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Original Article

Diversity in attachment devices of aquatic insects in a torrential hill stream of mid Himalaya

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Abstract: Present study was carried out to explore the morphological attachment devices od seven aquatic insect larvae or naiads (representing four orders of Ephemeroptera, Plecoptera, Trichoptera, and Hemiptera) along the swift water current of Binwa, a mid-Himalayan hill stream using scanning electron microscopy (SEM) images. Water current showed direct relation with slope/gradient and volume of water in stream. Samples were collected at four sites located at different attitude along the stream. The collected larvae or naiads have many morphological features, including adhesive pad, friction pad, serrated tarsal claw, bifid tarsal claw, tarsal claw with clamp, sucker, and serrated spines which serve as their adaptations for the fast-flowing waters. Principal Component Analysis revealed the importance of water current for assemblage and abundance of these aquatic insects, as it directly related to TDS, electric conductivity, turbidity and nutrients level in stream water, and cumulatively affect these aquatic macroinvertebrates. Similarities in structures of different species suggest the convergent evolution, while slight differences inferred as specializations for species specific niche of these organisms in stream habitat.

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Introduction

Streams play an important role in the ecology of rivers and reservoirs (Sharma, 2019). Hill streams of mid Himalayan region are swift, beside high-water velocity; support a vast community of the macroinvertebrates, in which aquatic insects dominate (Goswami and Singh, 2018). Aquatic insects represent just a fraction of class insect but taxonomically they are very diverse. They have remarkable ability to attach with the substratum and works of some workers in the line of attachment devices are worth mentioning (Nielsen, 1950; Merrit and Cummins, 1996; Nelson, 2009; Ditsche-Kuru et al., 2010). Little information is available on attachment devices used by insect larvae or naiads of western Himalayan streams, so the present study on aquatic insect fauna of Binwa stream has been undertaken. In this study, scanning electron microscopy (SEM) was made on *Baetis* sp., *Ecdyonurus* sp., *Baetis himalayana*, *B. bifurcatus*, *Cryptoperla* sp., *Rhyacophila* sp. and *Naucoris* sp. on tarsal claws and ventral side of these organisms, which were supposed to help in attachment on substratum. Adhesive apparatus, friction pads, tarsal claws with comb like serration and setae were reported, which might be useful for their survival by helping in attachment, clinging and resistance generation (frictional) in the swift waters.

Materials and Methods

Study area: For the present study, collections have been done from the Binwa, a hill stream of the Dhauladhar range in North-West part of India. From its head to its mouth, the Binwa stream covers a distance of about 48 km. Four observation/collection sites have been selected on the basis of altitudinal differences i.e. Kharli as S_1 (2822 m above msl), Baijnath as S_2 (945 m above msl), near Chobin as S_3 (746 m above msl) and Triveni as S_4 (572 m above msl) (Fig. 1, Table 1). The main habitat

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Table 1. Stations and associated geographic characteristics.

Observation sites	Elevation	Distance from previous station (in m)	Slope in Degree	Entrenchment Ratio	Width/Depth Ratio
Head of the river	3560	Nil	Nil	1.8	12.7
S_1 (Kharli)	2822	2200	18.55	1.4	8.8
S_2 (Baijnath)	945	18200	5.87	2.2	14.6
S_3 near Chobin	746	15700	0.689	1.6	12.5
S_4 (Triveni)	572	13200	0.94	2.3	15

