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Artículos

A genetic and chemical study of six Oedipodinae species (Orthoptera: Caelifera: Acrididae) from Algeria

Estudios genéticos y químicos de seis especies de Oedipodinae (Orthoptera: Caelifera: Acrididae) de Argelia

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Abstract: The Oedipodinae subfamily is the most species-rich and numerically abundant group of Acrididae in Algeria. Here, genetic and chemical data were used to characterize the identities of six species: *Oedipoda miniata mauritanica* (Lucas, 1849); *Acrotylus patruelis* (Herrich-Shaeffer, 1838); *A. insubricus insubricus* (Scopoli, 1786); *Sphingonotus .Neosphingonotus. azurescens* (Rambur, 1838); *Sphingonotus .Neosphingonotus. finotianus* (Saussure, 1885); and *Sphingoderus carinatus* (Saussure, 1888). Individuals were collected from two different sites in the Sétif region of northeastern Algeria. First, specimens were classified based on their morphological traits. Second, phylogenetic analysis was performed using DNA barcoding data (COI gene). Third, the specimens' cuticular hydrocarbons (CHCs) were analyzed. The phylogenetic analysis revealed clear distinctions among the species. The chemotaxonomic analysis highlighted CHC profile similarities between *S. azurescens* and *S. finotianus* and CHC profile differences between *A.patruelis* and *A. i. insubricus*. Compared to *S. azurescens* and *S. finotianus, S. carinatus* had .-alkanes and trimethylalkanes with much shorter carbon chains. The genetic and chemical data obtained here have helped clarify differences within and between genera. These data also enabled to clearly distinguish among the six species.

Keywords: Chemotaxonomy, DNA barcoding, Oedipodinae, Orthoptera, Phylogeny.

Resumen: La subfamilia Oedipodinae es el grupo de Acrididae más rico en especies y numéricamente más abundante en Argelia. Aquí, se utilizaron datos genéticos y químicos para caracterizar la identidad de seis especies: *Oedipoda miniata mauritanica* Lucas, 1849, *Acrotylus patruelis* (Herrich-Shaeffer, 1838), *A. insubricus insubricus* (Scopoli, 1786), *Sphingonotus .Neosphingonotus. azurescens* (Rambur, 1838), *Sphingonotus .Neosphingonotus. finotianus* (Saussure, 1885) y *Sphingoderus carinatus* (Saussure, 1888). Se recolectaron individuos de dos sitios diferentes en la región de Sétif, al noreste de Argelia. Primero, los especímenes se clasificaron en función de sus rasgos morfológicos. En segundo lugar, se realizó un análisis filogenético utilizando datos de códigos de barras de ADN (gen COI). En tercer lugar, se analizaron los hidrocarburos

cuticulares de las muestras. El análisis filogenético reveló claras distinciones entre las especies. El análisis quimiotaxonómico destacó las similitudes de perfil entre *S. azurescens* y *S. finotianus* y la diferencia de perfil entre *A. patruelis y A. i. insubricus*. El perfil de hidrocarburos cuticulares de *S. carinatus* fue diferente de los de *S. azurescens* y *S. finotianus* por .-alcanos y trimetilalcanos con cadenas de carbono mucho más cortas. Los datos genéticos y químicos obtenidos han ayudado a aclarar las diferencias dentro y entre los géneros. También pudieron distinguir claramente entre las seis especies.

Palabras clave: Código de barras de ADN, Filogenia, Oedipodinae, Orthoptera, Quimiotaxonomía.

INTRODUCTION

In living organisms in general, and in acrididians in particular, species have traditionally been identified based mainly on morphological criteria. However, although a species' morphology may be unique, its taxonomic identity comprises multiple facets. Indeed, according to Morin (1980), taxonomic identity is determined by several interdependent factors: morphology, genetics, physiology, and behavior. In 2005, Dayrat indicated that identification based exclusively on morphology (i.e., resulting in morphospecies) could lead to valid classifications but that the resulting species should be treated as hypothetical taxa whose identities required confirmation through different types of approaches and data. He proposed using a broader toolkit, including molecular methods and population genetics analysis, in lieu of relying exclusively on classical taxonomic techniques. Padial et al. (2010) suggested that characters can be classified by type: biochemical, molecular, morphological, behavioral, or ecological. Moreover, they emphasized that, although traditional methods may remain useful in many cases, taxonomy must become more interdisciplinary and adopt new approaches to define species if it wishes to become a modern discipline. More recently, chemotaxonomy has been used to separate out species that do not display clear morphological differences (Clément et al. 2001; Wicker & Bagnères, 2020).

