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Original Article

Description of *Pallisentis* (*Brevitritospinus*) *punctati* n. sp. (Acanthocephala: Quadrigyridae) from *Channa punctatus* in Bareilly, Uttar Pradesh, India

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Abstract

Background: The genus *Pallisentis* is an endoparasitic acanthocephalan inhabiting the intestinal walls. Hooks and spines of the worm are significant taxonomical and adaptive tools.

Methods: The parasites were fixed, dehydrated and examined under Olympus BX 53 Microscope with DIC attachment, digital camera and CELLSENS imaging system [Light microscopy (LM)] and fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, dehydrated, rotary-coated with gold palladium in NeoCoater 100-240V and examined in Neo JCM-6000 [scanning electron microscopy (SEM)].

Results: *P. punctati* n. sp. (prevalence 65.51%; mean intensity 3-6 par/host) is described. Females almost twice as large as males; proboscis hooks small; collar spine rows similar [16] and constant in both sexes but number of spines per row greater in females [22] than males [14]; trunk spine rows 28-39 (spines per row 14-18) in females and 20-26 (spines per row 10-12) in males. spine length of females almost twice as long as males, spines extend up to posterior testis in males and ¾ of total body length in females, Saefftigen's pouch present, nuclei in cement gland 10-11, seminal vesicle, bursa and egg size small. SEM indicated lack of micro sculptures, and spines embedded on pre-trunk and trunk. Sex-based differences apparent (hook sizes, greater number of spines per row and longer spines in pre-trunk and trunk of females). Male trunk spine was narrower and of lateral spine with characteristic hooked appearance.

Conclusion: A new species of *Pallisentis* based on LM and SEM is described, sexual diversity in hook and spine structure is reported.

Introduction

The Acanthocephalans form a compact group of endoparasites whose taxonomy has been debated for long. These parasites cause damage to host tissues due to highly developed proboscis hooks and body spination. The occurrence of *Pallisentis* Van Cleave, 1928, is common in fishes in many parts of the world. The present collection of *Pallisentis* from the intestine of *Channa punctatus* allowed the observation of the parasite under light and scanning electron microscopy.

The classification of *Pallisentis* has aroused interest amongst acanthocephalan taxonomists (1-9). A checklist of acanthocephalan fish parasites of India and Pakistan was provided (5). The acanthocephalan parasites of India were compiled (10) reporting 20 species of *Pallisentis*. However, proboscis hook size was used as valid criteria for classifying *Pallisentis* at the sub generic level (11). A total facelift was given to taxonomical approach incorporating new concepts in molecular taxonomy, gene sequencing and phylogenetic studies and currently, 32 species of *Pallisentis* under 3 subgenera [*Brevitritospinus* (10 species), *Demidueterospinus* (3 species) and *Pallisentis* (19 species)] are included (12). All species of *Brevitritospinus* except *P. (B) vietnamensis* are reported from India. *P. (B) fotedari* and *P. (B) mehrai* parasitize marine fishes, remaining *Brevitritospinus* species have been recorded from freshwater fishes.

Morphological details of the parasite examined under light microscopy have most often been used for studying parasites and are significant for morphological studies being extremely important in context to their taxonomic classification. SEM represents an important tool for studying the detailed surface topography of the parasite and its ability to provide three-dimensional images with high magnification allows a better understanding of the spatial relationships among surface structures. This technique is therefore acquiring importance for validating species and demonstrating differences between populations or races (13, 14).

The shape, number, and distribution of hooks on the proboscis are important characters used in taxonomy and classification (15). Poulin conducted quantitative analysis of interspecific patterns of investment in attachment structures in a taxonomic group of parasites for the first time (16).

The present study was undertaken to place the acanthocephalan parasite discovered from *Channa punctatus* in its proper taxonomic position based on LM and SEM and to examine further acanthocephalan attachment structures under SEM to give a proper insight to their structural and organizational pattern.

Materials and Methods

Host collection

Live specimens of the host fishes (n= 250) measuring 12-16 cm in total length were examined for acanthocephalans. The fish were procured from Nakatia and Deorania tributaries, offshoots of river Ramganga flowing 8 km from West to the South-East, separating Tehsil Aonla from the rest of the district or purchased from the local fish markets of Bareilly, Uttar Pradesh, India (28.35º N; 79.42º E). The fishes were transported to the Centre of Excellence laboratory of M.J.P. Rohilkhand University in large containers and maintained in aquaria.

Parasite study

Collection

Channa were desensitized, their whole intestines were incised carefully taking care not to damage any parasite and the contents teased carefully in 1% NaCl solution with a brush to expose the parasites under an Olympus stereozoom microscope.

Light microscopy

Pallisentis recovered thus were placed in distilled water for 24 h at 4°C for the proboscis to evert, fixed in 70% ethanol, transferred to fresh glycerin alcohol and placed in desicca-

tors for dehydration. Dehydrated worms were mounted on slides in anhydrous glycerine for routine examinations. Permanent slides were prepared by staining in carmine, dehydrated in ascending concentration of ethanol, cleared in xylene and mounted in Canada balsam. Parasites were digitally measured and photographed under Olympus BX 53 Microscope with DIC attachment, digital camera and CELLSENS imaging system. Prevalence of infection and mean intensity were calculated (17).

