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FIRST RECORD OF *ARGULUS JAPONICUS* (CRUSTACEA: BRANCHIURA) ON *CYPRINUS CARPIO* IN NEPAL, WITH ADDITIONAL NOTES ON MORPHOLOGY AND PREVALENCE OF *A. JAPONICUS* AND ITS TREATMENT

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ABSTRACT

The present study recorded the parasite Argulus japonicus (Crustacea: Branchiura) as the first discovery in Nepal. The description was based on using 11 males (4.12 mm average TL) and 5 females (4.18 mm average TL) of Argulus japonicus which were collected from Godawari fish farm, Lalitpur. Biological behavior of one summer old common carp (Cyprinus carpio) infected with Argulus japonicuswere sluggish movement, jumping and poor growth grown in earthen pond in winter at water temperature 15.5 ºC and pH 7.4. Heavily infected fishes showed brown-grey to reddish points throughout their body surface, especially on the caudal fins and on the body portion. Prevalence rate of this parasite was 80% in infected common carp fish with an average weight 261.80±85.12 g and length 22.9±4.9 cm. Argulus mean intensity count was averaged 10.2 per infected fish with relatively high infestation in body surface (2.8±3.9) followed by caudal fin (1.8±4.5) and dorsal fin (1.1±1.5). Prevalence rate of this parasite in treated common carp reduced by 95% and Argulids assembled in several regions of the fish was in average 0.1/treated fish by feeding Duroclean treated feed. Duroclean, an aquaculture drug was found effective to control this parasite. The effective dose of the drug was 0.3 g per kg fish feed and fed for 3 days consecutively with one day off and then again for 2 days.

Keywords: *Argulus japonicus*, ectoparasite, prevalence rate, duroclean

INTRODUCTION

The family Argulidae contains a valid Genus *Argulus* Muller, 1785, more commonly known as fish louse cause argulosis disease. Species of the *Argulus* are found distributed throughout the world (Fryer, 1968; Post, 1987; Rushton-Mellor, 1992) and 143 species in the Genus have been described (Anon, 2001), although many of these species are synonymous (Taylor *et al*., 2005). Further Poly (2008) has enlisted about 129 valid species, occurs around all continents, except Antarctica, in marine and estuarine (n= 44) and freshwater habits (n=85), in total, the diversity of freshwater Branchiura stands at 113 species. Among Crustacean parasite *Argulus japonicus, Argulus foliaceus* and *Argulus coregoni* arewidespread and the most documented species (Post, 1987; Taylor, *et al*., 2005).

In due course Argulosis disease is one of the major economic concerns in all phase of the aquaculture industry from production to marketing (Menezes *et al*., 1990; Taylor *et al*., 2006). Heavy infestations of *Argulus* parasite can induce significant morbidity and mortality in cultured fish population (Yamaguti 1963; Benz *et al*., 2001; Northcott *et al*., 1997; Wilson, 1902), both in

carp (Rahman, 1996) and trout fish (Menezes *et al*., 1990). In Nepal ectoparasite *Argulus* are currently considered to be one of the most commercially pathogenic parasite and known to infect a wide variety of fish. *Argulus* was reported as threat to production of Rohu (*Labeo rohita*) and Naini (*Cirrhinus mrigala*) and breeding performance of cultured carp fish (Mandal and Prasad, 2011). Pantha (1998) has reported annual losses about 15-20% loss in total fish production and 30-40% loss in fish seed production due to various disease problems every year which accounts an economic loss of about Rs 1513.3 million per year in Nepal. Since the *Argulus japonicus* is the first record for Nepal, therefore current study has initiated to assess the infestation rate,undertaken the morphological study of the adult stage of *Argulus japonicus* including determine the promising drug against this parasite.

