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SHORT COMMUNICATION

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Aparna Sureshchandra Kalawate, Shital Pawara, A. Shabnam & K.P. Dinesh

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SHORT COMMUNICATION

DNA barcode reveals the occurrence of Palearctic *Olepa schleini* Witt et al., 2005 (Lepidoptera: Erebidae: Arctiinae) from peninsular India with morphological variations and a new subspecies

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Abstract: The present study was taken up to report a new record of the tiger moth genus, *Olepa* Watson, 1980 from India along with the discovery of a new subspecies. Earlier the genus was thought to have restricted distribution range in South and South-East Asia until the report of *O. schleini* Witt, Müller, Kravchenko, Miller, Hausmann & Speidel from the Mediterranean Coastal Plain of Israel in 2005. The species identification and the new subspecies is proposed based on the combination of morphological studies, available literature comparisons, geographical distribution, DNA barcoding and its phylogeny. Morphological character crypticity and genital structure variations are well documented in the genus with 'bio-species' groups. DNA Barcoding data of mt COI has provided some resolution in sorting the problems of 'bio-species' groups of the genus in the past studies. In the present study, with the available mt DNA COI barcodes and newly generated barcodes genetic identity is confirmed for the species *O. ricini*, *O. schleini*, *O. toulgoeti* and *Olepa schleini chandrai* ssp. nov., with their phylogenetic relationships. Morphological variations within the *O. schleini* species complex are discussed with a new record of the species for India and a new subspecies description. With the first mt COI barcode phylogeny for the genus, comments are made on the taxonomic identity of the mt COI DNA barcodes available in the GenBank for the *Olepa* species from India.

Keywords: mt COI gene, Maharashtra, new record, *Olepa schleini chandrai* ssp. nov., Oriental region, tiger moth, Western Ghats.

Abbreviations: tl—total length | mw—maximum width | vl—length of vinculum | vw—maximum width of vinculum | jl—length of juxta | jw—maximum width of juxta | al—length of aedagus (excluding vesica) | aw—maximum width of aedagus.

The genus *Olepa* Watson, 1980 was previously considered monotypic, with a single species, *O. ricini* (Fabricius, 1775). In 1986, Orhant revised the taxonomy and systematics of the genus into two morphological species-groups of *O. ocellifera* (Walker, 1855) and *O. clavatus* (Swinhoe, 1885) and he described four new species, namely, *O. duboisi* Orhant, 1986, *O. anomi* Orhant, 1986, *O. koslandana* Orhant, 1986, and *O. toulgoeti* Orhant, 1986 from southern India and Sri Lanka. Subsequently, four more species were added to the genus, thereby making the total 11 from the World (see Orhant 2000, 2012; Witt et al. 2005; Dubatolov 2011). The caterpillars of this genus feed on various

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Competing interests: The authors declare no competing interests.

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crops such as cotton, castor, sunflower, sesame, maize, ivy gourd, brinjal, sweet potato, and banana (ICAR-NBAIR 2019).

Orhant (2000) divided *Olepa* into two morphological species group based on the shape of uncus and valvae, viz.: the *ricini* group (uncus and valvae narrow, pointed at tip mostly) and *ocellifera* group (uncus broad, triangular; valvae digitiform, apex rounded). He added *O. ricini*, *O. clavatus*, and *O. koslandana* to the *ricini* species group and *O. ocellifera*, *O. duboisi*, *O. anomi*, and *O. kakatii* Orhant, 2000 to the *ocellifera* group. This was further modified by Witt et al. (2005) who added *O. toulgoeti* to the *ricini* species group. The first set of DNA barcodes for the genus *Olepa* was provided for the species *O. ricini*, *O. toulgoeti*, and *O. schleini* by Witt et al. (2005) during the new species description of *O. schleini* from Israel.

During our studies in the peninsular India, we found some of our *Olepa* specimens were matching the morphological descriptions and male genital characters with the species of *O. schleini* from Israel. Due to the contrasting zoogeographical collection localities from Oriental and Palearctic regions and their geographical isolation, we subjected our samples of *Olepa* for DNA Barcode studies. The outcome of the studies is presented here as a new report of *O. schleini* from peninsular India with the proposal of a new subspecies of *Olepa*.

In the past studies, the intraspecies variability among the species of *Olepa* is well documented by Witt et al. (2005); in his words "Within the two groups, the species are slightly variable in habitus and genitalia so that some species are doubtful, especially in the *ocellifera* group". Also, *O. toulgoeti* which was synonymised by Singh & Singh (2013) under *O. clavatus* is reinstated herein based on distinct features of their genitalia and DNA study (Witt et al. 2005).

Material and Methods

The moth specimens were collected by using a light trap during night, and were euthanized by ethyl acetate vapors and dry preserved in fumigated entomological boxes for further study. The specimens were studied under Leica EZ4E stereomicroscope. The series of images obtained from the stereomicroscope was stacked using the CombineZP software (Hadley 2010). The geographic coordinates and altitude were obtained, possibly by a Garmin GPS. The maps of the collection locality were

