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Study on diversity and molecular systematics of *Stenopsyche kodaikanelensis* (Insecta: Trichoptera) and Phylogenetic lineage with Trichoptera and Lepidoptera in Kurangani stream

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Abstract: *This work incorporates two methods that macroinvertebrate bio-monitoring and DNA barcoding, in an effort to gain a greater understanding of how traditional taxonomic approaches and DNA barcoding, helps in interpretation of water quality and phylogenetic lineage of insects. The present work is carried out to find the effect of the phenomenon in the Kurangani streams from August 2018 to January 2019. Shannon and Simpson index were highest in November 2018 and lowest in January 2019 and it shows climatic changes which have an impact on insect's diversity. DNA barcoding of *Stenopsyche kodaikanelensis* which shows close relationship with *Chimarra sp* and the Indian species *S.kodaikanelensis* shows closer phylogeny with Chinese species *S.brevata*. By comparing the barcodes of Trichoptera and Lepidoptera in Kurangani falls, *Anisocentropus sp* (Trichoptera) is in closer lineage with *Anthene lycaenina* (Lepidoptera) and significantly tells that both arises from a common ancestor.*

Keywords: *Simpson index, Shannon index, Trichoptera, Lepidoptera, DNA barcoding.*

INTRODUCTION

Trichoptera which is also known as Caddisflies, they are one of the largest order in the freshwater aquatic insects. Normally they inhabit in the freshwater aquatic ecosystem and in saturated soil. More than 16,000 caddisflies species have been identified globally (Shiqi Luo *et al.* 2018). These trichoptera commonly share Lepidopteran ancestral characters. The trichopteran larva are aquatic subsequently it is used for monitoring anthropogenic effects and in biomonitoring surveys. Adult trichopteran flies live terrestrial life and they have setae in the wings. Trichopteran larva use materials from the environment to build the cases and retreats and this character which makes them the second most diverse order in the aquatic insects (Rios-Touma B *et al.* 2017). Stenopsychidae belongs to the phylogenetic family of Annulipalpia (Karl M. Kjer *et al.* 2001) and are normally fixed retreat makers. They mostly live in rivers and streams. Normally they are larger in size among the benthic macroinvertebrates and they are the keystone species in the river ecosystem (Rie Saito *et al.* 2018). There are also other families of trichoptera which includes Spicpalpia which are called

as cocoon makers (Weaver, 1984) and integripalpia which are called as tube case makers (Weaver and Morse, 1986). The measure of biodiversity of the ecosystem can be found only by studying the diversity indices of the particular habitat and this helps us to predict the climatic change and their effects due to global warming and various anthropogenic effects.

DNA barcoding is a molecular technique that sequences a short portion of mitochondrial DNA, the CO1 gene, to create a unique “barcode” (Hebert *et al.* 2003). Using the CO1 gene enables species level identification and it also helps in the cryptic species identification which looks morphologically similar and genetically variant, DNA barcoding studies which elucidate phylogenetic relationship among the species and genus level. Lepidoptera and Trichoptera which are called as sister orders because they arose from a single ancestor and most of the trichoptera share up to 20 common unique traits with Lepidoptera but not that much studies were made yet in this relationship of sister orders and in their lineage. Both Lepidoptera and Trichoptera are bioindicators of the ecosystem because they show and reflect to the stresses in the surrounding environment. This paper incorporates two methods includes macro invertebrate bio monitoring and DNA barcoding, in an effort to gain a greater understanding of how traditional taxonomic approaches compare to species identification by DNA barcoding.

Materials and Methods

Study area

The selected area for study is Kurangani falls. Kurangani – a small village at the base of the Eastern side of Munnar hills on the Western Ghats in Tamilnadu.

Insect collection

The aquatic insects were quantitatively sampled by using 1cm wide Kick-net (Burton and Sivaramakrishnan, 1993) with mesh size of about 1mm. Limited opportunistic collections (hand picking) were made to gather supplementary information on taxa richness. The organisms were then carefully picked from the net surface and were preserved immediately in 70% ethyl alcohol. These samples were transported to the laboratory for further processing. The Butterflies were collected using the sweep net.

Laboratory sorting, identification and enumeration

All specimens from each of the 3 sites in the stream were sorted and identified with the help of field guide by Sivaramakrishnan *et al.*, (1998).

Data analysis

The data analysis was done with the help of the PAST software to measure the various diversity indices.

Molecular protocols

Abdominal segments III–VI or legs of larvae and adults were used for DNA extraction. Genomic DNA was isolated from the legs using DNeasy® Blood and Tissue kit (Qiagen). PCR products were purified with the Qiagen QIAquick PCR Purification Kit and then sequenced by PCR thermal cycler (GeneAmp PCR system 9700, Applied Bio system, USA). Each individual DNA fragment was sequenced from both directions. COI sequences were aligned using ClustalX v2.1 (Larkin, M.A. *et al.* 2007).