Scanning Electron Microscopy

The parasites were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for 2.5 h at 4 °C and then in 1% osmium tetroxide in the same buffer for 2 h. To remove debris from proboscis hooks, the specimens were soaked in 20% glycerol for 30 h (18), dehydrated through ethanol series and dried to critical point. Specimens were mounted on metal specimen stubs, rotary-coated with gold palladium in NeoCoater 100-240V and examined in Neo JCM-6000 at an acceleration voltage of 15 kV and micrographs taken.

Results

Pallisentis punctati n. sp.

Pallisentis Van Cleave, 1928

Syns. *Devendrosentis* Sahay, Sinha and Ghosh, 1971
Farzandia Thapar, 1931
Neosentis Van Cleave, 1928
Saccosentis Tadros, 1966)

Diagnosis

Quadrigyridae, Pallisentinae

A classification dendogram of the species is provided in Fig. 1. The gastrointestinal tract of *C. punctatus* revealed the presence of an acanthocephalan parasite. It is assigned to the genus, *Pallisentis*. Light microscopy studies on the worm and SEM on attachment structures (hooks and spines) were performed.

General Description: Light Microscopy

(Figs. 2 & 3)

(All measurements average ± SE are given in Table 1)

(n= 20 ♂, 20 ♀)

Body tubular, elongated, cylindrical, spinose, vermiform consisting of proboscis, neck and trunk (Fig. 2A). Body is creamy white in color when alive. Length of ♂ approximately half of ♀. The worm has typical acanthocephalan character without digestive tract or mouth as apparent in *en face* preparation (Fig. 3A). Sexual dimorphism distinct. Maximum diameter in the region of the anterior rows of trunk spines. Fully protruded proboscis pyriform, armed with four circles of curved hooks (10 hooks in each circle) varying in size (Fig. 2B). Apical hooks are largest, basal are smallest, thorn-like (Fig. 2C). All circles of hooks on proboscis are equidistant and alternate (Fig. 3B). Each hook comprises of recurved blade and vertical root deeply buried in cuticle of presoma, ratio between blade and roots and angle between them gradually decrease from row I to row IV, those of the latter are thus most curved (Fig. 2C). Anterior knob-like portion of proboscis is followed by a small neck separated from proboscis by a transverse, muscular band, devoid of spines (Fig. 2D).

Proboscis sheath is thin, ovoid, single layered, elliptical muscular sac originates from base of proboscis and hangs down freely in the body cavity. Brain is composed of large, oval nerve ganglion, situated at posterior end of proboscis receptacle, nerve fibres are not visible. Lemnisci originate at base of proboscis, have long, slender, unequal arms extending up to tip and mid anterior testis in ♂ (Fig. 2A) and 5th and 8th row of trunk spines in ♀ (Fig. 2D). Retractor and protractor muscles are attached to sheath and with the body wall. Anterior girdle of collar immediately below neck has 16 circular rows each with short close-set collar spines (Fig. 3C), 14 in ♂ and 22 in ♀, followed by a short spineless area.

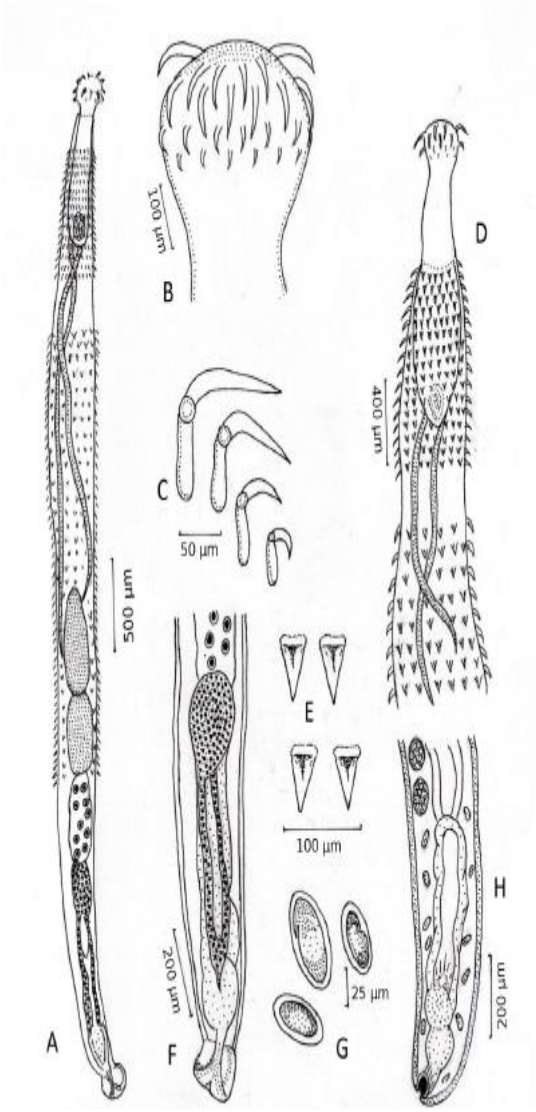

Fig. 2: Line diagrams of *Pallisentis* (*B.*) *punctati* n. sp.from *Channa punctatus* (Bloch).
A Holotype male. **B** Proboscis of allotype female. **C** Proboscis hooks (row I, II, III and IV). **D** Anterior region female showing collar and trunk. **E** Trunk spines. **F** Posterior region male showing cement gland (part), Saefftigen's pouch and bursa. **G** Eggs. **H** Posterior region female showing vagina, uterus, uterine bell and vulva. *Scale bar* = 500 µm in **A**, 100 µm in **B & E**, 50 µm in **C**, 400 µm in **D**, 200 µm in **F & H**, 25 µm in **G.**