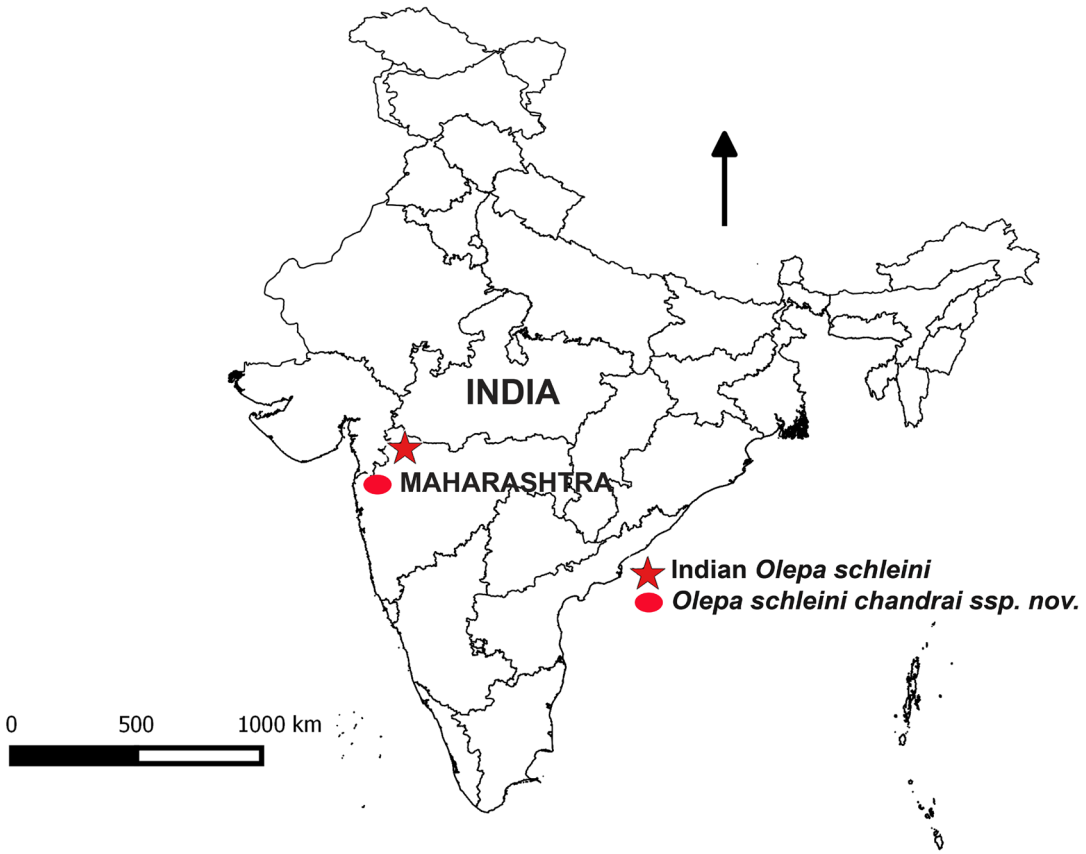

Figure 1. Collection sites of *O. schleini*: Indian *O. schleini* new record to India (star), *O. schleini chandrai* ssp. nov. (oval).

prepared using QGIS software. The details of collection locality are given under material examined and also shown in Figure 1. The identification of the specimen was done with the help of Orhant (1986) and Witt et al. (2005) terminology followed herein is after Orhant (1986, 2012). The genitalia of male and female were studied following Robinson (1976), and terminology is after Klots (1970), Orhant (1986, 2012), and Pekarsky (2012). The material examined is deposited in the Zoological Survey of India, Western Regional Centre, Pune, Maharashtra, India (ZSI-WRC).

DNA extraction, amplification, and sequencing

Whole genomic DNA was extracted from the dried leg and abdomen part of pinned preserved adults by modified Phenol-Chloroform method (Sambrook & Russell 2001) and the resultant pellet was eluted in 50µL of TE buffer pH 8.0, followed by DNA quantitation using Qubit 2.0 fluorometer by highly sensitive dsDNA assay kit. Approximately 578bp nucleotide portion of the mitochondrial COI gene was amplified using the primer pair LepCOI F, 5'-ATTCAACCAATCATAAAGATAT-3' and LepCOI R, 5'-TAAACTTCTGGATGTCCAAAAA-3' (Hebert et al. 2004). PCR reaction was performed in 25µL reaction volume comprising 12.5µL 2X Master Mix (Promega) DNAPolymerase, Reaction Buffer (pH 8.5), 400µM of each dNTPand 4mM MgCl2; 10µM of each forward and reverse primers, 1-5µL DNA (20-100ng) and nuclease free water to Q.S. Thermo cycling profile was as described by Hebert et al. (2003), with modifications, one cycle of 1min at 94°C; 10 cycles of 0.5min at 94°C, 1.5min at 45°C and 1.5min at 72°C; 30 cycles of 0.5min at 94°C, 1.5min at 51°C and 1min at 72°C and a final cycle of 1min at 72°C. PCR products were subjected to electrophoresis in 1% TAE gel stained with ethidium bromide (EtBr) and visualized under UV light via Gelstain Gel Documentation system. The amplified PCR product was purified using Invitrogen's Pure Link PCR Purification Kit. Purified PCR product was sequenced by Sanger's method on ABI 377 (Applied Biosciences) sequencer outsourced through M/S GeneMatrix, LLP, Pune.

Phylogenetic analysis

Generated sequences were initially aligned manually using MEGA version X (Tamura et al. 2011). With our sequences, mt COI DNA sequences for the species for the genus *Olepa* (21 sequences) were downloaded from the GenBank and aligned. Uncorrected pair-wise genetic distances (p-distances) were computed in MEGA version X (Tamura et al. 2011) to delineate our COI sequences from the rest of the *Olepa* sequences from GenBank. Final maximum likelihood (ML) tree was generated using raxmlGUI v1.3 (Silvestro & Michalak 2012). The ML tree (Figure 2) was obtained with 1,000 thorough bootstrap replicates under GTR+I model of nucleotide substitution using Akaike Information Criterion (-lnL = 1874.65; AIC = 3935.30) in jModel test (Posada 2008) and the consensus-generated tree was viewed using Fig. Tree v1.4.0, treating species of *Pygospila* as out-group (Table 1).

