Indian Journal of Entomology Online published Ref. No. e24322 DoI. No.: 10.55446/IJE.2024.2322

TAXONOMY AND DNA BAR CODING OF CACTUS MOTH *CACTOBLASTIS CACTORUM* (BERG)

Walija Fayaz[1], Imtiaz Ali Khan[1]* and Amjad Usman[1]

[1]Department of Entomology, The University of Agriculture Peshawar, Peshawar, Pakistan
*Email: imtiazkhanswb@gmail.com (corresponding author): ORCID ID 0000-0003-2428-5429

ABSTRACT

The cactus moth *Cactoblastis cactorum* (Berg) is endemic to the opuntia weeds around the world. Its larvae were used for biological control of both alien and native cactus weed in Pakistan in 1994 after its success in Australia. Its efficiency and side effects were not studied in Pakistan. In 2016, the moth severely devastated the cultivated *Opuntia ficus-indica* (L.) Mill in district Chakwal, Punjab, Pakistan. In this study, *C. cactorum* larvae were reported for the first time from Khyber Pakhtunkhwa province of Pakistan. Full-grown larvae (6th instar) were collected to study its classical taxonomy and DNA bar coding from various selected localities. For DNA bar coding, two DNA primers were used, where both the primers showed highest value for species identification.

Key words: *Cactoblastis cactorum,* taxonomy, DNA barcoding, invasive species, biological control, prickly pear cacti, *Opuntia*, Lepidoptera, Phycitinae, phylogenetic analysis, mtCo1

The cactus moth *Cactoblastis cactorum* (Berg) is one of the important species under family Pyralidae and subfamily phycitinae. Recently the first molecular phylogeny of the Phycitinae subfamily was made based on two regions of gene self-regulation, i.e., cytochrome oxidase and elongation factor 1alpha. These results showed two main groups of genera and the morphological characters seem to resemble these relations to some extent (Roe et al., 2015). Phycitinca moths are well known for their various environmental and economic impacts (Ahmad et al., 2017; Bae et al., 2017). Phycitinae, which feed on cacti, have been studied for ecological and biological control (Simonsen et al., 2008). The phycitine moth *Cactoblastis cactorum*, is a native of Argentina, Bolivia, Brazil, Paraguay, Peru, and Uruguay in South America. To biologically control weeds *C. cactorum* larvae from Argentina were released in Australia in the 1920's to eliminate the exotic prickly pear (Dodd, 1940; Legaspi and Legaspi 2010; McFadyen, 1985; Zimmermann et al., 2004). The moth was successful in controlling the *Opuntia* weed. Following this its in many locations larvae have been introduced including Pakistan (Legaspi and Legaspi, 2010; Zimmermann et al., 2004) and in Pakistan, it was confirmed in 2016 (Rafi et al., 2022). DNA bar coding has arisen as a useful means for the identification of animal species (Hebert et al., 2003; Tyagi et al., 2010; Virgilio et al., 2010). In a mtCo1 analysis five different haplotypes of *C. cactorum* were identified from 20 populations from Australia, Hawaii, the Caribbean, Mexico, the southeastern USA, and South Africa (Simonsen et al., 2008). According to Ashfaq et al. (2022) among 6,590 Barcode Index Numbers (BINs) more than half of these BINs in south Asia lie in Pakistan. The present study analysis its population from Pakistan.

MATERIALS AND METHODS

Six localities from each district were carefully selected based on wild cactus plantation from four selected divisions, namely Mardan, Peshawar, Malakand and Hazara of the Khyber Pakhtunkhwa Province of Pakistan. Infested cladodes were collected and dissected to detect the occurrence of cactus moth larvae. Collected materials were examined morphologically in the field with the help of a field microscope (Model#J2705), and magnifying lenses and with published literature (Folgarait et al., 2018; Karolina et al., 2021; Bennett and Miller, 2016; Walczak et al., 2024). DNA from larvae was extracted using a modified standard method and the protocol was optimized (Van Heesch et al., 2013; Mehmood, 2016). *C. cactorum* larvae were preserved in vials with 70% alcohol, PCR amplification of the extracted DNA was carried out, and sequencing was performed at the Canadian Centre for DNA Bar coding (CCDB), following the established protocols (Hebert et al., 2018; Ivanova et al., 2006; de Waard et al., 2019a; Vella et al., 2022). The amplified PCR products were run on a 1.5% agarose gel to check for successful amplification of DNA markers, and the same procedure

