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Molecular Identification of Ladybird Beetles (Coccinella: Coccinellidae) Using DNA Barcodes

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Abstract

DNA barcoding is a molecular genetics technique commonly applied for species identification based on the cytochrome c oxidase subunit I (COI) gene located on the mitochondrial DNA genome. Based on our analysis, the DNA barcodes were generated from 16 specimen of Coccinella (family Coccinellidae). All the samples were collected from diverse sites in Egypt and Libya. Morphological traits based on the number of dorsal spots, DNA extraction, PCR amplification of COI, sequencing, nucleotide BLAST, and phylogenetics analyses were used to classify the specimens. A phylogenetic tree was constructed using the maximum likelihood method, including 46 COI sequences (the study and NCBI sequences). Three Coccinella clusters were defined and classified as *Coccinella septempunctata*, *Coccinella novemnotata*, and *Coccinella undecimpunctata* each species clustered into a unique branch. DNA barcodes discriminated clearly against the analyzed species. This study validated the COI efficiency as a marker for DNA barcoding of insects (Coccinella, family Coccinellidae).

Keywords: Coccinella beetles, COI gene, molecular genetics, species ID, phylogeny

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1. Introduction

Ladybird beetles are members of the family Coccinellidae (Coleoptera), one of the dominant beetles globally a family of 360 genera that includes over 6000 described species [1]. A yellow, orange or red dorsal color is common, along with or without small black spots with lengths ranging from 0.8 to 18 mm [2]. They are predator insects and are commonly used as biological control agents against other insects in several crops, such as *C. septempunctata* versus *Harmonia axyridis* [3].

The species richness and evolutionary radiations of beetles are extraordinary and have been the subject of longstanding ongoing search [4, 5]. The diversity of this family is not attributed to a single factor but is mostly related to their specialist phytophagy ability on angiosperms [5-7]. Despite the taxonomic attention, the classification of this family below the family level has been unsuccessful. The taxonomic validation of *Coccinellinae* is often fraught with contradiction and unsupported morph-traits description [2].

Morphological identification and taxonomic assessment are challenging because characters for identification are dependent on adult reproductive structures and morphology [8-9]. The integration of DNA barcoding was a potential solution for morphology-based identification challenges. It is a standardized molecular identification protocol that has been used to identify animals at different life stages [10-12]. DNA barcodes are mostly suitable for taxa identification at the species level. It is considered the most appropriate methodology to solve the current issues in global diversity assessment and identification [13]. This identification system can provide a decent, timesaving, cost-effective, and accessible solution for species identification [10], as a drastic drop in the number of taxonomists and identification experts has been observed [14]. Based on several studies, the mitochondrial Cytochrome Oxidase Subunit I (COI) region was selected as a standard barcode for the kingdom Animalia, including the insects [15]. The effectiveness and practicability of the Molecular identification and DNA barcoding technique have been widely recognized among insects and other organisms [16-24]. However, barcoding studies on ladybird beetles require additional analysis, and the phylogenetic relationships of this family are poorly resolved. The main objectives of this study are to barcode species of *Coccinella* from Egypt and Libya using the mitochondrial COI gene, and to identify the ladybird species and diversity distributed along the northern coast.

2. Material and Methods

2.1 Sampling and morphological identification

Ladybird beetle specimens were captured from the agroecosystem in different locations in Egypt and Libya from June through Oct 2021 (Table 1). Both sweep-nets and hand-gathering were used to collect the ladybird beetles within a range of 100 m^2 (plot) for both sampling sites. The samples were preserved in 95% ethanol and stored at -20°C. For each specimen, the samples were identified to a species level under a stereomicroscope (Stemi 4) based on the elytral colour and the number of spots.

Table *1***.** Species identification table, including morphological inspection, common name, sample collection sites, and samples code.

Species	Morphological aspect	Common name	Proposed Scientific name	Location	Sample code
Sample 01	Seven black dorsal spots	Seven-spot ladybird	*Coccinella septempunctata*	Egypt	7S_Eg
Sample 02				Libya	7S_li
Sample 03				Libya	
Sample 04				Libya	
Sample 05	Nine black dorsal spots	Nine-spotted ladybird	*Coccinella novemnotata*	Egypt	9S_Eg
Sample 06				Egypt	
Sample 07				Egypt	
Sample 08				Egypt	
Sample 09				Egypt	
Sample 10				Egypt	
Sample 11	Eleven black dorsal spots	Eleven-spot ladybird	*Coccinella undecimpunctata*	Egypt	11S_Eg
Sample 12				Egypt	
Sample 13				Egypt	
Sample 14				Libya	11S_li
Sample 15				Libya	
Sample 16				Libya	

2.2 DNA extraction, PCR amplification, and sequencing

Ethanol-preserved specimens were cleaned with distilled water and then total genomic DNA was extracted from small-cut at their abdomen using the WizPrep™ gDNA Mini Kit (Cell/Tissue; Korea), according to the manufacturer's instructions, with a final elution volume of 50 ml. The integrity of each DNA was checked by 1% agarose gel electrophoresis and visualized under UV light using the Ingenius3 Gel documentation system (Syngene, UK).