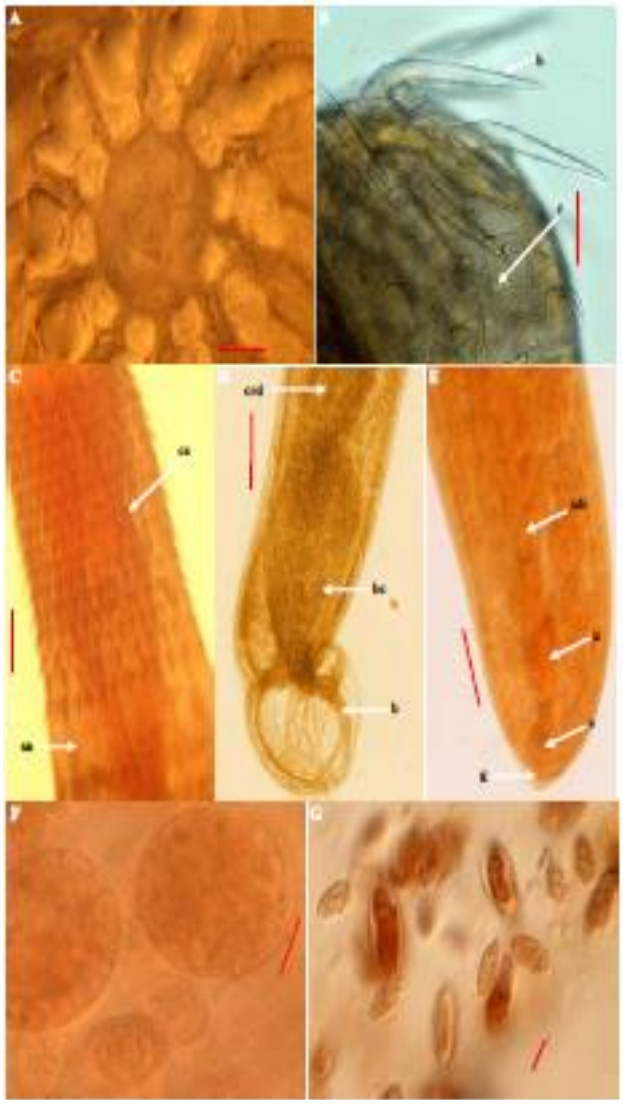

Fig. 3: Light microscopy of *Pallisentis* (*B.*) *punctati* n. sp.from *Channa punctatus* (Bloch).
A *en face* view. **B** Proboscis showing 4 rows of hooks (h) with roots (r). **C** Collar spines (cs) and spineless area (sa). **D** Posterior end of male showing cement reservoir duct (crd), bursal cap (bc) and bursa (b). **E** Posterior end of female showing gonopore (g), uterus (u), uterine bell (ub) and vagina (v). **F** Ovarian ball. **G** Discharged eggs. *Scale bar* = 15 µm in **A & F**, 50 µm in **B & E,** 100 µm in **C & D**, 20 µm in **G.**

Table 1: Measurements of *Pallisentis punctati* n. sp. from the intestine of *Channa punctatus* of Bareilly, India (All measurements are ± SE in mm)

