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DNA barcoding and morphological characterization of moth *Antoculeora ornatissima* (Walker, 1858) (Lepidoptera: Noctuidae), a new range record from western Himalayan region of India

Twinkle Sinha [1], P.R. Shashank [2] & Pratima Chaudhuri Chattopadhyay [3]

[1,2] Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, India
[3] Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh 201303, India
[1] twinklesinha1901@gmail.com, [2] spathour@gmail.com (corresponding author), [3] pchaudhuri@amity.edu

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Abstract: DNA barcoding of *Antoculeora ornatissima* (Walker, 1858) was done for the first time from India. Redescriptions of genitalia and diagnoses of genus and species are presented with images and illustrations.

Keywords: *Antoculeora ornatissima*, DNA barcoding, Lepidoptera, morphology, new range record, Noctuidae, Plusiinae, western Himalaya.

Noctuidae is one of the largest families of moths with more than 35,000 known species documented in the world. The subfamily Plusiinae (Lepidoptera: Noctuidae) is represented by approximately 500 species worldwide (Ronkay et al. 2008) of which 59 are reported in India (Shashank & Longjam 2014), which were grouped in three tribes, Abrostolini Eichlin & Cunningham, 1978, Argyrogrammatini Eichlin & Cunningham, 1978, and Plusiini Boisduval, 1928. Abrostolini is represented by two species, Argyrogrammatini by 33 species, and Plusiini by 24 species. Under the subfamily Plusiinae, the genus *Antoculeora* is represented by three species, namely, *A. yoshimotoi* (Ronkay, 1997), *A. locuples* (Oberthür, 1880), and *A. ornatissima* (Walker, 1858). Walker (1858) described *Plusia ornatissima* on a single female specimen from the southern Himalayan region (northern Hindustan). Later, *Antoculeora* was erected as a subgenus of *Erythroplusia* Ichinose, 1962 (Ichinose, 1973) and raised to a full genus by Chou & Lu (1979). Further, Oberthür (1880) described *P. locuples* for the island of Askold located in the Bay of Vladivostok, Russia. These two taxa were treated as synonyms by Staudinger & Rebel (1901), whose opinion was accepted by Hampson (1913) and Warren (1913), followed by all subsequent authors till Ronkay (1997). Kitching (1987) expressed his opinion that *A. ornatissima* might be a complex of closely related taxa but may also be a single species. A detailed history of generic classification and species changes was provided by Kitching (1987), Ronkay (1997), and Ronkay et al. (2008).

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Due to large intraspecific variation in this species and to resolve a few ambiguities of the species complex, a need for molecular work was suggested by Ronkay (1997) and Ronkay et al. (2008). This is the first effort to study the occurrence and DNA barcode of *A. ornatissima* from India.

Material and Methods

Sampling

For the present study, 12 specimens were collected from Chamoli and Katrain, two different localities of northern India. The collection of specimens were carried out by hand collection of larvae, which were reared to the adult stage in the Lepidoptera Laboratory, National Pusa Collection, Indian Agricultural Research Institute, New Delhi (NPC-IARI). Light traps using hanging cloth method were also used for collection of adult moths. Further, collected materials were processed by pinning, spreading, proper labelling, and preparation of wings and genitalia slides. All the specimens are preserved in NPC-IARI.

Morphology of adult moths

Genitalia of both male and female specimens were prepared and images were taken with a Leica DFC425C digital camera mounted on a Leica M205FA stereozoom microscope. The terminology used for male and female genitalia follows Klots (1970). Forewing length was measured from the outer edge of the tegula to the outer most edge of the apex.

DNA isolation, PCR amplification, and sequencing

The DNA easy blood and tissue kit (Quiagen GmbH, Germany) method was used to extract DNA from one to three legs of each adult. The DNA extraction method provided by Fukova et al. (2009) was followed. For mitochondrial cytochrome c oxidase subunit I gene analysis was used on the voucher specimens. The genomic DNA was visualized using 0.8% agarose gel and quantified by fluorometer using standard procedures. Depending upon the concentration, the DNA samples were diluted with molecular gradient water to get a working solution of 10–30 ng/µL. A portion of the total DNA was preserved in glycerol (10%) in -80°C for future reference purposes. The universal barcode primer described by Folmer et al. (1994) (LCO-5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'; HCO-5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') specific to mitochondrial cytochrome c oxidase subunit I gene (COI) was used in the present study. The optimized PCR conditions (per 25µL) using Taq DNA polymerase (Fermentas Inc., USA) were 2.5µL of 10 X PCR buffer with 2µL of 25mM $MgCl_2$, 0.5µL of 10mM dNTPs, 0.5µL each of forward and reverse primer, 1U of Taq, and 17µL of UltraPure water (Invitrogen). Thermocycler conditions were as follows: initial denaturation for 5min at 94°C followed by 35 cycles of denaturing for 30s at 94°C, annealing for 40s at 54°C and an extension time of 40s at 72°C, with a final extension for 5min at 72°C. PCR products were visualized on agarose gel after electrophoresis. Single bands were purified using a QIAquick PCR purification kit (Quiagen GmbH, Germany). Purified PCR products were sequenced directly in both directions using an automated sequencer (ABI prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) at SciGenom Lab, Cochin, India. COI sequences in FASTA format were processed and submitted to NCBI for GenBank accessions as per Shashank et al. (2014). Accession numbers for the five specimens are KY886404, KY886405, KY886406, KY886407, and KY886408.

