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Special Description of *Pallisentis (Brevitritospinus) allahabadii* Agarwal 1958 (Acanthocephala: Quadrigyridae) from *Channa punctata* (Bloch, 1793) by Scanning Electron Microscope

Channa punctata'dan (Bloch, 1793) Pallisentis (Brevitritospinus) allahabadii Agarwal 1958 (Acanthocephala: Quadrigyridae)'nin Taramalı Elektron Mikroskobu ile Özel Deskripsiyonu

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ABSTRACT

Objective: The aim of this study was to investigate the morphological features of acanthocephalans isolated from *Channa punctata* (Bloch, 1793).
Methods: Fishes collected alive from fish farms in Nadia District of West Bengal (India) were dissected for collection of acanthocephalans. Parasites isolated from intestine were fixed in formalin-aceto-alcohol and finally preserved in 70% ethanol for light microscopic studies and in 2.5% glutaraldehyde for scanning electron microscopy.
Results: The morphological characteristics of adult acanthocephalans were found to be similar to the original descriptions describing the basic characters of proboscis hooks, the spines of the collar and trunk region, and cement gland nuclei. New taxonomic characters were identified for the first time by scanning electron microscopy.
Conclusion: The acanthocephalan *Pallisentis (Brevitritospinus) allahabadii* Agarwal 1958 was described as an important parasite infecting fishes. The prevalence of the parasite was 23% and its adult stage was observed.
Keywords: Acanthocephalan, *Pallisentis (Brevitritospinus) allahabadii*, intestine, *Channa punctata*

ÖZ

Amaç: Bu çalışmanın amacı *Channa punctata*'dan (Bloch, 1793) izole edilen akantosefallerin morfolojik özelliklerini araştırmaktır.
Yöntemler: Balıklar Batı Bengal'in (Hindistan) Nadia Bölgesi'ndeki balık çiftliklerinden canlı olarak toplanmıştır ve akontosefallerin elde edilmesi için disekke edilmiştir. Bağırsaktan izole edilen parazitler formalin-aseto-alkol'de tespit edilmiş ve son olarak ışık mikroskobu çalışmaları için %70 etil alkolde, taramalı elektron mikroskobu (SEM) çalışmaları için %2,5 gluteraldehitte saklanmıştır.
Bulgular: Elde edilen ergin akantosefallerin morfolojik özellikleri; hortum kancaları, yaka ve gövde bölgesinin dikenleri ve sement bezi nukleuslarının temel karakterlerini tarif eden orijinal deskripsiyonlara benzer bulunmuştur. Yeni taksonomik karakterler SEM ile ilk kez teşhis edilmiştir.
Sonuç: Akantosefal *Pallisentis (Brevitritospinus) allahabadii* Agarwal 1958 balıkları enfekte eden önemli bir parazit olarak tarif edilmiştir. Parazitin yaygınlığı %23'tür ve ergin safhası bulunmuştur.
Anahtar Kelimeler: Akantosefal, *Pallisentis (Brevitritospinus) allahabadii*, bağırsak, *Channa punctata*

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Acanthocephalans are 'thorny' or 'spiny headed' worms with aquatic life cycles with fish as final or paratenic hosts and crustaceans as intermediate hosts (1). They are characterized by the presence of non segmented body, protrusible proboscis armed with hooks, body marked with transverse ridges (2). These adult parasites are found in the gastrointestinal tract in fishes acting as definitive hosts.

Van Cleave created the genus *Pallisentis* with *P. umbellatus* as its type species from a fresh water fish China (3). *Pallisentis* sp. belongs to Family Quadrigyridae which consists of two subfamilies, Quadrigyrinae (4) and Pallisentinae (4) and has been described frequently from fishes of family Channidae (5-8). Till recent times, 33 valid species of genus *Pallisentis* are known (9-11).

Although various scientists have worked on the taxonomy of acanthocephalans from freshwater and marine fish of India not much work has been done understanding their morphology and biodiversity from fresh water fish *Channa punctata* (Bloch, 1793) from West Bengal which would provide a database of parasites infecting fishes and extend the known geographic distribution of this species. This would help to outline requisite control measures for combating the spread of the parasite. This paper hence deals with a detailed description of *Pallisentis (Brevitritospinus) allahabadii* recovered from the intestine of *Channa punctata* by both light and scanning electron microscopy. New features which were not described earlier are hereby described in this study.

