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Mining bee *Andrena* (*Agandrena*) *agilissima* (Hymenoptera: Andrenidae): A new record from India with morphological and molecular notes

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Abstract: The mining bee *Andrena agilissima* (Scopoli, 1770), is recorded for the first time in India from the western agro-climatic zone of its Punjab state. This is the first account of morphological and molecular characteristics of *A. agilissima.* This new record now increases the number of mining bees known in India to 21. Taxonomic comments and metric values of 40 morphological characters have been presented. The mean values for body length, head width, compound eye length, median ocellus diameter, forewing length and hamuli number were 14.04±0.04 mm, 4.26±0.003 mm, 2.327±0.008 mm, 0.255±0.005 mm, 12.75±0.022 mm and 17.00±0.00, respectively. Using the standard barcoding protocols, cytochrome *c* oxidase subunit 1 marker (standard DNA barcode region) based 658 bp DNA barcode sequence of the species has been established, as a first step towards the DNA barcode library of solitary bees of Punjab. The barcode sequence generated for the species has been registered by Gen-Bank, National Centre for Biotechnology Information (NCBI) under accession 'KT960836' and Barcode of Life Data (BOLD) Systems under Barcode Index Number 'BOLD:AAY6909'. The floral sources for *A. agilissima* in Punjab are also provided. The results can be used to further study the races/ecotypes in different parts of country, habitat management studies, plant-pollinator interactions and in conservation programmes for the species. Further, the precise identification of *A. agilissima* and the inventory of its foraging plants would provide new opportunities for its potential use as pollinator of crops.

Keywords: Andrenidae, *Andrena agilissima,* DNA barcoding, Morpho-taxonomy, New species record

INTRODUCTION

The genus *Andrena* Fabricius (Andreninae: Andrenini) comprises of 1,526 described species worldwide (Ascher and Pickering, 2016) and is found throughout holarctic region, in south of Western Hemisphere upto Panama, where one species occurs in tropical lowlands; in Africa through the Eastern African highlands and south to the Cape of Good Hope, and in Asia to the mountains of southern India and of Malaysia (Michener, 2007). It was the fourth genus of bees to be proposed after *Apis* Linnaeus, 1758; *Eucera* Scopoli, 1770 and *Nomada* Scopoli, 1770. Currently 2,955 species in 49 genera of andrenidae are known in the world, but from India, only a single genus, *Andrena* has been reported so far (Ascher and Pickering, 2016). Most *Andrena* bees are solitary and a few are communal as *A. agilissima*. The *Andrena* bees are commonly known as sand or mining bees owing to their behaviour of building nests in soil preferably light sandy soils. There are 104 subgenera recognized under genus *Andrena* (Ascher and Pickering, 2016). The diversity of *Andrena* bees is relatively low in India with only 20 species (Ascher and Pickering, 2016) when compared to USA (1206), Mexico (526), Turkey (348) and Spain (219) and other parts of the world. The 20 species of *Andrena* in India included *Andrena aegyptiaca* Friese, 1899; *Andrena anonyma* Cameron, 1897; *Andrena arima* Cameron, 1909; *Andrena bellidoides* LaBerge, 1968; *Andrena burkelli* Bingham, 1908; *Andrena cineraria* (Linnaeus, 1758); *Andrena communis* Smith, 1879; *Andrena cussariensis* Morawitz, 1886; *Andrena flavipes* Panzer, 1799; *Andrena floridula* Smith, 1878; *Andrena fuscosa* Erichson, 1835; *Andrena gracillima* Cameron, 1897; *Andrena leaena* Cameron, 1907; *Andrena mephistophelica* Cameron, 1897; *Andrena morose* Cameron, 1897; *Andrena patella* Nurse, 1903; *Andrena pilipes* Fabricius, 1781 ; *Andrena rothneyi* Cameron, 1897; *Andrena rupshuensis* Cockerell, 1911 and *Andrena savignyi* Spinola, 1838.

