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A Survey of Eulophid Wasps (Hymenoptera: Chalcidoidea) Associated with Rice Ecosystems of Tamil Nadu

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Abstract

Surveys were conducted to explore the eulophid fauna in rice ecosystems of Tamil Nadu during 2015-16 in three different rice-growing zones *viz.*, western zone, Cauvery delta zone and high rainfall zone. In the present study, a total of 161 eulophid individuals were collected from rice ecosystems that represent 3 subfamilies, 8 genera and 14 species. The three subfamilies were Entodoninae, Eulophinae and Tetrastichinae. Alpha and beta diversity were computed for the three zones and the diversity indices (Simpson's index, Shannon-Wiener index, Pielou's index) revealed that the high rainfall zone as the most diverse zone, while western zone being the least. *Aprostocetus benazeer* Narendran was found to be the most abundant species in the rice ecosystem with a relative density of 12.4 per cent. On comparing the species similarities using the Jaccard's index in between the three sites taken in pairs, it was found that 66 per cent similarity between western and Cauvery delta zones and 42 per cent similarity between high rainfall and Cauvery delta zones and 35 per cent similarity between high rainfall and western zones.

Keywords: *Diversity, Hymenoptera, Chalcidoidea, Eulophidae, Rice Ecosystem, India.*

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Introduction

Rice is one of the most important grains for human nutrition, being the staple food of more than three billion people and cultivated across 112 countries covering every continent except Antarctica (Acosta *et al.,* 2017). Rice fields harbour a rich and varied fauna than any other agricultural crop (Heckman, 1979; Fritz *et al.,* 2011). The fauna is dominated by micro, meso and macro arthropods inhabiting the soil, water and vegetation sub-habitats of the rice fields. The different communities of terrestrial arthropods in the rice field include pests, their natural enemies (predators and parasitoids) and other neutral insects that inhabit or visit the vegetation as tourists (Heong *et al*., 1991). More than 800 species of insects are known to infest rice, of which about 20 species are of economic importance. Farmers generally rely on insecticides to combat pest problems of rice. Indiscriminate use of insecticides resulted in the loss of biodiversity of beneficial organisms like parasitic hymenopterans (Dudley *et al.,* 2005). Reducing the mortality of parasitic hymenopterans caused by insecticides is essential for greater sustainability in rice pest management (Heong and Hardy, 2009; Gurr *et al*., 2011). Parasitic hymenopterans especially eulophids are the best alternatives to pesticides. They show greater stability to the ecosystem than any group of natural enemies of insect pests because they are capable of living and interacting at lower host population level. To aid this means of pest control, it is essential that the diversity of parasitoids needs to be studied first (Dey *et al*., 1999).

The majority of Eulophidae are primary parasitoids of concealed larvae, especially those inhabiting leaf mines. The best known species attack Lepidoptera, but many species parasitize larvae of other insects living in similar concealed situations (such as Agromyzidae, heterarthrine Tenthredinidae and Curculionidae).

Other eulophids attack various gall-forming species of insects, eriophyid mites (Boucek and Askew, 1968) and also gall-forming nematodes (Berg *et al.*, 1990). Various other species collectively exhibit a great range of lifeways. A number of other eulophids develop as endoparasitoids in insect eggs. The diversity of eulophids associated with rice ecosystem is poorly studied and far from satisfaction especially in Tamil Nadu. Any additional knowledge in diversity, taxonomy and biology is of potential practical value. In this context, the present study was undertaken to explore the diversity of eulophid fauna in rice ecosystems of Tamil Nadu.

Materials and Methods

Sites of collection

The survey was carried out in the rice fields during 2015-16 in three different agro climatic zones of Tamil Nadu State *viz.*, western zone: Paddy Breeding Station, Coimbatore, 427 m, 10° 59' 43.24" N 76° 54' 59.22" E), Cauvery delta zone: Krishi Vigyan Kendra, Needamangalam, 26 m, 10° 46' 23.93" N, 79° 25' 0.96" E) and high rainfall zone: Agricultural Research Station, Thirupathisaram, 17 m, 8° 12' 16.70" N, 77° 26' 57.84" E). Collections were made for 20 consecutive days in each zone to give equal weightage and to minimize chances of variations in the collection. The time of sampling in each zone was decided by the rice growing season of the zone and the stage of the crop *i.e.*, 20 days during August- September, 2015 in western zone, October- November, 2015 in high rainfall zone and December, 2015 – January 2016, in Cauvery delta zone.

