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T Kubendran
High Altitude Regional Centre, Zoological Survey of India, Saproon, Solan - 721232, Himachal Pradesh, India

C Selvakumar
Zoological Survey of India, New Alipore - 700 053, Kolkata, West Bengal, India

Avtar Kaur Sidhu
High Altitude Regional Centre, Zoological Survey of India, Saproon, Solan - 721232, Himachal Pradesh, India

S Murali Krishnan
National Centre of Excellence on Statistical and Mathematical Modeling on Bioresources Management- MHRD, Thiagarajar College, Madurai-625 009, Tamil Nadu, India

Akhil Nair
High Altitude Regional Centre, Zoological Survey of India, Saproon, Solan - 721232, Himachal Pradesh, India

Correspondence
T Kubendran
High Altitude Regional Centre, Zoological Survey of India, Saproon, Solan - 721232, Himachal Pradesh, India

Diversity and distribution of Baetidae (Insecta: Ephemeroptera) larvae of streams and Rivers of the southern Western Ghats, India

T Kubendran, C Selvakumar, Avtar Kaur Sidhu, S Murali Krishnan and Akhil Nair

Abstract
The present research was conducted to study the diversity and distribution of Baetidae larvae (Insecta: Ephemeroptera) of hill streams/rivers of the southern Western Ghats, India. A total of 2982 individuals distributed among 10 genera and 25 species of baetid were collected from 60 sites of Tamil Nadu, Karnataka and Kerala during August 2009- July 2012 representing an environmental gradient. The present study describe the mesohabitat of sixteen baetid species and evaluate their responses to environmental degradation and water chemistry by means of biological measures (richness and abundance), in order to assess their potential capacity as indicators of these impacts. Most species were found predominantly associated with rock-strewn substrates, but some were associated with grasses and two species was found predominantly in lentic water bodies. Species distribution was influenced by the environmental gradient. In future able to identify which species were found in pristine versus the most impaired areas, therefore enabling to establish the sensitivity of each species among baetid.

Keywords: Ephemeroptera, baetidae, diversity, biomonitoring, Western Ghats, India

Introduction
The longitudinal distribution of aquatic insects within a river system is generally thought to be determined by a gradient of physicochemical parameters [1]. Although altitudinal/longitudinal patterns in the diversity and structure of aquatic insect communities are expected to be best explained by a large set of environmental factors, many studies have shown that just a few environmental variables explain most of the variability in assemblage structure. These factors include stream size [2, 3], velocity [4], substrate [5], temperature [6, 7], in-stream vegetation [3, 4] and large-scale catchment characteristics [8].

In recent decades, relationships between the physico-chemical environment characteristics of a stream and the species richness and community structure of aquatic insects inhabiting it have been actively investigated not only in Europe, North America and Australia but also in many other parts of the developing world [9]. However, knowledge about mayflies and other stream aquatic insects in India is fragmentary. Many previous publications on mayflies especially in developing countries have been purely descriptive and do not analyze the general relationships between environmental factors and biological communities. Mayflies (Ephemeroptera) are an important and abundant component of stream aquatic insect communities [10]. The spatial distribution of these organisms is known to be dependent on the altitudinal/ longitudinal gradients [11] as well as influenced by anthropogenic factors or stressors [12].

Although the mayfly fauna has been recently investigated in less explored regions like peninsular India and Western Siberia [13-15], the patterns of species richness and assemblage structure in relation to environmental parameters have not been examined. The aim of the present study was to investigate patterns of Baetidae species richness and assemblage structure and test their correlation to physical parameters associated with a stream/river altitudinal/ longitudinal environmental gradient. Measured parameters were altitude, longitude, steam width, depth, air temperature, water temperature, water current, dissolved oxygen, pH and bottom substrate. The samples used in the present analysis were collected in sixty sites of lotic ecosystems of the Western Ghats in the headwaters and downstream of the various streams and river basins.

2. Materials and Methods

2.1 Study area
The present study deals with the documentation of the diversity and distribution of Baetidae (Ephemeroptera) of southern Western Ghats (Plate 1).

2.2 Sampling states
Three south Indian states were selected within the latitudinal gradient of 8˚12' N- 13˚16'N˚, they are Tamil Nadu (TN), Karnataka (KA) and Kerala (KL) (Plate 1).

2.3 Sampling stations
The following 60 stations were selected for inventorying Baetidae (Ephemeroptera) diversity at higher taxa levels (Genera and species) in the streams of southern Western Ghats, India. Very brief descriptions of their locations of Western Ghats are summarized. However, the most common desirable of these stations as ideal sampling habitats are given separately.

2.4 General features of sampling stations
All stations have shared some common features as follow: 1. streams drain undisturbed forested areas or village level landscapes, 2. stream orders ranges from I to IV, 3. anthropogenic impacts on streams are either very much limited or totally absent, 4. their selection across three states is neither systematic nor stratified, 5. most of the streams consist of highly heterogeneous substratum with extreme degrees of habitat diversity and 6. most of them are perennial streams and very few are intermittent in flow.

2.5 Physico-chemical measurement
Width and depth of the streams were measured by using a marked pole and measuring tape at each station. Three replicates of altitude, longitude, steam width, depth, air temperature, water temperature and bottom substrate. Water current was recorded by using cork and stopwatch. The time taken for a propeller to stop was recorded and later used to calculate water current. Chemical analysis such as pH, dissolved oxygen (DO) of the water samples were determined by following APHA [16].

2.6 Percentage of substrate types
Substrates were classified by using Jowett method [17]. The following criteria: < 0.5 mm for mud/silt, 0.5-2 mm for sand, 2-64 mm for gravel, 65-256 mm for cobbles, and > 256 mm for boulders. For statistical analyses, substrate composition was converted to a substrate index adopted by Suren [18].

2.7 Sampling period
The stations were sampled during three different periods. Nearly, a third of the stations were studied during December 2011 (KA) while, another second were surveyed during March and November 2011 (KL). Remaining stations were sampled during August 2009 to July 2012 (TN).

2.8 Sampling season
Sampling is mostly done during the post monsoon season of the year. This is because earlier studies on south Indian streams have showed aquatic insect to be abundant at this time the annual cycle [13, 19].

2.9 Sampling frequency
All stations were sampled only once due to limitations in time, fund and feasibility related problems. These constraints do not permit sampling of each station for all seasons of an annual cycle. This kind of sampling frequency was most common in south Indian research on aquatic insects [19-21]. Most of the streams/rivers of Tamil Nadu are monthly sampled.

2.10 Sampling size
From each sampling stations five (n=5 samples were collected. Each sample consists of three replicates, which are pooled together as single sample. In fact, sample size is restricted by time availability, accessibility of streams in terms of travelling distance and water current velocity, fund and other relevant factors. However, five samples are ensured at any circumstances from each station for estimating and interpreting the biodiversity measures.

2.11 Sampling habitat
Sampling is mostly confined to riffle habitats of the selected stream ecosystems. Replicates were collected at three different riffles across the stream in each station. It has been well documented that riffles support maximum diversity of macroinvertebrates especially aquatic insects both in terns if abundance and taxa [13, 22, 23].

2.12 Sampling Design
Baetidae communities were sampled from 60 localities of the southern Western Ghats. Data on baetid communities were collected from 2009 to 2012. At each sampling spot, a stretch of approximately 10-15 km was chosen for collection of samples. Target habitats viz., run, riffles and pools were sampled from within this stretch. In addition to baetid species, environmental variables were analysed for each sampling spot.