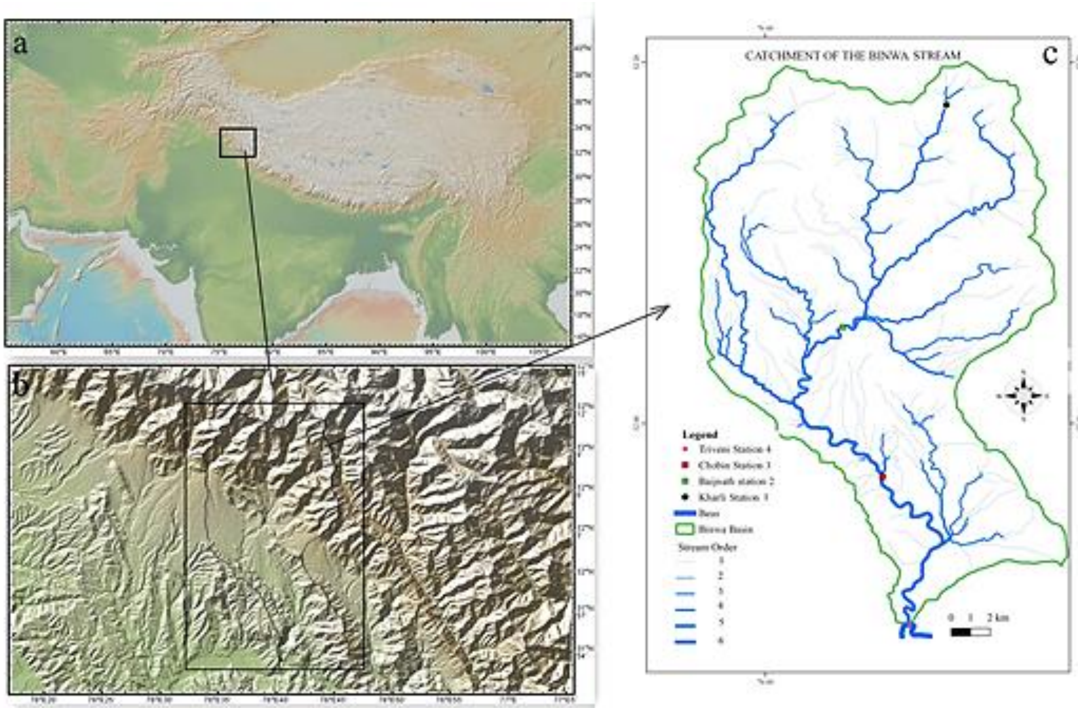

Figure 1. Map showing study area and catchment region of Binwa Stream.

is comprised of runs and riffles with some pools and a substrate of boulders and cobbles. In some regions, the riparian vegetation is dense, which adds allocathonous organic matter to the stream, and serves as feeding material for the larval forms. Drainage network in the study area, all the streams/Nullah have been ordered as per Strahler's scheme (1957) and Rosgen (1996). As longitudinal profile is constructed along the flow line of a stream, thus it effectively reflects the change in downward slope and knick points. Slope of the river bed governed the changes in characteristic of water current such as formation of run, riffles and pool. Similarly, the longitudinal profile of Binwa (Fig. 2) has been constructed from head (upstream Kharli) to its mouth (at Triveni) covering all four observation sites. This profile has been created by using 30 m Digital Elevation Model (DEM) of the study area and QGIS 3.4 software.

Field collection: Aquatic insects and water samples have been collected and observed on four sites for a period of two years i.e. March-2011 to Feb-2013. Water samples were analyzed for different parameters such as electric conductivity, TDS, turbidity, dissolved oxygen, pH, nitrates and phosphates (APHA, 1998). As the water current is the most important parameter for determining the diversity and abundance of aquatic insects in fast waters, therefore only the monthly average values of it given (Table 2). During the present study, water current measured in the field on monthly basis using a float, and the time of the float was noted between the known distance, and then water current was measured using formula $V = D/T$, where V = velocity, D = Distance traveled by

Table 2. Monthly average value of water current (cm/sec.) on four sites for study period 2011-13.

Observation sites	Study Period	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
S_1 (Kharli)*	2011-12*	-	93	102	110	109	105	83	73	65	-	-	-
	2012-13*	-	90	105	110	109	105	83	73	65	-	-	-
S_2 (Baijnath)	2011-12	86.45	90.5	95.7	98.5	124.8	120.5	115.6	105.4	82.5	80.12	80	85.7
	2012-13	88.5	91.5	95.8	98.3	129.8	124.5	114.6	103.4	83.5	79.23	81.2	84.3
S_3 near Chobin	2011-12	75.2	80.5	85.2	89.1	113	110.5	105.4	94	72.4	70	67.3	71.2
	2012-13	74.5	78.5	83.2	87.1	114.5	108.5	103.4	96.5	71.5	68.7	67.4	70.4
S_4 (Triveni)	2011-12	70	72.1	72.5	78.6	90.7	96.4	81	72.1	70.2	60.1	65.3	72.4
	2012-13	71	72.3	72.7	79.1	91.2	96.5	81.3	72.5	70.3	61.2	64.5	73.1

*Water samples were collected only for eight months (April-November) due to non-accessibility in winter months.