Phylogenetic analysis

Phylogenetic tree were constructed independently from COI sequences using MEGA software. The kimura 2 parameter (K2P model) was used with 1000 bootstrap values. Pairwise distances and within and between species divergences of COI nucleotides were calculated in MEGA v4.0 (Tamura, 2007) to provide comparisons among other species.

Results and Discussion

Sampling Trichoptera immature from August 2018 to January 2019 resulted in a total of 875 specimens belonging to 11 genera, 7 families were collected and 4 genera belonging to 4 families of butterflies were also

collected. Shannon index (Fig.1) was highest in November 2018 and lowest in January 2019. Simpson's index (Fig. 2) was highest in November 2018 and lowest in January 2019. Shannon and Simpson indices indicate the total amount of diversity in Kurangani stream. The diversity of the organisms were limited in the month of January due to lack of rainfall and this makes water scarcity among the stream and makes the conditions tough for the benthic macroinvertebrates to survive. The downstream (Site3) which shows less diversity indicates higher pollution in the downstream compared to mid (Site2) and upstream (Site1). The high scores of Diversity indices, such as those of Shannon index and Simpson's index, indicate that clean or unpolluted rivers support more diverse taxa, thus making them useful for detecting organic pollution (Lenat and Penrose, 1996).

DNA barcodes into bioassessment protocols would provide greater discriminatory ability than genus-level identifications and that this increased specificity could lead to more sensitive assessments of water quality and habitat. DNA barcoding has the further advantage that success extends across all life stages, allowing the association of immatures with adults (Mynott *et al.*, 2011). DNA barcoding was done to the *Stenopsyche kodaikanelensis* of Trichoptera and *Ypthima ceylonica*, *Hebomoia glaucippe*, *Anthene lycaenina* and *Pachliopta aristolochiae* of Lepidoptera. DNA barcodes of other genus of the trichoptera and BLAST sequences of *Stenopsyche kodaikanelensis* were collected from NCBI database. Phylogenetic analyses were made for Trichoptera and Lepidoptera.

DNA barcoding studies of trichopterans in Kurangani Streams (Fig. 4) shows that *Stenopsyche kodaikanelensis* shows monophyletic lineage to *Chimarra sp* which belongs to the family of Philopotamidae and it shows close resemblance with Stenopsychidae both in morphology and in retreat making and both of them comes under annulipalpia (Weaver and Malicky, 1994). The intergenetic diversity of stenopsychidae were also studied and this shows that *S.kodaikanelensis* which is an Indian endemic species which shows closer relationship with Chinese species *S.brevata* which mostly present in the Indochina peninsular area (Rie Saito *et al.*, 2018) and this concludes they both arises from a common ancestor and get diverged according to their environmental stress and environment but it is more diverged to that of *S.schmidi*, which is a Japanese species (Nozaki, T. and Shimura, N. 2015).

By comparing the case building behaviour and the wing pattern of the Trichoptera and Lepidoptera, many studies have revealed that both of them originate from a common ancestor and get diverged in the course of evolution. Both these order which belongs to the superorder Amphiesmenoptera and trichopteran adult flies looks similar to primitive moths. So we have done the phylogenetic analysis of Trichoptera and Lepidoptera collected from the kurangani stream. *Anisocentropus sp* which belongs to the family of calamoceratidae which comes under the intergipalpia which are transportable tube case makers and mostly these calamoceratidae comes under the superfamily of leptoceroidea (Weaver and Morse. 1986) which normally makes cases inside the leaves which closely resembles the life history pattern of Lepidoptera insects. The DNA study reveals that calamoceratidae family shows close relationship with *Anthene lycaenina* (Lepidoptera) which belongs to the order Lycaenidae and they both arose from the single cluster of ancestor.

Conclusions

The results of this study allows for a better understanding of the regional diversity and distribution of Trichoptera in kurangani stream. Such information will provide a solid basis through which we can obtain a better understanding of the structure and functioning of the complex ecosystem. More studies have to carry out such as like the mitochondrial COI and 16S genes, and the nuclear 18S and EF-1 α genes are recommended for insect molecular systematic comparisons (Caterino *et al.* 2000). More studies have to be carried out in comparison between the Lepidoptera and Trichoptera insect's life cycle patterns to know about how they evolve from the common ancestor. Molecular time clock where also be made with the help of the fossil species. Furthermore investigations will also need for the identification of cryptic species and their divergence patterns, so that more confusion among the systematists and taxonomists has been sorted out. We

can save any organism only when we know about the molecular, morphological and phylogenetic pattern of them, because these are potential bioindicators, so these studies are found to be crucial in saving the environment.

