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Species Diversity and Ecology of Drosophilids (Diptera: Drosophilidae) of Poonch, Jammu and Kashmir, India

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ABSTRACT

Present study was conducted from 2015 – 2017 to explore different region of Poonch, Jammu and Kashmir India for species diversity and ecology of drosophilids. Total 5,387 flies belonging to 28 species from 08 Genera viz *Drosophila, Lordiphosa, Scaptomyza, Mycodrosophila Hirtodrosophila, Impatiophila* (New genus), *Leucophenga*, and *Zaprionus* with dominance of Genus *Drosophila* were collected by using different methods. Various diversity indices, cluster analysis and Constancy were employed to analyze species occurrence.

Key words: Diversity, *Drosophila*, diversity indices, cluster analysis, Poonch.

INTRODUCTION

Since the times of Morgan, the species of genus *Drosophila* mainly *Drosophila melanogaster* forms a very good model organism in all aspects of biological research. Species of this genus has richly contributed to our understanding pattern of inheritance, variation, mutation and speciation. Studies have also been made on the population genetics of different species of this genus. *Drosophila* is also being used for the study of population fluctuations, as they are highly sensitive to slight environmental modifications that are reflected in the size of the natural population structure and ecology. Flies are small and have a short life cycle and are easy to collect and perform experiment in the laboratory. Most of drosophilid flies feed and breed on rotting fruits, flowers and fungal materials. These fact emphases the importance to explore and understand the drosophilid diversity and distribution pattern in different geographical regions of Indian subcontinent.

Studies on Indian drosophilids were initiated by Bezzi c.f (Sturtevant 1921). Most of our present knowledge on drosophilid fauna has been acquired only after 1964. The German Dipterist, Herman Lowe first used the family name Drosophilidae in his several papers published in 1862.

The first complete catalogue of the family (Wheeler, 1981) listed more than 2,500 species in 55 genera. The family Drosophilidae is comparatively large and divided into two subfamilies (Steganinae and Drosophilinae). The subfamily Drosophilinae is the most diversify one and includes 6,812 species of which *Drosophila fallen* alone consist of 1,192 recorded species (Bachli, 2016).

Along with the development of genetics, evolution and developmental biology in the *Drosophila,* taxonomic studies in the genera of the family drosophilidae are poorly explored in some region of India. Material for the present study was collected from different localities of Poonch, Jammu & Kashmir.

MATERIAL AND METHODS

Intensive field collection were undertaken at different localities viz, Loran located at an altitude of (2,416m a.s.l., 33˚48′ N and 74˚20′ E), Bafliaz (1,731m a.s.l., 33˚36 ′N and 74˚2′ E), ′Bhimber Gali (1,865 m a.s.l., 33˚33 ′N and 74˚14 ′E) and Sangiot (2,031m a.s.l., 33˚32 ′N and 74˚18′E) of Poonch Jammu and Kashmir.

Sample collection:
A large number of techniques were used for the collection of drosophilids. The following techniques were employed to collect the drosophilid from their natural habitats.

a. **Trap-bait method**: Milk bottles of 250 ml capacity containing ripened banana, mixed with yeast were tied to the twigs underneath small bushes at a height of two to four feet above the ground. The flies that were trapped in the bottles were transferred to the vials containing 70% ethanol for preservation.

b. **Net Sweeping method**: The drosophilid flies that are not easily attracted towards fermenting fruits were collected by net sweeping. Net sweeping was done on naturally occurring rotting fruits, flowers, mushrooms and leaves. The flies trapped in the net were transferred to the vials containing 70% ethanol for preservation.

c. **Directly with aspirator:** The drosophilid flies that show occasional appearance while resting or mating on flowers and mushroom were collected directly with aspirator.

Identification and morphological studies:
The collected flies were etherized, categorized and were identified under margined stereo zoom microscope and metric characters were measured with an ocular microscope. To observe the detailed structures of male and female terminalia, respective organs were dissected from the adult body and was cleared by warming in 10% KOH around 100°C for several minutes and then observed in a droplet of glycerol under a light microscope. *Drosophila* flies were identified by employing several keys (Sturtevant 1927; Thorckmorton, 1962, Gupta 2005) and online identification sites like (BioCIS 2004), (JDD 2014) and (FlyBase 2014).