MATERIALS AND METHOD

Collection of Host Fishes

Live host specimens *Channa punctata* (30-35 g) were collected from fish farms of Nadia district (23.4710 °N, 88.5565 °E) in West Bengal and were brought alive in parasitology laboratory, About 100 fishes were collected and then collection site, date of collection and number of host sampled were properly recorded. Specimens were identified, measured and were sampled for collection of parasites (12). As per CPCSEA instruction's protocol for experimentation on fishes, does not require approval from ethical committee.

Collection and Mounting of Parasites

Fishes were dissected and visceral organs were placed in petri dish containing normal saline (0.6% NaCl) to allow adhering parasites to be released. Acanthocephalans collected were thoroughly washed fixed in AFA fixative [alcohol (50%): formalin: acetic acid (100:6:2.5)] and after 24 hours preserved in glycerified 70% alcohol. Relative parameters were measured and identification was performed using selected identification keys (13,14).

Sample preparation for Scanning Electron Microscopic Study

The helminth parasites from infected fishes were collected and fixed in 2.5% glutaraldehyde solution prepared in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C. The samples were then postfixed in 1% osmium tetraoxide dehydrated through series of alcoholic grades followed by wash with absolute ethyl alcohol and amyl acetate mixture in 3:1, 2:2 and 1:3 ratios respectively and finally in 100% amyl acetate. Specimens were then mounted on stubs with double adhesive tape and coated with gold using a coater (Quorum, Q 150 TES). Coated samples were examined with a high-resolution scanning electron microscope (SEM) (Zeiss EVO-MA 10, Germany) operating at accelerating voltages of 15KV (15).

Illustrations and Measurements

Light microscopic photographs of parasite were taken by camera-mounted microscope (Olympus CX 41). All measurements are given in millimeters if not stated otherwise. Paratypes of the parasite were deposited in the parasitology laboratory, Department of Zoology, Krishnagar Govt College, West Bengal, India.

Statistical Analysis

Statistical analysis was conducted using SPSS software. The measurements and parameters are expressed in range, mean and standard deviation from the mean.

RESULTS

During the present study members of genera, viz. *Pallisentis* have been identified from fish. The system of classification adopted is based on that of Monks 2001 (16).

Phylum: Acanthocephala

Class: Eoacanthocephala Van Cleave, 1948

Order: Gyracanthocephala Van Cleave, 1936

Family: Quadrigyridae Van Cleave, 1920

Subfamily: Pallisentinae Amin, 1985

Genus: *Pallisentis* Van Cleave, 1928

Identifying Characters

Light Microscopy Observations

Body long, cylindrical and curved. Females longer than males. Proboscis short, cylindrical to globular with four circles of ten hooks each. Proboscis hooks slender and abrupt transition in size of hooks occurs from 2nd to 3rd row occurs (Figure 1). Each hook consists of a recurved blade, a horizontally directed root and handle sunk in the proboscis wall. Hooks of the first and second circle were large and stouter while basal hooks were small and curved (Figure 1). Proboscis separated from collar by as short unarmed neck (Figures 1 and 2). Ganglion near base of proboscis receptacle. Trunk spines are more widely spaced than collar spines gradually becoming smaller towards the posterior end (Figure 2). Lemnisci long, slender and cylindrical (Figures 2 and 3). Proboscis receptacle cylindrical to saccate longer than the proboscis with single layered muscular walls, reaching to second spinose region when the proboscis is introverted. Posterior to collar spines presence of an unspined region which is followed by 20-22 widely spaced rings of cuticular spines remaining part devoid of spines (Figure 3). Collar spines present just below the unspined neck region are curved and form an armature at the anterior position of the trunk (Figures 3 and 4). Collar spines arranged in 16 closely set rings with closely set collar spines near anterior extremity hence it is wider in the anterior end and narrows posteriorly (Figure 4). Cuticular spines were followed by trunk spines, separated by a small unspined region are not closely apposed like those of collar spines. Trunk spines triangular, larger, deeply embedded in the body wall are arranged in 17-20 circles, each

with 5-19 spines anteriorly. Female trunk with blunt posterior end. Uterus long and coiled, eggs numerous and oval in shape (Figure 5). Testes oval to cylindrical, contiguous, anterior testes larger than posterior. Cement gland long, cylindrical, syncytial containing a number of nuclei. Saefftigen's pouch present near cement gland. Bursa muscular and oval shaped (Figure 6).

