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Research Article

DNA barcoding of Indian species of genus *Lucilia* (Dip., Calliphoridae) with two new country records

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Abstract. *Lucilia* is a genus of flies causing myiasis and breeding in carrion making them a group of medical, veterinary and forensic importance. From India, the genus is represented by 8 species so far. Herein, we have reported two new records for the country (*Lucilia bismarckensis* Kurahashi and *Lucilia silvarum* (Meigen)) and provided an identification key to the known Indian species of the genus *Lucilia*. Cytochrome oxidase subunit 1 (COI) sequences for three Indian species were generated and analyzed along with seven publicly available sequences retrieved from the GenBank, using Maximum Likelihood analysis. We found that COI could be used as a reliable marker for DNA based identification of the group as the species in question showed enough interspecific divergence and formed well supported clades.

Keywords: Molecular identification, Forensic entomology, *Lucilia bismarckensis*, *Lucilia silvarum*

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Introduction

The genus *Lucilia* Robineau-Desvoidy, 1830, commonly known as green bottles, comprises mainly carrion breeders and species causing human and animal myiasis. The group is of great medical, veterinary and forensic importance as these flies are vectors of certain diseases and cause potential economic loss via sheep-strike in many countries every year (Wells *et al*., 2007). Moreover, members of this group are responsible for causing obligate primary myiasis and facultative myiasis, thus their biogeographical distribution across the globe needs to be monitored and updated regularly (Mirzakhanlou *et al*., 2025). The species of the genus *Lucilia* were earlier divided into number of genera namely *Phaenicia* Robineau-Desvoidy, 1863, *Bufolucilia* Townsend, 1919, *Phomonesia* Villeneuve, 1914 and *Roubaudiella* Seguy, 1925 (Williams *et al*., 2016). *Phaenicia* was erected on the bases of the yellow colour of the basicostal scale and presence of 3 acrostichial bristles (Zumpt, 1965). Similarly, *Bufolucilia* was erected to include facultatively parasitic species (Aubertin, 1933). *Phomonesia* and *Roubaudiella*, monotypic genera, were erected on the same species (Aubertin, 1933). Later, Zumpt (1965) synonymised all other genera under *Lucilia*.

From the Oriental Region, Senior-White *et al*. (1940) worked on this group and described nine species of which 6 were reported from India. Later, *Lucilia bazini* Seguy, 1934 and *Lucilia calviceps* Bezzi,1927 were added to the list (Bharti & Kurahashi, 2010). Of the 8 known species, *L. sericata* (Meigen, 1826) , *L. cuprina* (Wiedemann, 1830) and *L. porphyrina* (Walker, 1856) are primary facultative parasites; *L. illustris* (Meigen, 1826) and *L. ampullacea* Villeneuve, 1922 are secondary facultative parasites; *L. papuensis* Macquart, 1844 is saprophagous; whereas the behaviour of *L. bazini* and *L. calviceps* is unknown so far (Bharti & Kurahashi, 2010; Williams *et al*., 2016).

Keeping in view the importance of this group in veterinary and forensic contexts, morphological identification is a prerequisite for proper investigation. Moreover, the identification keys are based on adult representatives, and it is observed that the immature stages of these flies are generally encountered during homicide and myiasis but only handful of publications are available in this regard (ErzinÇlioğlu, 1987, Liu & Greenberg, 1989, ErzinÇlioğlu, 1990, Szpila *et al.*, 2024). Therefore, in such cases molecular data is the most common, preferred, and reliable method for DNA-based species identification of blow flies. A plethora of publications are available on the DNA based identification of blow fly species (Harvey *et al.*, 2003, Wallman *et al.*, 2005; Wells *et al.*, 2007; Reibe *et al.*, 2009; Liu *et al.* 2011; Boehme *et al.*, 2012; DeBry *et al.*, 2012,; Sonet *et al.*, 2013; GilArriortua *et al.*, 2013) but little is known from India (Bharti & Singh, 2017). The aim of this study is to provide an identification key to the known Indian species of the genus *Lucilia* and to verify whether the barcoding region of the COI gene could be used as a reliable tool for species identification. Moreover, we report two new records *i.e. Lucilia bismarckensis* Kurahashi and *Lucilia silvarum* (Meigen) from India.

