Acta entomologica serbica, 2020, 25(1): 1-11

UDC: 595.754(549.1)
DOI: 10.5281/zenodo.3889728

PHYLOGENETIC ANALYSIS OF RED COTTON BUG SPECIES (HEMIPTERA: PYRRHOCORIDAE) IN PUNJAB, PAKISTAN

WAQAR ALI KHAN[1,2*], SAFI UR REHMAN QAMAR[1,2,3*], JAM NAZEER AHMAD[2], MAYER L. CALMA[4] and ASAD ULLAH[1]

1 Department of Zoology, Wildlife, and Fisheries, University of Agriculture, Faisalabad 38000, Punjab, Pakistan
*E-mail: khanwaqarali0@gmail.com (corresponding author)

2 Department of Entomology, University of Agriculture, Faisalabad 38000, Punjab, Pakistan

3 Department of Applied Biological Sciences, Chulabhorn Graduate Institute, 54 Kamphaeng Phet 6 Road, Lak Si, Bangkok 10210, Thailand
*E-mail: ranasafi73@gmail.com (corresponding author)

4 Department of Biology, College of Science, University of the Philippines, Baguio 2600 Baguio City, Philippines

Abstract

Traditional techniques for identifying the red cotton bugs *Dysdercus koenigii* and *Dysdercus cingulatus* (Pyrrhocoridae: Hemiptera) depend on phenotypic and morphological data. These methods are suitable when species have well-defined structures and when huge taxonomic knowledge is at hand. In this study, the mitochondrial cytochrome oxidase I (COI) gene was selected for the molecular identification of *D. koenigii* and *D. cingulatus*. The sequence data showed that one species of the red cotton bug sample was identified as *Dysdercus koenigii* with 35-100% similarity to other *Dysdercus* species present in the NCBI database, while the other species was identified as *Dysdercus cingulatus* with 88-94% similarity to other red cotton bugs species present in the NCBI database. The phylogenetic analysis revealed that our *D. koenigii* Seq (>180319003-A02-102-DK-.ab1) shares the same cluster with four *D. koenigii* isolates (ZSI/SRC_I.28B, ZSI/SRC_I.28, GQ306227.1, KJ459924.1). While the *D. cingulatus* Seq (>180319003-A02-103-DC-.abl) shares a cluster with *D. cingulatus voucher* (RO_DC2015). This is the first molecular identification of red cotton bugs (*D. koenigii* and *D. cingulatus*) reported in Pakistan. Thus, in the current study, molecular identification has been accepted as a reliable method for the identification of these agriculturally important insect pests.

KEY WORDS: cytochrome oxidase I, red cotton bug, *Dysdercus koenigii*, *Dysdercus cingulatus*, phylogeny, taxonomy, molecular identification

Introduction

Agriculture is the pillar of the economy of Pakistan and most of the people are directly or indirectly associated with agriculture through manufacturing, handling and the supply of main agricultural products (Bakhsh *et al*., 2005). Pakistan is the fourth largest cotton-producing country worldwide after the USA, China and India. In Pakistan, the average harvest is 577.99 kg/ha, which is comparatively low compared to that of other cotton-growing nations (Bakhsh *et al*., 2005). Certain biotic and abiotic factors result in a low productivity of cotton in Pakistan. The most serious threat is the injury caused by different insect pests in cotton crops. More than 1326 insect species are reported to infest cotton crops worldwide (Atwal & Dhaliwal, 2015), including sucking insects like green leafhoppers, aphids, whiteflies, thrips, dusky cotton bugs, red cotton bugs, American bollworms, spotted bollworms, pink bollworms and tobacco caterpillars (Jaleel *et al*., 2014).

