Morphological and Molecular Analysis of some Bee Species of the Subgenus Taeniandrena (Hymneoptera: Andrenidae) from Northern Egypt

Isam M. Abu Zeid1, Mohamed A. Shebl2 and Ehab M.R. Metwali3,4*

1Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 8023 Jeddah, Saudi Arabia
2Department of Plant Protection, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt
3Department of Biological Sciences, Faculty of Science, University of Jeddah, 21959 Jeddah, Saudi Arabia
4Department of Botany, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt

*For correspondence: ehabmetwali@hotmail.com; ehabmetwali71@gmail.com

Abstract

Andrenids is one of those groups which have some taxonomic impediments. Andrena ovatula represents such taxonomic impediment with great individual variations in size, body length and even in coloration. Several species were collected and identified on the basis of morphological characters. This study involved DNA sequencing of COI region of the mitochondrial DNA genome of A. ovatula to determine the population genetics and bio-geographical structure from three different locations in Egypt (Alexandria (A), Monofia (M) and Suez Canal (S)). Each population had three females as a preliminary study of this group for barcoding in Egypt. All investigated samples belonged to nine mtDNA haplotype. The phylogenetic analysis showed that there are two groups of A. ovatula as some samples matched 100% with Andrena intermedia not known or recorded in Egypt so far. A large sampling scale of these species which is widely distributed in Asia, Europe and Africa could show more population differentiation. Further studies can help for more clarifications of the species identity of the genus Andrena worldwide. © 2019 Friends Science Publishers

Keywords: Solitary bees; Apoidea: Andrena; Barcoding; DNA sequences; mtDNA; Phylogenetic; Population genetics

Introduction

Andrenidae is one of the bee families, divided into four subfamilies, two of which (Andreninae and Panurginae) are widely distributed in the Palaearctic Region. Genus Andrena is one of the largest bee genera and is known as sand bees or solitary mining bees with over 3000 known species worldwide (Michener, 2007). Species of the genus Andrena fly from early spring to early summer (Dobrzynsky et al., 2005), and very few in summer or autumn seasons. Several species of Andrena are generalists on their floral resources and gather pollens from several plant species (polylectic species) but some others considered as oligolectic species (Shebl et al., 2015). Family Andrenidae is a monophyletic group and few efforts have been made to trace its phylogeny (Alexander, 1995; Larkin, 2002). The subgenus Taeniandrena is primarily Palaearctic including 23 medium size bee species, are hard to distinguish morphologically due to overlapping characters (Shebl, 2010). Andrena (Taeniandrena) ovatula (Kirby, 1802), one of the most common bee species of this subgenus; is a good pollinator of some cultivated crops, is widespread all over the world (Asia, Africa and Europe) with some known subspecies. In Egypt, this species is known across the country around the Nile Delta and other regions of the desert (Shebl et al., 2013; Shebl and Farag, 2015). Yet the phylogenetic position of this genus, as well as their relationships with other bee families has not been satisfactorily analyzed. Andrenidae is poorly studied group in terms of their molecular phylogeny. There were very few studies attempted to tackle this genus (Danforth, 1999). Mitochondrial cytochrome oxidase I (COI) is a mitochondrial protein-coding gene and has been tested successfully with a variety of insect phylogenetic analysis (generic and subgeneric levels) (Simon et al., 1994) and has been found suitable for obtaining a good resolution on the subgeneric level within bees. There are very few molecular and phylogenetic studies of the subfamilies, tribes, and genera of Andrenidae (Danforth et al., 2013). Recently, Kek et al. (2017) used mitochondrial cytochrome oxidase subunit I (COI) gene region to identify and classify bees.

The aim of the current study was to examine nucleotide diversity of COI mtDNA of A. ovatula collected from different locations in northern Egypt, ecotypes as the initial step of molecular characterization, taxonomic position and population differences among the individuals from different geographical areas. Moreover, it addresses other questions to
find out if there are any genetic differences between different geographical populations or not? This is the first time to study *Taenianandrena* using DNA sequences approach in Egypt, although there are some published data on some other subgenera (Larkin et al., 2005; Reemer et al., 2008), which could lead to further studies on the phylogeography, population genetics and DNA barcoding of Egyptian bees.

