh²

¹Department of Zoology, Shah Abdul Latif University Khairpur, Pakistan

²Department of Zoology, Government College University, Hyderabad, Pakistan

*Corresponding Author's email address: waheed.panhwar@salu.edu.pk

Article Received 20-04-2023, Article Revised 16-08-2023, Article Accepted 16-09-2023.

Abstract

In this paper, *Sitophilus oryzae*, *Sitophilus zeamais*, and *Sitophilus granarius*, three economically significant grain pests are thoroughly analyzed in terms of their morphological and molecular differences. The work provides critical insights into species identification, genetic differentiation, and adaptation by contrasting conventional morphological features and genetic approaches. The samples were collected from eight talukas of district Khairpur: Kingri, Kot Deji, Khairpur, Gamabat, Mirwah, Sobhodero, Nara, and Faizganj during the year 2018-2022. The samples were identified on the basis of morphology and extraction of DNA was done. The samples were sent for sequencing. The findings provide important evolutionary insights and aid in pest management. Additionally, the text describes the distinguishing physical characteristics of mature *Sitophilus granarius*, maize weevils, and rice weevils, emphasising size, colour, markings, and anatomical aspects. Understanding the differences among various weevil species is made easier by this information.

Keyword. Stored grains, weevils, Morphology, Molecular, Coleoptera

Introduction

The *Sitophilus* genus includes several significant stored grain pests, such as the rice weevil and the maize weevil, *Sitophilus oryzae* and *Sitophilus zeamais*. Due to their propensity for destruction and capacity to invade and harm stored grains, specifically rice and maize. These two species are of significant economic importance. However, due to overlapping characteristics in their identification, it is essential to distinguish between these two weevil species in order to design successful pest management plans and focused control actions (Hagstrum and Subramanyam, 2019). Some of the prevalent and harmful pests of stored cereals belong to the genus *Sitophilus* Schoenherr. The tropics, subtropics, and warm zones are home to large populations of the rice weevil *Sitophilus oryzae* (L.) and the maize weevil *Sitophilus zeamais* (Motsch.). Both may grow on a variety of grains, although *Sitophilus oryzae* prefers wheat while *Sitophilus zeamais* prefers maize (Bernal and Navarro, 2013). However, new research by Athanassiou *et al.* (2017) shows that although popular names of these species are connected to certain grains, it does not necessarily imply that these grains are the only the best

candidates for their development. Additionally, it is said that adults of *S. oryzae* and *S. zeamais* were much more numerous on rice than on maize. The FAO estimates that 10% of the world's grain is lost each year. Whole grain infestations can result in weight loss, fungal development, and quality loss due to an increase in free fatty acids (Trematerra, 2009). The invasion of these species could make it easier for mites, infections in stored products, and secondary colonizers to establish themselves. Effective control of these pests requires knowledge of their biology (Smith and Smith 1989). Few writers who have conducted biological research on specific *Sitophilus* species have noted variations in the fertility of *S. oryzae* on host plants such rice, wheat, sorghum, and maize. Additionally, it was shown that animals raised on wheat had shorter developmental times and longer life spans (Folmer *et al.*, 1994; Smith and Smith 1989; Smith and Navarro, 2017). This study also revealed specifics regarding *S. oryzae*'s developmental cycle's seasonal variations. According to Makarova and Kuzmina (2016), who conducted a comparative study, there is a significant variation in body size between the two *Sitophilus* species in terms of their distribution, body size, food

preferences, hibernating habits, field infestation, heating, developmental period, and flying ability. Despite their significant prominence as stored grain pests, Smith and Smith (1989) cites biological research of the *Sitophilus* species and claims that no current or thorough analysis whereby their physiology is contrasted has been presented. According to Shi *et al.* (2007), *S. oryzae* and *S. zeamais* have different developing periods. It was found that for *S. oryzae*, oviposition and the developmental period were more favourable in wheat than for *S. zeamais*. The biological characteristics of each of the three *Sitophilus* species were presented by Mason and McDonough in (2012).

The variation and differentiation between *S. oryzae* and *S. zeamais* have been extensively studied using morphological and molecular approaches. According to Cruickshank (2002), molecular markers utilized in phylogenetic investigations should have certain characteristics that make them appropriate for resolving the specific taxonomy level or ranking under examination. The requirement for an amino acid substitution or mutation frequency that impacts an appropriate balance among producing a sufficient number of useful (variables across sequences) sites and avoiding long saturating of the nucleotide locations, that could mask genetic communication and is one of the most essential of these (Mason and McDonough, 2012). To ensure that sufficient relevant changes are present during the relatively brief period that these genera have been diverging, studies looking at very closely related organisms will need genes with quick substitution rates (for example, at the species-level within a genus). In contrast, genes with slower substitution rates are needed when examining organisms that are more distantly related (such as at the family-level or above), as this will reduce the effects of saturation and provide evidence for these older divergences (Cognato and Sperling, 2000). Paralogous sequences are less likely to arise when a gene is present as a single copy or several homogenous forms on haploid chromosomes, rather than when different copies of the gene are present in distinct individuals. While each cell contains several copies of the mitochondrial genes. Originally, this gene has been used (though not exclusively) in smaller-scale phylogeny to investigate associations between newly diverging, rather closely related species due to its high substitution rate (Cognato and Sperling, 2000). Weevil classifications at the level of genera have used Cox1 sequences, either on their own or in combination with other genes (as in the scolytine genus *Ips* phylogeny), for example, an additional MtDNA gene and two nuclei genes. There may be less evolutionary help for internal nodes compared to peripheral clades if the studied group is too divergent and cox1 alone is ineffectively too saturated to resolve internal nodes.

Despite its limited utility in resolving phylogenies of distantly related species, Cox1 has been shown to be useful in other contexts. In this paper, we examine and discuss the research that has been done about the morphological and molecular differences between *Sitophilus oryzae*, *Sitophilus zeamais*, and *Sitophilus granarius*. Present study provides a thorough summary of the most recent research on the taxonomic, genetic, and evolutionary features of these three economically significant weevil species by synthesizing the results from numerous investigations.