Scanning Electron Microscopy Observations

The proboscis star shaped with a flat base with elevated apical structures in the middle but anterior tip unarmed. All circles of hooks on proboscis are equidistant and alternate (Figure 7). Proboscis hooks curved, slender, rooted and arises from the elevated rims on proboscis surface (Figures 7 and 8). Hooks of all the rows have thorns longer than roots which are deeply buried in the proboscis wall, wider posteriorly before narrowing towards the tip and does not bear any microsculptures, striations, grooves or protuberances (Figure 8). Proboscis bears sensory pits which leads to an unarmed neck. Proximal part of the spines form an armature while distal free end bears hooks (Figure 9). The transition from 2nd to 3rd row was abrupt, 3rd and 4th row hooks are almost half the size of the 1st and 2nd rows. Hooks of the first row was least curved while from second row the hooks were more curved and oriented towards to the posterior (Figures 9 and 10). Collar bears closely set cone shaped spines which tapers to a narrow tip and were anteriorly directed (Figure 11). Cuticular spines were arranged in parallel rows just below the collar spines which were followed by a short unspined region. The spines were conical in shape shorter than both proboscis hooks and collar spines and were widely spaced than collar spines. Trunk spines

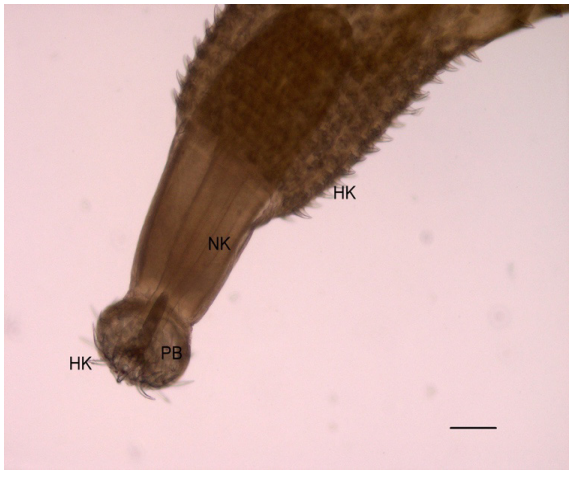

Figure 1. Light microscopic structures showing proboscis with hooks (HKS) and neck (NK). Scale bar- 100 µm, proboscis (PB)

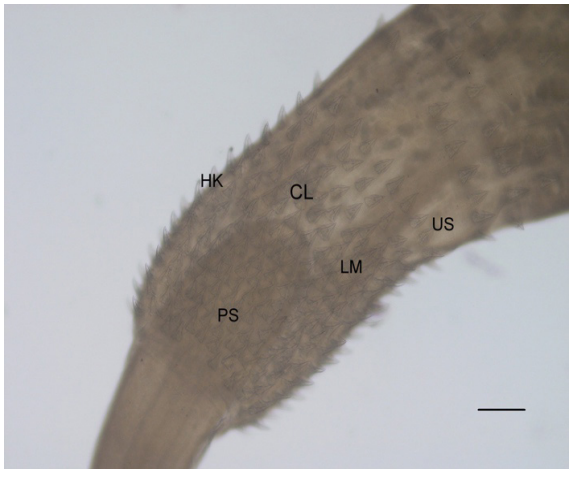

Figure 2. Light microscopic structures showing proboscis (PB), neck (NK), collar (CL), lemnisci (LM), proboscis sac (PS), trunk (TK) and trunk spines (TS) region. Scale bar- 167 µm