Methods of collection

A total of three different gadgets *viz.*, sweep net, yellow pan trap kept at ground level and yellow pan trap erected at canopy levels were employed. All the three gadgets were employed continuously for 20 days.

(a)Sweep Net

The net employed for collection was essentially similar to an ordinary insect net with 673 mm mouth diameter and a 1076 mm long aluminum handle. The frame can be fitted to one end of the handle. This facilitates easy separation of the frame. The long handle allows the net to be used as far as possible making the sweeping easier and effective. The net bag was made up of thin cotton cloth. It measures about 600 mm in length and has a well rounded bottom. The top of the bag which fits around the frame was made up of a canvas. The canvas was folded over the frame and sewed in position. Sweeping of vegetation was as random as possible from ground level to the height of the crop. Sweeping was done during early morning and late evening hours for about half an hour per day which involved 30 sweeps. One to and fro motion of the sweep net was considered as one sweep.

(b)Yellow pan traps kept at ground level

This trap was based on the principle that many insects are attracted to bright yellow colour. Yellow pan traps are shallow trays of 133 mm × 195 mm and 48 mm deep and were of bright yellow in colour. Altogether, twenty yellow pan traps were installed at ground level in each site on the bunds, half- filled with water containing a few drops of commercially available detergent (to break the surface tension) and a pinch of salt (to reduce the rate of evaporation and to prevent rotting of trapped insects). The spacing between traps was standardized as 1.5 m. The traps were set for a period of 24 hours (Example: traps set at 10 AM on one day were serviced at 10 AM on the following day).

(c)Yellow pan traps erected up to canopy level

Erected yellow pan traps were installed at the crop canopy by means of polyvinyl chloride pipes fitted below, with a screw attachment and were installed in 10 numbers per site in the same fashion as Yellow pan traps kept at ground level.

Preservation and identification of the specimens up to family level:

The parasitoids thus collected were preserved in 70% ethyl alcohol. The dried specimens were mounted on pointed triangular cards and studied under a Stemi (Zeiss) 2000-C and photographed under Leica M205 A stereozoom microscopes and identified through conventional taxonomic techniques by following standard keys. For future references all the identified specimens were submitted in Insect Biosystematics Laboratory, Tamil Nadu

Agricultural University, Coimbatore.

Measurement of diversity

1. Relative Density

Relative density of the species was calculated by the formula, Relative Density (%) = (Number of individuals of one species / Number of individuals of all species) X 100.

2. Alpha Diversity

Alpha diversity of the zones was quantified using Simpson's diversity Index (*SDI*) Shannon-Wiener index (*H'*), Margalef Index (α) and Pielou's Evenness Index (*E1*).

(a) Simpson's Index

Simpson's diversity index is a measure of diversity which takes into account the number of species present, as well as the relative abundance of each species. It is calculated using the formula, $D = \Sigma n(n-1)/N(N-1)$ where n = total number of organisms of a particular species and N = total number of organisms of all species (Simpson, 1949). Subtracting the value of Simpson's diversity index from 1, gives Simpson's Index of Diversity (SID). The value of the index ranges from 0 to 1, the greater the value the greater the sample diversity.

(b) Shannon-Wiener Index

Shannon-Wiener index (*H'*) is another diversity index and is given as follows: $H' = -\Sigma Pi \ln(Pi)$, where $Pi = S / N$; S = number of individuals of one species, N = total number of all individuals in the sample, ln = logarithm to base e (Shannon & Wiener, 1949). The higher the value of *H'*, the higher the diversity.