2.13 Collection and Preservation
The larvae of baetid were collected by taking kick net (mesh size; 0.5 to1.0 mm) sampling strategy of Balasubramanian [24]. The duration of each kick net operation was 2 minutes. The substratum viz., bed rocks, boulders and cobbles were vigorously disturbed strictly restricted to one m^2 area. All specimens from the net surface were carefully collected without any morphological damage using fine forceps or brush and preserved in 80% ethyl alcohol immediately.
Baetidae larvae are particularly fragile because the gills and terminal filaments detach from the body very easily. Therefore, when possible, series of specimens should be collected maximize the likelihood of obtaining intact specimens and accurate determinations. To minimize damage to specimens, it is best to collect mayflies into containers separate from other aquatic insects.

2.14 Identification
Collected samples were brought to laboratory and were examined under Olympus microscope and subsequently processed with Magnus live MIPS-USP No. 0672 (Olympus Private Ltd, India) and identified using standard taxonomic literature. Every effort was made to meticulously identify the Baetidae larvae at the species level, making use of scattered published literature on the Oriental realm and keys were constructed for the Western Ghats [25-37].

2.15 Preservation of voucher specimens
Voucher specimens of family Baetidae upto species level isolated from the samples without any morphological damage and preserved separately in 80% ethyl alcohol in glass vials.

Both immature as well as adult stages are preserved. They are labeled for their taxonomic hierarchy, natural colours, life stage, sampling stations, taxonomic traits and date of sampling. Microscopic slides are prepared for minute sub-microscopic traits of taxonomic significance.

2.16 Depository

The new species of Baetidae are deposited in Zoological Survey of India (ZSI), southern regional centre, Chennai and other species are deposited in Museum of Zoology, Thiagarajar College (MZTC), Madurai, Tamil Nadu, India, as reference collections of southern Western Ghats.

2.17 Data collection

From the taxonomic analysis of samples, the following primary data were collected for further analysis. They are estimates of number of genera and species recorded at each station and estimates of abundance and distribution of Baetidae family at each station.

2.18 Diversity indices and Cluster Analysis

The following diversity indices and Cluster analysis are performed from the primary data, which are collected from 60 study sites. They are Shannon-Weiner index, Simpson index, Berger-Parker Dominance index and Evenness by using PAST software.

3. Results

The present study focused on Baetidae diversity in streams/rivers of southern Western Ghats in three states of south India viz., Karnataka (KA), Kerala (KL) and Tamil Nadu (TN). KL and TN located between 8º and 12º N latitude while KA between 13 Nº S 16 Nº latitude.

The study streams ranged in altitude from 105 to 5445 m.s.a.l. and had a wide range of environmental conditions and the species richness from 0.0998 to 1.0 had a wide range of environmental conditions. The environmental parameters measured are shown in relation to altitude. Therefore, the curves are shown in Figs. (2, 3, 4, 5, abc) with respective correlation coefficient (r^2). Among the measured factors, only temperature was powerfully correlated with altitude (Fig. 2c; r^2=0.84). The relation of other factors to altitude were poorly weak, but stream width reached maximum values and water current and substrate size reached minimum values at low altitudes.

A total of 2982 individuals (Fig.1) distributed among 10 genera and 25 species of baetid were collected in the 60 study sites of the southern Western Ghats during 2009-2012. *Liebebiella vera, Labiobaetis soldani* and *Nigrobaetis paramakalyani* were the most abundant and ubiquitous genera. (Plate 2).

Table 1 represents the names and site codes of selected sampling stations. Of these 60 stations, 1-51, 52-54 and 55-60 were located in TN, KL and KA respectively. Maximum numbers of stations (51) are situated in TN followed by 6 in KA. KL is represented by least number of stations (3).

Fig 2,3,4,5(a,b,c) represent the physico-chemical parameters of the selected streams/rivers of southern Western Ghats. The selected streams run through moderately heterogeneous and highly heterogeneous substrate with less abundant boulders. The riparian vegetation is 20 to 80 %. The canopy covers 20 to 70 %. The width of the streams ranges from 7.5 m to 10.4m all streams. The depth of the selected streams ranges from 0.7 m to 1.5 m and 0.59 to 0.9 m. regarding the temperature, the selected streams have no higher fluctuation. A maximum atmospheric temperature (27°C) and minimum (21°C) are recorded during investigation. A maximum water temperature (23°C) and minimum (18°C) are noted during study period. The pH of the streams was from neutral to slightly basic ranged from 7.0 -7.8. The dissolved oxygen concentration in the streams ranged from 7.0 to 10.4 mg/L. Free carbon di - oxide concentration of the selected streams ranges from 1.0 to 1.1 are recorded during the investigation. The bed rock higher percentage 85% in site 5, boulders higher percentage 50% in site 52, pebbles higher percentage 40% in site 18, gravels higher percentage 50% in site 35 and sand higher percentage 94% in S1.

More number *Baetis acceptus* was collected in the site 29, 33 & 40 absent in site 1 & 53. More number of *B. conservatus* was collected in the site 8. Greatest number of *B. frequentus* was collected in the site 10 & 20. *Tenuibaetis frequentus* was collected in the site 39. *Cloeon bimaculatum* was collected in the sites 23. Maximum number of *Procloeon regularum* was collected in the site 44. *Chopralla similis* was highly collected in the site 13. *Choprella cylonensis* was largely collected in the site 3. *Indobaetis michaelohubbardi* was highly collected in the site 30. *Labiobaetis jacobusi* was largely collected in the site 43 and. *L. soldani* was collected in the site 26. *Liebebiella vera* was highly collected in the site S28. *Nigrobaetis paramakalyani* was highly collected in the site 7, 15 & 39. In other hand, some species are not recorded during investigation such as *Baetis dipsicus, B. fluitans, Cloeon bicolor, C. harveyi, C. kimminsi, C. marginale, C. taeniatum, Labiobaetis palmyrae, L. rubellum* and *Symbiocloeon madhyasthai* but these species are recorded in Western Ghats at earliest.

The baetids were found in all sampled substrates, with highest densities in the aquatic macrophytes. Most of identified taxa were found in high densities associated with vegetal substrates, such as the aquatic microalgae, macrophytes and marginal vegetation.

At the level of genera and species, the richness, Simpson's and Shannon indices were highest for the riffles and lowest for the pools. The abundance of *Labiobaetis geminatus, L. soldani*, *L. jacobusi* and *Nigrobaeis paramakalyani* were highest in the riffles and stream bed vegetations. But the *Baetis acceptus, B. conservatus, Tenubaetis frequestus, B. frequentus, Liebebiella vera* and *Indobaetis michaelohubbardi* were highest in the riffles not in stream bed vegetations. The genus *Chopralla similis* and *C. cylonensis* were highest in the rock and pebbles. The genera of *Cloeon* sp are only present in the pool or stream lentic water bodies. The maximum number of unique genera and species were found in riffles. There was no species unique to the pools except the genus *Cloeon* spp. Species turnover across the habitats shows that riffles and run are very similar in composition than pools.

Species dominance was range from 0.0880 to 0.555. The very low dominance value was recorded in site 19 and high value was recorded in site 57. Simpons's index was range from 0.0 to 0.912; the lowest Simpons's index value was recorded in site 1 and high value was recorded in site 19. Shannon's index was range from 0.0 to 2.44; the lowest Shannon's index value was recorded in site 1 and high value was recorded in site 23. Evenness was range from 0.5507 to 1.0; the lowest Evenness value was recorded in site 20 and high value was recorded in site 1, 54, 55 and 58 (Fig 4 and 5 abc).