Result and Discussions

Our mt COI DNA studies confirmed two genetically distinct populations within the *O. schleini* species complex with morphological variations. Since the mt COI DNA sequences for our collections of *O. schleini* populations from Nandurbar were matching 100% with the mt COI DNA sequences of *O. schleini* from Israel we are treating the *Olepa* specimens from Nandurbar as the new report of *O. schleini* from peninsular India. Another population of *O. schleini* from Palghar which is 0.6% divergent for mt COI DNA with the *O. schleini* from Israel is described here as a new subspecies under *O. schleini*. The morphological character variations across the *O. schleini* populations from Israel (Palaearctic regions) and peninsular India (Oriental region) are discussed below (see taxonomic details).

Geographically, the type locality of *O. schleini* from Israel and the present record of the species from peninsular India are separated by a minimum aerial distance of 4,000km. This forms the first report for an *Olepa* species having its distribution range in both the Oriental and Palearctic regions

In the taxonomic account the comparisons were made on the basis of morphological characters, male genitalia, aedagus, position and number of cornuti as these characters were considered for comparison by Orhant (1986, 2000, 2012), Witt et al. (2005), and Dubatolov (2011). The VIIIth abdominal sternite is also considered for comparison as per Orhant (1986, 2000, 2012) and Dubatolov (2011).

Taxonomic account

Family Erebidae Leach, [1815]

Subfamily Arctiinae Leach, [1815]

Tribe Arctiini Leach, [1815]

Genus *Olepa* Watson, 1980

Olepa Watson, 1980, *The Generic Names of Moths of the World* 2: 133.

Type species: *Alope ocellifera* (Walker, 1855) [=*Olepa ocellifera* (Walker, 1855)]

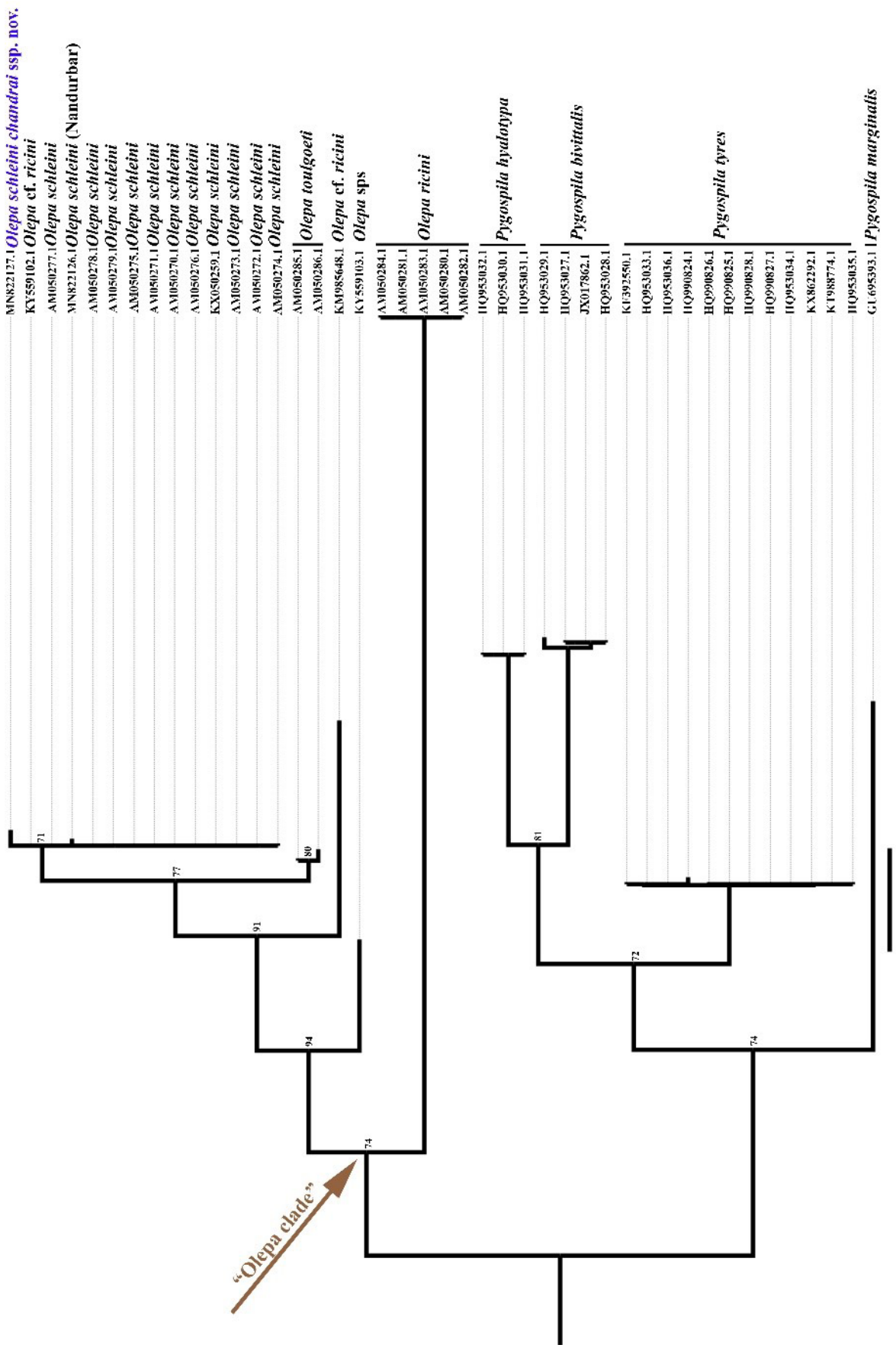

Figure 2. Maximum Likelihood (ML) tree for the species of *Olepa* based on the 578 bp of mitochondrial COI DNA gene sequences

***Olepa schleini* Witt, Müller, Kravchenko, Miller, Hausmann & Speidel, 2005**
(Image 1A–G)

Material examined: ZSI-WRC L-2028, 04.vii.2019, 01 male, Nandurbar (21.363N & 74.241E; 216m), Maharashtra, India, coll. Shital Pawara.

