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DIAGNOSTICS OF THE TWO SPECIES OF *AMMOPHILA* KIRBY FROM UZBEKISTAN

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ABSTRACT

This study taxonomically analyses the two spieces of ground digger wasps of the subfamily *Ammophilinae* and family Sphecidae of hymenoptera viz., Ammophila heydeni Dahlbom and *A. sabulosa* (Linnaeus) occurring in the Namangan region of Uzbekistan and provides a redescription. Molecular analysis of MtCo1 of these two species are also provided comparing of these and submitting accessiars in the NCBI Gen Bank.

Key words: Hymenoptera, Apocrita, Apoidea, Sphecidae, *Ammophila*, genus, species

The family of ground digger wasps, Apoidae and Sphecidae are distributed throughout the world. The family Apoidae currently includes 4 families, 276 genera, 9889 species. Of these there are 4 families, 101 genera, 2302 species in the Palearctic; and 3 families, 87 genera, 684 species in Russia (Belokobyls, 2017). Sphecidae is distributed mainly in arid, semiarid and tropical regions with 19 genera and 779 species, of these 13 genera and 260 species are in the Palearctic; and 11 genera and 68 species in Russia (Belokobyls, 2017). Ground digger wasps of the genus *Ammophila* Kirby 1798 live in open sunny areas with sparse vegetation. *Ammophila* is the most diverse genus of the tribe Ammophilini and 240 species have so far been identified (Pulawski, 2020). In his two recent revisions, Dollfuss (2013; 2015) listed 87 species for the Palearctic region and India, and 47 species for sub-Saharan Africa. To these, eight species have been added later (Danilov, 2015; 2018; Wang et al., 2017).

Wasps of the genus *Ammophila* have a large elongate body. Its species are most frequently encountered in open sunlit areas with sparse vegetation. Females dig holes in loose, usually sandy, ground, where they store lepidopteran or hymenopteran larvae (Lomholdt 1984, Bohart and Menke, 1976). Over the past decade, DNA-based identification has been adopted as a key tool for characterizing biological specimens (Hebert et al., 2016 a, b). To compare species composition among sites, to describe community organization, or to access previous knowledge related to the taxa encountered, specimens must first be identified. A quick and efficient approach is to cluster specimens into molecular operational taxonomic units or MOTUs (Blaxter et al., 2005). Indeed, the clustering of sequences combined with an interim taxonomy enables efficient characterization of biodiversity (Smith et al., 2013) and of species interactions (Clare et al., 2019). Yet, full realization of the value of such data relies on connecting as many MOTUs as possible to Linnaean taxonomy, because this makes it possible to connect species detected in DNA-based surveys to prior biological knowledge. Thus, the most efficient avenue for combining molecular data with taxon-specific knowledge involves populating reference databases with DNA barcodes annotated with Linnean taxonomy (Hebert et al., 2003). This study attempts in few species of *Ammophila* from Uzbekistan.

MATERIALS AND METHODS

During 2019-2022, materials were collected from the mountainous, foothill and plain regions of the Ferghana Valley. Wasps were collected using soil traps of Malaise, Merike, and Barber. Malaise trap used is three H-shaped interlocking plates and covers flush with the soil surface. In addition, entomological meshes of different sizes were also used (Pravdin, 1978). The net was swept over the surface of grasses, young shrubs and trees (with a quantitative score of 50 or 100) (Vinokurov, 2015). The collected material was preserved in 70% ethanol.The terminology for species' description follows Bohart and Menke, 1976, except the terms mesosoma and metasoma, which are used for the thorax including the propodeum (true abdominal segment I) and the

definitive abdomen (true abdominal segments II–X), respectively. To isolate DNA specimens were preserved in a 70% alcohol and males *A. heydeni* and *A. sabulosa*, were then dried over paper. DNA was extracted from the legs and stored at -20°C (ISSA, 2013). The PCR of these was performed using an automatic programmable cycler (PR-96E) using primers LEP-F-forward, 5-ATTCAACCAATCATAAAGATAT-3 and LEP-R-reverse 5-TAAACTTCTGGATGTCCAAAAA-3, following standard procedure (Paul, 2014). To determine the length of fragments, bacteriophage λ DNA hydrolyzed with PstI endonuclease and a special marker Ladder 3-1 from Axigen were used as a marker. Analysis of the obtained nucleotide sequence was carried out using computer programs Bioedit, Clustal W and DNAstarTM.