Materials and methods

Collection data of new records

The adult specimens of *Lucilia bismarckensis* were collected from pig and fish carcasses using a sweep net in Jaipur, Rajasthan, West Bengal and Assam. *Lucilia silvarum* was collected from fish baits in Jammu and Kashmir. The collected specimens were killed with ethyl acetate vapours and preserved in absolute alcohol for further analysis. Identification keys were used to identify the specimens (Kurahashi, 1987; Kurahashi & Thapa, 1994). All examined specimens are deposited in the Diptera Laboratory, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India.

DNA Extraction and PCR Amplification

A total of 10 species of genus *Lucilia* which occur in India were used for molecular analysis. DNA sequences from three species were successfully extracted and amplified in this study (PP980536, PP980525, OR492603), along with seven pre-existing public sequences retrieved from the GenBank (Table 1). Quigen DNeasy tissue kit (Qiagen, Inc., Valencia, CA) was used as per manufacturer's protocol to extract DNA from crushed head tissue. Each PCR reaction consisted of 10µl of 2x Master Mix (Promega Corporation, Madison, WI), 1 µl of each primer (10pmol/µl), 1–3 µl of template DNA (10–30 ng), and rest of water to make the total volume of 20 µl. DNA amplifications were carried out in a Master cycler Nexus Thermal Cycler (Eppendorf, Hamburg, Germany) using PCR protocols, as described in Wells & Sperling (2001). Sequencing was conducted by Eurofins Genomics (Bangalore, India). *Chrysomya thanomthini* (Kurahashi & Tumrasvin) (#PV203235) and *Hemipyrellia ligurriens* (Wiedemann) (#OR501396) were used as outgroups for analysis.

DNA sequence analysis

All sequences were aligned using default parameters in Muscle (Edgar, 2021), as implemented in MEGA X (Kumar *et al.*, 2016). Non-overlapping regions of sequences were trimmed, and the rest of the aligned sequences were used for pairwise distances analyses. For calculating pairwise nucleotide diversity, the pairwise deletion option with default parameters in MEGA X was used. Analysis was performed on 788bp of aligned sequences using Maximum Likelihood (ML) analysis in MEGA X (Kumar *et al.* 2016). Maximum Likelihood analysis was performed for 1000 bootstrap replications using the unweighted heuristic search option, ML optimality criterion and General Time-Reversible model with variable sites (GTR+I). The best fit model for ML analysis was selected using Akaike information criteria (AIC) in MEGA X software.

Results and Discussion

Taxonomic analysis

Herein, we report *Lucilia bismarckensis* Kurahashi, 1987 and *Lucilia silvarum* (Meigen, 1826) as new records from India. *L. bismarckensis* is a saprophagous species and thus can be used for determining a PMI in homicide cases. *Lucilia silvarum* (toad fly) has an interesting history with respect to its niche preference. Earlier records

associated this fly with amphibian myiasis (Zumpt, 1965; Bolek & Janovy, 2004) but later it was established that this fly is saprophagous and colonizes carcasses of birds and mammals including humans (Davies, 1999; Fremdt *et al.*, 2012; Bagsby *et al.*, 2024). This mistake was the result of misidentification as researchers misidentified *Lucilia bufonivora* Moniez, 1876 as *Lucilia silvarum* (Bagsby *et al.*, 2024). During present investigation *L. silvarum* was collected from fish bait, substantiating the recent findings to consider this species of forensic relevance, however more studies are needed to confirm the same.

Key to the Indian species of *Lucilia*

1. Post-sutural acrostichial: 3 …… (2)
- Post-sutural acrostichial: 2 …… (4)

2. Basicostal scale yellow, palpus bright orange, upper and lower calypter white in color …… (3)
- Basicostal scale brown or black; palpus brown or black, upper calypter pale and lower calypter brown in color …… *Lucilia silvarum* (Meigen)

3. Posterior slope of humeral callus with 0-4 hairs; abdomen arched in profile; sternites with tuft of long hairs; hypopygium prominent; parafrontalia bare or almost bare except for frontals and fronto-orbitals in female …… *Lucilia cuprina* (Wiedemann)
- Posterior slope of humeral callus with 6-8 hairs; abdomen not arched in profile, sternites without tuft of long hairs, hypopygium inconspicuous; parafrontalia in female with short decumbent bristles among frontals and parafrontals …….. *Lucilia sericata* (Meigen)

4. Upper and lower calypter white …… (5)
- Upper calypter white and lower calypter infuscated or both alar squama and thoracic squama infuscated …….. (6)