Molecular diagnosis: mt COI DNA sequences of ZSI/ENT/311 (GenBank accession number MN822126.1) were homologous with the sequences of *O. schleini* by Witt et al. (2005) without any nucleotide base pair differences.

Description: (Image 1A–B). Forewing length 46mm. Antennae brown and bipectinate, branches very small. Head and thorax dark grey-brown; collar bordered with yellow band, with two yellow ringed black spots; patagia with two pairs of big, same sized, yellow ringed black spots; one same bigger spot on middle of thorax. Abdomen coral, with eight dorsal and lateral black spots or bands; extremity of abdomen yellow; underside of abdomen yellowish. Legs fuscous brown, except extremity of femura and joints of tarsus yellowish. Forewing brown, with pale ringed dark spots, incomplete basal line; subbasal line very broad, curved, complete; median, subterminal and terminal lines macular, with variable spots; cilia greyish in between yellowish; underside same pattern. Hindwing light coral, somewhat yellowish near the coastal area, with three brown lines or bands; antemedial band thick, complete; median band incomplete; postmedial band with five blotches, interrupted in middle; underside same pattern as dorsal; cilia greyish in between yellowish.

Male Genitalia (Image 1 C–E). Total length of genitalia 5.82mm, maximum width 4.64mm. Genitalia heavily sclerotized. Uncus oblong, narrow than the other members, parallel sided (unlike Israelian *O. schleini*, where the base of uncus very narrow); apex tapering, blunt, surrounded by elongated thick setae. Valvae simple, very broad comparatively, curved in its half portion, long, central portion more broad, less narrowed and tapering in its distal portion ending in blunt apex; in an inflection directed against the uncus; the inner margin very broadly serrated at the tip, very distinctly patterned; the outer margin strongly serrated; the tip of the valvae not smooth, directed outward, convex. Juxta (length: 1.89mm, width: 1.56mm) longer, surai shaped. Vinculum (length: 0.772mm, width: 1.94mm) broad U-shaped, very shorter than tegumen, more than twice wider, uniformly sclerotized, with small outgrowth on its proximal end. Tegumen longer than uncus; broad inverted U-shaped, strongly sclerotised, comparatively smaller.

Aedagus (Image 1F). The length of aedagus: 4.34mm, width: 0.597mm. Short, thin, moderately sclerotized, minutely concave in the middle portion; with single, small, apical spine, apex acute; two-third portion of aedagus near apical spine highly sclerotized, remaining area opposite to it is membranous. Vesica membranous near base; subbasal diverticulum moderately scobinated, with two small patches of five, sclerotised cornuti; medial diverticulum heavily scobinated, with two short patches of eight to nine small, diverse cornuti, situated at posterior and anterior portion of diverticulum; the distal diverticulum, very heavily scobinated, with two patches of cornuti, situated medially and terminally, the medial patch consists of very minute 5–6 cornuti and the terminal, consists of comparatively big patch of small cornuti; a unique feature seen, is small patch of moderate sclerotisation present on the portion between the basal and the medial diverticulum on the ventral side.

VIIIth Abdominal sternite: the central plate of VIIIthAbdominal sternite is tridentate, with a very distinct pattern (Image 1G).

Measurement (in mm). tl: 5.82, mw: 4.64; vl: 0.772, vw: 1.94; jl: 1.89, jw: 1.56; al: 4.34, aw: 0.597.

Morphological variations within *O. schleini*: The forewing of adult of Indian *O. schleini* is with pale ringed macular bands (vs. without pale ringed spots on forewing of Israelian *O. schleini*). The subbasal band of forewing is broadest among all members of the group (it is normal, not very broad in Israelian *O. schleini*). The uncus of Indian *O. schleini* is narrow and parallel sided (which is very narrow at the base in Israelian *O. schleini*). The vinculum is broad U-shaped in Indian *O. schleini* which is of various shapes in Israelian *O. schleini* (see Witt et al. 2005) but not broad U-shaped. Juxta also differs significantly and is very broad in Indian *O. schleini*. The intraspecies variability is well supported and is in consistent with the study of Witt et al. (2005), wherein he stated the occurrence of intraspecies variability in this species and related taxa. The morphometric data conforms that the genitalial components of *O. schleini* is comparatively smaller than its subspecies.

Distribution: Israel; India (Nandurbar in Maharashtra).

***Olepa schleini chandrai* Kalawate, ssp. nov.**
(Image 1H–N)

urn:lsid:zoobank.org:act:5118E0A1-8041-41DB-9A83-551DD964022E

Holotype: ZSI-WRC L-2029, 07.x.2017, 1 male, Suryamal, (19.758N & 73.347E; 518m), Palghar District, Maharashtra, India, coll. V.D. Hegde and team, GenBank

Table 1. GenBank accession numbers for the species of *Olepa* and *Pygospila* used in the construction of Maximum likelihood tree.