Fragments of the mitochondrial COI barcode region (658 bp) were amplified using the universal primers HCO2198 and LCO1490 [25] for all samples including a positive sample (P: DNA extracted from another beetle sample that was previously amplified using the COI primers). Polymerase chain reaction (PCR) amplifications were conducted in a 25 µl volume including 12.5 µL of OnePCR™ master mix (Genedirex®, Taiwan), 1 µL of each primer (forward and reverse, each of 10 µM), and 1 µL of extracted DNA (~100 ng/µL). The thermal profile was as follows: an initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 30 s at 50 °C, extension for 90 s at 72 °C, and a final extension step at 72 °C for 10 min. Subsequently, all PCR products were checked using gel electrophoresis in 1.5% agarose and the purified fragments were then directly subjected to Sanger sequencing (Macrogen Inc., Seoul, South Korea) using the same primers as in PCR.

2.3 Sequence alignment and data analysis

DNA fragments in both directions were sequenced with sufficient overlap. After sequencing, the chromatograms obtained were evaluated, assembled, and edited using Geneious R10 [26]. The generated consensus sequences were identified using the BLAST search tool in the NCBI database applying default parameters. The sequences were aligned using the MAFFT aligner [27]. Pairwise genetic distance among sequences was calculated using maximum likelihood methods implemented in Geneious R10.

3. Results

3.1 Species abundance and composition

Based on the morphological aspect, 16 individual ladybird beetles were sampled from both countries. Three different species were recognized based on the number of spots, and two types with either melanic (black) or non-melanic (red) base colour of the elytra (Table 1, Fig. 1). Species were identified as *Coccinella septempunctata* (Seven-spot ladybird), *Coccinella*

novemnotata (Nine-spotted ladybird), and *Coccinella undecimpunctata* (Eleven-spot ladybird).

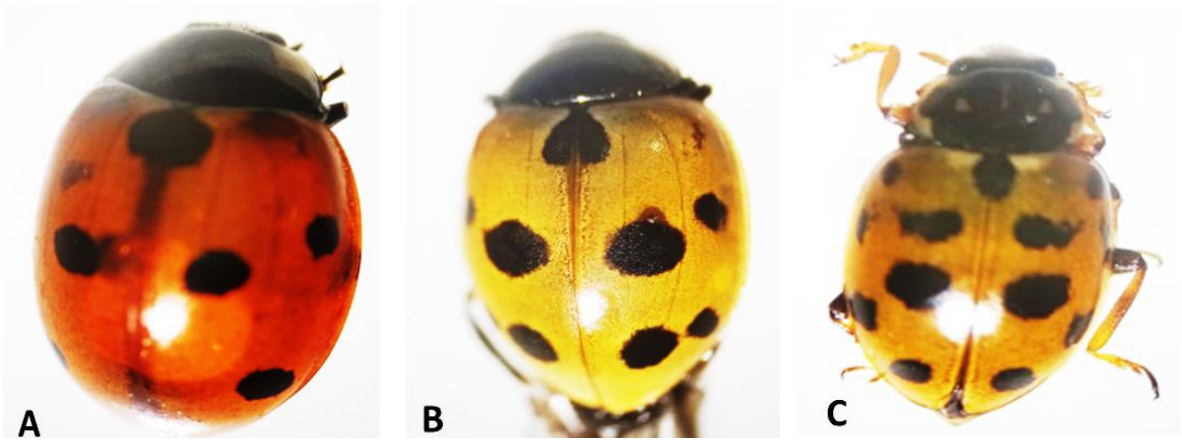

Figure 1. The appearance of the three species of Coccinellidae. **A)** *Coccinella septempunctata* (Seven-spot ladybird), b *Coccinella novemnotata* (Nine-spotted ladybird), and C) *Coccinella undecimpunctata* (Eleven-spot ladybird)

3.2 DNA extraction

The collected ladybird beetles were subjected to DNA extraction. All samples were successfully extracted with high concentration using 1% agarose gel electrophoresis (Fig. 2).