RESULTS AND DISCUSSION

Two species *Ammophila, A. heydeni* and *A. sabulosa* were identified using morphological (Fig. 1, 2).

Ammophila heydeni (Dahlbom 1845) (Fig. 1)

Material examined: Baimoq village of Chust district of Namangan region N 41.000204° E 71.234200°. 3♂, 1♀, collected from camel thorn (*Alhagi maurorum*).

Description: Female with clypeus straight and convex. Central anterior part of clypeus nearly straight between lateral angles or denticles; dotted lines small and sparse. The mesh microsculpture not clearly visible. Hair growth poorly developed and does not cover body. Feather covering poorly developed and does not extend over body. Forehead deeply sunken with a furrow in the middle. Eyes located almost parallel or slightly closer to the bottom; distance between hind eyes almost 1.5 less than the distance between one hind eye and the next eye. Tergium not long, twice as wide as long, with small lines and wrinkles on its surface. Middle tergite flat, transversely wrinkled, with small punctures; middle furrow appears weak or absent. Shield and posterior shield have thick longitudinal wrinkles **(**Kazenas, 1998**)**.

Male with clypeus slightly elevated, with dense silvery hairs and sparse pale hairs. There is a small hole in the middle of the front. The underside of the eyes are close together. The distance between the posterior eyes is almost 1.1 times less than the distance between one posterior and the next eye. Tergites have large dense punctures and hairs. There is no hole in the middle. The middle part of the chest is roughly pointed, with straight hairs. There are longitudinal wrinkles on the scutellum and posterior scutellum. The middle part of the intermediate segment has an uneven structure and short, semi-connected hairs. There are long feathers, the sides are shiny. The shoulder pads have thick hair. The length of first segment of connecting joint (wasp waist) is almost equal to length of the 1st and 2nd joints of hind leg. Head and chest are black, lower jaw and whiskers black. Loin is anterior and posterior (outside, the base black), the fore and middle calves, the base of the fore and middle paws are reddish-red, body length is 16–22 mm **(**Kazenas, 1998**)**.

Distribution: Russia: European part (Central, South

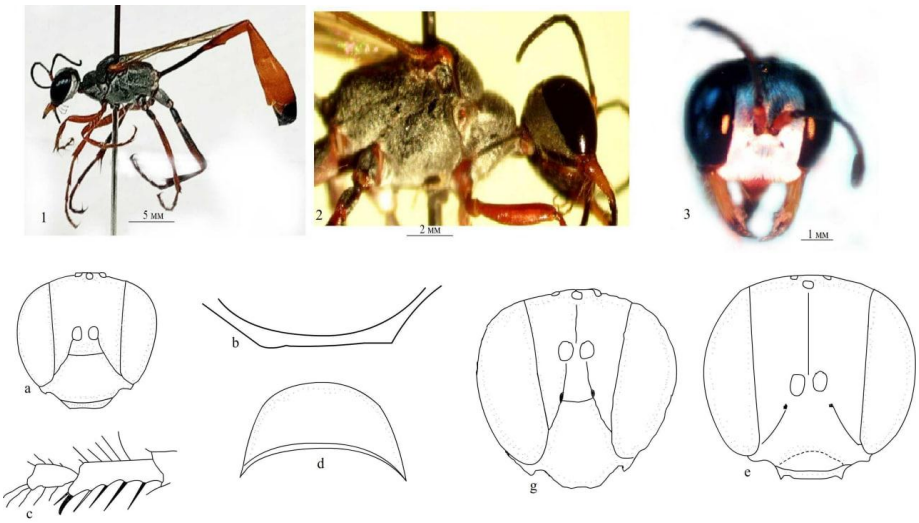

Fig. 1. *Ammophila heydeni*

1- general view 2- head-thoracic section 3- head front view. a, e - front edge of clypeus; b, g - head in front; c, e - pronotum from above; y-part of front wing.

