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DNA BARCODE FOR THE PARTHENOGENETIC SURINAM COCKROACH *Pycnoscelus surinamensis* (*Linnaeus, 1758*) (*Blattodea: Blaberidae*) FROM INDIA

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ABSTRACT

DNA Barcoding is one of the emerging tools in molecular identification of faunal diversity, specifically insect fauna. The Surinam cockroach, *Pycnoscelus surinamensis* is the only known roach to be obligatorily parthenogenetic, with reported haplotypes. *P. surinamensis* is well established in Indomalayan, tropical and subtropical regions and substantially documented from India with a phenetic approach. Herewith we report the first set of mt DNA barcode from a vouchered collection for the species from southern Western Ghats India. Discussions are made on the identity of two sequences each of *Blatteria* species and *Pycnoscelus* species reported from USA.

Contribution/Originality: This study contributes to the existing literature of *Pycnoscelus surinamensis* species from India with additional DNA barcode data from vouchered specimens for the species as well as the genus from the Western Ghats. Barcodes linked with the vouchered specimens forms the authenticated DNA reference library having utility in metabarcoding.

1. INTRODUCTION

In the trending era of 'DNA barcoding', the utility of short DNA stretches manifesting nucleotides divergence amongst species renders an intangible aid for precise identification and documentation of various life forms [1-3]. This nucleotide-based taxonomic research tool [3] have remarkably increased the pace of taxonomic discoveries facilitating access to Linnaean taxonomy for the experts and non-experts (for e.g. [4, 5]). Additionally, its usage has allowed the study of multifaceted heterogeneous questions outlining numerous paradigms [6]. Hebert's group [1] suggested the mitochondrial Cytochrome C oxidase subunit I gene (mt COI) as a barcode gene for the delimitation of animal taxa and it is widely accepted for delimitation of different invertebrate groups including Blattodea [1, 2]. Identification based on DNA barcodes solemnly depends on the DNA barcode reference library, whose caliber increases when the DNA barcodes are affiliated to a taxonomically identified vouchered specimen.

The order Blattodea is composed of roaches and termites [2, 7] of which roaches have a cosmopolitan distribution. Cockroaches are among the highly diverse insect groups with 4600 species/subspecies globally and 181 species in India [8] of which only 33 species (~18%) have DNA barcode data. The genus *Pycnoscelus* Scudder, 1862 is denoted by 15 species of which four species are known from India, *Pycnoscelus indicus* [9]; *Pycnoscelus nigra* [10]; *Pycnoscelus surinamensis* [11] and *Pycnoscelus tenebrigera* [12-14].

The species *Pycnoscelus surinamensis* belongs to the second largest cockroach family Blaberidae . It is an introduced species to India identified to have medical and economic importance [15, 16], native to the Sunda

Islands of the Malaya Archipelago with a predominant range of distribution in the Indomalayan region [16, 17]. The species has recently invaded large parts of the tropical as well as subtropical regions of the world due to human intrusions [15-17]. *P. surinamensis* is reported to have many parthenogenetic clonal forms from different regions including Florida, Australia, Panama, Hawaii, and Indonesia [18, 19]. The Surinam cockroach is known as peridomestic species worldwide [15, 17] and acts as plants pest, biological vector, and is known for ecological associations [15].

The species *P. surinamensis* is thought to have evolved from its bisexual intimate progenitor *P. indicus* for rapid establishment [16, 18, 19]. GenBank searches suggest no DNA Barcode data is available for the members of *Pycnoscelus* from India. Hence, as a part of our exercise to generate mt DNA barcodes and documentation of species diversity from the Western Ghats, we report the first mt DNA barcode for the species *P. surinamensis* from southern Western Ghats, India with a vouchered specimen deposited at the National Zoological Collections (NZC).

2. MATERIAL AND METHODOLOGY

Three specimens were collected below the leaf litter during the daytime from the Courtallam forest (N 8.9331; E 77.2674), Tamil Nadu, India on 26.viii.2019 by K.P. Dinesh and Party. Specimens were fixed and preserved in 70% alcohol for further studies. Images were procured using DSLR camera Figure 1. For molecular studies, tissue was derived from leg and thoracic muscles. Studied examples are deposited in the National repository of Zoological Survey of India, Western Regional Centre, Pune, Maharashtra, India with the register number ZSI/WRC/Ent-12/81.