In the Acridoidea superfamily (Orthoptera, Caelifera), a single family predominates: Acrididae. Within Acrididae the subfamily Oedipodinae is largely composed of species with brightly colored hind wings that are only visible during flight. When their wings are closed, individuals are camouflaged within the environment. In several studies carried out in Algeria (notably by Mekkioui & Mesli, 2010; Sofrane & Doumandji, 2016), it was found that Oedipodinae was the best inventoried taxonomic group because of its high levels of abundance. Similar results were obtained in the Maghreb (Usmani, 2008). In addition, it has been shown that many species belong to Oedipodinae based on the list of Acridoidea for northwestern Africa in general and for Algeria in particular; this list was established by Louveaux & Ben Halima (1987) and Louveaux et al. (2020). The current taxonomic approach for this group relies generally on morphological traits; however, it lacks the accuracy to unequivocally identify species. There is thus a need to develop complementary approaches in which additional, non-morphological traits are employed in species identification for this group.

Intensive research on Oedipodinae phylogeny and biogeography was carried out by Hochkirch & Husemann (2008), who examined representatives of the tribe Sphingonotini collected from the Canary Islands. Based on a molecular phylogeny, the researchers proposed synonymizing the genera *Wernerella, Pseudosphingonotus*, and *Neosphingonotus* with the genus *Sphingonotus*. Additional research in this vein was performed by Husemann et al. (2012) using two tribes of Oedipodinae (Bryodemini and Sphingonotini). Their findings illustrated that 1) external morphological characters are often unreliable when drawing conclusions about taxonomic relationships and 2) genetic data, such as DNA sequences, are more useful when defining higher-level taxonomic groups. Using the mitochondrial cytochrome oxidase I (COI) gene, Moussi et al. (2018) found that DNA barcoding is a valuable tool for identifying and delimiting species in many Oedipodinae genera. At present, COI-based barcoding is the most widespread method.

In addition, chemotaxonomy has been used by a number of researchers to study invertebrates, especially insects (e.g., Formicidae: Lucas et al., 2002; Blattidae and Termitidae: Said et al., 2005; *Rhinotermitidae:* Clément et al., 2001; and *Culicidae:* Amira et al., 2013). However, the chemical profiles of Acrididae in general and of Oedipodinae in particular, remain poorly described when compared to what has been done in other insect groups (e.g., Lockey & Oraha, 1990 and Grunshaw et al., 1990). In the above research, one of the most examined chemotaxonomic characters is the cuticular hydrocarbons (CHC) profile (see the review by Bagnères & Wicker-Thomas, 2010). The cuticle has two main layers: a thin, non-chitinous outer epicuticle and a thicker, chitinous inner procuticle. The epicuticle is waxy and composed of lipids comprising various hydrocarbons; the latter can be saturated (alkanes with saturated linear chains [n-alkanes]), methylated (methylalkanes), or unsaturated with double bonds (e.g., monoenes, dienes) (Lockey, 1988). According to Allen (1998), because epicuticular lipids reduce cuticular transpiration, they play a vital role in enabling arthropods to thrive in terrestrial environments. According to Bagnères & Wicker-Thomas (2010), many species and their populations can be identified based on their CHC profiles. Furthermore, several studies have shown that CHC profiles can also be used to differentiate between the sexes within species (e.g., mosquitoes: Amira et al., 2013).

With the aim of building a broader kit of taxonomic tools for identifying members of Oedipodinae, this study focused on six grasshopper species belonging to four Oedipodinae genera to assess differences within and among genera. Our specific research objectives were as follows: 1) to characterize species identity and relatedness using DNA barcoding and 2) to explore similarity in CHCs profiles within and among species.

MATERIAL AND METHODS

Sample collection

We collected 111 specimens from two sites in the Sétif region of northeastern Algeria. The first, El-Ourecia, is a wasteland (36° 14'43.5" N; 05° 24' 05.9" E; elevation 1040 m). Soil salinity is very low; the electrical conductivity (EC) level (1/5) is 0.182 deciSiemens per meter (dS/m). The second, Bazer Sakhra, is a sebkha (36° 04'18" N, 05° 39'56" E; elevation 915 m). Soil salinity is high (EC [1/15] = 18.43 dS/m). All specimens were frozen at -20 °C until their use in the analyses. Taxonomic identification was carried out using taxonomic keys that employ external morphology (Chopard (1943), Mestre (1988), Defaut (2006), Belmann & Luquet (2009), Fontana et al. (2019), and Louveaux et al. (2020)). Six species were identified: *Oedipoda miniata mauritanica* (Lucas 1849); *Acrotylus patruelis* (Herrich-Shaeffer 1838); *Acrotylus insubricus insubricus* (Scopoli 1786); *Sphingonotus (Neosphingonotus) azurescens* (Rambur 1838) (see specific discussion later); *Sphingonotus (Neosphingonotus) finotianus* (Saussure 1885); and *Sphingoderus carinatus*. (Saussure 1888). . Fifty-one individuals were used in the DNA barcoding analysis, where each species was represented by at least seven specimens. The only exception was *A. patruelis*, for which we only had a single specimen because it is rare in the study area (see Supp. Table I for details). All the grasshoppers (n = 111) were used in the chemotaxonomic analyses.