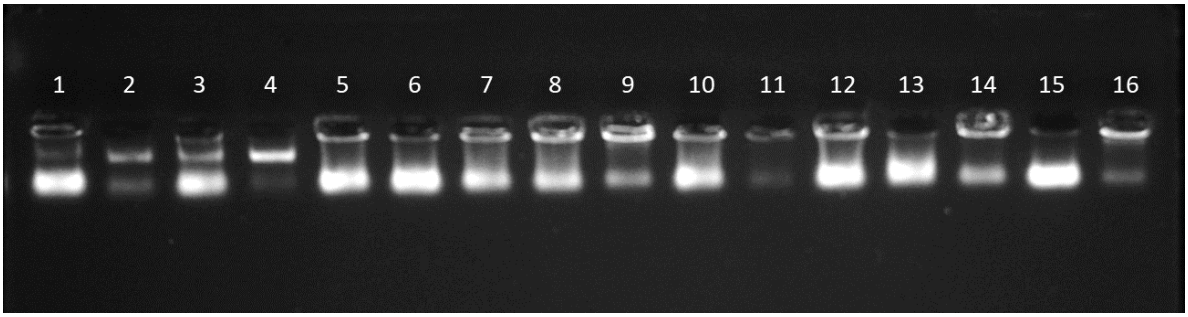

Figure 2. Agarose gel electrophoresis of total DNA isolated from 16 ladybird beetles.

3.3 PCR amplification, and sequence analysis

All COI sequences were amplified and sequenced across all samples representing the three identified species of ladybird beetles. The molecular size of the amplified COI sequences ranged from 551 to 658 bp (Fig. 3).

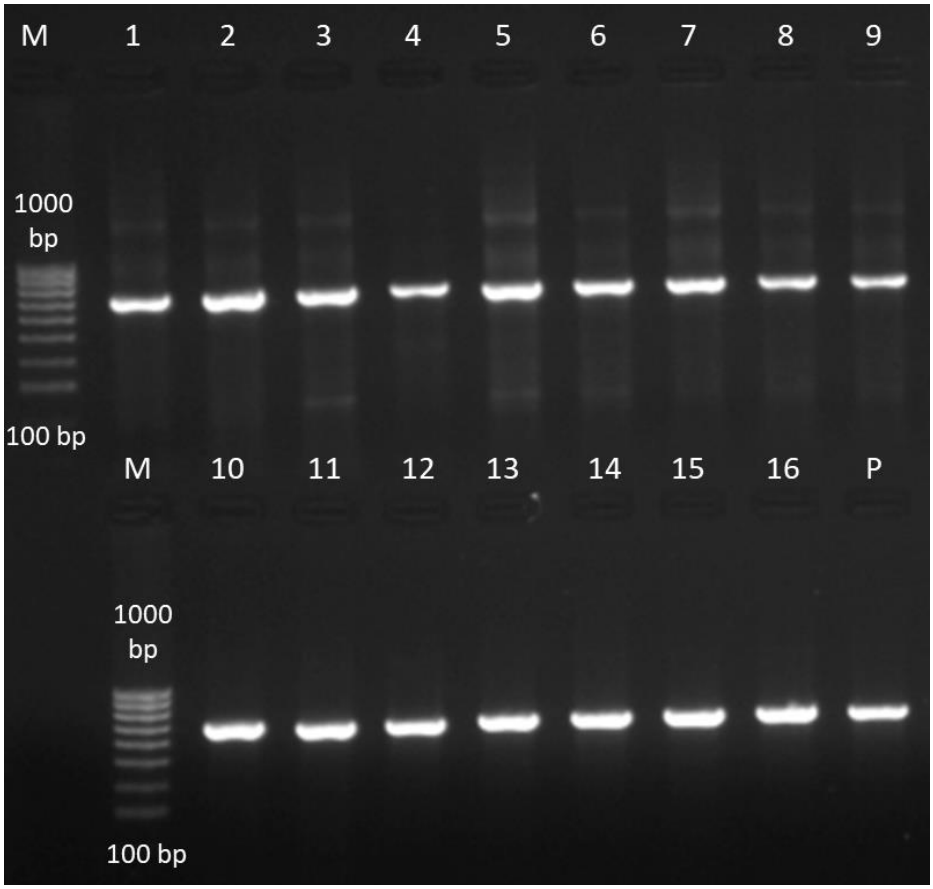

Figure 3. Gel electrophoresis of successfully amplified COI gene of 16 ladybird beetles. The DNA ladder (M) is shown in the first lane of each comb, and the last sample is a positive sample (P).

The mitochondrial COI sequences were blasted on the NCBI nucleotide database. The BLAST results shown by the COI differentiated all the samples down to species level (Table 2). In detail, samples from 1 to 4 matched *C. septempunctata* with a pairwise identity of 100%, samples from 5 to 10 matched *C. novemnotata* with a pairwise identity of 100%, and samples from 11 to 16 matched *C. undecimpunctata* with a pairwise identity ranged from 99.60 to

100%. Based on the BLAST results in Table (2), the top hit best-matched sequences were retrieved from the NCBI database and aligned with our results for all samples.