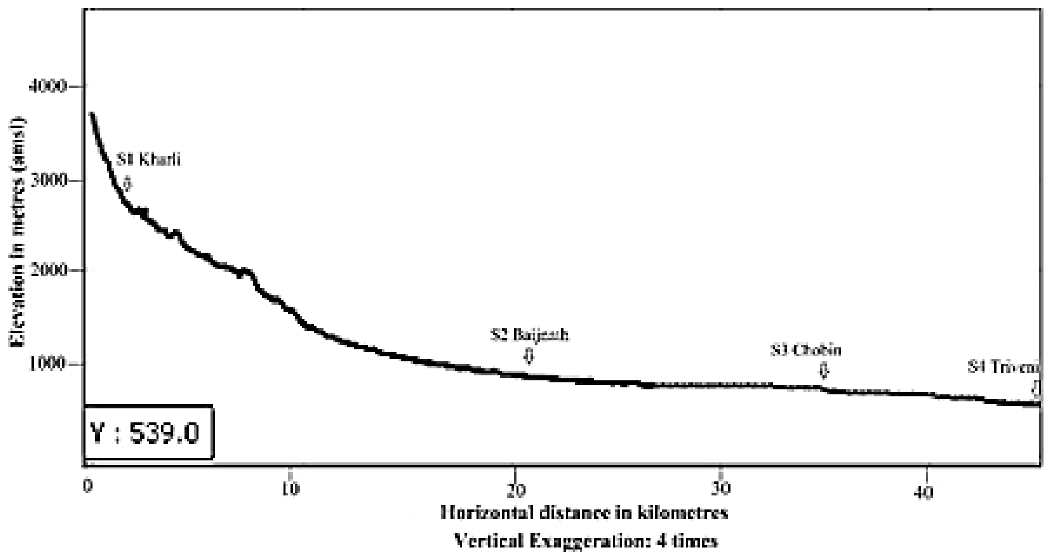

Figure 2. Generalized longitudinal profile of the Binwa Stream.

the float and T is the time taken by the float to travel the distance. Principal Component Analysis have been performed using SPSS.16 software to observe the influence of water current and associated parameters on abundance of aquatic insect naiads.

Naiads were collected from cobbles, large wood debris and other hard surfaces into surber sampler net by making disturbance in the substratum of the stream, unsettling them, and then collection in bucket and at last preserved in 4% formaldehyde solution. Laboratory sorting and identification of the insect larvae were accomplished based on Leckhmkuhl (1979), McCafferty (1981), Merrit and Cummins (1996) and Subramanian and Sivaramakrishnan (2007) with a binocular stereomicroscope. Samples were then prepared for SEM studies with the following procedure: Preserved specimens were washed with double distilled water (3-4 times) and dehydrated in different grades of acetone i.e. 50% (15 min), 70% (10 hrs), 90% (15 min), 100% (2 shifts each after 20 min), amyl acetate: acetone sol. (1:1) for 30 min. and shifted to amyl acetate sol. (30 min). Samples were subjected to critical point drying (CPD) and stubs of the samples were prepared. A fixed sample was mounted on metal stub, either made of aluminium or brass with the help of double stick tape. Care was taken to avoid trapping of air bubbles under the tape. Then the sample was coated with a thin layer (100 Å) of gold in a gold sputter coating unit. Finally, the sample was viewed under vacuum in the JEOL JSM-6100 scanning electron microscope at an accelerating voltage of 15-20 KV at low probe current. When not being examined, the specimens were stored in a desiccator. On the basis of field observations for attachment of organisms with the substratum, and the results inferred from

Table 3. Seasonal rainfall record (in mm) of Himachal Pradesh and Kangra for study period (2012).

Study Period	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
2012 in HP*	112.1	66.4	43.2	64.1	11.5	25.5	207.1	311.6	152.1	2.9	6.7	31.8
2012 in Kangra* (season wise)	216.5			100.5				1889.9			44.7	

*Data taken from India Meteorological Department, Ministry of Earth Science, Govt. of India (Kaur and Purohit, 2012).

