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Short note

A scanning electron microscopic study of *Argulus coregoni* Thorell, 1866 (Crustacea: Branchiura) parasitizing *Carassius auratus* (Linnaeus, 1758) from India

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ABSTRACT. During the survey, some *Argulus* or fish lice were isolated from the skin of *Carassius auratus* (Linnaeus, 1758). A comparison with previously described *Argulus* species was conducted and the specimen was identified as *Argulus coregoni* Thorell, 1866. Such infections can be a major threat to fish health. Mainly three species of *Argulus* infect *Carassius auratus* namely *A. japonicus* Thiele, 1899; *A. foliaceus* Linnaeus, 1758, and *A. coregoni* Thorell, 1866 which were originally reported from Europe. Although, the same three species were reported from India also. The present study aims to add knowledge to previous studies on the morphology of *A. coregoni* by scanning electron microscopic images and also adding a new locality for the parasite.

Keywords: *Argulus coregoni*, *Carassius auratus*, scanning electron microscopy

Introduction

The genus *Argulus* Muller, 1785 belongs to subclass Branchiura and class Maxillopoda [1]. These are common ectoparasites of Europe and Asia. More than 100 species of *Argulus* are distributed worldwide. They invade the skin and gills of the host and feed upon their body fluid, which damages the epithelium layer of the host's skin and makes them more susceptible to secondary infections [2]. Argulosis is a very common crustacean disease of ornamental fishes such as *Carassius auratus* (goldfish) and Koi carp [3]. Some of the common symptoms observed in the host are stunted growth, dark spots on the body, haemorrhage, anaemia, loss of scales, enhanced mucus production, lethargy, erratic swimming, such condition leads to increased production costs of cultured fishes [4]. In many cases, fishes were observed rubbing against surfaces in order to relieve irritation. Such infections may lead to even death of fish [5,6]. According to previous studies, it is clear that *Argulus* is not strictly host-specific as *A. coregoni* was reported from other hosts such as *Pleoglossus altivelis* (Salmoniformes: Plecoglossidae) from Honshu in 1995 [7], and from *Oncorhynchus mykiss* (rainbow trout), *Oncorhynchus masou* (masu salmon), *Oncorhynchus masou macrostomus* (amago salmon) and *Salvelinus fontinalis* (brook trout) from Japan.

The first description of *A. coregoni* was given by Thorell in 1866 on *Coregonus lavaretus* (Linnaeus, 1758) from Jemtland, Sweden [8]. In the year 1936 Tokioka redescribes the species as the previous data does not provide any information or image of scales on the periphery of the carapace, thorax, and maxillae [9]. Further, this species was described by [10–16].

Materials and Methods

Sampling of fishes

During the survey of fishes, fifteen specimens of *Carassius auratus* (total weight: 5–7 g, total length: 4–10 cm) and its types were collected from different aquaria shops in Lucknow, India (26.84°N 80.94°E) between January 2022 to March 2022. Hosts were identified using the keys provided by FishBase [17].