Table 2. *Blast results for the COI gene of Coccinellidae. Including sample code, percentage of pairwise %, GC content, the accession number, and organism name.*

Sample	Pairwise	GC	Accession	Organism
7S_Eg_01	100.00%	30.30%	GU013586	*Coccinella septempunctata*
	100.00%	30.80%	KR486755	*Coccinella septempunctata*
	99.40%	30.90%	MG847527	*Coccinella septempunctata*
	99.30%	30.70%	OU015577	*Coccinella septempunctata*
	99.30%	30.30%	OU015583	*Coccinella septempunctata*
7S_li_02	100.00%	30.90%	HM405499	*Coccinella septempunctata*
	100.00%	30.90%	HQ563281	*Coccinella septempunctata*
	100.00%	30.90%	KJ204125	*Coccinella septempunctata*
	100.00%	30.90%	KJ961952	*Coccinella septempunctata*
	100.00%	30.90%	MZ657700	*Coccinella septempunctata*
7S_li_03	100.00%	31.10%	HM405499	*Coccinella septempunctata*
	100.00%	31.10%	HM433745	*Coccinella septempunctata*
	100.00%	31.10%	HQ563281	*Coccinella septempunctata*
	100.00%	31.10%	KJ204125	*Coccinella septempunctata*
	100.00%	31.10%	KM845410	*Coccinella septempunctata*
7S_li_04	100.00%	31.10%	HM405499	*Coccinella septempunctata*
	100.00%	31.10%	HM433745	*Coccinella septempunctata*
	100.00%	31.10%	HQ563281	*Coccinella septempunctata*
	100.00%	31.10%	KJ204125	*Coccinella septempunctata*
	100.00%	31.10%	KM845410	*Coccinella septempunctata*
9S_Eg_05	100.00%	30.20%	KM844083	*Coccinella novemnotata*
	99.80%	31.00%	KR491458	*Coccinella novemnotata*
	99.80%	30.60%	KR486655	*Coccinella novemnotata*
	99.00%	30.70%	KR481900	*Coccinella novemnotata*
9S_Eg_06	100.00%	31.00%	KR481900	*Coccinella novemnotata*
	99.10%	31.00%	KR491458	*Coccinella novemnotata*
	99.10%	30.60%	KR486655	*Coccinella novemnotata*
	99.00%	30.20%	KM844083	*Coccinella novemnotata*
9S_Eg_07	100.00%	30.60%	KR486655	*Coccinella novemnotata*

	100.00%	31.00%	KR491458	*Coccinella novemnotata*
	99.80%	30.40%	KM844083	*Coccinella novemnotata*
	99.10%	30.90%	KR481900	*Coccinella novemnotata*
9S_Eg_08	100.00%	31.00%	KR486655	*Coccinella novemnotata*
	100.00%	31.00%	KR491458	*Coccinella novemnotata*
	99.80%	30.80%	KM844083	*Coccinella novemnotata*
	99.10%	31.40%	KR481900	*Coccinella novemnotata*

Cont. Table 2

9S_Eg_09	100.00%	31.00%	KR481900	*Coccinella novemnotata*
	99.10%	31.00%	KR491458	*Coccinella novemnotata*
	99.10%	30.60%	KR486655	*Coccinella novemnotata*
	99.00%	30.20%	KM844083	*Coccinella novemnotata*
9S_Eg_10	100.00%	30.60%	KR486655	*Coccinella novemnotata*
	100.00%	31.00%	KR491458	*Coccinella novemnotata*
	99.80%	30.40%	KM844083	*Coccinella novemnotata*
	99.10%	30.90%	KR481900	*Coccinella novemnotata*
11S_Eg_11	99.70%	30.20%	JF889781	*Coccinella undecimpunctata*
	99.70%	30.40%	JF889782	*Coccinella undecimpunctata*
	99.70%	30.40%	KJ965514	*Coccinella undecimpunctata*
	99.70%	30.20%	MW551370	*Coccinella undecimpunctata*
	99.50%	30.10%	KJ963757	*Coccinella undecimpunctata*
11S_Eg_12	100.00%	31.00%	KY569359	*Coccinella undecimpunctata*
	100.00%	31.00%	KY569360	*Coccinella undecimpunctata*
	100.00%	31.00%	KY838949	*Coccinella undecimpunctata*
	99.60%	30.60%	MW551370	*Coccinella undecimpunctata*
	99.10%	30.80%	KM445476	*Coccinella undecimpunctata*
11S_Eg_13	100.00%	30.60%	KM444805	*Coccinella undecimpunctata*
	100.00%	30.40%	KM452280	*Coccinella undecimpunctata*
	99.90%	30.40%	KM443366	*Coccinella undecimpunctata*
	98.90%	30.20%	JF889781	*Coccinella undecimpunctata*
	98.90%	30.20%	MW551370	*Coccinella undecimpunctata*
11S_li_14	100.00%	30.60%	KM444805	*Coccinella undecimpunctata*
	99.90%	30.40%	KM443366	*Coccinella undecimpunctata*
	99.90%	30.40%	KM452280	*Coccinella undecimpunctata*