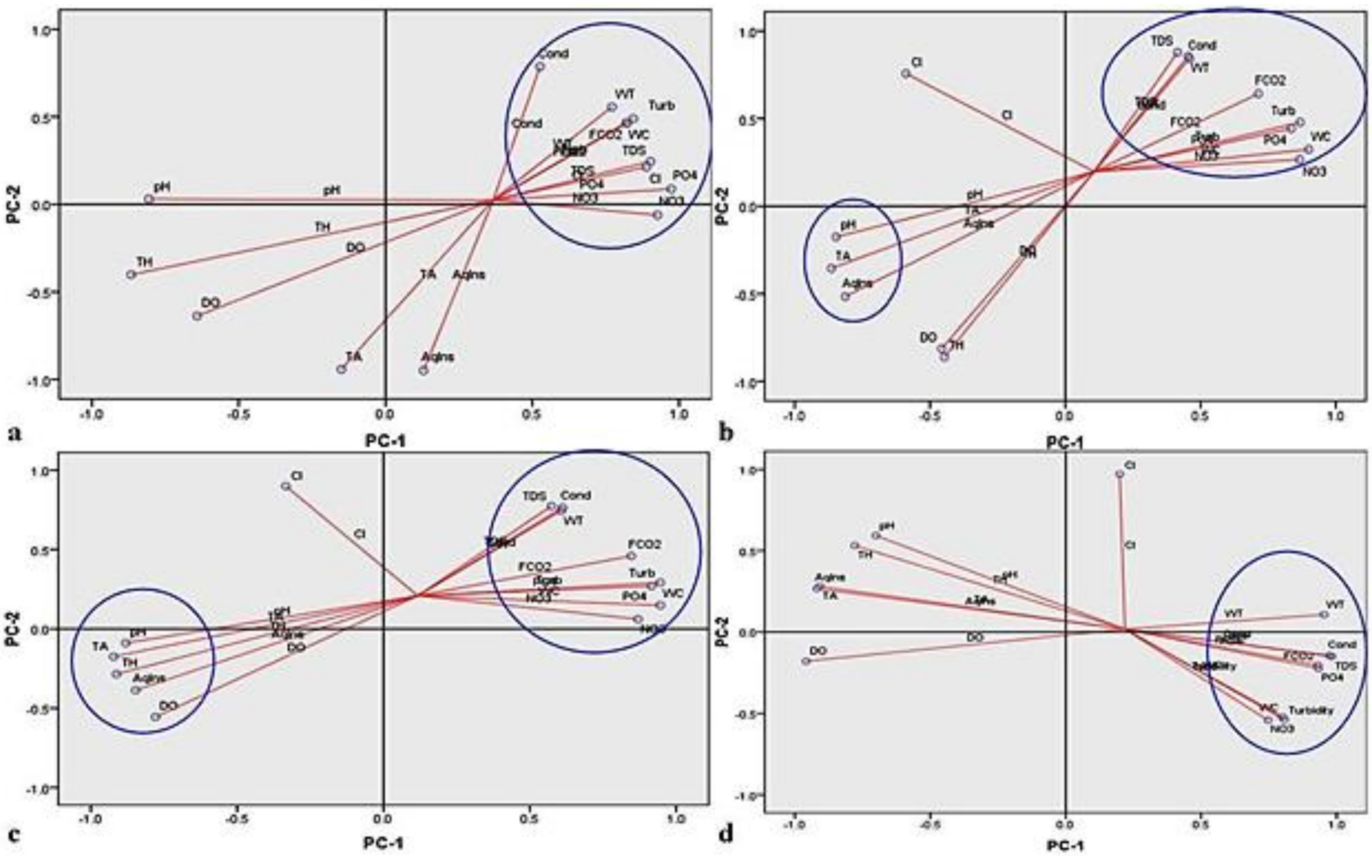

Figure 3. Principal Component Analysis (PCA) of different physico-chemical parameters and aquatic insects of Binwa stream: a. (S1), b. (S2), c. (S3), and d. (S4). (Abbreviations: DO dissolved oxygen, TA total alkalinity, TH total hardness, WT water temperature, WC water current, Cond. Electric conductivity, Turb. turbidity, TDS total dissolved solid and Aq.Ins. aquatic insects)

SEM studies, imaginative drawings have been drawn to show the suction generation, clinging and attachment mechanisms of these organisms with substratum.

Results

Longitudinal profile of the Binwa stream throws light on characteristics of terrain along the river bed (Fig. 2). Upstream of S_1 the slope of the stream bed was highest i.e. ~ 19° while between S_1 to S_2, S_2 to S_3, and S_3 to S_4 it was ~ 6°, 0.7° and 0.9°, respectively (Table 1). Beside longitudinal profile of channel, sinuosity (1.4), average width/depth ratio (12.7) and average entrenchment ratio (1.8) categorized Binwa stream as B3-type (Rosgen, 1996). As stream channel is narrower, high slope and have low sinuosity upstream, this implies high water discharge as well as high velocity (Table 1).

