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Morpho-molecular characterisation of two new and two previously reported species of *Acanthogyrus* (Acanthogyrus: Quadrigyridae) from freshwater fishes in India

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Abstract

Of the total 47 species in the subgenus *Acanthosentis,* 43 have been reported from the freshwater fishes of Asia. Amin *et al.* (2017) provided a key to the 23 species of the genus *Acanthogyrus* reported from the Indian subcontinent. The present study reports two new species: *Acanthogyrus bispinosa* n. sp. and *A. garciai* n. sp. from *Cirrhinus mrigala* Hamilton and *Labeo calbasu* Hamilton, respectively, and two previously described species: *A. golvani* Gupta and Jain, 1980 and *A. hereterospinus* Khan and Bilqees, 1990 from *L. rohita* Hamilton and *L. catla* Hamilton, respectively. *A. bispinosa* n. sp. comprises 3 circles of 6 proboscis hooks each. Trunk spines in *A. bispinosa* n. sp are divided into two groups: anterior and posterior separated by unarmed region, which has not been previously reported in the subgenus. Anterior spines are present in 7–8 and 7–10 circles in females and males, respectively, whereas posterior spines are in 23–28 and 31–38 circles in males and females, respectively. *A. garciai* n. sp. comprises 3 circles of 6–8 hooks each and a single set of trunk spines is present in *A. garciai* n. sp., comprising 35–42 and 25–45 circles in males and females, respectively. All four species were also characterised based on the 18S, 28S, and ITS1-5.8S-ITS2 rRNA molecular markers. The Bayesian inference tree generated based on these markers showed distinct identities of all the species, with a significant molecular divergence, ranging from 3.2 to 53.6%.

Introduction

The genus *Acanthogyrus* was erected by Thapar (1927), whereas the other sister genus *Acanthosentis* was created by Verma and Datta (1929). The genus *Acanthosentis* was later proposed as the subgenus under the *Acanthogyrus* by Golvan (1959). In the descriptions of Amin (1985) and Amin & Hendrix (1999) and the classification of the Acanthocephala provided by Amin (2013), the genera *Acanthogyrus* and *Acanthosentis* were treated as two subgenera under the genus *Acanthogyrus.* Both genera were differentiated on the basis of number of the proboscis hooks. This classification scheme has been accepted by taxonomists. However, the disagreements and conflicts regarding this synonymy and subgeneric classification continue to occur due to the morphological variability in the number of hooks between the subgenera (Amin 2005).

Currently, 50 species have been reported in *Acanthosentis,* including the recently described species *A. kenyirensis, A. terengganuensis,* and *A. tembatensis* (Mohd-Agos *et al.* 2021). However, some of the species in *Acanthosentis* have not been adequately described (Ru *et al.* 2022). Further, the molecular data of *Acanthogyrus* species are very scarce, and the sequences of only 4, 2, and 6 species based on 18S, 28S, and ITS1-5.8S-ITS2 rRNA molecular markers, respectively, are available in database.

The present study includes the detailed morphological characterisation of the two new species *Acanthogyrus bispinosa* n. sp. and *A. garciai* n. sp. from *Cirrhinus mrigala* Hamilton and *Labeo calbasu* Hamilton, respectively, and two previously reported species *A. golvani* Gupta and Jain, 1980 and *A. hereterospinus* Khan and Bilqees, 1990 from *L. rohita* Hamilton and *L. catla* Hamilton, respectively. Further, molecular data of all species based on the 18S, 28S, and ITS1-5.8S-ITS2 molecular markers have been generated. Additionally, the Bayesian inference phylogenetic trees have been generated to investigate the phylogenetic relationships within *Acanthogyrus* and among other sister taxa.

Materials and methods

Sample collection

A total of 55 fish species of *L. catla, C. mrigala, L. calbasu*, and *L. rohita* were investigated for acanthocephalan infections from 2021 to 2022 from two states in northern India viz., Himachal

Pradesh and Punjab. Of these, 3, 2, 4, and 8 fish species of *L. catla, C. mrigala, L. calbasu*, and *L. rohita,* respectively, were found infected. The fishes were procured from four different locations, including Beas River, Himachal Pradesh; Bhakra, Punjab (31.24°N, 76.26°E); Harike Wetland, Punjab (31.15°N, 74.97°E); and Ropar wetland, Punjab (31.0200°N, 76.5000°E). The dissection of the fish gut samples was performed at the collection site and in the parasitology laboratory, Department of Zoology, Panjab University, Chandigarh, India. The fish guts were dissected longitudinally with the help of surgical scissors in the petri plate containing tap water, and the intestinal canal was carefully examined for the parasitic infections. The parasites were carefully transferred to another petri plate, washed, and kept in normal saline (0.8%) for proboscis eversion. The worms were fixed in 70 and 100% ethanol for morphological and molecular characterisation, respectively.

Morphological characterisation

The worms were hydrated in the series of alcohol (50 and 30%) grades for 15–20 min each. The worms were then washed in the water and stained in Gower's carmine stain for 20–40 min. The worms were further dehydrated in the ascending series of alcohol for 10–20 min each, followed by 100% alcohol plus xylene for 5 min, cleared in xylene for 10–15 min, mounted in DPX mountant, and studied under the microscope (Magnus MLXi Plus, Magnus Optosystems, India). Whole worm line drawings were made with the aid of the projection microscope. The enlarged view of the different parts was drawn and measured using the Radical microscope RXLr-3T (Radical instruments, India) and Magnus MLXi Plus microscope (Magnus Optosystems, India), inclined with Magcam DC 10 MP camera with MagVision software. All measurements are in micrometers unless otherwise stated. Range is followed by the mean (in parentheses). Identification of the parasites was done by thoroughly studying the literature and the keys to the species by Amin (1987, 2005). The male and female specimens of the identified species were submitted to the High Altitude Regional Centre-Zoological Survey of India (HARC-ZSI), Solan, Himachal Pradesh, India, and voucher numbers were obtained.

Molecular characterisation

The genomic DNA extraction of the acanthocephalan worms preserved in 100% ethanol was performed using the Qiagen's DNeasy® Tissue Kit (QIAGEN, Hilden, Germany) per manufacturer's instructions. The appropriate quantity of extracted DNA template was subjected to the polymerase chain reaction (PCR) to obtain the amplified product for the 18S, 28S, and ITS1-5.8S-ITS2 molecular markers using the primers from Garcia-Varela *et al.* (2013), Garcia-Varela and Nadler (2005), and Rana and Kaur (2021). The 25 µl PCR reaction mixture comprised 2.5 µl 10X buffer, 1 µl dNTPS, 0.5 µl reverse and forward primer each, 0.3 µl Taq polymerase, 2–4 µl DNA template, and 16.2–18.2 µl molecular grade water. The PCR cycle included initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 552.9–55°C for 35 s, polymerisation at 72°C for 1 min, and final polymerisation at 72°C for 5 min. The amplified PCR products were delivered to Bioserve Biotechnologies India Pvt. Ltd. Hyderabad, Telangana, India for Sanger sequencing. The forward and reverse sequences for the respective molecular markers were obtained, and peak for each base pair, along with its Q value, was analysed manually by using the software Finch TV. The ends of the sequences were trimmed in the BioEdit. Forward and reverse sequences of the same sample of respective molecular markers were assembled together in the BioEdit, and a contig was obtained. The generated contig was subjected to Basic Local Alignment Search Tool to check the sequence similarity to the other sequences of the same group already present in the NCBI database. The sequences generated for each molecular marker of the identified species were submitted to the NCBI database to obtain the accession numbers. The comparable sequences from the database were downloaded, and multiple sequence alignment of the dataset was performed using CLUSTAL W in MEGA 7. All positions less than 95% site coverage in the dataset were eliminated. The genetic sequence divergence (p value) and the difference in the number of base pairs among the species were estimated using the software MEGA 7. The best fit models for the reconstruction of the phylogenetic trees were selected in the MEGA 7. The Bayesian inference trees were generated using the MrBayse3.2.7. Markov chain Monte Carlo (MCMC) chains were run until the average standard deviation of split frequency value reached less than 0.01.