Vegetation of collected sites:
The vegetation usually comprises of *Pinus roxburghii*, *Quercus* spp.*, Myrica esculnta,* and *Rhododendron species* forest.

Ecological Diversity:
Drosophilids are often called the "fruit flies", showing their common occurrence on fruits, flowers and mushroom for feeding, courtship and breeding. All juicy fruits are suitable for their larval development. These are the main food substrate for *Drosophila* mostly of the *immigrans, Zaprionus* and *Sophophora* groups. A number of flower and mushroom species are used by drosophilid flies. The *Impatiophila is* specialized on Impatiens flowers. *Scaptomyza himalayana* is commonly observed on *Prunus* flowers. The *Hirtodrosophila* and *Drosophila* genus generally observed on hard mushrooms of the Polyporaceae, growing on tree trunk and dead wood. Among other mushrooms *Mycodrosophila* sp. are the most attractive.

Data analysis:
Qualitative occurrence of species was verified using Occurrence Constancy Method (Dajoz, 1983). The constancy value (c) was calculated as ratio of the number of collections in which particular species occurred to the total number of collections done, and multiplying the result by 100. Species with index $c \geq 50$ were considered constant species. Accessory species were those with $25 \leq c < 50$. Accidental species had $c < 25$. Species that occurred in only one area were considered exclusive. The abundance, richness and diversity relationship of flies collected were assessed by Simpson's index of diversity (1-D), Shannon-Wiener (H) and Margelf (DMg) index. Jaccard index between a pair of communities was calculated to analyze community similarities.