Component part of parasite	Male	Female
Body (L x W)	4.777 ±0.226 X 0.377 ±0.013 (3.015-5.899X 0.307-0.461)	8.755±0.620 (5.472-14.791)X 0.625±0.024 (.461-0.820)
Proboscis (L x W)	0.108 ±0.001 (0.104-0.118)X0.109 ± 0.003 (0.090-0.120)	0.143±0.003 (0.126-0.180)X 0.164±0.003 (0.140-0.198)
Hook rows	4	4
No. of hooks/row	10	10
Hook 1 (L)	0.057 (0.057)	0.073±0.001 (0.068-0.090)
Hook 2 (L)	0.054 (0.054)	0.063±0.001 (0.061-0.078)
Hook 3 (L)	0.021 (0.021)	0.025 ±0.0007 (0.021-0.028)
Hook 4 (L)	0.018 (0.018)	0.018±0.0001 (0.014-0.021)
Neck (L x W)	0.216 ± 0.006 (0.190-0.255) X 0.112 ±0.001 (0.108-0.118)	0.419 ±0.019 (0.288-0.558)X 0.213±0.009 (0.162-0.273)
Proboscis sheath (L x W)	0.444 ±0.021(0.349-0.511) X 0.120 ±0.005 (0.090-0.129)	0.701 ±0.045 (0.284-0.939) X 0.167±0.008 (0.126-0.244)
Spineless area (L x W)	0.083 ±0.004 (0.054-0.100) X 0.262 ±0.012 (0.216-0.309)	0.142 ±0.006 (0.100-0.198)X 0.418±0.013 (0.338-0.522)
Collar (L x W)	0.360 ±0.004 (0.345-0.396)X0.237 ±0.009 (0.198-0.288)	0.507 ±0.017 (0.385-0.666)X 0.395±0.015 (0.298-0.504)
Spine rows	16	16
No. of spines/row	14	22
Spine (L x W)	0.024 (0.021-0.028)X 0.011 (0.010-0.014)	0.031 (0.025-0.046)X 0.017 (0.010-0.025)
Distance between adjacent spine	0.014 (0.010-0.019)	0.016 (0.010-0.021)
Trunk (L x W)	3.717 ±0.276 (2.376-5.335)X0.377 ±0.013 (0.307-0.461)	7.169 ±0.612 (4.018-13.098) X 0.625±0.024 (0.461-0.820)
Spine rows	20-26	28-39
No. of spines/row	12	14-18
Spine (L x W)	0.026 X 0.013 (0.021-0.028 X 0.010-0.018)	0.043(0.036-0.057)X0.021(0.014-0.025)
Distance between adjacent spines	0.036(0.25-0.72)	0.030(0.021-0.068)
Anterior Testis (LxW)	0.457±0.035 (0.374-0.684)X0.175±0.011 (0.133-0.216)	-
Posterior Testis (LxW)	0.393 ±0.041(0.370-0.648)X0.117±0.013 (0.140-0.241)	-
Distance of anterior testis from anterior end	1.949 (1.453-2.086)	-
Distance of posterior testis from anterior end	2.539 (1.898-2.907)	-
Cement gland (L x W)	0.523±0.088 (0.234-0.918)X0.171±0.018 (0.108-0.252)	-
Saefftigen's pouch (Lx W)	0.375 ±0.041(0.270-0.565)X0.152 ±0.012 (0.100-0.190)	-
Cement reservoir (L x W)	-	-
No. of nuclei in cement gland	10-11	-
Seminal vesicle (LxW)	0.378 ±0.030 (0.277-0.478) X 0.120 ±0.005 (0.108-0.140)	-
Bursa (L)	0.212 ±0.038 (0.102-0.345)	-
Pair of ducts from Saeffti-gen's pouch	0.383 (0.288-0.432)	-
Distance between body layer	0.017 (0.010-0.021)	0.020 (0.010-0.050)
Egg (L x W)	-	0.043±0.003(0.028-0.061)X0.018±0.001 (0.010-0.025)
Vagina (L x W)	-	0.053±0.004(0.036-0.079)X0.047±0.001 (0.039-0.054)
Uterus (L x W)	-	0.160(0.108-0.194)X0.074(0.054-0.104)
Uterine bell (L x W)	-	0.125±0.006(0.090-0.154)X0.081±0.007 (0.054-0.129)
Vulva (L x W)	-	0.048±0.003(0.025-0.072)X0.039±0.002 (0.025-0.054)
Ovarian balls (L x W)	-	0.052±0.001(0.039-0.064)X0.042±0.001 (0.025-0.054)

Trunk spines are conical, larger, more widely spaced than collar spines becoming progressively smaller towards the posterior with base deeply set in body wall making an acute angle (Fig. 2E). Anterior rows of trunk spines are closely set together with progressive increase in distance amongst adjacent spines and extend up to posterior testis (sometimes up till anterior end of cement gland) in ♂ and up to ¾ of total body length in ♀. Trunk spines are arranged in 20-26 rows with 10-12 spines per row in ♂ and 28-39 rows with 14-18 spines per row in ♀.

Male

Genitalia situated in posterior portion of body. ♂ genitalia has two elongated testes tandem and contiguous (Fig. 2A); anterior testis always slightly larger than posterior. Posterior testis more or less cylindrical with both ends blunt. From each testis, vas efferens runs downward to meet its fellow from other testis forming common sperm duct. Cement gland elongated, cylindrical, syncytial, and broad margin in contact with posterior testis with 10-11 nuclei. Saefftigen's pouch ('Markbeutal' of Saefftigen) pear shaped, bladder like, wider base directed anteriorly and proximates cement gland (Fig. 2F). Tip of pouch circular, directed posteriorly and a pair of long narrow ducts unite to open almost immediately at base of penis projecting into bursa. Saefftigen's pouch does not take carmine stain, thus appears light brown in color. Secretion of this gland facilitates eversion of bursa and helps in copulation (3). Seminal vesicle large, thin walled sac extending forward and opening behind at base of penis. Copulatory bursa equipped with muscular bursal cap (Fig. 2F) eversible, funnel-like with heavy musculature and everts outside through terminal aperture (Fig. 3D).

Female

Entire body cavity of mature worm filled with large number of eggs (Fig. 2G). Anterior end of uterus modified into uterine bell (Fig. 3E) and supported by a suspensor ligament (Fig. 2H). As ovary ripens, bursts, liberating spherical or oval ovarian balls (Fig. 3F) in body cavity. Guard cells inside and below uterine bell. Uterus long, flabby, convoluted tube beginning from end of uterine bell. Vagina thick, muscular, surrounded by sphincter muscles. Vulva, tubular posterio lateral. Fully developed eggs oval and freshly discharged eggs in various stages of development (Fig. 3G).

