9. Walkley, A. and Black, I. A., An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci*., 1934, **37**(1), 29–38.
10. Sankaram, A., *A Laboratory Manual for Agricultural Chemistry*, Asia Publishing House, Calcutta, India, 1966.
11. Olsen, S. R., Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture – Circular 939, 1954, pp. 1–9.
12. Schollenberger, C. J. and Simon, R. H., Determination of exchange capacity and exchangeable bases in soil – ammonium acetate method. *Soil Sci*., 1945, **59**(1), 13–24.
13. Vance, E. D., Brookes, P. C. and Jenkinson, D. S., An extraction method for measuring soil microbial biomass carbon. *Soil Biol. Biochem*., 1987, **19**, 703–707.
14. Alef, K., Soil respiration. In *Methods in Soil Microbiology and Biochemistry* (eds Alef, K. and Nannipieri, P.), Academic Press, London, UK, 1995, pp. 214–219.
15. Alef, K., Estimation of the hydrolysis of fluorescein diacetate. In *Methods in Soil Microbiology and Biochemistry* (eds Alef, K. and Nannipieri, P.), Academic Press, London, UK, 1995, pp. 232–233.
16. Brink Jr, Dubach, R. H. and Lynch, D. L., Measurement of carbohydrates in soil hydrolyzates with anthrone. *Soil Sci*., 1960, **89**, 157–166.
17. Kononova, M. M., *Soil Organic Matter: Its Nature, Its Role in Soil Formation and in Soil Fertility*, Pergamon Press, Oxford, London, 1966, 2nd edn, p. 554.
18. Prasad, J., Karmakar, S., Kumar, R. and Mishra, B., Influence of integrated nutrient management on yield and soil properties in maize–wheat cropping system in an Alfisol of Jharkhand. *J. Indian Soc. Soil Sci*., 2010, **58**(2), 200–204.
19. Shahina, T., Sammi Reddy, K., Vaishya, U. K., Singh, M. and Biswas, A. K., Changes in organic and inorganic forms of nitrogen in a Typic Haplustert under soybean–wheat system due to conjoint use of inorganic fertilizers and organic manures. *J. Indian Soc. Soil Sci*., 2010, **58**(1), 78–85.
20. Singh, R. N., Singh, S., Prasad, S. S., Singh, V. K. and Kumar, P., Effect of integrated nutrient management on soil fertility, nutrient uptake and yield of rice–pea cropping system on an upland acid soil of Jharkhand. *J. Indian Soc. Soil Sci*., 2011, **59**, 158–163.
21. Vipin Kumar and Singh, A. P., Long-term effect of green manuring and farmyard manure on yield and soil fertility status in rice–wheat cropping system. *J. Indian Soc. Soil Sci*., 2010, **58**, 409–412.
22. Bhandari, A. L., Walia, S. S. and Singh, T., Production sustainability of maize-wheat system in a Typic Ustipsamment soil as influenced by integrated nutrient sources. In Proceedings of International Conference on Managing Natural Resources for Sustainable Agriculture Production in the 21th Century, New Delhi, 2000, vol. 3, pp. 889–890.
23. Sawarkar, S. D., Khamparia, N. K., Thakur, R., Dewda, M. S. and Singh, M., Effect of long-term application of inorganic fertilizers and organic manure on yield, potassium uptake and profile distribution of potassium fraction in vertisol under soybean–wheat cropping system. *J. Indian Soc. Soil Sci*., 2013, **61**(2), 94–98.
24. Fereidooni, M., Raiesi, F. and Fallah, S., Ecological restoration of soil respiration, microbial biomass and enzyme activities through broiler litter application in a calcareous soil cropped with silage maize. *Ecol. Eng*., 2013, **58**, 266–277.
25. Kalaiyarasan, C. and Vaiyapuri, V., Effect of integrated nutrient management practices on seed yield and quality characters of sunflower (*Helianthus annuus* L.). *Int. J. Agric. Sci*., 2008, **4**(1), 231–233.
26. Raju, B., Rao, P. C., Reddy, A. and Rajesh, K., Effect of INM on nutrient uptake and seed yield in safflower. *Ann. Biol. Res*., 2013, **4**(7), 222–226.

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Genetic lineage of *Zeugodacus caudatus* (Diptera: Tephritidae) detected with *mtCOI* gene analysis from India

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***Zeugodacus caudatus* (Fabricius) is a pest of cucurbit plants. The present study was conducted to draw the relationship among Indian *Z. caudatus* populations with the other defined genetic lineage of the species. A total of 18 individuals' *mtCOI* gene sequences from 3 populations of India were analysed along with 34 individuals' *mtCOI* gene sequences from Malaysia, Indonesia, Thailand and China and generated 14 haplotypes. Phylogenetic study revealed the presence of distinct genetic lineage in *Z. caudatus* populations collected from India. The genetic distance between three distinct lineages of *Z. caudatus* was 0.057, 0.055 and 0.018 between Indonesia and Malaysia, India and**

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Indonesia, and India and Malaysia, respectively and also evident from phylogenetic analysis. Further, the mitochondrial cytochrome oxidase I (COI) gene sequences developed in this study will help detection and geographical distribution of new haplotypes and lineages of the species in future.

