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Contribution to the Knowledge of *Procloeon harveyi* Kimmins (Insecta: Ephemeroptera): Morphology and Ecology

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ABSTRACT

A detailed study was made on the morphology of larvae, male and female subimago and imago in their natural habitat in Kolkata (Calcutta, West Bengal, India). Important variation in colour patches was noted in imagos from natural and artificially raised individuals. Distribution and number of taxa under the genus show them to be predominantly Palearctic and Nearctic species. However, *Procloeon harveyi* is predominantly an Oriental species. Breeding experiment revealed the so far unknown mouth parts, legs and gill structures of the larval stage which are valuable taxonomic information to separate related species. A note on aquatic parameters of the breeding ecosystem is given. Fecundity and adult emergence at laboratory were measured. Observations on larval feeding, posture, gill ventilation and behaviour were made. Drawings of mouth parts and photography of larva, male and female imagos and male forceps are also provided.

Key words: *Procloeon harveyi*, life cycle, morphology, systematics.

INTRODUCTION

With more than seventy species, *Cloeon* Leach, 1815 is one of the most common and diversified genus of Baetidae; it possesses a worldwide distribution as it is only absent from the Neotropics. It colonizes freshwater with still or standing water; in ponds it can represent a great part of the biomass. *Procloeon* Bengtsson, 1915 was established for *Procloeon bifidum* (Bengtsson, 1912), an European species close to *Cloeon*; *Procloeon* encompasses 38 species mainly from Palearctic and Nearctic realms. Limits between *Cloeon* and *Procloeon* remains unclear and several species especially from Afrotropical and Oriental realms were assigned alternatively to one or the other genus depending on the authors and characters chosen. Several North American species previously assigned to *Cloeon* or *Centroptilum* Eaton, 1869 were

assigned to *Procloeon* without clear justification (McCafferty and Waltz, 1990). Gillies showed that African species assigned by Kimmins (Kimmins, 1960) to *Procloeon* based only on imaginal characters were indistinct from *Cloeon* at the larval stage (Gillies, 1962). Later on, he noticed that only the degree of development of the abdominal gills allows to separate adequately both genera, however, no reliable characters exist at the imaginal stage. Based on the structure of gills, we propose *Procloeon harveyi* to *Cloeon* with the new combination *Cloeon harveyi* (Kimmins, 1947). Customarily, the proportion of the hind tarsi of the imago was used to separate the two genera, but again this character seems subject to caution (Gillies, 1997).

During his survey of Mayflies from Kolkata (Calcutta) (October 1945 – January 1946), Kimmins (1947) collected five species of Baetidae, four of them being new to science. The female imagoes of *Procloeon harveyi* Kimmins, 1947 are characterized by the dark colour pattern in the pterostigmatic region as well the red-brown markings on terga II, III and VI. Till date, the larva of *Procloeon harveyi* remained unknown although Gillies (1949) suggested that the larva described by Ulmer as *P. bimaculatum* (Eaton, 1885) could be in fact *P. harveyi*. According to Gillies (1949), the larva described by Ulmer (Ulmer, 1939) is rather similar to *Cloeon simile* and thus the generic attribution was subject to caution. In the present study, rearing was made allowing secure association of the different stages. Based on this material, the larval stage of *Procloeon harveyi* is described; the generic attribution is discussed using both larval and imaginal characters. In the present study, the four weeks time is required to complete the life cycle which is shorter than observed by Gupta *et al.* (1993), Until the generic attribution is discussed herein, we keep the original assignment: *Procloeon harveyi.*

According to Gillies (1949), *Procloeon harveyi* is quite abundant in different parts of India as well as in other regions of the Oriental realms (Malaysia Peninsula, Hong-Kong and Thailand). Till date, there is no information on the biology of the present species.

MATERIAL AND METHODS

The specimens were collected in Kolkata (Calcutta), West Bengal, India (22° 34' 10" N / 88° 22' 10" E, alt. 97 Mtr MSL). Imagos were caught weekly at evening from the building wall, lamp post and sometimes during day from wall of wet toilets and in every case, there is a pond nearby from which they are assumed to have emerged. These ponds, adjoining bushy vegetation and human dwelling places probably build an ideal environment for the mayfly to adapt to urban ecosystem.