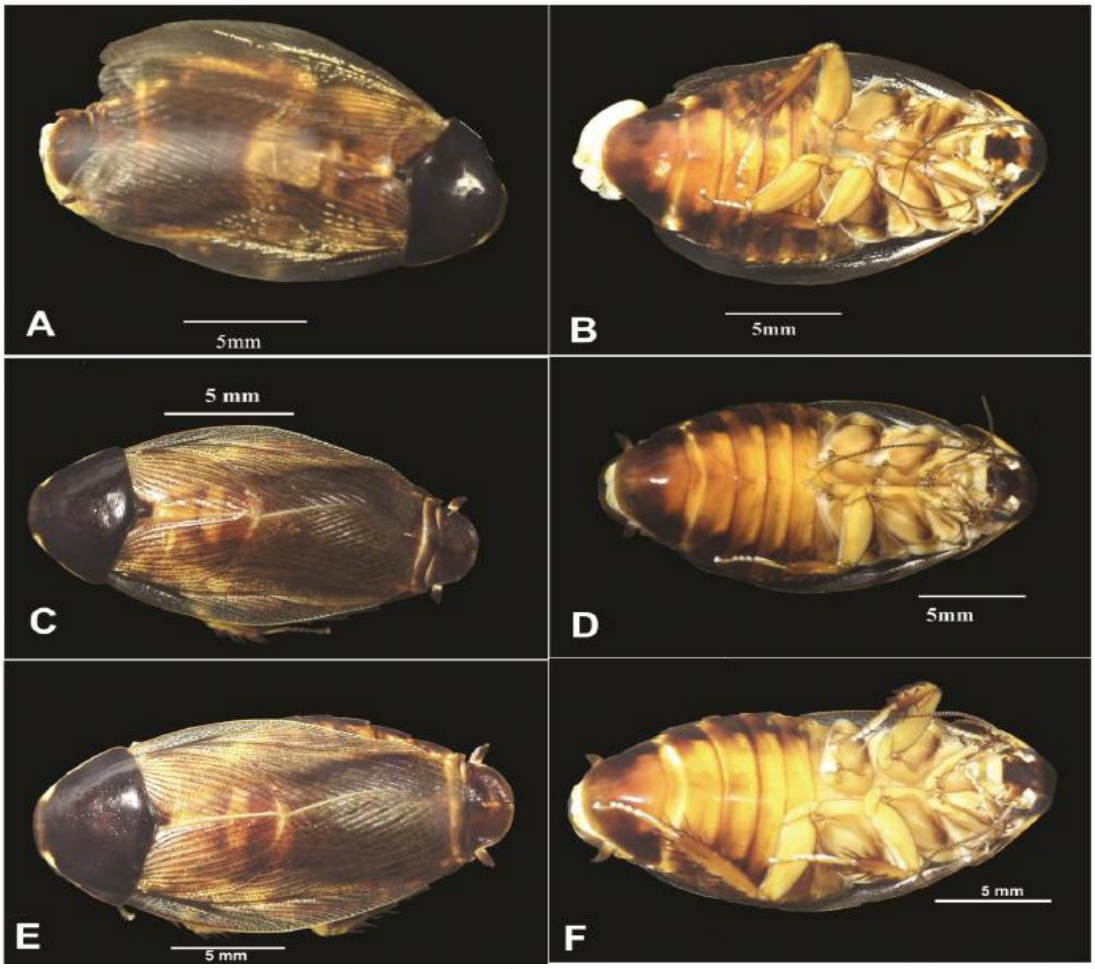

Figure 1. *Pycnoscelus surinamensis* from Courtallam forest, Tamil Nadu, India (A: Dorsal and 1 B: Ventral side of ZSI/WRC/Ent-12/81a; C: Dorsal and D: Ventral side of ZSI/WRC/Ent-2 12/81b; E: Dorsal and F: Ventral side of ZSI/WRC/Ent-12/81c).

Genomic DNA extraction and purification were completed by the DNeasy Blood and Tissue Kit (Qiagen) as per the manufacturer's protocol from the alcohol preserved tissue samples. The eluted DNA was quantitated by Qubit 2.0 fluorometer ds DNA HS Assay. PCR amplification was performed using universal COI primers, LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' [20] in 25µL reaction volume consisting of 12.5µL of Go Taq Hot Start Green Master mix (Promega) 2µL (100ng) of genomic DNA, 1µL (10pmol) of each primer and nuclease-free water up to *Q.S.* The thermal cycling profile was as per [1]. The amplified PCR product was verified on 1% agarose gel by electrophoresis stained by EtBr dye and visualized below UV light through Gelstain gel documentation system. Amplified PCR product was purified using Invitrogen's Pure Link PCR Purification Kit. Purified PCR product was sequenced bidirectionally by Sanger's method on ABI 377 (Applied Biosciences) sequencer.

3. RESULT AND DISCUSSION

The obtained sequences were manually verified for corrections and the mt COI gene sequences available for the species of *Pycnoscelus* were downloaded from the GenBank Table 1 and were aligned by Clustal W in MEGA X software [21]. For phylogenetic reconstruction, the Maximum Likelihood tree was built with RaxML [22] under the GTR+GAMMA+I model, with 1000 bootstrap replicates and the final consensus tree was visualized by FigTree v1.4.0 treating species of the closest subfamily as outgroups.