Taxonomic summary

Type host: Spotted snakehead fish (murrel) *Channa punctatus*
(Bloch, 1793) (Channidae)
Type locality: Bareilly, Uttar Pradesh, India (28.35ºN; 79.42º E)
Site of infection: Intestine
Type depository: ASCS MEB 44 (holotype male); ASCS FEM 105 (allotype female) paratype ASCS collection no. FEM 155)
The holotypes and paratypes are deposited in the "Centre of Excellence", Department of Animal Science, M.J.P. Rohilkhand University, Bareilly, U.P., India.
Etymology: The species is named after the name of the host fish.
Occurrence
Prevalence: 65.51%
Mean Intensity: 3-6 par/host
Subgenus diagnosis*:* *Brevitritospinus* Amin *et al.*, 2000

Remarks

Three subgenera of *Pallisentis* were erected (1) based on the number of hooks in each of the proboscis hook circles: *Farzandia* Thapar, 1931, with 10 hooks per circle, *Neosentis* Van Cleave, 1928 with 8 hooks per circle and *Pallisentis* Van Cleave, 1928 with 6 hooks per circle. However, due to inconsistency in the number of hooks per circle even within the same species, this system was rejected. The difference in the size of proboscis hooks in subsequent circles, size of the cement gland and the number of its giant nuclei, the shape and distribution of trunk spines and the pres-

ence or absence of Saefftigen's pouch were regarded to be more valid criteria to differentiate the subgenera (11). Accordingly, they differentiated 3 subgenera: *Demidueterospinus* Amin *et al*., 2000 with proboscis hooks in circle 2 about half as long as hooks in circle 1, cement gland usually small with few giant nuclei; *Brevitritospinus* Amin *et al*., 2000 with proboscis hooks in circle 3 about half as long as hooks in circle 2, cement gland usually small with few giant nuclei; and *Pallisentis* Van Cleave, 1928 *sensu stricto* with proboscis hooks gradually declining in size towards the posterior body; cement glands usually long with many giant nuclei. The characters of the present species fall under *Brevitritospinus* and is placed under this sub genus.

Recently, two species from the same host were described: *P. channai* and *P. vinodai* (19). However, the authors have not placed the species in any subgenus but based on the size of the proboscis hooks (first circle largest and basal row smallest) of both species, they appear to belong to *Pallisentis* and not to *Brevitritospinus* (11).

Ten species within the subgenus *Brevitritospinus*: *P.* (*B.*) *allahabadi* Agarwal, 1958; *P.* (*B.*) *cavasii* Gupta & Verma, 1980; *P.* (*B.*) *croftoni* Mital & Lal, 1981; *P.* (*B.*) *fasciata* Gupta & Verma, 1980; *P.* (*B.*) *fotedari* Gupta & Sinha, 1991, *P.* (*B.*) *guntei* Sahai et al., 1967; *P.* (*B.*) *indica* Mital & Lal, 1981; *P.* (*B.*) *jagani* Koul et al., 1991; *P.* (*B.*) *mehrai* Gupta & Fatma, 1986 and *P.* (*B.*) *vietanamensis* Amin et al., 2000 were identified (12).

Discussion

Morphometric traits of the present species when compared with other *P.* (*B.*) species indicated that it differs markedly from them in ♀ being almost twice as large as ♂; proboscis hook characteristics: being smaller than other species; hooks of the third row sharply reducing in size being less than half of those of second row; collar spine rows similar (16) and constant in ♂ and ♀ but the number of spines per row much greater in ♀ (22) than those of ♂ (14); trunk spine rows 28-39 in ♀ and 20-26 in ♂, never more than 1.5 times in the former, whereas number of spines per row are 14-18 in the former and 10-12 in the latter and spine length of ♀ almost twice as long as ♂. Lemnisci unequal, arms extending up to tip and mid anterior testis in ♂and 5th and 8th row of trunk spines in ♀. Anterior testis being slightly longer than posterior, presence of Saefftigen's pouch, number of nuclei in cement gland 10-11, bursa being small, set the *Brevitritospinus* species discovered from *Channa punctatus* apart from the described species and is thus designated as a new species, *Pallisentis* (*B.*) *punctati* n. sp. with the specific characters as given in this description.

SEM Studies on Attachment Elements of *Pallisentis punctati* n. sp. (Figs. 4, 5)

SEM studies on proboscis hooks indicated 4 rows of curved, sclerotized hooks, arranged alternately. Each circle of hook was almost equidistant from the next (31.2 – 32.3 µm) (Fig. 4A) and the tangential distance between first and fourth row hooks was 68.6 ± 4.2 µm. The hooks of the first row were least curved, those of the second and third rows progressively had longer horizontal parts prior to their vertical droop (Fig. 4B-E).