Results

Acanthogyrus bispinosa *n. sp*

Taxonomic summary

Class: Eoacanthocephala Van Cleave, 1936
Order: Gyracanthocephala Van Cleave, 1936
Family: Quadrigyridae Van Cleave, 1920
Subfamily: Pallisentinae Van Cleave, 1928
Genus: *Acanthogyrus* Thapar, 1927
Subgenus: *Acanthosentis* Verma and Datta, 1929
Species: *Acanthogyrus bispinosa* n. sp.
Type host: *Cirrhinus mrigala* Hamilton, 1822
Type locality: Beas River Himachal Pradesh, procured from fish market Sector 21, Chandigarh, India (30.7256 °N, 76.7758 °E)
Site of infection: Small intestine
Specimens submitted: Male holotype (HARC/ZSI/Ac-10) and female allotype (HARC/ZSI/Ac-10) were deposited in the High Altitude Regional Centre-Zoological survey of India, Solan, Himachal Pradesh.
Sequences generated: The sequences submitted to the NCBI database on the basis of 18S, 28S, and ITS1-5.8S-ITS have been allotted accession number OP541601, OP476684, and OQ371287, respectively.
Etymology: The specific name has been devised because of the arrangement of trunk spines in 2 sets.
Specimens examined: 5 males and 5 females

Morphological description (Figure 1)

Worms <7 mm in length, proboscis oblong, slightly longer than wider, apical organ with 2 nuclei. Three circles of proboscis hooks with solid central core, 6 hooks per circle, circles of proboscis hooks asymmetrical, nearly in perfect circles, hooks of 2nd circle equal to hooks of 3rd circle, very short hook roots, visible only in 1st circle. Proboscis receptacle twice the length of proboscis, with prominent cerebral ganglion at posterior end. Lemnisci paired, barely detectable. Trunk spines rose-thorn shaped with lobular base, divided into two groups: anterior and posterior separated by unarmed region. Posterior spines present to the posterior end in both sexes, irregular in posterior half in female. Posterior spines in full circles initially but lateral circles (2–4 (3) spines each) in posterior half of

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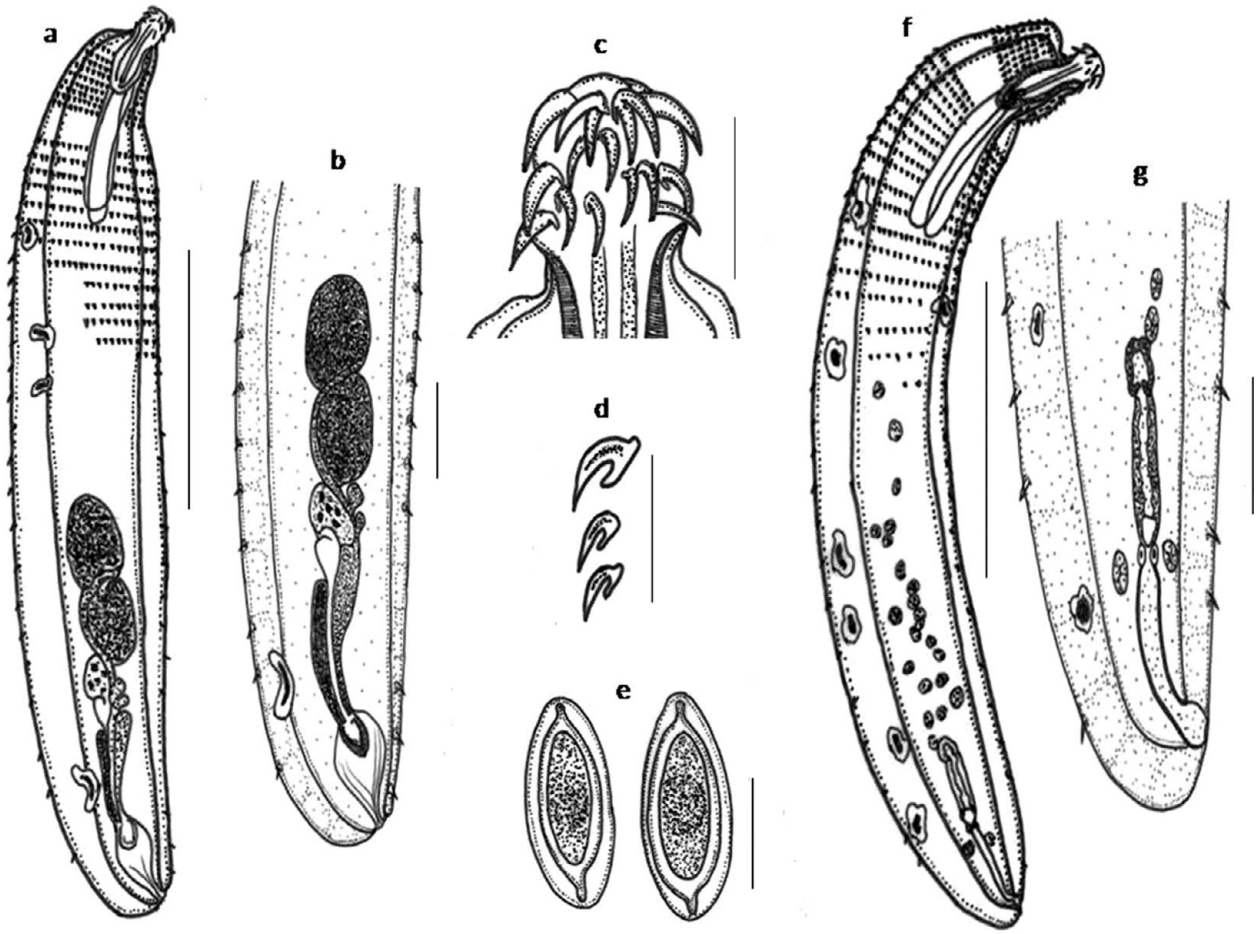

Figure 1. Line drawings of the specimens of *Acanthogyrus bispinosa* n. sp. a- male; b- posterior end of male; c- proboscis; d- proboscis hooks; e- mature egg; f- female; g- posterior end of female. Scale a- 1 mm; b- 200 μm; c- 100 μm; d- 100 μm; e- 10 μm; f- 1 mm; g- 200 μm.

trunk; 3–5 (4) lateral circles of closely spaced spines near the posterior extremity around the reproductive system in both sexes.

Male: Trunk 3.1–4.4 (3.75) mm long × 0.51–0.74 (0.62) mm wide. Proboscis 101.16–139.95 (120.55) long × 80.96–100.4 (90.68) wide. Proboscis hooks H1 54.87–66.105 (60.48) long × 11.39–14.53 (12.96) wide, H2 35.41–53.77 (44.59) long × 7.88–9.50 (8.69) wide, H3 35.89–51.49 (43.74) long × 7.27–8.15 (7.71) wide. Hook roots HR1 24.65–30.56 (27.6), HR2 17.56–22.05 (19.8), HR3 14.56–20.64 (17.6). Proboscis receptacle 220.08–225.95 (223.015) long × 91.50–92.82 (92.16) wide. Lemnisci L1 349.56–565.3 (456.43) long × 95.32–104.95 (100.13) wide, L2 340.54–548.32 (444.43) long × 94.98–117.44 (106.21) wide. Hypodermal nuclei 4–5 dorsal, none or 1 ventral. Anterior trunk spines 7–8 circles, 25–30 (27) spines in each circle. Posterior trunk spines 23–28 (25) circles, 30–40 (35) spines in each circle, present to posterior end posterior most circles lateral only (2 spines per circle). Testes globular, anterior testis 288.65–354.11 (321.38) long × 183.80–247.74 (215.77) wide, posterior testis 318.63–361.04 (339.83) long × 241.38–277.72 (259.55) wide. Cement glands contains 4–5 nuclei, measures 107.97–158.46 (133.215) long × 98.02–107.97 (102.99) wide. Cement reservoir 93.92–127.4 (110.66) long × 67.02–76.92 (71.97) wide. Vas efferens segmented in 2 sac-like structures above seminal vesicle. Seminal vesicle 196.53–276.4 (236.465) long × 52.73–110.00 (81.36) wide. Saefftigen's pouch tubular, 306.43–447.03 (376.734) long. Bursa 166.63–247.89 (207.26) long × 108.06–144.76 (126.41) wide. Gonopore terminal slightly bent towards ventral side.

Female: Trunk 3.2–6 (4.6) mm long × 1.9–4.1 (3.0) mm wide. Proboscis 120.38–137.1 (128.74) long × 100.61–104.6 (102.60) wide. Proboscis hooks H1- 55.87–69.9 (62.88) long × 13.04–16.95 (14.99) wide, H2- 44.39–53.63 (49.01) long × 10.34–13.29 (11.81) wide, H3- 42.27–52.25 (47.26) long × 9.29–12.55 (10.92) wide. Hook roots HR1 29.67–31.56 (30.61), HR2 19.44–21.05 (20.24), HR3 18.53–20.71 (19.62). Proboscis receptacle 237.47–271.04 (254.25) long × 86.83–108.49 (97.66) wide. Lemnisci L1 450.65–566.76 (513.70) long × 94.59–115.47 (105.03) wide, L2 450.45–567.38 (508.91) long × 95.01–114.93 (104.97) wide. Hypodermal nuclei 4–6 dorsal, none or 1 ventral. Anterior trunk spines 7–10 (8) circles, 23–30 (26) spines each circle. Posterior trunk spines 31–38 (34) circles, 24–38 (31) spines each circle, posterior most circles lateral with 2 spines per circle. Reproductive system 467.22–639.57 (553.39) long. Uterine bell short, 75.22–75.68 (75.45) long. Uterus moderately muscular, 166.81–220.29 (193.55) long × 38.97–52.51 (45.74) wide. Guard cells present at the junction of uterine bell and uterus. Vagina 225.69–343.60 (284.64) long. Vaginal sphincter at junction of uterus and vagina. Gonopore ventro-terminal. Egg 17.33–23.93 (20.63) long × 7.09–11.31 (9.2) wide, with three membranes, polar elongations of fertilisation membrane present.