A study was carried out at artificial environment to know the gradual development of larval stages up to adulthood.

Out of many live females, six females were reared successfully in aquaria. Specimens were caught by placing a wide mouthed test tube over the insect. The insect immediately move up along the tube and the open mouth is closed by cotton. They were either preserved in 75% alcohol or kept alive for rearing.

Rearing aquariums were 15 cm x 10 cm x 10 cm (height) and top was covered by a white sheet of cloth. Small amount of naturally grown algae taken from the

same pond was placed in each aquarium for feeding. The test tube containing single specimen was inserted in such way that the insect moves to the inside of the cloth cover to rest. It takes 1-2 days' observation for laying of eggs. For the study of gradual phases of hatching, these were collected immediately as well as at intervals of few minutes and fixed in alcohol.

The aquaria were placed in cool but naturally illuminated places for the growth of algae. The water at bottom of aquaria was carefully cleared by fine transparent rubber tube at 2-3 days' interval to maintain hygiene. The larval maturation, feeding periodicity, type of gill movement and ventilation frequency were observed. For these, the females were allowed to lay eggs in aquaria. The hatched out nymphs were fed with alga collected from the nearest pond. However, no aerator was used for the experiment.

Attention is paid to the so far undescribed larval structures and their gradual development, some information of male and female imagos and subimagos and also variation in colour patches in naturally and artificially raised individuals.

Measurements were done with ocular micrometer on alcohol fixed specimens. For observation on feeding, larvae were taken in a watch glass along with minute amount of algae. Water was sufficient for the larvae to remain underwater. Observation was done under binocular microscope. All measurements are in mm.

RESULTS

During present investigation on the bio-ecology of *Procloeon harveyi*, it appeared that the species is frequently observed during the premonsoon and monsoon periods in various stagnant waters in Kolkata (Calcutta, West Bengal, India). However, no method was employed to determine their frequency in those areas. Aquatic environmental factors were measured and associated aquatic fauna were collected and identified in natural environments where *P. harveyi* occurs.

Eggs

Each female lays eggs in a loose cluster and often seen to become scattered. Some eggs remain on water surface and they do not hatch at all and degenerates soon. Most of the eggs drop down to the bottom. The female dies soon after hatching and its posterior end starts decaying or is attacked by aquatic micro-arthropods or other organisms.

It appeared that on average, about 45 larvae are formed per female. The average number of adults emerging is only 5-6 per batch out of six such rearing events. Hatchability seems to be correlated with the prevailing temperature. The water temperature during study was 27°-28°C. The average numbers of larvae and adults were available from the study on the breeding of six females caught alive from nature and reared separately in aquaria. The methodology for the study of different stages was mentioned in materials and methods.

At laboratory, the eggs were seen to come out of genital pore in the form of brown oval (egg-like) mass. Eggs are tiny, white, elongated-oval with parallel sides. The

black spots of egg seen by light microscope are actually the large paired eye spots. In early nymphs these are clearly seen besides the three smaller ocelli.

Under microscope when light is passed through the egg, it appears granular with a clear area around below egg membrane. But when viewed under binocular with light over the egg, it appears whitish with two prominent black spots. Just before emergence, the body of the larvula is folded from metathoracic region. The outer membrane dissolved quickly and the bent abdomen became straight**.** The membrane dissolves at one or several points at a time to open up point of ruptures.

OBSERVATIONS AT DIFFERENT STAGES

Larva

Most of the time, the larva remains at the bottom of aquarium. Under water, it retains a curved position by keeping its belly down while front and back portions of the body are kept upwards. The larvae are seen to feed almost continuously with the assistance of forelegs. Gill ventilation, as seen under binocular, is a rhythm of several quick back and forward jerks of flaps followed by an interval of a few seconds. The number of such ventilation may even be single at times. During pause, the gills do not move at all. The larvae were observed to make nearly vertical movement to collect food and take rest for longer hours at intervals. They mostly move freely along the floor and less frequently move up along water column.