Upstream boulders were numerous, and relatively more angular and rougher. Roughness of boulder surface and angularity relatively decreased downstream. Additionally, the progressive decrease was observed in the average size of river bed stone especially downstream. At S_3 and S_4 cobbles and pebbles were dominating the river bed with gradual increase in the proportion of sand. Gradient of the

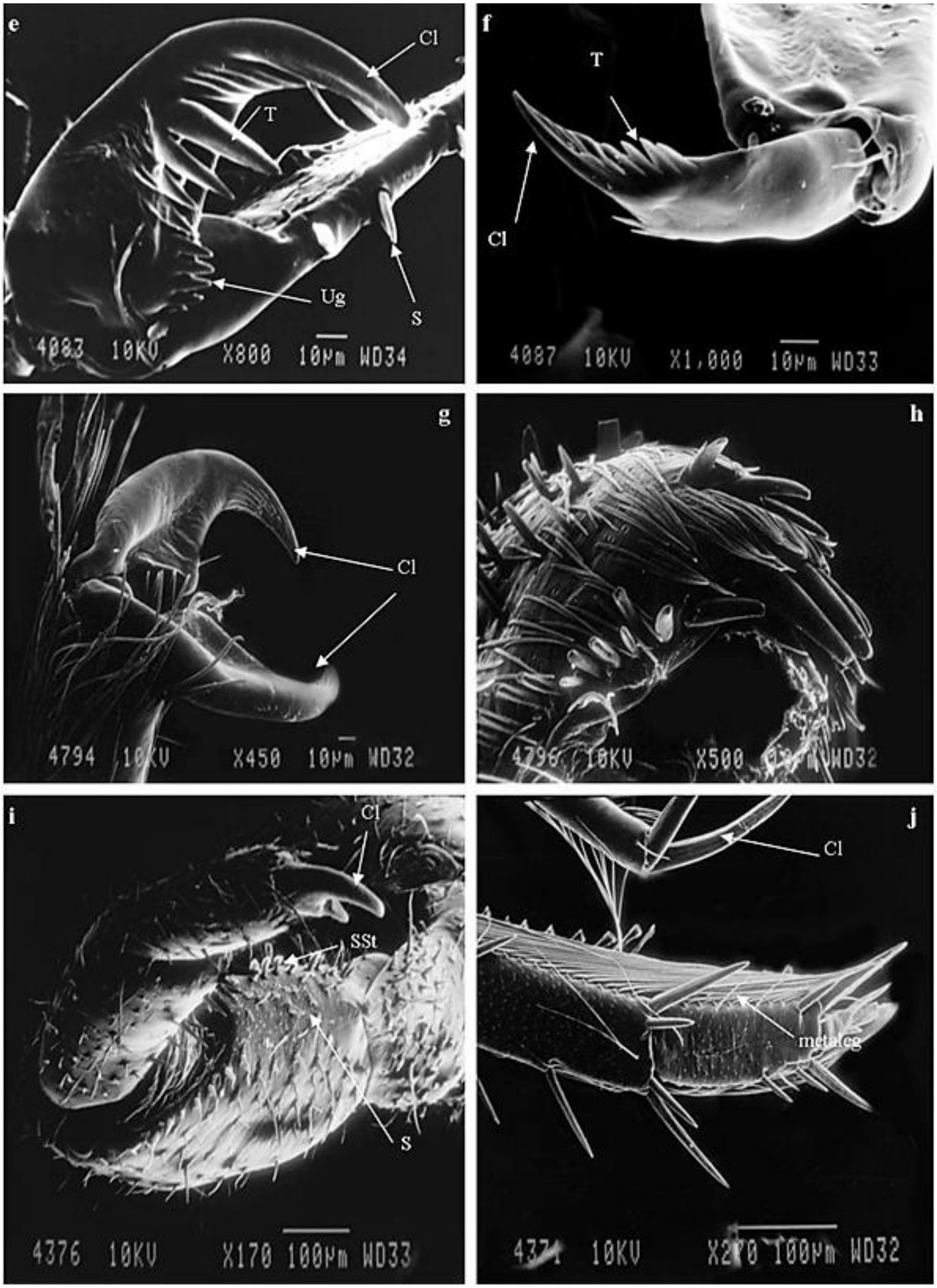

Figure 4. Different attachment devices in the studied aquatic insect naiads (e) *Baetis bifurcatus*, (f) *Baetis himalayana*, (g and h) *Cryptoperla* sp., (i) *Rhyacophila* sp., (i) *Naucoris* sp. and (j) *Baetis himalayana*. (Abbreviations: Cl-claw, S-spine, Ug-unguitractor, SSt-serrated setae)

stream bed is in direct relation with water velocity. It is evident from above observations that water velocity is very high in the stream, to prevent their wash away in swift waters of stream, insect naiads have slight modifications in their structures which might be used as adaptations. Water current in the stream was greatest during monsoon period (July-Aug) as more rain recorded during this time period