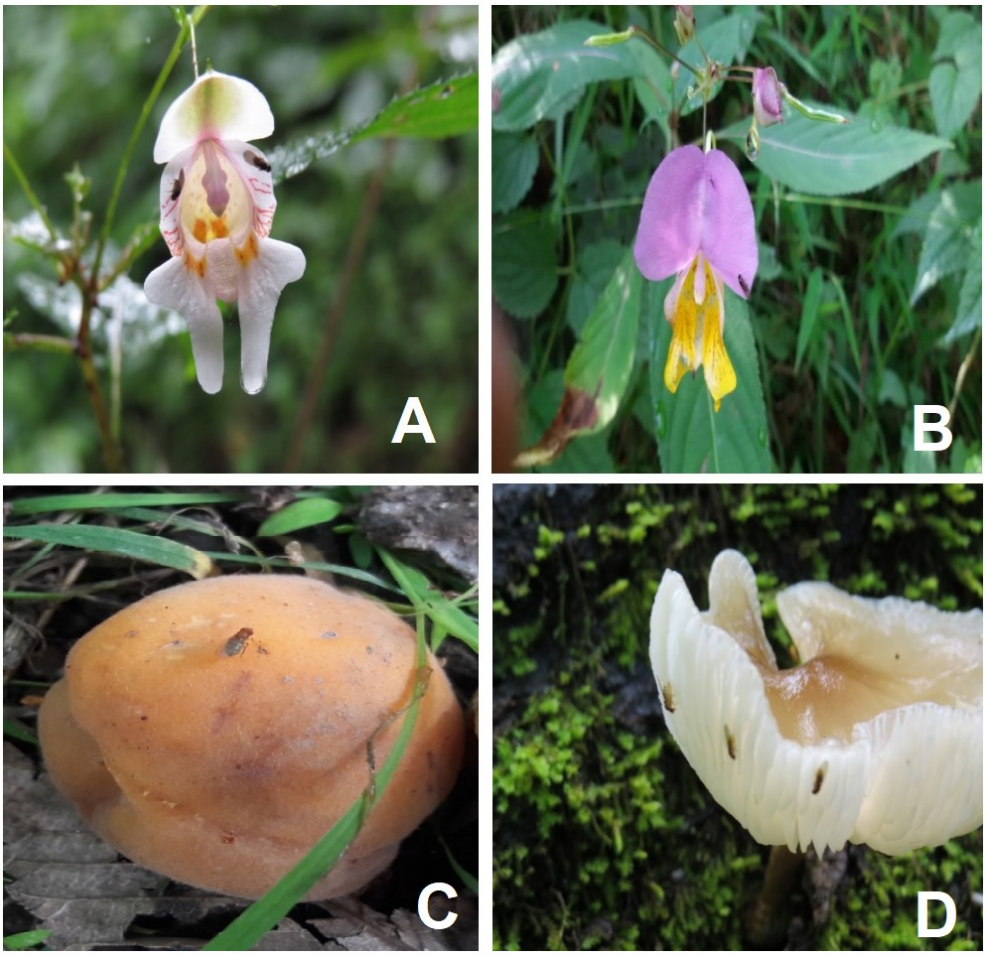

Fig. 1 Flies feeding on flowers (A and B), (C) fruit, (D) Mushroom.

SPSS 20.0 statistical package was used to determine the level of similarity of the 28 species of drosophilids collected and Hierarchical cluster analysis (Euclidean distances, Ward's method) was used to construct a single linkage Dendrogram.

RESULTS AND DISCUSSION

Table 1 shows the number of *Drosophila* species collected from different region of Poonch during 2015 to 2017. It revealed that 5,387 flies were collected consisting of a total 28 species; from 8 genera *viz Drosophila, Lordiphosa, Scaptomyza, Hirtodrosophila, Impatiophila, Leucophenga, Mycodrosophila,* and *Zaprionus.* With dominance of genus *Drosophila* including 13 species among which, 5 species belonged to subgenus *Sophophora,* 7 species belonged to subgenus *Drosophila* fallen and 1 species from subgenus *Dorsilopha* Sturtevant. Twelve species (*Drosophila nepalenesis, Drosophila bifasicata, Drosophila suzuki indicus, Drosophila melanogaster, Drosophila jambulina, Drosophila busckii, Drosophila immigrans, Drosophila parazonata, Drosophila novazonata, Drosophila painii, Scaptomyza himalayana, Zaprionus indianus,* and *Leucophenga albiceps,* were considered abundant and were collected from all four places These species describe generalist nature occupying broad niche i.e. no specificity towards one particular habitat and occurred across a range of habitats. Five species (*Hirtodrosophila sexivittata, Hirtodrosophila sp.* B4*, *Lordiphosa sp. B3*, Impatiophila sp. S4*, Impatiophila sp. S5*)* were collected from three places *(*Loran, LR, Bafliaz BF, Sangiot SG) and were not found from Bhimber gali (BG). Three species *Leucophenga sp*. L5*, *Hirtodrosophila fascipennis*, and *Hirtodrosophila sexvittata* were found only from one specific place. These drosophilid species are highly specific and were restricted to a specific type of habitat.

Constant species ($c \geq 50$) represented approximately 89% of the total collected species (25 out of 28). Three species were considered accessory (~ 11%). All genera showed constant species, except for the *Leucophenga* and *Hirtodrosophila* that indicates accessory species.