Keywords: Dacinae, fruit fly, haplotypes, population genetics.

TEPHRITID fruit flies are the most serious insect pests of horticultural crops causing enormous economic losses every year throughout the tropical and subtropical regions of the world[1–3].

The family Tephritidae comprises over 4448 species distributed in more than 481 genera, of which 800 species belong to the subfamily Dacinae[4–6]. Tropical Asia, Australia and South Pacific regions are the native places of *Bactrocera* and *Zeugodacus* species of subfamily Dacinae; whereas Africa and warm temperate areas of Europe and Asia are the home of few other species of these genera. Fruit fly species of the genus *Bactrocera* and *Zeugodacus* are pests of polyphagous plant having extensive distribution and wide range of climatic adaptability with high flying capacity and population potential and cause high economic losses in horticultural crops[7,8]. Members of subgenus *Zeugodacus* of the genus *Bactrocera* are mostly pest of cucurbits plants. Recently, *Zeugodacus* was elevated to the level of genus[9]. There are many pest species in the genus *Zeugodacus*: *Zeugodacus cucurbitae* (Coquillett), *Zeugodacus tau* (Walker), *Zeugodacus scutellaris* (Bezzi) and *Zeugodacus caudatus* (Fabricius)[3,10–12].

Zeugodacus caudatus (Fabricius) is presently renamed from *Bactrocera maculipennis* Doleschall, *Bactrocera caudata* (Fabricius) and *Chaetodacus caudatus* Fabricius[10,11]. *Z. caudatus* is identified with predominantly black scutum, yellow medial and lateral stripes on scutum; black line across mouth opening on the face and a coastal band of the wings expanded into spot apically[10].

Z. caudatus is distributed in Palearctic and Oriental regions of the world[13]. Its presence has also been reported from Brunei, China, India, Indonesia, Nepal, Malaysia, Myanmar, Taiwan, Thailand, Sri Lanka and Vietnam[1,10,13]. However, it had not been recorded from Australasian and Oceania regions[6,13,14]. *Z. caudatus* infests mainly the flowers of cucurbit plant.

Very limited genetic information of *Z. caudatus* is available between geographical regions of its range of distribution in comparison to other species of the genus, viz. *Z. cucurbitae* and *Z. tau*[12,15–18]. Recently, Lim *et al*.[13] suggested that distinct genetic lineages are present in *Z. caudatus* specimens collected from Malaysia and Indonesia with the analysis of *mtCOI* and *16S rDNA* gene sequences. Further, Yong *et al*.[14] reported that *Z. caudatus* population of the northern hemisphere (*Z. caudatus* samples from Malaysia and Thailand) was different from the southern hemisphere (*Z. caudatus* samples from Indonesia) with multigene phylogenetic analysis.

Precise resolution on species identification and characterization of different complex species/forms is often missing through morphological differences[17]. It can be enhanced by integration of molecular biology, i.e. DNA barcode using *mtCOI* gene sequences[17]. Phylogeographic structures and intra- and inter-specific relationships of different insect species including species of genus *Bactrocera* and *Zeugodacus* have been widely determined through robust evolutionary mitochondrial DNA based markers (COI gene sequences)[16–22]. The present study was conducted to examine the genetic variability and lineage of *Z. caudatus* species present in India using *mtCOI* gene sequencing and to determine their association in the context of divergent genetic lineages (cryptic species/sibling species) present in *Z. caudatus* populations.

Adult males of *Z. caudatus* were collected from three distant locations in India, viz. Mumbai (Maharashtra), Ranchi (Jharkhand) and Bhagalpur (Bihar) during 2012–2015. The individual of *Z. caudatus* adult fly was preserved in a separate vial with 800 µl of 95% ethyl alcohol at –25°C until genomic DNA isolation. Fruit fly specimens were identified as *Z. caudatus* based on available literature[1,23] and also the identity of specimens as *Z. caudatus* was confirmed by the fruit fly taxonomist M. L. Agarwal (Department of Entomology, Dr Rajendra Prasad, Central Agricultural University, Pusa, Bihar).