DNA barcoding

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. The antennae were used or, in their absence, the legs. Prior to the extraction, the samples were stored in 96% alcohol at 4 °C. The barcoding region of the COI gene (650 bp) was amplified using the modified primers described in Moulton et al. (2010): MDG-F (5'TYTCAACWAAYCAYAARGAYATYGG-3') and MDG-R (5'TADACTTCWGGRTGWCCRAARAATCA-3') for *Oedipoda miniata mauritanica*, *Sphingonotus (Neosphingonotus) azurescens*, *Sphingonotus (Neosphingonotus) finotianus*, and *Sphingoderus carinatus*. For *Acrotylus patruelis* and *A. insubricus insubricus*, COI-F and COI-R were used instead (Husmann et al., 2012). The PCR reaction mixture had a total volume of 40 µl and contained 2 ng of DNA template, 1 × Qiagen Multiplex PCR Master Mix, and 2 µM of each primer. PCR amplification was performed using an ESCO Swift Maxi® Thermocycler. The initial denaturation step at 95 °C (15 min) was followed by 35 iterations of the following cycle: 94 °C (30 s), 52 °C (1 min 30 sec), and 72 °C (2 min); there was a final extension step at 72 °C (10 min). The PCR products were sequenced by the GenoScreen Institute (http://

www.genoscreen.fr) using BigDye 3.1 and a 96-Capillary 3730 xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were inspected, corrected, and aligned using Geneious® Pro v. 5.6.6 (http://www.geneious.com/). They were then deposited in GenBank.

Phylogenetic analysis

The 51 aligned COI sequences were analyzed using neighbor-joining (NJ), maximum-likelihood (ML), and Bayesian inference (BI) methods. The NJ method was applied using PHYLO_WIN (Galtier et al., 1996). The ML method was applied using PhyML (Guindon & Gascuel, 2003). MrAIC was used to find the appropriate sequence evolution model for the data (GTR + G) (Nylander, 2004). BI was carried out using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001). The program was run for 15 million generations. No prior assumptions were made about the topology. All the sequences were analyzed using the COI reference sequences available for Oedipodinae in GenBank and BOLD (https://www.boldsystems.org/). These reference sequences are indicated in the phylogenetic tree (GenBank or BOLD accession code followed by species name). Two sequences were used for the outgroup: one from *Chortophaga viridifasciata* (JQ513034) and one from *Lilaea fuliginosa* (KM243529).

Analysis of cuticular hydrocarbons

CHCs were extracted from all the specimens using pentane (Blomquist et al., 1984); solvent quantity varied between 2 and 5 ml because it depended on specimen size. Immersion time was 10 min, and, during this period, the mixture was agitated several times. The resulting extract was evaporated under nitrogen flow. Between 40–80 µl of 10#5 g/ml of *n*-eicosane (*n*-C20) was added as an internal standard to calibrate the gas chromatograph (GC) profiles; the exact quantity again depended on specimen size. Then, 2 µl of the extract was injected into a GC (Agilent Technologies 6850, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and an apolar HP-1 capillary column (Agilent, 100% methylsiloxane; 30 m x 320 µm x 0.25 µm). The flame was supplied with air (450 ml/min) and hydrogen (40 ml/min). The carrier gas was helium at a pressure of 1.2 ml/min. The temperature of the detector as well as that of the injector was 250 °C. The oven's initial temperature was 70 °C. Temperature first climbed by 30 °C/min until it reached 150 °C; it then climbed by 5 °C/min to reach a final temperature of 320 °C. The injection occurred in splitless mode (30 sec), and the GC analysis cycle lasted 62 min. The CHCs were identified using a GC-mass spectrometer (MS) (Autosystem XL coupled with a TurboMass 5.4; Perkin Elmer, Waltham, MA, USA); an electron ionization setting of 70 eV was employed. The parameters of the GC cycle were the same as those described above. The mass spectra were identified using known

mass spectra from the literature (Lockey 1988; review in Blomquist & Bagnères, 2010).