Remarks

The present species shows the peculiar characteristics of the genus *Acanthogyrus* in having 3 circles of proboscis hooks, spinose trunk, and syncytial cement gland. Due to the presence of 6 hooks per

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Table 1. Morphometric comparison between *Acanthogyrus bispinosa* n. sp. and other closely related species of the genus

Species	*Acanthogyrus bispinosa* n. sp.	*Acanthogyrus oligospinus* Anantaraman, 1980	*Acanthogyrus bilaspurensis* Chowan, Gupta and Khera, 1987
Host	*Cirrhinus mrigala*	*Mystus gulio*	*Cirrhinus reba*
Locality	Himachal Pradesh, India	Tamil Nadu, India	West Bengal, India
Male length (mm)	3.1–4.4 (3.75)	4	3.9–5.6 (4.7)
Proboscis L x W (μm)	101.16–139.95 (120.55) × 80.96–100.4 (90.68)	150 × 70	120 × 110
Circles of proboscis hooks	3	3	3
Number of proboscis hooks in each circle	6	6	6
Hooks from anterior to posterior (μm)	H1 54.87–66.105 (60.48) H2 35.41–53.77 (44.59) H3 35.89–51.49 (43.74)	H1 50 H2 40 H3 40	H1 33 H2 24–28 (26) H3 24–28 (26)
Proboscis receptacle L × W (μm)	220.08–225.95 (223.015) × 91.50–92.82 (92.16)	400 × 90	210–430 (320) × 90–110 (100)
Lemnisci L × W (μm)	L1 349.56–565.3 (456.43) × 95.32–104.95 (100.13) L2 340.54–548.32 (444.43) × 94.98–117.44 (106.21)	–	490–780 (635) × 150–260 (205)
Hypodermal nuclei	Dorsal: 4–5 Ventral: none or 1	5–6 pairs	–
Circles of trunk spines	Anterior: 7–8 Posterior: 23–28 (25)	16–18 (17)	20–22 (21)
Anterior testis L × W (μm)	288.65–354.11 (321.38) × 183.80–247.74 (215.77)	380 × 230	510–660 (585) × 520–610 (565)
Posterior testis L × W (μm)	318.63–361.04 (339.83) × 241.38–277.72 (259.55)	260 × 203	520–700 (610) × 590–690 (640)
Cement gland L × W (μm)	107.97–158.46 (133.215) × 98.02–107.97 (102.99)	220 × 200	430–540 (485) × 330–460 (395)
Cement gland nuclei	4–5	–	–
Cement reservoir L × W (μm)	93.92–127.4 (110.66) × 67.02–76.92 (71.97)	150 × 90	410–930 (670) × 260–310 (285)
Saefftigen's pouch	306.43–447.03 (376.734)	–	360–710 (535) × 100–150 (125)
Seminal vesicle L × W	196.53–276.4 (236.465) × 52.73–110.00 (81.36)	–	–
Bursa L × W	166.63–247.89 (207.26) × 108.06–144.76 (126.41)	250 × 120	770–840 (805) × 330–360 (345)
Female length (mm)	3.2–6 (4.6)	4.4	6.03–7.12 (6.5)
Proboscis L × W (μm)	120.38–137.1 (128.74) × 100.61–104.6 (102.60)	–	200–220 (210) × 170–220 (195)
Hooks from anterior to posterior (μm)	H1 55.87–69.9 (62.88) H2 44.39–53.63 (49.01) H3- 42.27–52.25 (47.26)	–	H1 32–36 H2 24–28 H3 22–28
Proboscis receptacle L × W (μm)	237.47–271.04 (254.25) × 86.83–108.49 (97.66)	–	360–400 (380) × 140–150 (145)
Lemnisci L × W (μm)	L1 450.65–566.76 (513.70) × 94.59–115.47 (105.03) L2 450.45–567.38 (508.91) × 95.01–114.93 (104.97)	–	–
Hypodermal nuclei	Dorsal: 4–6 (5) Ventral: none or 1	–	–
Circles of trunk spines	Anterior: 7–10 (8) Posterior: 31–38 (34)	–	–
Reproductive system L (μm)	467.22–639.57 (553.39)	–	–
Uterine bell L (μm)	75.22–75.68 (75.45)	100	190–590
Uterus L (μm)	166.81–220.29 (193.55)	180	310–360
Vagina L (μm)	225.69–343.60 (284.64)	220	260–650
Egg L × W (μm)	17.33–23.93 (20.63) × 7.09–11.31 (9.2)	35 × 12	32 × 10

circle in proboscis, the present species belongs to the subgenus *Acanthosentis*.

According to the key to the species of the subgenus *Acanthosentis* provided by Amin *et al.* (2017), the present species shows closest similarity with *A. oligospinus* Anantaraman, 1980 and *A. bilaspurensis* Chowhan, Gupta, and Khera, 1987 in having same number of proboscis hooks in each circle and hooks of middle and posterior circles almost equal in size. The present species, however, differs from *A. oligospinus* based on the size of the proboscis, trunk supination, and size of the reproductive organs (Table 1). The

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length of the proboscis in male and female is shorter than *A. oligospinus*. Further, the trunk spines are present in the two zones separated by the spineless zone, which is an uncommon morphological trait in the genus *Acanthogyrus,* whereas in *A. oligospinus* a single set of trunk spines is present in the anterior half of the body. In the present species, the posterior testis is longer than the anterior testis, whereas in *A. oligospinus* the anterior testis is longer than the posterior testis. Also, the anterior testis in the present species is shorter than the anterior testis of *A. oligospinus,* whereas the posterior testis is longer than the posterior testis of *A. oligospinus*. The significant difference can also be observed in the size of the egg, which is smaller than *A. oligospinus*.

The present species differs significantly from the other morphologically similar species *A. Bilaspurensis* based on the trunk spination and size of the reproductive organs (Table 1). The trunk spines are divided into two separate zones and are present throughout the trunk, whereas in *A. bilaspurensis* 22–27 circles of trunk spines are present in the anterior half of the trunk. The size of testes is smaller than in the *A. bilaspurensis*. The size of female reproductive organs, including uterine bell, uterus, and vagina, and egg, is smaller compared to *A. bilaspurensis*.

Acanthogyrus garciai *n. sp*

Taxonomic summary

Class: Eoacanthocephala Van Cleave, 1936

Order: Gyracanthocephala Van Cleave, 1936

Family: Quadrigyridae Van Cleave, 1920

Subfamily: Van Cleave, 1928

Genus: *Acanthogyrus* Thapar, 1927

Subgenus: *Acanthosentis* Verma and Datta, 1929

Species: *Acanthogyrus garciai* n. sp.

Type host: *Labeo calbasu* Hamilton, 1822

Type locality: Ropar wetland, Punjab, India (31.0200 °N, 76.5000 °E)

Site of infection: Small intestine

Specimens submitted: Male holotype (HARC/ZSI/Ac-12) and female allotype (HARC/ZSI/Ac-13) were deposited in the High Altitude Regional Centre-Zoological survey of India, Solan, Himachal Pradesh.

Sequences generated: The sequences submitted to the NCBI database on the basis of 18S, 28S, and ITS1-5.8S-ITS have been allotted accession number OP541604, OP476687, and OQ430527, respectively.

Etymology: The specific name has been devised in the honour of Professor Martin Garcia-Varela, Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México for the remarkable contribution in the phylogeny of Acanthocephala.

Specimens examined: 6 males and 5 females.

Morphological description (Figure 2)

Worms <7 mm in length, proboscis oblong, longer than wider, apical organ with 1–2 nuclei. Three circles of proboscis hooks with 6–8 hooks per circle, circles of proboscis hooks, nearly in perfect circles, hook roots not observed. Proboscis receptacle more than twice the length of proboscis, cerebral ganglion at posterior end. Lemnisci paired, sac-like or spatulate. Hypodermal nuclei 2–4 (3) dorsal, ventral not observed. Trunk spines rose thorn shaped with stellate base, 21–32 (26) spines per circle in both sexes, maybe present throughout the trunk length, complete circles present to anterior half of trunk, incomplete circles may present in the middle of trunk, lateral circles of 2 spines each present in rest of trunk, spines in posterior half irregular, lateral circles of closely spaced spines near the posterior extremity around the reproductive system in both sexes.