	GenBank accession number	Species	Samples from	Source
1	MN822127.1	*Olepa schleini chandrai*	Palghar, Maharashtra, India	Current studies
2	KY559102.1	*Olepa* cf. *ricini*	India	Unpublished
3	AM050277.1	*Olepa schleini*	Israel	Unpublished
4	MN822126.1	*Olepa schleini*	Nandurbar, Maharashtra, India	Current studies
5	AM050278.1	*Olepa schleini*	Israel	Unpublished
6	AM050279.1	*Olepa schleini*	Israel	Unpublished
7	AM050275.1	*Olepa schleini*	Israel	Unpublished
8	AM050271.1	*Olepa schleini*	Israel	Unpublished
9	AM050270.1	*Olepa schleini*	Israel	Unpublished
10	AM050276.1	*Olepa schleini*	Israel	Unpublished
11	KX050259.1	*Olepa schleini*	NA	Ronka et al. (2016)
12	AM050273.1	*Olepa schleini*	Israel	Unpublished
13	AM050272.1	*Olepa schleini*	Israel	Unpublished
14	AM050274.1	*Olepa schleini*	Israel	Unpublished
15	AM050285.1	*Olepa toulgoeti*	India	Unpublished
16	AM050286.1	*Olepa toulgoeti*	India	Unpublished
17	KM985648.1	*Olepa* cf. *ricini*	Malappuram, Kerala, India	Unpublished
18	KY559103.1	*Olepa* sp.	India	Unpublished
19	AM050284.1	*Olepa ricini*	India	Unpublished
20	AM050281.1	*Olepa ricini*	India	Unpublished
21	AM050283.1	*Olepa ricini*	India	Unpublished
22	AM050280.1	*Olepa ricini*	India	Unpublished
23	AM050282.1	*Olepa ricini*	India	Unpublished
24	HQ953032.1	*Pygospila hyalotypa*	Australia	Unpublished
25	HQ953030.1	*Pygospila hyalotypa*	Australia	Unpublished
26	HQ953031.1	*Pygospila hyalotypa*	Australia	Unpublished
27	HQ953029.1	*Pygospila bivittalis*	Australia	Unpublished
28	HQ953027.1	*Pygospila bivittalis*	Australia	Unpublished
29	JX017862.1	*Pygospila bivittalis*	Australia	Haines & Rubinoff (2012)
30	HQ953028.1	*Pygospila bivittalis*	Australia	Unpublished
31	KF392550.1	*Pygospila tyres*	Australia	Hebert et al. (2013)
32	HQ953033.1	*Pygospila tyres*	Australia	Unpublished
33	HQ953036.1	*Pygospila tyres*	Australia	Unpublished
34	HQ990824.1	*Pygospila tyres*	Pakistan	Unpublished
35	HQ990826.1	*Pygospila tyres*	Pakistan	Unpublished
36	HQ990825.1	*Pygospila tyres*	Pakistan	Unpublished
37	HQ990828.1	*Pygospila bivittalis*	Pakistan	Unpublished
38	HQ990827.1	*Pygospila tyres*	Pakistan	Unpublished
39	HQ953034.1	*Pygospila tyres*	Australia	Unpublished
40	KX862292.1	*Pygospila tyres*	Pakistan	Ashfaq et al. (2017)
41	KT988774.1	*Pygospila tyres*	NA	Unpublished
42	HQ953035.1	*Pygospila tyres*	Australia	Unpublished
43	GU695393.1	*Pygospila marginalis*	Papua New Guinea	Unpublished

accession number MN822127.1.

Differential diagnosis: This subspecies could be distinguished from the other taxa in the genus by a combination of the following characters: thick median line of hindwing cojoined to antemedial line in some places; uncus long with blunt apex; valvae with slender apex, more recurved, the inflection directed against the uncus; juxta broader in its basal part and bigger; the central plate of VIIIth Abdominal sternite distinctly tridentate resembling a frill. The position of cornuti in the new subspecies is almost similar to *O. neumuthi* Orhant, 2012, except cornuti in the new subspecies is stronger, longer, denser, and present only in the subbasal region of vesica.

Molecular diagnosis: Pair wise genetic distance was 0.6% for the mt COI DNA sequences of *O. schleini chandrai* ssp. nov. from peninsular India and *O. schleini* from Israel; 1.8% to 2.% from *O. toulgoeti* and 25.4% from *O. ricini*. Due to limitations with mt COI DNA sequences further genetic studies are warranted to elevate the taxa to the species rank.

Description: (Image 1 H–I). Forewing length 48mm. Head and thorax dark grey-brown with admixture of olive-green scales; palpi brown; collar bordered with a yellow band and two black spots surrounded with whitish-yellow ring; patagia with two pairs of whitish-yellow ringed black spots, the front spot smaller; one same spot, bigger, on middle of thorax. Abdomen coral, with black bands, the first two are shorter, not reaching the lateral side, others reaching the lateral side of abdomen, lateral spots present, extreimity of abdomen black; underside of abdomen yellowish. Legs fuscous brown, except extremity of femura and joints of tarsus yellowish. Forewing moderately broad, apex rounded, fuscous brown, with several black spots surrounded by whitish-yellow ring, these spots sometimes fused to form band or line; basal and subbasal bands are not complete; antemedial band thick; median and subterminal bands complete; terminal line as a row of blackish dots between the veins; cilia brownish, in between patches of off-white. Hindwing light coral, costal area yellowish, with dark brown spots or blotches, these spots sometimes unites to form band or line; antemedial band thick, curved; thick median band cojoined to antemedial band in some places; postmedial band interrupted in the middle; thin marginal band; underside pattern of both the wings are exactly same as the dorsal; cilia brownish, in between patches of off-white.

