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Megaselia Scalaris (Loew) (Diptera: Phoridae) - A New Pupal Parasitoid Of The Invasive Banana skipper *Erionota Torus* Evans From India Confirmed By COI Gene Barcoding

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Abstract

Since its invasion of Kerala in 2014, the banana skipper *Erionota torus* Evans (Lepidoptera: Hesperiidae) has become a severe threat to banana farming. The present study reports phorid fly *Megaselia scalaris* (Loew), a prominent detritivorous insect, as a parasitoid attacking the pupa of Banana leaf roller *E. torus* Evans. This parasitoid was reported for the first time in the field population of banana leaf roller pupa collected from Pariyaram located at 12.0753N,75.3049E, Kannur district of Kerala, India, during August, 2019. By using both classical and molecular methods, we were able to confirm the species. The current research suggests that *M. scalaris* might be included to the list of parasitoid species that are known to exist for *E. torus* pupa. More research is needed to determine whether or not this phorid fly can be used as an efficient biocontrol agent.

Keywords: Scuttle fly, Megaselia scalaris, Pupal parasitoid, Banana leaf roller, Erionota torus, Biocontrol agent

Introduction

The banana skipper, *Erionota torus*, which was previously found only in northern and northeastern India, has now spread to peninsular India as a significant pest (Poorani et al. 2020).The various larval instars consumes leaves of practically all banana cultivars, causing significant damage. This limits the productivity by delaying fruit development, reducing bunch size, and restricting the normal use of the leaves. During the monsoon and post-monsoon seasons, the infestation rate increased from 10 to 40%, resulting in up to 50% loss of plant leaf area (Jaleel and Ghosh, 2020). In several ceremonies, banana leaves are utilized as plates in South India. Therefore, unless there are extreme circumstances, it is not advisable to use insecticides to handle the infestations (Deshmukh et al., 2018). Given the semi-perennial nature of the banana ecosystem and the crop's extended life cycle, biological management looks to be the best choice.

Poorani et al. (2020) have identified 12 insect parasitoids hitting the 'egg, larval, and pupal stages' of *E. torus*, which were raised and validated in surveys done in various Indian states between 2015 and 2018. The pupal parasitoids reported from India include *Brachymeria lasus* (Walker) (Hymenoptera, Chalcididae) from Kerala, *Xanthopimpla* (Sreedhar et al., 2020) from Karnataka, *Senometopia* sp., and *Winthemia sumatrensis* from South Karnataka (Sharanabasappa, 2018). Soumya et al., (2013) and Poorani et al. (2020) detected the incidence of an unidentified species of tachinid parasitizing the pupae of banana skippers from Kerala and Meghalaya, respectively.

'A worldwide and synanthropic scuttle fly, *Megaselia scalaris* (Loew), functions as a detritivore, parasite, facultative parasite, and parasitoid'. It has various dietary preferences (Costa, Jane, et al., 2007). The parasitic capability of *M. scalaris* against the invasive pest *Spodoptera frugiperda* was reported from China (Tang et al., 2021). The goal of this investigation is to record the first circumstance of *M. scalaris* in the pupae of *E. torus Evans* (Lepidoptera: Hesperiidae) in India and to confirm the species by DNA barcoding of the mitochondrial COI gene.

Methodology:

a. Study of percentage of parasitism of *M. scalaris* (Loew): The pupae along with their shelters were collected from different banana plantations in Pariyaram, Kannur (Kerala) by hand picking. All pupae were then removed from the plant along with the leaf roll and placed into separate plastic bags. The pupae were then placed in little plastic jars approximately 15 cm in diameter and 20 cm tall. Each jar contained only one specimen and was wrapped with muslin cloth. All plastic jars were kept in the raising chamber at a temperature of 27-30°C and a relative humidity of 80 percent. Any pupal parasitoids that emerged from the pupae were killed with ethyl acetate and transferred to vials comprising alcohol at a concentration of 70%. The pupal parasitoids were then put on rectangular cards (Noyes, 1982). A taxonomic researcher from ZSI Kozhikode, Kerala, helped us to identify the specimens that were collected. The voucher specimens were thereafter stored at the 'Zoology Museum of Research Laboratory, Government College, Kasaragod (Kerala)'. The percentage of pupal parasitism detected in the samples gathered was then computed. An average % of pupal parasitism was estimated using the following equation:

$$\text{Percentage of pupal parasitism} = \frac{\text{Number of pupae parasitized}}{\text{Total number. of examined pupae}} \times 100$$

b.Study of the biology of *M. scalaris* (Loew): The infected pupae of *E.torus* were kept in a 500 ml beaker covered with muslin cloth. They were then examined to record the time of larval emergence of the parasitoid . The adult morphology and morphometrics of *M. scalaris* were studied in the research laboratory of Zoology, Government College, Kasaragod. The morphology and morphometrics of the immature stages of the parasitoid, including egg, first instar, second instar, third instar larvae, pre-pupa, and pupa, were also studied using a Magnus stereo zoom microscope and repeated ten times. The adult flies were carefully observed to record their behavioral activities both in the field and under laboratory conditions.

c.Gene sequencing of COI gene of *M. scalaris* (Loew): 'Genomic DNA was extracted from the tissues using the NucleoSpin® Tissue Kit (Macherey-Nagel) according to the manufacturer's instructions. The quality of the extracted DNA was assessed using agarose gel electrophoresis. The PCR amplification was performed using a PCR thermal cycler (Applied Biosystems GeneAmp PCR System 9700). The sequencing reaction was performed in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) according to the manufacturer's instructions. The sequence quality was

assessed using Sequence Scanner Software v1 (Applied Biosystems). Geneious Pro v5.1 (Drummond et al., 2010) was used to align the retrieved sequences and modify them as needed'.

d. Submission of Sequences to NCBI GenBank

The properly aligned and analyzed sequences of *M. scalaris* (Loew) were deposited in GenBank through its submission portal using the submission tool after registering on the NCBI site at 'https://submit.ncbi.nlm.nih.gov/ or https://www.ncbi.nlm.nih.gov/genbank/submit/' and obtained valid accession numbers .

e. Phylogenetic Analysis and Evolutionary Lineage

Species determination and sequence comparison were carried out using the BLASTn tool in the GenBank database. The sequences were analyzed, and the percentage identity scores were compared with sequences of the same or closely related species. Identity scores below 91% were deemed insignificant, those between 92-96% were considered moderately significant, and scores between 97-100% were regarded as significant. Phylogenetic tree construction was performed using 'Neighbor-Joining' (NJ) (Saitou and Nei, 1987) and 'Maximum Likelihood' (ML) methods (Tamura et al., 2011). The Model test for Maximum Likelihood and Kimura 2-parameter (K2P) (Kimura, 1980) variations at the congeneric and conspecific levels were conducted using MEGA ('Molecular Evolutionary Genetics Analysis') Version 11 (Kumar et al. 2016), which is freely available for download. MEGA 11 was also used to calculate pairwise genetic divergences between nucleotide sequences, reconstruct phylogenetic relationships, and analyze evolutionary lineages. Bootstrapping of the evolutionary trees was performed following the methods of 'Felsenstein (1985) and Zharkikh and Li (1995)'.

Results

a. Percentage of parasitism of *M. scalaris*: In the current study, the scuttle fly *M. scalaris* (Loew) (Diptera: Phoridae) was detected as a facultative parasitoid of the pupa of banana skipper *E. torus* Evans for the first time in the field and laboratory reared pupae. When supplying fresh host pupa to the newly emerged adults, 100 % parasitism was observed. In the present study 0.4, 2.4 and 1.6 percent of parasitism were observed in the field during pre -monsoon, monsoon and post-monsoon seasons respectively (Figure. 1). Figure of normal pupa and infected pupa is shown in the figure 2. a and 2.b. Larval feeding of the parasitoid on the pupal tissue of the host is shown in the figure 3.