(c) Margalef Index

Species richness was calculated for the three zones using the Margalef index which is given as Margalef Index, $\alpha = (S - 1) / ln (N)$; S = total number of species, N = total number of individuals in the sample (Margalef, 1958).

(d) Pielou's Evenness Index

Species evenness was calculated using the Pielou's Evenness Index (*E1*). Pielou's Evenness Index, $E1=H'/ ln(S)$; H' = Shannon-Wiener diversity index, S = total number of species in the sample (Pielou, 1966). As species richness and evenness increases, diversity also increases (Magurran, 1988).

3. Beta Diversity

Beta diversity is a measure of how different (or similar) ranges of habitats are in terms of the variety of species found in them. The most widely used index for assessment of Beta diversity is Jaccard Index (JI) (Jaccard, 1912), which is calculated using the equation: JI (for two sites) = $j/(a+b-j)$, where j = the number of species common to both sites A and B, a = the number of species in site A and b = the number of species in site B. We assumed the data to be normally distributed and adopted parametric statistics for comparing the sites.

Statistical analysis

The statistical test ANOVA was also used to check whether there was any significant difference in the collections from three zones. The data on population number were transformed into X+0.5 square root before statistical analysis. The mean individuals caught from three different zones were analyzed by adopting Randomized block design (RBD) to find least significant difference (LSD). Critical difference (CD) values were calculated at five per cent probability level. All these statistical analyses were done using Microsoft Excel 2016 version and Agres software version 3.01.

Results and Discussion

In the present study, a total of 161 eulophid individuals were collected from rice ecosystems that represent 3 subfamilies, 8 genera and 14 species. The three sub families are Entodoninae, Eulophinae and Tetrastichinae. Altogether 8 species were collected and identified under the subfamily Tetrastichinae *viz.*, *Aprostocetus benazeer* Narendran, *A. harithus* Narendran, *A. malcis* Narendran, *Tetrastichus cupressi* Yang, *T. krishnieri* (Mani), *T. schoenobii* Ferriere, *T. howardi* (Oliff), and *T. tunicus* Narendran. Under the subfamily Eulophinae, four species were collected and identified *viz.*, *Euplectrophelinus* sp., *Hemiptarsenus* sp. and *Necremnus leucarthros* (Nees) and *Elasmus kollimalaianus* Mani. Under the sub family Entedoninae, *Closterocerus* sp. and *Pediobius inexpectatus* Kerrich were the two species collected in the present study. As on date, thirty-two species of eulophids were collected form rice ecosystems throughout India. Of which, *Euplectrophelinus* sp., *Hemiptarsenus* sp., *Necremnus leucarthros, Tetrastichus cupressi* and an undetermined species under the genera *Tetrastichus* were new additions (Daniel and Ramaraju, 2019).

The survey results revealed that the species richness was maximum (12) in high rainfall zone. The number of species collected from western and Cauvery delta zones was 07 and 08, respectively (Table 1). A total of 97, 41 and 23 eulophids were collected from high rainfall, western and Cauvery delta zones, respectively. *Aprostocetus benazeer* was found to be the most abundant species in the rice ecosystem with a relative density of 12.4 per cent. Species such as *A. benazeer, A. harithus, A. malicis, Closterocerus* sp. and *T. tunicus* were obtained only from high rainfall zone. Species such as *E. kollimalaianus, Euplectrophelinus* sp., *P. inexpectatus* and *T. cupressi* were common to all the three zones surveyed. *Hemiptarsenus* sp. and *N. leucarthros* were common to both western and Cauvery delta zones. Only one species named *T. howardi* was found common to both western and high rainfall zones. Two species *viz., T. krishnieri* and *T. schoenobii* were collected from Cauvery delta and high rainfall zones. *Tetrastichus cupressi, T. krishnieri* and *A. benazeer* were found to be predominant in western, Cauvery delta and high rainfall zones, with a relative density of 29.3, 30.4 and 20.6 per cent, respectively. The occurrence of four species *viz., A. benazeer, Closterocerus* sp., *P. inexpectatus* and *T. cupressi* were found to significantly differ between the zones as tested by ANOVA.