The present information on diversity, biology, colony organization, nesting characteristics and foraging plants of *Andrena* bees in India and the Punjab in particular, is inadequate. The present investigations were thus aimed at establishing precise morphological and molecular identification of *A. agilissima* as a first step towards an inventory of *Andrena* bees of Punjab and for their potential use as pollen vector of food crops in a planned manner.

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MATERIALS AND METHODS

Specimen collection: Specimens examined in the study were collected during the day time, while sweeping the flowers of *Brassica napus* L. and *Brassica juncea* (L.) Czern.in western agro-climatic zone (30° 25'6.9"N and 74°36'1.8"E) of Punjab, India at 275 a.s.l. during spring of 2014.

Morphometry: The measurements were made with image acquisition programmed zoom-stereo microscope (Olympus Cell^A Imaging Solutions for Life Science Microscopy). Five worker bees were used for recording the morpho-taxonomic data. Terminology and measurements here follow those of Michener (2007) and Ruttner (1988). The indices and their abbreviations used are as per given.

1) body length (BL), 2) head/ face length (HdL), 3) head width (HdW), 4) thorax length (ThL), 5) thorax width (ThW), 6) abdomen length (AbL), 7) abdomen width (AbW), 8) clypeus length (CL), 9) clypeus width (CW), 10) lower inter-orbital distance (LIOrD), 11) upper inter-orbital distance (UIOrD), 12) inter-orbital distance through antennal sockets (IOrD*as*), 13) clypeoantennal distance (CAD), 14) compound eye length (CEL), 15) compound eye width (CEW), 16) distance between antennal sockets (DbAS), 17) interocellar distance (IOD), 18) ocellocular distance (OOcuD), 19) antennocellar distance (AOD), 20) antennocular distance (AOcuD), 21) clypeocular distance (COcuD), 22) median ocellus diameter (MOD), 23) labrum length (LL), 24) labrum width (LW), 25) antennal socket maximum diameter (ASD), 26) scape length (SL), 27) scape diameter (SD), 28) pedicel length (PdL), 29) flagellum length (FgL), 30) 3rd flagellomere diameter (3FgmD), 31) forewing length (FwL), 32) forewing width (FwW), 33) hindwing length (HwL), 34) hindwing width (HwW), 35) jugovannal index (JVI), 36) hamuli number (HN), 37) hind tibia length (HTL), 38) hind basitarsus length (HbtL), 39) hind basitarsus width (HbtW) and 40) number of flagellomeres (FgmN). The observations pertaining to the bilateral body-parts such as eyes, antennae, legs and wings were taken on right side body part. The data on metric values is presented as Mean ± $S.E._{m}$.

DNA extraction and PCR reaction: The bee specimens were preserved in a DNA-friendly fashion by immersion in 100 percent ethanol and kept at -20°C in vertical deep freezer till DNA was isolated. DNA extraction was done using previously standardized CTAB method (Cubero *et al* 1999). CTAB was two per cent solution of cetyltrimethyl ammonium bromide (CTAB) in 100 mMTris.Cl (pH= 8.0), which additionally contained 20 mM of Na_2EDTA (pH=8.0) and 1.4 M NaCl. DNA isolation was carried using hind legs tissue of the bee. The primer pair, Forward - 5'ATTCAACCAATCATAAAGATATTGG3' and Reverse - 5'TAAACTTCTGGATGTCC AAAAAATCA3' were used to amplify 658 bp fragment of COI gene. All PCR amplifications were accomplished in a programmable DNA thermolcycler (Mastercycler Gradient - Eppendorf™) using the following PCR programme: Step 1: Initial denaturation at 94°C for 5 minutes (one cycle); Step 2: Initial denaturation at 94°C for 1 minute; Step 3: Primer annealing at 55 °C for 1 minute; Step 4: Primer extension at 72 °C for 2 minutes; Step 5: Repeated step 2 to 4 (35 cycles); Step 6: Final extension at 72 °C for 5 minutes and storing the PCR product at 4 °C. Each PCR product was subsequently gel purified. The purified DNA fragments were ligated into a 'PCR product cloning plasmid vector pTZ57R/T (Fermentas Life Sciences, USA)'. The ligation reaction product was transformed into *Escherichia coli* DH5-alpha host cells using 'InsTAClone™ PCR cloning kit (M/s Fermentas Life Sciences)' using manufacturer's protocol followed by custom sequencing from Xcelris Labs, Ahmedabad. The natural orientation of sequence was determined by aligning of sequence with the reported sequences (in GenBank database, www.ncbi.nlm.nih.gov/ pubmed/) for the species using 'Gene align function' of the DNA software program 'CLC Free Workbench ver 7.5. of CLC Bio A/S'.