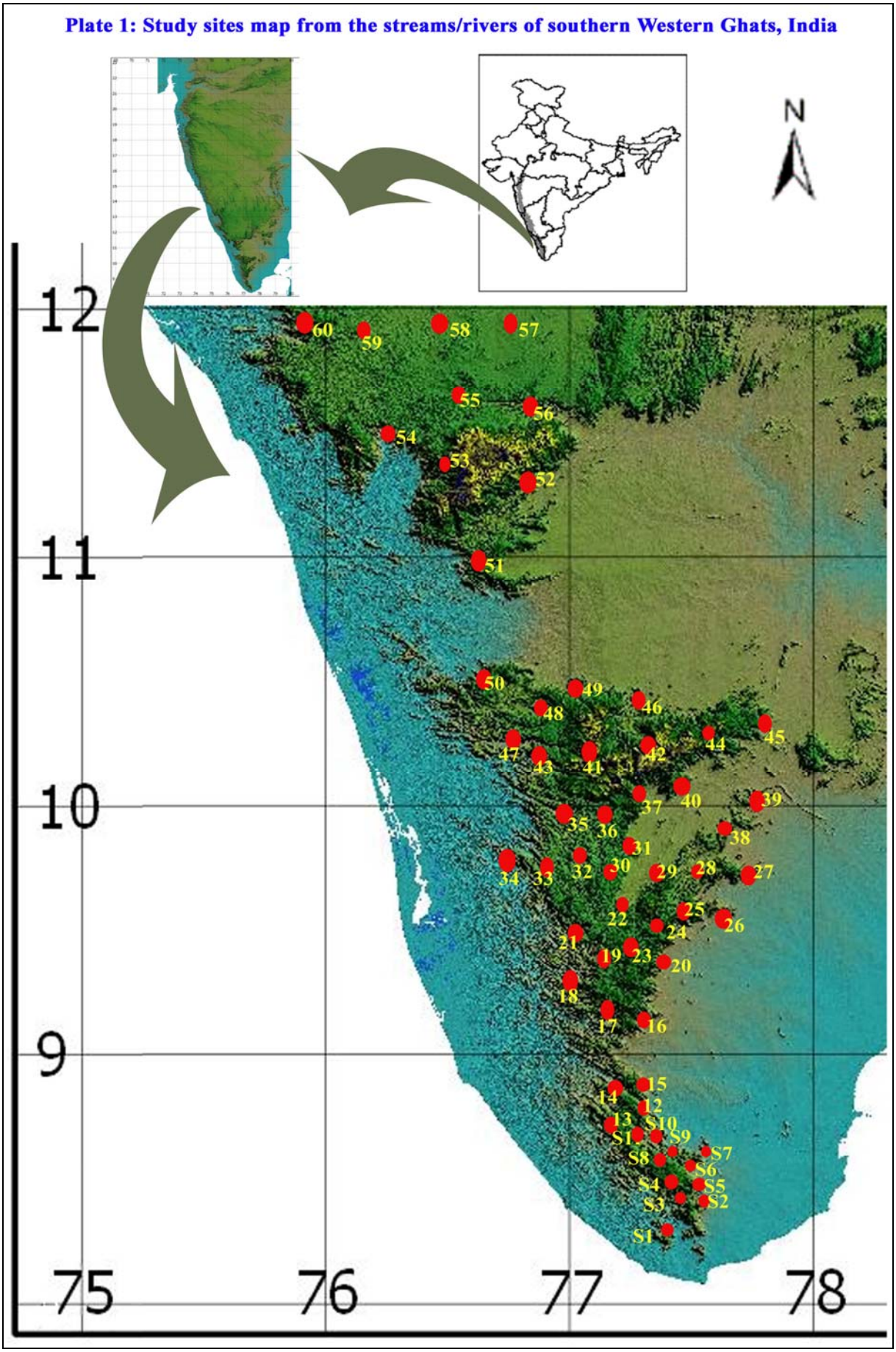
Plate 1: Study sites map from the streams/rivers of southern Western Ghats, India
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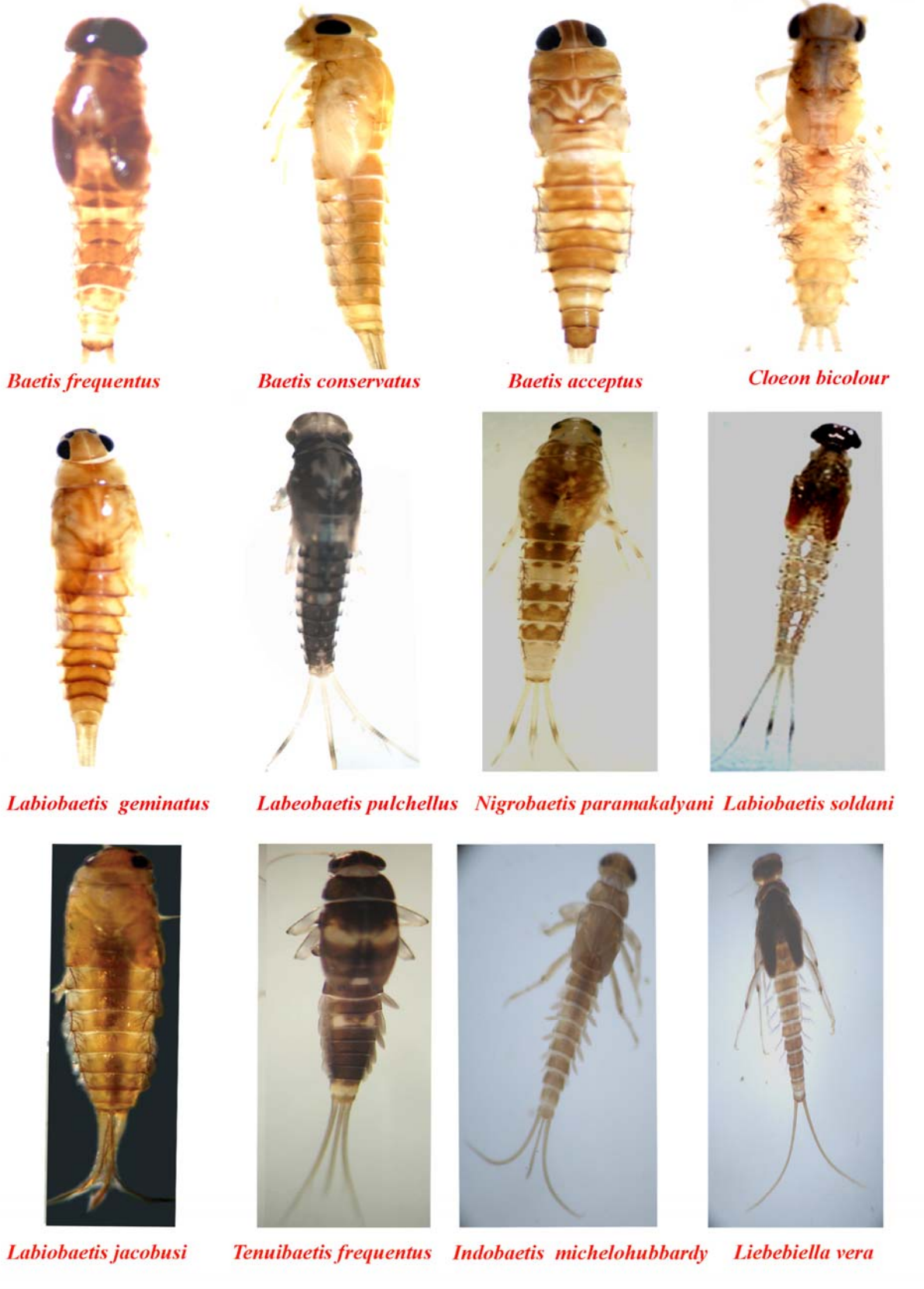


