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## Phylogenetic relationship of four species of *Sitophilus* (Coleoptera: Curculionidae)

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### Abstract

The present study provides details about the phylogenetic relationship of four species namely, *Sitophilus oryzae* (L.), *S. zeamais* (Motschulsky), *S. granarius* (Schoenherr), *S. linearis* (L.) on the basis of mitochondrial COI (Cytochrome oxidase I) gene as the marker. The study was done by amplifying the COI gene for the four species and then aligning and comparing their DNA sequences. The aligned DNA sequences were compared for evolutionary details with the help of MrBayes software. The phylogenetic relationships derived from molecular data act as an important tool in discriminating closely allied species and would act as a marker in providing reliable information on various patterns of variation, evolution and genetic diversity.

**Keywords:** Coleoptera, Curculionidae, Molecular diversity, Phylogeny, *Sitophilus*

### 1. Introduction

There are many species of *Sitophilus* such as *S. oryzae*, *S. zeamais*, *S. granarius*, *S. linearis*, *S. rugicollis* which comprise of almost 13 species and one of the fossil taxa <sup>[1]</sup>. In the present study, the molecular phylogeny was constructed by using partial mitochondrial COI gene. If not identical, all share relatively similar adult morphologies. The most economically important pests in the genus are *S. oryzae* and *S. zeamais* which attack the granaries and store houses more often. To investigate the relationship between the four species we related them to order Hymenoptera as outgroup <sup>[3]</sup>.

*Sitophilus* belongs to the subfamily Dryophthorinae (Coleoptera:Curculionidae) <sup>[2]</sup>. It is cosmopolitan. Antenna has a funicle of 6 segments and the scape of antenna reaches at least past anterior margin of eye. Elytra gradually tapered from base to apex, with apex broadly rounded. At the apex of elytra pygidium is exposed <sup>[2]</sup>. Tibiae with distinct subapical tooth on inner margin often giving pincer like appearance. Total body length (as measured from anterior margin of eye to elytral apex) less than 5 mm. No indentation at juncture of rostrum and head; Rostrum was weakly curved <sup>[2]</sup>.

### 2. Material and Methods

#### 2.1 Sample collection

The adult weevil samples were collected from different stored grains that too from different locations in and around Chandigarh. The collection was done during months of June, July and August in 2014 and 2015. The stored grains were collected from household products and also from grocery shop. The samples were preserved in 95% ethanol and kept in -20°C.



**Fig1:** *Sitophilus zeamais* (Motschulsky)

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**Fig 2:** *Sitophilus oryzae* (Linnaeus)



**Fig 3:** *Sitophilus granarius* (Schoenherr)



**Fig 4:** *Sitophilus linearis* (Linnaeus)

## 2.2 DNA extraction and sequencing

The adult sample was used for the DNA extraction by using the Bioline Isolate II Genomic DNA extraction kit following the manufacturer protocol<sup>[4]</sup>. The extraction product is stored at -20°C. The COI (Cytochrome oxidase I) gene was amplified

using the forward primer and reverse primers C1-N2191 (5'-CCAGGTAATAATATAAACTTC-3') and C1-J-1751 (5'-GGATCACCTGATATAGCATTCCC-3') respectively. A thermo cycler of Master Cycler (BioRad) kind was used for the PCR

**Table 1:** Standardised PCR conditions

Condition	Step	Temperature in °C	Time in minutes
1st Denaturation	Denaturation	94	3
35 cycles	Denaturation	94	1
	Annealing	47	1
	Elongation	72	1
	Final stage elongation	72	10

The PCR products were cleaned before sequencing by EXOSAP protocol. The Sequencing allowing the determination of the nucleotide sequence of a DNA fragment was carried out by Avantor using Sanger's sequencing protocol<sup>[5]</sup>.

## 2.3 Cleaning and Sequencing Alignment

The cleaning, adjustment and alignment of sequences were manually performed using Sequencher and BioEdit softwares. Sequence alignment allows seeing sites homology and highlighting variations in the obtained sequences. Phylogenetic trees were constructed by using MrBayes. Bayesian inference was performed with the software MrBayes(serial version 3.0B4 for windows)<sup>[6]</sup> with four simultaneous Markov Monte Carlo chains of 100,000 generations, sampling every 100 generations (resulting in 10,000 trees).