The relative percentages of the CHCs were calculated by performing GC analysis on the individual specimens; the cuticular mixtures were identified by performing GC-MS analysis on pools of specimens representing the same species. Principal component analysis (PCA) and hierarchical cluster analysis were carried out using the relative percentages of CHCs. An analysis of variance (ANOVA) was performed to test for intraspecific differences between the sexes (SPSS v. 18).

RESULTS

Phylogenetic results

The three phylogenetic methods yielded trees with congruent topologies that contained the same three major clades. Consequently, we have chosen to present only the BI tree (Fig. 1). The first clade was formed by all the specimens of *O. miniata* mauritanica. The second clade contained two well-supported monophyletic groups that corresponded to the two *Acrotylus* species. The third clade comprised the vast majority of the specimens representing the two *Sphingonotus* species as well as the specimens for *S. carinatus*. In the first clade (i.e., corresponding to O. m. mauritanica), four haplotypes were present that corresponded to four variable nucleotide sites. In the second clade, there were two groups. The first group contained a single haplotype (AA10) that was associated with *A. partuelis*; the second group contained six haplotypes (corresponding to eight variable nucleotide sites) that were associated with *A. insubricus* insubricus. In the third clade, there were three well-supported monophyletic groups that corresponded to the three species. The first group was composed of eight specimens divided into two haplotypes (corresponding to a single variable nucleotide site); this group was associated with the BOLD reference sequence for *Sphingonotus lucasii* (OBAL053-18). The second group was composed of nine specimens displaying a single haplotype that were associated with *S. (Neosphingonotus) finotianus*. The third group consisted of eight specimens divided into two haplotypes (corresponding to a single variable nucleotide site); this group was associated with *S. carinatus*.

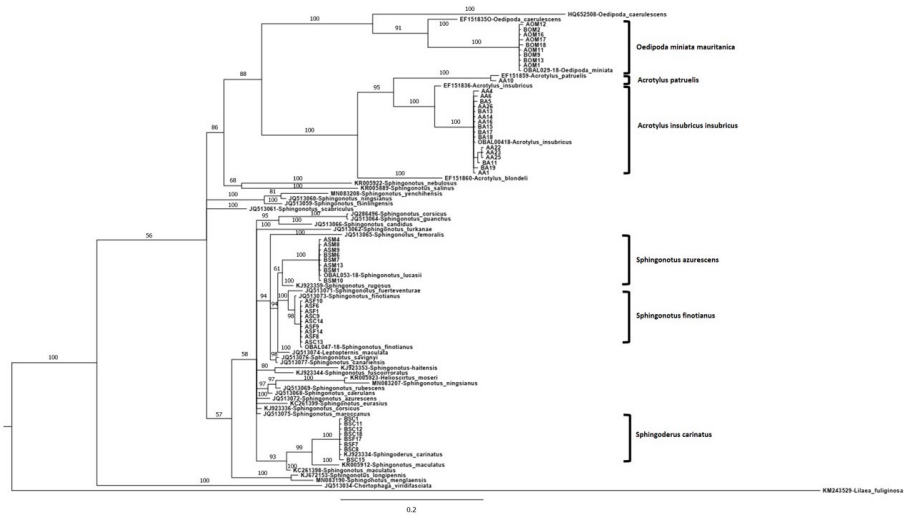

Fig. 1. Phylogenetic tree built using COI gene sequences and Bayesian inference. The branch support values are Bayesian posterior probabilities. The reference sequences are labeled (Genbank or BOLD accession code followed by species name). The brackets on the right side of the tree indicate the species identity of the specimens.

Analysis of cuticular hydrocarbons

Using the GC and GC-MS analyses, CHC profiles were obtained for the six study species (Fig. 2); they contained four CHC classes: n-alkanes, monomethylalkanes, dimethylalkanes, and trimethylalkanes. No unsaturated CHCs were seen (Supp. Table II).