### Material and Methods

#### Specimen Collection, Identification and Description

Bee individuals were collected by sweeping from different locations across Egypt during several field collections of bees from several plants such as *Triforium alexandrinum*, *Vicia faba* and *Brassica* spp. The specimens were killed, pinned and deposited at the sec author’s private collection. The specimens of Andrenids were collected from three major cities Ismailia (Eastern part of Egypt), Minoufia (Nile Delta) and Alexandria (Western part of Egypt) as shown in Fig. (1) during spring seasons in the years 2011-2017.

The specimens were identified based on the taxonomic key of the Egyptian species (Moustafa et al., 1979) then sorted out into three groups of *A. ovatula* based on locality (3 to 5 specimens per locality or per group, first group Minoufia 1-3; sec group Ismailia 4-6 and third group Alexandria 7-9. Specimens were examined and described using a stereoscopic microscope (OPTOMETRON D-81829). Photos of specimens were taken and illustrated using ADOBE PHOTOSHOP and PHOTOSCAPE software (Fig. 2). Male terminalia was pulled out from fresh captured specimens for examination and compared with the illustration published by Shebl (2010). The morphological terminology was adopted from Shebl and Tadauchi (2011).

Terms and abbreviations used are as follows: T: tergum, S: sternum, HL: head length, HW: head width, FL1: first flagellum length, FL2: sec flagellum length, FL3: third flagellum length.

#### DNA-extraction, Amplification, and Sequencing

DNA was extracted from wholly legs from each population. Standard protocol for animal tissues of DNeasy Tissue Kit (QIAGEN) was followed for DNA extraction. Concentration and purity of DNA of each sample was measured by a Nanodrop-photometer (IMPEN Germany). Primers used for PCR amplification in this study were 5’-CCACATTTATTTTGATTTTTTGG-3’ as a forward 5’-TCCAATGCACTATCTGCCATATT-3’ as a reverse (Petersen et al., 2007).

PCR amplification of the mitochondrial cytochrome oxidase I (COI) gene was performed in a total of 50 μl reaction volume using water, 10x PCR buffer, MgCl₂, dNTP (2 mM each), primers (10 pmol/L each), 0.6 U of Taq polymerase and 2 μL template DNA. The conditions for PCR were the following: initial denaturation (95°C, 3 min) followed by 35 cycles of denaturation (94°C, 45 s), primer annealing (56°C, 45 s), and DNA extension (72°C, 1 min).

#### Data Analysis

Sequences of COI mtDNA (590 bp) were aligned with published sequences of nine different haplotypes of *A. ovatula* populations. Sequence alignments were done using the Bio Edit 7.0.5 software (Hall, 1999). Neighbour Joining (NJ) analyses were performed using ClustalX software (Thompson et al., 1997). The number of haplotypes and their frequencies were determined both visually and with the program DnaSP version 4 (Rozas et al., 2003). DNAsp also estimated the following variables: haplotypic diversity (Hd), nucleotide diversity Pi (π), based on Nei (1987). Nucleotide diversity was interpreted as the average proportion of nucleotide differences between all possible pairs of sequences in the sample (Hartl and Clark, 1997), the mean number of pairwise nucleotide differences (K) according to Tajima (1983). The parameter theta (θ) is the proportion of...
nucleotide sites that are expected to be polymorphic in any sample from this region of the genome was estimated based on Hartl and Clark (1997). Our data was compared with the similar genome of the Gene Bank (International Nucleotide Sequence Database Collaboration).

Results

Systematic of the Species *Andrena ovatula* Kirby

*A. ovatula* is a widespread bee species that visits a wide range of flowers so the identity of the species is very important for any pollination study. So, it is important to provide full taxonomic characters to help non specialists to identify this species easily. This study provides a full description of both sexes of this species as it wasn't presented in previous literature dealing with Egyptian Andrenids.

