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Population Structure and Sex Ratio Studies in *Bilobella braunerae* (Dhervang 1981) (Collembola: Neanuridae) from Rubber Plantation of Southern Western Ghats, India

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Abstract: The population structure of *Bilobella braunerae* in rubber plantation shows presence of male, female and juveniles in all seasons. The females outnumbered males in all seasons. The presence of juveniles all-round the year shows that females are ovigerous and lay eggs during favourable conditions. The *Bilobella braunerae* being a epedaphic species reproduce sexually. For studying population structure and Sex ratio of *Bilobella braunerae* in Rubber plantation of Seethathode, Pathanamthitta district, Kerala state, India, samples were randomly collected every month. Hand sorting and light funnel extraction was adopted to extract *Bilobella braunerae*. Generally, the size and colour of the body of animals were used to identify the sexes. Female is longer and has wider abdomen compared to male. The female organisms are bright orange in colour with a size of about 3 mm -3.5 mm and the presence of an ovipositor on the ventral side of the fifth abdominal segment. Male usually having dull reddish color and size of 2 mm-2.5 mm. The study showed that the male female ratio is not 1:1. The Length range sex ratio observed showed that 0-1.5 cm range, male to female ratio was 1:1, but in 1.5-2.5 cm range, males outnumbered females and in 2.5-3 cm range females outnumbered males and above 3 cm length range, males were totally absent. In all these cases except 0-1.5 cm range critical chi-square value lesser than the computed chi-square value with $P<0.05$ indicating a deviation of the sex ratio from normal 1:1. Male survivorship is high in lower length range and female survivorship is more in upper length range in the studied habitat.

Keywords: *Bilobella braunerae*, Sex ratio, Length range sex ratio, Ovigerous, Epedaphic species, Rubber plantation

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Introduction

Collembolans have received little attention regarding reproductive biology and especially sex ratio. The sex ratio study is essential for understanding population dynamics as it indicates

the relative survival of males and females and thus the future breeding capacity of a population during environmental disturbances. Hal (1966) and Sharma and Kevan (1963) noted parthenogenesis in two collembolans *Tullbergia krausbaueri* and *Isotoma notabilis* and this was confirmed by Petersen (1971) by isolating eggs and rearing single individuals for numerous generations in laboratory cultures. Field studies have established that besides sexual reproduction parthenogenesis is common in collembolans. Most of the epedaphic species of collembola reproduce sexually while most of euedaphic species are parthenogenetic (Chahartaghi *et al*., 2006). According to Goto (1960) collembolan sex can be separated on basis of external genitalia. Sexual dimorphism is not morphological-- the shape of the genital opening which is transversally oriented in females; whereas males have longitudinal genital apertures. The individuals without a noticeable genital aperture were considered juveniles (Petersen, 1978; Hopkin, 2002).

The present study deals with population structure and sex ratio in *Bilobella braunerae* (*Dhervang 1981*)- (Collembola: Neanuridae) from Rubber Plantation of Southern Western Ghats.

Materials and Methods

For the population structure and sex ratio studies organisms were collected monthly from October 2016 to September 2017 between 8.00 am to 10.00 am, from Rubber plantation habitat in Seethathode, Pathanamthitta by random sampling method. The animals were sorted using a fine moistened camel brush into juveniles, males and females. Generally, the size and colour of the body of animals were used to identify the sexes. Female is longer and has wider abdomen compared to male. The female organisms are bright orange in colour with a size of about 3 mm -3.5 mm and the presence of an ovipositor on the ventral side of the fifth abdominal segment. Male were usually having dull reddish color and size of 2 mm-2.5 mm. Extracted organisms were identified using a Labomed Stereo Zoom Microscope for the presence or absence of ovipositor.

Statistical analysis:

Two-way ANOVA was performed to find out if any significant variation was there in the number of male, female, and juveniles of *B. braunerae* in various months and difference between population structure in various months. The chi-square test was conducted to find deviation of sex ratio from the normally expected ratio of 1:1 during different months and in different body length range. Ratio-test statistic was also computed for the sex ratio in different months and at different body length range.

Results

Adult individuals have orange colour and just after moulting they have a bright orange colour. Female organisms are more than male. Females are slightly bigger in size and have a broad abdomen and an ovipositor on the ventral side. Male *B. braunerae* has a narrow abdomen smaller than females (Fig.1).