Male: Trunk 3.22–4.0 (3.6) mm long × 0.66–0.83 (0.74) mm wide. Proboscis 91.09–129.04 (110.06) long × 81.51–117.9 (99.70) wide. Proboscis hooks H1- 53.82–65.79 (59.805) long × 11.77–12.96 (12.36) wide, H2- 51.47–54.61 (53.04) long × 7.15–11.62 (9.38) wide, H3- 43.17–53.86 (48.51) long × 6.69–11.22 (8.95) wide. Proboscis receptacle 224.33–254.29 (239.31) long × 63.90–70.55 (67.22) wide. Lemnisci L1 598.67 long × 85.71 wide, L2 570 long × 97.19 wide (one specimen). Trunk spines 35–42 (38) circles, 15.76–17.45 (16.6) long, 5–10 lateral circles of spines at posterior end of body. Testes paired, anterior testis 273.07–320.53 (296.8) long × 183.45–236.09 (209.77) wide, posterior testis 260.08–293.95 (277.015) long × 169.55–208.25 (188.9) wide. Cement gland contains 5–6 giant nuclei, measures 176.90–192.9 (184.9) long × 58.90–65.85 (62.375) wide. Cement reservoir 88.99–143.29 (116.14) long × 58.90–86.72 (72.81) wide. Seminal vesicle 224.24–274.40 (249.32) long × 62.27–100.77 (81.52) wide. Saefftigen's pouch drop-shaped, 282.89–314.74 (298.815) long. Bursa 130.85–162.49 (146.67) long × 123.42–157.48 (140.45) wide. Gonopore ventro-terminal.

Female: Trunk 3.4–4 (3.7) mm long × 0.68–0.95 (0.81) mm wide. Proboscis 91.09–106.38 (98.735) long × 61.51–100.9 (81.20) wide. Proboscis hooks H1- 59.14–67.4 (63.27) long × 10.56–14.63 (12.59) wide, H2- 44.9–58.91 (51.90) long × 7.22–12.53 (9.87) wide, H3- 42.82–57.67 (50.24) long × 6.94–10.54 (8.74) wide. Proboscis receptacle 198.22–327.66 (262.94) long × 61.52–95.95 (78.735) wide. Lemnisci L1 503.15–546.64 (524.89) long × 56.83–59.11 (57.97) wide, L2 421.31–508.96 (465.13) long × 56.17–62.08 (59.12) wide. Trunk spines 25–45 (35) circles, 14.77–17.69 (16.23) long, reaching anywhere to the anterior half or the posterior end, 3–5 (4) lateral circles of spines at the posterior end. Reproductive system 409.33–621.65 (515.49) long. Uterine bell short, 38.14–63.80 (50.97) long. Uterus muscular, balloon shaped, 170.56–268.89 (219.725) long × 49.77–75.43 (64.6) wide. Guard cells present at the junction of uterine bell and uterus. Vagina 200.63–288.96 (244.79) long, with vaginal gland. Vaginal sphincter at junction of uterus and vagina. Prominent vaginal bulb. 4–5 lateral circles of spines in posterior end around the gonopore. Gonopore ventro-subterminal. Egg 12.41–24.8 (18.60) long × 8.08–14.47 (11.27) wide.

Remarks

The present species shows the typical morphological characters of the genus *Acanthogyrus* in body shape, trunk supination, and arrangement of proboscis hooks. According to the key to species of the subgenus *Acanthosentis* provided by Amin (2005) and Amin *et al.* (2017), the present species having proboscis hooks gradually declining in length, distribution of trunk spines throughout the body, presence of vaginal gland and subterminal gonopore resembles closely with *A. thapari* Prasad, Sahay and Shambhunath, 1969 and *A. dattai* Podder, 1938.

The present species, however, differs from both its morphologically close species in many aspects (Table 2). The worms under study are shorter in total length than those of *A. dattai*. The average length of the proboscis hooks of all the circles is longer than *A. thapari* (H1- 48, H2- 42, H3- 32) and *A. dattai* (H1- 50, H2- 30, H3- 26). The proboscis and proboscis receptacle are significantly shorter in comparison to the *A. dattai* and *A. thapari* in both sexes. Lemnisci in case of the present species are shorter than both the morphologically comparable species. Further, 2–4 dorsal hypodermal nuclei are observed in the present species, whereas 1 dorsal and 2 ventral and 4–6 dorsal and 2 ventral hypodermal nuclei are

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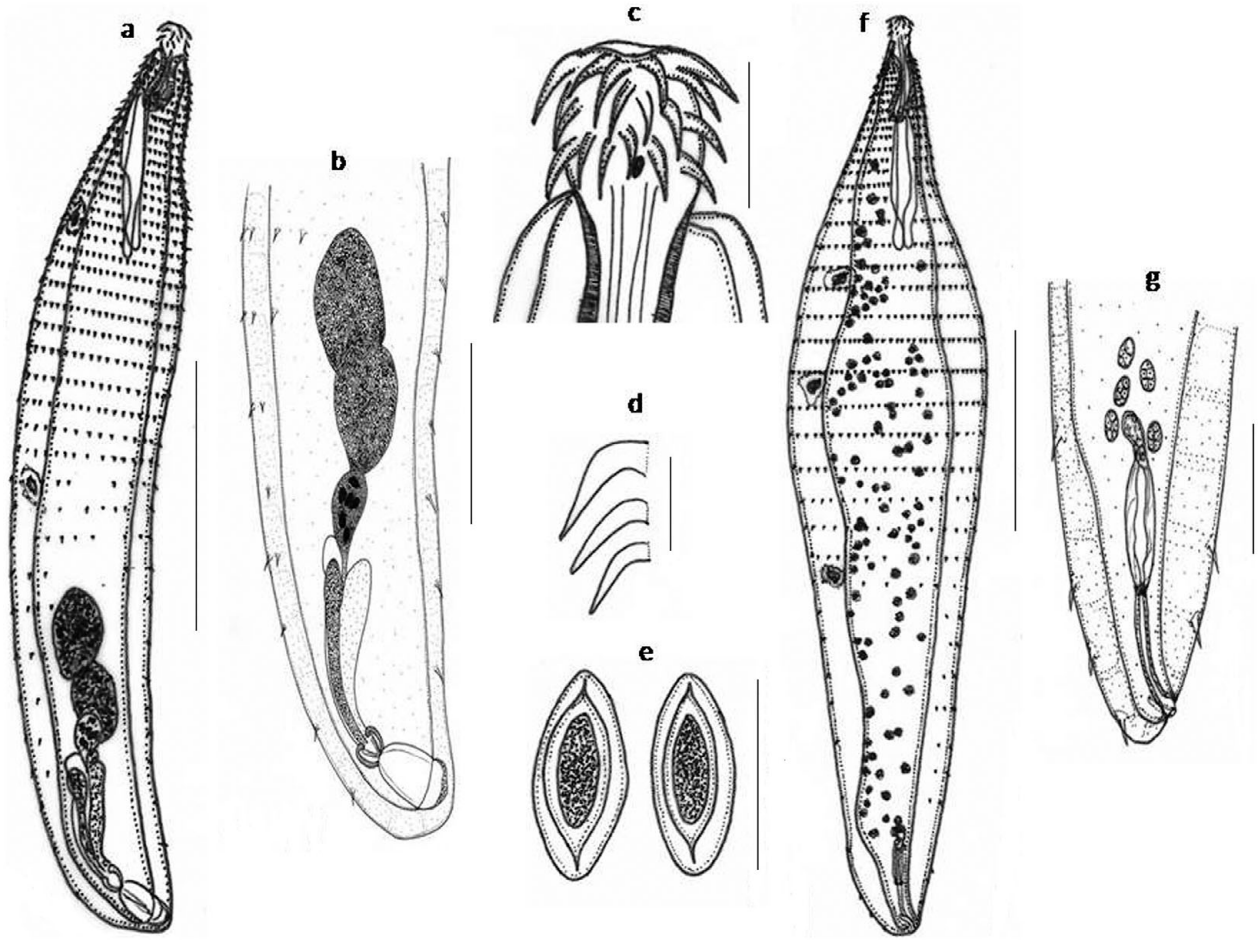

Figure 2. Line drawings of the specimens of *Acanthogyrus garciai* n. sp. a- male; b- posterior end of male; c- proboscis; d- proboscis hooks; e- mature egg; f- female; g- posterior end of female. Scale a- 1 mm; b- 300 μm; c- 100 μm; d- 50 μm; e- 20 μm; f- 1 mm; g- 250 μm.

reported in *A. thapari* and *A. dattai,* respectively. The size of male reproductive organs, including testes, cement gland, and seminal vesicle, are significantly smaller in the present species than other two species. The number of cement gland nuclei in *A. thapari* and *A. dattai* is 6–8, whereas in the case of present species 5–6 giant nuclei are observed. The female reproductive organs including vagina, uterus, and uterine bell of the present species are longer than the other two species. Uterus with coil near the uterine bell is observed in case of *A. thapari,* whereas no such coiling in uterus is observed in *A. dattai* and the present species. The female gonopore is subterminal located towards the ventral side in the present species, whereas gonopore is postero-ventral in *A. thapari* and *A. dattai.* Moreover, the present species have been isolated from *L. calbasu,* whereas the fish host of *A. thapari* is *Hilsa ilisha* and *A. dattai* is isolated from the multiple hosts, such as *Barbus ticto*, *B. stigma*, and *Puntius sophore.* Due to all above enlisted differences in the morphometric parameters, the above species is proposed as the new species under the genus *Acanthogyrus.*

Acanthogyrus golvani *Gupta and Jain, 1980*

Taxonomic summary

Class: Eoacanthocephala Van Cleave, 1936
Order: Gyracanthocephala Van Cleave, 1936
Family: Quadrigyridae Van Cleave, 1920
Subfamily: Pallisentinae Van Cleave, 1928
Genus: *Acanthogyrus* Thapar, 1927
Subgenus: *Acanthosentis* Verma and Datta, 1929
Species: *Acanthogyrus golvani* Gupta and Jain, 1980
Host: *Labeo rohita* Hamilton, 1822
Locality: Harike wetland, Punjab, India (31.15 °N, 74.97 °E)
Site of infection: Small intestine

Specimens submitted: Voucher specimens – male (HARC/ZSI/Ac-14a) and female (HARC/ZSI/Ac-14b) were deposited in the High Altitude Regional Centre-Zoological survey of India, Solan, Himachal Pradesh.