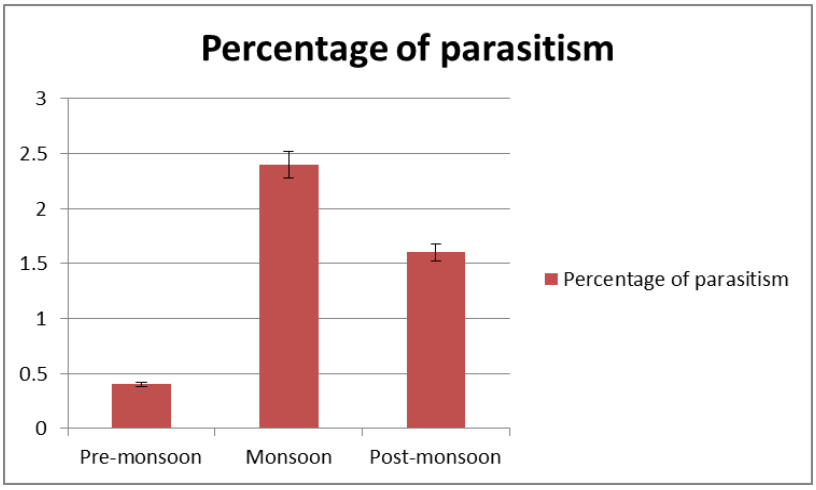

Fig.1. Percentage of parasitism of *M. scalaris* in the selected locality during the pre-monsoon, monsoon and post monsoon period.

Fig. 2. a. Normal Pupa

Fig. 2. b. Infested pupa

Fig. 3. Larvae feeding on pupal tissue

b. Biology of *M. scalaris* (Loew): *Megaselia scalaris* (Loew) infested pupae of *E.torus* were collected from the study area located at Pariyaram, Kannur. The analysis of parasitized pupae of *E.torus* (Fig.2b) confirmed that *M. scalaris* developed inside all parts including the head, thorax, and abdomen.The life cycle includes stages of egg, three larval instars, prepupa, and pupa.

i. Adult: The adult fly (Fig. 4. a. & and Fig. 4. b) is yellowish brown in colour, having many long evenly spaced bristles on the supra-antennal surface. The dorsal side of the thorax is brownish in colour. Small hairy bristles and 2–4 long ventral bristles are present in prothoracic pleura. The legs are paler, with enlarged femur, appeared darker apically, due to the presence of small dark brown hairs (Fig. 5). The halters are creamy yellowish in colour; the wings possess a densely fringed section along half of the costal margin (Fig. 6), with a darker brown abdomen. The mesopleuron has no distinct bristles, while the scutum possesses faint brown markings with a couple of long hairs at their posterior margin, and the scutellum also bears two pairs of bristles. The adult shows characteristic erratic movements in the rearing tube. Eggs are usually laid on the external surface of last instar larvae, pre- pupae and pupae. An average of 10 ± 5 eggs (n=30) were laid by a fly at a time and a mean number of 30±8 larvae (n=30) emerged from the dead pre-pupae and pupae of *E. torus*.


Fig.4. a. & 4. b. Adult *Megaselia scalaris*

Fig. 5. Adult L leg

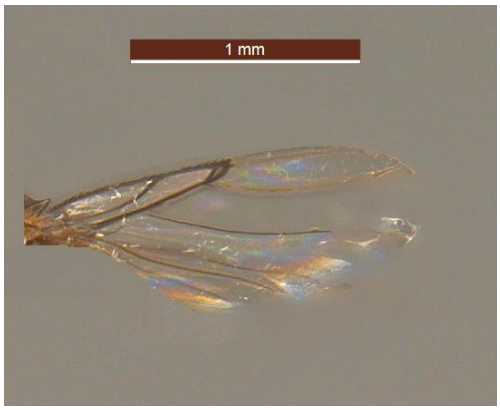

Fig. 6. Adult wing

ii. Eggs: Pale white coloured, small boat-shaped eggs are usually laid on the outer side of the pupa .Mean length of egg was 0.75±0.25 mm and the duration of eggs was1.5±0.5 days(Table. 1).