Table 1. Species diversity of drosophilids of Poonch collected during 2015-17

S. No.	Genus /Subgenus	Locality				
		Loran	Bafliaz (Surankote)	Bhimber gali	Sangiot	Total
Genus *Drosophila*						
Subgenus- *Sophophora* Sturtevant						
1	*Drosophila nepalenesis*	240	210	190	90	730
2	*Drosophila bifasicata*	70	66	39	28	203
3	*Drosophila suzuki indicus*	157	92	90	107	446
4	*Drosophila melanogaster*	109	70	24	73	276
5	*Drosophila jambulina*	24	18	13	17	72
	Total	**600**	**456**	**356**	**315**	
Subgenus- *Dorsilopha* Sturtevant						
6	*Drosophila busckii*	84	89	41	105	319
	Total	**84**	**89**	**41**	**105**	
Subgenus- *Drosophila* fallen						
7	*Drosophila immigrans*	114	63	51	77	305
8	*Drosophila repleta*	27	16	11	19	73
9	*Drosophila bizonata*	67	44	23	0	134
10	*Drosophila parazonata*	23	9	14	27	73
11	*Drosophila novazonata*	32	14	18	11	75
12	*Drosophila painii*	24	11	13	14	62
13	*Drosophila sp. B8**	34	45	0	0	79
	Total	**321**	**202**	**130**	**148**	
Genus *Hirtodrosophila* Duda						
14	*Hirtodrosophila trivittata*	96	0	0	0	96
15	*Hirtodrosophila sexivittata*	51	21	0	41	113
16	*Hirtodrosophila fascipennis*	0	70	0	0	70
17	*Hirtdrosophila* sp. *B4**	30	13	0	0	43
18	*Hirtodrosophila* sp. *B3**	45	21	0	30	96
	Total	**222**	**125**	**0**	**71**	
Genus *Scaptomyza* Hardy						
19	*Scaptomyza himalayana*	291	137	163	178	769
	Total	**291**	**137**	**163**	**178**	
Genus *Zaprionus* Coquillett						
20	*Zaprionus indianus* Gupta	274	179	99	249	801
	Total	**274**	**179**	**99**	**249**	
	Genus *Leucophenga* Mik					
21	*Leucophenga albiceps*	22	8	13	11	54
22	*Leucophenga* sp. *B1**	0	14	8	0	22
23	*Leucophenga* sp. *B3**	8	5	0	0	13
24	*Leucophenga* sp. *L5**	7	0	0	0	7
	Total	**37**	**27**	**21**	**11**	
Genus *Mycodrosophila* Oldenberg						
	Mycodrosophila gratiosa	30	12	0	19	61
	Total	**30**	**12**	**0**	**19**	
Genus *Lordiphosa* Basden						
25	*Lordiphosa sp. B3**	24	11	0	19	54
	Total	**24**	**11**	**0**	**19**	
Genus *Impatiophila Fu & Gao*						
26	*Impatiophila sp. S4**	78	28	0	70	176
27	*Impatiophila sp. S5**	65	16	0	76	157
	Total	**143**	**44**	**0**	**146**	
	Grand Total	**2026**	**1282**	**810**	**1261**	

Note: The species marked as * are not identified and are supposed to be new.

Table 2. The Absolute (A), Relative Abundance (R), and Constancy Value (*C*) of drosophilids collected from Poonch during 2015-2017

	Loran		**Bafliaz (Surankote)**		**Bhimber Gali**		**Sangiot**		*C*
	A	R	A	R	A	R	A	R	