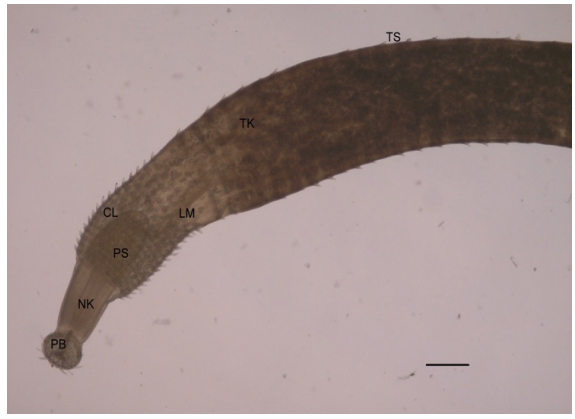

Figure 3. Light microscopic structures showing proboscis sac (PS) with lemnisci (LM), collar (CL) region with hooks (HK) and unspined region (US). Scale bar- 100 µm

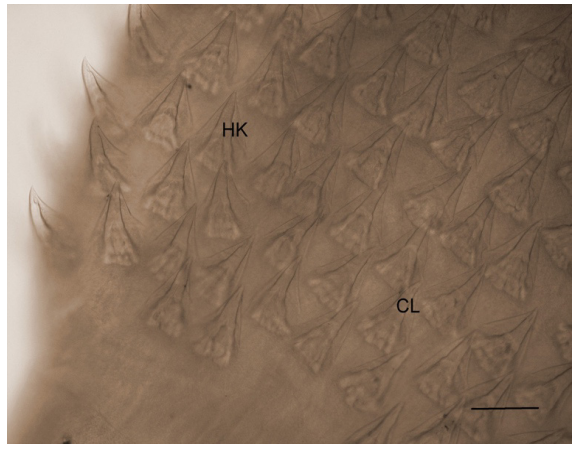

Figure 4. Light microscopic structures showing collar region (CL) with hooks (HK). Scale bar- 100 µm

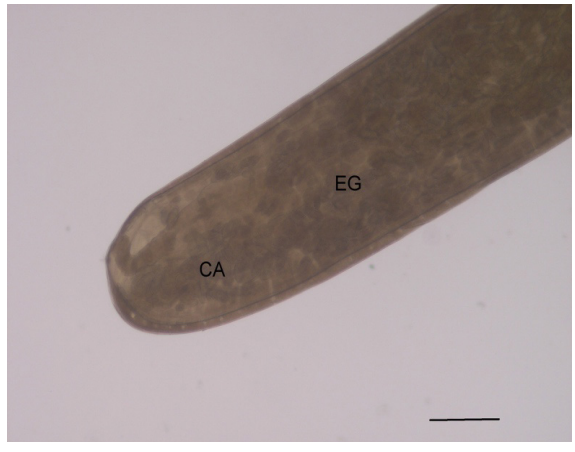

Figure 5. Light microscopic structures showing caudal (CA) region of female filled with eggs (EG).Scale bar- 500 µm

triangular shaped directed anteriorly and arranged transversely, anterior trunk spines larger in size than posterior ones. Toward the posterior region of the trunk the spines are more distantly spaced and fewer in number (Figure 12).

Description

Male: Worm medium size, cylindrical, spinose. Total body length 4.1-4.47 (4.38±0.65) in length and 0.260-0.400 (0.34±0.71) in width. Collar measuring 0.4392-0.536 (0.48±1.78) long 0.127-0.273 (0.23±3.4) wide at the base with 16 complete circles of collar

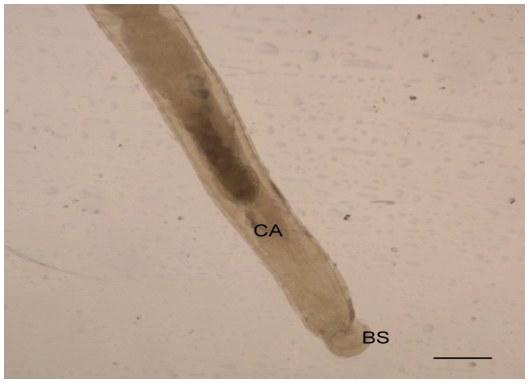

Figure 6. Light microscopic structures showing caudal (CA) region of male with oval shaped bursa (BS).Scale bar- 200 µm