MATERIALS AND METHODS

A total of 20 Common carp(*Cyprinus carpio*) of average weight 261±80 g and length 22.9±4.9 were collected from the earthen pond of Godawary fish farm, Lalitpur, at central Nepal for detection of *Argulus* infection in December 2015. Localities of fish farm at altitude N 27º35.989' and latitude E 085º23.246' and 1576 masl elevation. *Argulus* were removed from head, body, fins and caudal fin of common carp using brush and forceps and *Argulus* number/fish was noted and fixed in 70% ethanol. Clinical examination of collected fish was adopted using the methods as described by Lucky (1977) for the determination of any lesions or abnormalities on the external body surface. Identification and morphological study of sampled parasite *Argulus* was according Bykhovskaya Pavlovskaya *et al*., (1962) and Rushton-Mellor (1994). Description of identified *Argulus japonicus* was based on studies of their 16 adult specimens (males n=11, females n=5) (Table 1). The measurement of total length (TL), total width (TW), carapace length (CL), carapace length as percent of total length, abdomen length (AL), abdomen length as percent of total length and abdomen width (AW) of *Argulus japonicus* was taken in millimeters under light olympus microscope. Photos of male and female *Argulus japonicus,* were also takenunder olympus microscope (4x) using digital camera. Sex determination was accomplished as mentioned by Wilson (1902). Prevalence, mean intensity and mean abundance were calculated as following the formula suggested by Margolis *et al*., (1982):

$$\text{Prevalence (\%)} = \frac{\text{Number of individual infected fish}}{\text{Total number of fish examined}} \times 100$$

$$\text{Mean intensity} = \frac{\text{Number of collected parasite}}{\text{Number of infected fish}}$$

$$\text{Abundance} = \frac{\text{Number of parasite}}{\text{Number of fish examined}}$$

Efficacy of Duroclean (herbal extracts of satavari, copper salicylic acid, vitamin E and K) an aquaculture drug was tested on common carp as feed additive against *Argulus* parasite at research station: Godawary fish farm, Lalitpur district, Kathmandu. Further, drug verification trial was carried at farmer pond condition in Karaiya, Khaireni Nagarpalika-1, Chitwan central Nepal.

RESULTS

The present study recorded the *Argulus japonicus* as the first representative of the genus isolated from the common carp fish. *Argulus japonicus* is recognizedby the abdomen lobes which have nearly rounded terminal end (slightly pointed than *Argulus foliaceus*); covered marginally with small spines and not covered by carapace. Furthermore, the posterior incisures of abdomen reaches middle of the body and pair of respiratory areas occurs on the ventral surface of lateral lobe of the carapace, the anterior respiratory area was smaller and roughly ovular in shape and posterior was much larger kidney bean shaped.

Body length of male *Argulus japonicus*4.12±0.46 (3.35-4.68) mm, body width 2.39±0.29 (1.98-2.71) mm and abdomen length 1.03±0.10 (0.90-1.15) mm, comprising 25.02±1.17 (23.16-27.61) % of total length; its posterior lobes tapering to relatively rounded points. Carapace length is 3.08±0.43 (2.45-3.80) mm, comprising 74.62±3.40 (69.80-81.28) % of total length (Table 1). Cephalothoracic carapace extended beyond the beginning of abdomen and cover all four pairs of swimming legs (Fig. 1a, b). The males *Argulus japonicus* are more abundant than females (sex ratio=68.8%).

Table 1: Measurements of *Argulus japonicus* isolated from common carp

SN	Measurements (mm)	Male n=11	Female n=5
1	Total length (TL)	4.12 (3.35-4.68±0.46)	4.18 (3.58-4.75±0.46
2	Total width (TW)	2.39 (1.98-2.71±0.29)	2.35 (2.11-2.50±0.16)
3	Abdomen length (AL)	1.03 (0.90-1.15±0.10)	0.93 (0.78-1.05±0.11)
4	AL as % of TL	25.02 (23.16-27.61±1.17)	22.35 (21.28-23.33±0.86)
5	Abdomen width (AW)	0.60 (0.41-0.80±0.11)	0.56 (0.45-0.66±0.09)
6	Carapace length (CL)	3.08 (2.45-3.80±0.43)	3.26 (2.75-3.80±0.40)
7	CL as % of TL	74.62 (69.80-81.28±3.40)	78.56 (76.67-80.85±1.86)