RESULTS AND DISCUSSION

The new record of *A. agilissima* increases the number of mining bees known in India now to 21.

Systematics of species:

Family: Andrenidae Latreille, 1802

Subfamily: Andreninae Latreille, 1802

Genus: *Andrena* Fabricius

Subgenus: *Agandrena* Warncke

Andrena (Aganderna) agilissima (Scopoli, 1770)

Material examined: India (5 workers): 3 ♀♀, Muktsar, in western agroclimatic zone of Punjab, from *B. napus*, 25.ii.2014, 30°22'55" N and 74°38'19.9" E, 275 m a.s.l., coll.G.S. Makkar; 2♀♀, Muktsar, in western agroclimatic zone of Punjab, from *B. juncea*, 11.ii.2014, 30°25'6.9"N and 74°36'1.8"E;, 275 m a.s.l., coll.G.S. Makkar. Vouchers are deposited in the collection of Punjab Agricultural University Insect Museum and National Pusa collection (NPC), Indian Agricultural Research Institute (IARI), New Delhi. The identity of the species was confirmed by Dr Debjani Dey, Incharge of Insect Identification Service, NPC, IARI, New Delhi, India.

New record: India: Bhullar and Doda in Muktsar at 30°28′30″N 74°30′54″E, falling in western agroclimatic zone of Punjab.

Diagnosis: Workers of this species are conspicuous amongst other species of *Andrena* by several distinctive characters (Fig. 1-8) including shiny black body with tufts of velvety hairs on the facial fovea, on either side of thorax, on last abdominal tergite and on the femora of the third pair of legs. The wings have bluish reflections. Dimensions (Table 1): BL = 14.04±0.04 mm, HdL = 2.953±0.001 mm, HdW = 4.260±0.003,

Table 1. Measurements (mm) of workers of *Andrena agilissima* collected in India.

S.No.	Character measurement	*Mean ± S.E.$_m$
1	Body length	14.041±0.040
2	Head/Face length	2.953±0.001
3	Head width	4.260±0.003
4	Thorax length	4.990±0.004
5	Thorax width	3.292±0.004
6	Abdomen length	7.806±0.003
7	Abdomen width	4.286±0.003
8	Clypeus length (antero-posterior)	1.388±0.005
9	Clypeus width (maximum)	2.375±0.005
10	Lower inter-orbital distance	2.622±0.003
11	Upper inter-orbital distance	2.728±0.004
12	Inter-orbital distance through antennal sockets	2.867±0.022
13	Clypeoantennal distance	0.056±0.002
14	Compound eye length	2.327±0.008
15	Compound eye width	0.756±0.003
16	Distance between antennal sockets	0.497±0.004
17	Interocellar distance	0.383±0.002
18	Ocellocular distance	0.897±0.005
19	Antennocellar distance	0.779±0.002
20	Antennocular distance	0.880±0.002
21	Clypeocular distance	0.123±0.001
22	Median ocellus diameter	0.255±0.005
23	Labrum length	0.145±0.002
24	Labrum width	0.304±0.002
25	Antennal sockets maximum diameter	0.369±0.002
26	Scape length (Rt.)	1.061±0.009
27	Scape diameter (Rt.)	0.244±0.003
28	Pedicel length (Rt.)	0.199±0.004
29	Flagellum length (Rt.)	3.735±0.014
30	3rd flagellomere diameter	0.228±0.005
31	Forewing length (Rt.)	12.747±0.022
32	Forewing width (Rt.)	3.715±0.006
33	Hindwing length (Rt.)	9.362±0.019
34	Hindwing width (Rt.)	2.693±0.009
35	Jugovannal Index	65.609±0.110
36	Hamuli number	17.00±0.000
37	Hind-tibia length (Rt.)	3.700±0.007
38	Hind-basitarsus length (Rt.)	2.269±0.005
39	Hind-basitarsus width (Rt.)	0.383±0.002
40	Number of flagellomeres	10.000±0.000