Redescription of the Female

Color: Flagellum brownish beneath; mandibles black reddened apically; facial fovea brownish above, whitish below; wing membranes subhyaline, moderately brownish, veins and pterostigma brownish; femur, tibia and tarsus brownish, tibial spur yellowish.

Pubescence: Hairs on head sparse except antennal area with dense hairs, long, whitish; those on clypeus sparse, short, whitish, those on vertex dense, slightly long, whitish, those on genal area dense, whitish, long. Hairs on metasoma sparse, dense laterally, short, whitish; those on scutellum scantly, dense laterally, short, whitish, those on mesepisternum dense, long; whitish, propodeal corbicula developed, whitish, with internal simple, sparse hairs, trochanteral flocus perfect, whitish, femoral flocus dense, long, whitish, tibial scopa long, simple, silver whitish hairs. Hairs on metasomal terga T1,2 with interrupted whitish hair bands, T3,4 with completed whitish hair bands; caudal fimbria brownish; S2,5 with long, sparse, whitish subapical fimbriae.

Structure: Head length 2.7-3.3 mm, Head width 3.5-4 mm, HL/HW = 0.75-0.77. Vertex flat in frontal view, tessellate, shiny, with small PP, FL1<FL2<FL3, FL1<FL2+FL3, FL2<FL3 which are broader than long. Eyes with inner margins subparallelled. Supraclypeal area slightly flat rather convex, shiny, with small PP. Facial fovea long, broad, occupying 3/4 the space between the eye and lateral ocellus, much extending to a line at lower margins of antennal fossae. Face above antennal fossae with fine longitudinal rugulae and weak interrupal PP, shagreening. Facial quadrangle broader than long. Clypeus flattened, densely tessellate, punctate with slightly dense, obscure PP, with longitudinal median impunctate space. The process of labrum broad, trapezoidal, not emarginated apically.

Lower paraocular area punctate with dense, shiny with min PP. Malar space linear, broad. Genal area slightly broader than the eye, tessellate and shiny with sparse, min PP. Mesosoma: Pronotum with lateral suture and without humeral angle, densely tessellate, shiny with fine PP. Mesoscum densely tessellate, punctate, shiny with fine, obscure, small PP. Scutellum tessellate, punctate, shiny with dense, obscure, fine, small PP. Propodeal enclosure triangular, weakly indicated, rugulose basally, tessellate apically; dorsal face of propodeum roughened with fine, small PP. Mesepisternum tessellate, roughened, with fine, small PP. Vein 1°-m-cu meeting sec submarginal cell at the middle of the cell. Metasoma: Metasoma condensed oval, metasomal terga densely tessellate, punctate, nearly shiny, T1,4 with finely dense, FL1>FL2+FL3, FL2<FL3 which are longer than broad. Eyes with inner margins subparallelled. Supraclypeal area slightly flat rather convex, shiny, with small PP. Face above antennal fossae with fine longitudinal rugulae and weak interrupal PP, shagreening. Facial quadrangle broader than long. Clypeus flattened, weakly tessellate rather smooth, punctate with dense, obscure PP, with longitudinal median impunctate space. The process of labrum broad, trapezoidal, not emarginated apically. Lowerparaocular area punctate with dense, shiny with min PP. Malar space linear, broad. Genal area as broad as eye, weakly tessellate and shiny with min PP. Mesosoma: Pronotum with lateral suture and without humeral angle, densely tessellate, shiny with min, fine, sparse PP. Metoscutum densely tessellate, punctate, shiny with fine, obscure, small PP. Scutellum weakly tessellate, punctate, shiny with dense, fine, small PP. Propodeal enclosure triangular, weakly indicated, rugulose basally, tessellate apically; dorsal face of propodeum roughened with fine, small PP. Mesepisternum densely tessellate, with fine, small PP. Vein 1°-m-cu meeting sec submarginal cell at the middle of the cell. Metasoma: Metasoma condensed oval, metasomal terga densely tessellate, punctate, nearly shiny, T1,4 with finely dense,
small PP; posterior depressions of terga weakly indicated. S2-5 strongly tessellate and shiny with dense, min PP.