Among the three zones, more number of eulophids was collected from high rainfall zone with a mean number of 4.85 ± 1.04 eulophids per day. It is statistically superior to the western and the Cauvery delta zones which have a mean number of 2.05 ± 0.60 and 1.15 ± 0.39 eulophids per day, respectively (Table 2). The Simpson's index of Diversity was the highest for high rainfall zone (0.87), followed by Cauvery delta zone (0.84) and western zone (0.83). Similar trend was observed in Shannon-Wiener index also with 0.78, 0.79 and 0.94 for western, Cauvery delta and high rainfall zones, respectively. The values of Margalef index for the three zones revealed that maximum richness (2.40) was accounted for high rainfall zone followed by Cauvery delta zone (2.23) and western zone (1.16). The species evenness was maximum for western zone (0.40) and for the Cauvery delta and high rainfall zones, it was 0.37 and 0.38, respectively. On comparing the species similarities using the Jaccard's index in between the three sites taken in pairs, it was found that 66 per cent similarity between western and Cauvery delta zones and 42 per cent similarity between high rainfall and Cauvery delta zones and 35 per cent similarity between high rainfall and western zones. The host details of the all the collected Eulophids are tabulated (Table. 3). The fourteen species of eulophids that were collected are also presented (Plate 1).

Daniel *et al.* (2017, 2019b and 2020) obtained similar results by conducting experiments to assess the diversity of pteromalids, braconids and ichneumonids of rice ecosystems in Tamil Nadu. The species composition among elevational zones can indicate how community structure changes with biotic and abiotic environmental pressures (Shmida and Wilson, 1985; Condit *et al.,* 2002). Studies on the effect of elevation on species diversity of taxa such as spiders (Sebastian *et al.,* 2005), moths (Axmacher & Fiedler, 2008), paper wasps (Kumar *et al.,* 2008) and ants (Smith *et al.,* 2014) reported that species diversity decreased with increase in altitude. However, according to Janzen (1976), diversity of parasitic Hymenoptera is not as proportionately reduced by elevation as in other insect groups, a fact that is in support of our results. A similar study conducted by Shweta and Rajmohana, 2016 to assess the diversity of members belonging to the subfamily Scelioninae also declared that the elevation did not have any major effect on the overall diversity patterns. The elevational diversity gradient (EDG) in ecology proposes that species richness tends to increase as elevation increases, up to a certain point creating "diversity bulge" at moderate elevations (McCain and Grytnes, 2010). The elevation dealt with in this work ranged from 17-427 m which was not very high. So taking into account the scale and extent of elevational gradients, it can be said that species diversity and richness have not showed any correlation i.e. species diversity and richness were not proportional with that of elevation. Daniel and

Table 1. Comparison of Eulophidae collected from three rice growing zones of Tamil Nadu

Species	Zones						Total			
	Western		Cauvery Delta		High Rainfall					
	No.	%	No.	%	No.	%	No.	%	F	P
Aprostocetus benazeer	0	0.0	0	0.0	20	20.6	20	12.4	9.5	0.00
Aprostocetus harithus	0	0.0	0	0.0	4	4.1	4	2.5	2.9	0.06
Aprostocetus malcis	0	0.0	0	0.0	8	8.2	8	5.0	3.23	0.04
Closterocerus sp.	0	0.0	0	0.0	19	19.6	19	11.8	7.00	0.00
Elasmus kollimalaianus	6	14.6	1	4.3	3	3.1	10	6.2	1.31	0.27
Euplectrophelinus sp.	9	22.0	3	13.0	4	4.1	16	9.9	3.12	0.05
Hemiptarsenus sp.	4	9.8	1	4.3	0	0.0	5	3.1	0.76	0.47
Necremnus leucarthros	2	4.9	2	8.7	0	0.0	4	2.5	0.50	0.60
Pediobius inexpectatus	5	12.2	1	4.3	12	12.4	18	11.2	3.75	0.02
Tetrastichus cupressi	12	29.3	2	8.7	3	3.1	17	10.6	3.18	0.04
Tetrastichus krishnieri	0	0.0	7	30.4	6	6.2	13	8.1	1.96	0.14
Tetrastichus schoenobii	0	0.0	6	26.1	1	1.0	7	4.3	2.64	0.07
Tetrastichus howardi	3	7.3	0	0.0	2	2.1	5	3.1	1.52	0.22
Tetrastichus tunicus	0	0.0	0	0.0	15	15.5	15	9.3	1.50	0.23
Total No. collected	**41**	**-**	**23**	**-**	**97**	**-**	**161**	**-**	**-**	
Species Number	**07**	**-**	**08**	**-**	**12**	**-**	**14**	**-**		