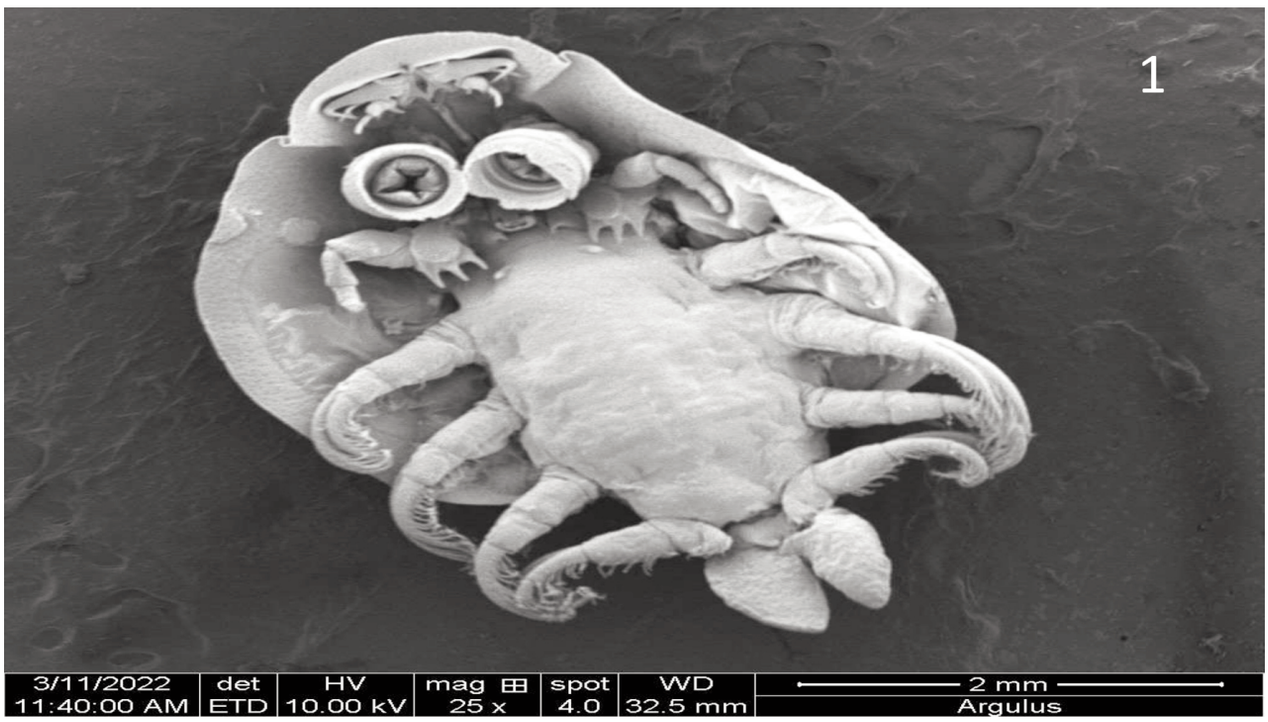

Figure 1. Whole body of *Argulus coregoni*

Fishes were transferred alive in the lab's aquaria. Firstly, the fish were examined macroscopically for any abnormal clinical signs of ectoparasitic infection. Then, they were screened under the stereomicroscope.

Parasite sampling

For examination of the parasite, the skin, fins, and gills of fish were screened and *Argulus coregoni* was isolated by forceps or brush. *Argulus* species were identified using morphological keys including length of the body, posterior lobes of carapace, abdomen, and posterior incisure of the abdomen. Some samples were processed for observing under scanning electron microscope.