	98.80%	30.20%	JF889781	*Coccinella undecimpunctata*
	98.80%	30.20%	MW551370	*Coccinella undecimpunctata*
11S_li_15	99.60%	29.90%	KM446139	*Coccinella undecimpunctata*
	99.50%	30.30%	KM444805	*Coccinella undecimpunctata*
	99.50%	30.10%	KM452280	*Coccinella undecimpunctata*
	99.30%	29.80%	MW551370	*Coccinella undecimpunctata*
	99.30%	30.10%	KM443366	*Coccinella undecimpunctata*
11S_li_16	100.00%	30.40%	JF889782	*Coccinella undecimpunctata*
	100.00%	30.40%	KJ965514	*Coccinella undecimpunctata*
	99.80%	30.20%	MW551370	*Coccinella undecimpunctata*
	99.70%	30.20%	JF889781	*Coccinella undecimpunctata*
	99.70%	30.10%	KJ963757	*Coccinella undecimpunctata*

3.4 Phylogenetic analysis

The alignment of the COI gene formed of all *Coccinella* samples under the study with matched sequences in the GenBank database was 557 bp in length, and the percentage of pairwise identity was 90.2; while the GC ratio was 31.1%. All the samples were utilized to construct a maximum-likelihood phylogenetic tree based on the obtained sequences along with the top hit results to infer the Coccinellidae species delimitation. The studied species displayed clades of conspecific sequences and showed a match between the present study and the GenBank (NCBI) database. Three main clads were produced, these clades were designated as *C. septempunctata*, *C. novemnotata*, and *C. undecimpunctata*. Each clade was clustered with a high bootstrap value, that showed next to each branch and the tree was rooted based on the outgroup of *Nephus quadrimaculatus* (Fig. 4).

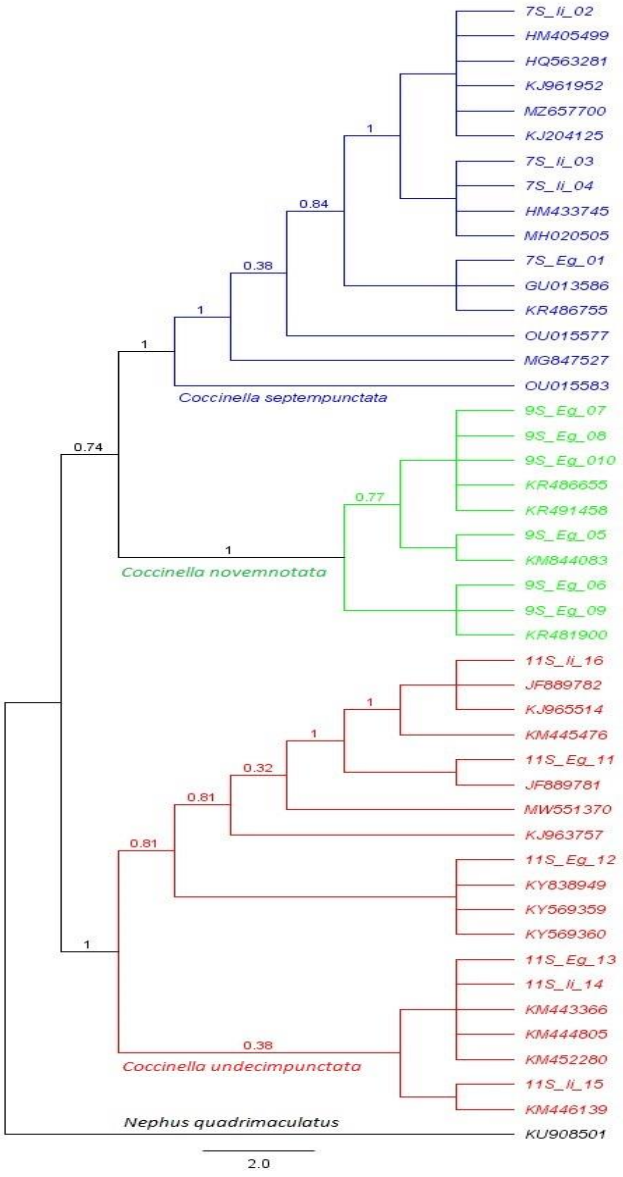

Figure 4. The phylogenetic tree between different species of *Coccinellidae* using the maximum-likelihood method based on the COI sequences.

