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First record of water mite larvae, *Hydrachna* sp. (Acari, Hydrachnidae) parasitism as quiescent nymphophan on two major aquatic insects of Coleoptera and Hemiptera from West Bengal and Odisha, India

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ABSTRACT: Occurrence of quiescent nymphophan of *Hydrachna* sp. attaching to the sternites and tergites of thorax and abdomen of water beetle (*Hydrophilus* sp.) and giant water bug (*Lethocerus* sp.) is reported for the first time from West Bengal and Odisha of India. After feeding from one to five weeks as a parasitic larva on its host, *Hydrachna* sp, stops feeding and enters a quiescent nymphophan (nymphochrysalid) stage of development in which the larva squeezes into its exoskeleton and forms a sac-like structure where metamorphosis occurs. By the means of gnathoma, it remains attached to the host body, casts off its exoskeleton, and within a short time, the developing nymph can be seen within it. The nymph comes out of a slit in the exoskeleton and assumes a free-living existence. These nymphochrysalids ranged in length from 682.17 to 2112.45µm with lateral stripes adorning their external integuments. Body appeared to be bottle shaped with pointed or rounded posterior end. Preferences of water mites for insect host body parts and seasons, infection intensity and prevalence were reported.

KEY WORDS: *Hydrachna*, water beetle, *Hydrophilus*, water bug, *Lethocerus,* morphometrics, infection intensity

INTRODUCTION

The Hydrachnidia (true water mites) are found in almost all freshwater ecosystems (Zawal, 2003a). In the life cycle of water mites, the larva, deutonymph and adult are the active stages (Di Sabatino *et al*., 2000; Zawal, 2008). While the larval stages of almost all species of water mite remain as ectoparasites on some specific aquatic insect orders, the other active stages are free living predators that are attached with the eggs and larvae of aquatic insects and micro crustacea (Reilly and Mccarthy, 1993; Di Sabatino *et al*., 2000; Smith *et al*., 2001; Fairn *et al.,* 2008). Depending on the species, larval water mites tend to attach to their hosts from 4 days to 6 weeks (Ihle and McCreadie, 2003; Zawal, 2003b). The host provides nutrients to the larval mites and triggers high dispersal capability to the mite population (Zawal, 2003a).

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Water mites are made up of 300 genera containing more than 5000 species but descriptions of their larvae and deutonymphs are rare found from the literature (Di Sabatino *et al*., 2000; Zawal, 2008). It has long been known that the host association of *Hydrachna* species is extended, that the protonymph (old terminology: nymphochrysalis, nymphophan) is spent attached to the host, and that the host groups for this genus are aquatic Coleoptera and aquatic Hemiptera (Soar and Williamson, 1925). The water mites of Hydrachnidiae have a cosmopolitan distribution and inhabits standing waters, in addition to aquatic hemipterans, it parasitizes aquatic coleopterans. The larvae of the water mites of the genus *Hydrachna* are observed as ectoparasites that parasitize on Noteridae, Dytiscidae, Heteroceridae, Hydrophilidae, Gyrinidae of Coleoptera (Davis and Brown, 1969; Biesiadka and Cichocka, 1994; Fairn *et al*., 2008; Hajizadeh and Hosseini, 2022), as well as Corixidae, Nepidae, Veliidae, Belostomatidae of Hemiptera (Davids, 1972; Hajizadeh and Hosseini, 2019; Zawal *et al*., 2013; Perez *et al.,* 2014; Abe *et al.,* 2015; Gerecke *et al.,* 2020) and they have strong selection power for their hosts preferring to attach to selected sites on the host's body, i.e. sternites and tergites of thorax and abdomen (Wainstein, 1980; Reilly and McCarthy, 1993; Biesiadka and Cichocka, 1994; Cichocka, 1995; Zawal, 2002, 2003a, b; Sánchez *et al*., 2015; Céspedes *et al*., 2019).

The present study is an attempt to highlight the host-parasite relationship of water scavenger beetle, *Hydrophilus* sp. and electric light bug, *Lethocerus* sp. with water mite larvae, *Hydrachna* sp. From the selected water body at the buffer zone of Kuldiha Wildlife Sanctuary Odisha and an aquatic body of Haldia industrial belt, West Bengal, India.

MATERIALS AND METHODS

Study area and study period: Water beetles belonging to the family Hydrophilidae and giant water bugs belonging to the family Belostomatidae were collected from Rissia dam, Kuldiha Wildlife Sanctuary, Odisha, and aquatic body of Haldia, West Bengal, India (Fig. 1). A total of six samplings in a gap of four months have been done, two of them from post-monsoon, another two from pre-monsoon and another two from monsoon season (December 2021 to August 2023).