was used as described above. The DNA ladder mix was also loaded on the gel to estimate the size of PCR products. In accordance with the established protocols, DNA extraction, amplification by PCR, and sequencing were carried out at the CCDB (DeWaard et al., 2019b; Hebert et al., 2018). Based on the aligned sequences, phylogenetic analysis was performed using MEGA 7 with maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP) (Kumar et al., 2016). By utilizing the Kimura-2 parameter, neighbor-joining tree with 1000 bootstrap was constructed (Swofford, 2002). Sequences were assembled, aligned, and edited using Codon Code Aligner before submission to BOLD. SMRT sequencing protocols were employed (Hebert et al., 2018). The resulting sequences were uploaded to mBRAVE (Multiplex Barcoding Research and Visualization Environment) for editing (sequence trimming, quality filtering, de-replication), identification, and generation of operational taxonomic units (OTUs). The edited sequences were subsequently exported to BOLD for BIN assignment and reference library development.

RESULTS AND DISCUSSION

The larvae were gregarious measuring 30-32 mm in lengths; brilliant orange or crimson with broken cross bands or rows of black dots; two SV setae were found in abdominal segments seven and eight (Fig. 1). The examined larvae were identified as *C. cactorum*, (Fig. 1). The genus *Cactoblastis* comprises of five species, namely *C. cactorum*, *C. Bucyrus* Dyar, *C. mundelli* Heinrich, *C. doddi* Heinrich, and *C. ronnai* (Brèthes). These are all together restricted to the South American countries, i.e., Argentina, Bolivia, Brazil, Paraguay, Peru, and Uruguay (Dodd, 1940; Heinrich, 1939; Mann, 1969; Zimmermann et al., 2007). These larvae can damage entire cactus plantations (Rose, 2009). It is a frequent pest of cactus species under the genera *Nopalea*, *Cylindropuntia*,and *Consolea* of the family Cactaceae (Rose, 2009) and can cause death of the whole plant (Rose, 2009). Recently, in two studies from the Nearctic region a connection was found between *C. cactorum* and the species of the genus *Hylocereus* (epiphytic cacti) (Galette, 2015). The most recent discovery of *C. cactorum* as a potential pest of *Hylocereus lemairei* Hook and *H. costaricensis* in Brazil was made (Hoshinoa et al., 2022). The adult *C. cactorum* moths are chracterised by colored spots on the fully formed 6th instar larva which is brilliant orange-red, 25-30 mm long, and has transverse broad bands that produce black dots (Habeck et al., 2016). Adults are difficult to distinguish with (Folgarait et al., 2018). With the aid of the morphology of completely grown larvae, the best method of species identification is simple (Habeck et al., 2016), but several species of the genus Cactoblastis' larvae were shown to be unique (Folgarait et al., 2018).

Fig. 1. Full grown larvae of *C. cactorum*

The results of the present study revealed relatively low genetic diversity in *C. cactorum* populations in Pakistan. A total of 27 haplotypes were identified based on the mtCOI sequences, with six haplotypes unique to Pakistan. Phylogenetic analysis showed that the *C. cactorum* populations in Pakistan clustered with haplo types from other countries, indicating multiple introductions of them into Pakistan. Population structure analysis indicated low levels of genetic differentiation among the *C. cactorum* populations in Pakistan, suggesting a high degree of gene flow among them. Phylogenetic relationship among the *C. cactorum* species accession after retrieving DNA sequences from NCBI-GenBank. BLAST (Basic Local Alignment Search Tool) was done. These revealed that 98%- 99% similarty for all the sequences of *Cactoblastis* spp. After BLAST, the nucleotide sequences of these species were aligned using MUSCLE alignment online software. The aligned data sheet had a maximum of 890 genetic characters, and after trimming, the extra and ambiguously aligned fragments from both 5' and 3' ends of the aligned data sheet's remaining 715 genetic characters were used for further analysis. The mean pair wise distances (MPD) of each species ranged from 0.012 to 32.18%. While differences in evolutionary rate among two categories, gamma distribution and invariant (+G+I), were recorded as 0.89 and 1.01. The ML tree was constructed with the highest log likelihood of -11567.2668and bootstrap-supported values below the branches (Fig. 2). Among 715 genetic characters or positions, 33 were conserved, 601were variable, 80 were parsimony informative, and was a single-tone site. The MP tree obtained with