Sequences generated: The sequences submitted to the NCBI database on the basis of 18S, 28S, and ITS1-5.8S-ITS have been allotted accession number OP541603, OP476686, and OQ371288, respectively.

Specimens examined: 6 males and 7 females

Remarks

The present specimens isolated from the fish host *L. rohita* shows the characteristic features of the subgenus *Acanthosentis* in having 3 circles of 6 proboscis hooks each, the shape of trunk, and distribution of trunk spines. The morphometry of the present species matches the description of *A. golvani* (Gupta and Jain 1980), showing similarity in almost every taxonomically important feature (Table 3). Additionally, the length of the female reproductive organs of *A. golvani* is provided for the first time. *A. golvani* has been previously reported from Punjab, India from *M. seenghala* and *L. rohita*, which is the same locality as that in the present study.

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Table 2. Morphometric comparison between *Acanthogyrus garciai* n. sp. and other closely related species of the genus

Species	*Acanthogyrus garciai* n. sp.	*Acanthogyrus dattai* Podder, 1938	*Acanthogyrus thapari* Prasad, Sahay and Sambhunath, 1969
Host	*Labeo calbasu*	*Barbus ticto* and *B. stigma*	*Hilsa ilisha*
Locality	Punjab, India	West Bengal and Bihar, India	Bihar, India
Male length (mm)	3.22–4.0 (3.6)	1.34–3.34 (2.34)	3.35–3.85 (3.6)
Proboscis L x W (μm)	91.09–129.04 (110.06) × 81.51–117.9 (99.70)	120 × 55	185–190 (187.5) × 110
Circles of proboscis hooks	3	3	3
Number of proboscis hooks in each circle	6-8	6	6
Hooks from anterior to posterior (μm)	H1 53.82–65.79 (59.805) H2 51.47–54.61 (53.04) H3 43.17–53.86 (48.51)	H1 50 H2 30 H3 26	H1 48 H2 40–44 H3 28–36
Proboscis receptacle L × W (μm)	224.33–254.29 (239.31) × 63.90–70.55 (67.22)	420 × 120	540–615
Lemnisci L × W (μm)	L1 598.67 × 85.71 L2 570 × 97.19	L1 680 × 56 L2 590 × 55	–
Hypodermal nuclei	Dorsal 2–4 (3) Ventral not observed	Dorsal 4–6 (5) Ventral 2	Dorsal: 1 Ventral: 2
Circles of trunk spines	35–42 (38)	–	
Anterior testis L × W (μm)	273.07–320.53 (296.8) × 183.45–236.09 (209.77)	480 × 310	430–600 (515) × 220–310 (265)
Posterior testis L × W (μm)	260.08–293.95 (277.015) × 169.55–208.25 (188.9)	290 × 275	430–570 (500) × 225–312 (268.5)
Cement gland L × W (μm)	176.90–192.9 (184.9) × 58.90–65.85 (62.375)	350 × 260	240–300 (270) × 100–220 (160)
Cement gland nuclei	5–6	6–8 (7)	6–8 (7)
Cement reservoir L × W (μm)	88.99–143.29 (116.14) × 58.90–86.72 (72.81)	130 × 120	110–150 (130) × 60–125 (92.5)
Saefftigen's pouch	282.89–314.74 (298.815)	–	–
Seminal vesicle L × W	224.24–274.40 (249.32) × 62.27–100.77 (81.52)	–	330–450 (390)
Bursa L × W	130.85–162.49 (146.67) × 123.42–157.48 (140.45)	–	120–125 (122.5) × 110–135 (122.5)
Female length (mm)	3.4–4 (3.7)	1.67–9.46 (5.56)	3.35–4.9 (4.12)
Proboscis L × W (μm)	91.09–106.38 (98.735) × 61.51–100.9 (81.20)	–	175–205 (190) × 125–162 (143.5)
Hooks from anterior to posterior (μm)	H1 59.14–67.4 (63.27) H2 44.9–58.91 (51.90) H3 42.82–57.67 (50.24)	–	–
Proboscis receptacle L × W (μm)	198.22–327.66 (262.94) × 61.52–95.95 (78.735)	–	630
Lemnisci L × W (μm)	L1 503.15–546.64 (524.89) × 56.83–59.11 (57.97) L2 421.31–508.96 (465.13) × 56.17–62.08 (59.12)	–	–
Hypodermal nuclei	Dorsal: 2–4 (3) Ventral: not observed	–	–
Circles of trunk spines	25–45 (35)	–	–
Reproductive system L (μm)	409.33–621.65 (515.49)	–	–
Uterine bell L (μm)	38.14–63.80 (50.97)	95	110
Uterus L (μm)	170.56–268.89 (219.725)	290	50
Vagina L (μm)	200.63–288.96 (244.79)	175	–
Egg L × W (μm)	12.41–24.8 (18.60) × 8.08–14.47 (11.27)	26 × _	–

Acanthogyrus heterospinus Khan and Bilqees, 1990

Taxonomic summary

Class: Eoacanthocephala Van Cleave, 1936
Order: Gyracanthocephala Van Cleave, 1936
Family: Quadrigyridae Van Cleave, 1920
Subfamily: Pallisentinae Van Cleave, 1928
Genus: *Acanthogyrus* Thapar, 1927
Subgenus: *Acanthosentis* Verma and Datta, 1929

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Table 3. Morphometric comparison of the present specimens of *Acanthogyrus golvani* Gupta and Jain, 1980 with the original description

Species	*Acanthogyrus golvani* Gupta and Jain, 1980 (present study)	*Acanthogyrus golvani* Gupta and Jain, 1980 (original description)
Host	*Labeo rohita*	*Mystus seenghala* and *Labeo rohita*
Locality	Punjab, India	Punjab, India
Male length (mm)	7–10 (8.5)	7.3–10.7 (9)
Proboscis L x W (μm)	127.68–172.64 (150.16) × 79.98–105.70 (92.84)	120 × 100
Circles of proboscis hooks	3	3
Number of proboscis hooks in each circle	6	6
Hooks from anterior to posterior (μm)	H1 61.31–80.41 (70.86) H2 45.06–59.85 (52.455) H3 35.61–55.81 (45.75)	H1 53–75 (64) H2 64 H3 58
Proboscis receptacle L × W (μm)	324.03–417.62 (370.825) × 124.54–161.94 (143.24)	125–127 (127) × 70
Lemnisci L × W (μm)	–	L1 900–920 (910) × 160–190 (175) L2 135–145 (140) × 160–170 (165)
Hypodermal nuclei	Dorsal 4–5 Ventral 1–2	Dorsal 5 Ventral 2–3
Circles of trunk spines	50–63 (56)	51–54 (52)
Anterior testis L × W (μm)	329.50–449.49 (389.495) × 209.88–300.61 (255.245)	1080–1270 (1175) × 690–840 (765)
Posterior testis L × W (μm)	300.40–388.81 (344.605) × 210.41–298.12 (254.265)	970–1050 (1010) × 760–790 (775)
Cement gland L × W (μm)	122.87 × 130.69	670–930 (800) × 490–510 (500)
Cement gland nuclei	4–6 (5)	6
Cement reservoir L × W (μm)	–	240–280 (260) × 210–270 (240)
Saefftigen's pouch	–	–
Seminal Vesicle L × W	–	630–870 (750) × 330–450 (390)
Bursa L × W	224.15–259.61 (241.88) × 179.77–185.21 (182.49)	–
Female length (mm)	8–14 (11)	9.9–11.7 (10.8)
Proboscis L × W (μm)	127.32–162.61 (144.965) × 115.09–148.40 (131.745)	–
Hooks from anterior to posterior (μm)	H1 61.35–87.35 (74.35) H2 48.09–58.18 (53.135) H3 45.42–53.57 (49.495)	–
Proboscis receptacle L × W (μm)	390.13–399.79 (394.96) × 119.92–136.8 (128.365)	140 × 70
Lemnisci L × W (μm)	–	L1 1550 × 1500 L2 1440 × 100
Hypodermal nuclei	Dorsal 4–5 Ventral 1–2	–
Circles of trunk spines	55–60 (52)	59
Reproductive system L (μm)	1018.39–1416.46 (1217.42)	1050
Uterine bell L (μm)	184.67–261.75 (223.21)	–
Uterus L (μm)	554.01–769.75 (661.88)	–
Vagina L (μm)	279.71–384.96 (332.335)	–
Egg L × W (μm)	–	13 × 7

Species: *Acanthogyrus heterospinus* Khan and Bilqees, 1990

Host: *Labeo catla* Hamilton, 1822

Locality: Bhakra Punjab, India (31.15 °N, 74.97 °E)

Site of infection: Small intestine

Specimens submitted: Voucher specimens – male (HARC/ZSI/Ac-15) and female (HARC/ZSI/Ac-15) were deposited in the High Altitude Regional Centre-Zoological survey of India, Solan, Himachal Pradesh.