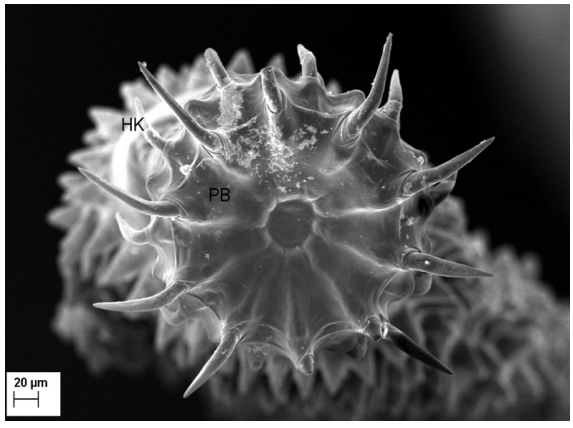

Figure 7. Scanning electron microscopic structures showing dorsal view of proboscis region (PB) with hooks (HK)

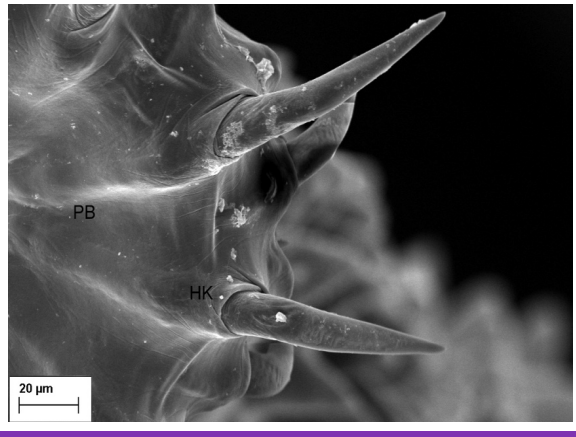

Figure 8. Scanning electron microscopic structures showing proboscis region (PB) with hooks (HK)

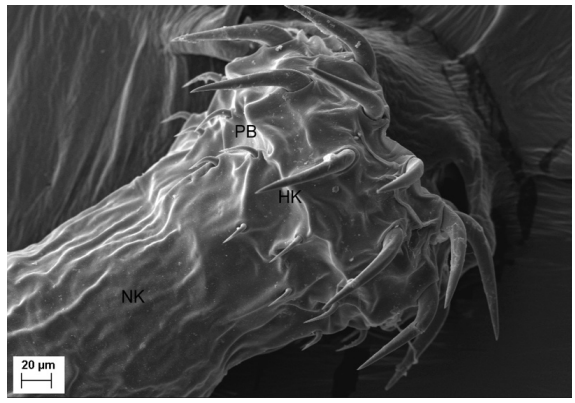

Figure 9. Scanning electron microscopic structures showing proboscis (PB) with hooks (HK), and neck (NK) region



Figure 10. Scanning electron microscopic structures showing lateral view of proboscis (PB) with hooks (HK), neck (NK) and collar region (CL)

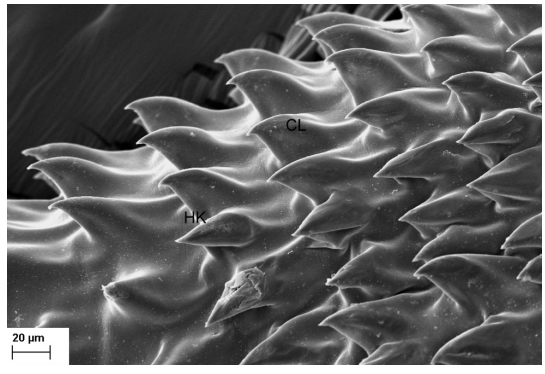

Figure 11. Scanning electron microscopic structures showing collar region (CL) with closely set conelike hooks (HK)