The red cotton bugs *Dysdercus cingulatus* (Fabricius, 1775) and *Dysdercus koenigii* (Fabricius, 1775) belong to the order Hemiptera and family Pyrrhocoridae (Holm *et al*., 1979; Pandey & Tiwari, 2011). They are emerging sucking cotton pests worldwide (Khan *et al*., 2019). The red cotton bug is also known as the "cotton strainer" because its infestation on the emerging cotton bolls spreads fungi on the underdeveloped seed, which then stains the fluff with a typical yellow color. As a result of red cotton bug infestation, seed weight, oil content and seed viability decline (Khan *et al*., 2019). The red cotton bug also acts as a vector for the transmission of various bacterial diseases such as boll abortion, boll rot, discoloration, early shade and premature opening in cotton crops (Natarajan *et al*., 2005). The identification of the pest is important for any successful Integrated Pest Management (IPM). The accuracy of identifying species is important. The traditional morphological and phenotypic methods involve long rearing times and dissection processes (mostly from 5 to 6 weeks) so in this case, PCR-based studies are useful and time-saving since they can identify the species even after the 24 h of oviposition (Zhu & Williams, 2002). The phenotypic or morphological identification of red cotton bugs is based on the morphology of femur, body color and different spotted segments. However, this morphological classification is lengthy, slow and can be complicated by phenotypic plasticity as well as demanding substantial skill and taxonomic proficiency. Therefore, molecular identification was suggested as a fast, simple and economical method based on unique, small and uniform gene regions for species identification, thereby accelerating the finding of new specimens (Hebert *et al*., 2003). Hebert *et al*. (2004) have confirmed that the COI section is suitable for discriminating among closely-associated species across varied animal phyla. COI gene markers have been used in molecular systematics and its base pairs is frequently used to study phylogenies. It has been studied in many agriculturally important insects and has been found suitable for the molecular identification of many insect pests. The insect's COI gene develops into one of the most common molecular markers in insect classification as a result of parental inheritance, high constancy and maintenance. (He *et al*., 2015; Park *et al*., 2011). The control of arthropod pests, especially biological control of the red cotton bug, has usually depended on phenotypic and morphological techniques for the identification of pest species (da Silva *et al*., 2014; Wang & Messing, 2003). In recent years, molecular methods have become significant in the management of insect pests. In addition to this, in some cases morphological techniques dominate in the precise recording of these insect pest (Malausa *et al*., 2010). Undeniably, outside the field, one advantage of molecular methods over phenotypic approaches is that they bypass the need for the professional insect information essential for morphological type variation and can be done in a wide range of laboratory locations (Stouthamer *et al*., 1999). Moreover, molecular techniques can facilitate the distinction between morphologically identical classes of insects, and their larval types. (Mills & Kean, 2010). Thus, the application of strong molecular investigative methods increase the chances of red cotton bug biological control programs. Molecular genetic data about insects help us to examine insect biosystematics and to know the evolutionary drifts that allow the insects to acquire pest status quickly. In the present study, the red cotton bugs *D. cingulatus* and *D. koenigii* were collected from different parts of Punjab and were analyzed using mitochondrial COI gene primers.

Materials and Methods

Sample Collection

In 2018, opportunistic sampling of red cotton bugs was done in different fields (cotton and okra) and from different parts (Sargodha, Faisalabad, and Multan) of Punjab, Pakistan (Table I). The initial or filed identification was mainly based on the morphology of femur, body color and different spotted segments of both *D. koenigii* and *D. cingulatus* species of red cotton bug (Fig. 1). The collected samples of red cotton bugs were stored in 95% ethanol and preserved at -20°C at Integrated Genomics Cellular and Developmental Biotechnology Laboratory, University of Agriculture Faisalabad, Pakistan.

Figure 1. Collected adults of red cotton bugs.

DNA extraction and PCR Analysis

For the gene sequencing, DNA was extracted from the whole body of the collected insects by using the cetyltrimethylammonium bromide (CTAB) method (Ahmad *et al*., 2018). A total of 9 samples were processed including one control sample (Table I). The quantification of DNA was done using a NanoDrop One/OneC instrument (Thermo Fisher scientific, QIAGEN N.V, Germany). PCR amplifications of partial cytochrome oxidase I (COI) gene were performed in a thermocycler (Peqstar, Germany) using the primers HCO-2198 (5´-TAAACTTCAGGGTGACCAAAAAATCA-3´) and LCO-1490 (5´-GGTCAACAAATCATAAAGATATTGG-3´) (Folmer *et al*., 1994). The initial denaturation as at 95°C for 5 min, followed with 40 cycles at 94°C for 40 s, annealing at 47°C for 40 s, extension at 72°C for 45 s and final extension at 72°C for 15 min.