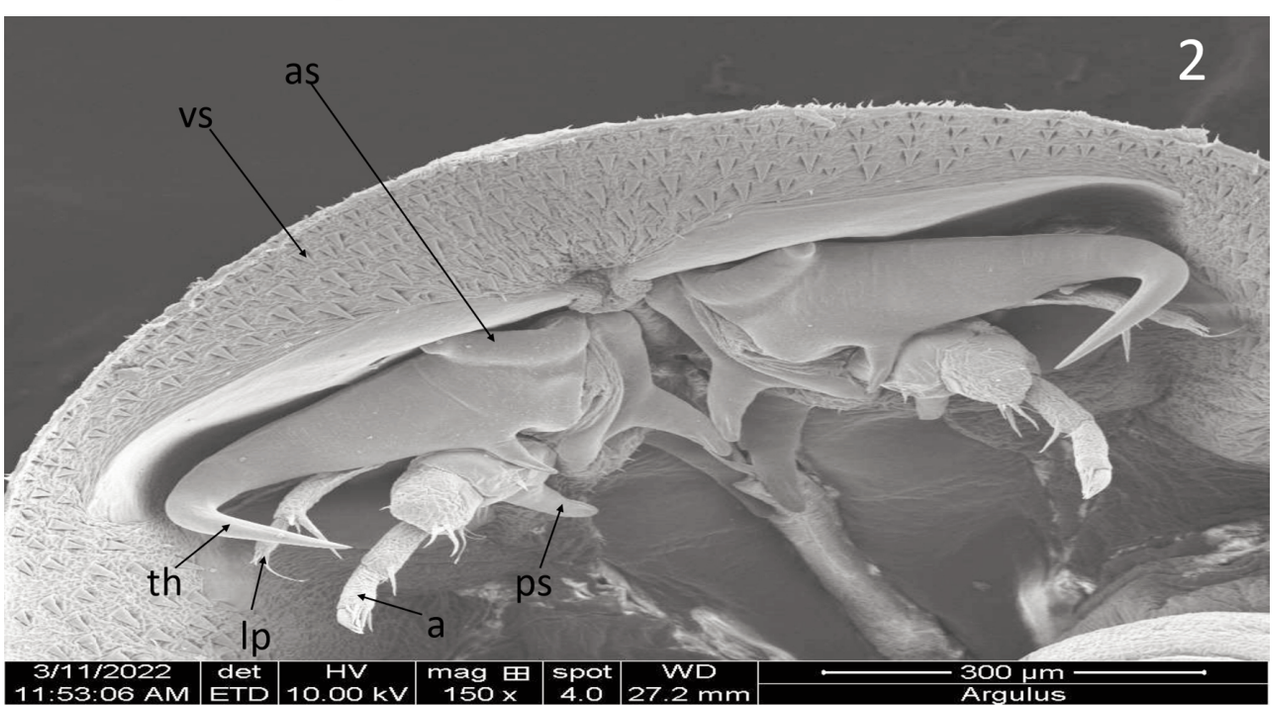

Figure 2. Dentate scales on ventral edge of the carapace (vs – ventral dentate scales, a – antennae, as – anterior spine, ps – posterior spine, th – terminal spine, lp – lateral projection

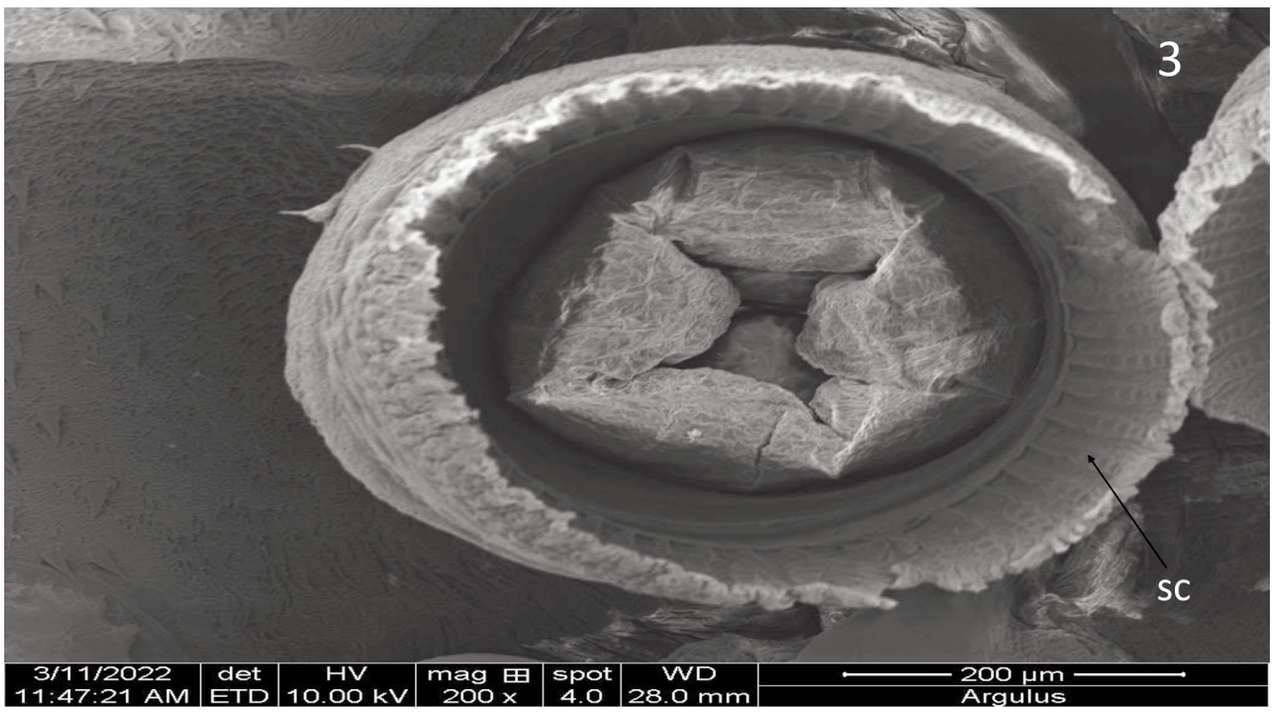

Figure 3. Sucker (sc – sclerite rods)

Scanning electron microscopy

Sample is washed in PBS buffer (2 times for 15 minutes), fixed in 2.5% glutaraldehyde (24–48 hours), post-fixed in 1% osmium tetroxide at 4°C, dehydrated in a graded ethyl alcohol series, dried by CO_2 critical point, coated with silver, examined under scanning electron microscope.