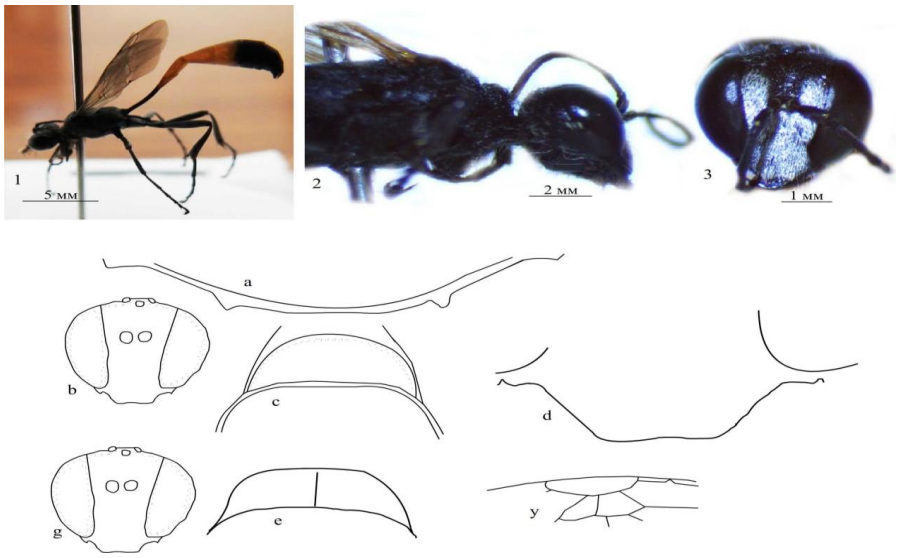

Fig. 2. *Ammophila sabulosa*
1- general view 2- head-thoracic section 3- head front view. a, e - front edge of clypeus; b, g - head in front; c, e - pronotum from above; y-part of front wing.

East, North Caucasus, Republic of Crimea), Ural region, Western Siberia (Omsk region, Novosibirsk region, Altai Territory), Europe (Western Europe, Northern Europe, Southern Europe). (Eastern Europe), North Africa, Armenia, Turkey, Syria, Jordan, Israel, Iran, Afghanistan, Central Asia, Kazakhstan, Mongolia, China (Belokobyls, 2017).

Remarks: Desert-steppe eurybiont species. It lives in all types of deserts, semi-deserts and steppes. Sometimes found in river valleys, but avoids dense thickets. Females build unicellular nests in the ground and feed these with several Geometridae or Noctuidae larvae in each cell (Kazenas, 1998).

Ammophila sabulosa (Linnaeus, 1758) (Fig. 2)

Material examined: Mirzaakhmedov village, Ulugnor district, Andijan region (N 40.738053° E 71.633611°, 4♂, 2♀, 4♂, 2♀), collected from camel thorn (*Alhagi maurorum*) 15.05.2021.

Description: Female with clypeus flat-convex, with a sharp edge on the sides. Eyes almost parallel inside and with very few dots and lines on the clypeus. Hairline on the top of head poorly developed. Distance between posterior eyes almost 1.9x less than the distance between one posterior and the next eye. Width of anterior scapula twice the length and no fossa in the center. Middle part of the chest has uneven fine lines. There are almost no wrinkles along the length, but on sides they can look blurry. Anterior part of scutellum has longitudinal wrinkles, relatively smooth and has several punctures. Center of the intermediate segment has a wrinkled structure, the microsculpture uneven, and there are long light hairs over the entire surface. On the chest, shoulders, sides of middle part of chest, sides of the intersegmental segment with dense silvery hairs (Kazenas, 1998).

Male with anterior part of clypeus almost free or slightly furrowed. Clypeus with dense setae and long pale hairs. Underside of eyes close together distance between posterior eyes almost 1.5x less than distance between one eye and the other eye closest to it. Length of the anterior part of chest is 2x shorter than width with a longitudinal fossa in the middle. On the middle part of the chest there are uneven large stripes (rarely in middle part) and thick micropunctures, mainly on the sides in the form of marginal and submarginal cells (Kazenas, 1998).

Distribution: Russia: European part, Ural region, Western Siberia, Eastern Siberia, Far East, Kamchatka region, Magadan region, Europe (Western Europe, Northern Europe, Southern Europe, Eastern Europe), North Africa, Turkey, Syria, Iran, Central Asia, Kazakhstan, Mongolia, China (North) (Belokobyls, 2017).