Table I. List of the selected samples of red cotton bugs (*D. koenigii*, *D. cingulatus*), their ratio, DNA concentration (using NanoDrop One/OneC for quantification of DNA), the field of collection and site of collection.

Samples	Purity Ratio of DNA	DNA concentration	Field of collection	Site of collection
RCB 1	1.899	580 ng/µL	Cotton	Multan
RCB 2	1.753	1078 ng/µL	Cotton	Sargodha (Bhalwal)
RCB 3	2.026	3445 ng/µL	Okra	Faisalabad
RCB 4	1.834	1108 ng/µL	Cotton	Sargodha (Bhalwal)
RCB 5	2.136	706 ng/µL	Cotton	Multan
RCB 6	1.098	961.0 ng/µL	Okra	Faisalabad
RCB 7	2.041	452.0 ng/µL	Cotton	Sargodha (Bhalwal)
RCB 8	1.320	817 ng/µL	Cotton	Sargodha (Bhalwal)

Gel electrophoresis analysis of PCR amplified product

Gel electrophoresis on 2% agarose gel was used for the verification of the existence of amplified genomic DNA. Under the UV-light SYNGENE Gel documentation system (Bio-Rad Lab., CA, USA), the PCR product was recognized. We observed distinct bands at 710 bp in our collected samples (Fig.2); the control sample was blank.

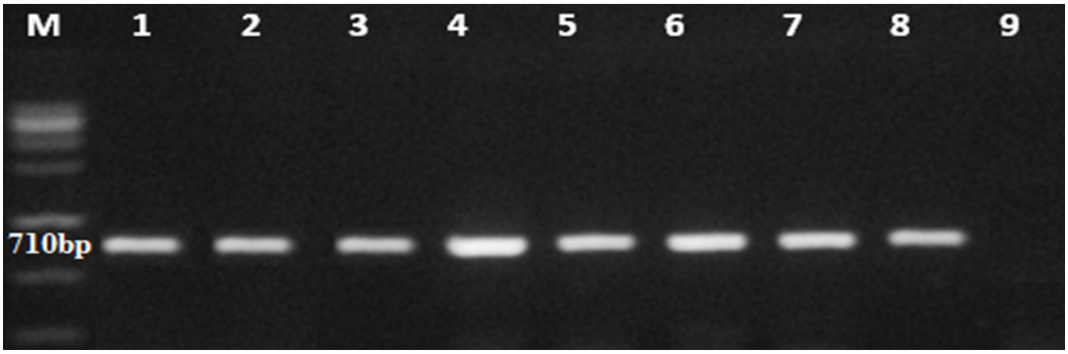

Figure 2. Gel electrophoresis of PCR amplified from DNA of *Dysdercus koenigii* and *D. cingulatus* using COI primer pairs. Lanes M – Molecular DNA ladders; Lane 9 – Control; Lane 1-4 – DNA of *Dysdercus koenigii*; Lane 5-8 – DNA of *Dysdercus cingulatus*.

Sequence Analysis

Sequencing was carried out using the services of M/S Macrogen (Korea). Lasergene v. 7.1 ((DNASTAR, USA) was used for the analysis of acquired sequences. CLUSTAL-W software was used for further alignment. Comparison between sequences available on the NCBI and the obtained sequences was done using the Basic Local Alignment Search Tool (BLAST). A pairwise alignment tree for our sample query (dendrogram) was constructed with available NCBI data. The studies were performed with software BLASTN 2.8.0+ employing a methodology for pairwise alignment for the construction of a phylogenetic tree. Evolutionary analysis was conducted in MEGA 6 software using the maximum likelihood estimation method.