All hooks were without any micro sculptures, striations or grooves but their margins had protuberances at a distance of 4.35 ± 0.25 µm (Fig. 5A). The hooks were deeply rooted in the proboscis wall, the socket, 4.05 ± 0.23 µm in diameter holding the stout hook, 3.50 ± 0.35 µm in diameter (Fig. 5B).

Cuticular spines embedded in the tegument were numerous starting from the anterior end of the metasoma (Fig. 5C). They were evenly distributed in parallel lines but each spine of the next row was neither placed just below nor alternately consistently Fig. 5C). They were shorter and stouter than the proboscis hooks,

broad at their base, pointed at the posterior end (Fig. 5D). Distance between two spines was unequal in different rows (tip: 46.9-106µm, base: 36.0-81.2µm) and even varied in the same row (tip: 83.5-102.0µm, base: 74.1-80.7µm) (Fig. 5D). Ventral surface maybe prominently eccentrically grooved (Fig. 5D & E), or smooth (Fig. 5F). The proximal (almost half) part of the spine remains embedded in the trunk, the remaining distal end being free, posterior end pointed and spiny (Fig. 5E) or hooked (Fig. 5F). Their numbers reduced towards the posterior end of the body. This was followed by a spineless area leading to trunk spines, conical, larger than pre-trunk spines and becoming smaller towards the posterior end. Distance between each circlet in trunk spines increase but the number decreases towards the posterior end. Lateral trunk spines form an acute angle with the body surface and two lateral spines maybe parallel to each other. The size of the ♂ and ♀ hooks differed being larger in ♀ in each row. Moreover, the ♀ pre-trunk spine was also not only longer but also the number of spines per row was greater (22) as compared to the ♂ (14).

The trunk spines again, the number of rows (28-39) and number of spines per row (14-18) in ♀ was greater to that in ♂ [(20-28) and (10-12) respectively]. The length of the ♀ trunk spine followed the same trend being longer (0.043 µm) than the ♂ (0.028 µm). Trunk spine in ♂ was narrower as compared to the broad ♀ spine. Measurements taken at 5, 10 and 15 µm from the tip in ♂ (3.26 ± 0.33, 5.38 ± 0.62, 7.21 ± 0.81 µm) and ♀ (3.78 ± 0.49, 7.46 ± 0.91, 12.0 ± 1.02 µm) trunk spines revealed a definitely broader trunk spine in ♀ indicating a ♂: ♀ ratio of 1:1.15, 1:1.38 and 1:1.66 at 5, 10 and 15 µm distance of the spine tip respectively, thus becoming definitely broader at the base in the ♀ spine. The lateral spine in the former had characteristic hooked appearance at a distance of 9.35 ± 0.85 µm in contrast to the smooth in the latter.

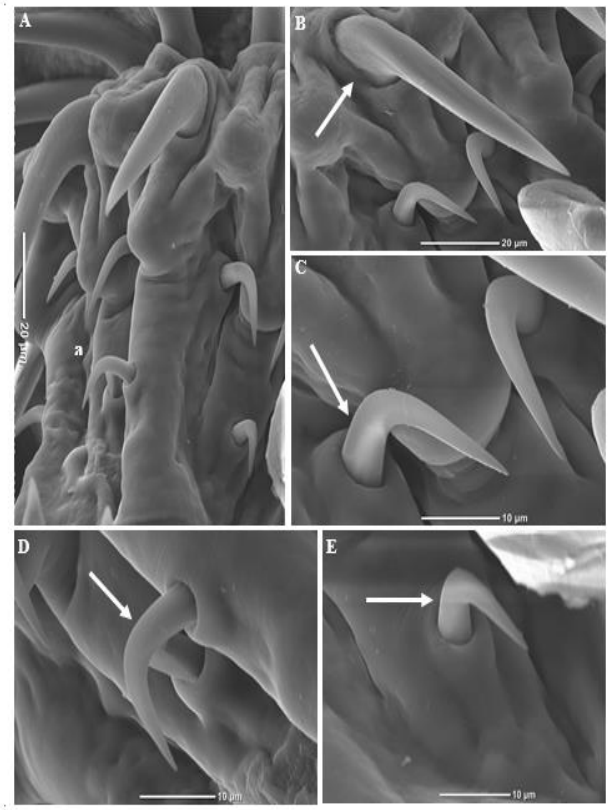

Fig. 4: Scanning electron microscopy of proboscis hooks of *Pallisentis* (B.) *punctati* n. sp. Circles of hooks. A All 4 rows. B First row. C Second row. D Third row. E Fourth row (Note the increasing length of the horizontal arm). *Scale bar* = 20 µm in A & B, 10 µm in C-E

Proboscis is a fluid filled structure, inserted into the gut wall of the host and the number and length of the hooks play an important role in anchoring the worm in a location. Morphometrics of hooks and spines are of key significance in the discrimination of closely related species of acanthocephalans. Congeneric species are often differentiated only based on subtle differences in proboscis armature. Proboscis hooks vary in size and shape, show morphometric variation from apex to base and can be measured easily, therefore studies on proboscis hooks and trunk spines of acanthocephalans are of prime importance

as their number, arrangement and structure are important interspecific diagnostic tools used in taxonomy and classification (20).