Materials and methods

Sitophilus species were collected for the current study from different of locations in Khairpur, Sindh, Pakistan. With the aid of farmers, the weevils were removed manually from palms that were affected. For this investigation, 210 adult weevils' total 105 males and 105 females, were collected.

Sample collection: *Sitophilus* species were collected from Khairpur, Sindh, Pakistan. The weevils were captured from infested palm trees using handpicking methods. The samples were collected from eight talukas of district Khairpur: Kingri, Kot Deji, Khairpur, Gamabat, Mirwah, Sobhodero, Nara, and Faizganj.

Morphometric measurements: A morphometric analysis of the weevils is performed by utilising a pair of calipers and a binocular stereo microscope to study their various parts of body i-e: abdomen length (al), Body length (l), pronotum width (pw) pronotum length (pl), head size (hs), the distance of the tip of the rostrum to insertion of the antennae (ta) , and abdomen width (aw) are all included (Mizzi *et al.*, 2009 Panhwar *et al.*, 2023)

Molecular study

DNA Extraction: The COI-1 (Cytochrome C Oxidase) gene is frequently used as a DNA barcode to distinguish between various species. Due to the conserved sequence of COI in conspecifics, it is employed to extract since it is found in mitochondria. The QIAamp DNA Minikit was used to help with the DNA extraction process. For the full extraction of mitochondrial and genomic DNA from tissues, utilize the QIAamp DNA Minikit (Black *et al.* 2001; Brown *et al.* 1997; Armstrong and Wratten, 1996).

Polymerase Chain Reaction (PCR): PCR amplification of the COI gene region was done. Created and picked suitable primers that amplify the COI gene's conserved sections in order to guarantee effective amplification in many species. For COI amplification in insects, commonly used primer pairs included HCO2198 and LCO1490.

PCR Amplification Protocol: Using the extracted DNA as a template, set up PCR reactions in a sterile setting. The following general cycling conditions were

utilized for PCR (particular parameters may differ depending on the thermocycler and primers used):

Initial denaturation: 3-5 minutes at 95°C Cycling (25-35 cycles):

Denaturation: 30 seconds at 95°C

Annealing: 30 seconds at the appropriate primer-specific temperature (typically around 50-60°C)

Extension: 1-2 minutes at 72°C

Final extension: 5-10 minutes at 72°C

DNA Sequencing: Send the PCR products for Sanger sequencing after purifying them. The COI gene's actual DNA sequences for each specimen of the weevil were be generated in this step.

Phylogenetic and Blast Analysis

Prior to sequencing, PCR-amplified products underwent purification. In order to sequence the cytochrome oxidase subunit-1 and internal transcribed spacer regions, forward and reverse primers were used. Weevils obtained from several locations within the Khairpur district were used to choose these areas. The Khairpur weevil sample-derived PCR products were effectively used to produce sequences. Sequences with useless or empty information were manually removed until the base pairs were aligned in their final position.

Results

Genus *Sitophilus* Schoenherr, 1838.

Type species: *Curculio oryzae* (Linnaeus, 1763):

Diagnosis. One of the notable pests of stored products, grains, and seeds are the fauna that make up the genus *Sitophilus*. *Sitophilus oryzae*, a significant pest of cereal grains and goods, is one of the most globally distributed species of rice weevil, and its larvae develop inside a seed kernel or similar items. In the genus *Curculio*, *Sitophilus oryzae* was initially proposed by Linnaeus. Subsequently, other synonymies were proposed. Last but not least, these stored grain pests were classified as *Sitophilus* by Schoenherr 1838. Due to its striking physical resemblances to the rice weevil, this species has historically been mistaken for another or thought to be a morph.

Morphological description of *Sitophilus oryzae* (Linnaeus, 1763): The pronotum, the shield behind the skull, is punctured with coarse, circular holes, and the head has a thin snout. The elytra have sharp punctures and deep lines. The snout is nearly one millimetre long. The prothorax or elytra are as long as the head including the snout. The elytra feature lines of holes inside transverse grooves, whereas the prothorax (the part of the body above the scalp) is heavily pitted. Weevil adults measure between 3.1 and 4.3 mm. The adult rice insect is a dark reddish-brown to black in color, with 4 faint red or yellow spots on the elytra (wing covers) and round or irregularly shaped pits on the thorax (Fig. 1).

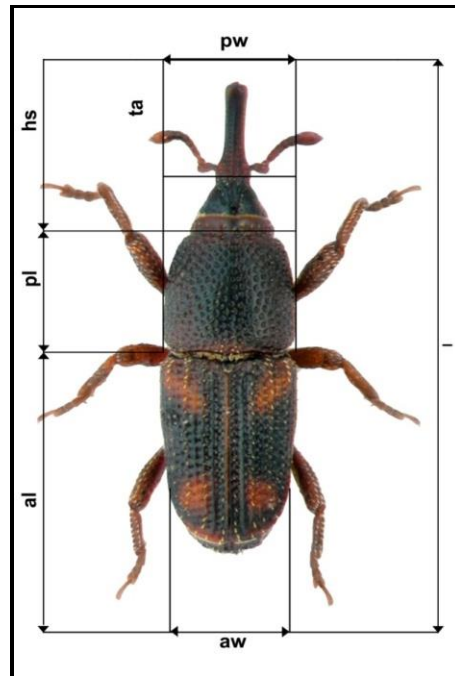


Fig. 1 Morphology of *Sitophilus oryzae*

Worldwide Distribution of *Sitophilus oryzae*:

Sitophilus oryzae is distributed in India, Brazil, Srilanka, Pakistan, New Zealand, Australia, China, Cuba, United Kingdom, Sweden, Spain, Canada, Poland, Germany, Russia, Italy, Africa, Egypt, Greece, Kenya, Malaysia, Israel and Iraq.