Male Genitalia (Image 1J–L). The total length of genitalia 6.00mm, width 5.44mm. Genitalia less sclerotised comparatively. Uncus fused with tegumen, oblong, narrow, tapering towards apex, with blunt apex, long, surrounded by elongated, thin, minute setae. Tegumen shouldered, longer than uncus, broad inverted U-shaped, moderately sclerotised. Valvae curved, long, central portion broad, cucullus narrowing and ending in a blunt apex, in an inflection directed against uncus, costa plough-like; the inner margins broadly serrated in the upper region, the outer margin minutely serrated, tip of valvae not smooth, directed outward, convex. Juxta (length: 1.72mm, width: 1.66mm) broader in its basal part and bigger. Vinculum (length: 0.797mm, width: 1.84mm) truncated U-shaped, very shorter than tegumen, uniformly sclerotized, two times wider, with moderate outgrowth on its proximal end.

Aedagus (Image 1M). Length: 5.10mm, width: 0.687mm; is closer to *O. neumuthi*. It is long, thick, crooked, with single large, stout, apical spine, the tip of spine rounded; two-third portion of the aedagus near apical spine more sclerotised than the rest of the aedagus, the ramianing area opposite to it, is membranous, not sclerotised, the whole aedagus except the portion mentioned, is minutely sclerotised; vesica membranous, with patches of scobination, armed with stout, very long cornuti in three patches present on the subbasal region, comparatively dense cornuti.

VIIIth Abdominal sternite: the central plate of it is distinctly tridentate, resembles a frill, present in double layer (Image 1N).

Measurement of genitalia (in mm). tl: 6.00, mw: 5.44; vl: 0.797, vw: 1.84; jl: 1.72, jw: 1.66; al: 5.10, aw: 0.687.

Etymology: The subspecies is named after Dr. Kailash Chandra, an eminent Entomologist and the Director of the Zoological Survey of India, Kolkata.

Remarks: The new subspecies is externally similar to *O. neumuthi* and belongs to the *ricini-schleini-neumuthi* complex. It differs from all congeners by the distinct cucullus, which is narrowing and ending in a blunt apex, (vs. cucullus of *O. ricini* and *O. neumuthi* always ending in a point or less rounded apex; in *O. schleini*, it is rounded). The inflection of valvae is directed towards the uncus in *O. ricini*, *O. neumuthi*, and *O. schleini*. Whereas, it is directed against the uncus in the new subspecies. The vinculum is truncated U-shaped, very shorter than the tegumen, with moderate outgrowth on its proximal end (vs. U-shaped in *O. ricini*, and *O. schleini* with comparatively large and moderate outgrowth, respectively; in *O. neumuthi* it is V-shaped, with small outgrowth).

The aedagus of the new subspecies is similar to the *O. neumuthi*. Aedagus of *O. schleini chandrai* ssp. nov. is thick, crooked and strong, with three patches of strong,