%- Relative Density, No.- Total number of individuals collected, F-Value, P-Value

Table 2. Diversity indices of Eulophidae from three rice growing zones of Tamil Nadu

Zones	Mean No. of Eulophidae collected/day	Std. Error	SID	*H'*	α	*E1*	β %
Western	2.05 (1.41)[b]	± 0.60	0.83	0.78	1.61	0.40	W and C – 66
Cauvery Delta	1.15 (1.15)[b]	± 0.39	0.84	0.79	2.23	0.37	C and H - 42
High Rainfall	4.85 (2.12)[a]	± 1.04	0.87	0.94	2.40	0.38	H and W - 35
S.ED	0.23	-	-	-	-	-	-
CD (p=0.05)	0.48	-	-	-	-	-	-

*Figures in parentheses are square root transformed values; In a column, means followed by a common letter(s) are not significantly different by LSD (p=0.05).

*SID- Simpson's Index of Diversity, *H'*- Shannon-Wiener Index, α- Margalef index, *E1*- Pielou's index, β-Beta diversity (Jaccard Index).

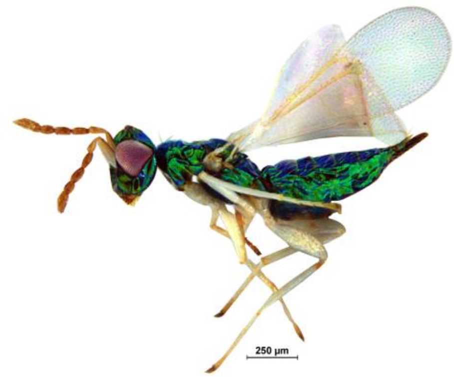
*W- Western Zone, C- Cauvery Delta Zone, H- High Rainfall Zone

Table 3. Eulophidae collected in the study along with their host

Parasitoid	Host	Reference
Aprostocetus benazeer *Aprostocetus harithus* *Aprostocetus malcis*	Cicadellidae Delphacidae Gryllidae Dytiscidae	Noyes, 2003
Closterocerus sp.	Agromyzidae	Edwards and La Salle, 2004
Elasmus kollimalaianus	Primary external parasitoids of the larvae of Lepidoptera or hyperparasitoids on them through various Hymenoptera	Gauthier *et al*., 2000

Euplectrophelinus sp.	Unknown	
Hemiptarsenus sp.	Agromyzidae	Thu and Ueno, 2002
Necremnus leucarthros	Chrysomelidae	Dosdall *et al*., 2007
Pediobius inexpectatus	Nymphalidae	Purnamasari and Ubaidillah, 2007
Tetrastichus cupressi	Eggs of Lepidoptera	Yang, 2006
Tetrastichus krishnieri		
Tetrastichus schoenobii		
Tetrastichus howardi		
Tetrastichus tunicus		

