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DNA barcoding and phylogenetic analysis of paederinae (Coleoptera: staphylinidae) in relation to morphological data using cox I sequences

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Abstract
Diversity analysis, DNA barcoding and phylogenetic analysis of Paederinae of north Kerala were performed. Cytochrome Oxidase I gene of 18 species of Paederinae were sequenced and deposited in NCBI (all new to the database). The present analysis confirms the current placement of Paederini and Lathrobiini as distinct tribes. The two major subtribes of Paederini namely Paederina and Cryptobiina formed as sister clades and the members of genera *Cephalochetes* and *Ochthephilum* shared monophyly which is in congruence with their morphologic characters as both of them have synapomorphic geniculate antennae. The clustering of species was similar based on morphology and molecular data and hence the robustness of the tree is ensured for better prediction of phylogeny which clearly demonstrates the advantage of using both the data simultaneously for phylogeny.

Keywords: molecular taxonomy, rove beetles, paederinae, phylogeny

1. Introduction
Staphylinidae, the largest family of Order Coleoptera, are known to be present throughout the world. As the most speciose beetle family they include 63137 species in 3870 genera, placed under 32 subfamilies (Grebennikov and Newton, 2009) [8] and more than 3000 species have been recorded so far from India (Sar and Hegde, 2015) [15]. Pioneer workers on rove beetles are Motschulsky (1858) [12], Kraatz (1859) [10], Fauvel (1895) [6] and Bernhauer (1915) [2]. Cameron's work on *Fauna of British India* series (1930, 1931, and 1932) described hundreds of new species. Paederinae is a predominant rove beetle subfamily consisting 3 tribes namely Paederini, Lathrobiini and Pinophilini as per the current classification (Schomann and Solodovnokov, 2016). The knowledge on Indian Staphylinid beetles remain negligible and their molecular data is obscure. Only a few studies are present on rove beetle taxonomy from south India especially from Kerala. Ascertaining the phylogeny using morphological characters is difficult in the case of rove beetles because of the high level of divergence among the members of same taxa. Rove beetles are well known for their role in biological control of agricultural pests. This study, the first effort of DNA barcoding of Staphylinids from India, includes 18 species of rove beetles coming under two tribes of the Subfamily Paederinae, collected from the north Kerala region including the Western Ghats biodiversity hotspot. The study also intends to check the utility of molecular techniques in the identification of rove beetles. Additionally, Phylogenetic relationship among the members of subfamily Paederinae was also examined based on their Cytochrome Oxidase I sequences.

2. Materials and Method
A total of 18 species of Staphylinid beetles of subfamily Paederinae belonging to 12 genera and two tribes were collected from various places of north Kerala using light traps (SAFS low intensity UV). Collected specimens were preserved in 80% ethyl alcohol. Identification up to species level was done with the help of Cameron, 1931. Complete genomic DNA was extracted from entire body tissue of individual samples using Origin® DNA extraction kit in accordance with the manufacturer's instructions and amplified by Polymerase Chain Reaction using the forward primer 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer 5'-TAAACTTCAGGGTGACCAAAAAATCA- 3' for their Cytochrome Oxidase subunit I (COI) gene. PCR reaction was performed in a 50 µl reaction volume containing 5 µl of template DNA, 5 µl of 10X reaction buffer (100 mM Tris pH 9.0,500 mM KCl, 15 mM MgCl2, 0.1% Gelatin), 1 µl of 10 mM dNTPs, 1 µl of each primer, 0.5 µl Taq polymerase (2.5 units) and nuclease free water. The thermal profile of COI amplification was 5 min at 95º C, and 35 cycles of 10 sec at 94º C, 1 min at 52º C and 45 sec at 72º C, followed by a final extension of 7 min at 72º C. The purified PCR products were sequenced using ABI 3730XL automated sequencer (Agrigenome, Kakkanad, Kerala) by dideoxy chain termination method (Sanger, 1975). Sequences were checked in BLAST tool (Altschul *et al.*, 1990) to find the similar sequences in the NCBI (www.ncbi.gov) database. Additional sequences for analysis were retrieved from NCBI database. The forward and reverse strands were aligned using Clustal W in BioEdit software to ensure the sequences are clear without any mismatches. Phylogenetic trees were constructed using multiple aligned sequences of partial Cytochrome Oxidase I gene using MEGA X (Kumar *et al.*, 2018). Best-fit nucleotide substitution model was selected from 24 models available in MEGA X (Kumar *et al.*, 2018) based on the minimum Akaike information criterion (AIC) value (Posada and Buckley, 2004) and Bayesian Information Criterion (BIC) value. Reliability of the Maximum likelihood phylogenetic tree was estimated using bootstrap values run for 1000 iterations.