DNA (genomic) of individual *Z. caudatus* was isolated from the preserved specimens using the cetyl trimethyl ammonium bromide (CTAB) method[24]. Each fly was crushed into fine powder individually in a sterile mortar and pestle. The material was then transferred to a microcentrifuge tube containing 300 µl of pre-heated (60°C) CTAB buffer made up of 1 M tris HCl pH 8.0, 5% CTAB, 5 M NaCl, 0.5 M EDTA pH 8.0 and 4% β-mercaptoethanol. The preparation was then incubated at 60°C for 30 min with gentle mixing at regular intervals. After incubation, 300 µl of premixed solution of chloroform and isoamyl alcohol (24 : 1) were added, mixed properly by inverting the tube several times and then the tube were centrifuged at 8000 rpm for 10 min to remove the aqueous phase. The aqueous phase of the content in tube was transferred to a new microcentrifuge tube and 150 µl pre-chilled isopropanol was added. Microcentrifuge tubes were kept for 20–30 min at –20°C for the precipitation of genomic DNA. Then, the tubes were centrifuged at 12,000 rpm for 12 min and the supernatant was decanted. The DNA pellet present in tubes was washed with 70% pre-chilled ethyl alcohol, kept for drying and then dissolved in 30 µl of Tris EDTA buffer (10 mM Tris HCl pH 8.0 and 1 mM EDTA) and stored at –20°C.

Primer pair UEA7 5′ TACAGTTGGAATAGACGTTGATAC 3′ (forward) and UEA10 5′ TCCAATGCACTAATCTGCCATATTA 3′ (reverse) for the amplification of mitochondrial *COI* gene developed by Lunt *et al.*[25] were

Table 1. Sample size (*n*), number of unique haplotypes (*H*), haplotype diversity (*Hd*), nucleotide diversity (π), average number of nucleotide differences (*K*), number of variable sites (*V*), uncorrected average pairwise distances between samples (*P*) and per cent nucleotide composition for *mtCOI* gene for different populations of *Zeugodacus caudatus*

Countries	*n*	*H*	*Hd*	π	*K*	*V*	*P* ± SE	%T	%C	%A	%G	Accession number
India	18	10 (H1–H10)	0.90196	0.00477	3.03922	15	0.005 ± 0.001	35.8	20.3	30.4	13.5	KT989670–KT989677, KU041685–KU041688, MF038800–MF038805
Malaysia*	24	2 (H11 & H13)	0.08333	0.00013	0.00013	1	0.000 ± 0.000	35.8	20.4	30.8	13.0	JN542416, JN542417, KP694327, FJ903493
Indonesia*	7	1 (H12)	0.00000	0.00000	0.00000	0	0.000 ± 0.000	34.9	20.9	30.3	14.0	JN542418, JN542419
China*	1	1 (H11)	–	–	–	n/c	n/c	35.8	20.4	30.8	13.0	GQ458048
Thailand*	2	2 (H11 & H14)	1.00000	0.00785	5.00000	5	0.008 ± 0.003	35.8	20.4	30.7	13.1	KP694328, AF423109

**mtCOI* gene sequences obtained from GenBank, NCBI; n/c, not calculated.

used to perform the polymerase chain reaction (PCR). The DNA amplification was carried out in 0.2 ml micro tubes with 20 µl reaction volume containing 2 µl 10× reaction buffer with 25 mM $MgCl_2$, 0.5 µl 10 mM $dNTP_S$, 1 µl 20 pmol of each primer, 0.2 µl 5U/µl *Taq* polymerase (all manufactured by HI-MEDIA India, Mumbai) and 2 µl 10 ng of DNA template. Amplification was carried out in Flexigene 9700 thermal cycler (QIAGEN India) with an initial DNA denaturation for 3 min at 94°C, followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and a final step of elongation at 72°C for 30 min. The amplification of targeted DNA fragment was confirmed with separation of PCR product in 2% (w/v) agarose gel using DNA electrophoresis with TAE buffer (1 mM EDTA, 40 mM Tris-acetate). Freeze-dried PCR products of *mtCOI* gene were custom sequenced (ABI PRISM 310™ Genetic Analyser, Applied Biosystems, USA) using the same primers pair (Xcelris Labs Limited, India and Scigenome, Kochi, India). Unique *mtCOI* gene sequences of *Z. caudatus* were deposited in GenBank with accession no. KT989670-KT989677 (Ranchi, India), KU041685-KU041688 (Bhagalpur, India), MF038800-MF038805 (Mumbai, India).

The 637-bp portion of *mtCOI* gene sequences from 52 *Z. caudatus* (18 *mtCOI* gene sequences from this study and 34 *mtCOI* gene sequences obtained from NCBI comprised of *Z. caudatus* populations from 5 countries) was aligned in MEGA ver. 6.0 software using ClustalW program[26]. Descriptive statistics, viz. haplotype diversity (*Hd*), number of haplotypes (*H*), average number of nucleotide difference (*K*) and nucleotide diversity (π) were calculated using Dnasp version 5.0 software[27].

A median-joining haplotype network was constructed to depict the evolutionary and geographical relationships among haplotypes using NETWORK version 4.6 software[28]. *Z. caudatus* mitochondrial haplotypes were colour coded with the country of origin of specimens to know the geographical genetic relationship among the population.