Sequences generated: The sequences submitted to the NCBI database on the basis of 18S, 28S, and ITS1-5.8S-ITS have been allotted accession number OP541602, OP476685, and OQ371286, respectively.

Specimens examined: 3 males and 8 females

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Table 4. Morphometric comparison of the present specimens of *Acanthogyrus heterospinus* Khan and Bilqees, 1990 with the original description

Species	*Acanthogyrus heterospinus* Khan and Bilqees, 1990 (present study)	*Acanthogyrus heterospinus* Khan and Bilqees, 1990 (original description)
Host	*Labeo catla*	*Labeo catla*
Locality	Punjab, India	Sindh, Pakistan
Male length (mm)	2.9–3.5 (3.2)	2.62–4.49 (3.55)
Proboscis L x W (μm)	88.98–107.04 (98.01) × 83.90–93.72 (88.81)	75–97 (86) × 90–120 (105)
Circles of proboscis hooks	3	3
Number of proboscis hooks in each circle	6	6
Hooks from anterior to posterior (μm)	H1 58.06–68.18 (63.12) H2 32.26–47.76 (40.01) H3 31.11–50.88 (40.995)	H1 55–59 (57) H2 37 H3 40–41 (40.5)
Proboscis receptacle L × W (μm)	189.57–277.85 (233.71) × 96.22	300–330 (315) × 120–140 (130)
Lemnisci L × W (μm)	L1 511.54–678.12 (594.83) × 98.56–143.88 (121.22) L2 562.09–689.16 (625.62) × 97.56–132.16 (114.86)	L1 760–960 (860) × 140–150 (145) L2 900–1090 (995) × 140–150 (145)
Hypodermal nuclei	Dorsal 4–6 (5) Ventral 2	–
Circles of trunk spines	27–39 (33)	30–36 (33)
Anterior testis L × W (μm)	349.32–376.71 (363.01) × 88.33–95.06 (91.95)	260–450 (355) × 310–400 (355)
Posterior testis L × W (μm)	313.58–324.60 (319.09) × 77.49–83.49 (80.49)	270–450 (360) × 300–340 (320)
Cement gland L × W (μm)	105.21–181.54 (143.375) × 84.82–115.76 (100.29)	110–150 (130) × 120
Cement gland nuclei	3 or more	many
Cement reservoir L × W (μm)	88.99–143.29 (116.14) × 58.90–86.72 (72.81)	110–150 (130) × 120
Saefftigen's pouch	247.97	330–420 (375)
Seminal vesicle L × W	–	250–360 (305) × 70–74 (72)
Bursa L × W	140.58–146.72 (143.65) ×103.87–106.69 (105.28)	330–420 (375) × 140–220 (180)
Female length (mm)	3.2–4.2 (3.7)	5.47–5.81(5.64)
Proboscis L × W (μm)	87.68–120.78 (104.23) × 55.11–85.32 (56.715)	100–120 (110) × 110–120 (115)
Hooks from anterior to posterior (μm)	H1 53.41–73.03 (63.22) H2 32.94–56.08 (44.51) H3 34.21–50.03 (42.12)	H1 64–66 (65) H2 38–43 (40.5) H3 44–46 (45)
Proboscis receptacle L × W (μm)	128.15–231.71 (179.93) × 65.25–82.99 (74.135)	–
Lemnisci L × W (μm)	L1 487.11–620.89 (554) × 78.44–99.15 (88.79) L2 465.65–598.13 (531.89) × 89.15–109.50 (99.32)	L1 620–910 (765) × 110–140 (125) L2 670–930 (800) × 120–140 (130)
Hypodermal nuclei	Dorsal 4–6 (5) Ventral 2	–
Circles of trunk spines	30–40 (39)	38–41 (39)
Reproductive system L (μm)	–	480–560 (520)
Uterine bell L (μm)	–	250–280 (265)
Uterus L (μm)	149.30–216.22 (182.76)	180–220 (200)
Vagina L (μm)	171.91–212.39 (192.15)	50–60 (55)
Egg L × W (μm)	11.90–16.63 (14.265) × 6.75–7.48 (7.115)	11–12 (11.5) × 7–7.4 (7.2)

Remarks

The present species shows the morphological characters of the subgenus *Acanthosentis* in body shape, trunk supination, and arrangement of proboscis hooks in 6 hooks per circle (Amin and Hendrix 1999). After studying the literature carefully, it was observed that the specimens recovered show morphological similarity with *A. heterospinus* (Khan and Bilqees 1990) from the same fish host *L. catla* from the Kalri Lake, Sindh, Pakistan. The present species shows similarity with *A. heterospinus* in a number of different taxonomically important features, including the total length of male and female, size of the proboscis hooks, number of trunk spines, and size of the reproductive organs (Table 4). The morphometric difference can be observed in the proboscis receptacle of male and the length of vagina in female. The size of

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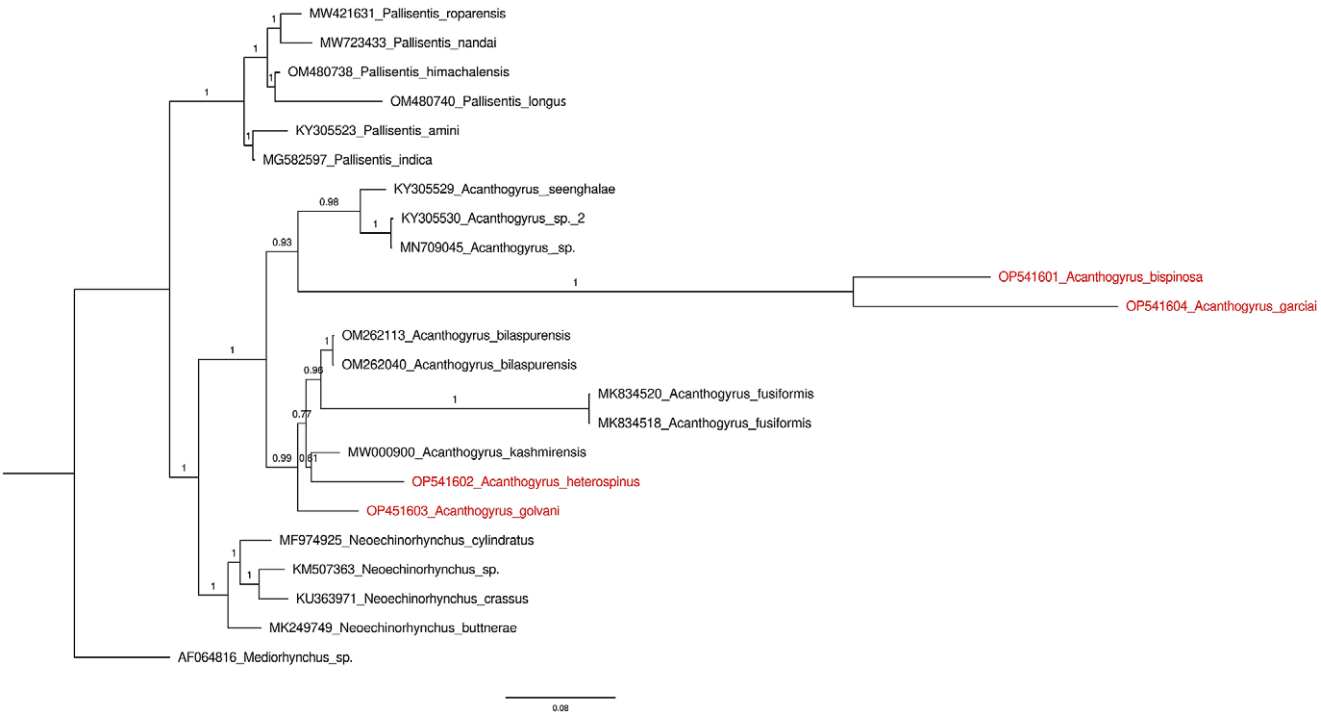

Figure 3. Bayesian inference phylogenetic tree of the genus *Acanthogyrus* inferred from 18S rRNA molecular marker. The numerical values near internal nodes represent Bayesian posterior probability values. The taxa for the sequences generated in the present study have been shown with red colour.