Population structure of B.braunerae in Rubber Plantation:

In Rubber Plantation also percentage of female was more compared to males and juveniles. The number of juveniles was less in all months. The percentage of juveniles ranged from 3% to 7% (Table 1; Fig. 2). Two-way ANOVA shows significant difference between the number of male, female and juveniles of *B.braunerae* ($F= 92.68263$, $F_{critical} = 3.443356779$; $P<0.05$) but no significant difference between population structure in various months ($F= 1.101297$, $F_{critical}= 2.258518357$; $P>0.05$) (Table 2). The population was always dominated by female organisms.

Sex Ratio of B. braunerae in Rubber plantation:

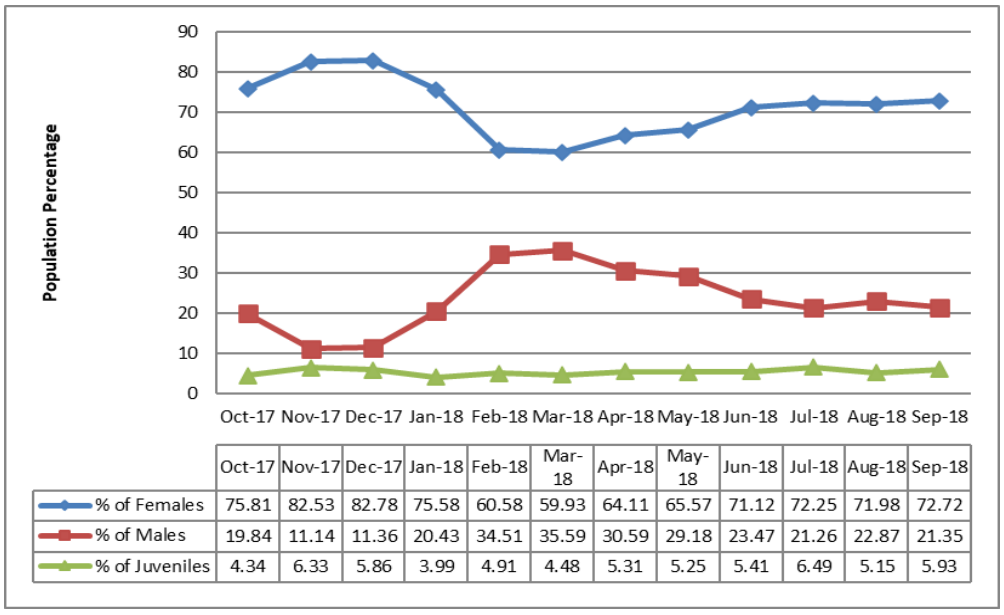
During post-monsoon season (October, November, December, and January) the percentage of females was approximately 88% and that of male was about 11% to 22%, female (62% to 69%) and and male (21% to 37%) during pre-monsoon season (February to May) and during the monsoon season (June to September)

(A) (B) (C)

Fig. 1: *Bilobella braunerae* - (A) Juvenile; (B) Adult; (C) Adult : Male (L) and Female (R).

Table 1: Population structure of *B. braunerae* in Rubber Plantation

Year	Month	Total no. of organisms inspected	No. of Females	No. of males	No. of Juveniles	% of Female	% of male	% of Juvenilles
2016	October	1290	978	256	56	75.813	19.844	4.341
2016	November	1185	978	132	75	82.531	11.139	6.329
2016	December	1092	904	124	64	82.783	11.355	5.860
2017	January	1302	984	266	52	75.576	20.430	3.993
2017	February	936	567	323	46	60.576	34.508	4.914
2017	March	826	495	294	37	59.927	35.593	4.479
2017	April	716	459	219	38	64.106	30.586	5.307
2017	May	610	400	178	32	65.573	29.180	5.245
2017	June	1257	894	295	68	71.121	23.468	5.409
2017	July	1110	802	236	72	72.25	21.261	6.486
2017	August	1242	894	284	64	71.980	22.866	5.152
2017	September	1316	957	281	78	72.720	21.352	5.927



	Oct-17	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18	May-18	Jun-18	Jul-18	Aug-18	Sep-18
% of Females	75.81	82.53	82.78	75.58	60.58	59.93	64.11	65.57	71.12	72.25	71.98	72.72
% of Males	19.84	11.14	11.36	20.43	34.51	35.59	30.59	29.18	23.47	21.26	22.87	21.35
% of Juveniles	4.34	6.33	5.86	3.99	4.91	4.48	5.31	5.25	5.41	6.49	5.15	5.93

Fig. 2: Population Structure of *B. braunerae* in rubber plantation.