4. Discussion

Taxonomic accuracy and species identification are the key factors to study a species ecology and evolution. Recently, systematics has exponentially increased by molecular phylogenetics due to the increasing ease of sequencing DNA, along with the reduction of irreplaceable taxonomical experts who retire with no replacements [28]. In this regard, The DNA barcoding technique was proposed for taxonomic classification and identification by

sequencing a standardized DNA sequence suitable for a large group of organisms. DNA barcoding using a partial sequence of the mitochondrial COI proved its ability for species-level identification in many animal groups [16, 29]. The effectiveness of the COI DNA barcoding has been applied and validated for species that possess distinct barcode arrays, with high divergences and low intraspecies variation [30, 31]. The sequence divergence between species, along with sequence conservation within species made the barcode COI sequence very specific.

Despite the Coccinellidae being considered one of a large cosmopolitan insect family, only a few formal phylogenetic analyses have been performed on this family. This study has validated the efficacy of the use of mitochondrial DNA COI barcodes for the identification of *Coccinella* species and their relationship. The result was in concordance with the work of Halim *et al*. [32], where the COI gene has been very useful in providing positive identification of *enosepilachna vigintioctopunctata* (Coleoptera: *Coccinellidae*). Additionally, Seago *et al*. [2], used the DNA barcoding technique to identify the major clades within Coccinellidae, revisited its classification, and identify the polyphagous diversification within this group. Lin *et al*. [29] explored the quality of DNA barcodes to identify and delimit the species of *Tanytarsus* genus (Diptera: Chironomidae). However, the validity of DNA barcoding mainly depends on the established references from taxonomically confirmed samples [33]. Therefore, the lack of reference DNA barcodes for 98% of the known insect species is considered one of the major limitations of this technique, which requires worldwide effort to enrich the database with taxonomically confirmed specimens.

The relationships identified among Coccinellidae are inconsistent and most nodes are poorly supported, thus making the affiliations doubtful [1]. However, in our analysis, the phylogenetic tree displayed significant separation between the three species of Coccinellidae species (*C. septempunctata*, *C. novemnotata*, and *C. undecimpunctata*); each species clustered into a unique branch and confirms its monophyly within the family.

In Conclusion, the present study highlighted the usefulness of the mitochondrial COI gene for *Coccinella* species identification and estimating genetic relationships, especially when the morphological characteristics are unreliability or inaccurate, or subject to ecological influences.

5. References

1. A. Magro, E. Lecompte, F. Magne, J. L. Hemptinne, and B. Crouau-Roy, “Phylogeny of ladybirds (Coleoptera: Coccinellidae): are the subfamilies monophyletic?”, Mol Phyl Evo 54(3): (2010), https://doi.org/10.1016/j.ympev.2009.10.022
2. A. E. Seago, J. A. Giorgi, J. Li, and A. Ślipiński, “Phylogeny, Classification and Evolution of Ladybird Beetles (Coleoptera: Coccinellidae) Based on Simultaneous Analysis of Molecular and Morphological Data”, Mol Phyl Evo 60(1): (2011), https://doi.org/10.1016/j.ympev.2011.03.015
3. T. T. Obrycki, and T. J. Kring, “Predaceous Coccinellidae in biological control”, Ann Rev Entom 43(1): (1998), https://doi.org/10.1146/annurev.ento.43.1.295
4. T. L. Erwin, Tropical forests: their richness in Coleoptera and other Arthropod species. The Coleopterists Bulletini, (1982), London.
5. B. D. Farrell, “Inordinate Fondness Explained: Why Are There So Many Beetles?”, Science, 281(5376): (1998), https://doi.org/10.1126/science.281.5376.555
6. N. Janz, S. Nylin, and N. Wahlberg, “Diversity Begets Diversity: Host Expansions and The Diversification of Plant-Feeding Insects”, BMC Evol Biol 6(1): (2006), https://doi.org/10.1186/1471-2148-6-4
7. D. D. McKenna, A. S. Sequeira, A. E. Marvaldi, and B. D. Farrell, “Temporal Lags and Overlap in The Diversification of Weevils and Flowering Plants”, Proc Nat Acad Sci 106(17): (2009), https://doi.org/10.1073/pnas.0810618106
8. W. M. Said, Z. M. Ahmed, T. R. Mohamed, "Morphological and Anatomical Studies on Some Taxa of Sub Family Malvoideae (Malvaceaes. l)", J Sci Res Sci 35.part 1: (2018) 371-389.
9. M. J. Madden, R. G. Young, J. W. Brown, S. E. Miller, A. J. Frewin, and R. H. Hanner, “Using DNA Barcoding To Improve Invasive Pest Identification at US Ports-Of-Entry”, PLoS One 14(9): (2019), https://doi.org/10.1371/journal.pone.0222291
10. P. D. Hebert, A. Cywinska, S. L. Ball, and J. R. DeWaard, “Biological Identifications Through DNA Barcodes”. Proc Roy Soc Lon. Ser B: Biol Sci, 270(1512): (2003), https://doi.org/10.1098/rspb.2002.2218
11. A. Frewin, C. Scott-Dupree, and R. Hanner, “DNA Barcoding for Plant Protection: Applications and Summary of Available Data For Arthropod Pests”, CAB Rev 8(018): (2013), https://doi.org/10.1079/PAVSNNR20138018
12. J. R. Wilson, P. Ivey, P. Manyama, and I. Nanni, “A New National Unit for Invasive Species Detection, Assessment and Eradication Planning”, South Afr J Sci 109(5): (2013), https://doi.org/10.1590/sajs.2013/20120111
13. K. W. Will, and D. Rubinoff, “Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification”, Cladistics, 20(1): (2013), https://doi.org/10.1111/j.1096-0031.2003.00008.x

