Morphological and Molecular Identification of *Oryctes* (Coleoptera: Scarabaeidae) Collected from Date Palm Plantations in Algeria[1]

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Abstract Light traps operated from May 2022 to August 2023 collected *Oryctes* beetles (Coleoptera: Scarabaeidae) in date palm (*Phoenix dactylifera* L.) plantations in El Oued, Algeria. Morphological examination of 105 adult *Oryctes* specimens collected from the plantations revealed them to be *Oryctes agamemnon arabicus* (Fairmaire). Molecular identification, using the cytochrome oxidase *c* subunit I (COI) marker, confirmed those identifications. Notably, MASH analysis revealed differing degrees of genetic similarity, suggesting nuanced genetic diversity within the population. Utilizing Kraken2 software and the COI database from the National Center for Biotechnology Information, we also determined a high classification rate of approximately 99% of reads for all samples, reinforcing the identity of the specimens as *O. agamemnon*. These results help advance taxonomic knowledge of the insect fauna of the region as well as supporting environmental conservation initiatives.

Key Words *Oryctes* species, date palm plantations, morphological identification, molecular analysis, genetic diversity

Date palm trees, *Phoenix dactylifera* L., are attacked by several coleopteran pests that inflict substantial harm to both the stem and root systems. Notably, within the North African and Middle Eastern regions, three species belonging to the genus *Oryctes* (Coleoptera: Scarabaeidae), namely *Oryctes agamemnon* Burmeister, *O. elegans* Hellwig, and *O. richteri* Petrovitz, reportedly infest date palm trees (Rochat et al. 2004). In addition to date palms, these rhinoceros beetles pose a threat to other economically important palm species including the oil palm (*Elaeis guineensis* Jacquin) and the coconut palm (*Cocos nucifera* L.) (Bedford et al. 2015). Other *Oryctes* species have been reported as causing significant economic losses in date palm cultivation, both in commercial production and in ornamental landscapes (Al-Jassany and Al-Saedi 2019, El-Shafie et al. 2020).

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The way a potentially invasive species is introduced into a novel habitat involves a variety of aspects like its geographic origin and frequency of introductory events and is a pivotal determinant affecting its success in new environs. Furthermore, this pathway significantly affects the demographic dimensions and genetic variety within the initial population, thereby underscoring its critical role in successful establishment (Lockwood et al. 2005).

The primary purpose of this study was to identify the *Oryctes* species that are attacking date palm in El Qued, Algeria. We proposed using a combination of morphological and molecular analyses, offering valuable insights for advancing taxonomic knowledge of these pests, thus providing a foundation for the development of effective pest management strategies and future ecological studies.

Materials and Methods

Sampling. *Oryctes* adult specimens were captured utilizing light traps operated in four oases situated in the communes of Taleb Larbi and Douar El Ma within the province of El Oued, Algeria. The global positioning system coordinates for the four oases were N 33°42′30.8″, E 7°31′12.0″; N 33°42′27.6″, E 7°31′17.5″; N 33° 22′06.8″, E 7°41′20.6″; and N 33°21′45.5″, E 7°41′09.6″. The collection period extended from May 2022 to August 2023.

Morphological identification. The morphological identification of adult specimens was conducted with 64 females and 41 males. The identification process involved examining and analyzing morphological characteristics specific to the Dynastinae subfamily of beetles. The morphological criteria used for classification were in accordance with the guidelines provided by Endrödi (1985). Paramera were placed in lactic acid for 24 h for further analysis and, followed by examination, images were captured using a Keyence 5000 digital microscope.

Molecular identification. Samples for molecular identification were sent to Macrogen Genome Center (Beotkkot-ro, Geumcheon-gu, Seoul, Republic of Korea). Total deoxyribonucleic acid (DNA) was extracted from the legs of each individual insect sample. A 680-base-pair segment of the cytochrome oxidase *c* subunit 1 (*COI*) gene was amplified utilizing the universal barcoding primer pairs LCO1490 (5′GGT CAACAA ATC ATA AAG ATA TTG G 3′) and HCO2198 (5′ TAA ACT TCA GGG TGA CCA AAA AAT CA3′) (Folmer et al. 1994).

DNA analysis was conducted to assess quantity, integrity, and the fragment size for short-read sequencing. Quantity of DNA was determined by the PicoGreen double-stranded DNA quantification method using Victor 3 fluorometry. Integrity of the DNA in the sample was assessed using gel electrophoresis. The library quality-control (QC) method was used to ensure the quality of libraries used for sequencing, and the library size check involved assessing the template size distribution of polymerase chain reaction (PCR)-enriched fragments using the 2100 bioanalyzer (Agilent, Santa Clara, CA, USA) equipped with a DNA 1000 chip. For Illumina libraries, quantitative (q)PCR quantification was conducted according to the Illumina qPCR quantification protocol guide to achieve optimal cluster densities on sequencing platforms. For PacBio libraries, the Qubit standard quantification solution and calculator were utilized to create a standard curve of fluorescence readings and to determine the library sample concentration.