Pair-wise distance measurements between individual sequences of *Z. caudatus* were performed using the *p* genetic distance model implemented in MEGA 6.0 software. *p* genetic distance model was chosen for these analyses with GenBank sequences which specifically addressed the level of divergence at the *mtCOI* gene of *Z. caudatus*. The phylogenetic relationships were established between different lineages of *Z. caudatus* with minimum evolution[29], maximum likelihood and neighbour-joining methods[30] using Kimura-2 parameters[31] as estimate of genetic divergence with *Zeugodacus cucurbitae* (HQ378218) and *Zeugodacus tau* (HQ378233) *mtCOI* sequences as outgroup species using MEGA 6.0 software. Branch truthness of phylogenetic trees constructed with different methods was assessed with bootstrap test[32] (1000 replications).

All 52 *mtCOI* gene sequences were aligned and used in the genetic analysis of *Z. caudatus* comprising seven populations from five countries of Asia (Table 1). The annotated final length of *mtCOI* gene sequences was 637 bp. The averaged *mtCOI* gene sequences' nucleotide composition was 35.7% T, 30.6% A, 13.3% G and 20.4% C with 45 parsimony informative sites, 5 singleton sites and a total of 50 variable sites. Basic descriptive genetic diversity results obtained from populations of five countries of *Z. caudatus* are presented in Table 1. The haplotype numbers per population (*H*) ranged from 1 to 10. Haplotype diversity (*Hd*) and nucleotide (π) diversity ranged from 0 to 1.0 and 0 to 0.00785 respectively. Highest *Hd* was found in *Z. caudatus* population of Thailand (1.0) followed by India (0.90196).

A total of 14 unique haplotypes from 52 individual sequences of 7 populations were identified from 3 geographic regions of Asia, viz. India, Malaysia and Indonesia (Table 1). Six haplotypes were shared by at least 2 individuals of *Z. caudatus* from the same or different populations and 8 haplotypes composed of only single individual sequences from 14 identified haplotypes. The most common haplotype was H11 followed by H12, H2 comprised of 25, 7 and 5 individuals respectively, from different populations of *Z. caudatus*. Haplotype 11 was shared by 3 populations of Malaysia, China and Thailand whereas haplotype H12 was found in only in Indonesian

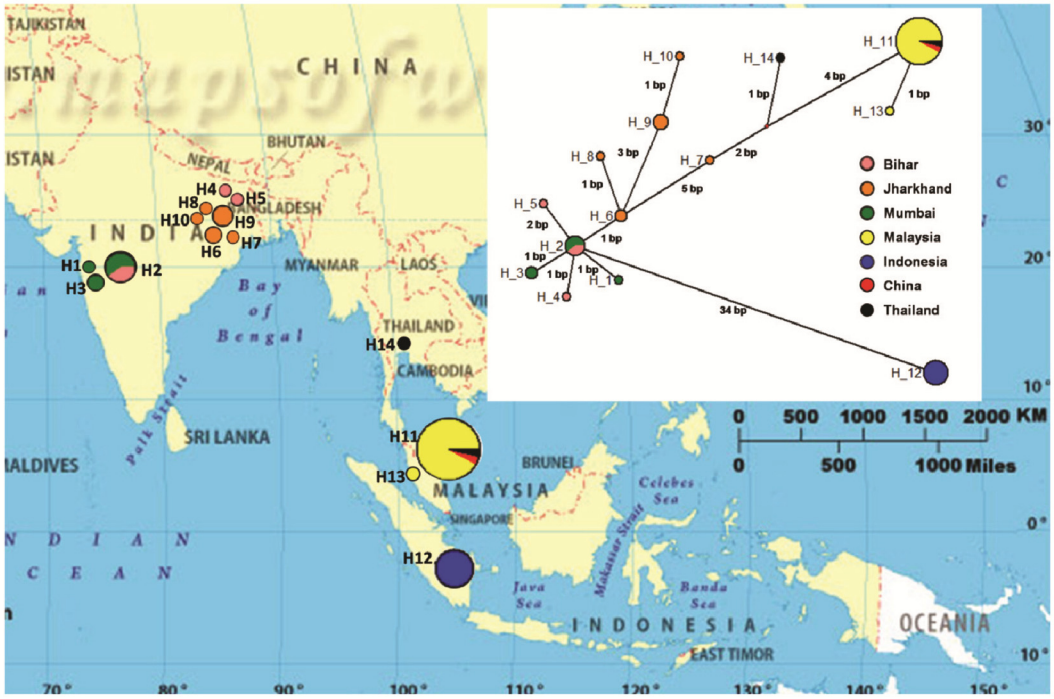

Figure 1. Geographical distribution and median joining network of haplotypes detected in *Zeugodacus caudatus* populations from different countries. Each circle represents a haplotype, and circle diameter is relative to haplotype frequency. Colours represent the geographical origin of the specimens.

population of *Z. caudatus*. Haplotype 2 composed of five individuals shared by two populations from India, i.e. Mumbai and Bhagalpur. When we compared the endemic/private haplotypes formation in each population of different regions, populations with a higher degree of endemic/private haplotypes formation were from India (10 haplotypes from 18 individuals) and Malaysia (2 haplotypes from 24 individuals). Only one haplotype was detected from seven individuals of *Z. caudatus* from Indonesia.