Male genitalia and male sternitesstrctreis are shown in Fig. 3-4. Specimens examined. [Egypt] 1 female, Suez, Amer Village., 18. iii. 2014 (M. Shebl); 1 female, Ismailia, Suez Canal University Campus., 17. i. 2018 (M. Shebl); 1 male, Ismailia, Suez Canal University Campus., 17. i. 2018 (M. Shebl); 2 males, Ismailia, Suez Canal University Campus., 14. i. 2014 (M. Shebl); 3 males, Ismaila, Masaid, El KantraGharb, 3.ii.2018.

Distribution. N Africa, Europe and Asia

Remarks: Add here morphological comparison of this species as you have re-described this species. Please compare your re-description with previous descriptions mentioning your additions and previous shortcomings of description.

Molecular Analysis

The molecular analysis of Andrena comprises nine individuals representing three different locations from Egypt (three individual per each region) as shown in (Fig. 1), based on differences between COI mtDNA sequences in A. ovatula from Egypt. Population from Minoufia (seq1, seq2, and seq3), Ismailia (seq4, seq5 and seq6) and Alexandria (seq7, seq8 and seq9) generated 20 parsimony substitutions. DNA sequences initially produced 850 bp of mitochondrial COI. Overall aligned sequences which were used for the analysis totaled 590 bp based on the shortest and clear one. The number of sequences were nine with 590 bp in length; total number of sites (excluding sites with gaps/missing data) were 590 out of which 570 were invariant (monomorphic) sites.

The variable (polymorphic) sites were 20; out of the five were singleton variable sites at (site positions): 135, 185, 222, 326 and 438 and 14 parsimony informative sites. Parsimony informative sites (two variants): 15 at site positions: 33, 99, 138, 315, 339, 378, 384, 393, 426, 459, 519, 523, 531 and 537 beside parsimony informative sites (three variants) at site positions: 108. However, there are no variable sites with four variants Fig. (5) and Table (1).

Based on nucleotide diversity seq1 differ from seq2 and 3 with 3 nucleotides (Minoufia population). Seq4 differ from seq5 and 6 (A. ovatula) with 6 nucleotides (Imsailia population). Seq7 differ from seq8 and 9 with 4 nucleotides (Alexandria population) as shown in Table (1). This shows some differences among the species or ecotype of A. ovatula from each population and population from Ismailia (seq4-, seq5 and 6) showed high nucleotide variation than other populations as appears from phylogenetic tree Figs. (6) and (7). From aforementioned information beside phylogenetic tree it clear that the population (seq4-, seq5 and seq6) from Ismailia seems to be highly diverged and isolated than other populations from Alexandria and Minoufia because of barrier and long distance between those areas and Ismaila (Fig. 6 and 7).

Number of Haplotypes, h: 9 and haplotype (gene) diversity, Hd: 1.000. The variance of Haplotype diversity was 0.003. The standard deviation of haplotype diversity was 0.052. Moreover, nucleotide diversity, Pi (π) was 0.015 and theta (θ) (per site) was 0.013. An average number of nucleotide differences, k: 8.78.

The amount of phylogenetically informative characters of the sequenced part of COI was noticeably very low (5.7 %) for Andrena.

Comparing the genomic sequences of A. ovatula using BLAST revealed that some species are matching with A. intermedia, the matching percentage is ranging from 99.49 to 100 % in the population collected from Alexandria and Minoufia while the Ismailia population is ranging from 99.2 to 99.8 % (Table 2).

Discussion

Taeniandrena Hedicke, 1933 is a primarily Palaearctic subgenus including 11 species from Europe, two species from North Africa, five species from Central Asia and 8 species from East Asia (Tadauchi and Xu, 2003). Almost all species resemble each other and very hard to differentiate morphologically. In addition, the subgenus has taxonomic impediment with some close species such as A. ovatula, A. wilkella, A. intermedia. etc. A. ovatula is one of the most
common species which have a wide range of distribution in Asia, Europe and Africa. Several subspecies were recognized in *A. o. ovatula* and *A. o. heliopolis* (Egypt), *A. o. poupilleri* (Libya and Crete) and *A. o. transcaspiensis* (Oystejnjuk, Grace, 2010; Shebl, 2010; Shebl et al., 2013).