Results

Redescription of *Antoculeora ornatissima* (Walker, 1858)

Systematic accounts

Family: Noctuidae Latreille, 1809

Subfamily: Plusiinae Boisduval, 1828

Genus: *Antoculeora* Ichinose, 1973

Genus: *Antoculeora* Ichinose, 1973

Type species: *Plusia ornatissima*.

The genus *Antoculeora* is characterized by its large size of 30–42 mm, with broader, acutely pointed forewings. Head and collar lateritius. Forewing with metallic sheen present, gamma mark larger and divided into two oval spots. Male genitalia with longer uncus, juxta sclerotised. Asymmetrical valvae with saccular extensions. Female genitalia with longer sacculiform corpus bursae with strongly ribbed appendix bursae. Ovipositor rather medium sized, weakly sclerotised, papillae anales rounded.

***Antoculeora ornatissima* (Walker, 1858)** (Images 1A,B)

Plusia ornatissima Walker, 1858: 1786. Type locality: northern Hindostan.

Cerviplusia wukongensis Chou & Lu, 1974: 73. Type locality: China.

Antoculeora ornatissima Goater, Ronkay & Fibiger, 2003.

Redescription: Forewing length of adults 36–40 mm. Spherical rounded medium-sized compound eyes. Frons and vertex copper-bronze coloured. Frons distinctly exceeding the eyes. Labial palpi upturned, third segment is pointed and small. Second segment of labial palpi is densely covered with reddish scales. Antennae of male and female filiform in shape. Metathoracic tuft