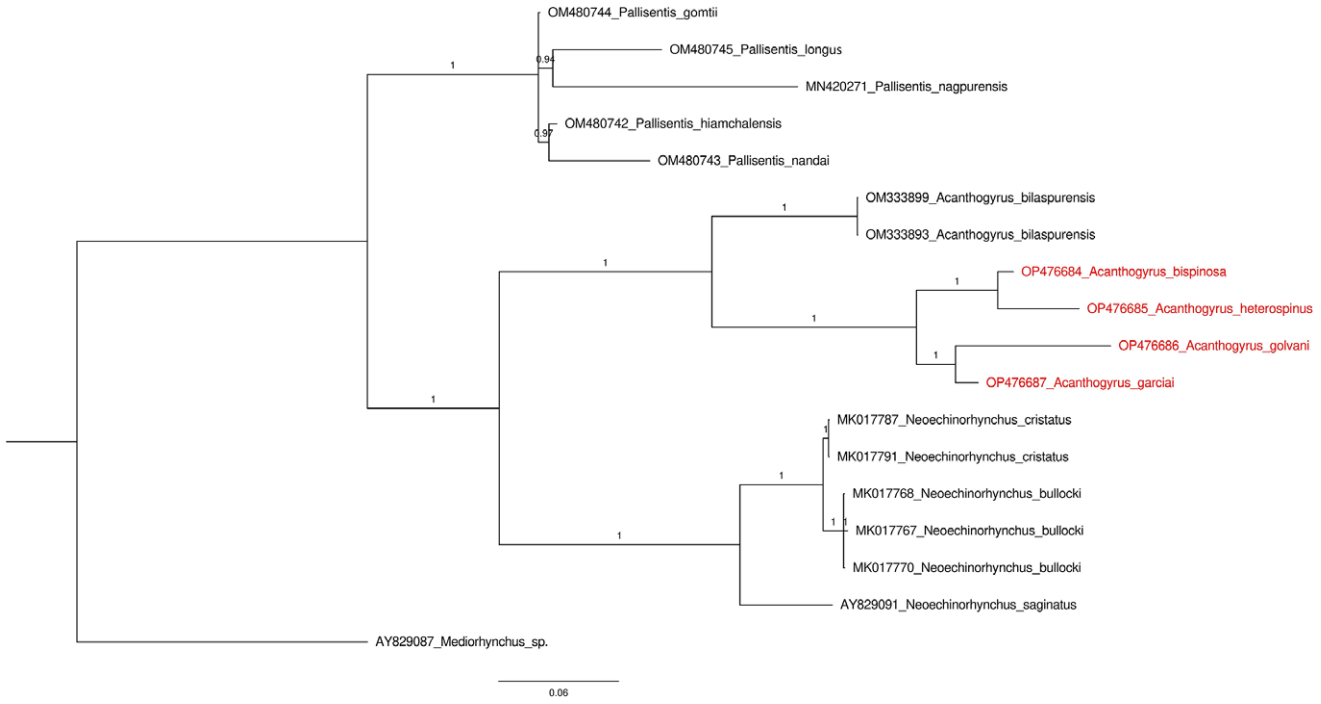

Figure 4. Bayesian inference phylogenetic tree of the genus *Acanthogyrus* inferred from 28S rRNA molecular marker. The numerical values near internal nodes represent Bayesian posterior probability values. The taxa for the sequences generated in the present study have been shown with red colour.

the proboscis receptacle in the present specimens is shorter than reported specimens in case of the male, whereas the size of proboscis receptacle in the female is not provided in the earlier description. Further, the length of vagina in the female is significantly longer in the specimens recovered in the present study in comparison to the earlier observations.

Molecular results

The Bayesian inference phylogenetic tree on the basis of the 18S rRNA molecular marker comprised a total of 23 taxa with 2112 characters, which included 12 sequences of genus *Acanthogyrus*, 6 sequences of the genus *Pallisentis*, and 4 sequences of

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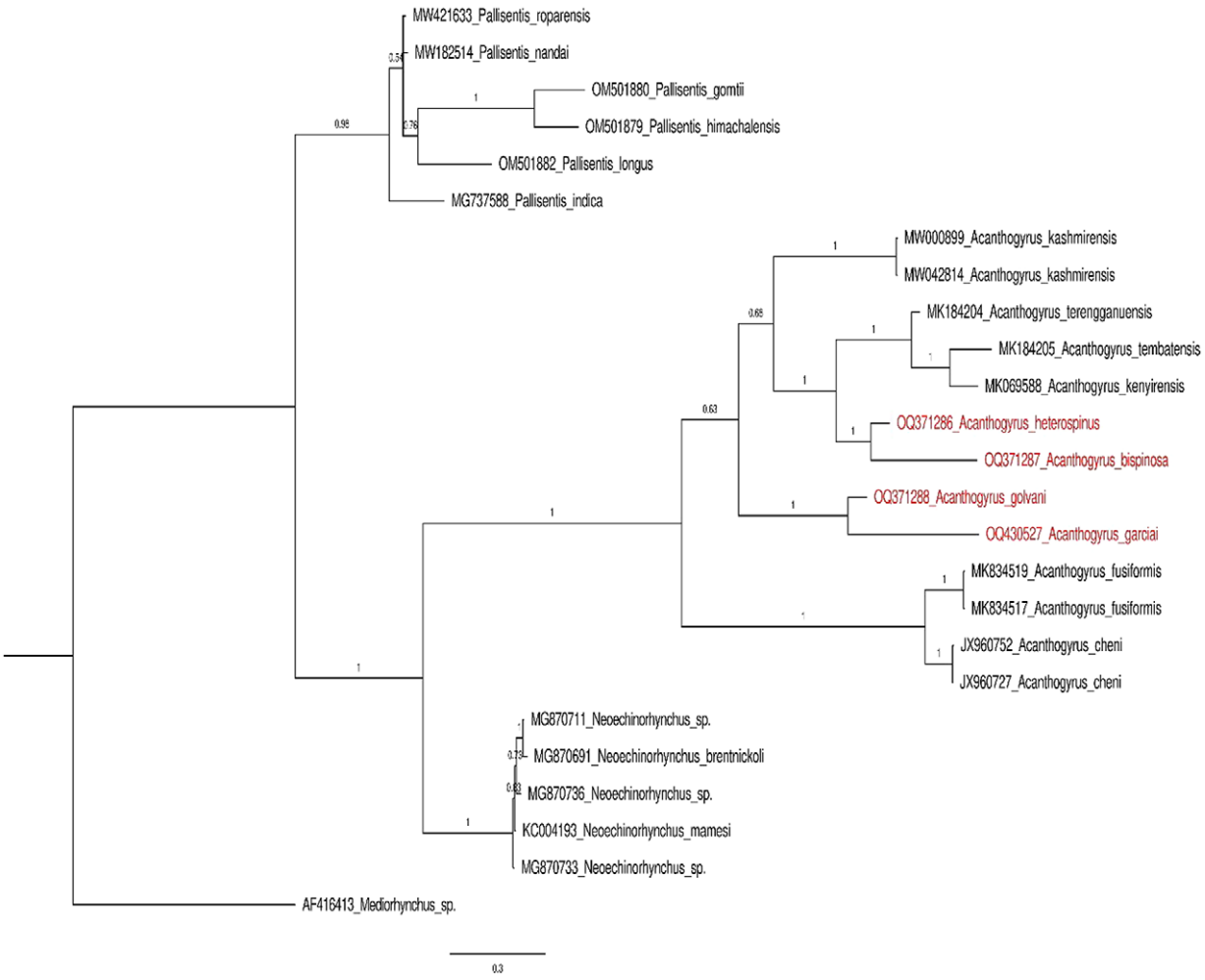

Figure 5. Bayesian inference phylogenetic tree of the genus *Acanthogyrus* inferred from ITS1-5.8S-ITS2 rRNA molecular marker. The numerical values near internal nodes represent Bayesian posterior probability values. The taxa for the sequences generated in the present study have been shown with red colour.

genus *Neoechinorhynchus*. The sequence of *Mediorhynchus* sp. (AF064816) was taken as an outgroup for the dataset. The tree initially bifurcated into 2 clades with the maximum posterior probability value. One of the clades comprised the sequences of the genus *Pallisentis,* whereas the other clade included the sequences of the genus *Acanthogyrus* and genus *Neoechinorhynchus* (Figure 3). The second clade was again bifurcated into 2 subclades, one of which comprised all the sequences of genus *Acanthogyrus,* whereas the other contained the sequences of the genus *Neoechinorhynchus.* All the sequences of *Acanthogyrus* were clustered in 2 clades. One subclade included the sequences of *A. seenghalae* (KY305529), the newly generated sequences of *A. bispinosa* n. sp. (OP541601) and *A. garciai* n. sp. (OP541604) along with the 2 unspecified sequences (KY305530 and MN709045). The other clade within *Acanthogyrus* included the sequences of *A. bilaspurensis* (OM262113 and OM262040), *A. fusiformis* (MK834520 and MK834518), *A. kashmirensis* (MW000900) along with the newly generated sequences of the species *A. heterospinus* (OP541602), and *A. golvani* (OP541603). The sequences of all *Acanthogyrus* species showed their distinct identities. The genetic divergence between the newly generated sequences of *A. bispinosa* n. sp. (OP541601) and *A. garciai* n. sp. (OP541604) is 21.0%. The genetic divergence between *A. bispinosa* n. sp. (OP541601) and *A. bilaspurensis* (OM262113 and OM262040) is 46.8%. The genetic divergence of *A. bispinosa* n. sp. with *A. fusiformis* (MK834518 and MK834520), *A. kashmirensis* (MW000900), *A. heterospinus* (OP541602), and *A. golvani* (OP541603) is 43.4, 40.3, 35.3, and 36.7%, respectively. Similarly, the genetic divergence of *A. garciai* n. sp. (OP541604) with *A. bilaspurensis* (OM262113 and OM262040), *A. fusiformis* (MK834518 and MK834520), *A. kashmirensis* (MW000900), *A. heterospinus* (OP541602), and *A. golvani* (OP541603) is 53.3, 46.0, 48.5, 39.9, and 39.3%, respectively.