***Sitophilus oryzae* distribution in Khairpur.:** This species is found extensively throughout the different

talukas of the Khairpur district. The taluka Gambat had the highest population of this species, then following Nara as well as Khairpur. The taluka Faiz Ganj has the lowest number of *Sitophilus oryzae*, followed by Kot deji as well as Mirwah. Besides, taluka Kingri as well as Sobho dero also had a medium population of *Sitophilus oryzae* (Fig. 2 & Table. 1).

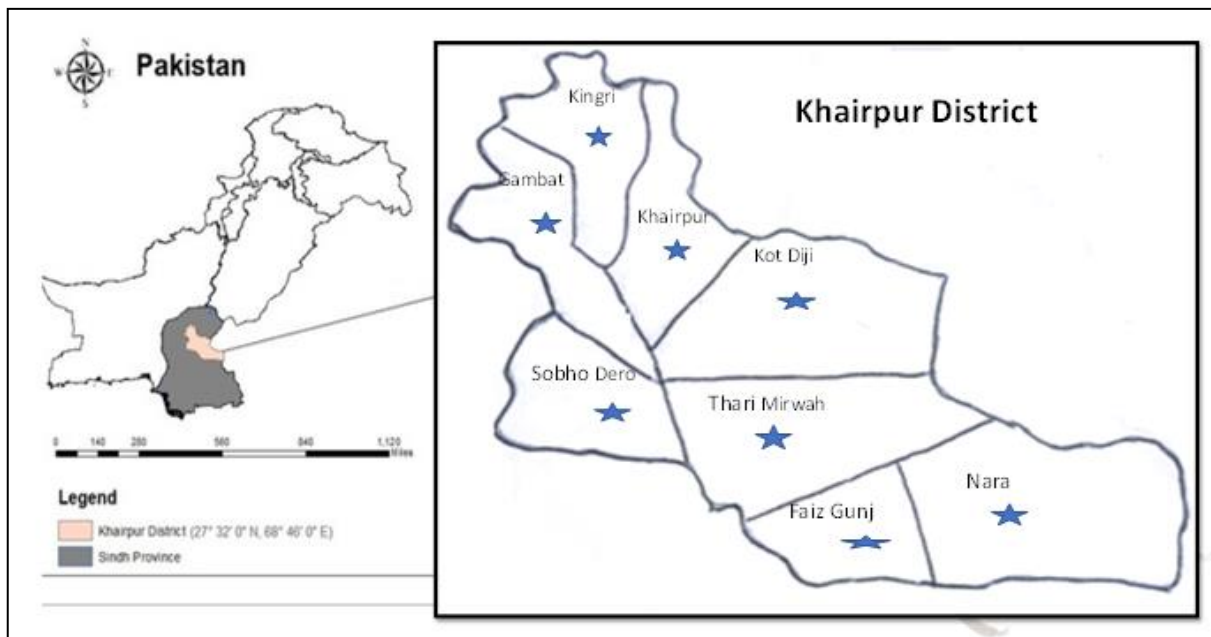


Fig. 2 *Sitophilus oryzae* distribution in Khairpur

Table 1 Distribution of *Sitophilus oryzae* in district Khairpur.

Sex	Mirwah	Khairpur	Kingri	Kot Diji	Gambat	Sobhodero	Nara	Faizganj
Female	14	12	9	14	13	18	10	16
Male	22	14	14	15	19	18	13	18
Total	36	26	23	29	32	36	23	34

Table 2 Morphometry of *Sitophilus oryzae* (Male)

Male (N=15)	Morphometrics Parameters (mm)						
	L	Al	Aw	Pl	Pw	Hs	Ta
Min	3.190	1.120	0.700	1.055	0.630	1.105	0.580
Max	4.210	1.890	0.910	1.340	0.820	1.230	0.733
Average	3.700	1.505	0.805	1.197	0.725	1.167	0.656

Table 3 Morphometry of *Sitophilus oryzae* (Female)

Female (N=15)	Morphometric Parameters (mm)						
	L	Al	Aw	Pl	Pw	Hs	Ta
Min	3.410	1.223	0.730	1.154	0.720	1.110	0.605
Max	4.320	1.950	0.890	1.210	0.834	1.240	0.810
Average	3.865	1.586	0.810	1.182	0.777	1.175	0.707

Phylogeny of *Sitophilus oryzae*: The Cytochrome Oxidase I (COI) gene's nucleotide sequence was taken from the *Sitophilus oryzae* and subjected to BLAST analysis. Then, relevant sequences from the Gene Bank were used for alignment by BioEdit computer programmes, and a tree of phylogeny was built in

MEGA6 to show the relationship of the *Sitophilus oryzae* with previously reported species in NCBI. The current analysis revealed strong similarities to the *Sitophilus oryzae* species, which was discovered in India and assigned the accession number (KF298426.1) in Fig 3.