Subimagos

There is now an observable moulting and a winged (spot-less), white subimago emerges out over the water surface. It is slender, with two-tails (cerci) and straight abdomen. At laboratory, the emergence of subimago takes place over water surface in the morning. At first a split is observed at the juncture of head and thorax through which the body curls out. Normally the individual then rests for about two hours before flying away to nearby buildings or trees where it starts the final moulting.

At laboratory, the moulting took place on the inside of the cloth covering the aquarium and the duration between final larval to subimago is only 2-3 hours. This subimago moults again into spotted-winged adult with dark red eyes. Just after moulting, its abdomen is still straight and curves later. The subimago was observed to wag its abdomen slowly and regularly like a pendulum. Such a movement is absent in adult.

Imagos

During summer, the individuals search for cooler place and was detected from inner wall of toilet at noon. Normally, mayfly is said to lay eggs and hence the term is retained here. So far no copulating pairs were observed in the surrounding of the site. The female lays eggs on to the water surface in a floating state. At this condition the wings, cerci, four posterior legs and abdomen are in contact with water while the front part of body and fore legs are kept above water.

Morphological description

The first instar larva is very minute without median caudal filament. In the cultured population, larvae with five or six pairs of gills were found. The entire digestive tract is filled by granular food material. Measurements are given in Table 1.

Table 1. Measurements of body and body parts at the three different larval stages (all measurements in mm).

		Early larva	Intermediate larva (no wing pad)	Mature larva, ♀ (with wing pad)
Total length		1.84	3.84	5.12
Head width		0.32	0.6	0.80
Head length		0.16	0.52	
Antenna		0.72	2.48	2.0
Width of thorax				
Length of thorax				
Fore leg	Total	0.72	1.36	1.76
	Femur		0.64	0.64
	Tibia		0.32	0.48
	Tarsi		0.40	0.64
Middle leg	Total	0.80	2.16	2.0
	Femur		0.64	0.76
	Tibia		0.72	0.60
	Tarsi		0.80	0.64
Hind leg	Total	0.88	1.41	2.0
	Femur		0.80	0.80
	Tibia		0.48	0.56
	Tarsi		0.56	0.64
Lateral Cerci		1.04		2.56
Median Caudal Filament		0.40		2.0
Width of gills		0.12-0.168		

The seven-gilled stage is crucial in several respects (Fig. 6). The development of median caudal filament coincides with the formation of seventh gill pair. The early seven-gilled larva shows gradual elongation of median circus (= caudal filament); while the length of median caudal filament is 0.40 mm, the cerci are 1.04 mm showing a comparative ratio of 0.38. All measurements were obtained from ten mature larvae.

The gills are flattened antero-posteriorly. Gills became smaller posteriorly. Each gill is contained in a transparent pouch called lamella and contains distinctly branched tracheae. The number of such trachea being 3-5 in anterior two gill lamellae but as many as 7-8 in middle lamellae (these gill lamellae are larger). At the base of a gill lamella, the lateral longitudinal trachea divides into two branches which ramify inside a lamella. The development of rudimentary wing pad is visible at this stage.

DESCRIPTION OF MATURE LARVA

Head. Uniformly light amber brown. Antennae yellow.

Dorsal surface of labrum (Fig. 1) with long setae scattered over the surface; ventral surface with row of 4 long pointed setae; distal margin bordered with feathered setae.

Right mandible (Fig. 2) with two sets of incisors only partially fused; prostheca slender with poorly developed denticles apically; tuft of abundant long setae between prostheca and mola.

Left mandible (Fig. 3) with two sets of incisors almost completely fused except apically; prostheca with three denticles and comb-shaped structure; tuft of setae between prostheca and mola present; thumb of mola in same direction as distal margin.