Remarks: Eurybiont species. It occurs in the mountains and on the plains in the area from forest to desert. Avoids sandy areas. Prefers river valleys, oases and mountain gorges in desert areas, open

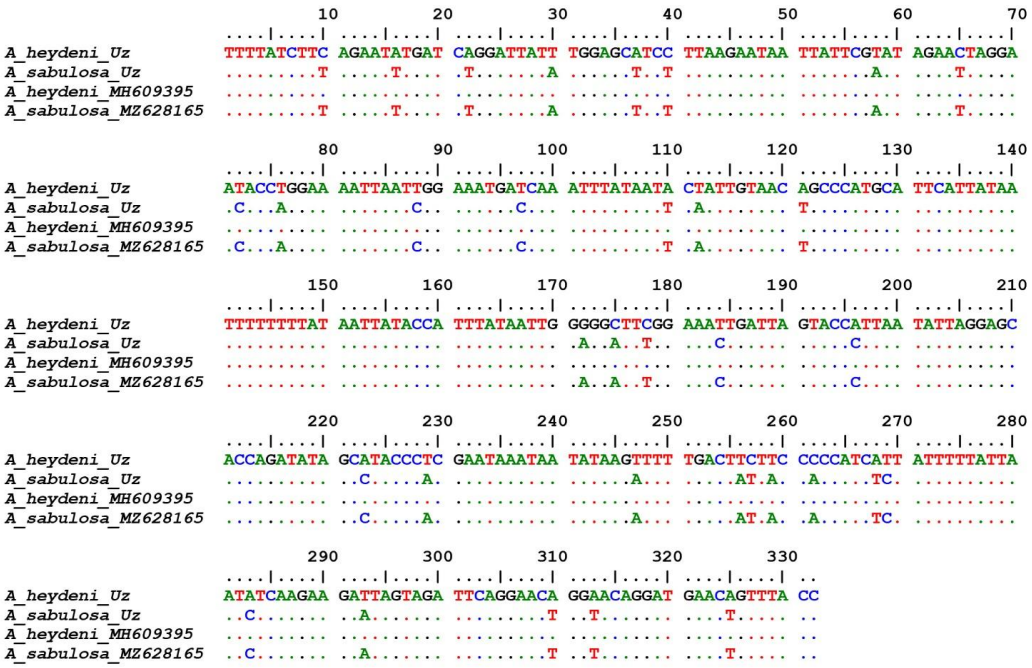

Fig. 3. Comparison of the nucleotide sequence of the mtDNA COI region of *A. heydeni and A. sabulosa* species *Ammophila* W. Kirby, 1798 based on sequencing material.

places in the forest zone. Females themselves build single-celled nests in solid soil. Butterfly caterpillars (Lepidoptera: Noctuidae, Geometridae, Limantriidae, Pieridae, Notodontidae, etc.) are prey. Builds a nest before hunting. One or two worms are placed in each nest. Adults feed on plant nectar. Found in settlements and other anthropogenic biotopes.

For molecular genetic studies, DNA was isolated from the legs and whiskers of males. The results revealed from nucleotides with 302 base pairs mtCO1 of *A. heydeni* and *A. sabulosa*.

These were *A. heydeni* (accession number: MN609395) and *A. sabulosa* (introductory number: MZ628165) submitted to NCBS (https://blast.ncbi.nlm.nih.gov). There was an exchange of 34 nucleotides between the nucleotides of *A. heydeni* and *A. sabulosa* (Fig. 3).

No differences were found between *A. heydeni* and *A. sabulosa* nucleotides from the National Center for Biotechnology Information. 11.2% nucleotide differences in the COI mRNA branch of *A. heydeni* and *A. sabulosa* species studied from a molecular genetic point of view were revealed.

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AUTHOR CONTRIBUTION STATEMENT

Kadirov T ilhomjon and Akhmedova Y U Zukhra collected entomological samples, morphological identification and identification of species, Khudoyberdieva O Marifat and Amirov O Oybek molecular genetic research.

CONFLICT OF INTEREST

The study was carried out with the consent of the authors and they have agreed to publish this article in the Indian Journal of Entomology.

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