The Bayesian inference phylogenetic tree generated on the basis of the 28S rRNA molecular marker included a total of 18 taxa in the dataset with 3083 characters. The dataset comprised 6 sequences of *Acanthogyrus,* 5 sequences of *Pallisentis*, and 6 sequences of *Neoechinorhynchus.* The sequence of *A. maroccanus* (MK953673) has not been included in the dataset due to the very large genetic divergence of the sequence in the whole dataset which might lead to long branch biasness of the analysis. The sequence of *Mediorhynchus* sp. (AY829087) was taken as an outgroup for the analysis. The phylogenetic tree initially bifurcated into 2 clades. One clade included the sequences of the genus *Pallisentis*, and the other clade included the sequences of the genera *Acanthogyrus* and *Neoechinorhynchus*, which are further clustered into 2 distinct subclades (Figure 4). All the sequences of *Acanthogyrus* were clustered within a single clade and further clustered into 2 subclades. The one subclade included the sequences of *A. bilaspurensis* (OM333899

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and OM333893), and the other subclade included the sequences of the newly generated sequences of *A. bispinosa* n. sp. (OP476684), *A. heterospinus* (OP476685), *A. golvani* (OP476686), and *A. garciai* n. sp. (OP476687). The sequences generated in the present study showed the distinct identities of all the species described. The genetic divergence between the newly generated sequences of *A. bispinosa* n. sp. (OP476684) and *A. garciai* n. sp. (OP476687) is 4.6%. The genetic divergence between *A. bispinosa* n. sp. (OP476684) and *A. bilaspurensis* (OM333899 and OM333893) is 8.2%. The genetic divergence of *A. bispinosa* n. sp. (OP476684) with *A. golvani* and *A. heterospinus* is 8.4 and 3.2%, respectively. Similarly, the genetic divergence between *A. garciai* n. sp. and *A. golvani* (OP476686) and *A. heterospinus* (OP476685) is 5.5 and 6.9%, respectively.

The Bayesian inference phylogenetic tree generated on the basis of the ITS1-5.8S-ITS2 molecular marker in the study included a total of 25 taxa in the dataset with 1539 characters. The dataset comprised 13 sequences of *Acanthogyrus,* 6 sequences of *Pallisentis*, and 5 sequences of *Neoechinorhynchus.* The sequence of *Mediorhynchus* sp. (AF416413) was taken as an outgroup for the analysis. The tree bifurcated initially into 2 clades. One clade included all the sequences of *Pallisentis*, and the other branch comprised the sequences of *Acanthogyrus* and *Neoechinorhynchus* (Figure 5). The sequences of *Acanthogyrus* are clustered again into two subclades. The first subclade included isolates of *A. bispinosa* n. sp. (OQ371287)*, A. garciai* n. sp. (OQ430527), *A. golvani* (OQ371288), and *A. heterospinus* (OQ371286) along with the sequences of *A. kashmirensis* (MW000899 and MW042814), *A. terengganuensis* (MK184204), *A. tembatensis* (MK184205), and *A. kenyirensis* (MK069588). The other subclade included the sequences of *A. cheni* (JX960572 and JX960727) and *A. fusiformis* (MK834517 and MK834519). The genetic divergence between the newly generated sequences of *A. bispinosa* n. sp. (OQ371287) and *A. garciai* n. sp. (OQ430527) is 46.6%. The genetic divergence of *A. bispinosa* n. sp. (OQ371287) with *A. golvani* (OQ371288), *A. heterospinus* (OQ371286), *A. kashmirensis* (MW000899 and MW042814), *A. terengganuensis* (MK184204), *A. tembatensis* (MK184205), *A. kenyirensis* (MK069588), *A. cheni* (JX960572 and JX960727), and *A. fusiformis* (MK834517 and MK834519) is 39.6, 23.6, 39.1, 32.9, 40.9, 38.6, 48.4, and 49.2%, respectively. Similarly, the genetic divergence of *A. garciai* n. sp. (OQ430527) with *A. golvani* (OQ371288), *A. heterospinus* (OQ371286), *A. kashmirensis* (MW000899 and MW042814), *A. terengganuensis* (MK184204), *A. tembatensis* (MK184205), *A. kenyirensis* (MK069588), *A. cheni* (JX960572 and JX960727), and *A. fusiformis* (MK834517 and MK834519) is 26.6, 42.7, 44.0, 44.5, 51.4, 50.3, 53.6, and 52.6%, respectively.

Discussion

The present study reports 4 species of the genus *Acanthogyrus* of which two species, *A. bispinosa* n. sp.and *A. garciai* n. sp., are proposed as new from *C. mrigala* and *L. calbasu,* respectively, and the other two, *A. heterospinus* and *A. golvani*, are redescribed from *L. catla* and *L. rohita,* respectively. In this study, *A. bispinosa* n. sp. and *A. garciai* n. sp. have been placed in the subgenus *Acanthosentis. A. bispinosa* n. sp. shows two sac-like structures above the seminal vesicle, which may be the segments of vas efferens. Similar structures have also been reported in *A. kashmirensis* where the sperm duct is divided into 3 segments (Amin *et al.* 2017). Notably, two cement glands have also been mentioned in the description of *A. Acanthogyrus,* which probably can be the rudiments of the cement gland in immature adults (Petrotschenko 1956).

The molecular data of 4 species generated on the basis of 18S rRNA, 28S rRNA, and ITS1-5.8S-ITS2 molecular markers show the distinct identity of respective species. The sequences of the different *Acanthogyrus* species generated in this study are well nested among the other sequences of the genus. Notably, the molecular data of *Acanthogyrus* are almost negligible in comparison to the morphologically described species. To date, only 8 sequences representing 4 species (excluding present study) on the basis of the 18S rRNA molecular marker including 2 unspecified sequences, 3 sequences (excluding present study) representing 2 species on the basis of the 28S rRNA molecular marker, and 53 sequences representing 3 species (excluding present study) on the basis of the ITS1-5.8S-ITS2 molecular marker including 2 unspecified sequences and 46 sequences of the same species, *A. chenni,* are present in the database (to make the phylogenetic tree brief, only 2 sequences of *A. chenni* have been included in the dataset). All the sequences of *Acanthogyrus* have been observed to be clustered in 2 clades. Additionally, the Bayesian tree constructed on the basis of 18S, 28S, and ITS1-5.8S-ITS2 molecular markers showed the clustering of the sequences of *Aacnthogyrus* and *Neoechinorhynchus* in a single larger clade which then bifurcates into two subclades genus-wise. This clustering depicts that genus *Acanthogyrus* is phylogenetically closer to the genus *Neoechinorhynchus,* which has also been reported by Gautam *et al.* (2020) and Ru *et al.* (2022). Notably, the limited molecular data of *Acanthosentis* possess a problem in elucidating phylogenetic relationships of this group with other genera (Amin *et al.* 2019). However, due to the unavailability of the sufficient data, it would be too early to deduce any conclusive results on the phylogenetic relationships within the genus as well as among these genera. Moreover, the molecular data of the species belonging to the subgenus *Acanthogyrus* remain unavailable. The confusion among the species due to the variable morphological features and subgeneric classification cannot be justified unless either the detailed examination of material is conducted or more descriptive morphological data are provided along with the molecular characterisation of the previously reported species.

Conclusively, additional molecular data are required to deduce the evolutionary relationships of this acanthocephalan group. Further, the question regarding the monophyletic or paraphyletic origin of the species of *Acanthosentis* also warrants additional analysis. The data presented in this study will be helpful in evaluating the phylogenetic relationships of genus *Acanthogyrus* and higher taxa in the future.

Data availability. The datasets generated during the current study are available from the corresponding author on reasonable request.

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Competing interest. The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical standard. Ethical clearance has been obtained from the Institutional Animal Ethics Committee (IAEC) of Panjab University (Approval no.: PU/45/99/CPCSEA/IAEC/482).

Consent for publication. All authors agree to publish this manuscript.

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