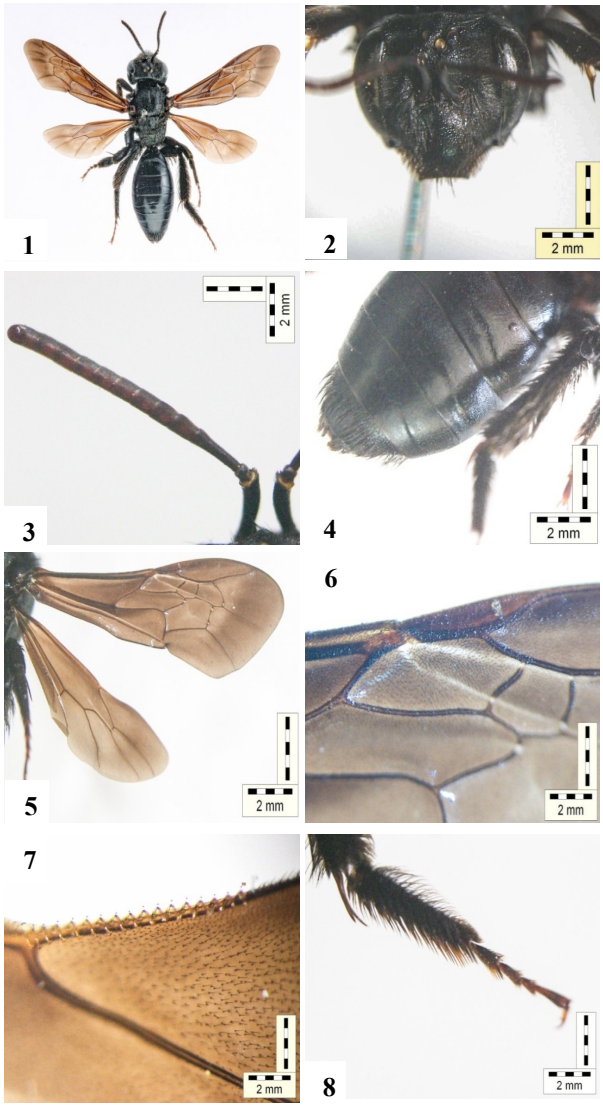

Fig. 1-8. *Worker of Andrena agilissima (Scopoli 1770). 1. Mounted view. 2. Frontal view of head. 3. Antennal scape, pedicel and flagellomeres. 4. Tufts of velvety hairs on last abdominal tergites. 5. Fore and hind wing. 6. Stigma and prestigma. 7. Hamuli of hindwing. 8. Velvety hairs on femora of hind legs.*

CEL = 2.327±0.008, CEW = 0.756, MOD = 0.255±0.005, 3FgmD = 0.228±0.005, FwL = 12.75±0.022 and FwW = 3.715±0.006 mm. The other important measurements (means in mm) of the species were LIOrd = 2.62, IOrD*as* = 2.87, IOD = 0.38 and OOcuD = 0.89. The hamuli number in the species was 17. Hind tibia 1.63x longer than hind basitarsus, hind basitarsus 5.92x longer than wide, compound eye 3.078x longer than wider, abdomen 1.005x longer than head and thorax combined, stigma broader than prestigma (measured to wing margin). Dehon *et al.* (2014) studied morphological characterisitics of a male andrenid bee, *Andrena antoinei* Michez & De Meulemeester sp. nov. and reported head 2.56 mm long, 2.30 mm wide; compound eyes 1.62 mm long, 0.53 mm wide; scape 0.4 mm long; pedicel 0.27 mm long; Forewing 5.94 mm long, 1.53 mm wide.