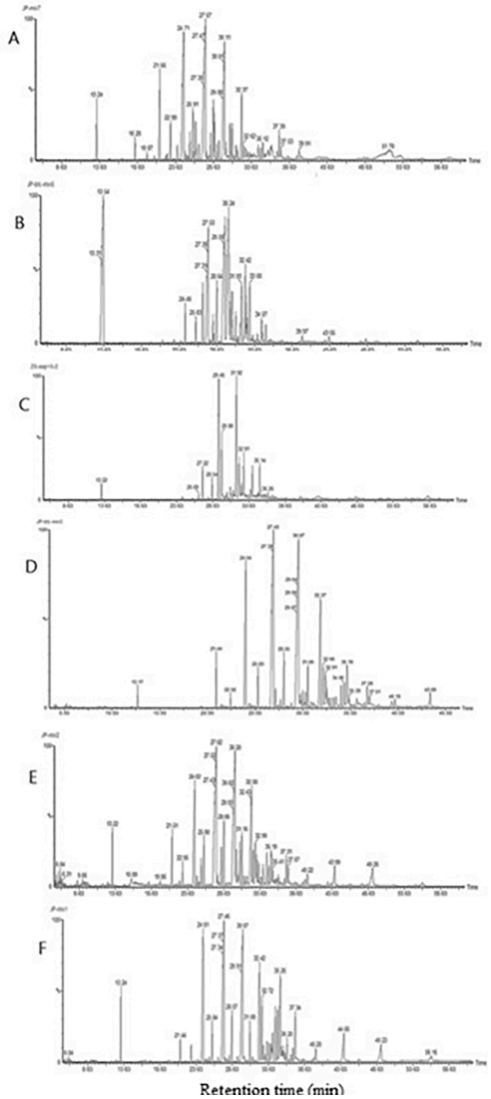

Fig. 2. Total ion chromatograms (GC-MS) for *Oedipoda miniata mauritanica* **(A),** *Acrotylus insubricus insubricus* **(B),** *Acrotylus patruelis* **(C), S***phingonotus azurescens* **(D),** *Sphingonotus finotianus* **(E), and** *Sphingoderus carinatus* **(F).**

Within species, individuals displayed certain similarities in compound classes, although relative compound quantities differed (Fig. 3).

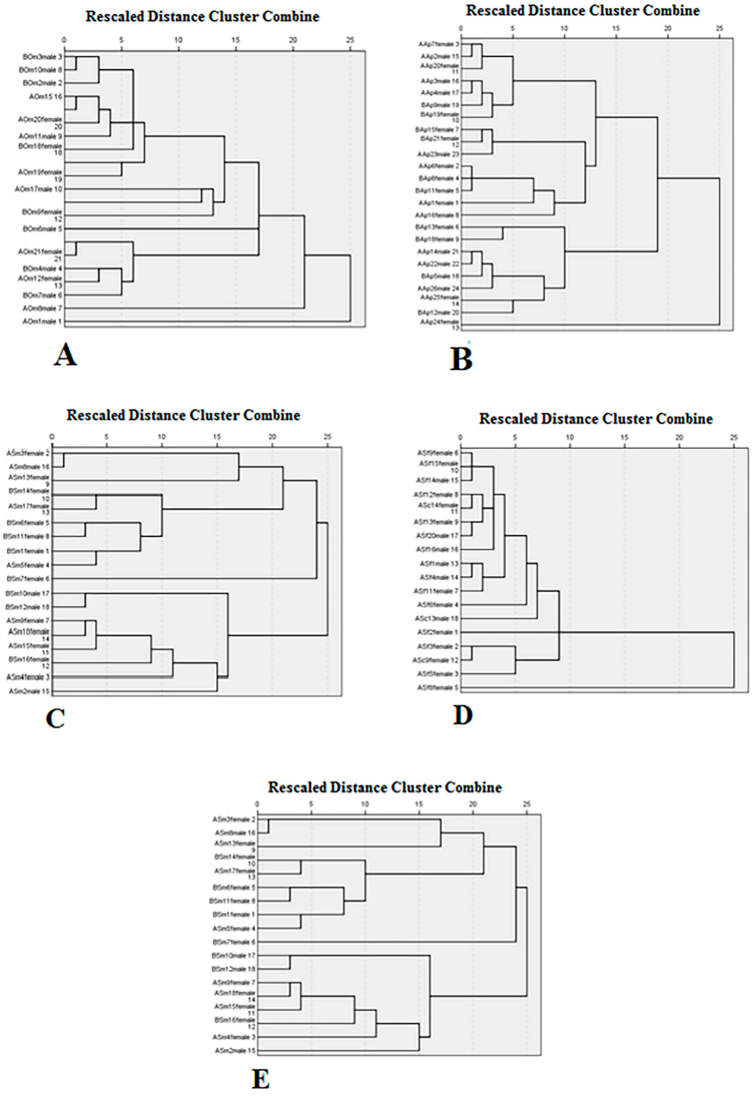

Fig. 3. Hierarchical cluster analysis of the CHC profiles of *Oedipoda miniata mauritanica* (A), *Acrotylus insubricus insubricus* (B), *Sphingonotus azurescens* (C), *Sphingonotus finotianus* (D), and *Sphingoderus carinatus* (E).