14. U. Jinbo, T. Kato, and M. Ito, "Current progress in DNA barcoding and future implications for entomology", Entom Sci, 14(2): (2011), https://doi.org/10.1111/j.1479-8298.2011.00449.x

15. P. D. N. Hebert, M. Y. Stoeckle, T. S. Zemlak, C. M. Francis, and C. Godfray, "Identification of birds through DNA barcodes", PLoS biol 2(10): (2004), https://doi.org/10.1371/journal.pbio.0020312

16. S. M. E. Ahmed, Y. G. Fiteha, H. T. Elhifnawy, M. Magdy, A. Mamoon, N. Hussien and M. A. Rashed, "DNA Barcoding for Identification of Some Fish Species (Sparidae) in Mediterranean Sea Area", J Sci Res Sci 38 (1): (2021), https://doi.org/10.21608/jsrs.2021.210686

17. S. M. Saleh, N. O. Ehsan, S. Y. Mohamed, T. R. Mohamed, "Molecular Genetic Variability of Some Deciduous Fruit Rootstocks in Egypt", J Sci Res Sci 35.part 1: (2018), https://doi.org/10.21608/jsrs.2018.26787

18. M. M. Al-Rasheed, E. A. H. Abdel-Moaty, A. A. Al-Hazzani, "Genetic and Molecular Analysis of Seed Coats and Pollen Grains of *Phaseolus vulgaris* L. During Their Developmental Stages", J Sci Res Sci 35.part 1: (2018), https://doi.org/10.21608/jsrs.2018.46878

19. E. M. Abbas, M. Ismail, A. El-Ganainy, F. S. Ali, "First DNA Barcoding-Based Inventory of Suez Gulf Fishes In Egypt And Its Implication For Species Diversity", J Ichthy 61: (2021), https://doi.org/10.1134/S0032945221030012

20. J. Kjærandsen, "Current State of DNA Barcoding of Sciaroidea (Diptera)—Highlighting the Need to Build the Reference Library", Insects 13(2): (2022), https://doi.org/10.3390/insects13020147

21. P. R. Shashank, N. L. Naveena, N. N. Rajgopal, T. A. Elliott, K. Sreedevi, S. Sunil, and N. M. Meshram, "DNA barcoding of insects from India: Current status and future perspectives", Mol Biol Rep, 49: (2022), https://doi.org/10.1007/s11033-022-07628-2

22. T. Tsuji, H. Gotoh, S. Morita, J. Hirata, Y. Minakuchi, T. Yaginuma, and T. Niimi, "Molecular Characterization of Eye Pigmentation-Related ABC Transporter Genes in The Ladybird Beetle *Harmonia axyridis* Reveals Striking Gene Duplication of The White Gene". Zoo Sci, *35*(3): (2018), https://doi.org/10.2108/zs170166

23. S. Zheng, Y. Li, X. Yang, J. Chen, J. Hua, and Y. Gao, "DNA Barcoding Identification of Pseudococcidae (Hemiptera: Coccoidea) Using The Mitochondrial COI Gene", Mitochondrial DNA, 4(2): (2019), https://doi.org/10.1080/23802359.2019.1685160

24. B. Bukowski, S. Ratnasingham, P. E. Hanisch, P. D. Hebert, K. Perez, J. deWaard, and D. A. Lijtmaer, "DNA Barcodes Reveal Striking Arthropod Diversity and Unveil Seasonal Patterns of Variation In The Southern Atlantic Forest", PloS one, 17(4): (2022), https://doi.org/10.1371/journal.pone.0267390

25. O. Folmer, M. Black, W. Hoeh, R. Lutz, R. Vrijenhoek, "DNA Primers For Amplification of Mitochondrial Cytochrome C Oxidase Subunit I From Diverse Metazoan Invertebrates", Mol Mar Biol Biotech, 3: (1994), 294–299.