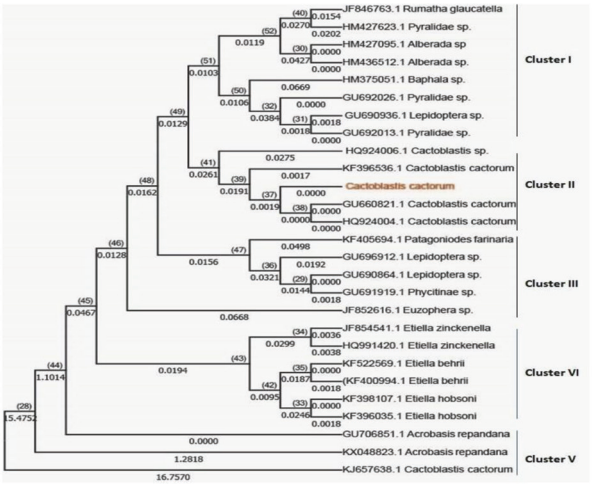

Fig. 2. Maximum Likelihood in the phylogenetic tree of *C. cactorum* generated using the Tamura and Nei method, bootstrap values are shown above the relevant branches. The sequence of the current study is highlighted in red

length =3444 has boot strap-supported values above the branches (Fig. 3). The consistency index (CI), retention index (RI), and composite index were recorded as 0.288261, 0.643373, and 0.174490, respectively. The effective sample size (ESS) was recorded as 18 using Bayesian analysis. Maximum parsimony, maximum likelihood, and neighbour joining approaches were employed to construct a phylogenetic tree by using the software Bio Edit, Mega-7, and the online MUSCLE software. The maximum likelihood phylogenetic tree was constructed into five clades, i.e., I, II, III, VI, and V. Our newly sequenced insect species from Pakistan were cluster edintoclade II; in this clade, 5 species were gathered (Fig. 4). Whereas *C. cactorum* showed similarity with *C. cactorum* species (GU660821.1 and HQ924004.1) having bootstrap values (MLM = 99% and branch length 0.0191). The maximum parsimony analysis was used to construct the phylogenetic tree

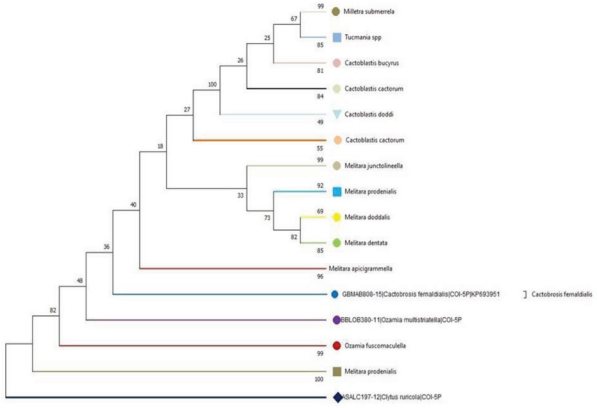

Fig. 3. Maximum Parsimony phylogenetic tree of *C. cactorum* generated using neighbor joining analysis