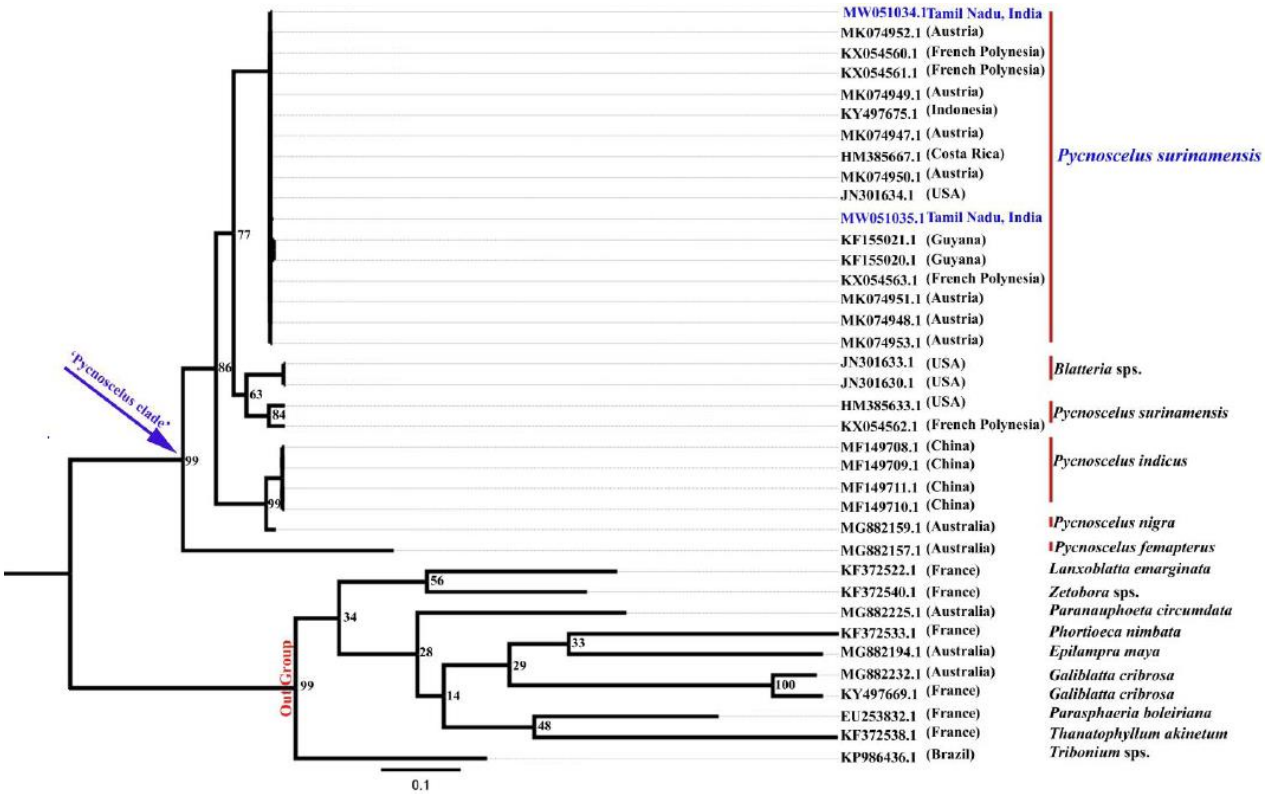

Figure 2. Maximum Likelihood tree for the members of *Pycnoscelus* based on the 588 bp of mt COI DNA Table 1.

Species identity was confirmed as *Pycnoscelus surinamensis* based on the Basic Local Alignment Search Tool (BLAST) search of National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) with 100% match for the DNA barcodes generated in our study. In the preliminary phylogenetic tree our samples formed a monophyletic clade Figure 2 with *Pycnoscelus surinamensis* sequences reported from China, Indonesia, Austria, and USA Table 1 complying with parthenogenesis. The *P. surinamensis* clade Figure 2 had specimen representations from, India, Austria, French Polynesia, Indonesia, Costa Rica, USA, and Guyana supporting the distribution range

of the species to be cosmopolitan. The two sequences (HM385633.1, KX054562.1) from the USA were making a separate sub-clade. Additionally, JN301633.1 from the USA forms a sub-clade with JN301630.1 from French Polynesia Figure 2 intimating potential haplotypic forms of *Pycnoscelus surinamensis*.

Table 1. mt COI DNA sequences used for building Maximum Likelihood tree.