Table 2: Two-way ANOVA between months and between population structure of *B.braunerae* in various months

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Months	218954.3	11	19904.94	1.101297	0.404652	2.258518
Between population	3350309	2	1675154	92.68263	1.92E-11	3.443357
Error	397630	22	18074.09			

the percentage of male was 22% to 24% and female population was 75% to 77%. As the chi square value is < 0.05 level of significance, hence, there is a significant difference in sex-ratio of male and female *B. braunerae.* As the chi square value is < 0.05 level of significance, hence the male to female sex-ratio varies from 1:1. The sex ratio varied from 1: 7.4 to 1: 1.68. The population of females was more than males throughout the year (Table 3).

Sex ratio of B.braunerae in different body length range in Rubber Plantation:

The body length range from 0-1.5 mm and 1.5-2.5 mm, the percentage of males was 51% and 66% whereas that of female organisms was 48% and 33%, respectively. There was no significant difference in the sex ratio of males and females in the length range of 0-1.5 mm as the Chi-square value was > 0.05 level of significance. On the other hand, the length range of 1.5-2.5 mm and 2.5-3.5 mm shows a significant difference in sex ratio as the Chi-square value is < 0.05 level of significance. Consequently, sex ratio of *B. braunerae* varies considerably from the expected ratio of 1:1 in the 1.5 mm to 3.5 mm length range in Rubber Plantation (Table 4).

Discussion

Bilobella braunerae is an epedaphic collembolan with male and females in the population. Chahartaghi *et al*. (2006) confirmed that mostly in epedaphic collembolans male and female populations are present whereas most of the euedaphic species are parthenogenetic. The temporal and spatial habitat fluctuations in the environment favor sexual reproduction (Waxman and Peck, 1999). Vrijenhoek (1979) observed vacillation of sexual and parthenogenetic reproduction in many invertebrate taxa. Population structure in *B. braunerae* was found to be dependent on soil factors especially moisture and climatic conditions (Sutton, 1968). Choudhuri (1961) observed that temperature can remarkably modify the sex ratios in *Onychiurus imperfectus,* and males are usually short-lived than females. In the population of *Onychiurus imperfectus,* if eggs are reared at 20^0C or above, more males than females and below 14^0C more females than males. The predominance of females consequently may be the result of intermittent parthenogenesis pooled with the normally longer life of adult females, and environmentally formed anomalous sex ratios. Lee *et al*. (2016) observed no significant temperature effect on the sex ratio of *Yuukianura szeptyckii* and females outnumbered males in the population.

In the present study number of organisms collected during the post-monsoon season (October to December) was very high followed by the monsoon season in all habitats under study. The juveniles, adult male and female organisms were obtained in all monthly collections. The presence of juveniles during the pre-monsoon season was comparatively less. The shortage in the number of juveniles during pre-monsoon may be attributed to vertical migration to find more suitable edaphic conditions for its survival. The population of females was more than males in all

Table 3: Sex Ratio of *B. braunerae* in Rubber plantation

Year	Month	Total no. of organisms inspected	No. of Females	No. of Males	% of Females	% of Males	X^2	P<0.05	Ratio of Male to female
2016	October	1234	978	256	79.251	20.745	7.204E-94	P<0.05	1 : 3.82
2016	November	1110	978	132	88.103	11.891	3.03E-142	P<0.05	1: 7.40
2016	December	1028	904	124	87.934	12.066	1.00E-130	P<0.05	1: 7.29
2017	January	1250	984	266	78.726	21.281	1.090E-91	P<0.05	1 : 3.69
2017	February	890	567	323	63.702	36.291	2.86E-16	P<0.05	1: 1.75
2017	March	789	495	294	62.734	37.263	8.31E-13	P<0.05	1: 1.68
2017	April	678	459	219	67.698	32.307	3.051E-20	P<0.05	1: 2.09
2017	May	578	400	178	69.201	30.796	2.6075E-20	P<0.05	1: 2.247
2017	June	1189	894	295	75.183	24.817	1.3576E-67	P<0.05	1: 3.038
2017	July	1038	802	236	77.263	22.739	4.3458E-69	P<0.05	1: 3.398
2017	August	1178	894	284	75.892	24.105	1.1472E-70	P<0.05	1: 3.147
2017	September	1238	957	281	77.301	22.696	2.9029E-82	P<0.05	1: 3.405