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Declaration of Interest

The authors report no conflict of interest.

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Table 1. List of trichoptera and Lepidoptera collected from Kurangani falls

TRICHOPTERA	Rhyacophilidae	<i>Rhyacophila sp</i>
	Philopotamidae	<i>Wormaldia sp</i>
		<i>Chimarra sp</i>
	Stenopsychidae	<i>Stenopsche kodaikanelensis</i>
	Polycentropodidae	<i>Polycentropus sp</i>
	Hydropsychidae	<i>Hydropsyche sp</i>
		<i>Cheumatopsyche sp</i>
		<i>Potamyia sp</i>
		<i>Herbertorossia sp</i>
	Lepidostomatidae	<i>Goerodes sp</i>
	Calamoceratidae	<i>Anisocentropus sp</i>
LEPIDOPTERA	Papilionidae	<i>Pachliopta aristolochiae</i>
	Nymphalidae	<i>Ypthima ceylonica</i>
	Pieridae	<i>Hebomoia glaucippe</i>
	Lycaenidae	<i>Anthene lycaenina</i>

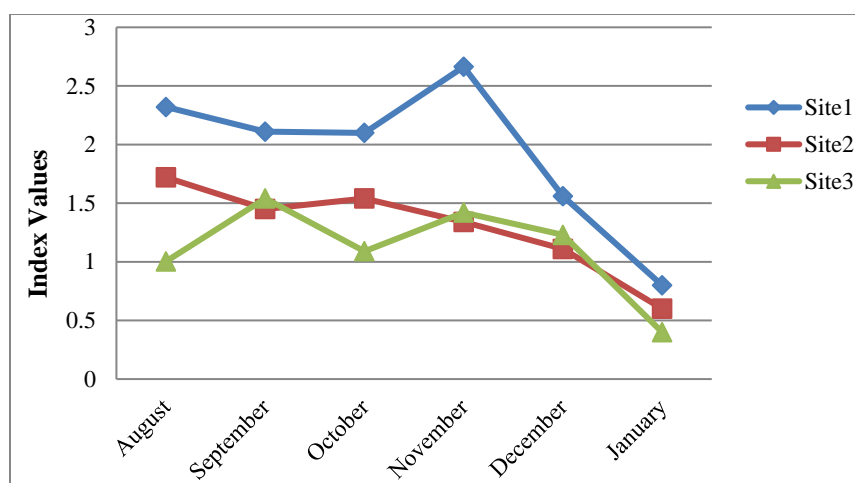


Figure 1. Shannon index values of aquatic insects in stream of Kurangani

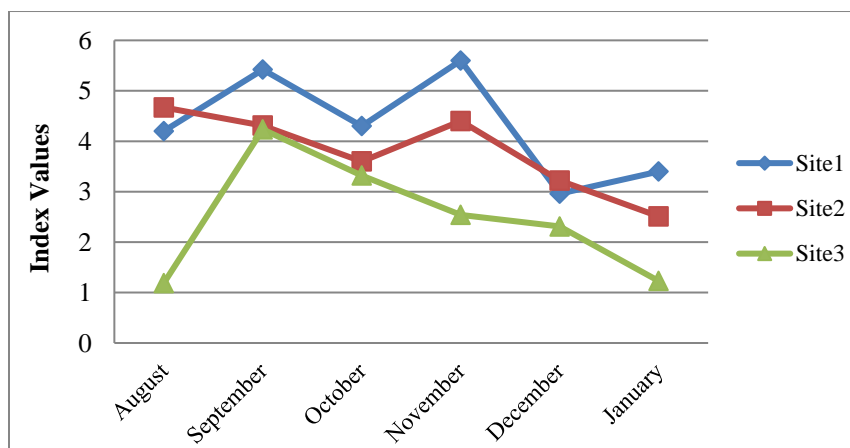


Figure 2. Simpson index values of aquatic insects in stream of Kurangani

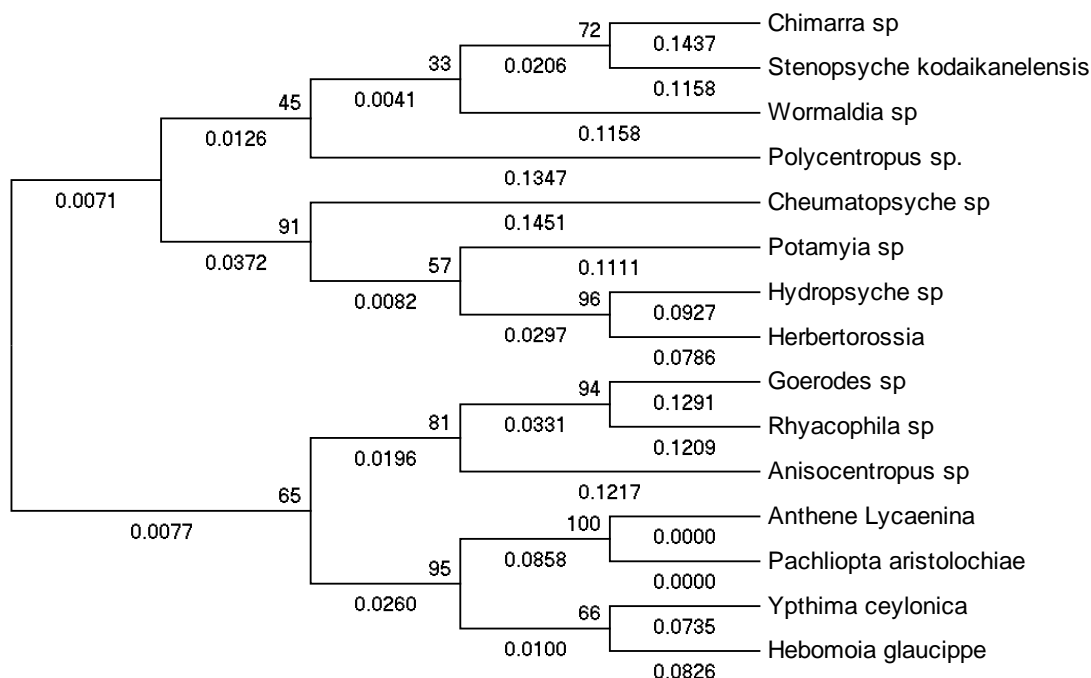


Figure 3. Phylogenetic tree of order Trichoptera and Lepidoptera in Kurangani stream

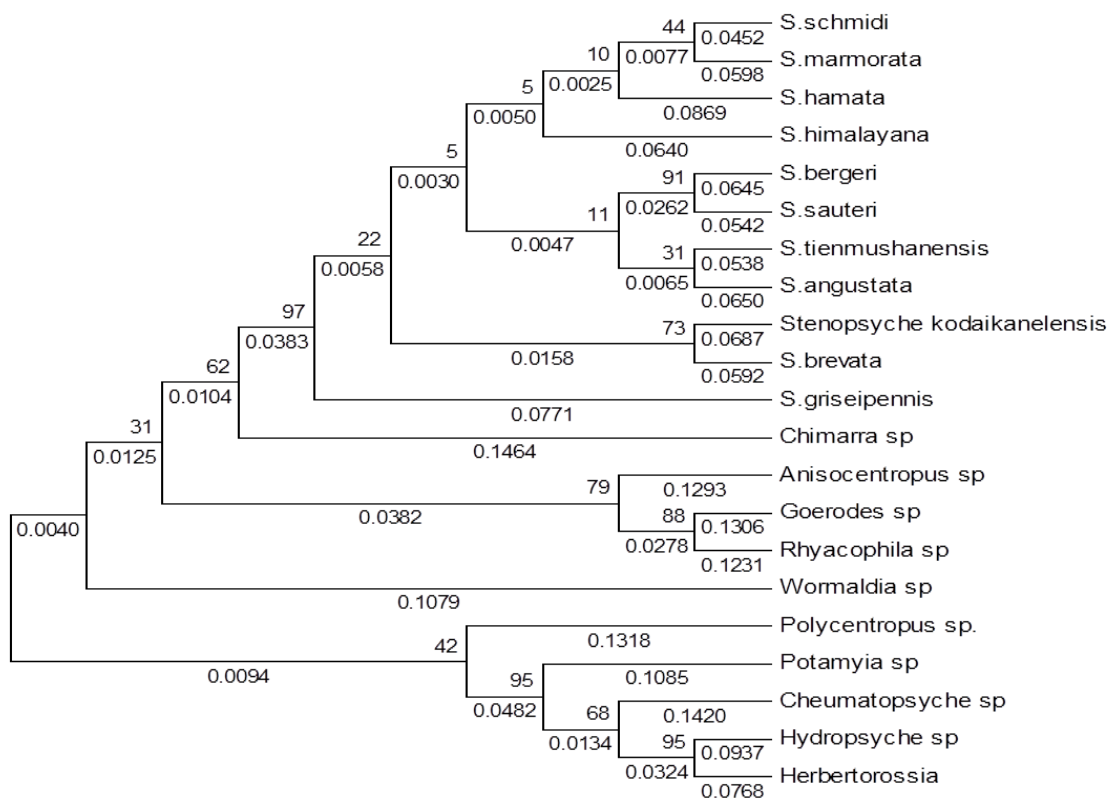


Figure 4. Phylogenetic tree of *Stenopsyche kodaikanelensis* in comparison with other trichoptera in Kurangani stream