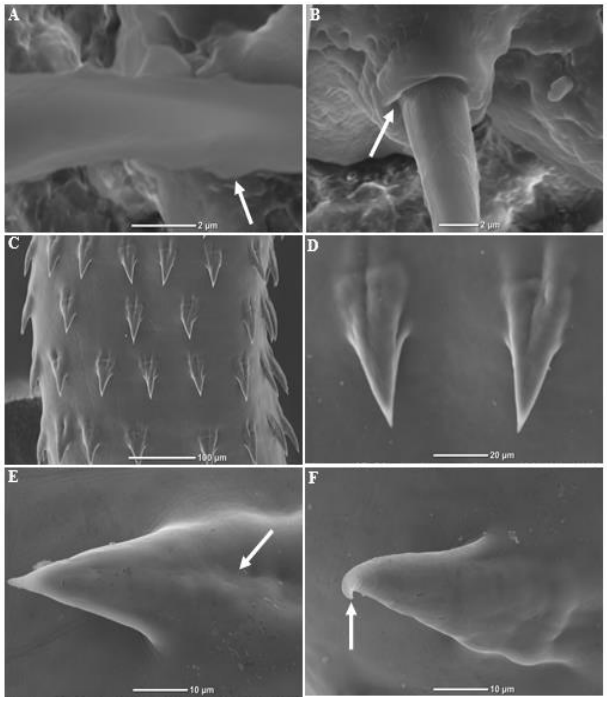

Fig. 5: Scanning electron microscopy of attachment structures of *Pallisentis* (*B.*) *punctati* n. sp. **A** Surface of proboscis hook showing protuberances. **B** Deeply rooted proboscis hook ; **C** Cuticular spines. **D** Magnified cuticular spines showing broad base and pointed ends. **E** Ventral surface of spine showing groove and pointed spiny end.. **F** Spine with hooked end (*Scale bar* = 10 µm). *Scale bar* = 2 µm in **A & B**, 100 µm in **C**, 20 µm in **D,** 10 µm in **E.**

Attachment traits used for acanthocephalan diagnostics include the number of longitudinal rows of hooks on the proboscis; the number of hooks per row; and the mean hook length. Hooks assist in securing the proboscis into the gut wall and the spines play a secondary role in attachment (21). However, detailed information on their ultra-structure (22) is still wanting. Meristogram analyses of morphological variants in hook patterns have been utilized to distinguish species of *Echinorhynchus* (15) and *Pomphorhynchus* (20). Trunk spines are presumably different from proboscis hooks in the absence of a hollow core and site of origin. Reduction and variability of trunk spines in *Corynosoma cetaceum* and the role of physical constraints on attachment were reported (22). Quantitative analyses of interspecific patterns of investment in attachment structures in a taxonomic group of parasites were performed for the first time (16). Unique striations were observed on hook surface of *Dentitruncus truttae* (23) which were lacking in the present species. SEM comparison with the hooks of other Palaeacanthocephala species (*Acanthocephalus anguillae, A. clavula, Echinorhynchus truttae, Telosentis exiguus, Acanthocephaloides propinquus, Pomphorhynchus laevis, Polymorphus minutes*), with two species of Eoacanthocephala, (*Neoechinorhynchus rutili, N. emydis*) and with a Polyacanthocephala, (*Polyacanthorhynchus kenyensis*) indicated that in these species, micro sculptures in the form of striations were absent and our findings fall on similar lines.

The development and morphology of acanthocephalan proboscis hooks has been a matter of limited concern. An updated review on origin, development, and morphology of larval and adult hooks in several species of Acanthocephala belonging to Palaeacanthocephala, Eoacanthocephala, and Archiacanthocephala was provided (24) which carries consensus in general.

Many acanthocephalans, (but not all), possess trunk spines, presumably functioning as a secondary holdfast device (25). There are examples where spines are present on the fore trunk only [e.g., *Dentitruncus truttae* (23)] or on both fore- and hind trunk [e.g., *Telosentis exiguus* (26); *Corynosoma cetaceum* 22)]. In the present species, spines were present on both fore- and hind trunk thus falling in the latter category.

The presence of large number of spines both on the fore-trunk and hind-trunk and well developed proboscis hooks in *P. punctati* n. sp. depicts its efficient adaptation for effec-

tive attachment to help maintain close contact with the host's intestinal mucosa. Its successful persistence in the gut wall probably accounts for its appreciable prevalence in the host fish examined.

Lack of sexual dimorphism with reference to hooks and spine size has been reported (23) which is contrary to our findings. On the other hand, it has been postulated that investment strategies on attachment may differ not only between congeneric acanthocephalan species but also even between sexes of the same species (24). The well-marked differences in the ♂ and ♀ attachment elements of the present worm clearly indicate sexual dimorphism in *P. punctati* n. sp. and based on the structure, number and arrangement of the attachment elements, these characters maybe effectively utilized for differentiating the male and female worms.