In *Oedipoda miniata mauritanica* and *S. azurescens*, n-alkanes were more common in females; in *S. finotianus*, *S. carinatus*, and *Acrotylus insubricus insubricus*, they were more common in males. Monomethylalkanes were also generally more common in females. However, in *S. azurescens* and *S. carinatus*, they were more common in males. There were only minor differences in dimethylalkanes between the sexes. That said, dimethylalkanes were far more prominent in females in *A. i. insubricus* and *S. carinatus*. Across all six species, trimethylalkanes were generally more common in males. The only exceptions were seen in S. carinatus (more common in females) and in *A. i. insubricus* (no sex-specific difference) (Fig. 4).

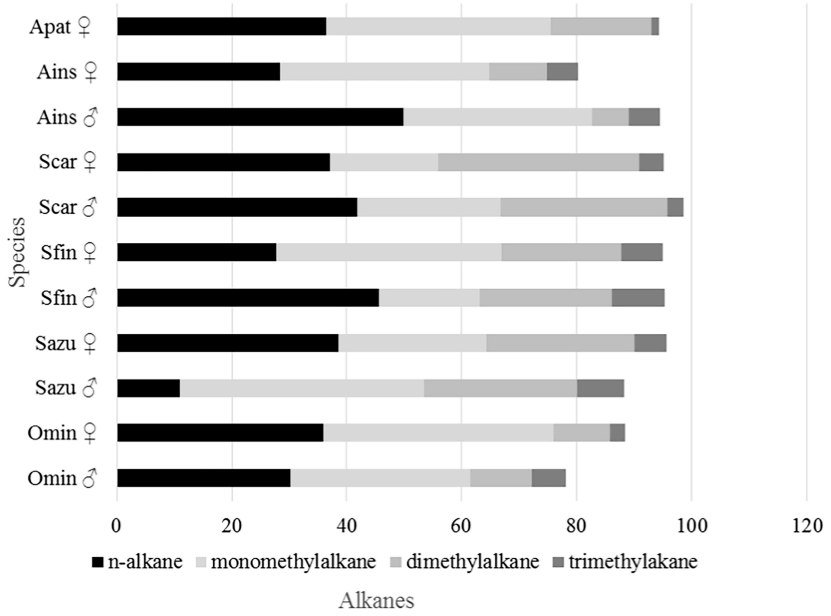

Fig. 4. Relative percentages of the different CHC classes observed in *Oedipoda miniata mauritanica* **(Omin),** *Acrotylus insubricus insubricus* **(Ains),** *Acrotylus patruelis* **(Apat),** *Sphingonotus azurescens* **(Sazu),** *Sphingoderus carinatus* **(Scar), and** *Sphingonotus finotianus* **(Sfin).**

In general, in *Oedipoda miniata mauritanica*, *Acrotylus patruelis* and *A. insubricus insubricus*, the most prominent CHC classes were the monomethylalkanes and n-alkanes. The latter were most prominent in *S. azurescens*, *S. finotianus*, and *S. carinatus.*

Comparing relative CHC levels among species, n-alkanes were more common in S. azurescens; monomethylalkanes were more common in *Acrotylus patruelis*; and dimethylalkanes were more common in *S. carinatus*; and trimethylalkanes were more common in *S. finotianus*.

A PCA was carried out using 64 variables, which corresponded to the CHC profiles of all the specimens (i.e., the GC-MS date (Table I), and the identities of the six study species. Taken together, axis 1 and axis 2 explained 53.18% of the total variance (33.04% and 20.14%, respectively; Fig. 5). Three clusters formed largely along taxonomic lines: one with individuals from *Oedipoda miniata mauritanica*; another with individuals from both *Acrotylus insubricus insubricus* and *A. patruelis*; and a third with individuals from *S. azurescens*, *S. finotianus,* and *S. carinatus*. Furthermore, *O. m. mauritanica* had a very distinct profile, characterized by monomethylalkanes, dimethylalkanes, and trimethylalkanes with much shorter carbon chains. The clustering of *S. azurescens*, *S. finotianus*, and *S. carinatus* can be explained by the similarities in their CHCs classes, namely the mono- and dimethylalkanes. (Table I).