Results

Taxonomic summary

Phylum: Arthropoda, Subphylum: Crustacea, Class: Maxillopoda, Subclass: Branchiura, Order: Arguloida, Family: Argulidae, Genus: *Argulus*

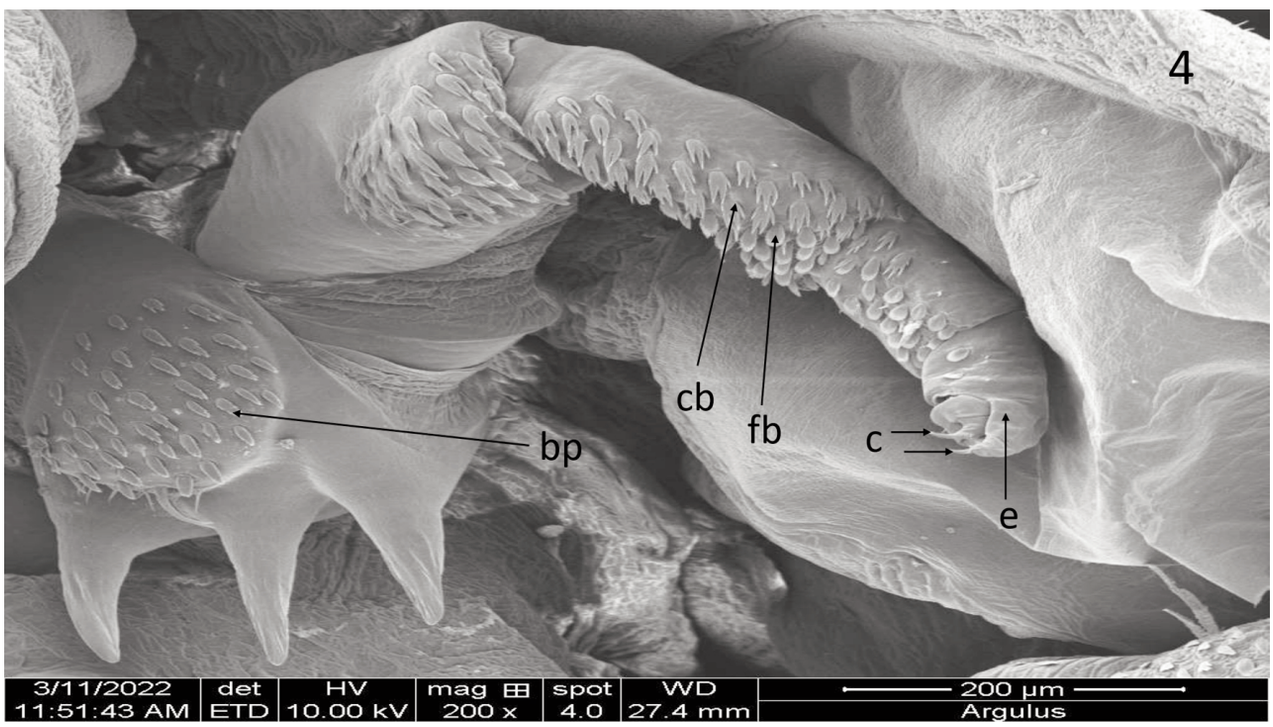

Figure 4. Maxillae (bp – basal plate, e – blunt fused extension where maxillae terminate, c – claws, cb – coarsely bristled scales, fb – finely bristled scales)