Sample collection and fixation: A net with a mesh size of 0.5mm was used to collect specimens. The collected specimens were fixed and preserved in situ with ethyl alcohol 70 per cent solution. In the laboratory, they were cleaned with a small paint brush and each specimen was closely observed under microscope (Nikon SMZ 745T) for study of parasite larvae attached to different body parts. The site specific occurrences and the number of parasitic mites on the host body have been also recorded. Ectoparasites were separated from their host bodies and mounted in glycerine jelly for detailed morphological studies. All the morphometric measurements and photographs of parasites were made using Carl Zeiss Axiovert A1.Mat Inverted Advance Binocular Research Microscope.

RESULTS AND DISCUSSION

Description of parasite life stages: There are seven distinct stages in the life cycle of *Hydrachna* water mites, that are: i) eggs, ii) active larval stage, iii) parasitic larva, iv) quiescent nymphophan, v) nymph, vi) quiescent telescophan, and vii) adult (David Lou Kass, 1962). Free-living larvae emerge from the eggs. Following a brief period of free-swimming, the larvae become parasitic when they connect themselves to aquatic insects by the means of gnathosoma. They develop from the parasitic larvae to nymphophans that stay affixed to the host insects and undergo a metamorphosis process into nymphs during this period. The nymphs soon break off their sac like container and become visible as free living nymphs. After a short period, according to Crowell (1957), the nymph attaches to algae or any substrates, and a second pupal stage, quiescent teliphan, appears in which the final adult characteristics develop. Once the quiescent teliphan stage ends, the sexually mature adult beign to emerge.

Morphological description of quiescent nymphophan: The organism stop feeding after one to five weeks as a parasitic larva on its host, and enter a quiescent nymphophan (nymphochrysalid)

Table 1. Occurrences of water mite larvae *Hydrachna* sp. on Coleopteran and Hemipteran hosts

Month	Host no.		Infested host no.		Prevalence (%)		Parasite no.		Average intensity		Body location	
	Hy	Le	Hy	Le	Hy	Le	Hy	Le	Hy	Le	Hy	Le
December, 2021	8	0	1	0	12.5	-	1	0	1	-	Hind tarsus (right)	-
April, 2022	13	1	3	1	23	100	6	98	0.46	98	Foreleg coxa, pronotum, metasternal process	Scutellum, wings, prosternum, coxal region
August, 2022	2	0	0	0	0	-	0	0	0	-	-	-
December, 2022	9	0	0	0	0	-	0	0	0	-	-	-
April, 2023	9	0	2	0	22.2	-	4	0	0.44	-	Hind tarsus (left), foreleg coxa, prosternum	-
August, 2023	1	0	0	0	0	-	0	0	0	-	-	-

Hy – *Hydrophilus*, Le – *Lethocerus*

Table 2. Morphometric measurements of parasitic larvae (in µm) (n=5)

Characters	Range	Mean	SD
Total Body Length	682.17-2112.45	1418.98	616.15
Gnathosoma length	215.71-242.52	230.35	11.42
Idiosoma length	466.46-1869.93	1188.62	604.87
Gnathosoma width at sucker	70.46-72.61	71.29	0.86
Maximum width of Gnathosoma	121.36-141.60	130.82	7.99
Maximum width of idiosoma	227.38-685.78	431.86	177.11
Width of idiosoma at posterior most region	122.96-242.70	183.31	46.47
Eye length	30.07-48.04	39.70	7.30
Eye width	18.06-35.17	26.89	6.68
Total length of eye and anterior eye plate	45.15-80.58	64.02	14.23

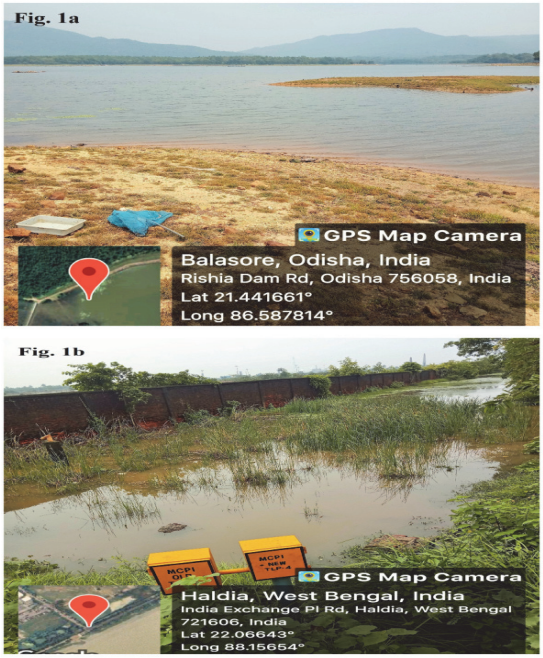

Fig. 1 The study area: a) Rissia Dam, Kuldiha WLS, Odisha and b) aquatic habitat at Haldia Industrial Belt, Purba Medinipur, West Bengal, India