Genus *Drosophila*									
Subgenus- *Sophophora* Sturtevant									
Drosophila nepalenesis	240	0.118	210	0.163	190	0.234	90	0.071	100
Drosophila bifasicata	70	0.034	66	0.051	39	0.048	28	0.022	100
Drosophila suzuki indicus	157	0.077	92	0.071	90	0.111	107	0.084	100
Drosophila melanogaster	109	0.053	70	0.054	24	0.029	73	0.057	100
Drosophila jambulina	24	0.011	18	0.014	13	0.016	17	0.013	100
Total	**600**		**456**		**356**		**315**		
Subgenus *Dorsilopha* Sturtevant									
Drosophila busckii	84	0.041	89	0.069	41	0.05	105	0.083	100
Total	**84**		**89**		**41**		**105**		
Subgenus- *Drosophila* fallen									
Drosophila immigrans	114	0.056	63	0.049	51	0.062	77	0.061	100
Drosophila repleta	27	0.013	16	0.012	11	0.013	19	0.015	100
Drosophila bizonata	67	0.033	44	0.034	23	0.028	0	0	75
Drosophila parazonata	23	0.011	9	0.007	14	0.017	27	0.021	100
Drosophila novazonata	32	0.015	14	0.01	18	0.022	11	0.008	100
Drosophila painii	24	0.011	11	0.008	13	0.016	14	0.011	100
*Drosophila sp. B8**	34	0.012	45	0.035	0	0	0	0	50
Total	**321**		**202**		**130**		**148**		
Genus *Hirtodrosophila* Duda									
Hirtodrosophila trivittata	96	0.047	0	0	0	0	0	0	25
Hirtodrosophila sexivittata	51	0.025	21	0.016	0	0	41	0.032	75
Hirtodrosophila fascipennis	0	0	70	0.054	0	0	0	0	25
Hirtdrosophila sp. *B4**	30	0.014	13	0.01	0	0	0	0	50
Hirtodrosophila sp. *B3**	45	0.022	21	0.016	0	0	30	0.023	75
Total	**222**		**125**		**0**		**71**		
Genus *Scaptomyza* Hardy									
Scaptomyza himalayana	295	0.145	137	0.106	163	0.201	178	0.141	100
Total	**295**		**137**		163		**178**		
Genus *Zaprionus* Coquillett									
Zaprionus indianus	278	0.137	179	0.139	99	0.122	249	0.197	100
Total	**278**		**179**		**99**		**249**		
Genus *Leucophenga* Mik									
Leucophenga albiceps	22	0.01	8	0.006	13	0.016	11	0.008	100
Leucophenga sp. *B1**	0	0	14	0.01	8	0.009	0	0	50
Leucophenga sp. *B3**	8	0.003	5	0.003	0	0	0	0	50
Leucophenga sp. *L5**	7	0.003	0	0	0	0	0	0	25
Total	**37**		**27**		**21**		**11**		
Genus *Mycodrosophila* Oldenberg									
Mycodrosophila gratiosa	30	0.014	12	0.009	0	0	19	0.015	75
Total	**30**		**12**		**0**		**19**		
Genus *Lordiphosa* Basden									
Lordiphosa sp. B3*	24	0.011	11	0.008	0	0	19	0.015	75
Total	24		11		0		19		
Genus Impatiophila Fu & Gao									
Impatiophila sp. S4*	78	0.038	28	0.021	0	0	70	0.055	75
Impatiophila sp. S5*	65	0.032	16	0.012	0	0	76	0.06	75
Total	**143**		**44**		**0**		**146**		
Grand total	**2026**		**1282**		**810**		**1261**		