Results and Discussion

Sufficient information about the agricultural importance of red cotton bugs depends on the correct identification of the insects. The gold standard for any strong taxonomic system is its capability to provide consistent and correct species identification (Hebert *et al*., 2003). In the current study, we were able to recover and align the targeted COI portion from both the red cotton bug species studied.

The nucleotide sequencing of DNA fragments was analyzed and aligned through BLASTN with sequence data of red cotton bugs (RCB) previously reported in the NCBI. The reference accession numbers of sequences used for alignment were RCB (MK038750.1, MK038749.1, GQ306227.1, KJ459924.1, NC042437.1, KU242579.1, KP898253.1, EU427335.1, MG838358.1, KX523445.1, MG665971.1, and MG665884.1). In base-pair sequence alignment, the homology of red cotton bug species was compared with previously reported NCBI database through a BLAST option. Pairwise alignment of the constructed dendrogram tree indicated that our red cotton bug (*D. koenigii)* Seq (>180319003-A02-102-DK-.ab1) shared the same cluster with *D. koenigii* isolates ZSI/SRC_I.28B (MK038750.1), *D. koenigii* isolates ZSI/SRC_I.28, (MK038749.1) *D. koenigii* (GQ306227.1) and *D. koenigii* (KJ459924.1) (Table II).

Table II. Assessment of similarity of cytochrome oxidase I (COI) gene fragment of red cotton bug *D. koenigii* with that of gene bank available in NCBI.

Query	Subject	Maximum score	Overall score	Coverage of query	E.value	Similarity	Accessions
>180319003-A02-102-DK-.ab1	*Dysdercus koenigii* isolate ZSI/SRC	1201	1201	97%	0.0	*100*	MK038750.1
	D. koenigii	1201	1201	97%	0.0	100	MK038749.1
	D. koenigii	1133	1133	97%	0.0	98.89	GQ306227.1
	D. koenigii	1114	1114	94%	0.0	99.35	KJ459924.1
	D. evanescens	979	979	95%	0.0	93.843	NC042437.1
	D. evanescens	970	970	98%	0.0	94.035	KU242579.1
	D. cingulatus	966	966	96%	0.0	93.214	KP898253.1
	D. cingulatus	934	934	96%	0.0	91.385	EU427335.1
	D. evanescens	808	808	96%	0.0	95.285	MG838358.1
	D. fuscomaculatus	769	769	96%	0.0	88.786	KX523445.1
	Notonecta lutea	673	673	96%	0.0	35.34	MG665971.1
	N. lutea	673	673	96%	0.0	35.34	MG665884.1

While other species *D. cingulatus* voucher, *D. cingulatus evanescens*, *Dysdercus fuscomaculatus*, *Notonecta lutea* and *Notonecta lutea* of the same genus have different clusters (Fig. 3). Among these species *Notonecta lutea* was used as an out species to compare *Dysdercus cingulatus* (RO DC2015) and *D. fuscomaculatus* as maximum likelihood similarities was found in both species. Both species share the maximum amount of COI/COX1 sequence as they originate from common or same ancestor base joining (Fig. 3).