stage of development, where in the larva shrink into its exoskeleton and forms a sac-like structure where metamorphosis takes place. By the use of gnathosoma, they eling to their host and cast off theor exoskeleton eventually revealing the growing nymphs inside. The nymph comes out of an opening in the exoskeleton and appear a free-living existence. These nymphochrysalids measured from 682.17 to 2112.45µm. in length and the external integuments of the larvae are ornamented with lateral stripes. Body appeard to be bottle shaped with pointed or rounded posterior end (Fig. 4).

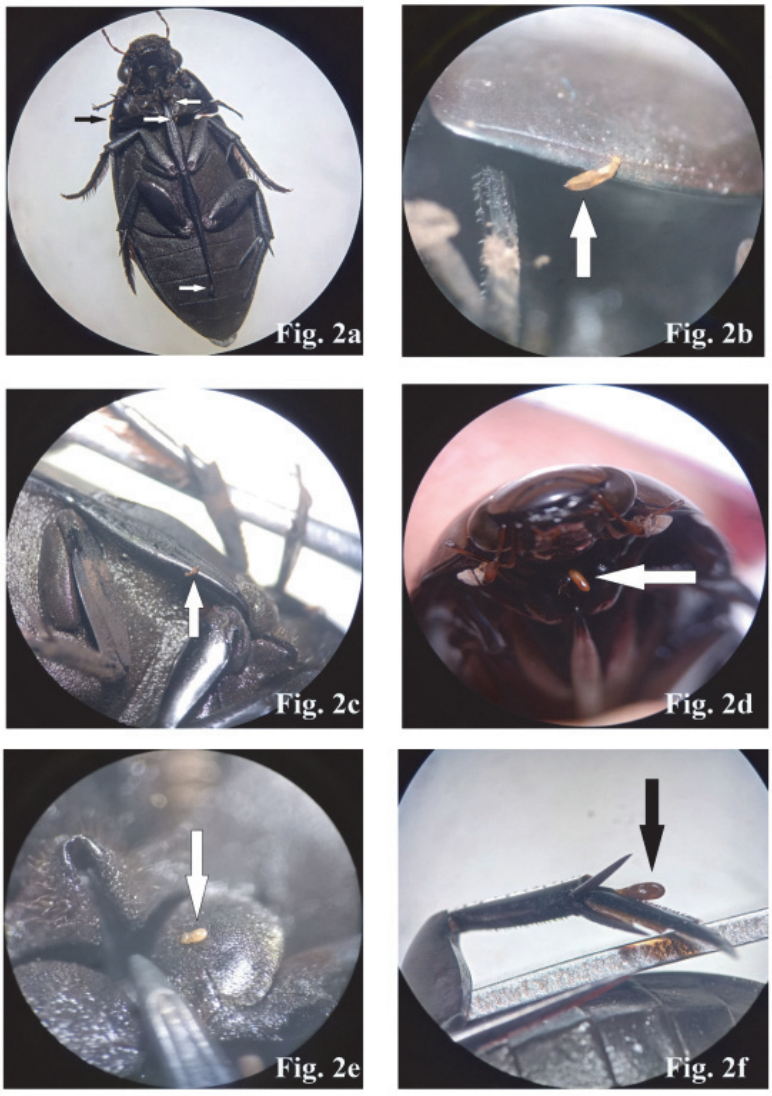

Fig. 2 a) *Hydrophilus* sp. with parasites *Hydrachna* sp.; b)-f) Different positions of *Hydrachna* larva on host (*Hydrophilus* sp.) body parts: b) edge of pronotum, c) anterior portion of metasternal process, d) raised portion of prosternum, e) another position of prosternum, and f) hind tarsus

Occurrences of water mites on host bodies: Water mite parasitism on aquatic insects hosts belonging to the family Hydrophilidae (Coleoptera) and Belostomatidae (Hemiptera) was investigated and they were all in resting stage (nymphochrysalis). A total of 42 beetle specimens of Hydrophilidae and one bug specimen of Belostomatidae were collected, among which six were infested with 11 parasites that were attached on the surfaces of the foreleg coxa, prosternum, pronotum, metasternal process, hind tarsus of the beetle body and one bug was infested with 98 parasites that were attached on dorsal side of wings and ventral side of head and thorax (Table 1, Figs. 2, 3).