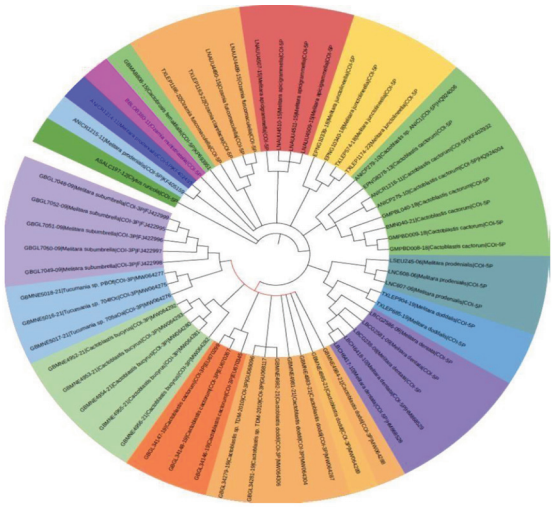

Fig. 4. Neighbour joining in the phylogenetic tree of *C. cactorum* generated using the neighbor joining method

among *C. cactorum* species. The MP tree resulted in 3 clades, i.e., clades I, II, and III. The newly sequenced *C. cactorum* was clustered into clade III. In clade III, a total of 6 species were clustered and showed affinity with (GMPBD009-18 Pakistan Punjab, BOLDAAA7177 Mexico, GMPBD008-18| Pakistan. Punjab, ID Unpublished|Mexico |BOLD:AAA7177, ID Unpublished|Mexico. Quintana Roo|BOLD:AAA7177, BMN040-21| Pakistan. Baluchistan) Khyber Pakhtunkhwa Swabi (Fig. 3). The phylogenetic study revealed similarity of all sequences having boots trap values (ranges=75%-95%). The evolutionary history was inferred using the neighbor-joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% boots trap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in units of the number of base substitutions per site. The differences in composition bias among sequences were considered in evolutionary comparisons. This analysis involved 58 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd, and noncoding. All ambiguous positions were removed for each sequence p air (pair wise deletion option) (Fig. 3).

Among *C. cactorum*, the NJ tree resulted in 14 clusters; *C. cactorum* was grouped in clade II with the other four *Cactoblastis* and showed similarities with (HQ924006.1, KF396536.1, GU66082.1,

andKF39666.1) (Fig. 2). The evolutionary history was inferred using the neighbor-joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The differences in composition bias among sequences were considered in evolutionary comparisons. This analysis involved 58 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding (Fig. 4).

An ideal DNA characterization should be short, making it easy for recovery, and have sufficient information to provide maximal species discrimination (Kress et al., 2005; Yan *et al.,* 2015). In this study, two DNA primers were used, and all primers showed the highest value for species identification. The present study provides the current distribution of *C. cactorum* in various localities of districts in selected divisions of Khyber Pakhtunkhwa, based on mitochondrial DNA markers.

ACKNOWLEDGEMENTS

The authors acknowledge the support (specie confirmation) of Dr. Muhammad Islam, National Agricultural Research Center (NARC) Islamabad, Pakistan. Muhammad Ashraf (also raised *C. cactorum* larvae), Muhammad Tahir and Majid Bilal (NARC) for the sample collection at survey and Mr. Muhammad Israr to provide transport during surveys.

FINANCIAL SUPPORT

The current research project was financially supported by the Department of Entomology, The University of Agriculture Peshawar.

AUTHOR CONTRIBUTION

Fayaz W designed and carried out the experiments project and prepared an initial draft of the manuscript. Khan I A and Usman A revised the initial draft and helped in designing and overall supervision of the project.

CONFLICT OF INTETERST

No conflict of interest.

REFERENCES

Ahmad T, Sarwar Z M, Ijaz M, Sajjad M, Binyamen M. 2017. Biodiversity and faunistic studies of the family Pyralidae (Lepidoptera) from Pothwar Region, Punjab, Pakistan. Pakistan Journal of life Sciences 15(2): 126-132.

Ashfaq M, Hebert P D N. 2016. DNA bar codes for bio-surveillance: Regulated and economically important arthropod plant pests. Genome 59(11): 933-945.