The *mtCOI* median-joining network generated using NETWORK program displayed a simple genealogy of *Z. caudatus* for the entire population set of three major populations, i.e. India (Mumbai, Bhagalpur and Ranchi), Malaysia and Indonesia (Figure 1). Indian populations of *Z. caudatus* were found as connecting link between the *Z. caudatus* population of Malaysia and Indonesia (Figure 1). Most of the haplotypes detected in the present study were region-specific, i.e. haplotypes H1-H10 were from the Indian region of *Z. caudatus*. Whereas H11-H13 were from Malaysia, Thailand and China. Haplotype H14 was from Indonesian population of *Z. caudatus*.

Phylogenetic relationships were constructed with sequences available in GenBank and the *Z. caudatus* sequences generated in this study. Most of the Indian specimens of *Z. caudatus* form separate clades in the phylogenetic tree from other *Z. caudatus* lineages described earlier from Malaysia and Indonesia with high bootstrap support value (99) reconstructed with three different methods (Figures 2–4). The *p* genetic distance among isolates of *Z. caudatus* from different countries varies from 0.004 to 0.059 (Table 2). Highest *p* genetic distances were found between the populations of Indonesia and other countries isolates of *Z. caudatus*. Also, the *p* genetic distance between different lineages of *Z. caudatus* varied from 0.018 to 0.059 (Table 3).

Z. caudatus is a pest of cucurbit plants infesting mostly flowers and immature fruits of the plant[10]. The species from genus *Bactrocera* and *Zeugodacus* of the family Tephritidae formed many species complexes[6,33]. Morphological characteristics sometimes have proven to be of limited help, particularly in identifying the sibling/cryptic species present in the species complex[14]. An earlier study on the genetic relationship of *Bactrocera* species from India to other countries showed similarity with the reported sibling/cryptic species from other parts of the world[17]. The present study reports the presence of distinct genetic lineage of *Z. caudatus* in India on the basis of *mtCOI* gene sequences. Genetically, *Z. caudatus* forms two different lineages present separately in two different hemispheres of the world[14]. Indian populations of *Z. caudatus* form a separate clade in the phylogenetic reconstruction by different methods with earlier studied population of *Z. caudatus* from Indonesia and Malaysia including China and Thailand[13,14] with *mtCOI* gene sequences and robust bootstrap support. *mtCOI* gene has already been in use to study and analyse the genetic diversity and resolution of cryptic species complex

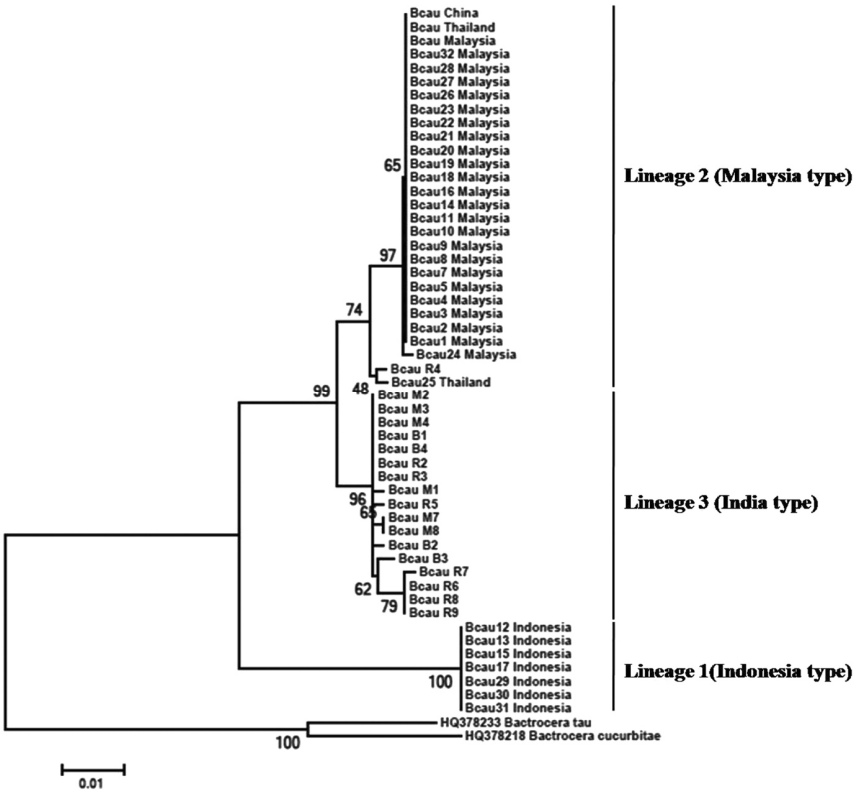

Figure 2. Phylogenetic tree constructed with minimum evolution method[29] for the lineages present in *Zeugodacus caudatus* populations. Values near nodes present the boot strap support value. Sequence data of *Zeugodacus tau* and *Zeugodacus cucurbitae* are used as out groups.