Plate 2. Some Baetidae species are collected from southern Western Ghats, India

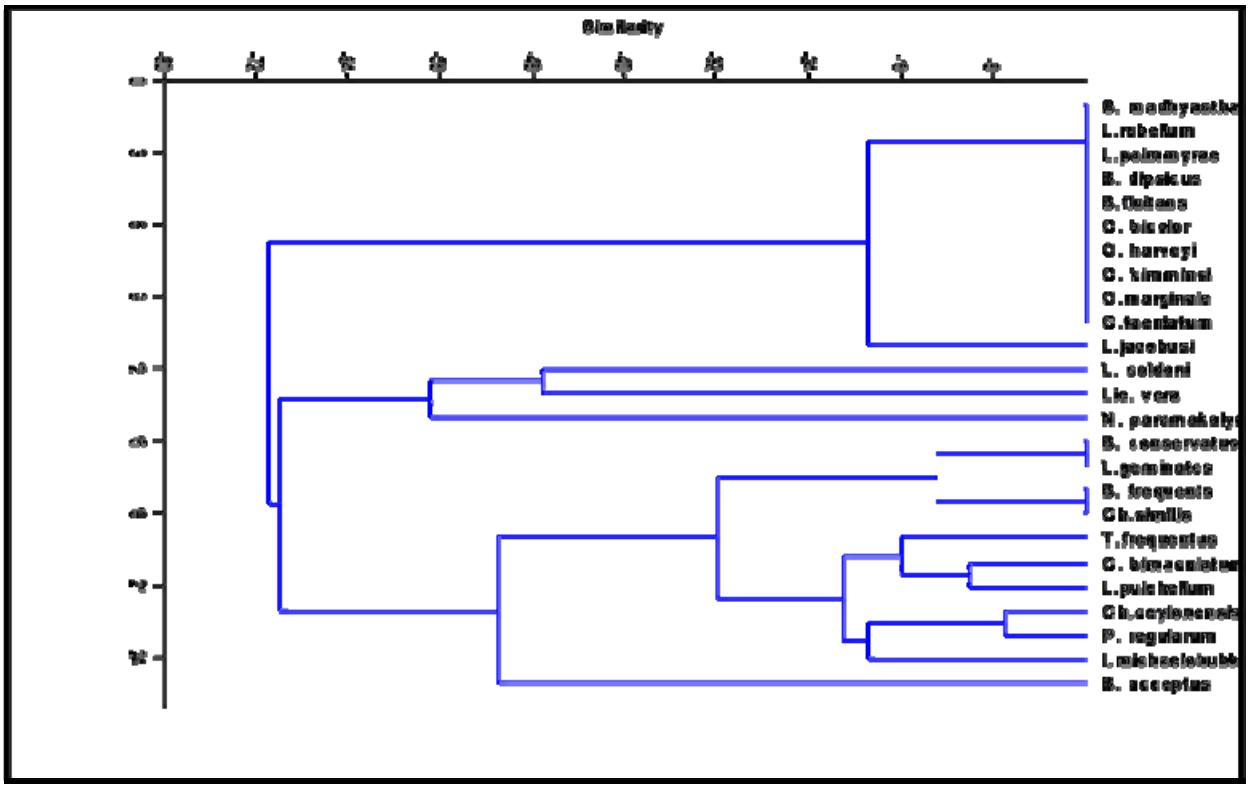
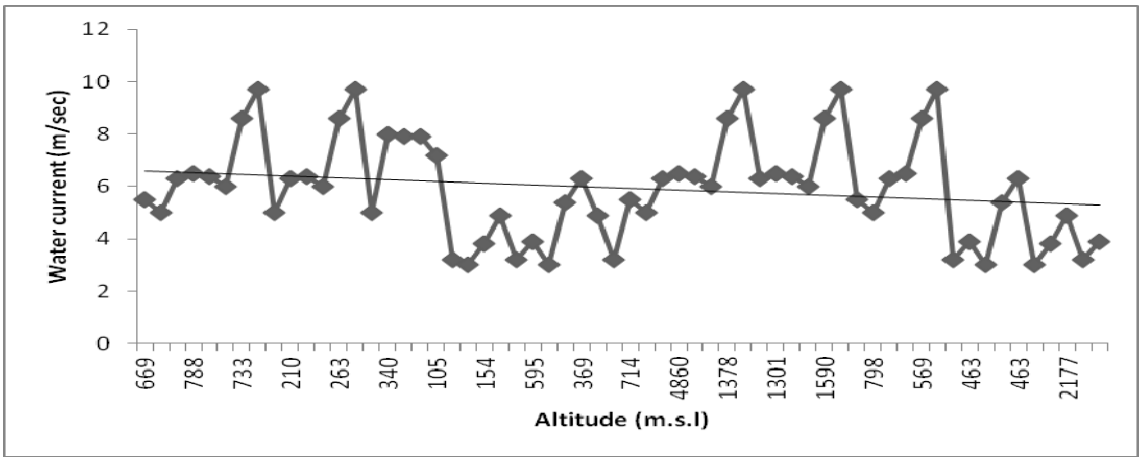
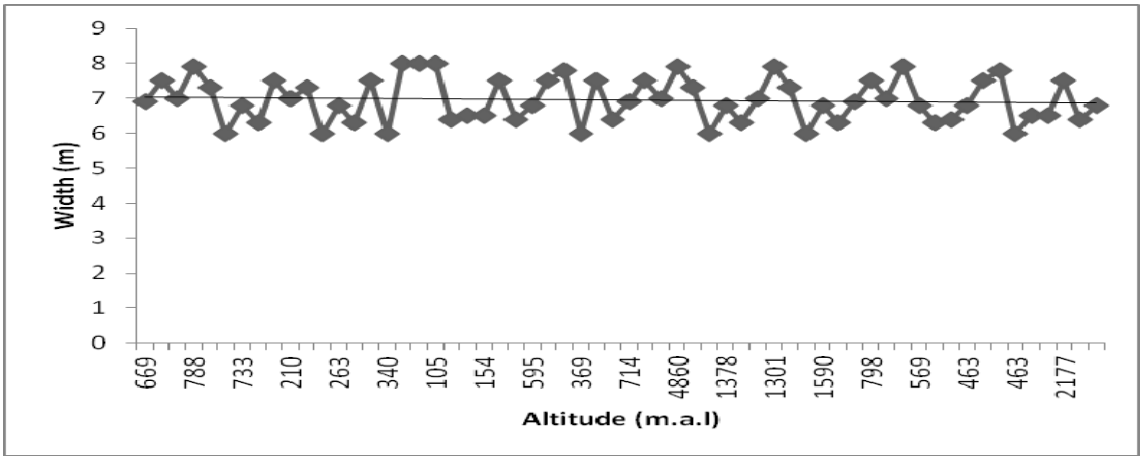


Fig 1: The cluster analysis of the total number of individuals of genus and species collected from streams/rivers of southern Western Ghats during the period 2009-2012



(Fig. 2 a)