3. Results

Nineteen COI sequences of 18 species of rove beetles representing 12 genera were obtained with all the sequences with length above 500 bp. No stop codon or frame shifts were detected indicating that sequences were not pseudogenes (NUMTs). All the sequences, except that of *Paederus fuscipes*, were first submissions of Staphylinids from India and also new to NCBI as we could not find conspecific sequences while running BLAST nucleotide search. But the search resulted in many of our sequences matching with the congeneric species of GenBank. This stipulate that COI sequences are useful in determining the genus if the database is robust. Even if the sequence of a species is not available in NCBI the nearest match will be from the same family. As in other insect groups (Sabir *et al.*, 2019) codon structure of Cytochrome Oxidase 1 of Staphyinids collected were AT biased (Adenine 29.6%, Thymine 37.4%, Cytosine 17.7% and Guanine 15.3%).

K2P divergences within and between genera and tribe were calculated and compared with the sequences present in the online portals and the results were summarized in Table 3. A notable increase in K2P divergence was found across different taxonomic levels.

Table 1: List of staphylinids of North Kerala with voucher and NCBI accession number

Sl. No.	Organism	Voucher No.	Place of Collection	Accession No.
1.	*Acanthoglossa hirta*	SB18	Parambil Bazar, Kozhikode	MH614378.1
2.	*Astenus indicus*	SB12	Thenjipalam, Malappuram	MN515409.1
3.	*Cephalochetus brunneus*	SB40	Thenjipalam, Malappuram	MN515415.1
4.	*Cephalochetus elegans*	SB31	Cherupuzha, Kannur	MN515407.1
5.	*Charichirus chinensis*	SB35	Kodumbu, Palakkad	MN515410.1
6.	*Cryptobium nilgiriense*	SB86	Padikkal, Malappuram	MN882068.1
7.	*Lithocharis uvida*	SB52	Pattambi, Palakkad	MN882066.1
8.	*Medon andrewesi*	SB92	Aralam, Kannur	MN882067.1
9.	*Ochthephilum extraneum*	SB25	Parambil Bazar, Kozhikode	MH614379.1
10.	*Ochthephilum filum*	SB53	Kakkayam, Kozhikode	MN638771.1
11.	*Ochthephilum sanguinolentum*	SB01	Medical College, Kozhikode	MH614376.1
12.	*Ochthephilum sanguinolentum*	SB10	Karuvanchal, Kannur	MH614377.1
13.	*Paederus fuscipes*	SB38	Musliyarangadi, Malappuram	MH025910.1
14.	*Paederus hingstoni*	SB60	Kozhippara, Kozhikode	MN515414.1
15.	*Pseudolathra caffra*	SB85	Padikkal, Malappuram	MN515412.1
16.	*Scopaeus beesoni*	SB43	Tholpetty, Wayanad	MT009021.1
17.	*Scopaeus bicuspis*	SB33	Ranipuram, Kasaragod	MT009022.1
18.	*Scopaeus obscuripes*	SB61	Nelliampathi, Palakkad	MN638769.1
19.	*Stiliderus crassus*	SB69	Athirappilly, Thrissur	MN638770.1

Table 2: List of Cytochrome oxidase I sequences of staphylinids retrieved from NCBI for phylogenetic analysis