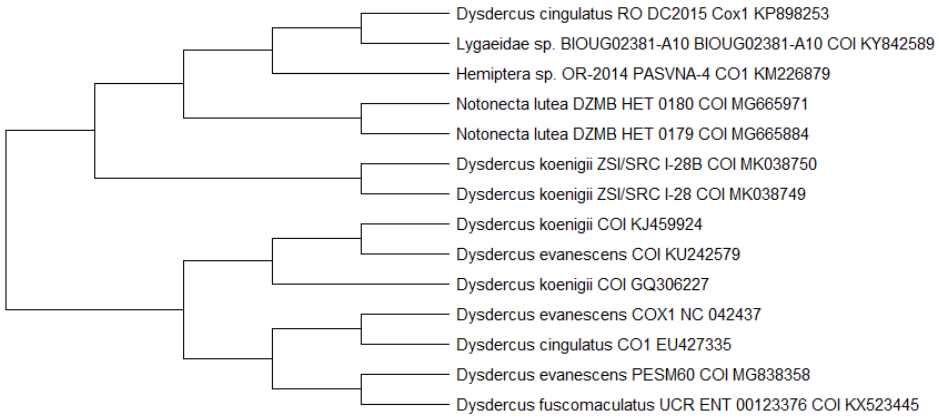

Figure 3. The evolutionary history was inferred by using the maximum likelihood estimation method and the Tamura-Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (-9230.96) is shown. Initial trees for the heuristic search were obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with the superior log likelihood value. This analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1534 positions in the final dataset. Evolutionary analyses were conducted in MEGA 6.

Our second sample of red cotton bug (*D. cingulatus*) Seq (>180319003-A02-103-DC-abl) shares a cluster with *Dysdercus cingulatus* voucher RO_DC2015, while on the other hand, *D. koengii* isolates ZSI/SRC_I.28B, *D. koenigii* isolates ZSI/SRC_I.28, *Dysdercus koenigii*, *D. evanescens* and *D. fuscomaculatus* belong to same genus but have different clusters of COI sequence. This difference leads them to move away from the base hierarchy (Fig. 4).

In the homology sequence study, *D. koenigii* showed 100% similarity among the same samples collected from different sites. On the other hand, *D. koenigii* shows, 100% similarity for *Dysdercus koenigii* isolate ZSI/SRC (MK038750.1), 100% for *D. koenigii* (MK038749.1), 98.89% for *D. koenigii* (GQ306227.1) 99.35% for *D. koenigii* (KJ459924.1), 93.843-% for *D. evanescens* (NC042437.1), 94.035-% for D. evanescens (KU242579.1), 94.214-% for *D. cingulatus* (KP898253.1), 91.385-% for *D. cingulatus* (EU427335.1), 95.285-% for *D. evanescens* (MG838358.1), 88.786-% for *D. fuscomaculatus* (KX523445.1), 35.34-% for *Notonecta lutea* (MG665971.1) and 35.34-% for *N. lutea* (MG665884.1) respectively, from NCBI database. Our results, when compared with a previously conducted study (Ahmad *et al*., 2019) on dusky cotton bug *Oxycarenus hyalinipennis* OH-1 and *O. hyalinipennis* OH-2, showed 100% and 99% similarity index related to our results. The similarity index from (da Silva *et al*., 2014) on hemipteran mealybug *ferrisia meridionalis* is also 99-% which is closely related to our findings. The studies on *F. gill* (Gullan *et al*., 2003) shows the similarity of 99-% related to that of our results on red cotton bug. The pairwise alignment also revealed 93.846-% (NCO42437.1), 94.035- % (KU242579.1), 93.214- % (KP898253.1), 91.385- % (EU427335.1), 95.285- % (MG838358.1) 88.787- % (KX523445.1), 85.34- % (MG665971.1 %) and 85.34- % (MG665884.1) similarity respectively, with other species of the same genus (Table II).

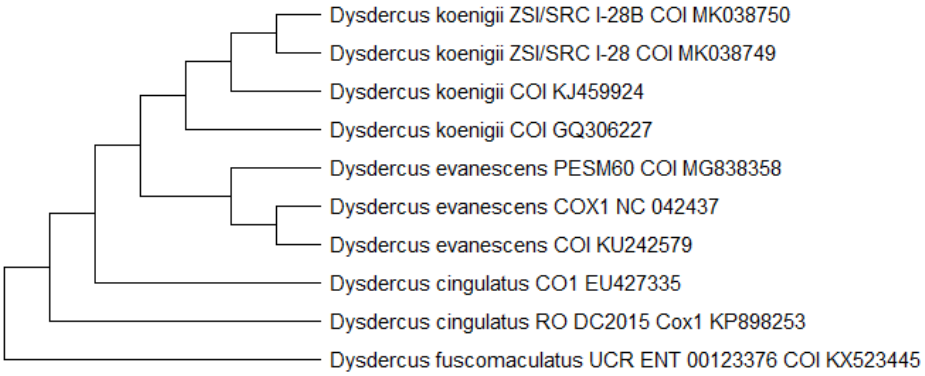

Figure 4. The evolutionary history was inferred by using the maximum likelihood estimation method and Tamura-Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (-2778.73) is shown. Initial trees for the heuristic search were obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with the superior log likelihood value. This analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1393 positions in the final dataset. Evolutionary analyses were conducted in MEGA 6.

