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Morphological characters and DNA barcodes to separate *Oenopia sauzeti* Mulsant and *O. mimica* Weise (Coleoptera: Coccinellidae), two externally similar lady beetles from the Indian subcontinent

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ABSTRACT: *Oenopia sauzeti* Mulsant and *O. mimica* Weise (Coleoptera: Coccinellidae) are externally very similar and commonly misidentified species of lady beetles distributed in the Indian subcontinent. Diagnostic characters including male genitalia are illustrated for these species to facilitate their identification. The *cox1 mtDNA* sequences of *O. sauzeti* and *O. mimica* (658 bp) had only 89% similarity upon pair-wise alignment, which distinguished them with 75 nucleotide differences, thus confirming that these are distinct species. DNA barcodes with accession numbers AGIMP042-15 for *O. sauzeti* and AGIMP043-15 for *O. mimica* were obtained.

KEY WORDS: *Oenopia*, Coccinellidae, DNA barcodes, morphology, Indian subcontinent

INTRODUCTION

Oenopia sauzeti Mulsant (1866) and *O. mimica* Weise (1902) are externally very similar sympatric species distributed in the Indian subcontinent (Mader, 1935; Miyatake, 1985; Poorani, 2002a, b). Of these two, *O. sauzeti* is fairly common and widely distributed throughout north, northwestern and northeastern India, Pakistan, Nepal, Bhutan, China and in parts of Southeast Asia. *Oenopia mimica* has a much more restricted distribution and is confined to the higher altitudes of Eastern Himalayas in India, Nepal, and Bhutan.

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Mader (1935) illustrated both species and described their diagnostic characters, particularly body size and elytral colour patterns. He observed that "*O. mimica* is as big as the smallest specimens of *O. sauzeti*". Iablokoff-Khnzorian (1979) synonymized *O. sauzeti* and *O. mimica*, perhaps misled by their external similarity. Miyatake (1985) restored them as valid species based on his studies on collections from Nepal Himalayas and summarized the morphological differences between the two species with illustrations. These two species were also illustrated and keyed in Poorani's (2002b) review of Indian species of *Oenopia*. Still, they continue to be misidentified in many Indian collections, with *O. mimica* nearly always wrongly identified as *O. sauzeti*, the more common and abundant species. Kapur's (1958) habitus and male genitalia figures for *O. sauzeti* were in fact those of *O. mimica*. It is also likely that molecular sequences for these two species could be based on wrong morphological identifications. This is illustrated by the fact that in the website of Barcode of Life Database (BOLD), the photograph of *O. sauzeti* is featured in the species page for *O. mimica* (from Pakistan).

We characterized the two species by their *cox1mtDNA* gene sequences and generated DNA barcodes as additional tools of diagnosis in conjunction with the already documented morphological characters. In this paper, we provide a complete illustrated account of the known morphological differences between the two species coupled with DNA barcodes, which should be useful in separating them.

MATERIALS AND METHODS

(i) Morphological studies

The examined specimens of *O. sauzeti* and *O. mimica* are deposited in the reference collections of the National Bureau of Agricultural Insect Resources, Bangalore. Photographs of morphological characters and genitalia were taken using a Leica M205A stereo microscope and composite images were generated from image stacks by Combine ZP software. The images were touched up for clarity and plates prepared in Photoshop Elements 11.

(ii) Amplification of *mtDNA COXI* gene and DNA barcoding

Card mounted specimens of *O. sauzeti* and *O. mimica* collected from Uttarakhand and Sikkim, respectively (<6 months old), were morphologically identified and used for DNA extraction and sequencing of 5' end of *cox1 mtDNA* (cytochrome c oxidase subunit 1 gene corresponding to the standard animal DNA barcoding locus) in the Molecular Entomology laboratory at NBAIR, Bangalore.

Genomic DNA was extracted from a single adult using QiagenDNeasy® kit, following the manufacturer's protocols. Specimens of both species were retained as voucher specimens after DNA extraction at NBAIR, Bengaluru. The DNA thus obtained was subjected to polymerase chain reaction (PCR) following standard protocol as described by Hebert *et al*. (2003). The following primers were used: forward primer (LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3'), and reverse primer (HCO 2198 5'-

TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions were carried out in PCR tubes obtained from M/s Tarsons, Kolkata, India, following manufacturer's protocol, using de-ionized distilled water. The amplified products were analyzed on 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001). The amplified products were sequenced in an automated sequencer (ABI Prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) using primers both in forward and reverse directions.