5. Eyes separated by little less than 3rd antennal segment, frons obliterated for short space; abdominal segments III and IV without dark marginal band …… *Lucilia illustris* (Meigen)
- Eyes holoptic, separated at point of closet association by a distance slightly more than the width of ocellar triangle; abdominal segments III and IV with marginal bands, at most with a trace of very narrow median dark stripe …… *Lucilia bismarckensis* Kurahashi

6. First pair of post-sutural acrostichial setae situated anterior to second pair of dorsocentral setae in dorsal view …… …… 7
- First pair of post-sutural acrostichial setae situated at level or slightly posterior to second pair of dorsocentral setae in dorsal view …… 8

7. Abdomen without distinct black marginal bands, wings faintly and uniformly tinged with brown, which deepens slightly at the base …… *Lucilia porphyrina* (Walker)
- First abdominal segment greenish black, rest with black marginal bands, wings hyaline …… *Lucilia ampullacea* Villeneuve

8. Upper calypter creamy with a tuft of yellowish white hairs at the inner lower margin, lower calypter pale, brownish on disc, occiput with more than two irregular rows of black post ocular setae …… *Lucilia bazini* Seguy
- Upper calypter fuscous brown, usually with a tuft of blackish-brown hairs at inner lower margin …….. 9

9. Body metallic blue, narrowest part of male frons broader than the distance between two posterior ocelli, parafacialia broader than the width of third antennal segment in female …… *Lucilia papuensis* Macquart
- Body metallic green, narrowest part of male frons distinctly narrower than the distance between both posterior ocelli; parafacialia as broad as, or narrower than the width of third antennal segment in female …….. *Lucilia calviceps* Bezzi