Sl. No.	Species	Accession No.	Country
1.	*Astenus gracilis*	HQ953976.1	Germany
2.	*Astenus pulchellus*	KU919604.1	Germany
3.	*Homaeotarsus pimerianus*	JF887727.1	Canada
4.	*Lathrobium fulvipenne*	HQ953726.1	Germany
5.	*Lathrobium impressum*	KU919314.1	Germany
6.	*Lithocharis nigriceps*	KU916608.1	Finland
7.	*Lithocharis nigriceps*	KU917362.1	Germay
8.	*Medon apicalis*	KT780658.1	London
9.	*Medon fusculus*	HQ953660.1	Germany
10.	*Paederus fuscipes*	KU913530.1	Germany
11.	*Paederus riparius*	KU912611.1	Poland
12.	*Paederus schoenherri*	HQ954585.1	Germany
13.	*Pinophilus latipes*	HQ582746.1	Germany
14.	*Pinophilus parcus*	HQ582750.1	Germany
15.	*Rugilus rufipes*	KU919485.1	Germany
16.	*Scopaeus gracilis*	KM439385.1	Austria
17.	*Scopaeus gracilis*	HQ953589.1	Germany
18.	*Scopaeus laevigatus*	KU917026.1	Germany
19.	*Sunius melanocephalus*	KM441753.1	Czech Republic
20.	*Staphylininae* sp. (Outgroup)	KM847280.1	Canada

Table 3: Sequence divergences (K2P) at various taxonomic levels

	Range	Mean dist.	S.E.
Intra species divergence	0.0–1.0	0.05	0.05
Intra generic sequence divergence	7.5–20.9	16.29	2.10
Intra tribe sequence divergence	20.2–24.2	22.20	2.00
Inter generic sequence divergence	16.5–29.1	24.00	2.30
Inter tribe sequence divergence	22.0–27.9	25.53	2.10

4. Discussion

The phylogeny analysis confirms the current placement of Paederini and Lathrobiini as distinct tribes because the intra tribe divergence of Paederini is less than between Paederini and Lathrobiini. There is a remarkable difference in nucleotide substitution patterns among Paederini and Lathrobiini (Fig.3). Tribe Pinophilini showed monophyly

and common ancestry with other two tribes (Lathrobiini + Paederini) and this is evident in their morphological characters also (Herman, 1981) [9]. The members of Lathrobiini showed polyphyletic relationships among subtribes but kept much distance from members of Paederini in the Phylogenetic tree in congruence with the morphology-based study. Synapomorphic characters of tribe Lathrobiini include nasale with seta Fl2 short and swollen at the base, ligula with branched microtrichia and anterior margin of buccal cavity with cuticular process arranged in rows and the shared characters of members of Paederini include nasale with unmodified seta Fl2, ligula with microtrichia not branched and anterior margin of buccal cavity with cuticular processes not arranged in rows (Frania, 1986) [7]. Within Paederini, our study reveals two distinct monophyletic subtribes namely Paederina and Cryptobiina. The genera *Ochthephilum* and *Cephalochetus* are here confirmed as sister taxa with strong support. This agrees with the earlier suggestion of Schulke and Smetana, 2015 that *Cephalochetus* is indeed a member of Cryptobiina. Geniculate antennae, narrow anterior tarsi without adhesive setae underneath, and the structure of the aedeagus of *Cephalochetus* also support this placement. The clustering of species was similar in both molecular and morphology-based analysis and hence the robustness of the study is ensured for better prediction of phylogeny.