Sequences obtained were checked for homology and frame shifts by using NCBI-BLAST and ORF finder. As no insertions, deletions or stop codons were observed in 2nd frame of DNA, sequences were chosen from ORF finder for submission to GenBank. The sequences were submitted to GenBank and the accession numbers obtained were uploaded to the project Agriculturally Important Insects of India (AGIPM) at Barcode of Life Database Systems (BOLD Systems, http://www.boldsystems.org) and DNA barcodes were generated under the following process IDs AGIMP of NBAIR, Bangalore.

RESULTS AND DISCUSSION

(i) Morphology

Oenopia sauzeti and *O. mimica* share the same overall external color scheme and the general pattern is superficially similar. They can be separated by the pronotal marking, elytral pattern, sculpturing on elytra and genitalia. Brief comparative diagnostic accounts of both species are given here with illustrations to facilitate easy identification based on external characters and male genitalia.

***Oenopia sauzeti* Mulsant (Figs. 1, 4, 6, 10–12)**
Oenopia sauzeti Mulsant, 1866: 281.
Oenopia sauzeti: Crotch, 1874: 158.-Kapur, 1963: 27.-Gordon, 1987: 19.-Yu, 2009: 100.
Gyrocaria sauzeti: Miyatake, 1967: 76; 1985: 15.-Poorani, 2002b: 103.

Diagnosis: Length: 3.40–4.60 mm. Ground colour (Fig. 1) of head and pronotum creamy yellow, elytral colour variable from creamy yellow to bright lemon yellow. Head black in female, yellow in male. Pronotum with a hat-shaped black marking (Fig. 4) on posterior margin, its posterolateral ends never reaching posterolateral corners of pronotum. Elytral pattern (Fig. 1) as illustrated, median sutural spot broad, distinctly transverse-quadrate and rectangular, occasionally with rounded edges. Elytral punctures distinct, interspaces between elytral punctures more or less smooth (Fig. 6) to alutaceous, without any microsculpture. Male genitalia (Figs 11, 12) diagnostic, with penis guide of tegmen deeply and narrowly parabolic (Fig. 11), penis (Fig. 12) with an elongate capsule having distinct arms.

Distribution: *Oenopia sauzeti* is distributed in India (Assam, Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Manipur, Meghalaya, Nagaland, Mizoram, Punjab, Sikkim, Tripura, West Bengal, Uttar Pradesh, Uttarakhand), Pakistan, Nepal, Bhutan, Myanmar, Thailand, Laos,

Vietnam, Taiwan and China. It is very common in all the northeastern states of India. In northern India, it appears to be more prevalent in higher elevations and cooler climes and rarely found in the plains. It was introduced in North America for controlling balsam woolly aphid [*Adelges piceae* (Ratzeburg)], but did not establish (Amman & Speers, 1964; Mitchell& Wright, 1967).

Hosts: It feeds mainly on aphids and also whiteflies. Agarwala and Ghosh (1988), Irshad (2001) and Poorani (2002) listed some of the common hosts of this species. Some of the common hosts documented are as follows: **Hemiptera**: **Adelgidae**: *Adelges* spp. on conifers. **Aphididae**: *Aphis gossypii* Glover, *A. craccivora* Koch, *A. pomi* De Geer, *A. spiraecola* Patch, *Acyrthosiphon pisum* (Harris), *Chaitophorus* sp., *Hyadaphis* sp., *Metopolophium dirhodum* (Walker) (as *Macrosiphum graminum* Theobald), *Sitobion rosaeiformis* (Das), *Myzus obtusirostris* David, Narayanan & Rajasingh, *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* L., *Sipha maydis* (Passerini), *Schizaphis graminum* (Rondani), *Sitobion avenae* (F.); *Sarucallis kahawaluokalani* (Kirkaldy)) (label data). **Aleyrodidae**: *Aleurolobus barodensis* (Maskell), *Neomaskellia andropogonis* Corbett, *Neomaskellia* sp. **Cicadellidae**: *Evacanthus repexus* Distant (Cicadellidae). **Acari**: *Tetranychus* sp.

***Oenopia mimica* Weise (Figs. 2, 3, 5, 7-9)**

Oenopia mimica Weise, 1902: 505.-Iablokoff-Khnzorian, 1979: 70 (as synonym of *O. sauzeti*).-Mader, 1935: 343.-Poorani, 2002b: 104.

Gyrocaria mimica: Miyatake, 1985: 16.
Oenopia sauzeti sensu Kapur, 1958: 331.