Morphometric measurement: Total body length (gnathosoma length: 215.71-242.52µm and idiosoma length: 466.46-1869.93µm) ranges from 682.17-2112.45µm; maximum width of gnathosoma ranges from 121.36-141.60µm and minimum width of gnathosoma (sucker area) ranges from 70.46-72.61µm; maximum width of idiosoma ranges from 227.38 to 685.78µm and minimum width of idiosoma

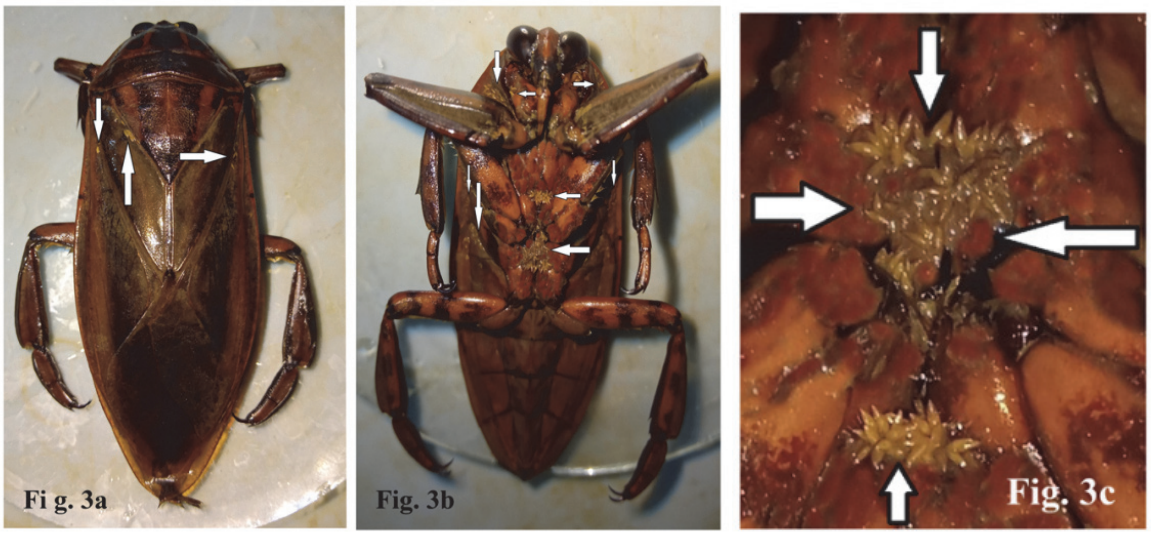

Fig. 3 *Lethocerus* sp. with parasites *Hydrachna* sp. a) Dorsal side: junction between scutellum and wing and on the wing (hemelytra), b) Ventral side: maximum parasites were found in the coxal region of mid and hind leg, some were found in prosternum and some were in the ventral side of hemelytra, and c) focused view of parasites in coxal portion

(posterior most region) ranges from 122.96 to 242.70µm; eye length ranges from 30.07-48.04µm; eye width ranges from 18.06 to 35.17µm; total length of eye with anterior eye plate ranges from 45.15 to 80.58µm (Table 2).

Preferences of water mites for host body parts: The prevalence of *Hydrachna* sp. on the head, prothorax, meso- and metathoraxes, abdomen, fore legs, mid legs and hind legs for six infested adult *Hydrophilus* sp. and one *Lethocerus* sp. were calculated (Table 1). In case of *Hydrophilus* sp., among 11 parasites, three were found in forelegs, three on hind legs, two on pronotum, one on prosternum, and two on metasternal process. In case of *Lethocerus* sp., 17 parasites were found on dorsal side (on wings) and 81 were found on ventral side (head and thorax) (Fig. 3). Preference for a particular attachment site on a host aquatic insect has been noted for the mites' larvae. Lanciani (1970) enumerated the attachment sites on several genera under the families Dytiscidae and Hydrophilidae. In this study, it was observed that maximum number of parasites were attached to the ventral parts of the beetles and minimum to the dorsal sides. This fact is also true for the family Belostomatidae.

Parasite load of a single beetle indicates that mites may be deliberately selecting unparasitized hosts or hosts with only a few parasites. Comparisons of the frequency distribution of the number of mites per host revealed that attachment was not random. Work done by Nielsen and Davids (1975) indicates that mites actively select the sites on a host. Data in Table 1 offer one explanation for such selectivity.