Amplicon sequencing was performed on four samples using the COI marker. The analysis included the data QC and trimming of the data, followed by the

taxonomic classification and quantification from the reads using a database of COI sequences. The obtained results were used to calculate alpha- and beta-diversity indices at the genus and species level and to calculate the MASH distance among the samples, which measures the similarity between two or more genomes. MASH distance also can be used to measure the similarity between samples on the basis of marker genes such as *16S*, *18S*, or *COI*, often referred to as "MASH-based marker gene analysis" or "MASH screening."

Results

Morphological analysis. The morphological analysis results demonstrated that all 105 adult specimens of *Oryctes* collected by the light traps in the four oases were the subspecies *O. a. arabicus* (Fairmaire). The identified specimens exhibited the following distinctive characteristics that conformed to the taxonomic criteria outlined by Endrödi (1985):

- *Oryctes agamemnon* (Burmeister). The clypeus is deeply and broadly emarginate, with sharply pointed anterior angles. The pronotum knob is bidentate in males and unidentate in females, with a smooth or finely punctate surface posteriorly. In males the pronotal cavity is large; in females it is smaller. The areola apposita is relatively strongly impressed and remains undivided from the anterior wrinkled area. The elytra are smooth or very finely and irregularly punctate, with only the sutural stria being distinct. The prosternal process is tall, and its apex is densely setose. The stridulatory ridges on the basis are more pronounced, though fine toward the posterior. The paramera are slender, and their apices are narrow and not dilated. The specimens ranged between 24 and 40 mm.
- *Oryctes agamemnon arabicus* (Fairmaire). The individuals in this subspecies are generally smaller, most $<$35 mm in length. Additionally, the elytra exhibit fine and distinct punctation (Figs. 1, 2). The morphological measurements of *O. a. arabicus* specimens show variations within and between sexes. Males had a mean body length of 30.53 mm (standard deviation [SD] $=$ 1.24), head width of 5.82 mm (SD $=$ 0.36), thorax width of 12.81 mm (SD $=$ 0.51), elytra width of 14.72 mm (SD $=$ 0.53), and horn length of 5.61 mm (SD $=$ 0.58). In contrast, females displayed slightly smaller dimensions, with a mean body length of 29.16 mm (SD $=$ 2.42), head width of 5.80 mm (SD $=$ 0.48), thorax width of 12.18 mm (SD $=$ 1.07), elytra width of 14.12 mm (SD $=$ 1.23), and horn length of 3.15 mm (SD $=$ 0.41). The head width, thorax width, and elytra width also exhibited variations within each sex. When comparing measurements between sexes, males generally displayed larger body length, width, and horn length than females. However, there were exceptions, such as in the case of elytra width, where females had slightly larger measurements than males.

Molecular analysis. The total number of reads generated, the guanine–cytosine (GC) and adenine–thymine (AT) content percentages, as well as the quality percentage of the base calls 20 (Q20) and 30 (Q30) for the four analyzed samples showed that sample 03 exhibited the highest number of sequenced bases (72.5 million), whereas sample 01 had the lowest (50.9 million). Sample 03 also had the highest number of reads (240,762), whereas sample 01 had the fewest (169,302). The GC content ranged from 38.9% to 39.5%, with sample 01 having the highest GC percentage. The AT content was between 60.5% and 61.1%. All samples exhibited sequencing quality, with Q20 percentages ranging from 79.7% to 80.8%

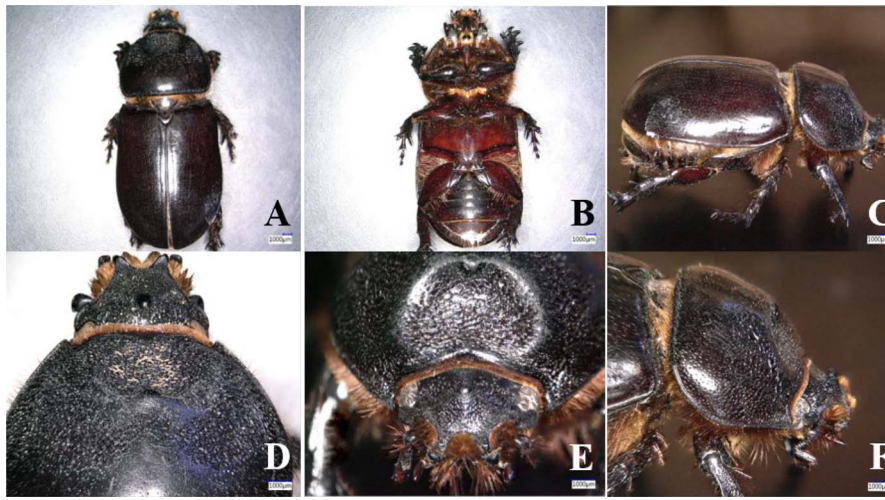

Fig. 1. Female *Oryctes agamemnon arabicus* dorsal view (A); ventral view (B); lateral view (C); head in dorsal view (D); head in frontal view (E); head in lateral view (F).