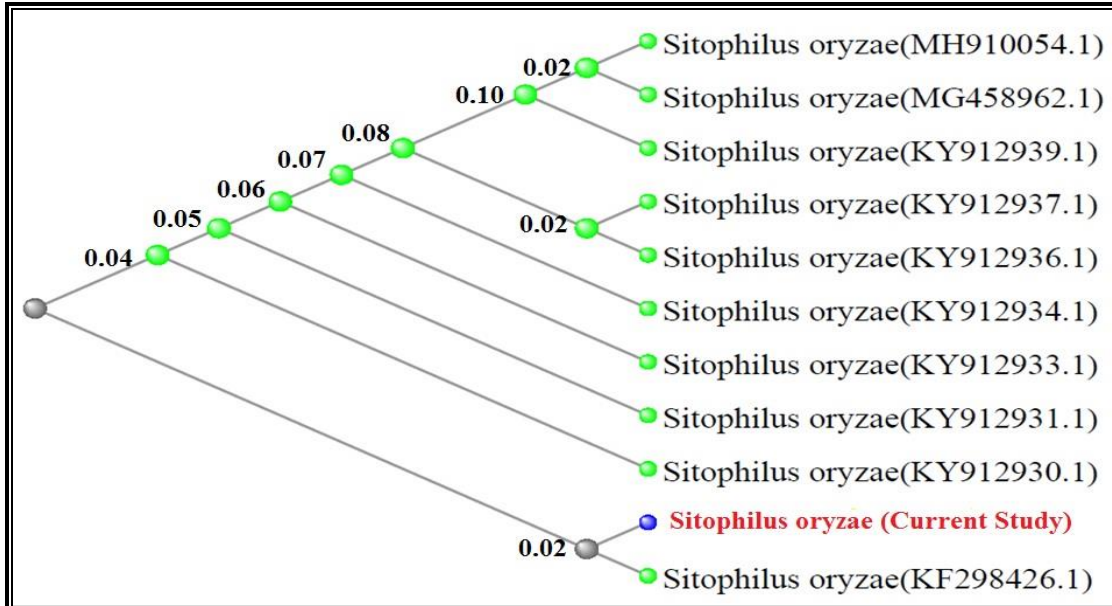


Fig. 3 Phylogeny of rice weevils (*Sitophilus oryzae*)

Morphological description of *Sitophilus granarius* (Linnaeus, 1875): *Sitophilus granarius* adults can range in length from 2.9 to 3.9 mm, while 3 to 4 mm is most common. They display the Curculionidae family's distinctive rostrum and elbowed antennae. They have an evenly polished tint ranging from reddish brown to lustrous black. Short, robust yellow hairs are

sporadically distributed throughout the body. The head extends into a thin snout. In comparison to females, men have closer and more pronounced punctures on the dorsal surface of the rostrum. There are prominent round punctures on the prothorax. Adults are unable to fly because they lack wings (Fig 4).

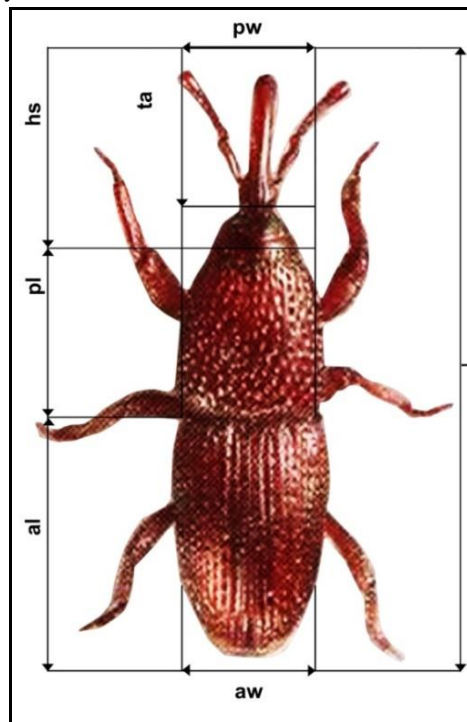


Fig. 4 Morphology of *Sitophilus granarius*

Worldwide Distribution of *Sitophilus granarius*:
This species occurs in Africa, Germany, Pakistan,

Poland, Cuba, Malaysia, Iraq, Brazil, Egypt, Australia, United Kingdom, India, Italy, Sweden, Canada,

Hungry, China, Russia, Spain, Argentina, Sri Lanka, France, New Zealand and Greece.

Sitophilus granarius distribution in Khairpur: *Sitophilus granarius* is found extensively throughout the different talukas of the Khairpur district. The taluka Nara had highest population of this species, then

following Kot diji then Kingri. The taluka Khairpur had the lowest concentration of *Sitophilus granarius*, which proceeded by Mirwah as well as Faizganj. Additionally, taluka Gambat and Sobho dero both had a medium population of *Sitophilus granarius* (Fig.5 & Table. 4).