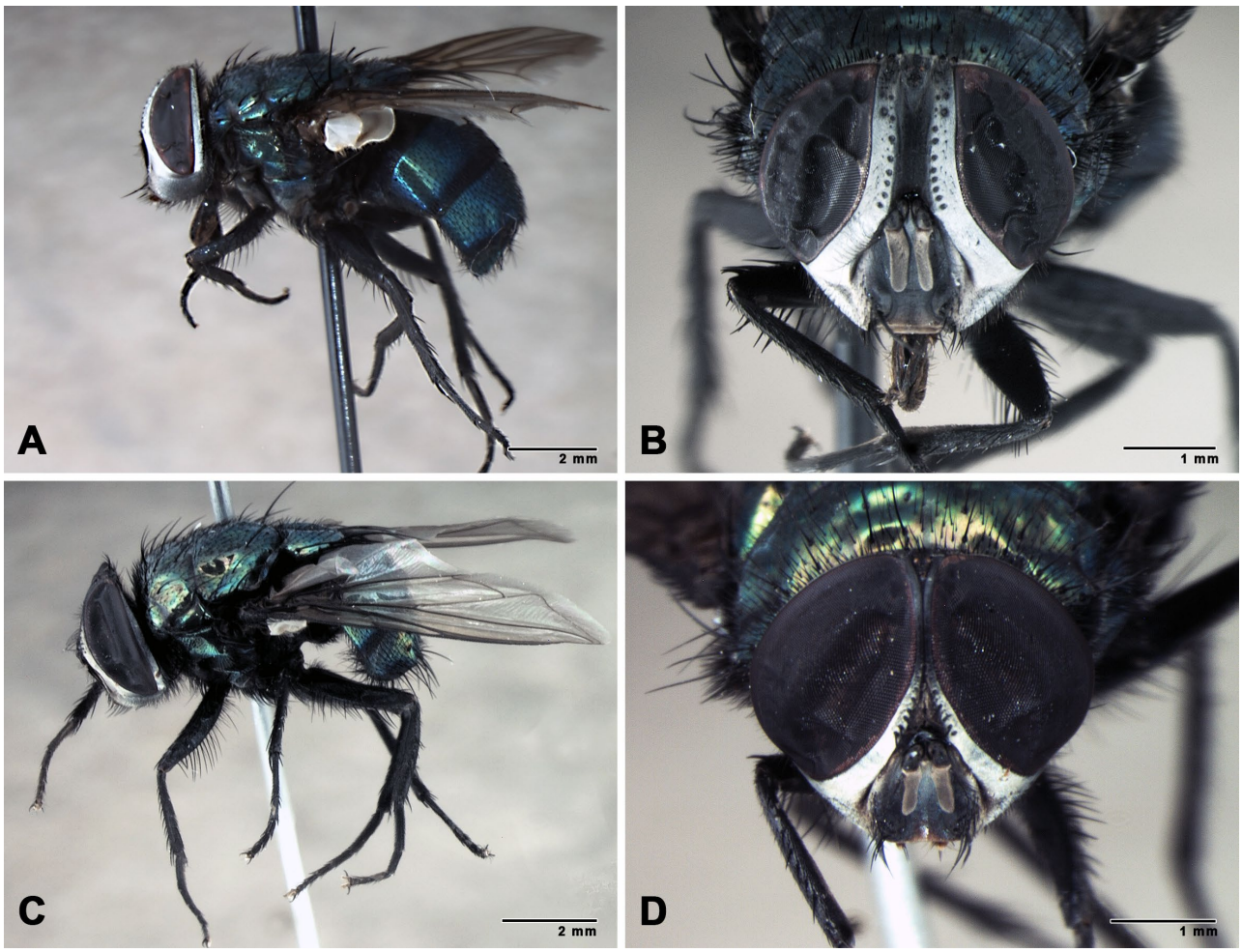

Fig. 1. *Lucilia bismarckensis* (a) profile female (b) head female (c) profile male (d) head male (Scale bar: A=2mm; B=1mm; C=2mm; D=1mm)

Lucilia bismarckensis Kurahashi, 1987 (Fig. 1)

Material Examined: 8♀, 9♂, India, West Bengal, Buxa Tiger Reserve, 26.9167° N, 89.9167°′ E, 867m, 25.x.2023; India, West Bengal, Hasimara, 26.7309° N, 89.3506° E, 109m, 27.x.2023; India, Assam, Bongaigaon,26.5030° N, 90.

Diagnostic characteristics: Upper calypter white with a tuft of yellowish white hairs, humeral hairs black, anterior pair of acrostichial at level with second pair of post-sutural dorsocentrals, eyes in male closely approximated but separated by the width of ocellar triangle, parafacialia usually grey dusted, body 6.0-9.0 mm in length.

Lucilia silvarum (Meigen, 1826) (Fig. 2)

Material examined: 4♀, 6 ♂, India, Jammu and Kashmir, Hokersar, 34.090° N 74.710° E, 1584m, 15.x.2022, India, Jammu and Kashmir, Hafthrada, 34.450° N 74.060° E, 1950m, 27.viii.2022, India, Jammu and Kashmir, Uri, 34.080° N 75.030° E, 1721m, 10.vi.2023.

Diagnostic Characteristics: A dark base of wing, subcostal sclerite without setulae, tergite 1+2 black in color, large bristles on the hind border of tergite 4 in females and tergite 3 in males, eyes separated by the width of third antennal segment in male, 3 postsutural acrostichal bristles.

Evolutionary Divergence: The main purpose of this study was to test the reliability of the COI gene for the molecular identification of Indian species of the genus *Lucilia*. The pairwise distances of 12 species were calculated (Table2) and it was observed that the interspecific divergence ranged from 0.61% (for *L. sericata* and *L. cuprina*)

to 10.18% including the outgroup (for *Chrysomya thanomthini* and *L. silvarum*). The low intraspecific divergence between *L. cuprina* and *L. sericata* (0.6%) in the present study could be attributed to the high external morphological similarity of the two species. Moreover, introgression and presence of natural hybrids of the two species have been reported by other researchers (Wallman *et al.*, 2005; DeBry *et al.*, 2010; Williams & Villet, 2013).

It is known that interspecific divergence is low in insect orders and is rarely beyond 2% in all animal groups (Hebert *et al.*, 2003). A wide barcoding gap observed in this study suggests that except *L. sericata* and *L. cuprina*, all species tested have enough COI sequence divergence for reliable species identification.

Molecular analysis: We analysed our data using ML to obtain a robust tree (Fig. 3). All the species included in this study (except *L. sericata* and *L. cuprina*) were monophyletic and can be reliably identified using COI barcodes. The ML tree (Fig. 3) clearly divided the Indian species of genus *Lucilia* into two major groups. The first group comprises of species of old-world lineages and are saprophagous (*L. papuensis, L. bismarckensis, L. ampullacea, L. illustris, L. calviceps, L. bazini, L. porphyrina*). Primary and secondary facultative parasites (*L. cuprina, L. sericata and L. silvarum)* constitute the second group of the ML tree.