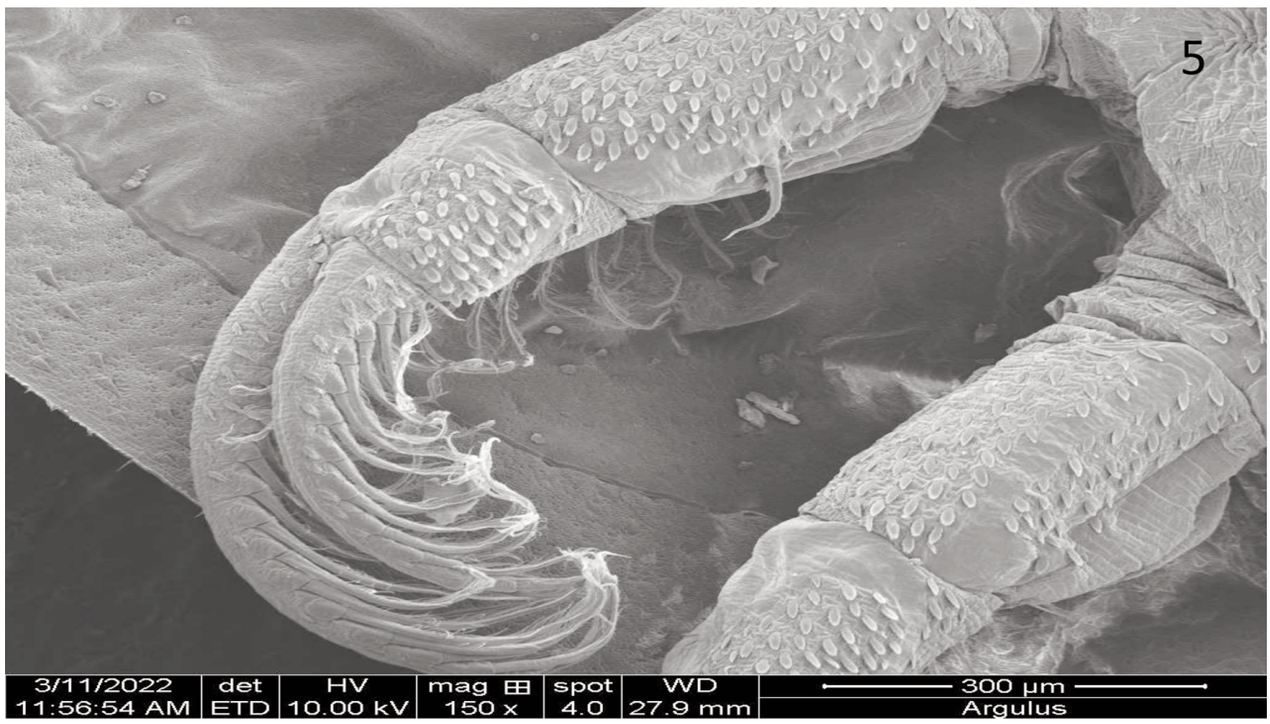

Figure 5. Swimming legs

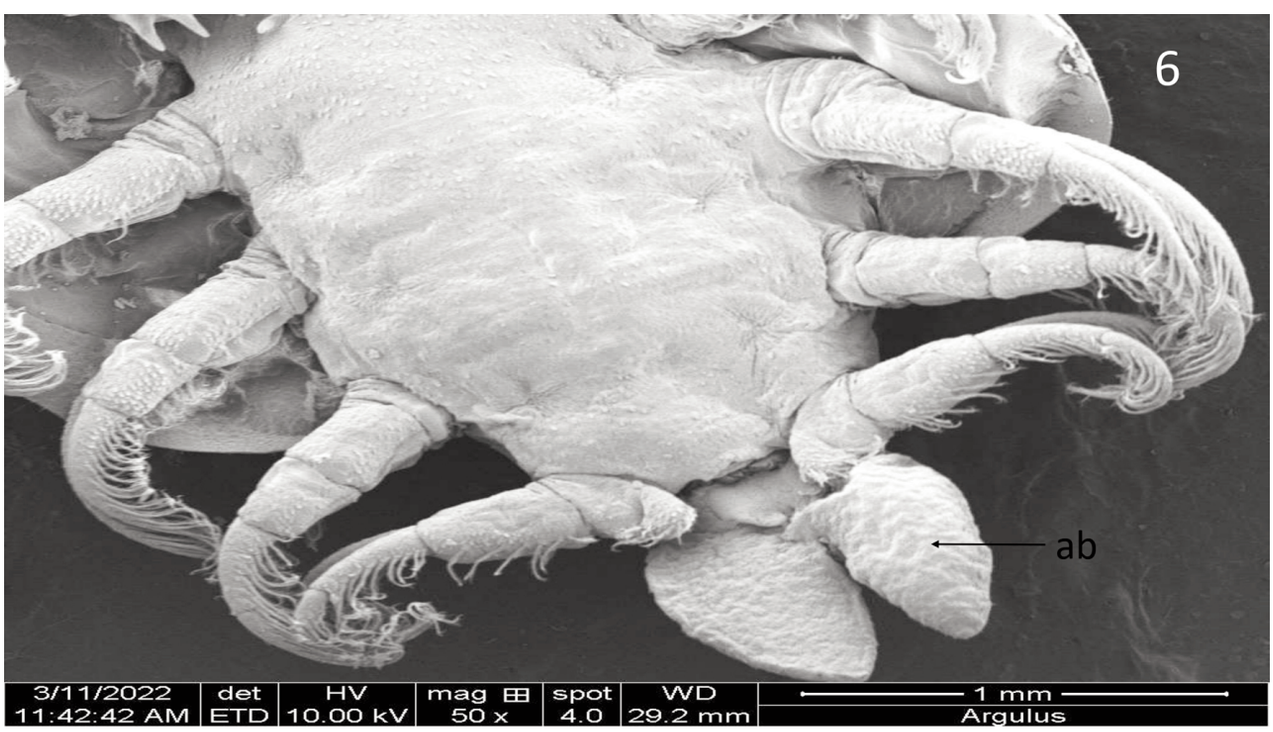

Figure 6. Abdomen (ab)

Argulus coregoni, Figs 1–8

Type host and locality: *Coregonus lavaretus* (Linnaeus, 1758); Jemtland, Sweden

Additional host and locality: *Carassius auratus* (Linnaeus, 1758); Lucknow (26°52′12″N; 80°55′20″E), Uttar Pradesh, India

Infection site: Skin and gills

Prevalence of infection: 20% (3 out of 15 fishes) were infected with the intensity of 1–2 parasites/infected host

The prevalence of infection is very low as infected fishes contain 1–2 *Argulus* specimens. All parasites were collected from the body and fins of the host and were not detected in the gills. *A. coregoni* was