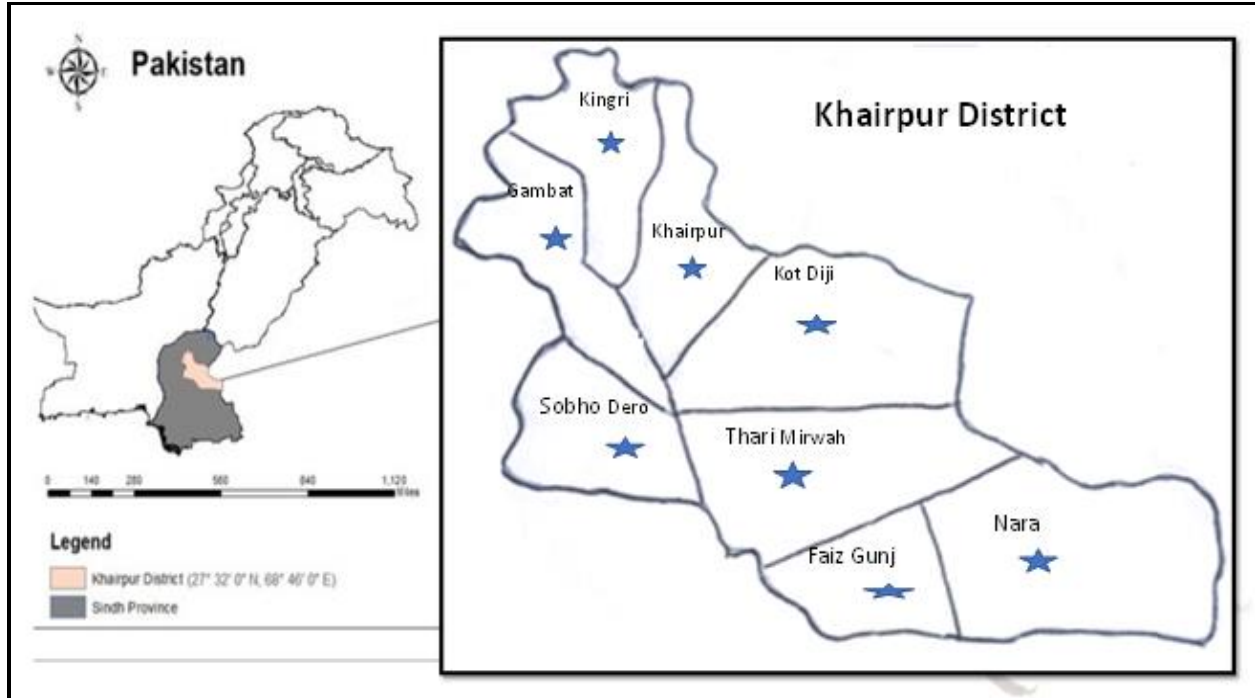


Fig. 5 *Sitophilus granarius* distribution in Khairpur

Table. 4 Distribution of *Sitophilus granarius* in district Khairpur

Sex	Mirwah	Khairpur	Kingri	Kot Diji	Gambat	Sobhodero	Nara	Faizganj
Male	14	08	18	19	22	19	24	21
Female	18	19	22	21	17	19	17	15
Total	32	27	40	40	39	38	41	36

Table 5 Morphometry of *Sitophilus granarius* (Male).

Male (n=15)	Morphometric Parameters (mm)						
	L	Al	Aw	Pl	Pw	hs	Ta
Min	2.980	1.050	0.734	1.010	0.722	1.003	0.490
Max	3.890	1.433	0.823	1.230	0.890	1.323	0.710
Average	3.435	1.241	0.778	1.120	0.806	1.163	0.600

Table 6 Morphometry of *Sitophilus granarius* (Female).

Female (n=15)	Morphometric Parameters (mm)						
	L	Al	Aw	Pl	Pw	Hs	Ta
Min	3.115	1.102	0.820	1.012	0.785	1.090	0.530
Max	3.990	1.534	0.980	1.010	0.950	1.430	0.812
Average	3.552	1.318	0.900	1.011	0.867	1.260	0.671

Phylogeny of *Sitophilus granarius*: *Sitophilus granarius* provided the nucleotide sequence for the

Cytochrome Oxidase I (COI) gene, which was then subjected to BLAST analysis. Relevant sequences from

the Gene Bank were used for alignment by BioEdit software, and a phylogenetic tree was created in MEGA6 to show the relationship of *Sitophilus granarius* with previously reported species in the NCBI (National Centre for Biotechnology Information). The

results of the current study revealed strong genetic similarities to the *Sitophilus granarius* species found in the USA, accession number (MG458963.1) depicted in Figure 6.

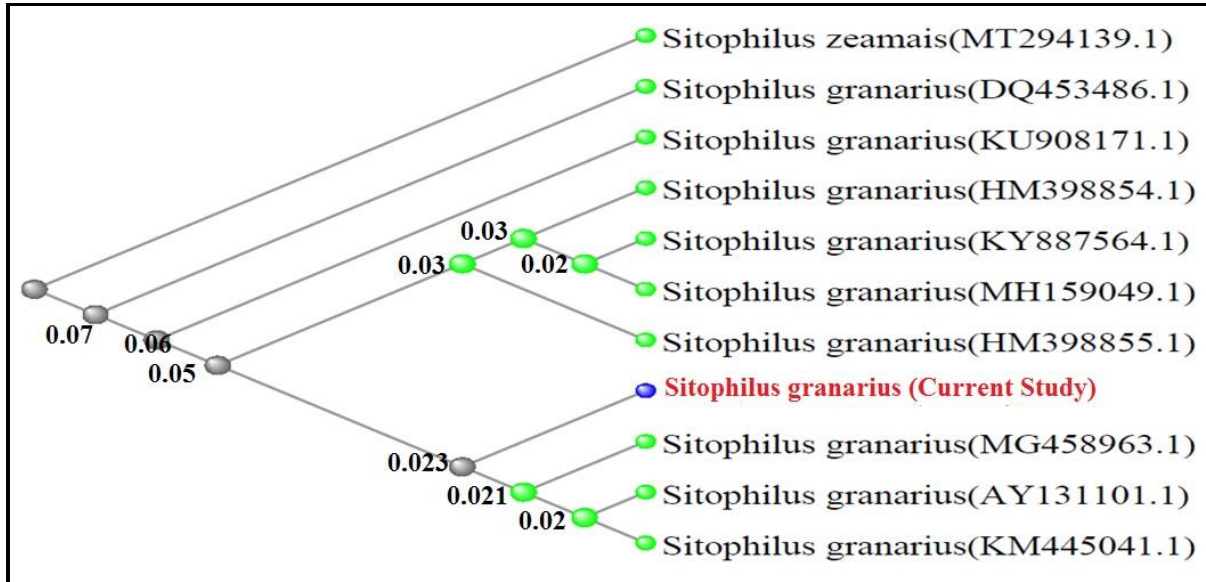


Fig. 6 Phylogeny of wheat weevil (*Sitophilus granarius*)

Morphological description of *Sitophilus zeamais* Motschulsky 1855: On the wing coverings, the reddish patterns are more distinct. The maize weevil is a small, variable-sized snout beetle with an average length of four millimetres. It can range in colour from dark reddish brown to practically black, and the back normally bears four faint reddish or yellowish patches. Under its wing covers, the maize weevil has fully grown wings and is able to fly with ease. With the exception of a smooth, narrow strip running down the centre of the dorsal (top) side, the thorax is heavily pitted and punctured with slightly irregularly shaped holes (Fig. 7).

Worldwide Distribution of *Sitophilus zeamais*.

This species occurs in Australia, Korea, Africa, India, Russia, Egypt, Japan, Yemen, Italy, Syria, Saudi Arabia, Sri Lanka, Pakistan, Malaysia, China, Germany, Brazil, Iraq, New Zealand, Greece, Canada, Poland, Cuba, Sweden and United Kingdom.