Morphologically: Indian species of genus *Lucilia* could be broadly categorized into two groups based on the number of acrostichial bristles. The species with three acrostichial bristles are *L. silvarum, L. cuprina* and *L. sericata* which also formed a separate clade of facultative parasites in molecular analysis. The second group with two acrostichial bristles comprises *of L. bazini, L. papuensis, L. bismarckensis, L. ampullacea, L. illustris, L. calviceps,* and *L. porphyrina* constitute the old-world lineage of the ML tree. A similar trend was observed in the phylogenetic analysis carried out by Williams *et al.* (2016) wherein different degrees of parasitism were phylogenetically clustered. However, the researchers concluded that none of these parasitic behaviours are limited to any geographical area and is an outcome of phylogenetic diversification within *Lucilia* (Williams *et al.*, 2016).

In conclusion, it was observed that the COI gene showed enough genetic diversity between the Indian species of genus *Lucilia* and thus could be used as a reliable marker for species delimitation. However, as *L. sericata* and *L. cuprina* showed a low intraspecific divergence, morphological characters such as male genitalia should be considered in such cases. A great deal of work is still required to build a reliable reference database for DNA-based identification of forensically important blowflies from different regions of India.

Table.1. NCBI GenBank accession numbers of species of the genus *Lucilia* used in our molecular analysis along with their distribution in India and across the globe, and "country of origin" of the sequence

No.	Species	Distribution in India	Global Distribution	Accession Numbers	"Country of origin" of the sequence
1.	*Lucilia sericata*	Widely distributed	Cosmopolitan	KX893339	India
2.	*Lucilia cuprina*	Widely distributed	Afrotropical, Australasian, Oriental, Palaearctic, Nearctic	KX053869	French Polynesia
3.	*Lucilia porphyrina*	Widely distributed	Australasian, Oriental, Palaearctic	KX893338	India
4.	*Lucilia bazini*	West Bengal, Himachal Pradesh, Punjab, Uttarakhand	Oriental, Palaearctic	PP980536	India
5.	*Lucilia illustris*	West Bengal	Oriental, Palaearctic, Nearctic	PP980525	India
6.	*Lucilia bismarckensis*	Rajasthan, Assam, West Bengal	Australasian, Oriental	JN014874	Malaysia
7.	*Lucilia papuensis*	Assam, Arunachal Pradesh, Jammu and Kashmir, Kerala, Meghalaya, Tamil Nadu, West Bengal	Australasian, Oriental	KX893335	India
8.	*Lucilia calviceps*	Himachal Pradesh	Australasian, Oriental	JN014875	Malaysia
9.	*Lucilia silvarum*	Jammu and Kashmir	Oriental, Palaearctic, Nearctic	OQ611440	Germany
10.	*Lucilia ampullacea*	Widely distributed	Palaearctic and Oriental	OR492603	India