Table 4: Sex ratio of *B.braunerae* in different body length range in Rubber Plantation

Length in mm	Total no. of organisms inspected	No. of Males	No. of Females	% of Males	%of Females	X^2	P<0.05
0-1.5	135	70	65	51.8518	48.1481	0.66695	P>0.05
1.5-2.5	267	178	89	66.666	33.333	5.13E-08	P<0.05
2.5-3.5	290	76	214	26.206	73.793	5.33E-16	P<0.05

months irrespective of season and the number was more when the moisture content of soil was relatively high that is during post-monsoon, monsoon seasons than the pre-monsoon. The sex ratio of male to female was 1:3 and 1:4 during monsoon (June to September) and post monsoon (October to January) seasons while in pre-monsoon (February to May) it was 1:1.5. The sex ratio of collembolan in the litter layer was more female prejudiced (Chahartaghi *et al*., 2006). In *Smithurides aquaticus*, the shorter life-span of males than females may have led to female liable sex ratios as confirmed by Falkenhan (1932). The presence of juveniles all around the year suggests that females are ovigerous and carry eggs throughout the year and lays it during the favorable conditions when the moisture content is moderately high in the soil.

B. braunerae shows a marked deviation from the expected sex ratio of 1:1 all throughout the period of study. The male to female sex ratio is 1: 1 in animals (Nair, 1984). and an unexpected rise in the number of one sex is frequent in insects (Nair, 1978). Generally, in terrestrial collembolans it is taken into consideration that sexes are approximately equal in the population of juveniles, and in later stages of lifecycle one of the sexes becomes predominant (Materna, 2002). Sex determination in *B. braunerae* can be done when an organism grows to its maximum size of 2-3 mm. Generally, females are larger and more bright

orange in colour compared to males. The chi-square test clearly shows the deviation of sex ratio from the normally expected ratio of 1: 1. The number of male organisms was found to be more in small size range from 0-1.5 mm and 1.5 -2.5 mm. Geiser (1934) and Wilkins (1978) are of the view that parasitic castration of female collembolans in juvenile stage may be the reason for a less number of females in small body length size. In this study male population is high in the body length range of 0-1.5 mm and 1.5 mm to 2.5 mm. The females outnumbered males in the body length range of 2.5 mm -3.5 mm, in all the studied habitats all throughout the year. This is the nature's survivor strategy for the collembolan as it is prey for larger microarthropods because of its nonchitinous cuticle (Heeley, 1941).

The sex ratio affects the population prototype of *Bilobella braunerae*. Above the body length of 3 mm males are totally absent. In a microhabitat with a 2-3 mm length range, a large number of mature females are present along with subadult and adult males. The adult males are visible in the 2.5 mm to 3 mm length range and sub adult males in 1.0 mm to 2.0 mm. The role of the sub-adult males in population propagation is limited. Low fecundity and hatching rate are observed when breeding occurs between sub adult males and adult females. This might be to check the population size. In the body length of 1.5 mm to 2.5 mm, the sex ratio of males is more than females in all habitats. In the case of 0-1.5 mm range, the male-female ratio is 1:1 but in 2.5-3.5 mm body length sex ratio of female inclined. The number of male organisms is high in the lower body length range and that of females in the upper length range. This is true in the case of all habitats studied.

According to Sutton (1984) collembolans breed continuously all year round. One or two peak breeding season is observed during a year depending upon the edaphic conditions. In collembolans the breeding season is followed by a period of egg diapauses (Leinaas and Bleken, 1983). Detsis (2000a), Ponge (2000), and Potapov *et al.* (2017) reported that during summer the vertical migration leads to low population density. The absence of males in higher length class intervals is due to the short-life span of male insects.

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