iii. Larvae: A single pupa of *E.torus* may be infested by more than one fly and an average of 45±3 (n= 10) larvae (Fig. 7) were observed in an infected pupae. Larvae emerged 1.5±0.5 days (n=30) after the eggs were laid. The larvae are transparent and gelatinous white in colour. Three larval instars with average duration of 2±1 days (n=30) for first two each and 3±1days (n=30) for third instar. The length of the first, second and third instar larvae were 2±1 mm, 3.5±0.5 , 4±1 mm.(n=30) respectively. The larvae feed ravenously on tissues of the host.

iv. Pupa: The puparium was dorsoventrally flattened with light brownish in colour with a set of long respiratory horns (Fig. 8.a & 8.b.). The pupa has two pairs of spiracles at each end. The prepupal stage is completed in approximately 2 ± 1 days (n=30), while the pupal stage lasts around 6 ± 1 days (n=30).

Table. 1. Length in mm and duration in days of different stages of *Megaselia scalaris.*

Stage	Length in mm	Duration in days
Eggs	0.75±0.25	1.5±0.5
First instar larva	2±1	2±1
Second instar larva	3.5±0.5	2±1
Third instar larva	4±1	3±1
Prepupa	4±1mm	2 ± 1
Pupa	4±1mm	6±1

Fig. 7 Early instar larvae

Fig. 8.a. Puparium

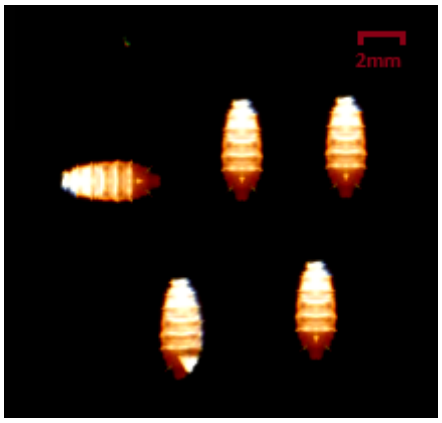

Fig. 8. b. Pupae

c. Gene sequencing of COI gene of *M. scalaris* (Loew): The mitochondrial COI gene barcoding by partial sequencing of *M. scalaris* conducted during the different trials in the study period yielded a 645 bp linear DNA product.

d. Submission of sequences in GenBank: The amplified sequence was deposited to GenBank with accession number OL655409.1. Figure 6 presents a distance tree comparing the COI region of the present sequence with sixteen other deposits from GenBank. A BLAST search confirmed that our sequence belongs to *M. scalaris*, showing 99.84% homology with the sequence KX266968.1 from Kenya, 99.53% identity with KX832635.1 from China, 99.52% homology with KX832632.1 from China, and 99.38% similarity with sequences KX832638.1 from South Korea, MT396272.1 from Korea, and KX832636.1 from China, as listed in the NCBI database (Table 3).The sequences obtained and deposited in the current study were most similar to *M. scalaris* (KX832638.1) from China.

e. Phylogenetic analysis and evolutionary lineage:

The phylogenetic tree was created using the 'Fast Minimum Evolution method' (Fig. 9) and the 'Neighbor-Joining method' (Fig. 10) with 'MEGA 11' software.

Table. 3. Hit table comparing the sequence ID OL655409.1. with sequences of *M. scalaris* and other nearest related species retrieved from NCBI

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Megaselia scalaris	1192	1192	100%	0	100.00%	OL655409.1
Megaselia scalaris	1170	1170	100%	0	99.38%	KX832638.1
Megaselia scalaris	1170	1170	100%	0	99.38%	MT396272.1
Megaselia scalaris	1170	1170	100%	0	99.38%	MT396271.1
Megaselia scalaris	1164	1164	99%	0	99.38%	KX832636.1
Megaselia scalaris	1164	1164	100%	0	99.23%	MT396301.1
Megaselia scalaris i	1164	1164	100%	0	99.23%	MT396291.1
Megaselia scalaris	1164	1164	100%	0	99.23%	MT396282.1
Megaselia scalaris	1162	1162	98%	0	99.53%	KX832635.1
Megaselia scalaris	1162	1162	98%	0	99.53%	KX832634.1
Megaselia scalaris	1158	1158	98%	0	99.53%	KX832633.1
Megaselia scalaris	1158	1158	98%	0	99.53%	KX832630.1
Megaselia scalaris	1153	1153	98%	0	99.53%	KX832637.1
Megaselia scalaris	1153	1153	98%	0	99.53%	KX832631.1
Megaselia scalaris	1153	1153	97%	0	99.84%	KX266968.1
Megaselia scalaris	1147	1147	97%	0	99.52%	KX832632.1
Megaselia scalaris	1147	1147	100%	0	98.76%	MT251292.1
Megaselia scalaris	1147	1147	100%	0	98.76%	MT396354.1
Megaselia scalaris	1147	1147	100%	0	98.76%	MT396342.1
Megaselia scalaris	1147	1147	100%	0	98.76%	MT396299.1
Megaselia scalaris	1147	1147	100%	0	98.76%	MT396280.1