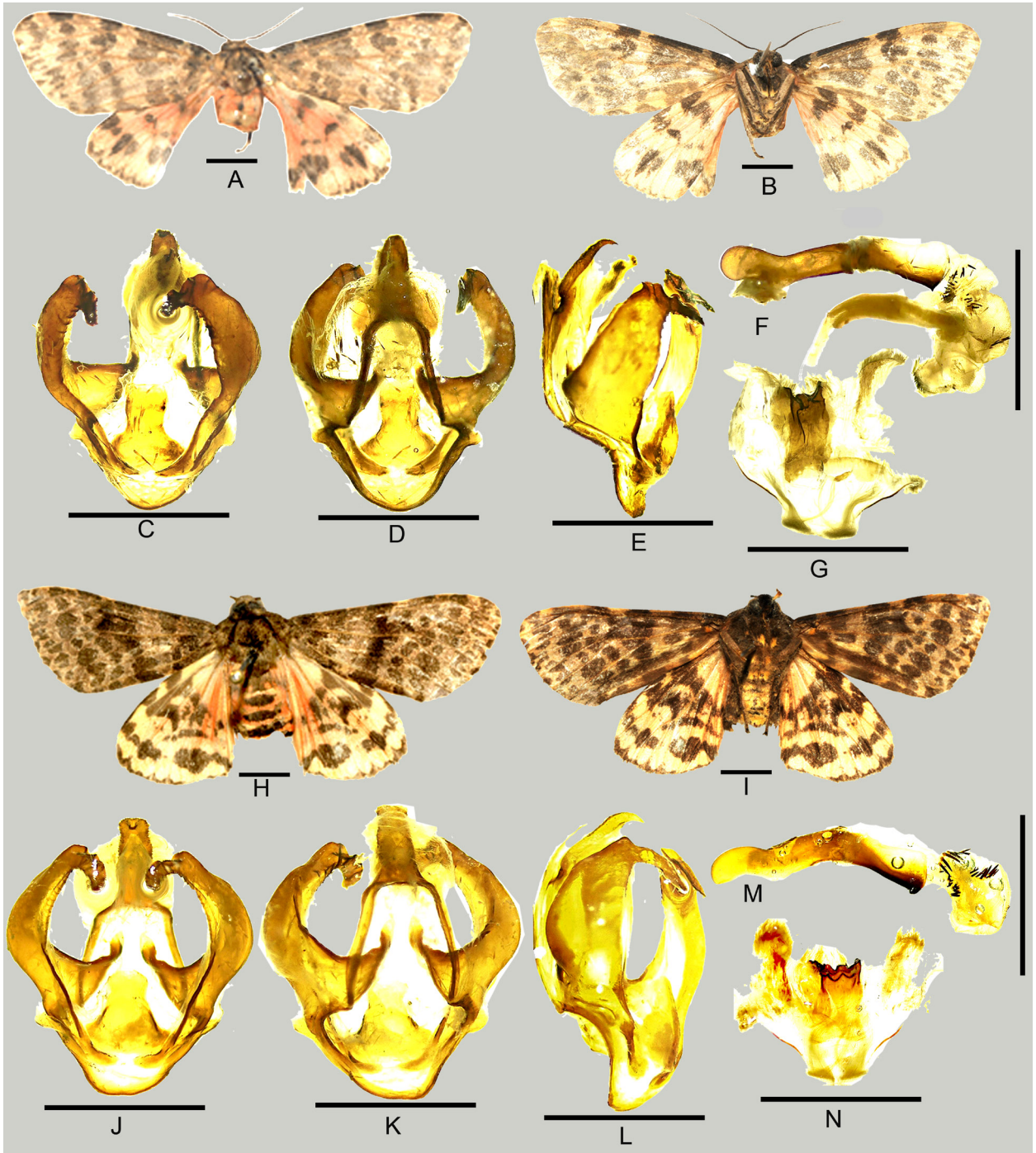

Image 1. A–G, *Olepa schleini* from India (A—habitus, dorsal | B—habitus, ventral | C—male genitalia in ventral view | D—male genitalia in dorsal view | E—male genitalia in lateral view | F—aedagus | G—VIIIth Abdominal sternite) | H–N—*Olepa schleini chandrai* ssp. nov. (holotype) (H—habitus, dorsal | I—habitus, ventral | J—male genitalia in ventral view | K—male genitalia in dorsal view | L—male genitalia in lateral view | M—aedagus | N—VIIIth Abdominal sternite). Scale bars = 1mm (C–G, J–N); 5mm (A–B, H–I).

longer and dense cornuti in the subbasal region of vesica (vs. almost straight aedagus, presence of three patches of modest cornuti in the basal region in *O. neumuthi*; in *O. schleini* and *O. ricini* there are 5–6 patches of cornuti).

Distribution: Known only from the type locality: Suryamal, Palghar District (Western Ghats), Maharashtra, India.

The present study is indicative of the species richness of this genus which was underestimated due to its complex cryptic nature and wide range of distribution. A

Table 2. Genetic distance (in percentage) matrix for the mt COI DNA among the DNA Barcode available species of *Olepa*.

	Olepa schleini	*Olepa schleini chandrai*	*Olepa toulgoeti*	*Olepa ricini*
Olepa schleini				
Olepa schleini chandrai	0.60			
Olepa toulgoeti	1.60	2.30		
Olepa ricini	25.90	25.90	25.40	

total of 21 mt COI sequences of *Olepa* were downloaded from the GenBank, with our two mt COI sequences on the phylogenetic tree five clear sub-clades could be discerned within the larger 'Olepa clade'. Our sequences of *Olepa* species formed a monophyletic clade with the species of *O. schleini*, over all genetic distance within the '*Olepa schleini* species clade' was up to 0.6%. Since our sequence for *O. schleini* (Nandurbar) was matching 100% with the 11 *Olepa schleini* sequences of Witt et al. (2005) we have treated our sample of *O. schleini* from Nandurbar, Maharashtra as the first record of *O. schleini* to Indian sub-continent extending its range of distribution from Israel in spite of slight morphological differences (see taxonomic discussion above).

Our *Olepa* species collected from Palghar, Maharashtra is showing a very shallow genetic divergence of 0.6% from *O. schleini* populations of India and Israel with high morphological divergence. Hence, *O. schleini* collections from Palghar has been proposed as a subspecies (*O. schleini chandrai* ssp. nov.) of *O. schleini* due to high morphological divergence (i.e., hindwing pattern, distinct cucullus and the position of cornuti in the new subspecies) and shallow genetic distance (Table 2) warranting further molecular studies. Spatially *O. schleini* from Nandurbar and *O. schleini chandrai* ssp. nov. from Palghar are 180km apart. Shallow genetic divergence of 1.8% was earlier reported between the species *O. ricini* and O. *toulgoeti* by Witt et al. (2005).

Interestingly, sequence of *O. ricini* (KY559102.1) from India matches 100% with the sequences *O. schleini* (Figure 2), where voucher specimen studies are warranted to understand the morphological divergence. Also, the sequence of *O. ricini* (KM985648.1) from Malappuram, Kerala do not match either of the sequences of *O. ricini* or *O. schleini*, where morphological studies of voucher samples are warranted for the exact identity of the species. Likewise, the voucher specimen of the sequence of *Olepa* sp. (KY559103.1) from India needs examination to justify the species name for the sequence. As of now among the 11 species of *Olepa* available globally mt COI DNA gene data are available specifically only for *O. toulgoeti*, *O. ricini, O. schleini*, and *O. schleini chandrai* ssp. nov. Voucher specimen studies on the two lineages of *O.* cf. *ricini* (KM985648.1) and *Olepa* sp. (KY559103.1) could provide information on the mt COI DNA barcode data for the known species of *Olepa* or it may be a potential new species awaiting formal description. As stated earlier, the new subspecies is proposed on the basis of the following set of characters: it possesses a thick median line of hindwing cojoined to antemedial line in some places; uncus long and valvae with slender apex, more recurved, the inflection directed against the uncus; the central plate of VIIIth abdominal sternite distinctly tridentate resembling a frill. The position of cornuti in the new subspecies is almost similar to *O. neumuthi*, except cornuti in the new subspecies is stronger, longer and denser and present only in the subbasal region of vesica.