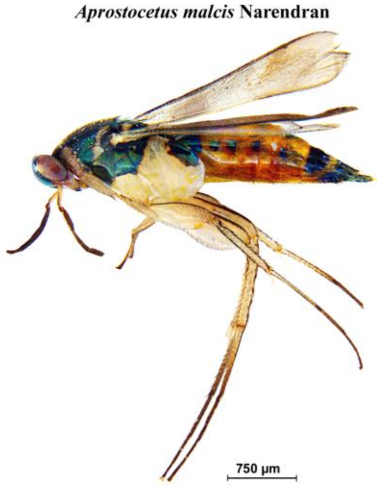
***Aprostocetus benazeer* Narendran**



***Aprostocetus harithus* Narendran**

***Aprostocetus malcis* Narendran**

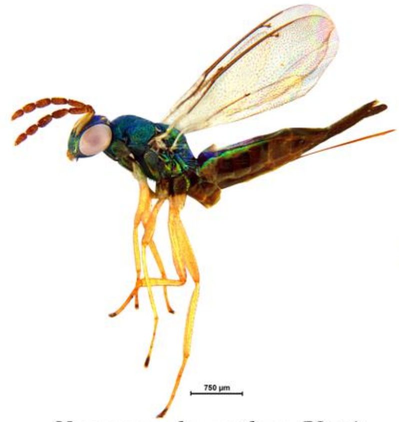
***Closterocerus* sp.**



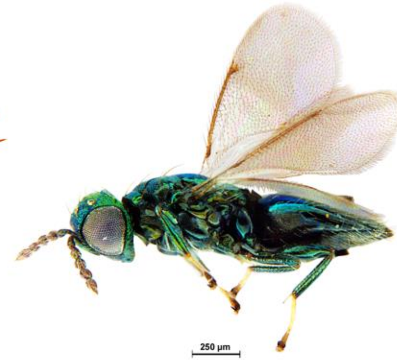
***Elasmus kollimalaianus* Mani**

***Euplectrophelinus* sp.**

***Hemiptarsenus* sp.**



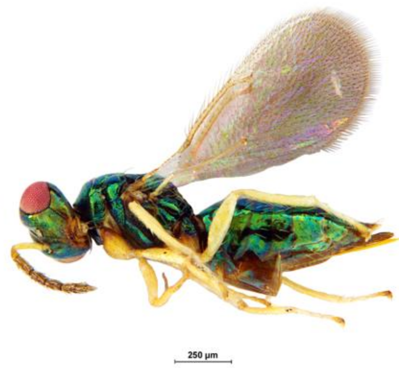
***Necremnus leucarthros* (Nees)**



***Pediobius inexpectatus* Kerrich**

***Tetrastichus cupressi* Yang**

***Tetrastichus krishnieri* (Mani)**



***Tetrastichus schoenobii* Ferriere**

***Tetrastichus howardi* (Oliff)**

***Tetrastichus tunicus* Narendran**

Plate 1. Fourteen species of Eulophidae collected from three rice growing zones of Tamil Nadu

Ramaraju (2017; 2020a & b) assessed the diversity of Chalcididae, Platygastroidea and parasitic Aculeatea, among three rice growing tracts of Tamil Nadu and concluded that there was no correlation between elevation and species richness. This fact supports our present study.

Studies on the altitudinal variation of parasitic Hymenoptera assemblages in an Australian sub-tropical rainforest by Hall *et al.* (2015) did not record any distinct assemblage at each altitude, at the morphospecies level, even though there was a clear separation between 'upland' and 'lowland' assemblages. To detect minute changes in species assemblages, species level sorting is found to give the best result (Grimbacher *et al.,* 2008). The area under cultivation turns out to be a very important factor with respect to abundance and species density in rice fields (Wilby *et al.,* 2006). The number of species in a habitat increases with increase in area (Gotelli and Graves, 1996). Only few studies have demonstrated the importance of different varieties in attracting the natural enemies (Scutareanu *et al.,* 1997; De Moraes *et al.,* 1998; Thaler, 1999; Kessler and Baldwin, 2001; Lou *et al.,* 2005; Rasmann *et al.,* 2005; Daniel *et al.,* 2019a, d). Lack of success in biological control programs has often been caused by high mortality of parasitoids due to climatic extremes (Daniel *et al.,* 2019c). Therefore, more researches like this should be encouraged to understand the underpinnings between varietal preferences, climatic conditions and parasitoid diversity.

Conclusion

This study reveals the diversity of eulophids of three different rice ecosystems of Tamil Nadu. The reasons for the significant changes in diversity of these parasitoids and their host insects are to be further studied. There is much scope for research to be taken on these aspects.

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