Preferences of water mites for season: The highest abundance of aquatic beetles and parasites was seen in pre-monsoon season and the prevalence was recorded (23 and 22.2% respectively for April 2022 and April 2023). Post-monsoon season shows the medium abundance of beetles and very few parasites and prevalence (12.5 and 0% respectively for December 2021 and December 2022). During monsoon season, although got very few beetles, but there were no parasites. On the other hand, one *Lethocerus*, that is called giant water bug (8cm long), shows the host for much higher parasite prevalence (100%) in pre-monsoon of 2022. Greatest parasitism rates were found in the spring and early summer, when the beetle and bug populations are high or increasing (Aiken and Wilkinson, 1985; Aiken, 1985a), affording the mite

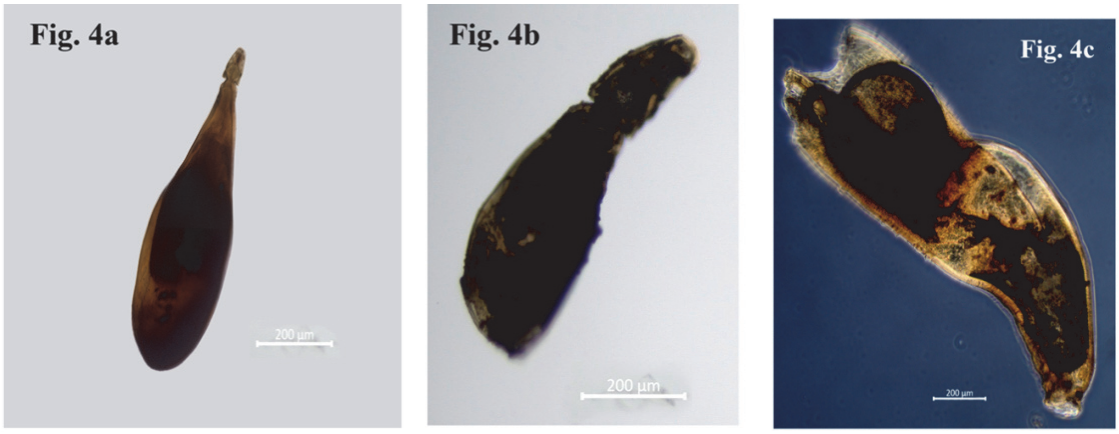

Fig. 4 Different positions of the isolated *Hydrachna* parasites: a) dorsal view with gnathosoma and idiosoma, b) lateral view with distinct gnathosoma and idiosoma, and c) lateral view with prominent sucker. Scale bars: 200µm.

Fig. 5 Microscopic view of: a) gnathosoma with sucker, four pairs of limb buds and internal structures, and b) position of eye and anterior eye plate. Scale bars: 50µm.

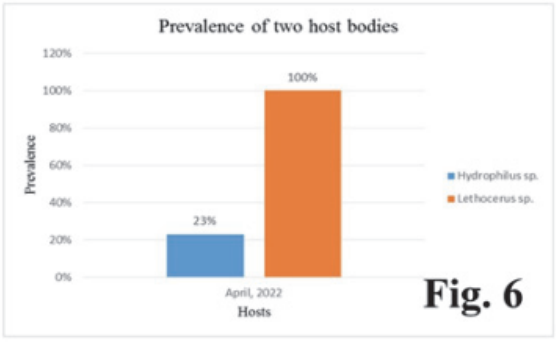

Fig. 6 Comparison of prevalence in water beetle, *Hydrophilus* sp. and giant water bug, *Lethocerus* sp.

higher numbers of potential hosts. When hosts are scarce in monsoon (July and August), most mites completed their larval growth and are in nonparasitic stages (Table 1). The study reveals the same fact that the higher parasitism (most of the hosts and parasites) was found in pre-monsoon season (April 2022 and 2023), lower in post-monsoon (December 2021 and 2022) and none in monsoon (August 2022 and 2023) season.

Infection intensity and prevalence: The infection prevalence of this study is somehow low in *Hydrophilus* sp. of Coleoptera and much higher in *Lethocerus* sp. of Hemiptera (Fig. 4, Table 1). Previous studies showed that the host specificity varies according to the species considered: *Hydrachna geographica* and *H. inermis* were found only on Dytiscidae, *Hydrachna leegei* and *H. incognita* only to species of Hydrophilidae whereas *Hydrachna crassipalpis* parasitizes beetles belonging to the both families. The number

of larvae, the intensity of infection and the prevalence of parasitism were higher in Dytiscidae than in Hydrophilidae of Coleoptera. This was the result of the different strategy of infection (Zawal, 2002). But this study reveals the infection of *Hydrachna* larvae on *Hydrophilus* sp. of Hydrophilidae, along with *Lethocerus* sp. of Belostomatidae.

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