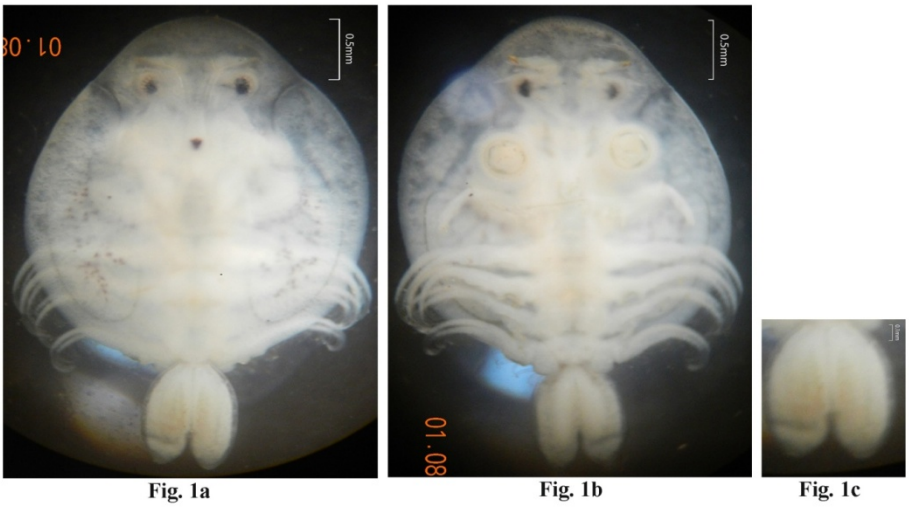

Fig. 1a Fig. 1b Fig. 1c

Fig. 1: Male of *Argulus japonicus* Thiele 1900 viewed under a light microscope (4x)
(**1a** dorsal view, 1**b** ventral view;1c abdomen with a pair of testis (3.75 mm TL, 0.90 mm AL)

Body of female *Argulus japonicus* is slightly elongate, with length 4.18±0.46 (3.58-4.75) mm and body width 2.35±0.16 (2.11-2.50) mm. Abdomen length 0.93±0.11 (0.78-1.05) mm, comprising 22.35±0.86 (21.28-23.33) % of total length; its posterior lobes tapering to relatively rounded points. Carapace length is 3.26±0.40 (2.75-3.80) mm, comprising 78.56±1.86 (76.67-80.85) % of total length (Table 1). Cephalothoracic carapace extended beyond the beginning of abdomen and cover all four pairs of swimming legs (Fig. 2a, b).

Fig. 2: Female of *Argulus japonicus* Thiele 1900 viewed under a light microscope (4x)
(2a dorsal view; 2**b** ventral view; 2**c** abdomen with a pair of spermetogeneis (4.05 mm TL, 0.91 mm AL))

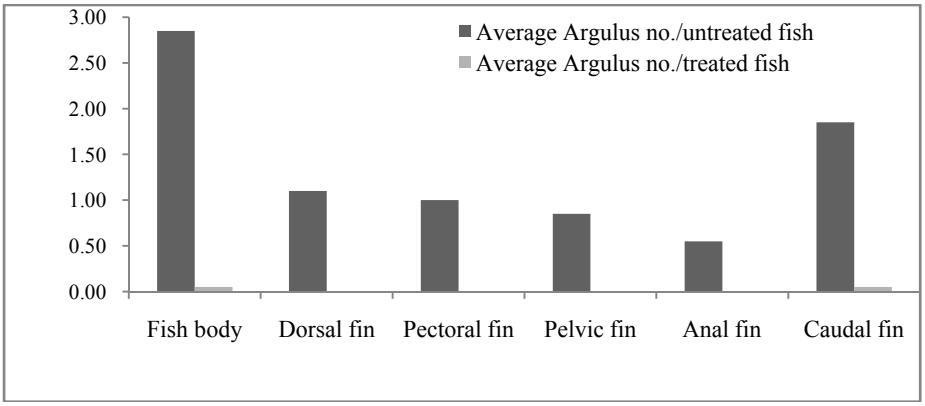

Fig. 3: Average *Argulus* no. assembled in body and fins of common carp before and after treatment