***Sitophilus zeamais* distribution in Khairpur.**

Sitophilus zeamais is distributed throughout the different talukas of the Khairpur district. The taluka Nara had the highest population of this species, then Kingri as well as Sobhodero. The taluka Khairpur had the lowest number of *Sitophilus zeamais*, which was subsequently pursued by Mirwah then Gambat. Besides, taluka Kot deji and Faizganj both have a medium population of *Sitophilus zeamais* (Fig.8 & Table.7)

Phylogeny of *Sitophilus zeamais*: The cytochrome oxidase I (COI) gene's nucleotide sequence was taken from *Sitophilus zeamais* and analysed using BLAST analysis. Following that, pertinent sequences obtained from the Gene Bank had been utilised for alignment by BioEdit programmes, and a tree of phylogeny was generated in MEGA6 to show the relationship of *Sitophilus zeamais* with previously identified species in NCBI. The current analysis revealed strong similarities to the *Sitophilus zeamais* species described from China and assigned the accession number (MT294139.1) in Figure9

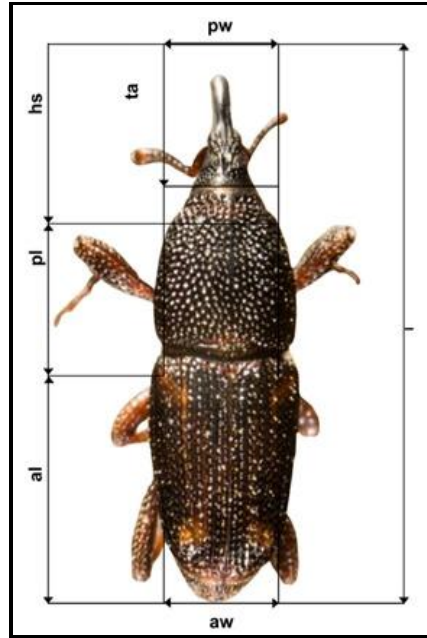


Fig. 7 Morphology of *Sitophilus zeamais*

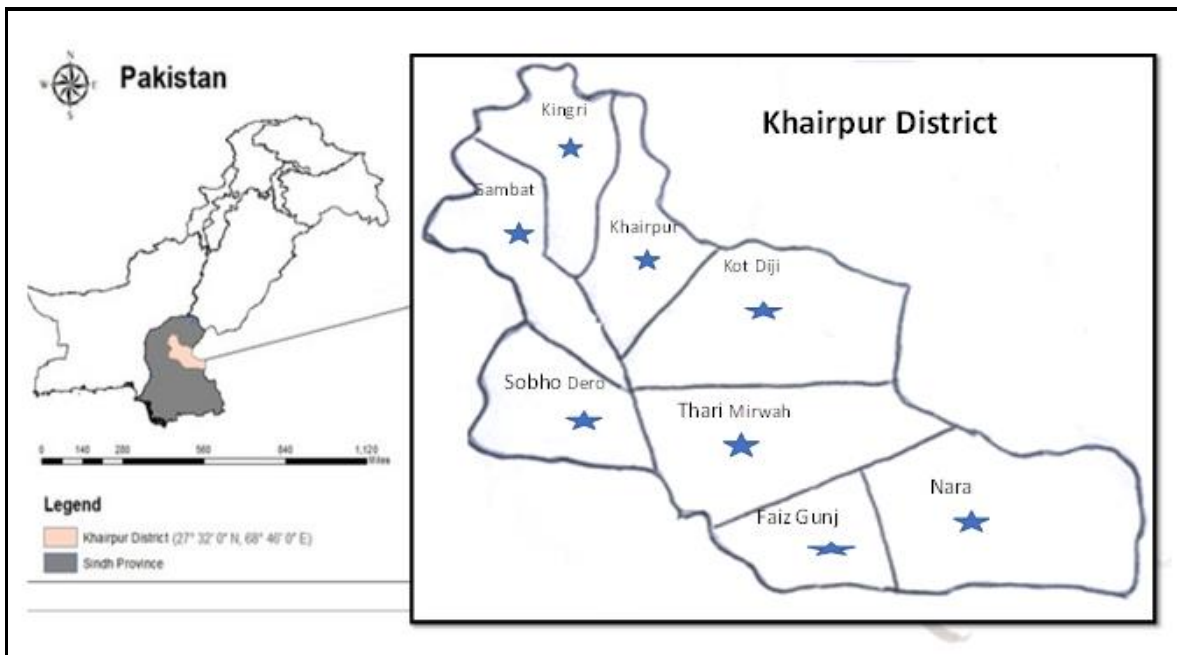


Fig. 8 *Sitophilus zeamais* distribution in Khairpur

Table 7 Distribution of *Sitophilus zeamais* in district Khairpur

Sex	Mirwah	Khairpur	Kot Diji	Kingri	Gambat	Sobhodero	Nara	Faizganj
Male	16	16	17	18	15	16	18	19
Female	15	13	21	15	17	19	19	14
Total	31	29	38	33	32	35	37	33

Table 8 Morphometry of *Sitophilus zeamais* (Male).

Male (N=15)	Morphometric Parameters (mm)						
	L	Al	aw	Pl	Pw	Hs	ta
Min	3.780	1.430	0.734	1.150	0.722	1.230	0.610
Max	4.310	1.112	0.900	1.104	0.890	1.120	0.810

Average	4.045	1.271	0.817	1.127	0.806	1.175	0.71
---------	-------	-------	-------	-------	-------	-------	------

Table 9 Morphometry of of *Sitophilus zeamais* (Female).