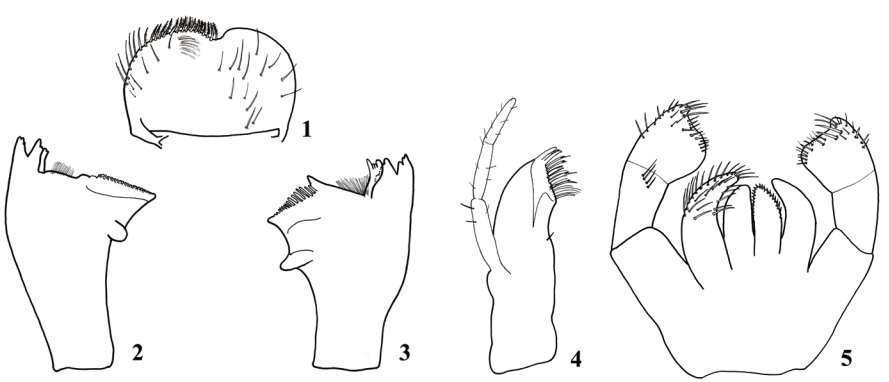
Hypopharynx with a broad lingua, apically with minute stout setae; superlingua slightly longer than lingua.

Maxillae (Fig. 4) with 4 fine and elongated denticles, none of them opposed to others; palp 3-segmented.

Labium (Fig. 5) with glossae shorter than paraglossae; margin of glossae with short and stout setae; paraglossae falcate with long and stout setae apically, ventrally with a few long setae, dorsally with only a row of long stout setae; labial palp 3-segmented; segment III broader than the second, apically falcate, distal margin with stout pointed setae, the apical ones longer.

Thorax. Amber brown.

Legs uniformly light yellow, femora and tibiae with a pale brown band distally. Femora scantily, tibiae moderately and tarsi densely covered by setae. Fore-femora with 12-15 setae on dorsal and ventral margins; ventral margin with additional stout, curved and pointed setae more abundant in proximal half; lateral margin of fore femora with few setae; but mid-femur with only ventral row of setae laterally bare. Tibiae dorsally with few long and thin setae; lateral margin almost bare except few scattered setae; ventral margin with shorter pointed setae, apically longer and more abundant. Distal end of middle and hind tibiae with two setae.



Figs. 1-5. Larval structures of *Cloeon harveyi*: 1: labrum (left : ventral; right : dorsal). 2: right mandible. 3: left mandible. 4: right maxilla. 5: labium.

Tarsal claws elongated, approximately 0.6x length of tarsi, with 2 rows of small teeth.

Length of fore and middle femora equal; tibiae and tarsi of middle leg longer than those of fore and hind legs.

Abdomen. Coloration amber brown with two yellow sublateral spots more or less well defined; well developed on terga IV and V, smaller and more central on terga VI to VIII, fused on tergum IX, tergum X yellow; terga IV and V generally with an additional brown central spot.

Terga with scale bases, almost bare; posterior margin with pointed alternately long and short spines.

Sterna without scale bases; posterior margin smooth, without spines.

Abdominal segments I to VII without lateral spines, segment VIII with 5 to 7 lateral spines; segment IX with 6 to 8 lateral spines.

Gills I to VI with two lamellae, the second lamella rounded and well-developed, gill VII single and smaller, gill II largest than others, tracheation dark brown.

Median caudal filament slightly shorter than cerci; with very thin setae on both sides; base with two black spots. Cerci with a black band at each joint with thin black or blackish setae; setae at inner margins only, absent in proximal region and becoming longer in segments away from the middle. Measurements are given in Table 1.

DESCRIPTION OF MALE SUBIMAGO

General colouration: pale blackish with tinge of green; tergite V distinctly blackish; tergites VII and IX without brick red patches. Turban eye bright brown with a distal white rim; disc and collar part (upright portion) lighter brown.

Forceps very short, straight and rod like. Measurements are given in Table 2.

DESCRIPTION OF FEMALE SUBIMAGO

General colouration: abdomen whitish except paired longitudinal brownish mark in each segment. Wings with basal distinct spot; postero-marginal setae present; distal pterostigmatic spot very pale. Mesonotum dorsally brown with four whitish ovoid patches and a white median line. Dorsally metanotum pale brown, with anteriorly incomplete and posteriorly complete thin black line. Abdomen dorsally brown, with deep black patches on segment II; segments V to VIII gradually widened. All legs uniformly whitish without any pattern. Cerci long and spotted (unlike male subimago). Measurements are given in Table 2.