The pairwise alignment of our query *D. cingulatus* among the samples showed close similarity to the hemipteran species. likewise *Oxycarenus hyalinipennis* reported from the USA (Nagoshi *et al*., 2012) showed 100-%, *O. laetus* from India (Habeeb *et al*., 2011) showed 99-% similarities to our findings. As both of these species belong to order Hemiptera. Therefore, both share common cluster that lead to similarity. Moreover, no exact literature or previous work related to red cotton bugs was found, so, the literature related to genus *Oxycarenus* has been cited due to its hemipteran order and close similarity to our results. While, referred to NCBI gene data bank, closely related similarity of 94.79- % (MK038750.1), 93.91- % (MK038749.1), 93.91- % (GQ306227.1), 93.35- % (KJ459924.1), 93.846- % (NCO42437.1), 94.035- % (KU242579.1), 99.21- % (KP898253.1), 93.385- % (EU427335.1), 94.285- % (MG838358.1) and 88.80- % (MG838358.1) has been found respectively, with other species of the similar genus (Table III).

The phylogenetic tree showed that altogether the *D. koenigii* belonging to Hemiptera, being bunched/clustered with each other on genus level have a high query coverage score from 94-98% (Table II). Our entry of red cotton bug *D. cingulatus* showed 97-98% query coverage after being clustered with Hemipteran and other species of same genus (Table).

Table III. Similarity of cytochrome oxidase I (COI) gene fragment of red cotton bug *D. cingulatus* with that of gene bank available in NCBI.

Query	Subject	Maximum score	Overall score	Coverage of query	E.value	Similarity	Accession
>180319003-A02-103-DC-.ab1	*Dysdercus cingulatus* isolate ZSI/SRC	766	766	97%	0.0	*94.79*	MK038750.1
	D. koenigii	766	766	97%	0.0	93.91	MK038749.1
	D. koenigii	968	968	97%	0.0	93.91	GQ306227.1
	D. koenigii	966	966	98%	0.0	93.35	KJ459924.1
	D. evanescens	1000	1000	98%	0.0	93.846	NC042437.1
	D. evanescens	996	996	98%	0.0	94.035	KU242579.1
	D. cingulatus	1216	1216	98%	0.0	99.21	KP898253.1
	D. cingulatus	959	959	98%	0.0	93.385	EU427335.1
	D. evanescens	797	797	94%	0.0	94.285	MG838358.1
	D.fuscomaculatus	800	800	98%	0.0	88.80	KX523445.1

Conclusion

Molecular genetic data about insects help us to understand their biosystematics and to know the evolutionary drifts that allow insects to acquire pest status quickly. In the current study, red cotton bugs *D. cingulatus* and *D. koenigii* were collected from various parts of Punjab and were analyzed using mitochondrial COI gene primers. Our sequence will assist as a high-class DNA barcode for these two species of red cotton bug.

Acknowledgments

We are thankful to Dr. Jam Nazeer Ahmad for allowing us to conduct this work in the Integrated Genomics Cellular Developmental and Biotechnology Laboratory, Department of Entomology University of Agriculture Faisalabad, Punjab and providing us with the materials required for this research work.

Author Contribution

Waqar Ali Khan designed and conducted the experiment, Safi Ur Rehman Qamar conducted the phylogenetic analysis using mega software, Jam Nazeer Ahmad supervised the experiment, Asad Ullah and Waqar Ali Khan collected samples and Mayer L. Calma reviewed the article.