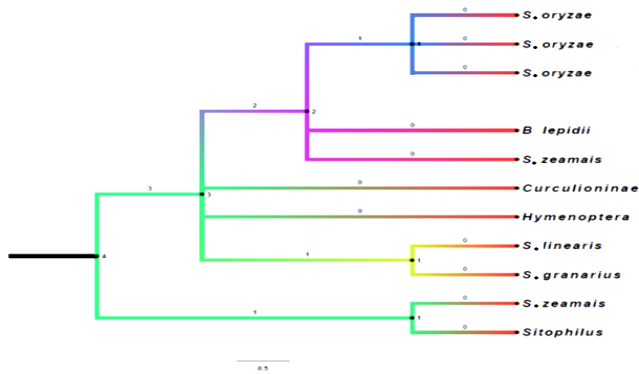
## 2.4 Statistical analysis

After the amplified DNA was sequenced it was then aligned and cleaned in Sequencher and BioEdit software so as to get an aligned data set. The other species that were compared belongs to subfamily Curculioninae, order Hymenoptera (outgroup). The Phylogenetic tree was formed by using software MrBayes. The tree file of MrBayes was viewed in FigTree v 1.4.2 to see the various parameters<sup>[6]</sup>.

The numbers above the branches show the posterior probabilities of the corresponding node in the Bayesian inference. In Bayesian inference the monophyletic group diverged into two branches. Phylogenetic trees for this method were visualized from Fig tree version 1.3.1 software. The phylogenetic tree analysis was conducted in connection with the robustness of bootstraps or the value of posterior probabilities for Bayesian inference. The cladogram was generated with the clade credibility values. Each clade within the tree is given a score based on the fraction of times that it appears in the set of sampled posterior trees, and the product of these scores are taken as the tree's score. The tree with the highest score is then the maximum clade credibility tree.

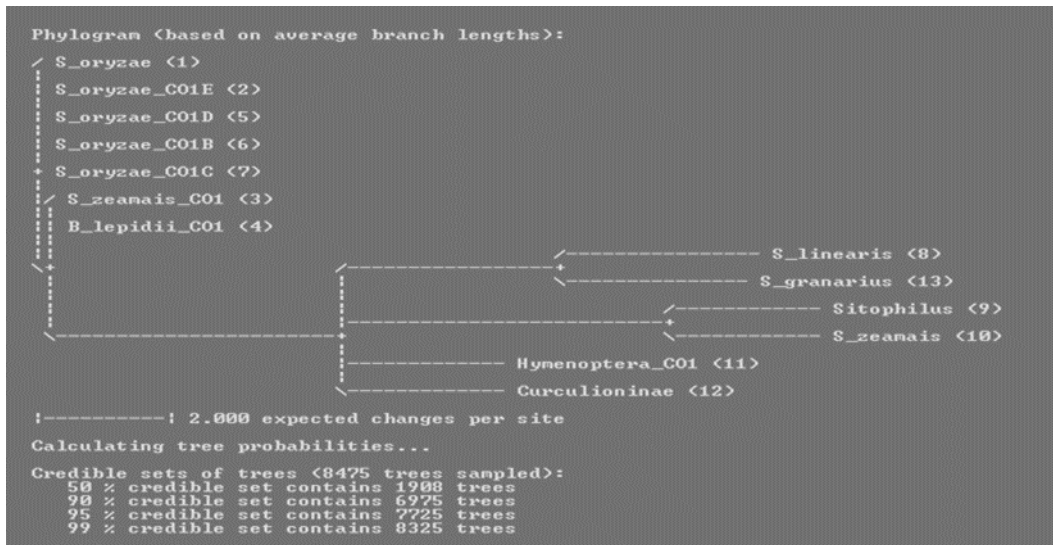
## 3. Results and Discussion

The phylogeny lineages have been discussed by Farell, 1998 for the beetles. The subfamily classification for the true weevils is a point of discussion. The Bayesian tree for four species of *Sitophilus*, in the present study, clarified the common ancestry. The tree also suggests the common ancestor for the two orders Coleoptera and Hymenoptera. The subfamily Curculioninae has separated from Dryophthorinae (*Sitophilus*) for different evolutionary lineage<sup>[5]</sup>. The diversification of Curculioninae and Dryophthorinae subfamilies has originated from the common ancestor<sup>[1]</sup>.