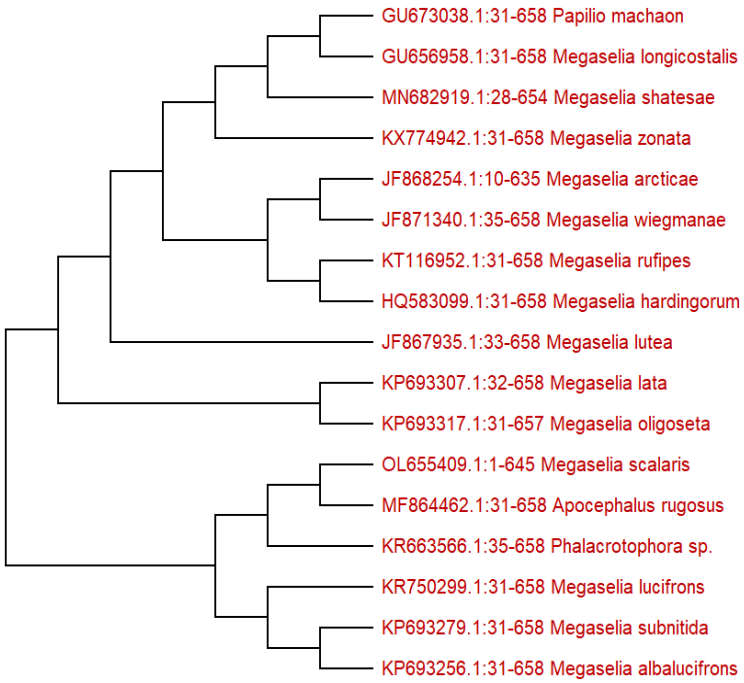

Fig.9 .Phylogenetic tree of *M. scalaris* (Loew) constructed by fast minimum evolution method