Significance of the Use of Scanning Electron Microscope for the Study of Acanthocephalans

Light microscopy is an important tool for studying all morphological aspects of acanthocephalans because these structures are very significant in the context of their classification. Traits commonly employed for acanthocephalan diagnosis are proboscis receptacle, number, size and arrangement of proboscis hooks and trunk spines, shape and size of lemnisci, testes, ovary, cement gland, number of nuclei in cement gland, presence or absence of Saefftigen's pouch and are regarded as characteristics of taxonomic importance and have to be explicitly studied under the light microscope. The scanning electron microscope is an additional tool for the study of acanthocephalans as it provides three-dimensional images with high magnification that facilitates to understand the spatial relationships among surface structures. The attachment elements (hooks and spines) have since long been used as valid taxonomic tools for differentiating subgenera and species of acanthocephalans. It is commonly used to distinguish species that appear morphologically identical when examined under light microscope (14, 27). The present venture was therefore undertaken to supplement light microscopy with SEM to provide valid information on the taxonomically important attachment elements, which demonstrated sex-based differences at the ultra-structural level.

Conclusion

Channa punctatus is infected with a new species of acanthocephalan parasite, *P. punctati* n. sp. This is the first record of *Pallisentis* from Bareilly, Uttar Pradesh, India. SEM studies showed species-specific attachment organs. Scanning Electron microscopy of hooks and spines of male and female worms, exhibit sexual dimorphism.

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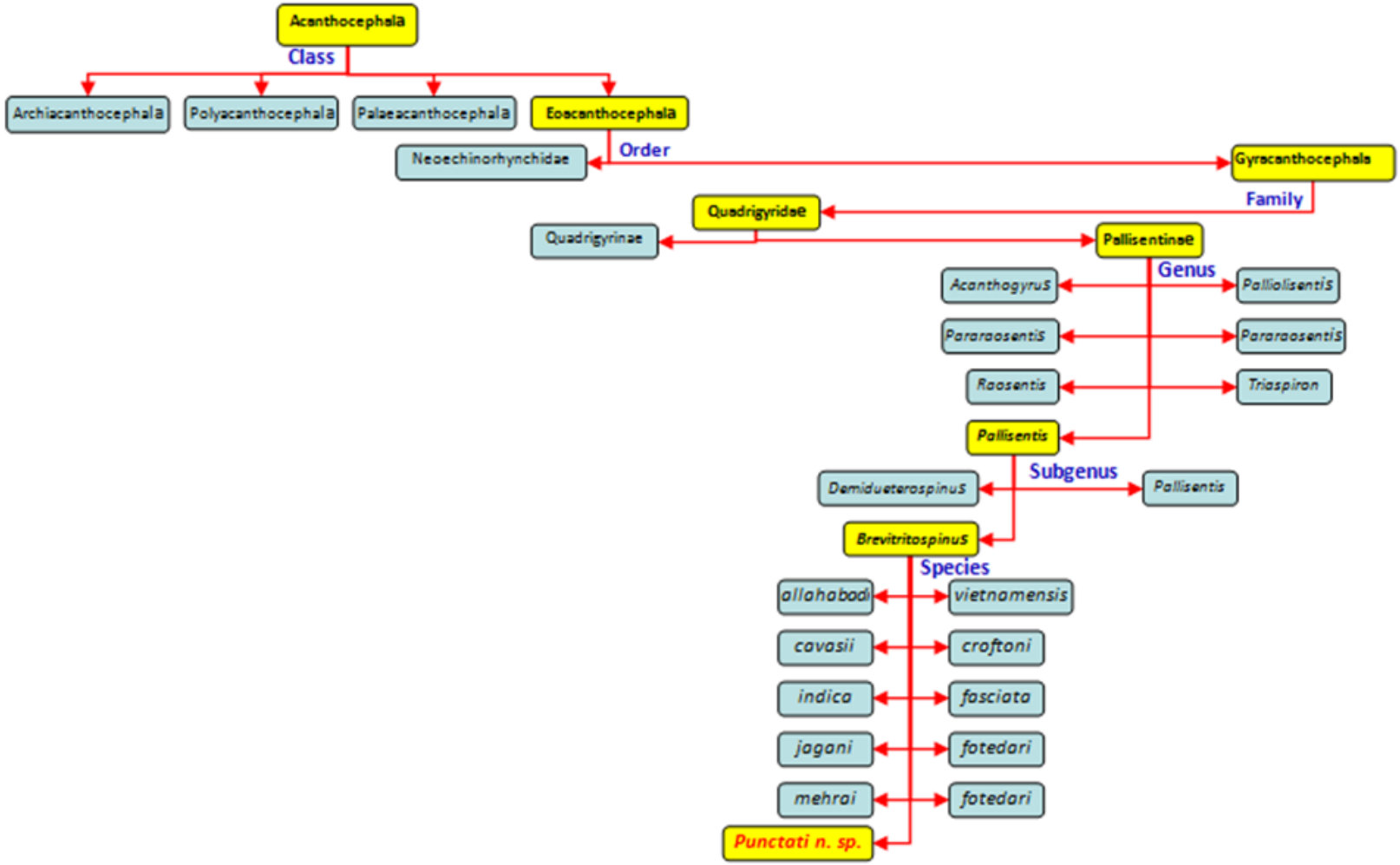

Fig. 1: Classification dendogram of *Pallisentis* (*B.*) *punctati* n. sp. (Classification adopted from Amin, 2013)