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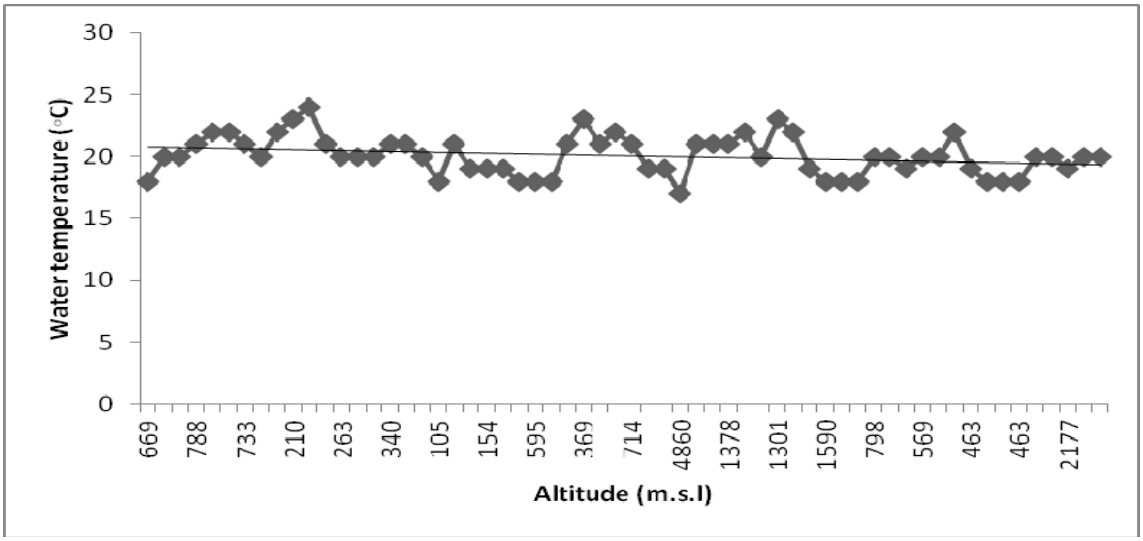
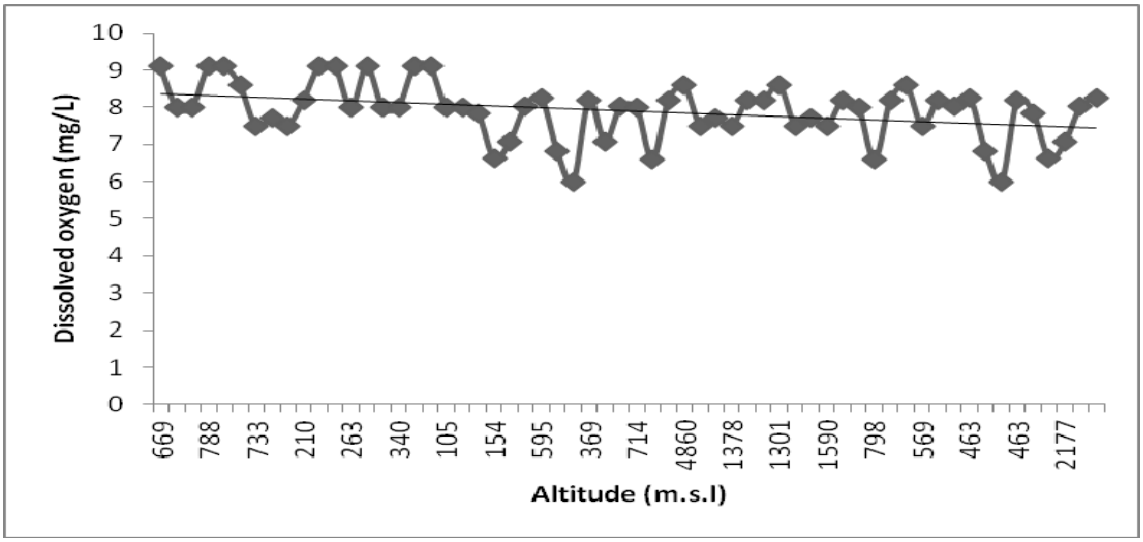
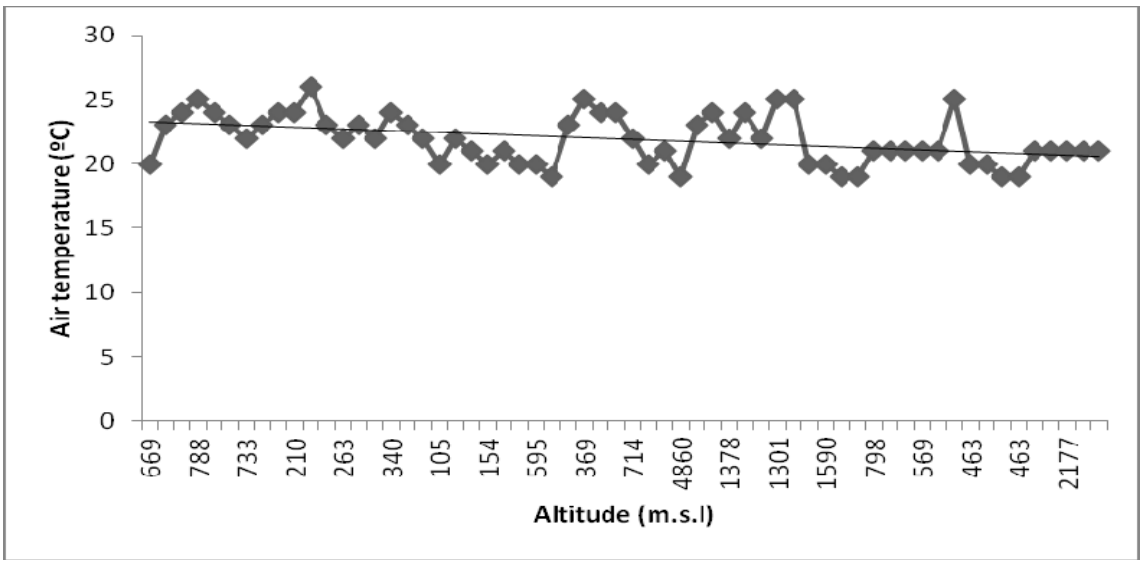


(Fig 2 c)

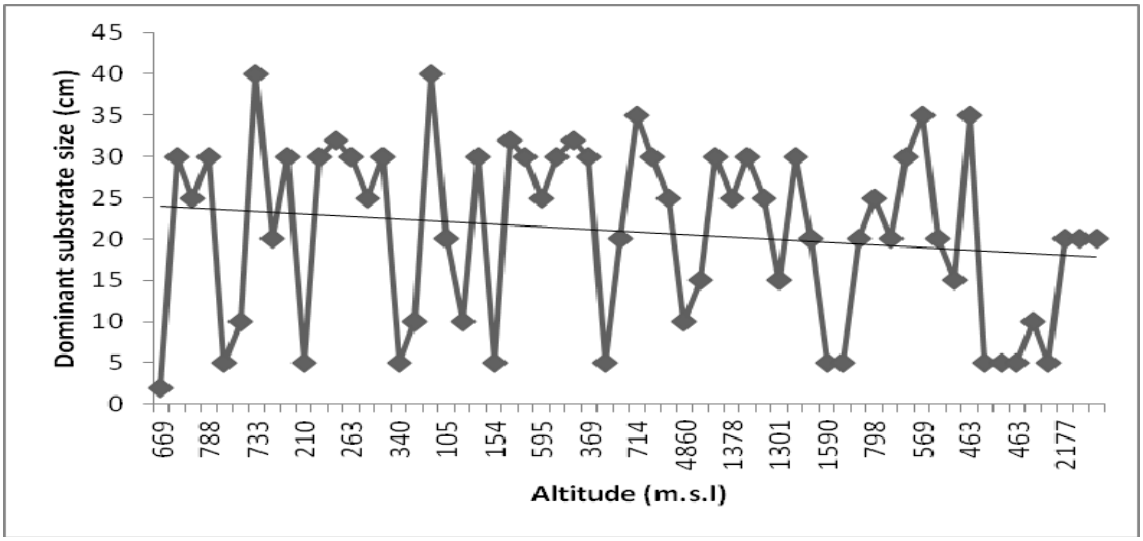
Fig 2 (a,b,c): Environmental parameters of the studied watercourses in relation to absolute altitude (m.s.l): (a) water current (m/sec) (r^2)=0.002 $p<0.01$; (b) width (m) (r^2)=0.98 $p<0.01$ and (c) water temperature (ºC) (r^2)=0.98 $p<0.01$; (log^{+1} transformed and squared Pearson's correlation coefficients (r^2) are given.