Conflict of interest

There is no conflict of interest between the authors.

References

Ahmad, J. N., Jafir, M., Javed, M. W., Maqsood, S., & Ahmad, S. J. (2019). Molecular identification and sequence analysis of dusky cotton bug, *Oxycarenus hyalinipennis* (Hemiptera: Lygaiedae) infesting cotton field in Pakistan. *Pakistan Journal of Zoology, 51*(2), 789-792.

Ahmad, J. N., Mushtaq, R., Ahmad, S. J. N., Maqsood, S., Ahuja, I., & Bones, A. M. (2018). Molecular identification and pathological characteristics of NPV isolated from *Spodoptera litura* (Fabricius) in Pakistan. *Pakistan Journal of Zoology*, *50*, 2229-2237.

Atwal, A., & Dhaliwal, G. (2015). *Agricultural pests of South Asia and their management*: Kalyani Publishers, 487 pp.

Bakhsh, K., Hassan, I., & Maqbool, A. (2005). Factors affecting cotton yield: a case study of Sargodha (Pakistan). *Journal of Agriculture & Social Sciences, 1*(4), 332-334.

da Silva, V. C. P., Bertin, A., Blin, A., Germain, J.-F., Bernardi, D., Rignol, G., Botton, M., & Malausa, T. (2014). Molecular and morphological identification of mealybug species (Hemiptera: Pseudococcidae) in Brazilian vineyards. *PloS one, 9*(7), e103267.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294-299.

Gullan, P., Downie, D., & Steffan, S. (2003). A new pest species of the mealybug genus *Ferrisia* Fullaway (Hemiptera: Pseudococcidae) from the United States. *Annals of the Entomological Society of America, 96*(6), 723-737.

Habeeb, S., Kumar, S., Sanjayan, K., Anuradha, V., & Praveena, A. (2011). Comparative evolutionary analysis of insects using mitochondrial genes. *International Journal of Current Research, 3*(11), 66-73.

He, Y. B., Zhan, R. L., Sun, G. M., Wu, J. B., & Zhao, Y. L. (2015). Phylogeography of pink pineapple mealybugs, *Dysmicoccus brevipes* (Cockerell) reveals the history of pineapple introduction and cultivation in China. *Genetics and molecular research: GMR, 14*(3), 9890-9897. doi:10.4238/2015.August.19.23

Hebert, P. D., Cywinska, A., Ball, S. L., & Dewaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313-321.

Holm, L., Pancho, J. V., Herberger, J. P., & Plucknett, D. L. (1979). *A geographical atlas of world weeds*: John Wiley and Sons, 391 pp.

Jaleel, W., Saeed, S., Naqqash, M. N., & Zaka, S. M. (2014). Survey of Bt cotton in Punjab Pakistan related to the knowledge, perception and practices of farmers regarding insect pests. *International Journal of Agriculture and Crop Sciences, 7*(1), 10.

Khan, S. M., Ali, S., Nawaz, A., Bukhari, S. A. H., Ejaz, S., & Ahmad, S. (2019). Integrated pest and disease management for better agronomic crop production. In *Agronomic Crops* (pp. 385-428). Springer, Singapore. https://doi.org/10.1007/978-981-32-9783-8_19

Malausa, J., Blanchet, A., Bon, M., Cheyppe-Buchmann, S., Groussier-Bout, G., Jones, W., Pickett, C., Ris, N., Roche, M., & Thaon, M. (2010). Introduction of the African parasitoid *Psyttalia lounsburyi* in south of France for the classical biological control of *Bactrocera oleae*: will hybridization affect establishment and population growth? *International Organization for Biological and Integrated Control of Noxious Animals and Plants (OIBC/OILB), West Palaearctic Regional Section (WPRS/SROP) Dijon France, 53*, 49-55.

Mills, N. J., & Kean, J. M. (2010). Behavioral studies, molecular approaches, and modeling: methodological contributions to biological control success. *Biological Control, 52*(3), 255-262.