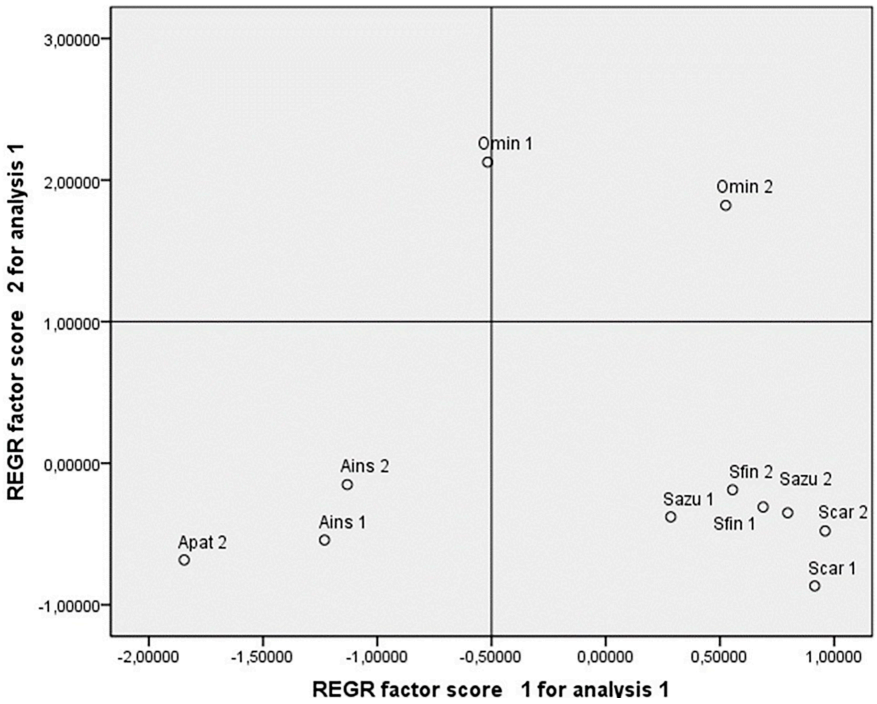

Fig. 5. Principal component analysis of the CHC profiles of *Oedipoda miniata mauritanica* (Omin), *Acrotylus insubricus insubricus* (Ains), *Acrotylus patruelis* (Apat), *Sphingonotus azurescens* (Sazu), *Sphingoderus carinatus* (Scar), and *Sphingonotus finotianus* (Sfin). The results for males (1) and females (2) are shown.

Species	*n*-alkanes	Mono	Di	Tri
Omin	23–39	23–39	29–39	35–39
Ains	23–37	26–35	27–35	30–34
Apat	27–37	28–35	31–35	30–36
Sazu	23–39	25–39	25–39	33–39
Sfin	23–39	25–39	25–39	33–39
Scar	24–39	25–39	25–39	33–37

Table I. Carbon chain length range for the cuticular hydrocarbon classes seen in *Oedipoda miniata mauritanica* (Omin), *Acrotylus insubricus insubricus* (Ains), *Acrotylus patruelis* (Apat), *Sphingonotus azurescens* (Sazu), *S. finotianus* (Sfin), and *Sphingoderus carinatus* (Scar).

Sex-specific differences in CHCs profiles were seen in *Oedipoda mauritanica* and *S. carinatus* (ANOVA: $p < 0.05$) but not in *S. azurescens*, *S. finotianus*, *Acrotylus insubricus* insubricus, or *A. patruelis*.

DISCUSSION

There is a need to expand the taxonomic toolkit for identifying species in Oedipodinae. To this end, we explored the ability of a combined approach—using both genetic and chemical data—to differentiate among six study species.

Phylogenetic results

The COI-based phylogenetic analysis generally confirmed our morphology-based classifications. Indeed, the sole member of the second clade in the phylogenetic tree was identified as *Acrotylus patruelis*. Thus, the difference between *A. patruelis* and *A. insubricus* was clear from the barcoding data.

Individuals that were morphologically identified as belonging to *S. azurescens*, *S. finotianus*, and *S. carinatus* occurred in three well-supported monophyletic groups in the third clade. Consequently, questions are raised about the *S. azurescens* (JQ513072) and *S. lucasii* (OBAL053-18) reference sequences—are those species assignments actually correct? Our specimens that grouped genetically with the *S. lucasii* reference sequence were morphologically classified as *S. azurescens*. The latter species is identified based on the following traits. It has a pronotum that is nearly smooth, weakly rough, and finely punctuated. The pronotum's median carina is prominent in the prozone but reduced to a single line in the metazone. The species' posterior femurs have a dark inner side with two clear bands. The upper carina of the posterior femur descends in its apical half. The posterior tibiae are yellowish white with a bluish tinge. The tegmina have a broad brown spot in their basal third and a narrow brown stripe in their middle. The wings have a broad brown fascia that varies in length; it starts at the middle of the anterior wing edge and closely follows the edge. Given that our specimens clearly displayed these traits, we assumed that they were indeed *S. azurescens*.