Bae Y S, Paek M, Qi M. 2017. Insect fauna of Korea. Arthropoda: Insecta: Lepidoptera: Pyralidae (PhycitinaeI) Pyralid Moths II. National Institute of Biological Resources Ministry of Environment, Korea 16(15): 148.

Berg C. 1885. *Zophodia cactorum* Berg. Analesdela Sociedad Cientifica Argentina, *19*, 276.

Brèthes J, 1920. Type species: *Neopyralisronnai* Brèthes, original designation. Anales Sociedad Rural Argentina 5: 284.

De Waard J R, Ratnasingham S, Zakharov E V, Borisenko A V, Steinke, D, Telfer A C, Perez K H J, Sones J E, Young M R, Levesque-Beaudin,V, Sobel C N, Abrahamyan A, Bessonov K, Blagoev G, de Waard S L, Ho C, Ivanova N V, Layton K K S, Lu L, Manjunath R, McKeown JT. A, Milton M A, Miskie R, Monkhouse N, Naik, S, Nikolova N, Pentinsaari M, Prosser S W J, Radulovici A E, Steinke C, Warne C P, Hebert P D N. 2019a. A reference library for the identification of Canadian invertebrates:1.5 million DNA barcodes, voucher specimens, and genomic samples. Scientific Data 6(1): 308.

DeWaard J R, Levesque-Beaudin V, deWaard S L, Ivanova N V, McKeown J T A, Miskie R, Naik S, Perez K H J, Ratnasingham S, Sobel C N, Sones J E, Steinke A E, Telfer A C, Young A D, Young M R, Zakharov E V, Hebert P D N, Wilson J J. 2019b. Expedited assessment of terrestrial arthropod diversity by coupling Malaise traps with DNA barcoding. Genome 62(3): 8595.

Dodd A P, 1940. The biological campaign against prickly pear. Commonwealth Prickly Pear Board, Brisbane, Australia: pp.1-177.

Heinrich C. 1939. The cactus feeding Phycitinae: a contribution toward a revision of the American pyralidoid moths of the family Phycitidae. Proceedings of United States National Museum 86: 331-413.

Folgarait P J, Montenegro G A, Plowes R M, Gilbert. 2018. A study of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in its native range: further insights into life cycle, larval identification, developmental parameters, natural enemies, and damage to the host plant *Opuntia ficus-indica* (Caryophyllales: Cactaceae). Florida Entomologist 101(4): 559-572.

Galette A S, 2015. Host range expansion of the argentine cactus moth, *Cactoblastis cactorum* on to dragon fruit, *Hylocereus* spp. Master Thesis for the degree in Agricultural Sciences, Florida Agricultural and Mechanical University. 80 pp.

Habeck D H, Bennett F D, Miller C. 2016. Cactusmoth, *Cactoblastis cactorum* (Berg) (Insecta: Lepidoptera: Pyralidae). Department of Entomology and Nematology, UF/IFAS Extension. EENY-056, Original publication date August 1998. Revised September. 2012, April 2013 and June 2016.

Hebert P D N, Braukmann T W A, Prosser S W J, Ratnasingham S, deWaard J R, Ivanova N V, Janzen D H, Hallwachs W, Nai S, Sones J E, Zakharov E V. 2018. A Sequel to Sanger: ampli consequencing that scales. BMC Genomics 19: 219.

Hebert P D N, deWaard J R, Zakharov E V, Prosser S W J, Sones J E, McKeown T A, Mantl B, Salle J L, 2013. A DNA 'Barcode Blitz': Rapid digitization and sequencing of a natural history collection. PLoS ONE 8(7): 685-690.

Hoshinoa A T, Androciolib H G, Caviglioneb J H, Aulerb P A M, Menezes

Juniora A O. 2022. First Record of *Cactoblastis cactorum* Berg, 1885 (Lepidoptera: Pyralidae) in *Hylocereus lemairei* (Hook.) Brittonand Rose and *H. costaricensis* (F. A. C. Weber) Britton and Rose (Cactaceae) in Brazil. Brazilian Journal of Biology 82: e238020.