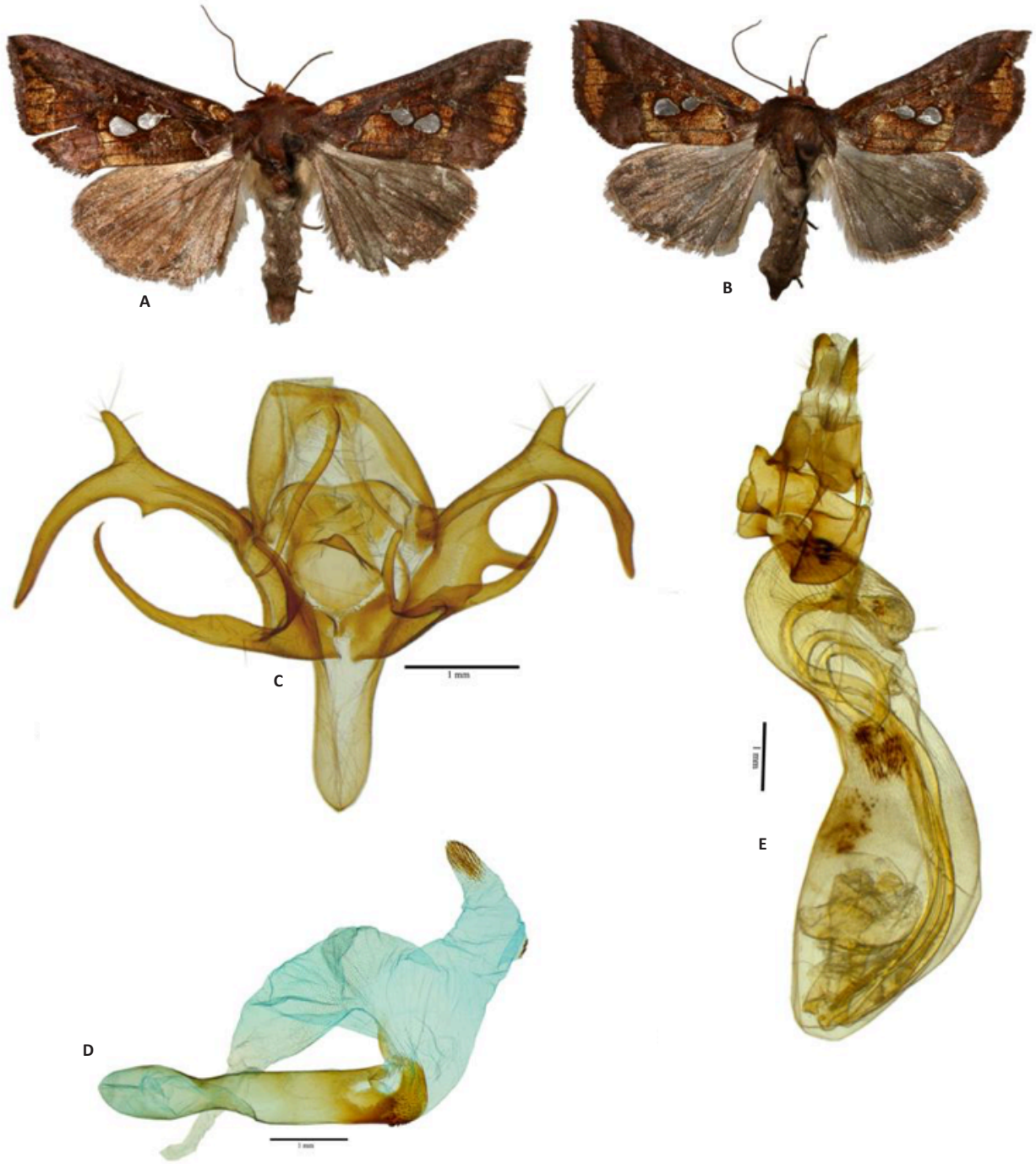

Image 1. *Antoculeora ornatissima* (Walker, 1858): A - adult male, B - adult female, C - male genitalia, D - aedeagus, E - female genitalia

well developed, looks like a pair of horns and generally reddish-orange coloured. Meso- and metathorax densely covered with hairs. Foreleg, midleg, and hindleg are similar in both males and females. Femur and tibia of foreleg covered with brownish-orange hairs. Tarsi full of spines. Two pairs of spurs present on hind leg. Abdomen light brown coloured. Abdominal tuft well developed. Two prominent crest present on abdomen. Anal tuft present. Orange coloured scales present on the back portion of the entire abdomen. Forewing triangular, subfalcate and metallic shimmering present on the termen region and post-medial region. Antemedial line golden coloured and ends at the stigma. Stigma mark is like two large oval spots with silver colour filled into it. Comma-like structure present above the gamma mark, the best identification feature of this species. Hindwing

aeneous brown coloured. Forewing with 12 veins, vein SC and R1 are free. RS2+RS3 joined by a short vein to discal cell. Vein M2 present near the middle of the discal cell. Veins RS1 and RS2 connected with short vein to form an areole. Anal vein separately present. Hindwing with nine veins present. RS1 joined with M1 (RS1+M1). M2, M3, and CuA1 originate from the same base stalk. Two anal veins A1 and A2 freely present.

Male genitalia (Image 1C,D): Male genitalia robust. Valvae with longer projections outwards. Fultura inferior rather low, apical part more or less broadly triangular. Tegumen arms are extremely swollen anteriorly and, in dorsal view, diverge at 180°. The valvae have projections that can interlock itself when valvae are closed. Vinculum U-shaped, clavus longer in shape. Aedeagus with carina terminated in a broadly half-moon-shaped plate covered with short but strong teeth at base (and sometimes in medial third); basal part of vesica with two diverticula bearing bundles of short, spiniform cornuti, one of them usually long and tubular, second much smaller, often without spinules; distal part of vesica with scobinate walls but without spinulose field.

Female genitalia (Image 1E): Corpus bursae lobular. Proximal third of corpus bursae a large, spacious sac, covered partly with fine, short, hair-like spiculi. Medial third of corpus bursae only slightly wrinkled. Ductus bursae smaller and twisted. Diverticule of corpus bursae sclerotised, proximal papillae anales asymmetrical. Ostium bursae form a heavily sclerotized, double complex. Posterior part smaller, flattened, more or less rectangular with deeply incised proximal margin; anterior part huge, axe-head-shaped with pointed postero-lateral tip. Cervix bursae elongated, folded.

Material examined: 1901–1904, 19.v.2015, 2 males, 2 females, Chamoli, Uttarakhand, India, 30.242^{0}N & 79.614^{0}E, coll. Shashank; 1905, 04.vi.2014, 1 male, Palampur, Himachal Pradesh, India, 32.129^{0}N & 76.538^{0}E, coll. Pathania; 1906–1908, 05.ix.2007, 2 males, 1 female, Srinagar, Jammu & Kashmir, India, 34.117^{0}N & 74.776^{0}E, coll. Rajesh; 1909–1912, 21.ix.2016, 2 males, 2 females, Katrain, Himachal Pradesh, India, 32.097^{0}N & 77.135^{0}E, Shashank.

Global distribution: India (Uttarakhand, Himachal Pradesh, Jammu & Kashmir, and Sikkim), Pakistan, China, Japan, and Russia.

Conclusions

Our study highlights the occurrence of species *A. ornatissima* from India. Molecular evidence along with morphology confirm the presence of this species. There was no molecular data available for this species from India, which is also its type locality. DNA barcodes provided in this study will help in the accurate diagnosis of this species from India.

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