According to Chopard (1943), *S. azurescens* has been confused with a neighboring species, notably with *S. lucasii*. Louveaux et al. (2020) reiterated that confusion between these species may occur and that their distribution ranges need to be confirmed. The species appear to occur in different regions of North Africa (e.g., northern and southern Algeria). They are geographically close.

Moussi et al. (2018) stated that there was a match between the molecular and morphological classifications of many Oedipodinae species; however, *Sphingonotus* remained a problematic taxon in their phylogenetic analysis. These authors interpreted this result as showing that COI-based species delimitation remains difficult, at least for most Sphingonotini genera, perhaps because of nuclear copies of mitochondrial sequences (pseudogenes), even though DNA barcoding is a valuable tool for identifying species in these genera. They suggested additional genetic data may be required to achieve better levels of resolution. Here we assume our eight specimens as *S. azurescens* rather than as *S. lucasii*.

Analysis of cuticular hydrocarbons

The length of the CHC carbon chains observed in this study are consistent with those seen in previous research on insects. According to Nelson & Blomquist (1995), this length ranges approximately from 21 to

50 carbons, and the shorter chain compounds are volatile. In general, the number of methylated compounds was high, and there was a remarkable absence of unsaturated products (alkenes). These results agree with those obtained for *Locusta migratoria* cinerascens (Genin et al., 1986).

The cluster analysis revealed species specific variation in CHC profiles. Some past studies (Nielsen et al., 1999; Buczkowski et al., 2005; Parkarsh et al., 2008; Bontonou et al., 2013) have found that variation in CHCs can be linked to factors such as age, diet, habitat, and diverse environmental characteristics, while other studies have seen no such correlation (Toolson, 1982; Vander Meer et al., 1989; Dahbi et al., 1996). Consequently, CHCs could contribute to creating a species-specific chemical signature.

The PCA results highlighted the profile similarities among *S. azurescens*, *S. finotianus*, and *S. carinatus*. The three species formed a single cluster that was distinct from the cluster formed by Oedipoda miniata mauritanica and from the cluster formed by *Acrotylus insubricus insubricus* and *A. patruelis*. These results show that, although there were similarities, the CHC profiles were nonetheless relatively species specific, notably in their number of compounds. The clusters arising from the chemical data generally corresponded to the clusters arising from the genetic data.

There was a significant sex-specific difference in relative CHC percentages in *S. carinatus* and *Oedipoda miniata mauritanica* but not in *S. azurescens*, *S. finotianus, Acrotylus insubricus insubricus*, or *A. patruelis*. However, it could be that more volatile compounds not analyzed here are also at play. Sexual selection could be involved (Thomas & Simmons, 2008), given that sexual dimorphism in CHC profiles was seen in only some species and that profile variation was seen within genera. An explanation could be that hydrocarbons are involved in reproduction, acting as sex pheromones as they do in other insect taxa, such as mosquitoes (Amira et al., 2013), *Drosophila* (Bontonou et al, 2013), *Blattella germanica* (Morakchi et al., 2010), and cricket *Teleogryllus oceanicus* (Thomas & Simmons, 2008).

In this study, we successfully explored intraspecific diversity within *Acrotylus insubricus* and used our combined taxonomic approach to distinguish between the two *Acrotylus* species. For *Sphingonotus finotianus* and *S. carinatus*, we increased the taxonomic specificity and showed that the species display clear genetic and chemical differences. We also helped flesh out the genetic and chemical taxonomy of the two other study species.

The genetic and chemical data we obtained have helped clarify differences within and between genera. They were also able to clearly distinguish between the six study species. Our findings indicate that such multifaceted analyses could make a significant contribution by complementing current morphotaxonomic techniques and thus help clarify species systematics.

CONCLUSION

In this study, we successfully explored intraspecific diversity within *Acrotylus insubricus* and used our combined taxonomic approach to distinguish between the two Acrotylus species. For *Sphingonotus finotianus* and *S. carinatus*, we increased the taxonomic specificity and showed that the species display clear genetic and chemical differences. We also helped flesh out the genetic and chemical taxonomy of the two other study species.

The genetic and chemical data we obtained have helped clarify differences within and between genera. They were also able to clearly distinguish between the six study species. Our findings indicate that such multifaceted analyses could make a significant contribution by complementing current morphotaxonomic techniques and thus help clarify species systematics.

Supplementary materials

Supp. Table (pdf)

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