Female (N=15)	Morphometric Parameters (mm)						
	L	Al	aw	Pl	Pw	Hs	ta
Min	3.950	1.612	0.930	1.122	0.785	1.334	0.710
Max	4.320	1.634	0.980	1.254	0.950	1.430	0.910
Average	4.135	1.623	0.955	1.188	0.868	1.382	0.81

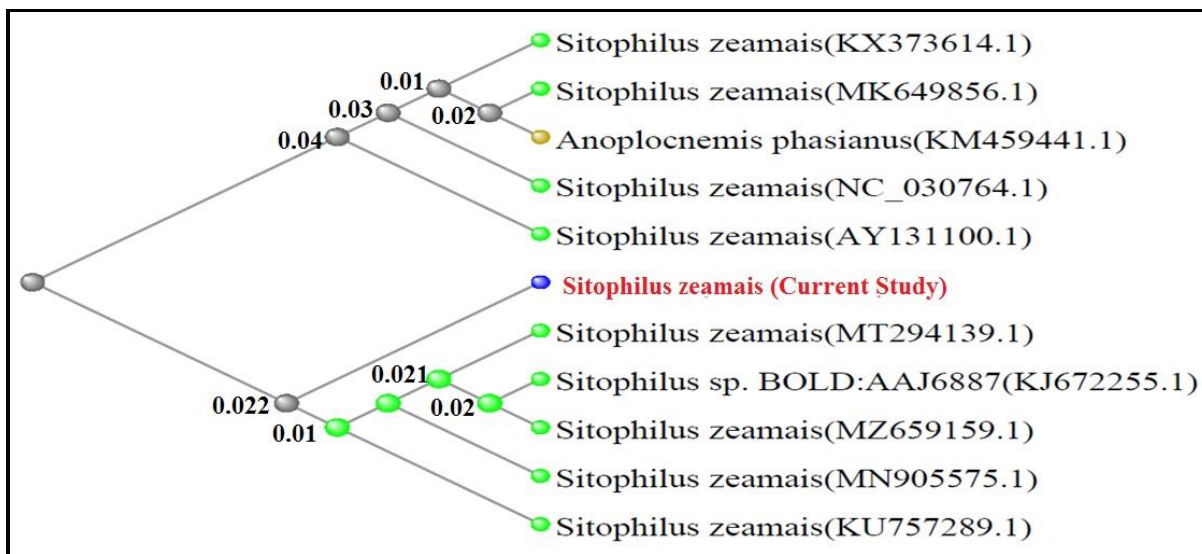


Fig. 9 Phylogeny of maize weevils (*Sitophilus zeamais*)

Discussion

Weevils are members of the superfamily Curculionidea, which also includes several widely recognized Coleoptera species. Several weevils possess snouts that are the same length as their bodies (Farrell *et al.*, 2001). Most weevil species that prey on grains belongs to genus *Sitophilus*. Weevil infestations are periodic or temporal and fluctuates between numerous months of the year, according to various survey programmes (Wakil *et al.*, 2021). During the present study *Sitophilus* species were collected from eight talukas of district Khairpur: Kingri, Kot Deji, Khairpur, Gamabat, Mirwah, Sobhodero, Nara, and Faizganj. A total of 210 samples of weevils were captured from infested palm trees using by handpicking methods. The samples were identified into 03 species: *Sitophilus oryzae*, *Sitophilus zeamais*, and *Sitophilus granarius*. Zafar *et al.*, (2021) while studying the diversity of weevils in Gujrat, Pakistan collected 315 specimens of weevils and reported 03, i-e: *Sitophilus granarius*, *Sitophilus zeamais*. They also noted that diversity was consistent throughout, temperature and humidity had an impact on abundance. The abundance rose along with these two characteristics. August having the highest abundance. More female weevils than male weevils were present. Between the two factors and

diversity, a positive link developed. Most of weevils, at larvae and adults stage, feed on plants. Weevils are significant agricultural pests because they chew holes in fruits, nuts, and other plant material. The majority of the adult weevils possess a long rostrum and use to help ovipositing the eggs (deposit eggs) in plant tissue. Others feed on decaying wood or bark, while certain wood-borers eat ambrosia fungus (Farrell *et al.*, 2001). Infestations of stored grain insects can lead to decreased weight, quality, earnings, and seed viability. The majority of these insects about 75% are coleopterans, and the most destructive species of storage insects are found in the genera *Sitophilus* and *Tribolium*. The economically important and widespread bug *Sitophilus* species consumes stored grains. The outermost layer of the grain is not the preferred food source for the larvae, which causes a large loss of protein as well as vitamins. The adults of weevils mostly consume its endosperm lowering the amount of carbohydrates present. Insects which attack the germ particularly will suffer a greater loss in germination (DalBello *et al.*, 2000). Besides, morphological features. Cox1 has emerged as the preferred gene for DNA barcoding initiatives. It's critical to differentiate among phylogeny, which aims at reconstructed history of evolution, and barcoding, and whose goal is to identify species. Cox1 is

especially well suited for this because it is present in all eukaryotes, has a high mutation rate, and lacks introns. Additionally, there are universal primers that make it relatively simple to amplify the gene across many lineages. It has been demonstrated to be reliable and economical. Cox1 sequences have recently been employed with effectiveness for automatic species finding and delimitation (Hebert *et al.*, 2003). During the present study the Cytochrome Oxidase I (COI) gene's nucleotide sequence was taken from the *Sitophilus oryzae*, *Sitophilus granarius*, and *Sitophilus zeamais* were subjected to BLAST analysis. Then, relevant sequences from the Gene Bank were used for alignment by BioEdit computer programmes, and a tree of phylogeny was built in MEGA6 to show the relationship of the *Sitophilus oryzae* with previously reported species in NCBI. The current analysis revealed strong similarities to the *Sitophilus oryzae* species, which was discovered in India and assigned the accession number (KF298426.1). *Sitophilus granarius* provided the nucleotide sequence for the Cytochrome Oxidase I (COI) gene, which was then subjected to BLAST analysis. Relevant sequences from the Gene Bank were used for alignment by BioEdit software, and a phylogenetic tree was created in MEGA6 to show the relationship of *Sitophilus granarius* with previously reported species in the NCBI (National Centre for Biotechnology Information). The results of the current study revealed strong genetic similarities to the *Sitophilus granarius* species found in the USA, accession number (MG458963.1). The cytochrome oxidase I (COI) gene's nucleotide sequence was taken from *Sitophilus zeamais* and analysed using BLAST analysis. Following that, pertinent sequences obtained from the Gene Bank had been utilised for alignment by BioEdit programmes, and a tree of phylogeny was generated in MEGA6 to show the relationship of *Sitophilus zeamais* with previously identified species in NCBI. The current analysis revealed strong similarities to the *Sitophilus zeamais* species described from China and assigned the accession number (MT294139.1). This study has confirmed the status of *Sitophilus* species both on morphology and molecular basis.