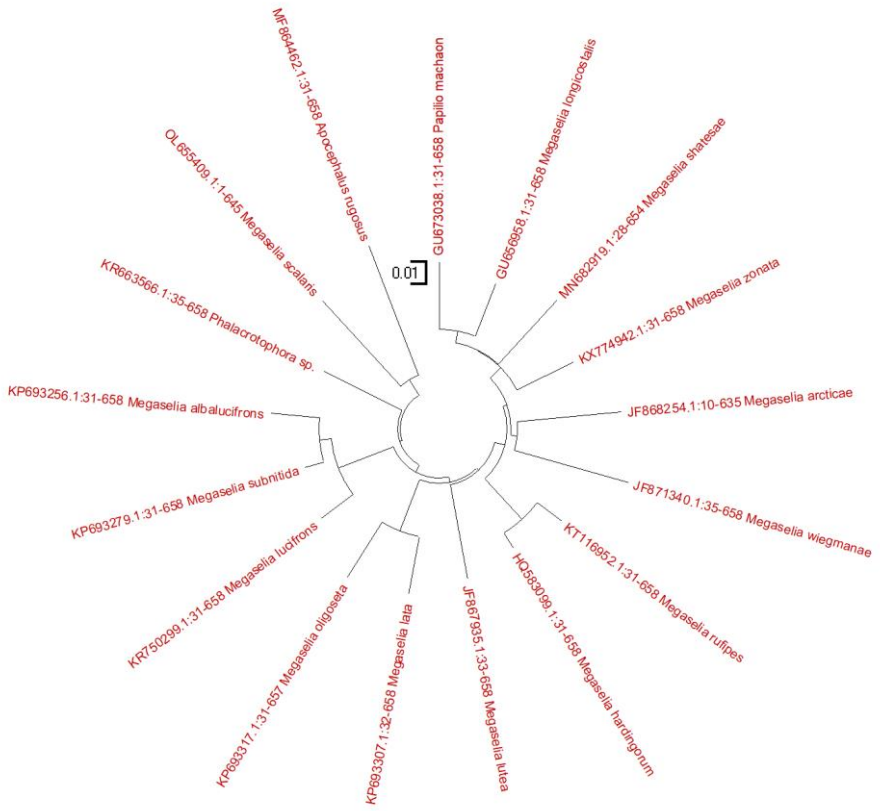

Fig.10. Phylogenetic tree of *M. scalaris* (Loew): constructed by neighbour joining method

Discussions:

Since *Erionota torus* invaded peninsular India, numerous investigations have been conducted to identify potent natural enemies capable of parasitizing different stages of *E. torus*. Parasitoids targeting the eggs, larvae, and pupae of *E. torus* have been reported (Poorani et al., 2020, Sharanabasappa et al., 2018 and Sreedhar et al., 2020). '*Brachymeria lasus* (Walker) (Hymenoptera: Chalcididae)' is a prevalent parasitoid in India, frequently found in association with various lepidopteran pupae, including *E. torus* (Poorani et al., 2020). Additionally, unidentified tachinid species have been reported as pupal parasitoids of *E. thrax* and *E. torus* (Cock, 2015; Soumya et al., 2013; Poorani et al., 2020). In the present investigation, the phorid fly *M. scalaris* was identified infesting the pupae of *E. torus* collected from different infested areas in Kannur district, Kerala. Both morphological and molecular approaches, including DNA barcode identification, confirmed *M. scalaris* as a pupal parasitoid of *E. torus*. According to Disney (1998), this fly is widely distributed across various geographical regions and typically feeds on decaying organic matter. Andreotti et al. (2003) documented the occurrence of *M. scalaris* as a parasitoid of *Boophilus microplus* in Brazil. Cham et al., (2018) identified this fly as an opportunistic parasitoid attacking honey bees in Cameroon. The initial report of *M. scalaris* infesting the pupae of *Palaeosepsis* sp. was made by Marchiori (2020) from Brazil. Tang et al. (2020) stated that *M. scalaris* infested the invasive insect pest *Spodoptera frugiperda* Smith in China. Deshmukh et al. (2021) showed the first report of *M. scalaris* as a parasitoid of *S. frugiperda* (Lepidoptera: Noctuidae).

However, the potential use of *M. scalaris*s as biological control agents is still unknown and requires future studies. Emphasizing biological control using natural enemies can reduce pesticide use and support the execution of operational 'Integrated Pest Management (IPM)' techniques for managing *E. torus* infestations globally, wherever necessary. This underscores the need for more research and exploration of different parasitoids and their subsequent development into effective biocontrol agents for managing *E. torus*.

Conclusion

In the current investigation, the phorid fly '*Megaselia scalaris*' (Loew) (Diptera: Phoridae) was identified and reported for the first time as a facultative parasitoid of the pupa of banana leaf skipper *E. torus*, both in the field and the laboratory. This identification was confirmed by gene barcoding of *M. scalaris.* The current study proposes that '*M. scalaris*' might be involved in the list of parasitoid species of *E. torus;* though upcoming research is inevitable to confirm it as an effective biocontrol agent. Thus, the current study offers a new perspectives for implementing management strategies against this hazardous invasive banana pest.