Diagnosis: Length: 3.0–4.3 mm, usually much smaller than *O. sauzeti*. Basic colour scheme (Fig. 2) similar to that of *O. sauzeti*, ground colour of head and pronotum creamy yellow, of elytra bright lemon yellow to creamy yellow. Head black in female, yellow in male. Pronotum with a black macula (Fig. 3) positioned on posterior margin similar to *O. sauzeti*, but its outer edges posteriorly extended, touching posterolateral corners of pronotum. Elytral pattern (Fig. 2) basically similar to that of *O. sauzeti*, except median sutural marking distinctly more elongate, gradually dilated and oval in the middle, and narrowed towards both ends. Elytral punctation (Fig. 5) distinctive, punctures somewhat finer, placed farther apart and slightly less dense compared to those in *O. sauzeti*, with conspicuous microsculpture in interspaces between elytral punctures. Male genitalia (Figs 8, 9) diagnostic, with penis guide of tegmen more widely emarginate and somewhat broadly v-shaped (Fig. 8), penis (Fig. 9) and penis capsule distinctly stouter.

The elytral pattern in *O. mimica* is also similar to that of *O. smetanai* Canepari (1997), another species distributed in the Nepal and Indian Himalayas. *Oenopia smetanai* is even rarer than *O. mimica* and can be distinguished from the latter by its much smaller size (only 2.8–3.0 mm long), pronotum with a pair of oblique oval median spots and the male genitalia (illustrated by Poorani, 2002).

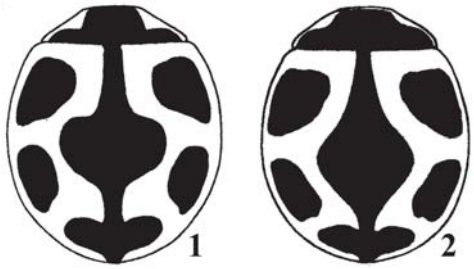


Fig. 1. *Oenopia sauzeti*: Adult, dorsal view; Fig. 2. *Oenopia mimica*: adult, dorsal view

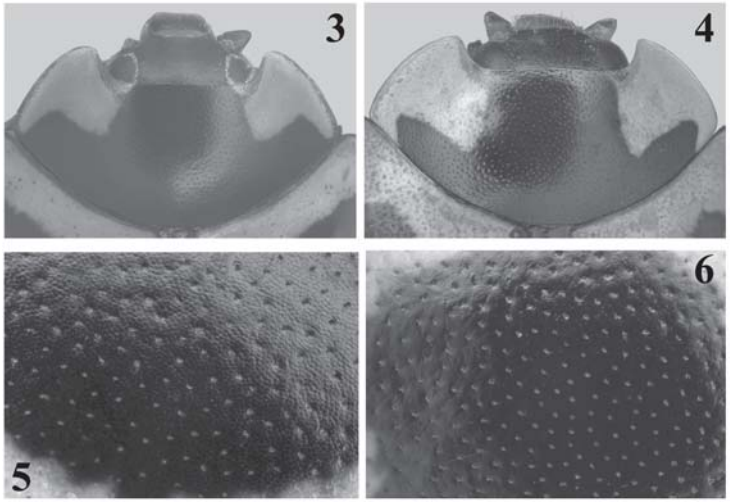
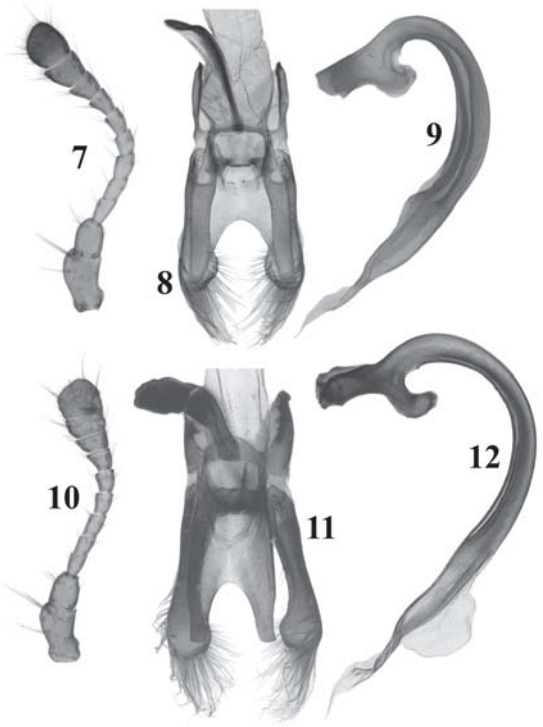


Fig. 3. Pronotal marking in *Oenopia mimica*; Fig. 4. Pronotal marking in *O. sauzeti*. Fig. 5. Elytral punctation in *O. mimica;* 6. Elytral punctation in *O. sauzeti.*

Distribution: *Oenopia mimica* is more or less confined to the upper reaches of Himalayas (Arunachal Pradesh, Himachal Pradesh, Sikkim, Uttar Pradesh) and is also known from Nepal, and Bhutan. There are some unconfirmed reports of its occurrence from Pakistan.