Figure 3. Phylogenetic tree constructed with maximum likelihood method based on Kimura-2 parameter model[31] for the lineages present in *Zeugodacus caudatus* populations. Values near nodes present the boot strap support value. Sequence data of *Zeugodacus tau* and *Zeugodacus cucurbitae* are used as out groups.

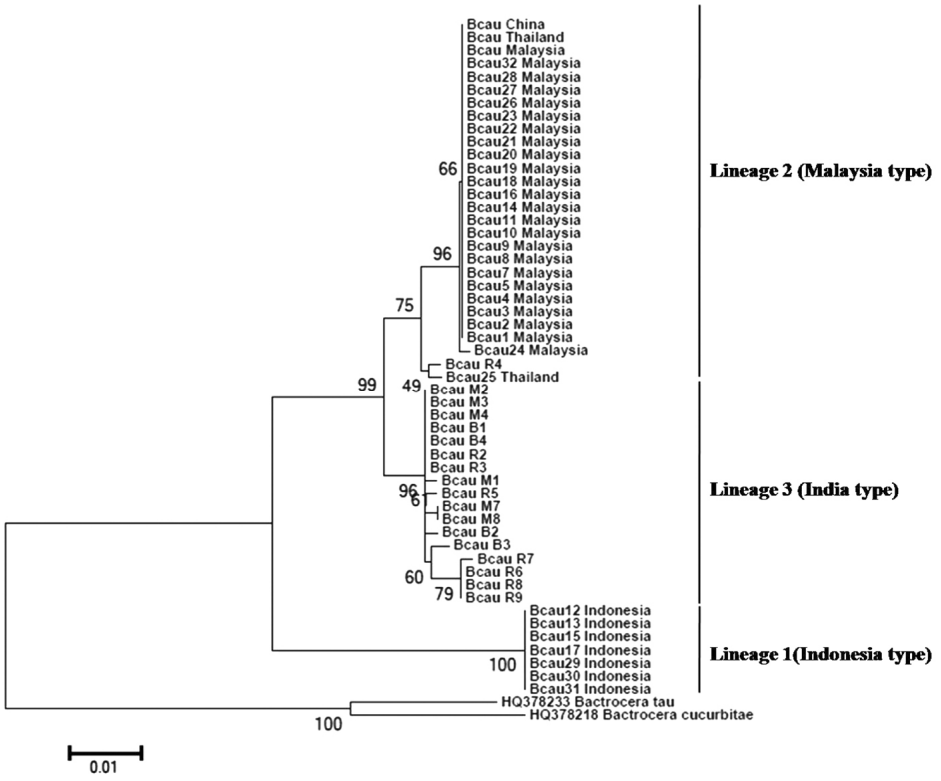

Figure 4. Phylogenetic tree constructed using neighbor-joining method[30] with Kimura-2 parameters[31] for the lineages present in *Zeugodacus caudatus* populations. Values near nodes present the boot strap support value. Sequence data of *Zeugodacus tau* and *Zeugodacus cucurbitae* are used as out groups.

Table 2. Estimates of evolutionary divergence (*p* distance) between *Zeugodacus caudatus* populations from various geographical regions

	p distance				
Populations of *B. caudata*	India	Malaysia	China	Thailand	Indonesia
India	**0.0048**	0.005	0.005	0.004	0.009
Malaysia	0.018	**0.0001**	0.000	0.002	0.009
China	0.018	0.000	**n/c**	0.002	0.009
Thailand	0.015	0.004	0.004	**0.0078**	0.009
Indonesia	0.056	0.056	0.057	0.059	**0.000**

p distance between group is shown below the diagonal. Standard error estimate(s) are shown above the diagonal. The analysis involved 52 nucleotide sequences. Within group *p* distance are shown in diagonal with bold letter.

among tephritid fruit flies[7,12,15,16,18,19,21]. Distinct genetic lineage has already been reported from *Z. tau* and *Zeugodacus ascita* based on *mtCOI* gene sequences[18,34]. In the present study, we designated here the three distinct genetic lineages of *Z. caudatus* as lineage 1: Indonesia type, lineage 2: Malaysia type and lineage 3: India type. Lineage 2, i.e. Malaysia type was also comprised of *B. caudatus* populations from Thailand, China and one individual from India (Ranchi). The presence of distinct genetic lineage in Indian population of *Z. caudatus* was also confirmed by *p* value of genetic distances generated for the whole population in this study. The *p* genetic distance value of *Z. caudatus* of India and Indonesia was 0.056, whereas India and Malaysia, including China and Thailand, varied from 0.015 to 0.018. The calculated *p* distance among a population of *Z. caudatus* is similar to the earlier studied population of *Z. caudatus*[13,14]. This can also be reproduced with the mitochondrial haplotype network construction among individuals of *Z. caudatus*. Median-joining network clearly showed the presence of different genetic lineage in *Z. caudatus* population from India (Figure 1). The Indian population of *Z. caudatus* shared 10 haplotypes out of total 14 haplotypes detected in the global population analysis. Earlier, Lim *et al.*[13] detected only two *mtCOI* gene haplotypes in *Z. caudatus* population collected from Malaysia and Indonesia.