Conclusion

Present study concluded that based on their morphology and DNA makeup, the current study has determined that the three species of the genus *Sitophilus* that are commonly referred to as stored grain pests are actually three distinct species. Additionally, this is the only piece of its kind to come from Pakistan's Sindh to the best of our knowledge. Hopefully, the results of this study will act as a starting point for future researchers that are interested in the *Sitophilus* fauna.

References:

- Athanassiou, C. G., Kavallieratos, N. G., & Campbell, J. F. Competition of three species of *Sitophilus* on rice and maize. *PLoS One*, **12**(3), e0173377. (2017).
- Armstrong, K. F., & Wratten, S. D. The use of DNA analysis and the polymerase chain reaction in the study of introduced pests in New Zealand. *Systematics Association Special Volume*, **53**, 231-264. (1996).
- Bernal, J. S., & Navarro, S. The evolutionary history of weevils of the genus *Sitophilus* (Coleoptera: Curculionidae): a molecular phylogenetic approach. *Molecular Phylogenetics and Evolution*, **67**(1), 92-104. (2013).
- Black, W.C.I.V., Baer, C.F., Antolin, M.F & DuTeau, N.M. Population genomics: genome-wide sampling of insect populations. *Annu. Rev. Entomol.*, **46**: 441-69. (2001).
- Brown, R.J., Malcolm, C.A., Mason, P.L. & Nichols, R.A. Genetic differentiation between and within strains of the saw-toothed beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) at RAPD loci. *Insect Mol. Biol.*, **6**: 285-289. (1997).
- Cognato, A.I, Sperling, F.A.H. Phylogeny of *Ips* DeGeer species (Coleoptera: Scolytidae) inferred from mitochondrial cytochrome oxidase I DNA sequence. *Molecular Phylogenetics and Evolution* **14**, 445-460. (2000).
- Cruikshank, R.H Molecular markers for the phylogenetics of mites and ticks. *Applied Aracology* **7**, 3-14. (2002).
- DalBello, G., Padin, S., Lastra, C. L., & Fabrizio, M. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. *Journal of stored products research*, **37**(1), 77-84. (2000).
- Farrell, B. D., Sequeira, A. S., O'Meara, B. C., Normark, B. B., Chung, J. H., & Jordal, B. H. The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution*, **55**(10), 2011-2027. (2001).
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**(5), 294-299. (1994).
- Hagstrum, D. W., & Subramanyam, B. Stored-Product Insect Resource. Elsevier. (2019).
- Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: *Biological Sciences*, **270** (1512), 313-321. (2003).

- Makarova, O. L., & Kuzmina, V. V. Population genetic structure of the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), inferred from mitochondrial and nuclear DNA sequences. *European Journal of Entomology*, **113**(1), 42-49. (2016).
- Mizzi S., Dandria D., Mifsud D., & Longo S. The red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) in Malta (Coleoptera: Curculionidae). *Bulletin of the Entomological Society of Malta*, **2**: 111-121. (2009).
- Mason, L.J., & McDonough, M. Biology, behavior, and ecology of stored grain and legume insects in: Hagstrum, D.W., Philips, T.W., Cuperus, G (Eds.), stored product protection. Kanas State University Agricultural Experiment Station and Cooperative Extension Service, pp.7-20. (2012).
- Panhwar, W. A. ., Khatri, I., & Soomro, F. (2023). Records of Orthoptera in Fauna of British India within present boundaries of Pakistan. *Journal of Applied Research in Plant Sciences* , **4**(02), 567–570.
- Shi, Z, Du, J, Yang, W, Zhang Y, Zhang, Z, & Wang, D. Comparative analysis of mitochondrial genomes reveals mitochondrial DNA differentiation and species-specific molecular markers between *Sitophilus oryzae* and *Sitophilus zeamais*. *International Journal of Molecular Sciences*; **21**(8):2954. 2020
- Smith, C, & Smith, R. Morphological characteristics and identification of rice and maize weevils (*Sitophilus* spp.) infesting stored grain. *Journal of Stored Products Research*. **25**(3):135-139. 1989
- Smith, P. T., & Navarro, S. Morphological and molecular variation within and between strains of *Sitophilus oryzae* (Coleoptera: Curculionidae). *Journal of Economic Entomology*, **110**(5), 2093-2103. (2017).
- Trematerra, P. Preferences of *Sitophilus zeamais* to different types of Italian commercial rice and cereal pasta. *Bulletin of Insectology*, **62**(1), 103-106. (2009)
- Wakil, W., Kavallieratos, N. G., Ghazanfar, M. U., Usman, M., Habib, A., & El-Shafie, H. A. Efficacy of different entomopathogenic fungal isolates against four key stored-grain beetle species. *Journal of Stored Products Research*, **93**, 101845. (2021).
- Zafar, H., Afsheen, S., Bhatti, A. R., Malik, M. F., Zia, S. A., Mubeen, S., & Aziz, S. S. Diversity of weevils in rice mills of Tehsil Gujrat, Punjab, Pakistan. *Pure and Applied Biology (PAB)*, **10**(3), 751-760. (2021).

Publisher's note: PJBT remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. To

view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>