Kress W J, Wurdack K J, Zimmer E A, Weigt L A, Janzen D H. 2005. Use of DNA barcodes to identify flowering plants. Proceedings of National Academy of Sciences 102(23): 83698374.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Data sets. Molecular Biology and Evolution 33(7): 1870-4.

Legaspi Jr B C, Legaspi J C. 2010. Field-Level validation of a CLIMEX Model for C*actoblastis cactorum* (Lepidoptera: Pyralidae) using estimated larval growth rates. Quantitative Ecology: 395-377.

Mahlerová K, Jakubec P, Novák M, Růžička J. 2021. Description of larval morphology and phylogenetic relationships of *Heterotemna tenuicornis* (Silphidae). Scientific Reports 11: 16973.

Mehmood S A. 2016. Analysis of species diversity of Odonata in Hazara region of Pakistan through conventional and molecular approaches. Ph.D. thesis Department of Zoology Hazara University Mansehra - Pakistan.

Rafi M A, Pavulaan H, Islam M, Ashfaq M, Kamran H, Fayaz W, Parveen G N, Sultana R, Zia A, Ahmed W, Ullah Q, Qasim M, Naz F, Ahmed N, Khan M T, Saeed M, Khan J H. 2022. Establishment of the invasive cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) in Pakistan: A potential threat to cultivated, ornamental and wild *Opuntia* spp. (Cactaceae). The Taxonomic Report of the International Lepidoptera Survey 10(10): 33 pp.

Rose R. 2009. Eradication of South American cactus moth, *Cactoblastis cactorum*, from 11 Parishes in Southeastern Louisiana. Environmental Assessment, United States Department of Agriculture: 10 pp.

Roe A D, Simonsen T J, Scholtens B, Sperling F A H, Weller S J. 2015. Phycitinae Phylogeny based on two genes, with implications for morphological trait evolution and Heinrich's tribal classification (Lepidoptera: Pyralidae). Journal Lepidopteran Society 69(3): 157-172.

Simonsen T J, Brown R L, Sperling F A H. 2008. Tracinganinvasion: Phylo geography of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in the United States based on mitochondrial DNA. Annales of Entomological Society of America 101(5): 899-905.

Swofford D L. 2010. PAUP: phylogenetic analysis using parsimony (and other methods) (version4.0b10).

Van Heesch S, Kloosterman W P, Lansu N, Ruzius F P, Levandowsky E, Lee C C, Zhou S, Goldstein S, Schwarts D C, Harkins T T, Guryev V, Cuppen E (2013). Improving mammalian genome scaffolding using large insert mate-pair next-generation sequencing. B M C Genomics 14: 257.

Vella A, Mifsud C M, Magro D, Vella N. 2022. DNA barcoding of Lepidoptera species from the Maltese Islands: New and additional records, with an insight into Endemic Diversity. Diversity 2022 14(12): 1090.

Virgilio M, Backeljau T, Nevado B, Meyer M D. 2010. Comparative performances of DNA bar coding across insect orders. BMC Bioinformatics 11: 206.

Walczak K, Pape T, Wallman J F, Szpila K, Grzywacz A. 2024. To see the unseen: notes on the larval morphology and systematic position of *Achanthiptera* Rondani (Diptera: Muscidae). Arthropod Systematics & Phylogeny 82: 305-22.

Yan L J, Liu J, Möller M, Zhang L, Zhang X M, Li D Z, Gao L M, 2014. DNA barcoding of *Rhododendron* (*Ericaceae*), the largest Chinese plant genus in biodiversity hot spots of the Himalaya-Hengduan Mountains. Molecular Ecology Research 5(4): 932–944.

Zimmermann H G, Bloem S, Klein H. 2007. Biology, history, threat, surveillance andcontrol of the cactus moth, *Cactoblastis cactorum*. http://www. conabio.gob.mx / invasoras / images /4 /4e / OIEABOOK-5dic07.pdf (accessed4-VII-2011).

(Manuscript Received: June, 2024; Revised: July, 2024;
Accepted: August, 2024; Online Published: September, 2024)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e24322