(Fig 3 a)

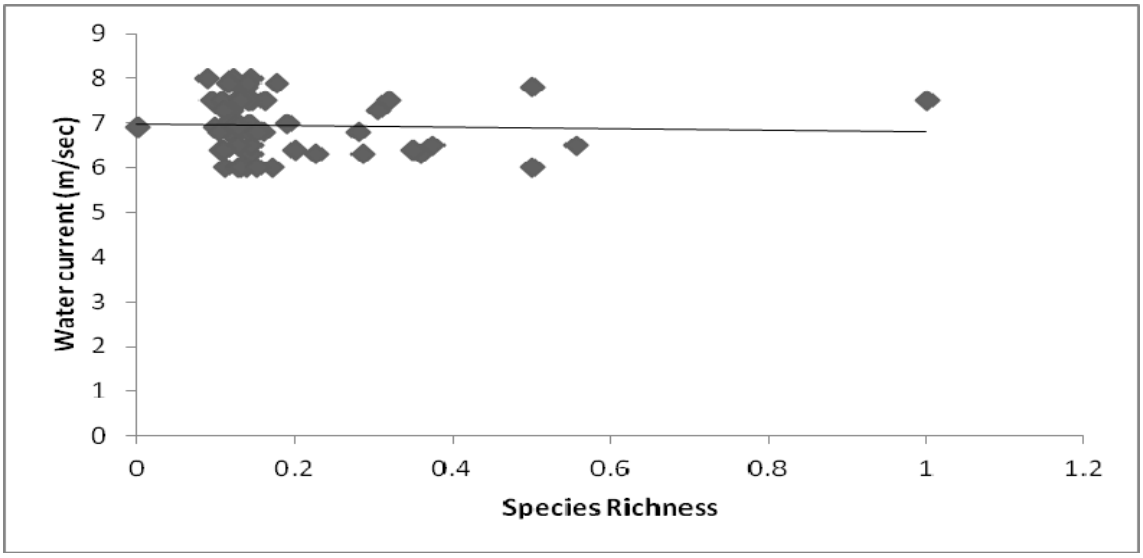


(Fig 3 b)

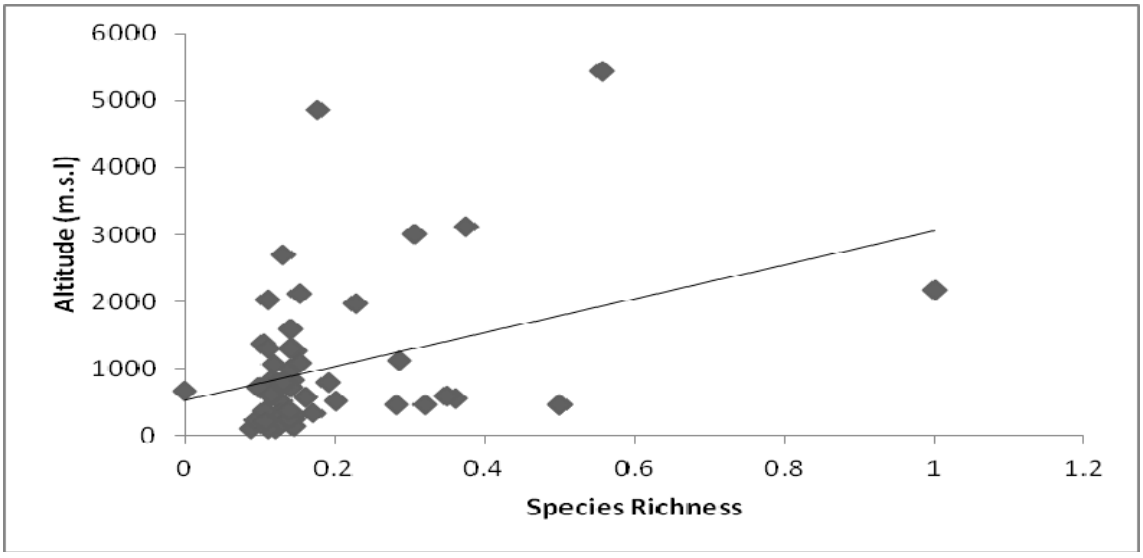


(Fig 3 c)

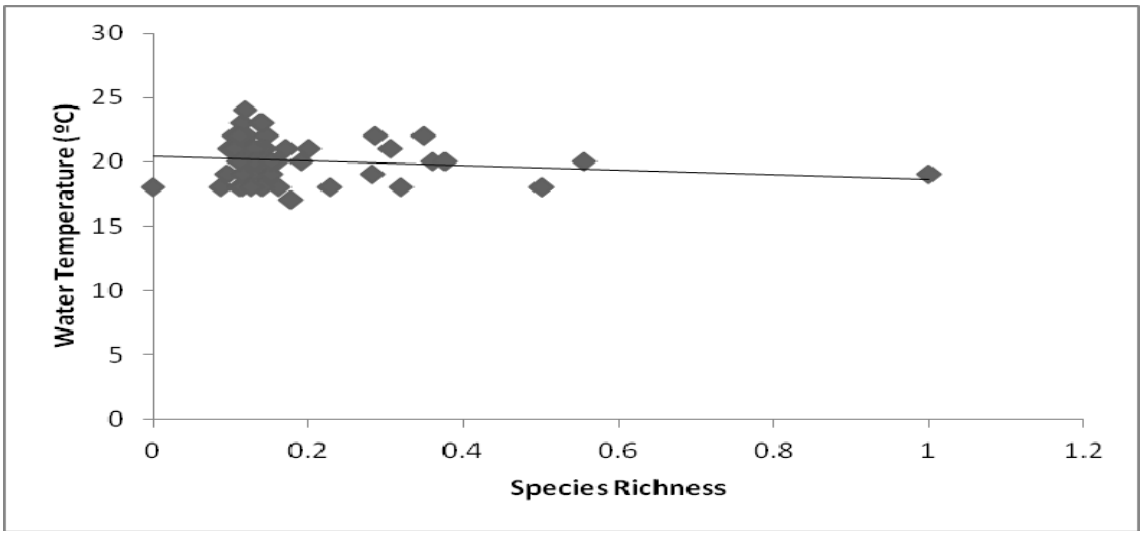
Fig 3 (a,b,c): Environmental parameters of the studied watercourses in relation to absolute altitude (m.s.l): (d) air temperature (ºC) (r^2)=0.98 p<0.01; (e) substrate dominance (cm) (r^2)=0.98 p<0.01 and (f) dissolved oxygen (mg/L) (r^2)=0.98 p<0.01; (log^{+1} transformed and squared Pearson's correlation coefficients (r^2) are given.