Male: Known from other parts of world but not yet recorded from India**.**

Geographical distribution: The species was previously known from most of Europe, in the Near East and in North Africa with around 332 specimen records vide bee specimen record database of American Museum of Natural History (Anonymous, 2015). The activity period of the species in palearctic region extends from April to July. In West Mediterranean region, the species is present in Slovenia in the Soča valley, near Strunjan (sub-Mediterranean region) and Maribor (sub-Pannonian re-

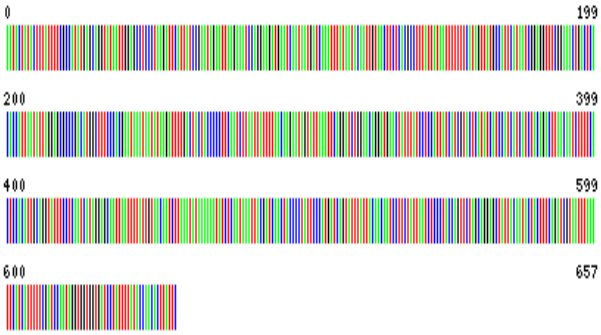

Fig. 9. *Illustrative DNA barcode of Andrena agilissima based on COI gene based sequence.*

gion), with activity period from May to June.

Nesting biology: Excavate the nests in burrows in the ground. This bee is a communal bee where about 5-50 females share a nest entrance. Several females of the same generation share the same nest, dug in sandy soil, on the river banks or on steep walls. Usually the nests have a common entrance, but each individual has its own brood cells with its egg. It is univoltine species.

Floral associations: It is oligolectic bee and thus collect pollen from only a few flowering plants. We collected this species foraging on *B. napus* and *B. juncea* flowers. Females carry pollen on the hind tibia, hind femora, and on the trochanter, that carriy a group of long hairs forming another basket, the floccus. Nectar is transported to the nest internally in the crop, as in other bees. However, Giovanetti *et al.* (2006) reported its feeding only on pollen of a few genera of Cruciferous vegetables (Brassicaceae species, as *Brassica napus*, *Brassica rapa*, *Raphanus raphanistrum*, *Barbarea vulgaris* and *Sinapis* species).

Molecular characterization: DNA barcoding offer a highly precise means of species identification using mitochondrial gene, cytochrome *c* oxidase I (COI) (Hebert *et al*., 2003). We generated 658 bp DNA barcode of *A. Agilissima* by using protocols discussed earlier. The sequence composition was Adenine (222), Guanine (74), Cytocine (136) and Thymine (226). The edited sequence of *T. iridipennis* was put in a BLAST (Basic Local Alignment Search Tool) search to compare our sequence with GenBank database of sequences to identify the database sequences that resemble our sequence. Based on this alignment, *A. helvola*, *A. tibialis, A.nigroaenea and A. nigrospina* showed 89.26, 89.21, 88.94 and 88.94% sequence similarity, respectively to *A. agilissima.* The COI gene based sequence of this species has been made available in GenBank, NCBI (http://www.ncbi.nlm. nih.gov/) under accession 'KT960836' and Barcode of Life Data Systems (BOLD) (http://www.boldsystems.org) under BIN 'BOLD:AAY6909'. The illustrative DNA barcode of the species is presented in Fig. 9.

Conclusion

A. agilissima is morphologically distinguishable from *A. Savignyi* Spinola, another common andrenid species of our region, though both have common host range and activity period in Punjab. However, *A. agilissima* is relatively more aggressive forager of *Brassica* flora. The present state of knowledge of diversity, biology, colony organization, nesting characteristics and foraging plants of andrenid bees of India and the Punjab in particular, is inadequate, and thus, systematic investigations are desired to completely understand all these attributes for realizing higher honey harvests as well as for their commercial utilization as pollen vectors in *Brassica* centred agro-ecosystem. DNA barcoding established mitochondrial COI based sequence database for precise species level identification. Additional collections and investigations are needed to completely understand the diversity, distribution and floral associations of andrenids in Punjab and for their potential utilization for effective crop pollination.

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