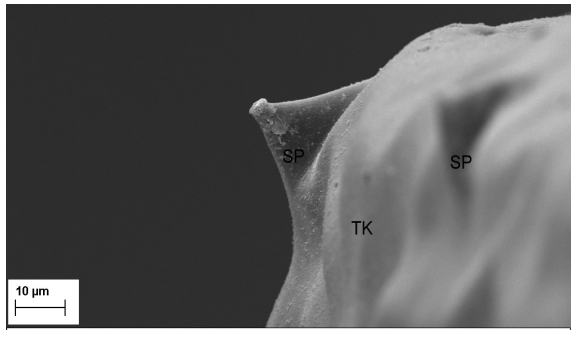

Figure 12. Scanning electron microscopic structures showing trunk with triangular shaped trunk spine (SP), trunk (TK)

spines, each with 14-16 spines. Proboscis globular in shape,widest anteriorly, and narrowing posteriorly into a long, prominent neck. Proboscis with conspicuous and rooted hooks in 4 circles of 10 hooks each (Figure 1). Proboscis 0.140-0.157 (0.149±2.81) long, 0.145-0.167 (0.158±17.74) wide anteriorly. Length of proboscis hooks from anterior to posterior, 1st circle 0.076-0.084 (0.08±3.45), 2nd circle 0.052-0.067 (0.059±2.75), 3rd circle 0.024-0.027 (0.0255±0.17), 4th circle 0.0195-0.024 (0.020±4.45). Neck long, robust, almost 1/3rd as long as a receptacle, 0.097-0.103 (0.10±4.52) long, 0.111-0.130 (0.12±1.60) in width posteriorly. Proboscis receptacles 0.292-0.439 (0.40±3.11) long by 0.156-0.195 (0.18±1.21) wide. Lemnisci cylindroid, longer than the receptacle and unequal sized, lemnisci (left) 1.1122-1.695 (1.365±23.07) long, 0.195-0.207 (0.201±0.75) wide posteriorly. Lemnisci right 1.56-1.756 (1.69±1.27) long, 0.025-0.029 (0.027±14.07) wide. Trunk spines in 17-18 circles, each with 5-12 spines. Reproductive system in posterior, slightly 2/3rd of the trunk. Anterior testes measures 0.410-0.590 (0.520±11.07) in length and 0.17-0.225 (0.19±5.04) in width while posterior testis measures 0.342-0.605 (0.51±9.27) in length and 0.118-0.224 (0.182±1.07) in width thus anterior testes being slightly larger in size than posterior one. Vas deferens arises from each testis moves downward in close association with cement gland, cement reservoir and finally joins bursa. Cement gland elongated, cylindrical, syncytial, and in contact with posterior testis. Cement reservoir measures 0.273-0.312 mm in length and 0.185 mm in width. Saefftigen's pouch oval, sac like placed in proximity with cement gland open by a narrow tubular duct into the bursa, where duct of cement reservoir ends. Bursa muscular, oval shaped and eversible.

Female: Females larger than males. Total body length 8.0-8.5 (8.14±1.98) long and 0.510-0.585 (0.54±0.36) wide. Collar measuring 0.601-0.639 (0.614±2.27) long, 0.303-0.371 (0.33±14.14) wide at the base, collar spines in 16 complete circles each with 16-18 spines. Proboscis 0.201-0.230 (0.214±1.98) long by 0.180-0.210 (0.195±0.34) wide anteriorly. Neck 0.255-0.270 (0.2614±2.17) long, 0.150-0.210 (0.181±12.42) wide posteriorly. Proboscis receptacle 0.380-0.410 long (0.39±2.27), 0.260-0.290 (0.614±2.61) wide. Lemnisci 0.970-1.0 long by 25-32 wide posteriorly. Trunk spines in 17-20 circles, each with 5-14 spines. Reproductive system 0.700-1.52 long with cylindrical shaped uterine bell 0.320-0.370 long and 0.87-0.110 wide. The uterus was 0.360-0.400 long and 0.080-0.100 thick. Vagina 0.085 long, 0.040 wide. Body cavity filled with numerous eggs. Eggs were elliptical in shape, 0.034-0.065 long, 0.020-0.025 wide.