DESCRIPTION OF MALE IMAGO (Fig. 8)

Upper part of turban eye close; deep brown while collar light brown. Fore wings with a basal dark brown, nearly L-shaped spot; without minute round spot (as described for female imago) in angle of this L-shaped spot. Wing venation same as female (see below) except distal pterostigmatic spot hyaline; area anterior to radial vein is pale brown being prominent towards distal part.

Fore femur brownish (appears to be banded); all segments of four posterior legs bear distal dark brown spot.

Abdomen translucent white, with considerable dark brown marking. Tergite I with lateral reddish patch, tergites II and III with paired distinct triangular patches in postero-lateral angles, tergites IV and V with a narrow apical line interrupted centrally, tergite VI with a triangular patch in each apical angle, tergite VII with a pair of less defined reddish patch, tergites VIII and IX brightly brick-red, tergite X with a patch but not trifid (Fig. 6 in Kimmins, 1947). Forceps fuscous brown; segment short and spherical (Fig. 9). Ventrally, entire body and forceps white; pair of reddish brown conically elongated and divergent bands present on either side of midline on sternites VIII and IX. Measurements are given in Table 2.

Table 2. Measurements of body and body parts of male and female imagoes and subimagoes (all measurements in mm). *Turban eye included

Parameters		Imago ♂	Imago ♀	Subimago ♂	Subimago ♀
Total length		4.64		4.36	
Head width*		1.2	0.88	0.96	
Head length		0.8		0.48	
Antenna		1.04		0.88	
Width of thorax		1.12	1.36		
Length of thorax		1.6			
Fore wing		4.4	4.4		
Fore leg	Coxa	0.16	0.4		
	Femur	0.72	1.12	0.64	0.80
	Tibia	0.8	1.04	0.72	0.88
	Tarsi	0.8	0.8	0.88	0.64
Middle leg	Coxa	0.16	0.32		
	Femur	1.12	0.88	0.8	0.88
	Tibia	1.04	1.04	0.4	0.80
	Tarsi	0.64	0.68	0.4	0.56
Hind leg	Coxa	0.16			
	Femur	1.12	1.04	0.8	
	Tibia	1.04	1.04	0.48	
	Tarsi	0.64	0.64	0.48	
Forcep		0.4	xx		
Cerci			10.0	6.4	5.2

DESCRIPTION OF FEMALE IMAGO (Fig. 7)

Body dorsally with small to large brown spots; ventrally entire thorax bright white; abdomen ventro-medially with dark brown line.

Eyes rounded; ash or white (alcohol preserved). Posterior edge of vertex concave (so that the posterior angle of each eye is rounded and projects posteriorly. Scapus and pedicel white with distal end of 1st segment with deep brown spot, flagellum dark brown, appearing nearly black; length of antenna 1.5x head width.

Forewing: basal area with dark brown mark. Costa with a concavity towards middle part of wing; costal margin finely serrated; subcosta and radial veins together up to distal concavity then separate till wing margin. Anterior median (MA) and posterior median (MP) separated near base, with four incomplete longitudinal veins. Anterior cubital (CuA) and posterior cubital (CuP) separated from their origin with two incomplete longitudinal veins. Two incomplete longitudinal veins between CuP and anal vein (A). Black to brownish-black L-shaped spot at base of costal, sub-costal and radial veins. Round pale brown spot proximal to L-shaped spot. Areas bounded by wing margin and sub-costa and by sub-costa and radial veins faint brown (similar colour pattern observed in rearing females but lighter). Marginal antero-distal region with characteristic dark-brown pterostigmatic patch, paler in rearing females (Fig. 2 in Kimmins 1947).

Pronotum brownish, very short. Mesonotum longitudinally appears 4-lobed; lateral lobes separated from adjacent median lobe by white lines. Lateral sides of lateral lobes with longitudinal white bands. Metanotum shorter than mesonotum. 1st abdominal segment bears a pair of dark brown spot dorsally.

Fore femora reddish brown, strong built; inside with three paler spots. Middle and hind femora ecru with a distal transverse reddish-brown stripe. All tibiae and tarsi pale brown.