Nagoshi, R. N., Paraiso, O., Brambila, J., & Kairo, M. T. (2012). Assessing the usefulness of DNA barcoding to identify *Oxycarenus hyalinipennis* (Hemiptera: Oxycarenidae) in Florida, a potentially invasive pest of cotton. *Florida Entomologist, 95*(4), 1174-1182.

Natarajan, K., Rajendran, T., & Rathinavel, K. (2005). Pest management in cotton in DUS experimentation. *DUS testing in cotton. All India Coordinated Cotton Improvement Project, Central Institute for Cotton Research Regional Station, Coimbatore, India*, 124-135.

Pandey, J., & Tiwari, R. (2011). Neem based insecticides interaction with development and fecundity of red cotton bug, *Dysdercus cingulatus* Fab. *International Journal of Agricultural Research, 6*(4), 335-346.

Park, D.-S., Suh, S.-J., Hebert, P. D., Oh, H.-W., & Hong, K.-J. (2011). DNA barcodes for two scale insect families, mealybugs (Hemiptera: Pseudococcidae) and armored scales (Hemiptera: Diaspididae). *Bulletin of entomological research, 101*(4), 429-434.

Stouthamer, R., Hu, J., van Kan, F. J., Platner, G. R., & Pinto, J. D. (1999). The utility of internally transcribed spacer 2 DNA sequences of the nuclear ribosomal gene for distinguishing sibling species of *Trichogramma*. *BioControl, 43*(4), 421-440.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution, 10*(3), 512-526.

Wang, X.-g., & Messing, R. H. (2003). Intra-and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryon*i (Hymenoptera: Braconidae), parasitoids of tephritid fruit flies. *Biological Control, 27*(3), 251-259.

Zhu, Y. C., & Williams, L. (2002). Detecting the egg parasitoid *Anaphes iole* (Hymenoptera: Mymaridae) in tarnished plant bug (Heteroptera: Miridae) eggs by using a molecular approach. *Annals of the Entomological Society of America, 95*(3), 359-365.

ФИЛОГЕНЕТСКА АНАЛИЗА ВРСТА ВАТРЕНИХ СТЕНИЦА (HEMIPTERA: PYRRHOCORIDAE) У ПАНЏАБУ, ПАКИСТАН

ВАКАР АЛИ КАН, САФИ УР РЕХМАН КАМАР, ЈАМ НАЗЕР АХМАД, МАЈЕР Л. КАЛМА и АСАД УЛАХ

Извод

Традиционалне технике идентификације ватрених стеница *Dysdercus koenigii* и *Dysdercus cingulatus* (Pyrrhocoridae: Hemiptera) зависе од фенотипских и морфолошких података. Ове методе су погодне када врсте имају добро дефинисане структуре и када је при руци огромно таксономско знање. У овом истраживању, ген за митохондријску цитохром оксидазу 1 (COI) изабран је за молекуларну идентификацију *D. koenigii* и *D. cingulatus*. Подаци о секвенци показали су да је једна врста узорка ватрене стенице идентификована као *Dysdercus koenigii* са 35-100% сличности са другим врстама рода *Dysdercus* присутним у бази гена NCBI, док је друга врста идентификована као *Dysdercus cingulatus* са 88-94% сличности са другим врстама ватрених стеница присутних у бази NCBI. Филогенетска анализа открила је да наш *D. koenigii* Seq (>180319003-A02-102-DK-.ab1) дели исти кластер са четири изолата *D. koenigii* (ZSI/SRC_I.28B, ZSI/SRC_I.28, GQ306227.1, KJ459924.1). Док *D. cingulatus* Seq (>180319003-A02-103-DC-.abl) дели кластер са *D. cingulatus* ваучером (RO_DC2015). Ово је прва молекуларна идентификација ватрених стеница (*D. koenigii* и *D. cingulatus*) забележених у Пакистану. Стога је у тренутној студији молекуларна идентификација прихваћена као поуздана метода за идентификацију ових важних инсеката у пољопривреди.

Received: April 4th, 2020
Accepted: May 30th, 2020