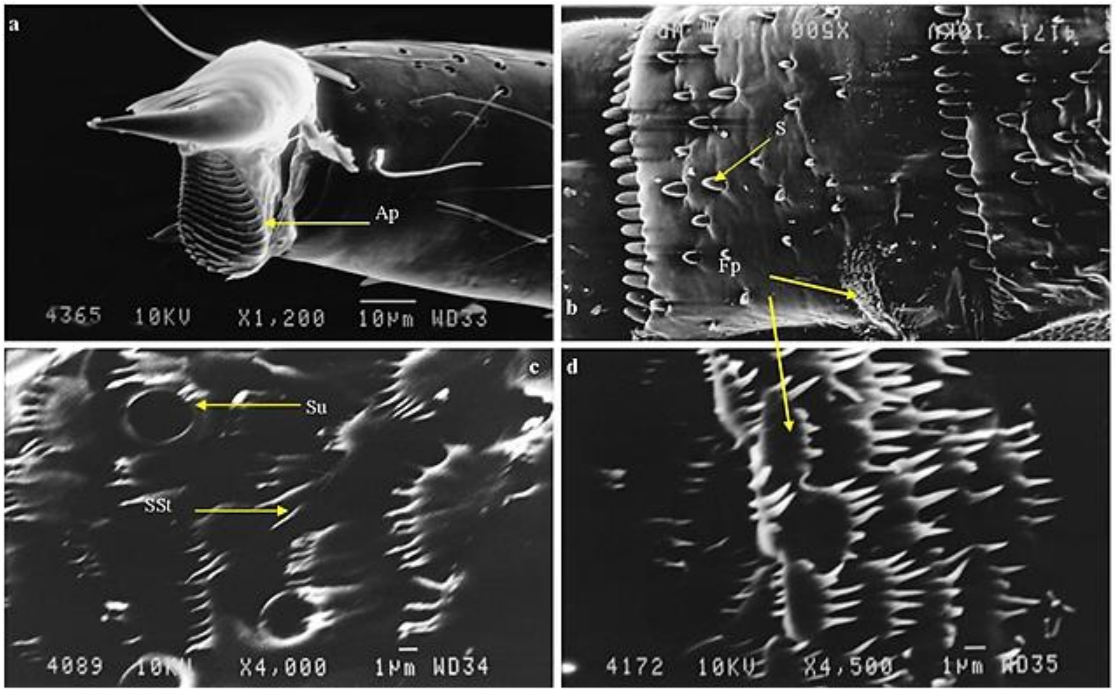

Figure 5. Different attachment devices in the studied aquatic insect naiads (a) *Baetis* sp., (b and d) *Ecdyonurus* sp. and (c) *Baetis himalayana*. (Abbreviations: Ap-adhesive apparatus, Su-sucker, SSt-serrated setae, Fp-friction pads, S-spine)

of year (Tables 2, 3).

PCA shows the clustering of factors influenced by water current, as their maxima in same season when water current observed highest. Surface runoff brings with it so many nutrients (NO_3 and PO_4), ions and solid waste into the stream during monsoon. Therefore, during monsoon high water current is responsible for increased values of electric conductivity, TDS and turbidity. Aquatic insects inversely affected by these factors. Abundance of aquatic insects observed in winter when water velocity was moderate, which pose less danger of their wash away and at high DO due to low water temperature (Fig. 3).

Scanning electron microscopy of tarsal claws with serration on inner side, and a sensilla near the upper surface of claw of *Baetis bifurcatus* and *B. himalayana* are represented in Figure 4e, f. In plecopteran, *Cryptoperla* sp. has strong, paired (bifid) tarsal claw (Fig. 4g) and proleg claw (Fig. 4h). In trichopteran, *Rhyacophila* sp. bears tarsal claw with clamp like structure (Fig. 4i); hemipteran, *Naucoris* sp. has long, thin, paired mesoleg and metaleg tarsus (Fig. 4j). Friction pads in *Ecdyonurus* sp. on lateral sides, constituted of numerous serrated setae (Fig. 5b, d). In *B. himalayana*, suckers and serrated setae are reported on ventral side near the external gills (Fig. 5c). Adhesive apparatus in *Baetis* sp. that is used to make stronger contact with rough substratum (Fig. 5a), is reported first time.