Sr no.	Gen Bank Acc. No	Locality	Species name as per Gen Bank	Publication details as per NCBI
1	MW051034.1	Tamil Nadu, India	*Pycnoscelus surinamensis*	This study
2	MK074952.1	Austria: Vienna	*Pycnoscelus surinamensis*	Zangl, et al. [23]
3	KX054560.1	French Polynesia	*Pycnoscelus surinamensis*	Ramage, et al. [24]
4	KX054561.1	French Polynesia	*Pycnoscelus surinamensis*	Ramage, et al. [24]
5	MK074949.1	Austria: Styria	*Pycnoscelus surinamensis*	Zangl, et al. [23]
6	KY497675.1	Indonesia: Sumba island	*Pycnoscelus* sps.	Unpublished
7	MK074947.1	Austria: Styria	*Pycnoscelus surinamensis*	Zangl, et al. [23]
8	HM385667.1	Costa Rica	*Pycnoscelus surinamensis*	Unpublished
9	MK074950.1	Austria: Vienna	*Pycnoscelus surinamensis*	Zangl, et al. [23]
10	JN301634.1	USA	*Pycnoscelus surinamensis*	Unpublished
11	MW051035.1	Tamil Nadu, India	*Pycnoscelus surinamensis*	This study
12	KF155021.1	Guyana	*Pycnoscelus* sps.	Unpublished
13	KF155020.1	Guyana	*Pycnoscelus* sps.	Unpublished
14	KX054563.1	French Polynesia	*Pycnoscelus. surinamensis*	Ramage, et al. [24]
15	MK074951.1	Austria: Vienna	*Pycnoscelus surinamensis*	Zangl, et al. [23]
16	MK074948.1	Austria: Styria	*Pycnoscelus surinamensis*	Zangl, et al. [23]
17	MK074953.1	Austria: Vienna	*Pycnoscelus surinamensis*	Zangl, et al. [23]
18	JN301633.1	USA	*Pycnoscelus surinamensis*	Unpublished
19	JN301630.1	USA	*Pycnoscelus surinamensis*	Unpublished
20	HM385633.1	USA	*Pycnoscelus surinamensis*	Unpublished
21	KX054562.1	French Polynesia	*Pycnoscelus surinamensis*	Ramage, et al. [24]
22	MF149708.1	China	*Pycnoscelus indicus*	Ma, et al. [25]
23	MF149709.1	China	*Pycnoscelus indicus*	Ma, et al. [25]
24	MF149711.1	China	*Pycnoscelus indicus*	Ma, et al. [25]
25	MF149710.1	China	*Pycnoscelus indicus*	Ma, et al. [25]
26	MG882159.1	Australia	*Pycnoscelus nigra*	Bourguignon, et al. [26]
27	MG882157.1	Australia	*Pycnoscelus femapterus*	Bourguignon, et al. [26]
28	KF372522.1	France	*Lanxoblatta emarginata*	Legendre, et al. [27]
29	KF372540.1	France	*Zetobora* sps.	Legendre, et al. [27]
30	MG882225.1	Australia	*Paranauphoeta circumdata*	Bourguignon, et al. [26]
31	KF372533.1	France	*Phortioeca nimbata*	Legendre, et al. [27]
32	MG882194.1	Australia	*Epilampra maya*	Bourguignon, et al. [26]
33	MG882232.1	Australia	*Galiblatta cribrosa*	Bourguignon, et al. [26]
34	KY497669.1	France	*Galiblatta cribrosa*	Unpublished
35	EU253832.1	France	*Parasphaeria boleiriana*	Legendre, et al. [28]
36	KF372538.1	France	*Thanatophyllum akinetum*	Legendre, et al. [27]
37	KP986436.1	Brazil	*Tribonium* sps.	Legendre, et al. [29]

The species *Pycnoscelus surinamensis* is sufficiently documented in India from the states Andhra Pradesh, Arunachal Pradesh, Chhattisgarh, Delhi, Manipur, Karnataka, Maharashtra, Meghalaya, Orissa, Rajasthan, Sikkim, Tamil Nadu, Tripura, and West Bengal [8, 12, 13] based on morphological studies and checklists without any genetic or DNA barcode data. Therefore, the occurrence of any potential haplotype or genetic variant for the parthenogenetic species is yet unexamined. As the evolution of thelytokous parthenogenenis is understudied the *Pycnoscelus* taxon is complicated to understand [18]. Therefore, the contribution of molecular data in this particular taxon is of immense value for delimiting the species complex and their evolutionary history.

The present report of *Pycnoscelus surinamensis* Figure 1 from Courtallam forest (Tamil Nadu) forms the first vouchered based DNA barcode record for the species as well as for the genus from India. To precisely validate the occurrence of any potential haplotype or genetic variant, large-scale DNA-based molecular studies are warranted. The current DNA barcode data generated for *Pycnoscelus surinamensis* from India forms baseline data for the DNA barcoding archives of the species and is expected to have utility in taxonomy, systematics, and metabarcoding other than biodiversity documentation.

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