and Q30 percentages between 70.0% and 70.1%. These sequencing statistics underscore the quality of the data, laying a solid foundation for further analyses and reliable results from the study (Table 1).

Taxonomic classification and quantification. Kraken2 software was used with a database of COI sequences (National Center for Biotechnology Information downloaded in June 2021) to perform the classification of the trimmed reads and quantify the organisms in the samples. A minimum confidence score of 0.1 was set for the analysis. The classification rate was about 99% of the reads for all the samples, and all samples showed the presence of *O. agamemnon* and a very low number of reads from contaminants, such as *Homo sapiens*, which were removed. Significant variations in the number of reads assigned to *O. agamemnon* among the different samples occurred. Sample 03 had the highest number of assigned reads with 79,684 sequences, suggesting that this sample was abundant in *O. agamemnon* sequences. In contrast, sample 01 and sample 04 had relatively lower numbers of assigned reads, with 55,684 and 67,388 sequences, respectively. When comparing sample 02 and sample 03, we observed that sample 02 had 70,090 assigned reads, slightly lower than the count in sample 03 (Table 1).

Diversity indices. These results consistently showed low diversity in the samples, thus strongly indicating that each sample consisted of a single genus and species. Importation of the read counts matrix in the R software and subsequent analysis with the Phyloseq package at the genus and species levels yielded the most abundant genus and species in each sample.

The alpha diversity indices for genus and species show consistent results across all samples. The Bray–Curtis distance was 0 for all the samples. Furthermore, the observed and Chao1 indices were identical for all samples, indicating that each sample contained a single genus and species. The Chao1 index, which estimates

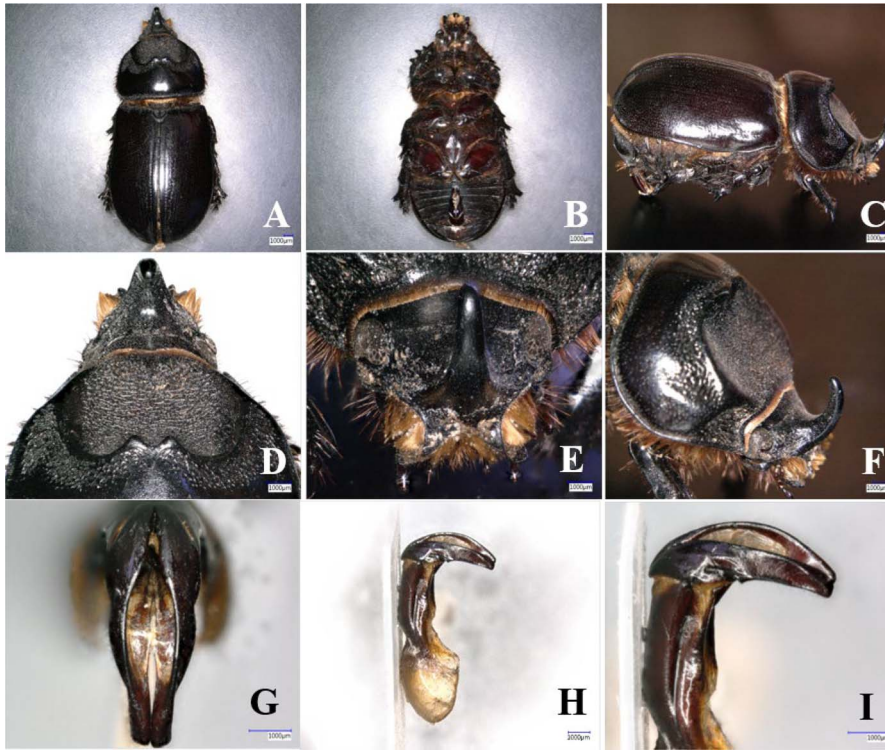

Fig. 2. Male *Oryctes agamemnon arabicus* dorsal view (A); ventral view (B); lateral view (C); head in dorsal view (D); head in frontal view (E); head in lateral view (F); paramera in caudal view (G); paramera in lateral view (H); paramera in lateral view (I).

the total number of genera and species in the community, also confirms that the diversity is relatively low, with an estimated richness of one genus and one species. These Chao1 values were consistently 0 across all samples, suggesting that the estimation of standard error for the Chao1 index was negligible and further reinforces the confidence in the observed low diversity within the samples. The Shannon index, which takes into account both richness and evenness of species, also was consistent at 1.37 for all samples, indicating relatively low diversity and relatively even distribution of the single species present in each sample.