Table 3. Estimates of evolutionary divergence (*p* distance) between lineages of *Zeugodacus caudatus*

Lineages of *B. caudata*	*p* distance		
	Lineage 1	Lineage 2	Lineage 3
Lineage 1 (Indonesia Type)	**0.000 ± 0.000**	0.056 ± 0.008	0.053 ± 0.008
Lineage 2 (Malaysia Type)	0.057 ± 0.009	**0.001 ± 0.000**	0.015 ± 0.005
Lineage 3 (India Type)	0.055 ± 0.005	0.018 ± 0.009	**0.004 ± 0.001**

Uncorrected *p* distance with standard error between lineage are shown below the diagonal. Corrected *p* distance with standard error between lineages are shown above the diagonal. The analysis involved 52 nucleotide sequences. Within lineage *p* distance is shown in diagonal with bold letter.

Further, two more *mtCOI* gene haplotypes were reported by Yong *et al.*[14] in *Z. caudatus* population studied from Indonesia, Malaysia, China and Thailand. Yong *et al.*[14] also reported that *Z. caudatus* lineages present in the northern and southern hemispheres are distinct from each other. The present analysis also supports Yong *et al.*[14] report on the presence of distinct lineages of *Z. caudatus* in the northern and southern hemisphere of the world, as no one haplotype was found similar to the haplotype present in the population of *Z. caudatus* from the southern hemisphere (Indonesia). Similar reports on the presence of distinct lineages, species complex and cryptic species/ sibling species in the same species of fruit fly with *mtCOI* gene sequences have also been established earlier. Overall 8 species/subspecies have been reported in the species complex of *Z. tau* around the world[15]. With the result of present study and support from the earlier studies[13,14], we suggest presence of three sub-species/type in *Z. caudatus* species complex localized in different parts of the world and can be recognized as lineage 1: Indonesia type, lineage 2: Malaysia type and lineage 3: India type. Formation of distinct genetic lineages may be the result of isolation of different geographical population of species long back after origin. The notion is supported by the biology of the insect as adults of these flies breed on the flower of cucurbit plants and movement of larvae with flowers to long distances is not possible even through human-mediated transportation. Earlier, many fruit fly species which infested fruits of the host plants originated at one place and are currently distributed in many countries of the world without forming genetically distinct lineage has been explained that the large distance movement of the species was through human-mediated activities as fruits were used to carry by human during travelling to different places[16,18].

In conclusion, *Z. caudatus* populations present in India are genetically different from the *Z. caudatus* populations present in other parts of the world, thus revealing the presence of sibling species/cryptic species in *Z. caudatus*. The present study supports earlier reports on *Z. caudatus* that more distinct lineages may be present in other regions of the world which need to be studied. Also, the data generated in this study would be supportive in the future study on *Z. caudatus* species complex.