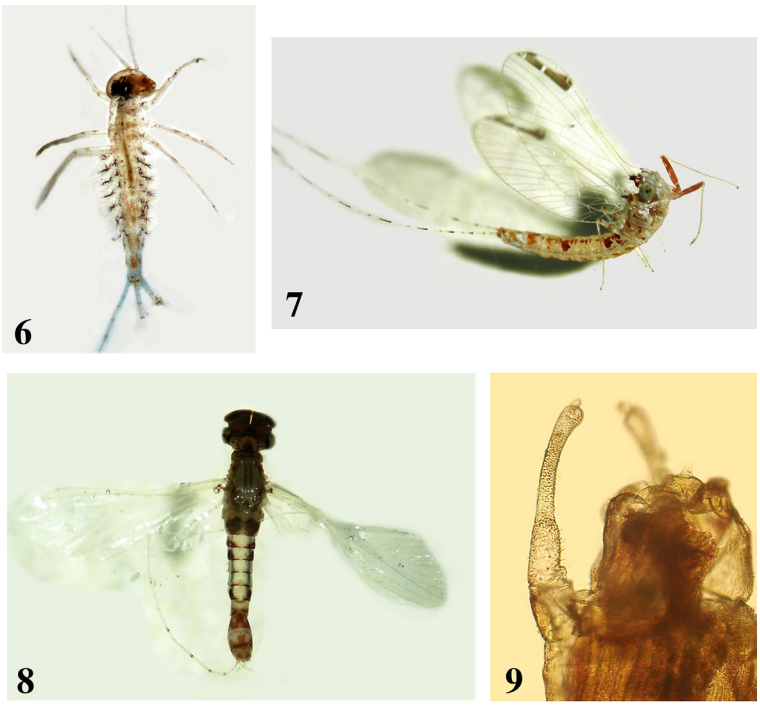
Abdominal tergites I, IV, V, VII and VIII with minute, feeble and pale brownish; abdominal tergites II, III and VI with broad and very prominent dark reddish brown lateral spots (Fig. 10 in Kimmins 1947)**.** Measurements are given in Table 2.

DISCUSSION

This insect breeds throughout the year. From random surveys, the species seems to be less abundant during hot summer days. They are only seen in cooler parts of the surroundings. Fewer were seen during too hot or colder evenings while they were frequent after post-monsoon and post-winter periods. Imagos were observed to increase in number during the month of August and the population declined from late October. In laboratory, the adult lifespan for females were 4-5 days and about four weeks time is required to complete the life cycle; the life cycle is shorter than observed by Gupta *et al.,* (1993), but they did not indicate with which species they made their experience. However, we could not measure the life span for males and they are supposed to die after mating. The eggs come out of genital pore in the form of brown ovoid mass.

At laboratory, the emergence of adult is nearly 11% which is assumed less in nature due to presence of natural predators.

Gillies (1949) indicated that several Oriental species of *Cloeon* and *Procleon,* including *C. bicolor*, *P. bimaculatum* and *P. harveyi* are known to be ovoviviparous; however Ulmer (Ulmer 1913) did not consider *Procloeon bimaculatum* as strictly ovoviviparous as he indicated that the eggs hatched two minutes after being laid. In the present study, *Procloeon harveyi* is striclty oviparous.



Figs. 6-9. Larva and imagos of *Cloeon harveyi*: 6: young larva. 7: female imago. 8: male imago. 9: forceps.

Some species viz. *Cloeon dipterum* are known to make burrow among submerged vegetation (McCafferty, 1983) which, however, has not been stated for the present species.

The absence of median circus and presence of 5-6 pairs of gills is distinctive feature of early larvae. At seventh gill stage, the larva is most active with more ramification of trachea showing high metabolic activity.

There are at least four phases or step-wise changes at seven-gilled stage. These phases do not correspond to true instar but to morphological changes. Indian *Cloeon* were recorded to present between 10 and 14 larval instars (Gupta *et al*., 1993). The early larva has gill lamellae without ramification of trachea inside it. Their intestine is also clear. In the second phase, the gills show ramification of trachea, clear intestine and no wing pad. In the third phase, the ramification of trachea becomes maximum, intestine is full of algal food and wing rudiments develop. Interestingly, only four pairs of the lateral lamellae undergo maximum development and rest appears to be reduced.