Taxonomic Affinity:

Type Host: *Channa punctata* (Bloch, 1793)

Site of Infection: Intestine

Type Locality: Nadia (23.4710 °N, 88.5565 °E), West Bengal

Number of Specimens: Thirty one acanthocephalan from 23 fishes, 100 hosts were examined

Prevalence and Intensity: 23/100 (23 %), 1.34 (1-2 specimens per fish)

Specimens Deposited: Paratypes bearing numbers PR/IK/A-03 and PR/IK/A-11 were deposited in Parasitology laboratory, Department of Zoology, Krishnagar Govt College, West Bengal, India

DISCUSSION

P. (*B.*) *allahabadii* was first described by Agarwal 1958 (6) from *Channa punctata* in Allahabad, India but the description was without any illustration. The original description lacked details of the measurement and transition of the proboscis hooks moreover there was no mention of Saefftigen's pouch. Collar spines with 15-18 circles with 20-25 each in females and 8-12 each in males, length 0.028-0.057 mm were described. Later Jain and Gupta 1979 (7) have redescribed the species and the detailed measurements, transition of proboscis hooks of *P*. *allahabadii* clearly demarcate it's from *P. ophiocephali, P. nagpurensis, P. nandai*. Jain and Gupta (7) have mentioned collar spines 15-16 circles of 12-16 each in both sexes but there is no mention of its dimensions. Moreover Agarwal (6) had mentioned trunk spines in males 21-25 circles of 1-12 each measuring 0.038 mm, in females 32-36 circles of 1-18 each but Jain and Gupta (7) have reported 20-26 circles of 2-14 each in both sexes. Soota and Bhattacharya (11) had proposed *P*. *allahabadii* to be junior synonym of *P. colisai* but Amin et al. (18) have made *P.* (*B.*) *allahabadii* the type species of the subgenus *P*. (*Brevitritospinus*). The morphometrics of the parasite obtained corresponds to the data provided by Jain and Gupta (7). Gupta et al. (17) have recently described two new species *P. channai* and *P. vinodai* from the intestine of *Channa punctata* (Bloch, 1793) from the River Gomti at Lucknow based on light microscopic studies. *P. channai* n. sp. described on the basis of only male specimens exhibits similarity with *P. (B.) allahabadii* in having proboscis armed with 4 circles of hooks, each with 10 hooks, collar spines arranged in 16 closely set rings, each with 16 hooks and trunk spines arranged in twentyone circlets, each with 14-18 spines although there are marked dissimilarities like differences in the dimension of proboscis hooks. Lemniscus, collar and trunk spines *P. vinodai* n. sp shows marked dissimilarity from *P*. *allahabadii* as proboscis bears four circle each with eight hooks, collar spines arranged in fourteen closely set rings and having trunk spines arranged in twentyseven circlets, each with 16-18 spines.

The present investigation emphasizes on scanning electron microscopy study which is being reported for the first time and in place of which provides detailed description of the specimen which was not provided earlier. SEM studies reveal various detailed morphometric structures which were never explored and described like proboscis and hooks shape, arrangement of proboscis hooks and trunk spines. Dimensions of the neck, collar spines and trunk spines are studied which were not reported earlier by Jain and Gupta (7). The dimensions of the proboscis, 1st and 2nd proboscis hook length in the present study was found to be higher then that observed by Jain and Gupta (7). Number of trunk spine also differed significantly. In *Pallisentis* (*Neosentis*) *celatus* Van Cleave (3) proboscis armed with posterior hooks were shortest, 28-36 mm, Amin and Hendrix (19). The hooks were arranged at alternative levels and not in a circle as described by Agarwal (6) and Jain and Gupta (7). Similar observations were also reported by Gupta et al. (17) while describing *Pallisentis* (*Brevitritospinus*) *punctati*. Absence of microsculptures, striations, grooves or protuberances in the proboscis hooks was observed in the present sample although Gupta et al. (17) reported presence of protuberances in hooks of *Pallisentis* (*Brevitritospinus*) *punctati* at a distance of 4.35+0.25 μm.

CONCLUSION

The description of *Pallisentis* (*B.*) ***allahabadii*** is greatly enhanced by the new information provided for the first time with SEM images which would enhance the database of parasites infecting *C. punctata* and thus help in formulating measures which would implemented in controlling the spread of the parasite.

* Ethics

Ethics Committee Approval: As per CPCSEA instruction's protocol for experimentation on fishes, does not require approval from ethical committee.

Informed Consent: There are no human patients in the study.

Peer-review: Externally and internally peer-reviewed.

* Authorship Contributions

Concept: I.K., Design: G.G., I.K., Data Collection or Processing: I.K., Analysis or Interpretation: I.K., Literature Search: I.K., Writing: I.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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