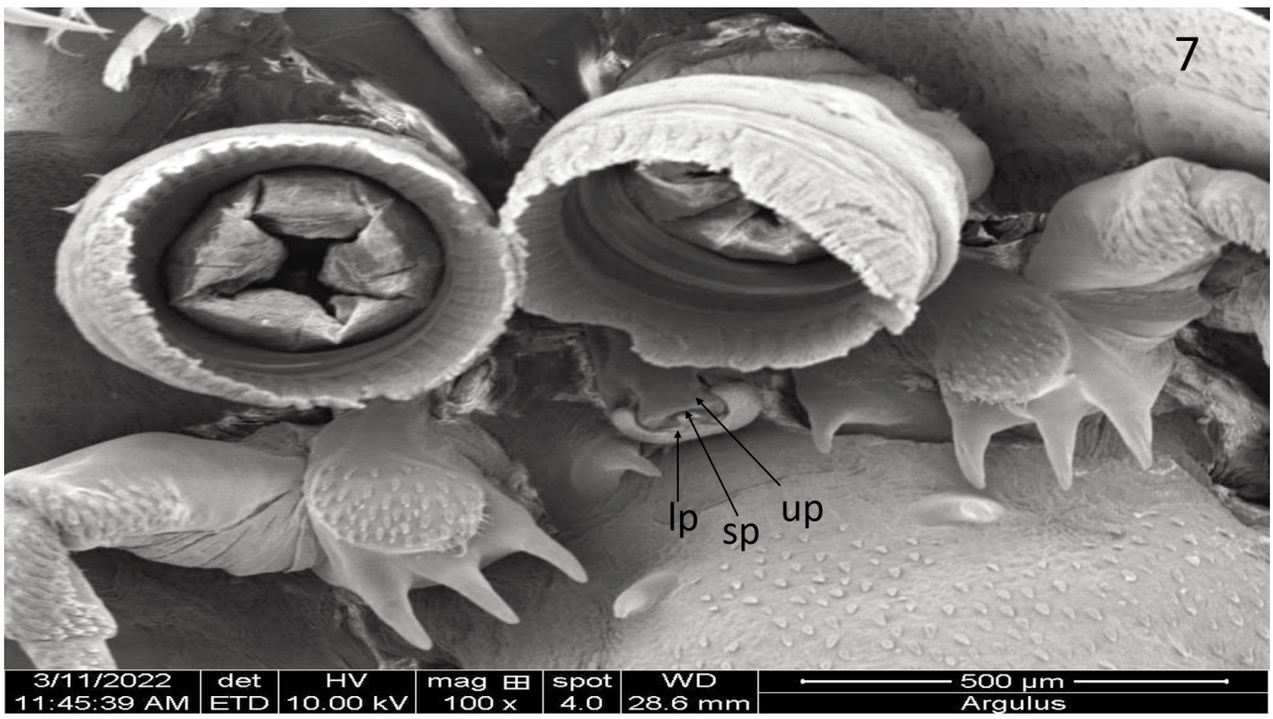

Figure 7. Mouth (up – upper lip, lp – lower lip, sp – sensory protrusions)

recognizable by its characteristic acuminate lobes. After examination of scanning electron microscopic images, a much clearer picture of the parasite is obtained. According to observation, the ventral edges of the carapace possess strong dentate scales, facing toward the posterior side. Antennule divided into two segments – proximal and basal, where the proximal part lacks ornamentation and the basal part bears a large posterior spine, the second bears an anterior spine, the third bears a postmedian spine, and the last podomere terminates in a hook. These spines vary in size. The antennae bear a sharp and short spine on the basal podomere, and the rest of the podomeres bear setae.

The proboscis terminates into the mouth which is bordered by an upper lip and a crescent-shaped lower lip. First maxillae were modified into sucking discs; the structure is supported by overlapping small sclerite rods. The second maxilla consists of five segments and the basal podomere bears a basal segment armed with a plate of scale having three sharp, finger-like projections. The basal plate has triangular scales, third and fourth segments at the anterior part have scales. The terminal part of the maxilla had two-sharp claws. Four pairs of swimming legs have biramous terminal ends, nearly of the same size except for the fourth pair which is relatively shorter. These legs are made up of endo and an exopodite and had scales on the ventral side.

The abdomen is bilobed containing acuminate lobes with no spines. Posterior incisures in the abdomen reach beyond the middle in the case of *A. coregoni*. The observed specimen in our study is a female *Argulus* as there is no accessory piece present on the second, third, and fourth swimming legs, which is one of the characteristic and distinguishing features of female.

Discussion

Chanda et al. [18] reported *Argulus* species from goldfish, Kaur et al. [19] from Koi carp, Saha and Bandyopadhyay [20] reported three species of *Argulus* namely *A. japonicus* Thiele, 1899; *A. foliaceus* Linnaeus, 1758 and *A. coregoni* Thorell, 1866 in red cap oranda from India.

These species can be distinguished by their physical appearance as *A. coregoni* were comparatively larger in size and have the characteristic acuminate lobes [21] without marginal spines and incisures of the abdomen reach beyond the middle, while *A. japonicus* and *A. foliaceus* have round lobed abdomen with marginal spines. Although, *A. japonicus* is a little more pointed than *A. foliaceus* but still less than *A. coregoni* [20]. Posterior lobes of the carapace in *A. coregoni* do not extend beyond the beginning of the abdomen similar to *A. foliaceus*, but in the case of *A. japonicus* posterior lobes of the carapace reach beyond the middle of the abdomen.