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References

1. Abdul Jaleel, K., S M Ghosh,S.M., 2020. Biology and damage of Banana skipper *Erionota torus* (Evans) from Malabar region of Kerala. Indi-and Jou.Entomology. 82 (3) , pp 429-434 .
2. Carlos Henrique Marchiori.,2020. First occurrence of *Megaselia scalaris* (Loew) (Diptera: Phoridae) in pupae of *Palaeosepsis* sp. (Díptera: Sepsidae) in Brazil. Qeios. doi:10.32388/LV51P8.
3. Jane Costa,Carlos,E., Almeida,Gleidson,M.,Esperanca,Nínive Morales,Jacenir ,R., dos, S., Mallet Teresa, C.M., Goncalves,Angelo, P., do Prado., 2008. First record of *Megaselia scalaris* (Loew) (Diptera: Phoridae) infesting laboratory colonies of *Triatoma brasiliensis* Neiva (Hemiptera: Reduviidae). Neotropical Entomology [online]. 2007, v. 36, n. 6 [Accessed 24 August 2022] , pp. 987-989. . ISSN 1678-8052. https://doi.org/10.1590/ S1519-566X2007000600026.
4. Cham,D.T.,Fombong, P.N., Ndegwa, L.W. Irungu, E. Nguku, and Raina,S.K.,2018)."*Megaselia scalaris* (Diptera: Phoridae), an Opportunist Parasitoid of Honey Bees in Cameroon," *African Entomology* 26(1), 254-258, . https://doi.org/10.4001/003.026.0254
5. Deshmukh, S.S., Kiran, S., Naskar, A., Palam Pradeep, Kalleshwaraswamy ,C. M., & Sharath K. N., 2021.First record of a parasitoid, *Megaselia scalaris* (Diptera: Phoridae) of fall armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) from India. Egypt J Biol Pest Control 31, 94 . https://doi.org/10.1186/s41938-021-00439-w
6. Disney, R., 2008. Natural history of the Scuttle fly, *Megaselia scalaris*. Annu. Rev. Entomol. 53, 39–60.
7. Felsenstein, J.,1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39: 783-791.
8. Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. Dec;16(2):111-20. doi: 10.1007/BF01731581. PMID: 7463489.
9. Kumar, S., Stecher, G. and Tamura, K., 2016. MEGA7: Molecular Evolutionay Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870-1874.
10. Poorani,Janakiraman, Balakrishnan, Padmanaban,Deshmukh,Sharanabasappa,Thanigairaj,R.,Ragesh, Gavas., 2020. A review of the pest status and natural enemy complex of Banana skipper *Erionota torus* Evans in south India and its management. Indian Journal of Entomology. 82, 479-492.
11. Saitou, N. and Nei, M., 1987. The Neighbour-Joining Method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4), 406-425.
12. Sharanabasappa,Kalleshwaraswamy, C .M., Maruthi, M. S., Shima H.,2018. Population dynamics and new record of larval parasitoids, *Senometopia* sp. and *Winthemia sumatrensis*

(Townsend) (Diptera:Tachinidae) on banana skipper, *Erionota torus* Evans (Lepidoptera:Hesperiidae) from South Karnataka. Journal of Biological Control 31(1).

13. Shreedhar Chatter, Roopa, S., Patil, H., Basappa and Akkamahadevi, Agasimani.,2020. Status and population dynamics of banana leaf roller, *Erionota torus* Evans in hill ecosystem of Uttara kannada, Karnataka, india. J. Exp. Zool. India Vol. 23, No. 2, pp. 1107-1112.
14. Tamura, K., Peterson, D., Peterson, N. and Kumar, S.,2011. MEGA5:Molecular Evolutionary genetics Analysis using Maximum Liklihood, Evolutionary Distance and maximum Parsimony methods. Molecular Biology and Evolution, 28(10): 2731- 2739.
15. Tang, Y., Li, Q., Xiang, L.Gu, R.,Wu, Y., Zhang, Y., Bai, X., Niu,X., Li, T., Wei, J., 2021. First Report on *Megaselia scalaris* Loew (Diptera: Phoridae) Infestation of the Invasive Pest *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in China. Insects,12,65. https://doi.org/10.3390/insects12010065
16. Zharkikh , A., and Li, W. H.,1995. Estimation of confidence in phylogeny: the complete-and-partial bootstrap technique. Molecular Phylogenetics and Evolution 4(1): 44-63.