Table 3. Shannon index (H′), Simpson index of diversity (1-D), and Margelf index (DMg). Highlighted boxes and bold numbers indicate highest and lowest values, respectively

Place	H′	1- D	DMg
LR	2.85	0.92	3.28
BF	2.78	0.91	3.49
B.G	**2.24**	**0.85**	**2.20**
SG	2.61	0.90	2.66

Table 4. Jaccard (J) index between pairwise populations Loran (LR), Bufliaz (BF), Bhimber Gali (B.G), and Sangiot (SG). Highlighted box indicates the highest Jaccard value.

	LR	BF	B.G	SG
LR	-	-	-	-
BF	0.85	-	-	-
B.G	0.555	0.615	-	-
SG	0.769	0.769	0.636	-

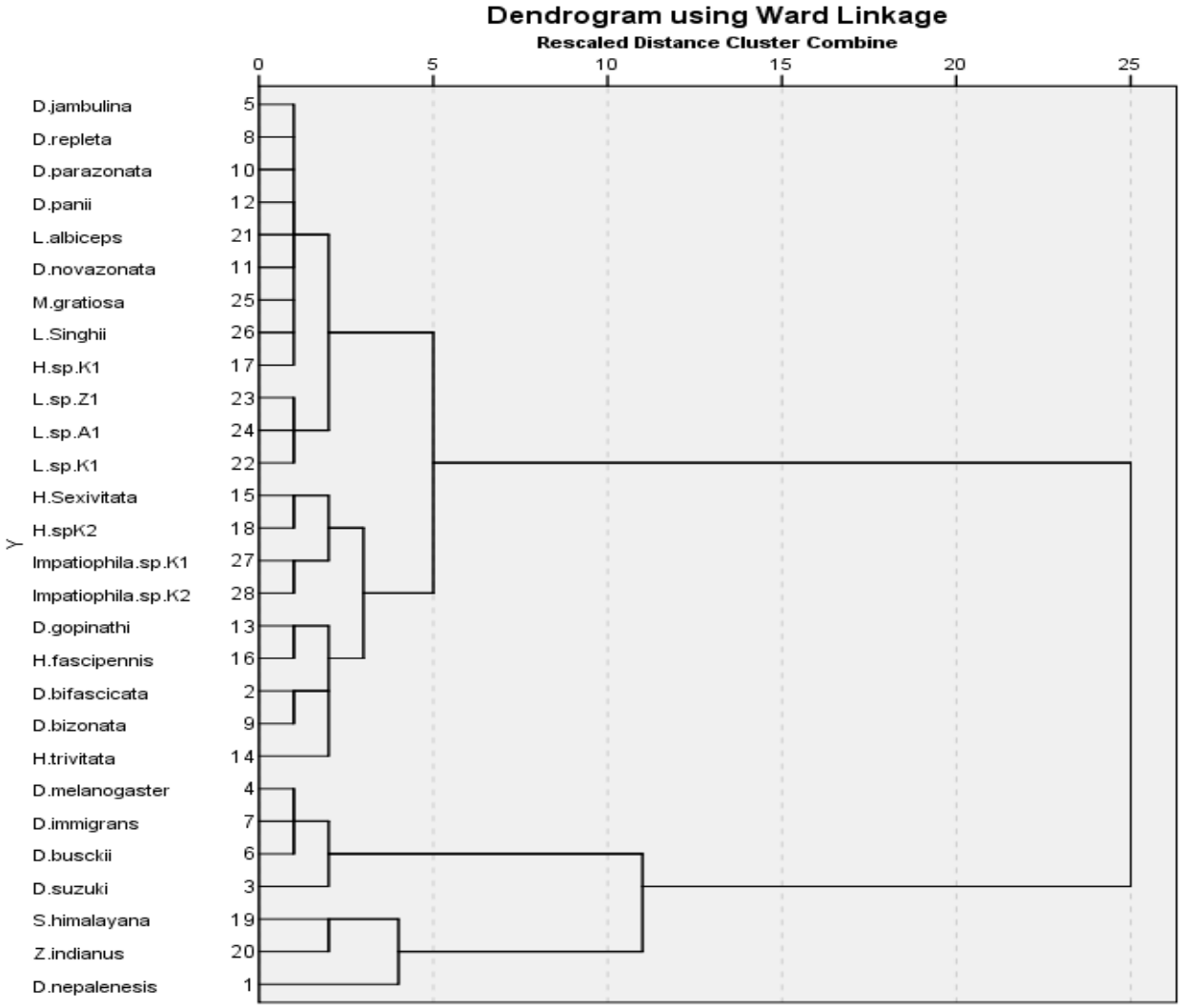

Fig. 2 Cluster analysis, of drosophilid species (Dendrogram using, Euclidean distance and Ward,s method)

The value of Shannon-Wiener, Simpson, Berger Parker and Margelf indices (Table 3) indicate the abundance, richness and diversity of drosophilids flies. According to the diversity indices Loran showed to be more diverse generating the greater value of Shannon-Wiener and Simpson's index of diversity and intermediate value of Margelf index. The Bhimber Gali shows least value of Shannon-Wiener and Simpson Index of diversity proved to be less diverse. While Bafliaz and Sangiot shows intermediate value of Shannon-Wiener index and Simpson index of diversity. The density or richness of species depends on the number of biotic and abiotic factors encountered in the seasons. A change in relative frequency of different species from season to season due to changes in natural environment was reported by (Guruprasad *et al.,* 2010). It is known that changes in temperature and rainfall affect viability, fertility, developmental time and other factors that influence the rate of population growth and survival (Torres and Madi-Ravazzi 2006).

The pair wise comparison using the Jaccard index (Table 4) of all four sampling sites showed that Bafliaz and Loran had the highest similarity (J= 0.857) .While Loran and Bhimber Gali showed least similarity among all comparison (J = 0.555). The similarity Pattern obtained in this comparison emphasis those two sites Loran (LR) and Bafliaz (BF), share more or less similar type of vegetation, temperature and Humidity.

Cluster analysis revealed that there are 2- major Clusters of species. Cluster first is divided into 2- sub clusters consisting of 21 species. Out of 21 species, sub cluster 1consist of 12 species, Sub cluster 2 consists of 9 species. Cluster 2 is divided into 2 sub clusters; sub cluster 1 consists of 4 species and sub cluster 2 consists of 3 species. Cluster 1 consist of rare species which are found less in number where as cluster 2 consist of abundant species which are dominantly found.

CONCLUSION

The present study indicates that the Poonch is rich in drosophilids fauna consisting of 8 genera and 28 species. It is an evident that the insects contribute much to the ecological welfare. Insect conservation has been recognized as vital for sustainable world in view of their critical role in conservation of ecosystem. Still there are other areas in this region which are unexplored. Further studies will be very helpful in understanding their ecological pattern and speciation.

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