Andrenidae has been a challenging group in view of phylogeny, the known subfamilies has wide-ranging from two to five subfamilies (Almeida, 2008). The results related to molecular analysis showed that (1) *Andrena* from Ismailia (Eastern part of Egypt), contain six highly divergent mitochondrial lineages. The difference in the nucleotide sequence occurred naturally due to various causes of intrinsic and extrinsic variations. These variations called mutations or reshuffling of existing genes during sexual reproduction (Gregory, 2009) resulting in changes in the genome and consequently the formation of new species or sub-species. The different sequences between selected *A. ovatula* are only 3, 6 and 4 for populations from Minoufia (Nile Delta), Ismailia (Eastern part of Egypt), and Alexandria (Western part of Egypt), respectively. Moreover, some genetic variation of any species displays undergoing genetic variation, a process which can be driven by ecological, evolutionary or historical factors (Oliveira et al., 2004). Also, our results suggest that *A. intermedia* is newly recorded from Egypt around river Nile and western part as shown in Table (2). Although some bee fauna were studied extensively morphologically in some regions but the discovery of new species and new records of bees is still running in the Palaearctic region unclear (Shebl et al., 2016; Engel et al., 2017; Shebl et al., 2018).

We recommend further genomic and proteomic studies to support the results of this study and to confirm the status of *A. intermedia* and *A. ovatula*. This information indicates that COI will provide more help to unify the above concept. A further study incorporating more genetically markers is needed to substantiate the findings of this research work. This study will contribute to increase the awareness, importance and effectiveness of using DNA barcodes in species level identifications of Egyptian bees. This protocol has been used successfully in several cases of bees and revealed some cryptic species of some bee groups (Carman and Packer, 1996; Pederson, 1996; Danforth et al., 1998; Zayed and Packer, 2007; Rehan and Richards, 2008; Schaefer and Renner, 2008; Sheffield et al., 2009).

Here, with our recent finding, probably the species of *A. ovatula* identified morphologically has some species complex and more species of the same subgenus could be discovered. So, with more sampling efforts from several countries the speciation and subspeciation of this group might be clarified.

### Conclusion

The Middle East, including North Africa and Saudi Arabia, is a very rich area with bee fauna. Although many species of Andrenids are known in Egypt as well as in North Africa and Saudi Arabia, but still there is limited data and lack of information about Andrenidae family.
Table 2: Identity of the *Andrena* species form Egypt, checked with the bold engine using BALST

<table>
<thead>
<tr>
<th>Sequence matching percentage</th>
<th><em>A. intermedia</em></th>
<th><em>A. ovata</em></th>
<th><em>A. wilkella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Minoufia</td>
<td>99.83%</td>
<td>96.92%</td>
<td>97.08%</td>
</tr>
<tr>
<td>Minoufia</td>
<td>99.83%</td>
<td>97.00%</td>
<td>96.9%</td>
</tr>
<tr>
<td>Ismailia</td>
<td>100%</td>
<td>96.92%</td>
<td>97.08%</td>
</tr>
<tr>
<td>Ismailia</td>
<td>97.44%</td>
<td>98.8%</td>
<td>95.21%</td>
</tr>
<tr>
<td>Ismailia</td>
<td>97.78%</td>
<td>99.15%</td>
<td>95.9%</td>
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<tr>
<td>Ismailia</td>
<td>97.95%</td>
<td>98.97%</td>
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<tr>
<td>Alexandria</td>
<td>100%</td>
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<td>Alexandria</td>
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<tr>
<td>Alexandria</td>
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Therefore, more sampling efforts might help to clarify the status of *A. ovata* complexity. Based on the results presented in this study, *A. intermedia* is a newly recorded bee species in Egypt based on COI which was identified previously as *A. ovata* based on morphological characters. So this research will encourage some similar studies on bees in Egypt and other countries such as Saudi Arabia.

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References


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