Discussions

The diversity and abundance of aquatic insects' naiads in torrential waters is determined by two main factors (i) water current and (ii) substratum. The biological significance of rate of water current has been emphasized by many workers (Thorp and Covich, 2001; Jindal et al., 2012). Water current is also influenced by two factors viz. (1) rate of precipitation that determines volume, and (2) slope of the substratum. High water current poses physical and mechanical danger to larvae and it also wash away the nutrients (Nelson, 2009; Jindal et al., 2012).

In the upper parts of the studied catchment, terrain is very rugged and steep. Steepness of the slope decreases dramatically downstream so as the water velocity. From its head to its mouth, the Binwa covers a distance of about 48 km. However, in this relatively short distance it experiences an impressive fall of about 3100 m (3560 m elevation at its head and 539 m at its mouth). Therefore, it experiences a vertical fall of about seven meters for a horizontal distance of every 100 m, as one moves downstream from the head of the river. In terms of slope, the average slope of the river bed is about 4°. However, this fall in elevation is not uniform along the entire stretch of the river as is evident. Fall in the elevation of river bed is the highest in the upper most segment of the stream (upto a distance of ~4 km from the head). It is moderate in middle part (from a distance of ~4 to 15 km) and the least in lower part of the profile (downstream from a distance of ~15 km from head to the mouth of the river). In other words, gradient of stream bed is highest in the upstream and it decreases exponentially downstream, however, there is slight increase in the gradient of river bed at the terminal part of catchment which may be attributed to deep vertical erosion by relatively much larger Beas river near the mouth of Binwa Stream. Evidence in this regard is provided by presence of ~60 m high river terrace at the mouth of the stream. As a result, to keep pace with level of trunk stream i.e. Beas River, Binwa has excavated the earlier mentioned river terrace by down cutting it and hence created relatively steeper slope. The stream slope/gradient decreases from upstream to downstream, this is in consonance with Singh et al. (1993).

On the basis of our findings, three types of approaches have been reported, and inferred from field observations and literature consulted as: (i) clinging devices (tarsal claw), (ii) frictional devices (different type of setae and friction pads), and (iii) fixing devices (sucker). Hora (1930) while studying the torrential fauna of the Indian subcontinent, reported particular forms and shapes (dorso-ventraly flat and streamlined body) and curved claws in aquatic insects.

In *B. bifurcatus* and *B. himalayana* tarsal claws have comb-like serration on inner side, which might increase the surface area for attachment on rough substratum, and a sensilla was noticed near the claw, help to judge the environment. Similar serrated claws have been reported in *Epeorus assimillis* as an attachment device with rough surface of substratum

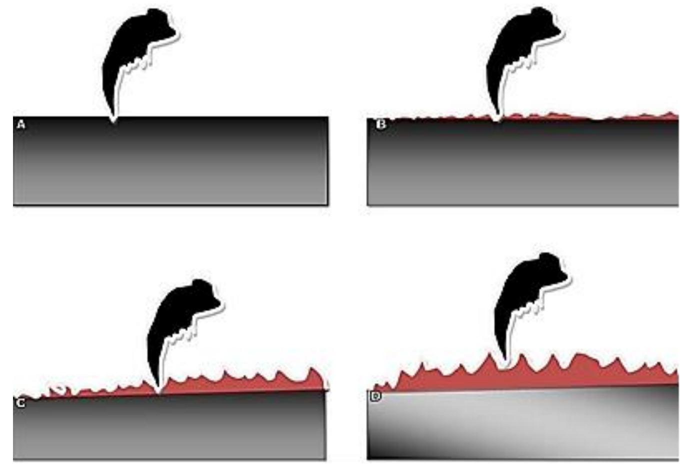

Figure 6. Interactions between tarsal claw and different type of substrate ranging from smooth to rough.

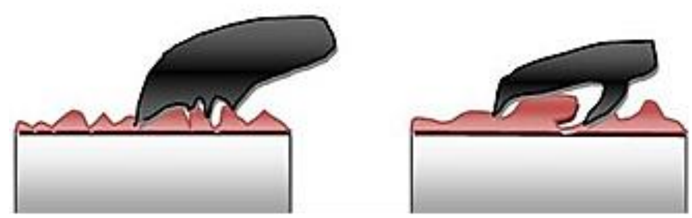

Figure 7. Interactions of tarsal claw of ephemeropterans and trichopteran naiads with rough substratum.

(Ditsche-Kuru et al., 2010).