(Fig 4 a)

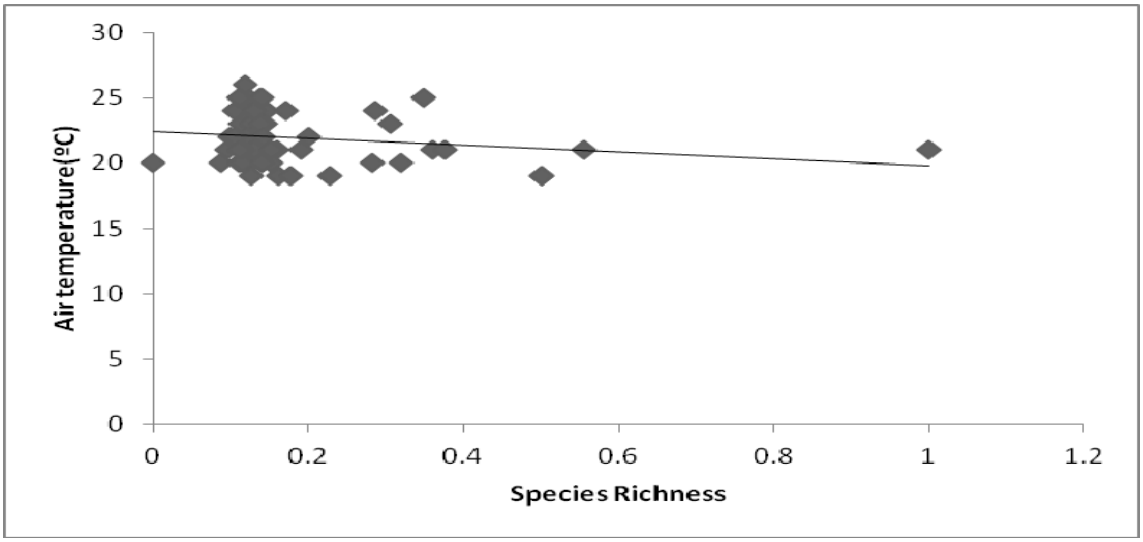


(Fig 4 b)

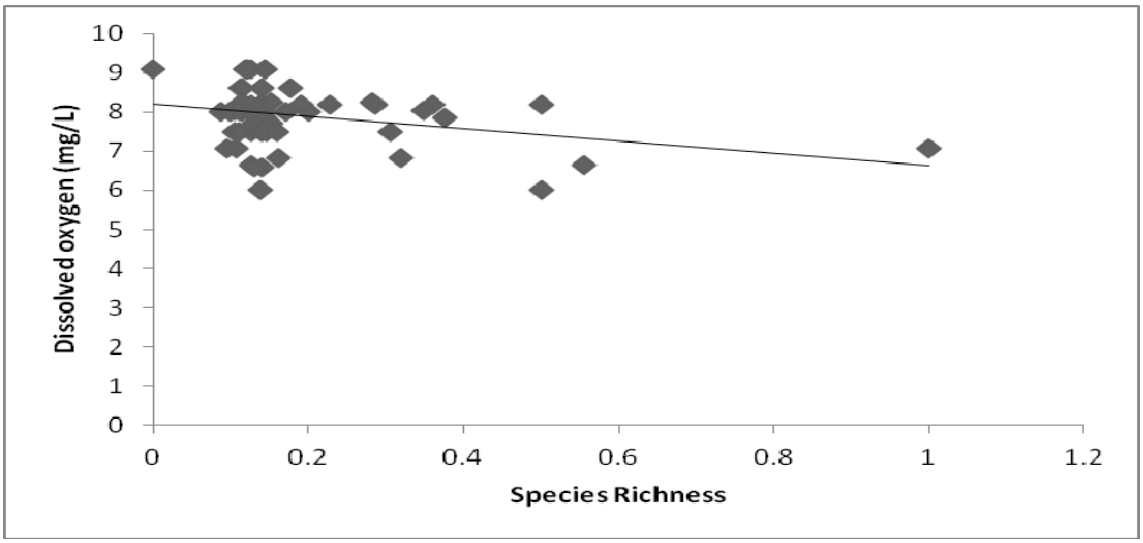


(Fig 4 c)

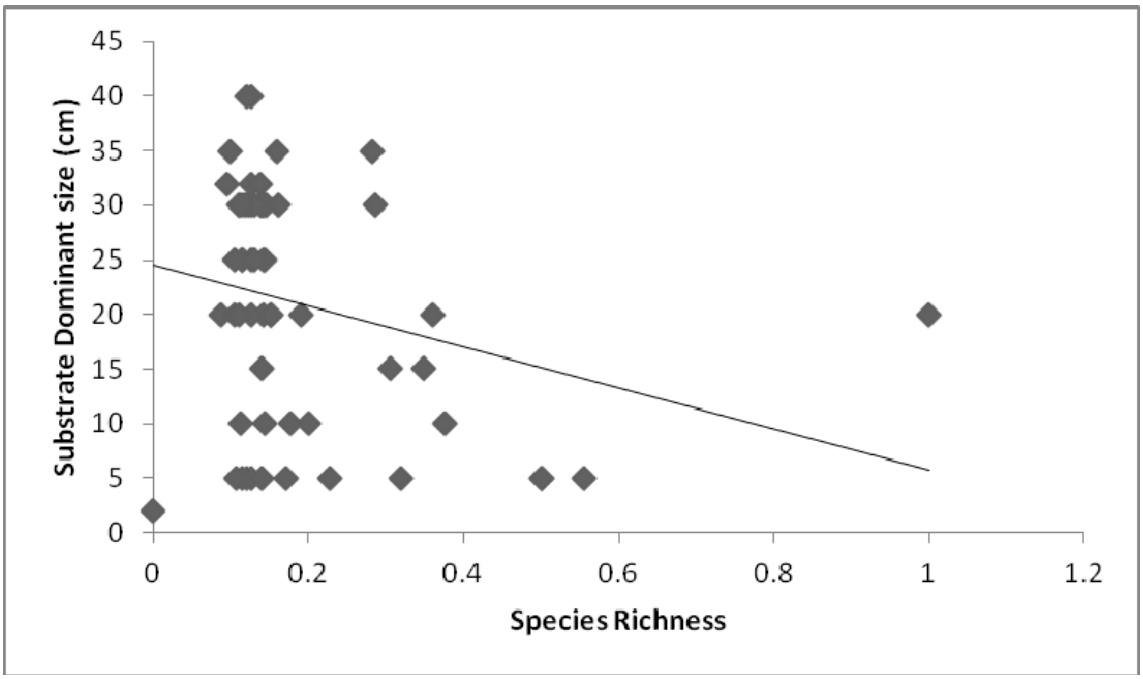
Fig 4 (a,b,c): Baetidae species richness (number of species per site) in relation to the main environmental parameters: (a) altitude (m.s.l) (r^2)=0.845 $p<0.01$; (b) water current (m/sec) (r^2)=-0.024 $p<0.01$ and (c) water temperature (ºC) (r^2)=0.83 $p<0.01$; (log^{+1} transformed and squared Pearson's correlation coefficients (r^2) are given.