This study forms the first report for the *O. schleini* described from Palearctic region having its distribution in Oriental region which is around 4,000km apart invoking many zoogeographic question of species distribution spatially. Understanding the genetic heterogeneity and morphological divergences within the species of *Olepa* from the studies of Witt et al. (2005) and the current study, there could be many more new species awaiting formal description which can be justified through spatial sampling and DNA studies.

References

Ashfaq, M., S. Akhtar, M.A. Rafi, S. Mansoor & P.D. Hebert (2017). Mapping global biodiversity connections with DNA barcodes: Lepidoptera of Pakistan. *PLoS ONE* 12(3): e0174749. https://doi.org/10.1371/journal.pone.0174749.

Dubatolov, V.V. (2011). A new *Olepa* Watson, 1980 species from South India (Lepidoptera, Arctiidae). *Atalanta* 42 (1/2) : 136–137.

Fabricius, J.C. (1775). Systema Entomologiae, sistens Insectorum Classes, Ordines, Genera, Species, Adiectis Synonymis, Locis, Descriptionibus, Observationibus. Kortii, Flensbvrgi et Lipsidae [= Flensburg and Leipzig]: [32]+832pp.

Hadley, A. (2010). Combine ZP software, new version. Available from: http://www.hadleyweb.pwp.blueyonder.co.uk/CZP/News.htm (accessed 16 August 2018).

Haines, W.P. & D. Rubinoff (2012). Molecular phylogenetics of the moth genus *Omiodes* Guenée (Crambidae: Spilomelinae), and the origins of the Hawaiian lineage. *Molecular Phylogenetics and Evolution* 65(1): 305–316.

Hebert, P.D., A. Cywinska, S.L. Ball & J.R. Dewaard (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270(1512): 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert, P.D., E.H. Penton, J.M. Burns, D.H. Janzen & W. Hallwachs (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences* 101(41): 14812–14817. https://doi.org/10.1073/pnas.0406166101.

Hebert, P.D., J.R. Dewaard, E.V. Zakharov, S.W. Prosser, J.E. Sones,

J.T. McKeown, B. Mantle & J. La Salle (2013). A DNA 'barcode blitz': rapid digitization and sequencing of a natural history collection. *PLoS ONE* 8(7): e68535. https://doi.org/10.1371/journal.pone.0068535

ICAR-NBAIR (2019). *Olepa ricini* (Fabricius) National Bureau of Agricultural Insect Resources. Available from: http://www.nbair.res.in/insectpests/credits.php (accessed on 18 September 2019).

Klots, A.B. (1970). Lepidoptera, pp. 115–130. In: Tuxen, S.L. (ed.). Taxonomist's glossary of genitalia in insects. Munksgaard, Copenhagen, 359pp.

Orhant, G. (1986). Deuxième contribution à l'étude des lépidoptèreshétérocères du sud-estAsiatique (1) Le complexed'espèces "*ricini* Fabricius" (Arctiidae: Arctiinae). *Bulletin Society Science National France* 50: 9–22.

Orhant, G. (2000). Unnouvel *Olepa* de l'Inde (Lepidoptera, Arctiidae, Arctiinae). *Lambillionea*100 (2): 269–270.

Orhant, G. (2012). *Olepa neumuthi* new species from Thailand (Lepidoptera: Arctiidae). *Bulletin Society Entomology Mulhouse* 68(4): 61–64.

Pekarsky, O. (2012). Two new species of *Pseudohadena* Alphéraky, 1889 from Kazakhstan (Lepidoptera, Noctuidae, Xyleninae). *ZooKeys* 187: 9–34. https://doi.org/10.3897/zookeys.187.2661

Posada, D. (2008). J Model Test: Phylogenetic Model Averaging. *Molecular Biology and Evolution* 25: 1253−1256. https://doi.org/10.1093/molbev/msn083

Robinson, G.S. (1976). The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. *Entomologist's Gazette* 27: 127–132.

Rönkä, K., J. Mappes, L. Kaila & N. Wahlberg (2016). Putting Parasemia in its phylogenetic place: a molecular analysis of the subtribe Arctiina (Lepidoptera). *Systematic Entomology* 41(4): 844–853.

Sambrook, J. & D. Russell (2001). *Molecular Cloning: A Laboratory Manual*, 3rd Edition. 619 Cold Spring Harbor, 2100pp.

Singh, N. & J. Singh (2013). Review of the genus *Olepa* Watson (Lepidoptera: Erebidae: Arctiinae). *Tinea* 22(4): 272–277.

Silvestro, D. & I. Michalak (2012). Raxml GUI: A graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335−337. https://doi.org/10.1007/s13127-011-0056-0

Swinhoe, C. (1885). Proceedings of the general meetings for scientific business of the Zoological Society of London, 295pp.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731−2739. https://doi.org/10.1093/molbev/msr121

Walker, F. (1855). List of the specimens of Lepidopterous insects in the collection of the British Museum 3: 583−775.

Witt, T.J., G.C. Müller, V.D. Kravchenko, M.A. Miller, A. Hausmann & W. Speidel (2005). A new *Olepa* species from Israel (Lepidoptera, Arctiidae). *Nachrichtenblatt der Bayerischen. Entomologen* 54(3/4): 101–115. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.463.9720&rep=rep1&type=pdf

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