**Fig 5:** Bayesian tree of the four species of *Sitophilus* deduced from COI sequences.

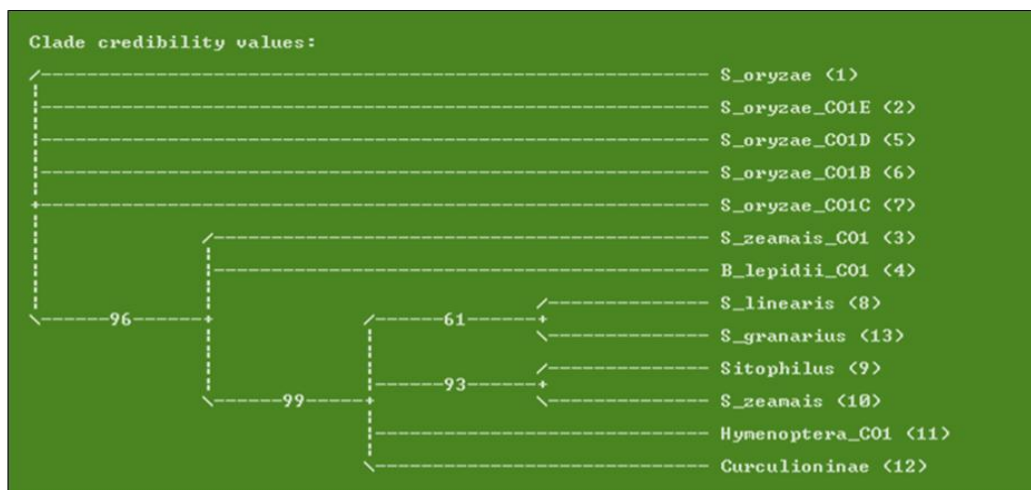
According to the Bayesian inference phylogram based on branch lengths had a scale of 2 expected changes per site. Thus, according to the phylogram (Fig. 5), *S. granarius*, *S. linearis* on one hand and *S. oryzae* must have a difference of at least 6 changes per site. And for *S. oryzae* and *S. zeamais* expected change comes out to be 8 changes per site. The Phylogram (Fig. 6) was generated on the base of the average branch length. The phylogram showed the sequence matching with a scale of 2 changes per site. *S. oryzae* and *S. zeamais* had a very slight difference between their lineages while *S. linearis* and *S. granarius* both show closeness in their phylogeny.



**Fig 6:** A phylogram for four species of *Sitophilus*.

The cladogram (Fig. 7) was generated by the clade credibility values. These values show the evolutionary time scale. Comparing these values for all the four species the *S. linearis*

and *S. granarius* are observed to be recent in evolution as compared to *S. oryzae*.



**Fig 7:** A cladogram with four species of *Sitophilus* and outgroup.

Although *Sitophilus* is a major pest of stored grains the four species discussed in the present study have diversified their host grains. The evolutionary trends have shown different host preferences such as *S. zeamais* infest maize grains and *S. oryzae* prefers rice grains [7]. So, the diversification of the four species also involves the reason of changed host preferences [8].

#### 4. Conclusions

In the present study the effort has been made to study the phylogenetic differences between the four closely related species of *Sitophilus*. The four species of *Sitophilus* studied here have been proved to be evolved from the common ancestor. Thus they belong to the same genera. All the four species follow a different lineage for further evolution. The

molecular analysis for CO1 gene of four species of *Sitophilus* shows that the four species are related with a little difference which is very difficult to make out from the morphology. The two major pest *S. oryzae* and *S. zeamais* have appeared to be related more closely than the other two species.

## 5. Acknowledgements

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