Specific difference between male and female of sampled *Argulus japonicus* included that the abdomen of the male contained the elongated a pair of testes, which were milky white with sperm in live specimens and dark in preserved specimens (Fig. 1a, b, c), while the abdomen of the female contained a pair of round spermathecae which was dark in color (Fig. 2a, b, c).

A total of 164 specimens of *Argulus japonicus* were isolated from one summer old common carp (Table 2). Prevalence rate of this parasite was 80% in infected fish. Argulids were assembled in several regions of the fish. *Argulus* mean intensity count was averaged 10.2 per infected fish with relatively high infestation on body surface (2.85±3.9) followed by caudal fin (1.85±4.5), dorsal fin (1.1±1.6), pectoral fin (1.0±1.92), pelvic fin (0.85±1.87) and anal fin (0.55±1.23) (Table 2, 3; Fig.

3). Fish infected with *Argulus japonicus* showed sluggish movements, jumping and poor growth in winter at water temperature 15.5 ºC and pH 7.4.

Table 2: Individual number of *Argulus* parasiteattachedin different body parts of common carp fish, n=20 in December 2015

SN	Total weight of Common carp (g)	Total length of common carp (cm)	*Argulus* counts on body surface	*Argulus* counts on Dorsal fin	*Argulus* counts on Pectoral fin	*Argulus* counts on Pelvic fin	Argulus counts on Anal fin	*Argulus* counts on Caudal fin	Total *Argulus* no/infected fish
1	470	29.1	4	0	8	7	3	2	24
2	190	23.8	2	1	3	5	3	2	16
3	280	25.5	0	0	0	0	0	0	0
4	302	25.9	5	5	0	0	0	20	30
5	268	26.1	18	2	3	1	1	2	27
6	360	29.5	5	3	1	0	4	6	19
7	266	26.2	1	0	0	0	0	0	1
8	380	11.8	1	0	0	0	0	0	1
9	172	21.2	0	0	0	0	0	0	0
10	168	26.3	1	0	0	0	0	0	1
11	150	19.5	0	0	0	0	0	0	0
12	262	23.6	1	2	0	0	0	0	3
13	325	10.4	2	0	1	2	0	0	5
14	260	25	4	0	0	0	0	2	6
15	142	19.1	0	0	0	0	0	0	0
16	295	25.1	2	0	1	0	0	0	3
17	248	22.1	3	1	2	0	0	0	6
18	250	23.1	3	4	1	0	0	1	9
19	148	20.5	2	3	0	1	0	1	7
20	300	25.2	3	1	0	1	0	1	6
Total	20		57	22	20	17	11	37	164
Av	261.80	22.95	2.85	1.1	1.0	0.85	0.55	1.85	8.2
SD	85.12	4.93	3.91	1.55	1.92	1.87	1.23	4.51	9.63

Table 3: Infestations value of *Argulus* parasite on the common carp fish

Season	No. of fish examined	No. of fish infected	No. of collected parasites	Prevalence %	Mean Intensity	Abundance
Winter	20	16	164	80	10.25	8.2

Besides, heavily infected fishes had some clinical symptoms as brown-grey to reddish points throughout their body surface, especially on the caudal fins and on the body portion. As a control measure, Duroclean was found effective against this parasite. Prevalence rate of this parasite in common carp reduced by 95% and Argulids were assembled in several regions of the fish that was 0.1/treated fish by feeding Duroclean treated feed. Although assembled argulids was 8.2/infected

fish (Table 2, 3; Fig. 3). The effective dose of the drug was 0.3g per kg fish feed and fed for 3 days consecutively with one day off and then again for 2 days.