Parasitic infection in fishes can cause high

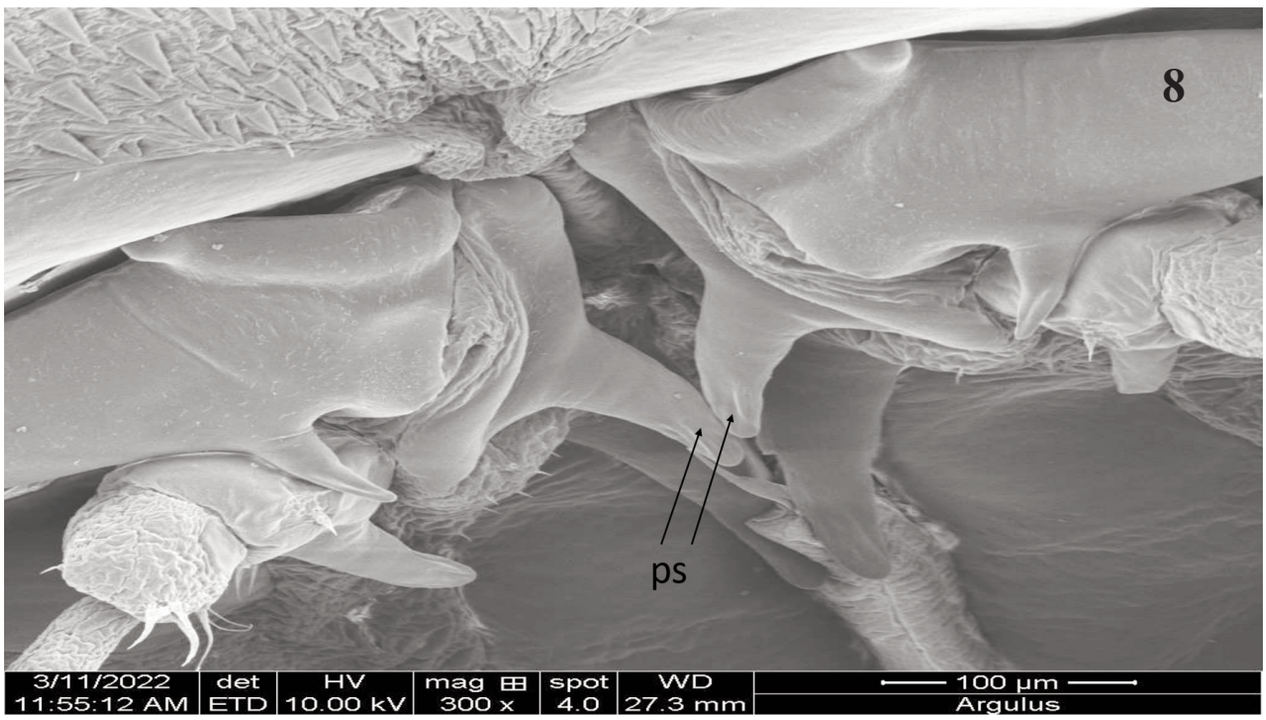

Figure 8. Focussed image of posterior spine (ps)

commercial losses in both the aquaculture [22] and fisheries industries [23]. The body of *Argulus* is divided into 3 regions namely the head, thorax, and abdomen. The head is somewhat fused with the thorax and body is covered by a flat, horseshoe-shaped carapace. Parasite possesses structures like maxillipeds, preoral sting, and basal glands. Thorax is four segmented and each bearing one pair of swimming legs. Due to their size, older stages of *Argulus* can be diagnosed with the naked eye and are visible moving on the host or swimming in the water. In addition to living on the host, *Argulus* sp. spends a significant amount of time swimming freely. This study concluded that infection of *Argulus* decreases at high temperatures. After infecting the host, *Argulus* releases some toxic enzymes in the body of the host resulting in irritation and inflammatory lesions. Such infections affect the aquaculture industry and may cause economic loss and mortality. It reduces fish performance such as loss of scales and color change in scales and many other aspects were observed that ultimately affect the market value of these fishes. *Argulus* possess a direct life cycle and are dioecious i.e., both male and female forms are distinct. They generally attach to the host and develop on it. Female *Argulus* possesses spermathecae where sperms get stored, and the male has large testes.

After *Argulus* infection is detected in the host, management and treatment are recommended due to the rapid escalation of infection. To avoid infestation, screening and quarantine of imported fish are very important. Insecticides have been used for a long time period to control the infection, such as emamectin benzoate, potassium permanganate, formalin [24], and organophosphate [3]. Although, insecticides are highly toxic for fish as well as for humans. Usually, antiparasitic agents are non-biodegradable [25] and affects the environment also. For example, malachite green which is used in aquaculture is banned in many countries because of its highly toxic effect.

Infection of *Argulus* is contagious and spreads rapidly from one fish to another. Importers should take note of the risks associated with infection and the potential diseases it transmits. These ectoparasites can survive without fish for hours and can infect other fish species also as these are not strictly host-specific. Stress conditions including mishandling, overcrowding of the fish population, poor quarantine conditions, poor sanitation, and poor bio-security can accelerate the disease out breaks. Proper management of *Carassius auratus* (goldfish) is necessary, in order to minimize the economic losses.

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