1. White, I. M. and Elson-Harris, M. M., *Fruit Flies of Economic Significance: Their Identification and Bionomics*, CAB International, Wallingford, UK, 1992, p. 601.
2. Clarke, A. R. *et al*., Invasive phytophagous pests arising through a recent tropical evolution radiation: The *Bactrocera dorsalis* complex of fruit flies. *Annu. Rev. Entomol.*, 2005, **50**, 293–319.
3. Prabhakar, C. S. *et al*., Distribution and developmental biology of fruit flies infesting cucurbits in north-western Himalaya. *J. Ins. Sci*., 2009, **22**, 300–308.
4. Agarwal, M. L. and Sueyoshi, M., Catalogue of Indian fruit flies (Diptera: Tephritidae). *Orient. Insects*, 2005, **39**, 371–433.
5. Fletcher, B. S., The biology of Dacine fruit flies. *Annu. Rev. Entomol.*, 1987, **32**, 115–144.
6. Drew, R. A. I., The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. *Mem. Queensland Mus*., 1989, **26**, 1–521.
7. Muraji, M. and Nakahara, S., Phylogenetic relationships among fruit flies, *Bactrocera* (Diptera, Tephritidae), based on the mitochondrial rDNA sequences. *Ins. Mol. Biol*., 2001, **10**, 549–559.
8. Prabhakar, C. S., Biodiversity of fruit flies (Tephritidae: Diptera) and utilization of gut bacteria in their management. Ph D thesis, CSK Himachal Pradesh Krishi Vishavavidyalaya, Palampur, Himachal Pradesh, India, 2011.
9. De Meyer, M. *et al*., A review of the current knowledge on *Zeugodacus cucurbitae* (Coquillett) (Diptera, Tephritidae) in Africa, with a list of species included in *Zeugodacus*. *Zoo Keys*, 2015, **540**, 539–557.
10. Kapoor, V. C. *et al*., Taxonomy and biology of economically important fruit flies of India. *Isr. J. Entomol*., 2005, **35–36**, 459–475.
11. Prabhakar, C. S. *et al*., Fruit fly, *Bactrocera scutellaris* (Bezzi): a potential threat to cucurbit cultivation under low and mid hills of Himachal Pradesh. *Pest Manage. Econ. Zool*., 2007, **15**, 181–185.
12. Hu, J. *et al*., Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae), from China and Southeast Asia. *Genetica*, 2008, **134**, 319–324.
13. Lim, P.-E. *et al*., Distinct genetic lineages of *Bactrocera caudata* (Insecta: Tephritidae) revealed by *COI* and *16S* DNA sequences. *PLoS ONE*, 2012, **7**, e37276.
14. Yong, H.-S. *et al*., Multigene phylogeography of *Bactrocera caudata* (Insecta: Tephritidae): Distinct genetic lineages in Northern and Southern Hemispheres. *PLoS ONE*, 2015, **10**, e0129455.
15. Jamnongluk, W. *et al*., Molecular phylogeny of tephritid fruit flies in the *Bactrocera tau* complex using the mitochondrial COI sequences. *Genome*, 2003, **46**, 112–118.
16. Prabhakar, C. S. *et al.*, Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) based on mitochondrial cytochrome oxidase (COI) gene sequences. *Genetica*, 2012, **140**, 83–91.

17. Prabhakar, C. S. *et al*., Population genetic structure of the pumpkin fruit fly, *Bactrocera tau* (Walker) (Diptera: Tephritidae) in Himachal Pradesh, India. *Biochem. Syst. Ecol*., 2013, **51**, 291–296.
18. Boontop, Y. *et al.*, Signatures of invasion: using an integrative approach to infer the spread of melon fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae), across Southeast Asia and the West Pacific. *Biol. Invasions*, 2017, doi:10.1007/s10530-017-1382-8.
19. Smith, P. T. *et al*., Phylogenetic relationships among *Bactrocera* species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol*., 2003, **26**, 8–17.
20. Nardi, F. *et al*., Population structure and colonisation history of the olive fly *Bactrocera oleae*. *Mol. Ecol*., 2005, **14**, 2729–2738.
21. Wan, X. *et al.*, The oriental fruit fly, *Bactrocera dorsalis*, in China: origin and gradual inland range expansion associated with population growth. *PLoS ONE*, 2011, **6**, e25238.
22. Choudhary, J. S. *et al.*, Genetic analysis of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) populations based on mitochondrial *cox1* and *nad1* gene sequences from India and other Asian countries. *Genetica*, 2016, **144**, 611–623.
23. Drew, R. A. I. and Raghu, S., The fruit fly fauna (Diptera: Tephritidae: Dacinae) of the rainforest habitat of the Western Ghats,
India. *Raffles Bull. Zool.*, 2002, **50**, 327–352.
24. Augustinos, A. A. *et al.*, Detection and characterization of *Wolbachia* infections in natural populations of aphids: Is the hidden diversity fully unraveled? *PLoS ONE*, 2011, **6**, e28695.
25. Lunt, D. H. *et al.*, The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Ins. Mol. Biol*., 1996, **5**, 153–165.
26. Tamura, K., MEGA6: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., 2013, **30**, 2725–2729.
27. Librado, P. and Rozas, J., DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 2009, **25**, 1451–1452.
28. Bandelt, H. J. *et al*., Median-joining networks for inferring intra-specific phylogenies. *Mol. Biol. Evol*., 1999, **16**, 37–48.
29. Rzhetsky, A. and Nei, M., A simple method for estimating and testing minimum evolution trees. *Mol. Biol. Evol.*, 1992, **9**, 945–967.
30. Saitou, N. and Nei, M., The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*., 1987, **4**, 406–425.
31. Kimura, M., A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*., 1980, **16**, 111–120.
32. Felsenstein, J., Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 1985, **39**, 783–791.
33. Baimai, V., Cytological evidence for a complex of species within the taxon *Bactrocera tau* (Diptera: Tephritidae) in Thailand. *Biol. J. Linn. Soc*., 2000, **69**, 399–409.
34. Jamnongluk, W. *et al*., Molecular evolution of tephritid fruit flies in the genus *Bactrocera* based on the cytochrome oxidase I gene. *Genetica*, 2003, **119**, 19–25.

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