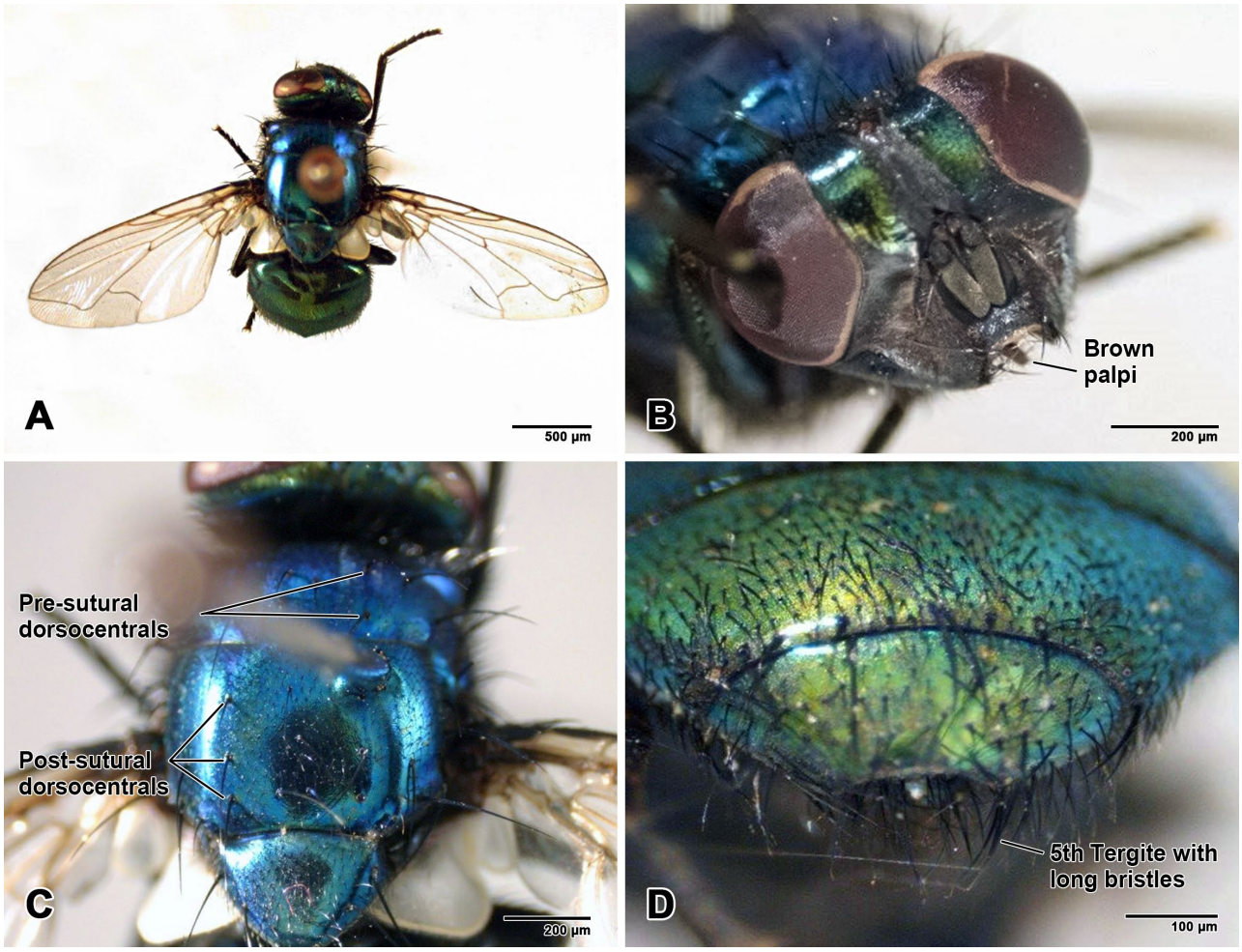

Fig. 2. *Lucilia silvarum* (A) habitus (B) head, frontal view (C) thorax showing two pre-sutural and three post-sutural dorsocentral setae (D) fifth tergite with long marginal bristles (Scale bar: A=0.5mm; B=0.2mm; C=0.2mm; D=0.1m

Table 2. Percentage intra- and interspecific pairwise sequence divergences of blowfly species occur in India.

	C. thanomthini	*L. porphyrina*	*L. bazini*	*L. calviceps*	*L. illustris*	*L. bismarckensis*	*L. papuensis*	*L. ampullacea*	*H. ligurriens*	*L. silvarum*	*L. cuprina*	*L. sericata*
Chrysomya thanomthini		90.8	90.8	90.8	90.56	91.19	90.35	90.4	91.51	89.82	90.58	91.75
Lucilia porphyrina	90.8		94.06	93.28	93.79	92.89	92.93	92.89	93.47	93.62	94.38	94.32
Lucilia bazini	90.8	94.06		94.78	94.68	94.01	94.16	94.16	93.18	93.01	93.01	93.65
Lucilia calviceps	90.8	93.28	94.78		96.81	96.82	96.33	97.15	92.41	93.16	93.31	94.01
Lucilia illustris	90.56	93.79	94.68	96.81		97.47	96.94	96.94	92.69	93.01	93.62	94.55
Lucilia bismarckensis	91.19	92.89	94.01	96.82	97.47		98.51	98.7	92.79	93.77	94.68	95.03
Lucilia papuensis	90.35	92.93	94.16	96.33	96.94	98.51		98.78	92.53	93.67	94.14	94.57
Lucilia ampullacea	90.4	92.89	94.16	97.15	96.94	98.7	98.78		92.48	93.31	93.62	94.29
Hemipyrellia ligurriens	91.51	93.47	93.18	92.41	92.69	92.79	92.53	92.48		93.16	94.53	95.11
Lucilia silvarum	89.82	93.62	93.01	93.16	93.01	93.77	93.67	93.31	93.16		96.05	96.35
Lucilia cuprina	90.58	94.38	93.01	93.31	93.62	94.68	94.14	93.62	94.53	96.05		99.39
Lucilia sericata	91.75	94.32	93.65	94.01	94.55	95.03	94.57	94.29	95.11	96.35	99.39	