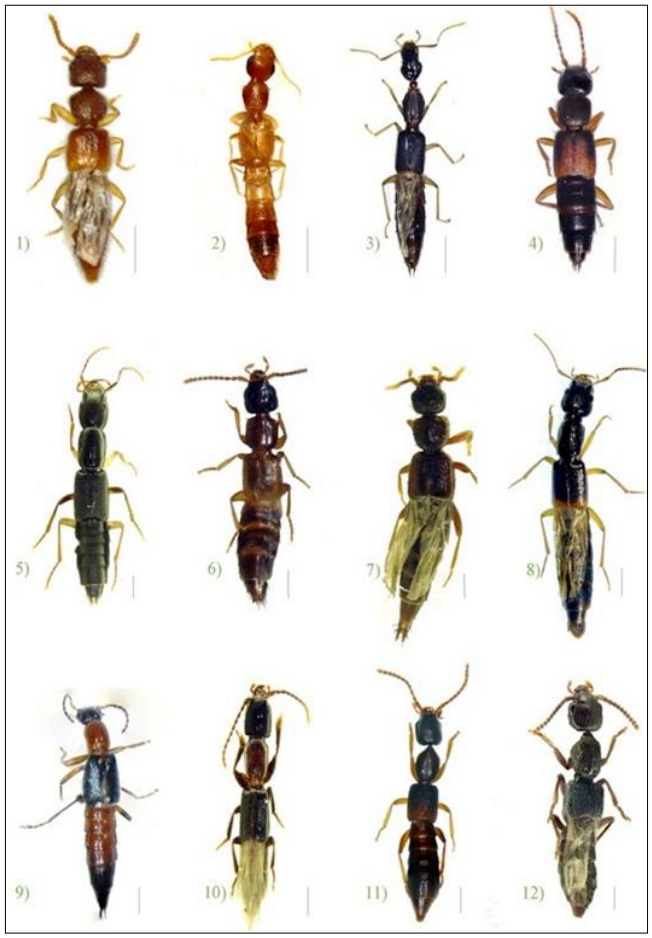

Fig 1: Rove beetles of North Kerala representing 12 genera of Subfamily Paederinae –1. *Acanthoglossa hirta*, 2. *Astenus indicus*, 3. *Cephalochetus brunneus*, 4. *Charichirus chinensis*, 5. *Cryptobium nilgiriense*, 6. *Lithocharis uvida*, 7. *Medon andrewesi*, 8. *Ochthephilum sanguinolentum*, 9. *Paederus fuscipes*, 10. *Pseudolathra caffra*, 11. *Scopaeus beesoni*, 12. *Stiliderus crassus* Scale bar 1mm

Fig 2: Map of Kerala showing the collection sites for the present work

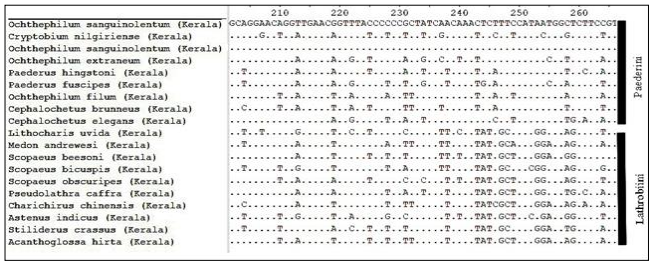

Fig 3: Multiple sequence alignment showing difference in nucleotide substitution among two tribes of Paederinae present in Kerala

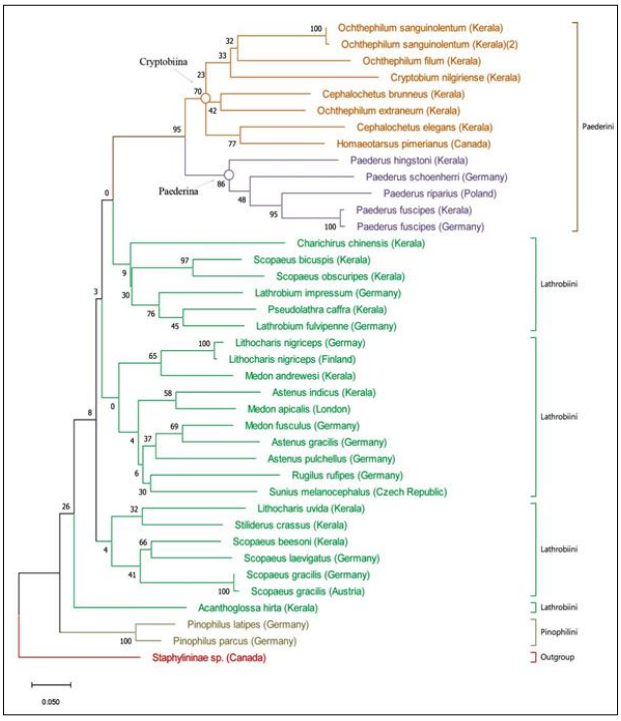

Fig 4: Phylogenetic tree of Paederinae based on Cytochrome Oxidase I sequences using Maximum likelihood method

5. Conclusion

Maximum likelihood analysis in the present data confirms that DNA barcoding is helpful in species level identification of rovebeetles. Eventhough staphylinidae is the most speciose family of Coleoptera it is unfortunate that the barcods of staphylinids from India are hardy present in database till date. The study contributes significantly to the barcode data of by adding sequences of staphylinids from North Kerala including the Western ghat regions.

The clustering of species in the Phylogenetic tree based on the Cytochrome Oxidase I sequences was found to be similar to that of morphology-based studies and hence the phylogeny prediction of Paederinae is more robust.

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Disclosure statement

The authors declare that they have no competing interests.

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