DISCUSSION

Argulus japonicus was originally described by Thiele (1900) locality from China and further discovered in Europe, North America, Australia and Africa (Taylor *et al*., 2005). Poly (2008), mentioned that *Argulus japonicus* Thiele, 1900 has been introduced from east/southeast Asia to all other continents, except Antarctica as well as currently discovered from central Nepal. *Argulus japonicus* has been studied by several authors such as Ikuta and Makioka (1997a) (described the structure of adult ovary and oogenesis); Gresty, *et al*., (1993) (described the structure and function of cephalic appendages); Tokioka (1936) and Lutsch and Avenant-Oldewage (1995) (described the larval stages); Tam (2005) (elucidate the pathogenecity of *Argulus japonicus*); Avenant Oldewage and Swanepoel (1993) (described the male reproductive system and mechanism of sperm transfer) and Ikuta *et al*. (1997b) (described the eggshell in *Argulus japonicus*). Rushton-Mellor (1994) described the shape of respiratory areas and abdomen as species specific of Genus *Argulus*. Additionally, Rushton-Mellor (1994) has mentioned the carapace lobes covers the all four pairs of swimming legs particularly in *Argulus japonicus*.

During current study, the morphometric comparison of collected *Argulus japonicus* was made with its closely related congeners; *Argulus coregoni* and *Argulus foliaceus.* The specimens of *Argulus japonicus* are distinguished with *Argulus coregoni* (Thorell 1866) by nearly rounded abdomen lobes (vs. sharply pointed abdomen lobes), by posterior lobes of cephalothoracic carapace extended beyond the beginning of abdomen (vs. posterior lobes of cephalothoracic carapace not extended beyond the beginning of abdomen), by the posterior incisures of abdomen reaching the mid-line (vs. posterior incisures of abdomen reach beyond middle of the abdomen) and by the body length 3.35-4.75 mm (vs. 12 mm body length) (Yildiz andKumantas, 2002; Saha and Bandyopadhyay, 2015). *Argulus japonicus* distinct with *Argulus foliaceus* (Linne, 1758), by nearly rounded abdomen lobes (vs. rounded lobes of abdomen), by the posterior incisures of abdomen reaching the mid-line (vs. the posterior incisures not reaching the mid-line), by posterior lobes of cephalothoracic carapace extended beyond the beginning of abdomen (vs. posterior lobes of cephalothoracic carapace not extended beyond the beginning of abdomen) (Yildiz and Kumantas, 2002; Saha and Bandyopadhyay, 2015).

Argulus japonicus is an opportunist parasite (Shafir and Oldewage, 1992; Avenant-Oldewage, 2001). Infestation with this parasite can reach severe proportions in a very short time which result in catastrophic fish deaths (Menezes *et al*., 1990; Northcott *et al*., 1997; Avenant-Oldewage 2001; Taylor *et al*., 2006). Bower-Shore, 1940; Shimura *et al*., 1983; Ahne, 1985; Moravec, 1994 and Bandilla *et al*., 2006 has mentioned that *Argulus* causes increase the susceptibility of secondary infections to its host. Against *Argulus* parasite, several chemicals such as salt (NaCl) (Singhal *et al*., 1986), formaldehyde (Rydlo, 1989), potassium permanganate (Singhal *et al*., 1986; Jafri and Ahmed, 1994), quicklime (Jafri and Ahmed, 1994; Ahmed, 2004), organophosphorus (Chandra *et al.*, 2004) and Gammexane (Singhal *et al*., 1986) have been found applied in aquaculture industry. In the current study efficacy of Duroclean was found promising to minimize *Argulus* number/infected fish (*Argulus* reduced from 8.2 to 0.1/ infected fish). Studies are warned to identify

the interaction between environmental conditions and outbreak of *Argulus japonicus* for developing better management practices of carp aquaculture.

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