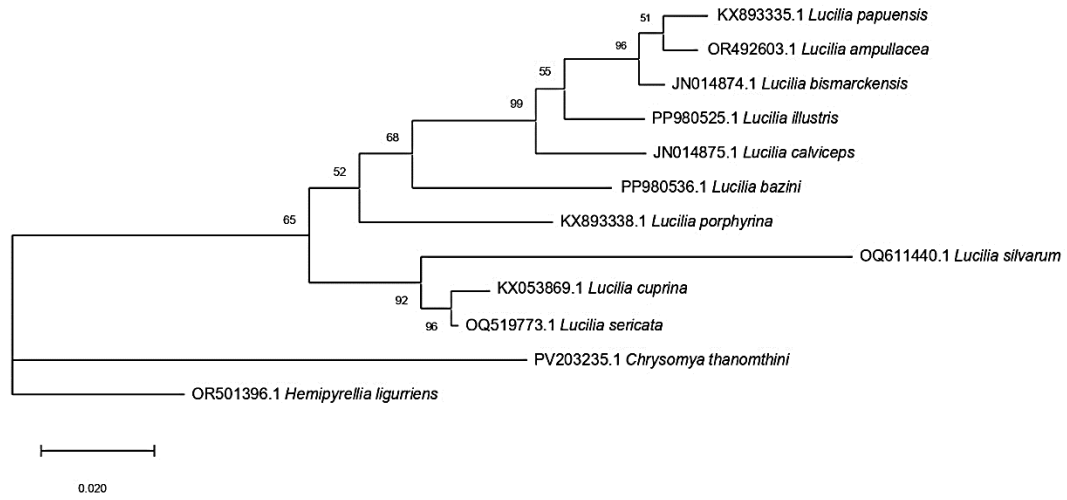

Fig. 3. Maximum Likelihood tree showing relationships among species of genus *Lucilia*. The tree was constructed using GTR+I model. The numbers near the nodes represent bootstrap value of each node in the tree.

Author's Contributions

Meenakshi Bharti: Conceptualization; methodology; formal analysis; investigation; draft preparation; final review and edit; visualization; supervision; project administration and funding acquisition. **Tanveer Ahmed Dar**: Methodology; formal analysis; investigation; draft preparation; final review and edit.

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Data Availability Statement

All data supporting the findings of this study are available within the paper. The sequence data obtained in this study were deposited in the GenBank database under accession numbers (PP980536, PP980525, OR492603). The specimens examined in this study are deposited in the first author's collection at the Department of Zoology & Environmental Sciences, Punjabi University, Patiala, Punjab, India.

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Ethics Approval and Consent to Participate

Insects were used in this study. All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by the author.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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Research Article

بارکدینگ DNA گونه‌های هندی جنس *Lucilia* (Dip., Calliphoridae) به همراه دو رکورد جدید کشوری

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چکیده: *Lucilia* جنسی از مگس‌ها است که باعث ایجاد میاز و تولید مثل در لاشه حیوانات می‌شود و همین امر آنها را به گروهی با اهمیت در دامپزشکی و پزشکی قانونی تبدیل کرده است. این جنس تاکنون در هند با ۸ گونه شناخته شده است. در اینجا، دو رکورد جدید شامل گونه های *Lucilia bismarckensis* Kurahashi و *Lucilia silvarum* Meigen برای هندوستان گزارش می‌شود و یک کلید شناسایی برای گونه‌های شناخته شده هندی جنس *Lucilia* ارائه گردیده است. توالی‌های زیر واحد ۱ سیتوکروم اکسیداز (COI) برای سه گونه تعیین گردید و این توالی ها به همراه هفت توالی متناظر این ناحیه ژنی که از بانک ژن اخذ شده بود با استفاده از آنالیز حداکثر احتمال، تجزیه و تحلیل شدند. این بررسی نشان داد که COI می‌تواند به عنوان یک نشانگر قابل اعتماد برای شناسایی مولکولی این گروه استفاده شود، زیرا گونه‌های مورد نظر، واگرایی بین گونه‌ای کافی را نشان دادند و کلادهای قابل اعتمادی تشکیل دادند.

کلمات کلیدی: شناسایی مولکولی ، حشره‌شناسی قانونی، *Lucilia bismarckensis*،*Lucilia silvarum*

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