(Fig 5 a)



(Fig 5 b)



(Fig 5 c)

Fig 5 (a,b,c): Baetidae species richness (number of species per site) in relation to the main environmental parameters: (d) air temperature (ºC) (r^2)=0.100 p<0.01; (e) substrate dominant size (cm) (r^2)=0.161 p<0.01 and (f) dissolved oxygen (mg/L) (r^2)=0.055 p<0.01; (log^{+1} transformed and squared Pearson's correlation coefficients (r^2) are given.

Table 1: Name of the study sites and their code in the southern Western Ghats during August 2009 - July 2012

S. No	Code	Name of the Site	S. No	Code	Name of the Site
1	S1	S.T Mangadu	31	S31	Upper Kurangani
2	S2	Thatchan pudavu	32	S32	Kurangani
3	S3	Nambi kovil	33	S33	Lower Kurangani
4	S4	Nambi kovil Checkpost	34	S34	Bodimettu
5	S5	Neterikkal	35	S35	Moonar
6	S6	Parapalar	36	S36	Karadikudi
7	S7	Anaikidanku odai	37	S37	Suruli
8	S8	Kombaiyar	38	S38	Veerapandi
9	S9	Kakachiyar	39	S39	Kumbakarai
10	S10	Gothaiyar	40	S40	Varusanadu
11	S11	Manjolai	41	S41	Valiparai
12	S12	Nalumukku	42	S42	Perumalmalai
13	S13	Manimuthar	43	S43	Moolaiyar
14	S14	Karumandiaman temple	44	S44	Gundar
15	S15	Kannikatti odai	45	S45	Upper Alagarkovil
16	S16	Panathertham falls	46	S46	Alagarkovil
17	S17	Karaiyar	47	S47	Lower Alakarkovil
18	S18	Servalar	48	S48	Perumalodai
19	S19	Sorimuthu Ayyanar temple	49	S49	Karanthamalai
20	S20	Agasthiyar falls	50	S50	Thiruvedagam
21	S21	Thalaianai	51	S51	Peranai
22	S22	Kallidai kurichi	52	S52	Highvavis
23	S23	Gorakkanathar temple	53	S53	Megamalai
24	S24	Kallar	54	S54	Vattaparai
25	S25	Sivasailam	55	S55	Iravangalar
26	S26	Gadana river Alwarkurichi	56	S56	Thalakaveri
27	S27	Ramanathi Dam	57	S57	Kudremukh
28	S28	Ramanathi Alwarkurichi	58	S58	Sringeri I
29	S29	Courtrallam	59	S59	Sringeri II
30	S30	Old Courtrallam	60	S60	Sringeri III

Table 2: Total Baetid species are collected form sixty sites of sreams/rivers of southern Western Ghats during August 2009 - July 2012.

S. No	Genus and Species	Total number of species collected in all sites
1	*Baetis acceptus*	259
2	*Baetis conservatus*	195
3	*Baetis dipsicus*	0
4	*Tenuibaetis frequentus*	116
5	*Baetis fluitans*	0
6	*Baetis frequentus*	208
7	*Cloeon bicolor*	0
8	*Cloeon bimaculatum*	100
9	*Cloeon harveyi*	0
10	*Cloeon kimminsi*	0
11	*Cloeon marginale*	0
12	*Cloeon taeniatum*	0
13	*Chopralla ceylonensis*	144
14	*Chopralla similis*	208
15	*Indobaetis michaelohubbardi*	163
16	*Labiobaetis geminatus*	195
17	*Labiobaetis palmyrae*	0
18	*Labiobaetis pulchellum*	90
19	*Labiobaetis rubellum*	0
20	*Labiobaetis jacobusi*	19
21	*Labiobaetis soldani*	386
22	*Nigrobaetis paramakalyani*	329
23	*Liebebiella vera*	433
24	*Procloeon regularum*	137
25	*Symbiocloeon madhyasthai*	0
	Total Numbers	2982

4. Discussion

The present study focused southern Western Ghats for the following reasons. 1. Tamil Nadu, Karnataka and Kerala states were least studied in terms of number of stations in the past [23], 2. Studies on aquatic insects of southern Western Ghats in Tamil Nadu were focused only on order odonata and

mayflies [21, 23, 24] and 3. Systematic studies on Baetidae are very limited in the Western Ghats [35-38].

This study is the first of its kind exclusively focusing on diversity and distribution of the species of larval Baetidae in southern peninsular India. In the present study, 25 species were recorded, comprising approximately 90% of the overall species list known for the territory investigated. Over a relatively large range of altitudes (105 m (m.a.s.l)) and variations in related environmental factors, maximum species richness was found at low altitudes; at places with high water temperatures, relatively slow water current, medium stream widths, and medium- small particle size of the dominant substrate and in the presence of macrophytes.

The trends in baetid species richness found in the present study in southern Western Ghats are in accordance with previously reported results for tropical-zone streams [23]. A number of empirical studies have shown that taxonomic richness of Baetidae, and steam aquatic insects in general, increases with a decrease in absolute altitude and corresponding changes in related environmental factors [20, 21, 39, 40]. When an entire stream system is considered, the dependence of taxonomic richness on altitudinal/longitudinal stream gradient is usually hump-shaped, with maximum taxonomic richness observed near the lowland end of the stream system [41]. The "stream/river continuum concept", well-known ecological theory, predicts that the taxonomic richness of aquatic communities will change with stream size, reaching a maximum, in mid-order stream; whereas in headwaters or large rivers taxonomic richness will be relatively low [1, 42].

The high level of taxonomic richness observed in medium-size watercourses is frequently attributed to the pronounced habitat heterogeneity of these water bodies because heterogeneous conditions can support species with different ecological preferences [43]. Several studies have shown that spatial heterogeneity of the stream bottom is the prime factor governing the taxonomic richness of stream aquatic insects [22, 44-47]. Other factors, such as temperature [42], flow, substrate stability [48] and substrate type [18] have also been found to be important parameters influencing the taxonomic richness of stream aquatic insects. However, it is difficult to understand the importance of separate environmental factors, as the factors are normally inter-correlated.

The high level of baetid species richness observed in the present study in the medium- size plain streams (stream width 6 m) can probably be explained by the relatively high level of habitat heterogeneity in these water courses. However, spatial heterogeneity was not directly quantified and related to species richness patterns. Among the analyzed environmental parameters, altitude and temperature were better predictors of species richness than other recorded variables, although the environmental characteristics considered were all more or less inter-correlated. Hence, it is only possible to conclude that taxonomic richness of baetid species is strictly dependent on the entire altitudinal/longitudinal gradient of environmental factors.

Dissolved oxygen analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous solution. The dissolved oxygen was lower in some polluted water and higher in undisturbed water bodies. Dissolved oxygen levels change and vary according to the time of day, the weather and the temperature [49].

The present investigation provides only baseline information on patterns in the community structure of Baetidae (Ephemeroptera) in southern Western Ghats streams. Further investigations especially focused on Eastern and Western Ghats streams of mayflies in this extremely large and poorly investigated territory are necessary to develop a detailed understanding of the relationship between biological communities and environmental factors.

5. Conclusion

The family Baetidae from this study can be proposed as an indicator of water quality and ecosystem health primarily because of its presence in both the polluted and unpolluted reaches of the stream/river. However, it appears to be sensitive to pollution as species richness and numbers are significantly reduced at downstream sites. The genus *C. bimaculatum* and *P. regularum* are tolerant to organic pollution. The results of this study allow for a better understanding of the regional diversity and distribution of Baetidae in the Western Ghats streams and rivers. However, research involving the entire benthic macroinvertebrates will be better to understand overall structure and function of streams and rivers of the southern Western Ghats with special reference to bio-assessment aspects.

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