26. M. Kearse, R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, and A. Drummond, "Geneious Basic: An Integrated And Extendable Desktop Software

Platform For The Organization And Analysis of Sequence Data", Bioinformatics, 28(12): (2012), https://doi.org/10.1093/bioinformatics/bts199

27. K. Katoh, and D. M. Standley, "MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance And Usability", Mol Biol Evol 30(4): (2013), https://doi.org/10.1093/molbev/mst010
28. P. Chattopadhyay, G. Banerjee, and N. Banerjee, "Distinguishing Orchid Species by DNA Barcoding: Increasing The Resolution of Population Studies in Plant Biology", OMICS: J Integ Biol, 21(12): (2017), https://doi.org/10.1089/omi.2017.0131
29. X. Lin, E. Stur, and T. Ekrem, "Exploring Genetic Divergence in A Species-Rich Insect Genus using 2790 DNA Barcodes", PloS one, 10(9): (2015), https://doi.org/10.1371/journal.pone.0138993
30. M. Hajibabaei, G. A. Singer, P. D. Hebert, and D. A. Hickey, "DNA Barcoding: How It Complements Taxonomy, Molecular Phylogenetics and Population Genetics", Tre Genet, 23(4): (2007), https://doi.org/10.1016/j.tig.2007.02.001
31. R. D. Ward, T. S. Zemlak, B. H. Innes, P. R. Last and P. D. Hebert, "DNA Barcoding Australia's Fish Species", Philo Trans Roy Soc B: Biol Sci, 360(1462): (2005) https://doi.org/10.1098/rstb.2005.1716
32. M. Halim, A. Aman-Zuki, M. A. Mohammed, and S. Yaakop, "DNA Barcoding and Relationships of Eight Ladybugs Species (Coleoptera: Coccinellidae) That Infesting Several Crops from Peninsular Malaysia", J Asia-Pac Ento, 20(3): (2017). https://doi.org/10.1016/j.aspen.2017.05.009
33. R. S. Purty, and S. Chatterjee, "DNA barcoding: an effective technique in molecular taxonomy", Austin J Biotechnol Bioeng, 3(1): (2016), 1059.

الملخص العربي

التعريف الجزيئي لخنافس أبو العيد (Coccinella: Coccinellidae) باستخدام تقنية تكويد الحمض النووي

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الملخص العربي

تكويد الحمض النووي هو تقنية وراثية جزيئية تُطبق عادةً لتحديد الأنواع بناءً على جين السيتوكروم سي أوكسيديز I (COI) الموجود على الجينوم الميتوكوندير ي. بناءً على تحليلنا، تم إنشاء أكواد DNA من 16 عينة تنمي الى جنس Coccinella (عائلة Coccinellidae). جمعت جميع العينات من مواقع مختلفة من مصر وليبيا. تم استخدام الصفات المورفولوجية بناءً على عدد البقع الظهرية، واستخراج الحمض النووي، وتضخيم جين الـ COI باستخدام تفاعل البلمرة المتسلسل (PCR)، وقراءة تتابعات الجين، ومقارنته بقواعد البيانات لتصنيف العينات. تم إنشاء شجرة علاقات القرابة باستخدام طريقة maximum likelihood، باجمالي 46 عينة شاملة العينات التي تم دراستها واخري مسجلة على قواعد البيانات من دراسات سابقة للمقارنة. تم تحديد ثلاث مجموعات من Coccinella وتصنيفها على أنها *Coccinella septempunctata* و *Coccinella novemnotata* و *Coccinella undecimpunctata* كل نوع متجمع في فرع فريد. وقد ميزت اكواد الحمض النووي بشكل واضح الأنواع التي تم تحليلها. أثبتت هذه الدراسة كفاءة تتابعات الـ COI كجين يصلح لتكويد الحمض النووي لانواع حشرات أبو العيد (Coccinella ، عائلة Coccinellidae).

الكلمات المفتاحية: حشرات ابو العيد، جين سيتوكروم اوكسيديز، وراثة جزيئية، تعريف الأنواع، علاقات القرابة