Hosts: *Oenopia mimica* is known to feed on *Adelges* spp. on silver fir, spruce and other coniferous vegetation; *Taoia indica* (Ghosh & Raychaudhuri) (label data). Host records from published literature are suspect and not included here.

Notes: Parts of Crotch's (1874) description of *Oenopia sauzeti* appear to match *O. mimica* better than *O. sauzeti.* His description of "thorax black, anterior angles with a quadrangular whitish spot, the inner angle produced to a point on the disc, outer portion prolonged to the posterior angle of the thorax" can be broadly applied to both species, but fits *O. mimica* more than *O. sauzeti.* Weise (1902) described *O. mimica* much later. It is not clear if the original type



Figs. 7–9. *Oenopia mimica*: 7. Antenna; 8–9. Male genitalia: 8. Tegmen, ventral view; 9. Penis; Figs 10–12. *Oenopia sauzeti*: 10. Antenna; 11–12. Male genitalia: 11. Tegmen, ventral view; 12. Penis.

series of *O. sauzeti* had any specimens of *O. mimica* also and Crotch's (1874) description is probably a result of his having examined more than one species in the material available to him. Gordon (1987) designated a lectotype for *O. sauzeti* (deposited at University of Cambridge, Crotch Collection), but did not mention anything about this.

Miyatake (1985) did not mention the difference in elytral sculpture between the two species, though it is the major distinguishing feature of *O. mimica*. The male genitalia are diagnostic for both species. The female genitalia in *O. sauzeti* and *O. mimica* are similar with the spermatheca differentiated into a distinct cornu, nodulus and ramus with a well-defined infundibulum, but the shape of the infundibulum is diagnostic for each species (see Poorani, 2012 for illustrations). Besides these characters, the antenna is also useful in separating the two species. In *O. sauzeti*, antennomeres 9 and 10 are distinctly transverse and the club is short and compact (Fig. 10). In *O. mimica*, antennomeres 9 and 10 are only slightly broader than long or nearly as broad as long (Fig. 7) and not transverse and the club is distinctly more elongate.

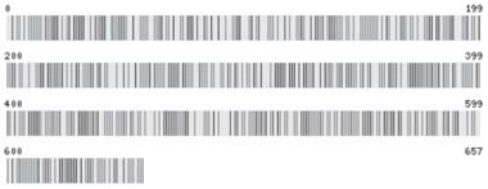
(ii) *cox1 mtDNA* **sequences and DNA barcodes**

The *cox1 mtDNA* gene sequence of 658 bp was obtained for both *O. sauzeti* and *O. mimica*. The *cox1 mtDNA* sequence of *O. sauzeti* from India had 98% similarity with that of another *O. sauzeti* isolate LBB41 (from China), confirming that they were conspecific. *Oenopia mimica* had 87% similarity with *Calvia quatuordecimguttata* (from Germany), an unrelated genus and species. The *cox1 mtDNA* sequences of *O. sauzeti* and *O. mimica* had only 89% similarity upon pair-wise alignment, which distinguished *O. sauzeti* from *O. mimica* with 75 nucleotide differences, thus confirming that these are distinct species.

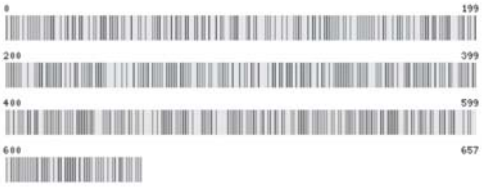
The GenBank accession numbers for the sequences of *O. sauzeti* and *O. mimica* were KR349051 and KR349052, respectively. Both the sequences were submitted to BOLDSYSTEMS and DNA barcodes obtained with accession numbers AGIMP042-15 for *O. sauzeti* and AGIMP043-15 for *O. mimica*. Species boundaries are established following a 2% divergence criterion (Hebert et al., 2003), based on the assumption that *cox1 mtDNA* divergences usually do not exceed a 2% divergence within a known species, whereas different species generally show a greater degree of divergence. Going by this criterion, the *cox1 mtDNA* sequences clearly separate *O. sauzeti* and *O. mimica*. Though the morphological differences between *O. sauzeti* and *O. mimica* are distinctive enough, these may be too subtle for the so called economic entomologists and the illustrations given here and the DNA barcodes should prove more useful for them in separating these two species.

DNA barcodes of *Oenopia sauzeti* and *O. mimica*

>*Oenopia sauzeti* _AGIMP042-15_ KR349051



>*Oenopia mimica* _AGIMP042-15_KR349052



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