MASH distance. The MASH distance was calculated to assess the relationship among the four samples on the basis of their sequence similarity. The lowest distance was observed between samples 04 and 02, whereas the greatest distance occurred between samples 03 and 01. The MASH distance values ranged from 0.00599405 to 0.014339, indicating that the genetic divergence between the samples was relatively low. A smaller MASH distance would suggest a greater similarity in sequence composition between compared samples (Table 2).

Table 1. Molecular analysis of *Oryctes agamemnon* collected from date palm in four oases in El Qued, Algeria.

Sample Identification	Total Bases (Base Pairs)	Total Reads	Assigned Reads	GC* (%)	AT** (%)	Q20† (%)	Q30‡ (%)
Sample 01	50,959,902	169,302	55,684	39.5	60.5	80.8	70.1
Sample 02	63,344,246	210,446	70,090	38.9	61.1	79.7	70.1
Sample 03	72,469,362	240,762	79,684	39.1	60.9	80.3	70.1
Sample 04	60,358,326	200,526	67,388	39.0	61.0	80.8	70.0

* Guanine–cytosine content.
** Adenine–thymine content.
† Quality score to base call 20.
‡ Quality score to base call 30.

Discussion

The morphological and molecular analyses of *Oryctes* specimens collected by light trap from the oases of El Oued, Algeria confirmed that all specimens belong to the subspecies *O. a. arabicus.* The morphological identification was strongly supported by molecular analysis utilizing the COI marker, which confirmed the presence of *O. agamemnon* in all samples. MASH analysis also indicated varying degrees of genetic similarity among the specimens, suggesting subtle genetic diversity within the population.

As an introduced species, *O. agamemnon* is expected to extend its range into other biogeographical habitats. In these expanded ranges, it will encounter various selective pressures, including abiotic and biotic factors as well as host plant availability (Prentis et al. 2008). Our results, beyond the identity of the scarabaeid beetles collected, will provide valuable insights into the presence and distribution of *O. agamemnon* genetic sequences within the data set, forming a basis for further analysis of its population dynamics. Alpha diversity analyses at both the genus

Table 2. MASH distance matrix in molecular analysis of *Oryctes agamemnon* collected from date palm in four oases in El Qued, Algeria.

Sample A	Sample B	Distance	*P*-value	Shared *K*-mers
2	1	0.00859207	0	3,062/5,000
3	1	0.014339	0	2,310/5,000
3	2	0.0112925	0	2,673/5,000
4	1	0.00887982	0	3,017/5,000
4	2	0.00599405	0	3,514/5,000
4	3	0.0113995	0	2,659/5,000

and species levels reveal a high degree of homogeneity in community composition, suggesting low species richness and evenness. However, contextual ecological factors may influence this low diversity. Overall, the MASH distance analysis enhances our comprehension of genetic diversity and relationships within the samples, setting the stage for further research on community dynamics in the ecosystem.

As highlighted by multiple researchers (Hartl et al. 1993, Suchentrunk et al. 2000), the observed genetic convergence among invasive insect pest populations may be attributed to a "genetic bottleneck" phenomenon. This suggests that the invasive population likely originated from a limited number of individuals, isolated from a larger gene pool within their initial habitat (in this case, the United Arab Emirates). Such genetic bottlenecks are presumed to reduce the genetic diversity within the invading population (Dlugosch and Parker 2008).

Bulman et al. (2005) expounded on the genetic adaptive capacity of an invasive species and its corresponding genetic diversity within its expanded range. This adaptability was found to be contingent upon three factors: the duration of the reproductive cycle, abiotic environmental conditions, and the presence of suitable host plants. Our results also have significant implications for advancing taxonomic understanding, pest management, and conservation efforts, emphasizing the need for further research to explore the ecological and genetic factors contributing to genetic variability in this population.

In conclusion, we have not only provided a comprehensive characterization of these pests of date palm in Algeria, but also uncovered subtle variations in their morphology and nuanced genetic diversity within the population. The use of advanced tools like COI marker and MASH analysis has reinforced these initial findings, with a high classification rate supporting the dominance of *O. agamemnon*. More important, our research goes beyond mere taxonomy, shedding light on the morphological and genetic intricacies of these insects. The outcomes of this research hold significance for advancing taxonomic knowledge, pest management, supporting environmental conservation efforts, and facilitating future ecological studies in the region.

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