The seventh gilled stage is highly mobile. They have frilled gill for vigorous respiration. At the end of the last larval instar, the subimago is already visible through the transparent cuticle. The median caudal filament is empty at the end of the last larval instar as the subimago doesn't posses this appendice. At the end of this stage, the entire gut is empty.

Systematics

In *Cloeon* and *Procloeon,* the female imago is the easiest stage for secure specific attribution (Gillies, 1949), and these two genera constitute an exception among the Baetidae. The pattern of the abdominal tergites as well the colouration of the costal and subcostal areas of the forewings are reliable and constant characters for a specific identification. Female imago of *Procloeon harveyi* is characterized by the reddish brown lateral spot on the abdominal tergites II, III and VI as well as the forewings with a dark brown pterostigmatic area. It can be easily separated from *Cloeon viridis* Kimmins, 1947*, Cloeon virens* Klapalek, 1905 and *Cloeon coomani* Navás, 1934 who presents greenish marginal pigments in forewings (Gillies, 1949); from *Cloeon bengalense* Kimmins, 1947, *Cloeon bicolor* Kimmins, 1947, *Cloeon marginale* (Hagen, 1858) which have the complete costal and subcostal area middle to dark brown (Chopra, 1924; Kimmins, 1947; Gillies, 1949); finally *Cloeon juliae* Gillies, 1949, *Cloeon siccum* Gillies, 1949 and *Cloeon septimum* Gillies, 1949 have the forewings unpigmented (Gillies, 1949). Male imagos have almost unpigmented forewings and pattern of abdominal terga less contrasted than female; the gonopods can however be useful for specific identification. The larval stage of Oriental *Cloeon* is still virtually unknown. When larvae were mentioned, they are not attributed to a species (Chopra, 1924). Only Ulmer (1939) associated a larva with a described species. This association remains doubtful as not based on rearing; Gillies (1949) suggested that the larvae may belong to a species described subsequently such as *P. harveyi*. Comparison to the figures 376 to 381 in Ulmer (1939) with our material does not allow confirming or rejecting that the larvae are conspecific.

According to Gillies (1997), the main morphological difference between the larvae of *Cloeon* and *Procloen* in the degrees of development of the gills. Larvae of *Cloeon* possess lower lamellae rounded, upper lamellae as long as broad and upper lamellae V and VI at least half of the size of the lower lamellae. Larvae of *Procloeon* possess lower lamellae elliptical, and upper lamellae when present much longer than broad, and upper lamella V and VI minute or absent. In the material observed, the lower lamellae are rounded and the upper lamellae are also rounded and present on abdominal segments I to VI. On the basis the gills, *Procloeon harveyi* conform to the definition of *Cloeon.* Therefore, we propose the reassignment of this species to *Cloeon* with the new combination *Cloeon harveyi* (Kimmins, 1947). The female imago of *Procloeon bimaculatum* presents high similarities with *Cloeon harveyi*; the larva stage associated by Ulmer (1939) to this species is rather controversial. Final attribution can be only made after examination of larvae undoubtedly associated with imago.

PARAMETERS OF THE HABITAT

The pond is abundantly colonized by larvae of *Cloeon harveyi*. This pond is used for pisciculture and fertilizer is added annually probably to encourage zooplanktons. No specimens were collected in polluted ponds. The physico-chemical parameters of the Bank Plot Lake, Jadavpur, Kolkata (Calcutta), India, from Late July to Late September, 2008 are shown in Table 3.

Table 3. Physico-chemical parameters of the Bank Plot Lake, Jadavpur, Kolkata (Calcutta), India, from Late July to Late September, 2008

Dissolved O_2	5.168 mg/L
Dissolved CO_2	4.789 mg/L
Alkalinity	196.45 mg HCO_3^-/L
pH	6.8
Zooplankton	*Cyclops, Metacyclops, Cypris* and *Brachionus*
Water temperature at 8.00 A.M.	26^{O}C
Air temperature	27-29 ^{O}C
Relative air humidity	88%

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