In plecopteran, *Cryptoperla* sp. have strong, paired tarsal claw and proleg claw that increase the area and reach for anchoring on substratum and applying in unsettling the substratum during detritivore feeding, respectively. *Rhyacophila* sp. possesses a tarsal claw with clamp like structure for helping in clinging and holding the rough substratum. Hemipteran, *Naucoris* sp. has long, thin, paired mesoleg claw that can help in clinging and climbing the macrophytes, and metaleg tarsus act as oar during swimming. An imaginative diagram showed how the tarsal claws of ephemeropterans and trichopteran employed for clinging and holding the rough substratum in Figures 6 and 7). Similar claws and hooks have been reported in caddisfly (Waringer, 1993), mayfly (Kellog and Kellog, 1994), and in stonefly (Gorb, 1996; Merrit and Cummins, 1996; Downes et al., 2000; Beutel and Gorb, 2001; Nelson, 2009) as attachment devices on substratum.

Friction pads were observed in *Ecdyonurus* sp. on

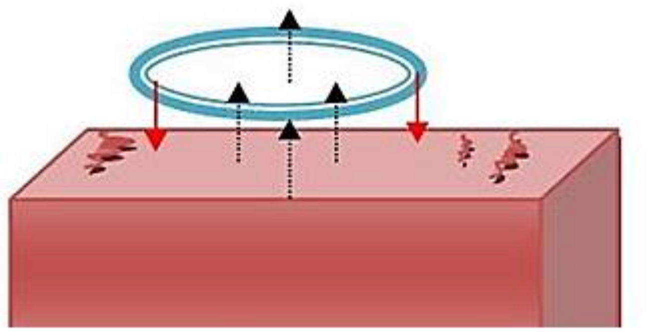

Figure 8. Image of sucker operating on the smooth surface as in *Baetis himalayana*.

lateral sides, constituted of numerous serrated setae, that can increase the frictional force and prevent the wash away by withstanding the water drag. In *B. himalayana*, suckers and serrated setae are found on ventral side near the external gills. The sucker help in attachment by generating suction, while serrated setae form fine contact with minute irregularities/unevenness of substratum and prevent weakening of suction by water rinsing (avoid water to reach upto sucker rim, as water weak the contact of rim with substratum) the sucker (see the imaginative diagram in Fig. 8). In Himalayan hill stream, similar sucker was reported in *Isoperla* sp. (Johal et al., 2011). The adhesive apparatus is reported first time in *Baetis* sp., and is used to make firm contact with exposed rough substratum during sitting or resting position.

As these observations were made on immature stages of aquatic insects, so these claws are not sexual dimorphism. It is clear that tarsal claw can easily cling to rough surface than smooth surface and roughness increases their chances of survival. Most of these structures such as serrated setae, spines, sucker, adhesive apparatus and tarsal claws exert a cumulative effect to fix/attach these naiads with the substratum, and with stand the water force (Jindal et al., 2012). While during winter, when water current is low their abundance observed in the stream (Sharma and Dhanze, 2012; Jindal and Singh, 2013).

Presence of similarity in the structures, such as tarsal claws, sucker, spines and serrated setae, represents convergence for same habitat. While slight variations such as tarsal claw of ephemeropteran have comb-like teeth on inner side, a clamp like structure for holding on inner side of trichopteran claw, bifid or paired tarsal claw in plecopteran help these organisms to explore different niches in the stream such as riffles, pools and swift velocity region. Ephemeropterans with comb-like serration on inner side of tarsal claw and trichopteran with clamp like structure enable them to explore the riffle section of the stream where water velocity is high and they can roam on the exposed surface of rock to water current. While elongated and slender shaped meso-leg tarsal claw help hemipteran to climbing, and meta-leg modified for swimming. Adhesive pad and friction pads help in fixing the organisms on substratum and resisting the water drag. Most of these organisms inhabit the upstream (high slope, more water current) and the centre of the channel (high velocity with more volume) region. As this section (centre of channel) has least chance of predation, maximum chances to get food (as wash away material with high velocity), might be the reason for their adaptation to high water velocity. These structures are modified and arranged in such a way, so that naiads can cling with minute crevices, increase their area of attachment, increase the frictional forces and help to fix these organisms on substratum.

Conclusion

Entire course of Binwa Stream from its head to middle part is riffled and water flow is turbulent and very rapid. Studied insects have developed many morphological characteristics namely adhesive pad, friction pad, serrated tarsal claw, bifid tarsal claw, tarsal claw with clamp, sucker, and serrated spines that act as adaptation mechanisms against the swift waters and serve as resistance increasing, hooking and vacuum generating devices. While adhesive apparatus reported for the first time in *Baetis* sp. of Western Himalayan stream and it cumulatively increases the survival rate of these insect naiads in swift waters of western mid Himalaya.

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