

Research Paper


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Taxonomic summary of *Schyzocotyle* (Cestoda: Bothriocephalidae) with a redescription of *Schyzocotyle nayarensis* (Malhotra, 1983) from India

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Abstract

In this study, we use an integrative taxonomic approach to redescribe *Schyzocotyle nayarensis* (Malhotra, 1983) (Cestoda: Bothriocephalidae), based on newly collected specimens from the type-host *Raiamas bola* (Hamilton, 1822) (Cypriniformes: Danionidae) in Fulbari, Siliguri, West Bengal, India. The detailed morphological assessment, from whole mounts, histology and scanning electron microscopy, offers additional insights into the scolex structure, vitelline follicles, and egg morphology. Molecular data from this and previous studies corroborate the identity and systematics of *S. nayarensis* as a bothriocephalid closely related to the Asian Fish Tapeworm, *Schyzocotyle acheilognathi* (Yamaguti, 1934). This study elucidates the historical context and taxonomic ambiguities surrounding *S. nayarensis*, emphasizing the key role of the scolex in both generic and species identification. Amendments to the diagnosis of *Schyzocotyle* Akhmerov, 1960 are proposed. A differential diagnosis of the two valid species within the genus, namely *S. acheilognathi* and *S. nayarensis*, is also provided. An evaluation of the taxonomic status of *Bothriocephalus teleostei* Malhotra, 1984, and *Capooria barilii* Malhotra, 1985 suggests that they may be *S. nayarensis*. Finally, we posit that none of the ten species of *Ptychobothrium* Lönnberg, 1889 described from Indian freshwater teleosts belong to this genus but instead appear to be a mix of species belonging to *Schyzocotyle*, *Senga* Dollfus, 1934, and possibly even Proteocephalidae La Rue, 1911; all require further study based on newly collected, properly fixed specimens and an integrated taxonomic approach. Finally, future survey studies may reveal hidden diversity of *Schyzocotyle* species in Indian cyprinoids.

Introduction

The genus *Schyzocotyle* Akhmerov, 1960 (Cestoda: Bothriocephalidea) was originally proposed by Akhmerov (1960) for the type species, *S. fluviatilis* Akhmerov, 1960. Dubinina (1982) suppressed *Schyzocotyle* by synonymising its type and only species *S. fluviatilis* with *Bothriocephalus acheilognathi* Yamaguti, 1934. More recently, however, Brabec *et al.* (2015) resurrected *Schyzocotyle*, based on a molecular phylogenetic analysis of Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008 that provided strong support for the genus. At present, *Schyzocotyle* comprises two species: the well-known *Schyzocotyle acheilognathi* (Yamaguti, 1934) as the type species and the lesser-known *Schyzocotyle nayarensis* (Malhotra, 1983) Brabec, Waeschenbach, Scholz, Littlewood & Kuchta, 2015, described from India (Brabec *et al.*, 2015).

Schyzocotyle acheilognathi (the Asian Fish Tapeworm) has been reported on all continents except Antarctica, earning the distinction of being one of the most successful invasive helminths (Kuchta *et al.*, 2018). The cosmopolitan distribution of this parasite highlights its ability to infect various fish hosts across different orders and families (Choudhury & Cole, 2011; Brabec *et al.*, 2015; Kuchta *et al.*, 2018).

Schyzocotyle nayarensis was described by Malhotra (1983) as *Ptychobothrium nayarensis*, from two cyprinoid hosts, trout barb, *Barilius* (= *Raiamas*) *bola* (Hamilton, 1822) (Danionidae), and snowtrout, *Schizothorax richardsonii* (Gray, 1832) (Cyprinidae), from the Pauri-Garhwal district, Uttarakhand, India. Kuchta & Scholz (2007) synonymised this species with *Bothriocephalus* (= *Schyzocotyle*) *acheilognathi* based on morphological similarities, a conclusion supported by Kuchta *et al.* (2008a), but without examining specimens. Several years later, Brabec *et al.* (2015) sequenced new tapeworm material from *Raiamas bola* in West Bengal, resurrected *Schyzocotyle*, and proposed a new combination, *Schyzocotyle nayarensis*, but without a morphological study.

Recognizing the need for an integrative taxonomic approach to the species composition of *Schyzocotyle*, we present the results of a comparative study of the two species of the genus using morphology and molecular analyses. In particular, the taxonomic status of *S. nayarensis* is

critically evaluated and the species is redescribed based on freshly collected specimens from one of two original fish hosts, *Raiamas bola*, in West Bengal, India. Additionally, we discuss the taxonomic status of *Bothriocephalus teleostei* Malhotra, 1984, *Capooria barilii* Malhotra, 1985, and 10 species of *Ptychobothrium* Lönnberg, 1889, described from Indian freshwater fishes (Phad, 1983; Malhotra, 1984a, 1985; Wadhawan, 1985; Kadam, 1993; Ghosh, 2013; Deshmukh *et al.*, 2015, 2016; Barshe, 2018; Bhure *et al.*, 2019; Gaikwad, 2019; Nanware *et al.*, 2019).

Materials and methods

Field study and sample collection

Tapeworms were collected from the intestine of *Raiamas bola* at Fulbari (Mahananda River basin) in Siliguri, West Bengal, India. Fresh fish were purchased from local fishermen. Live fish were killed by dorsal pithing (including spinal cord) and severing the spinal cord immediately behind the head. Fish were dissected 1 to 2 h after collection to avoid decomposition and ensure that fresh specimens were collected. Tapeworms were cleansed in 0.9% NaCl solution (saline). After separating a small portion (the posterior-most proglottids) of the worm and storing it in 100% molecular grade ethanol, the remaining (anterior) corresponding portion of the worms were placed in a Petri dish containing a small volume of saline and fixed by pouring hot (almost boiling) 4% formaldehyde solution over them (see Chervy, 2024). Some worms were directly placed in 100% molecular grade ethanol for further molecular study and few entire worms were fixed with hot 4% formaldehyde solution to get the full body measurements.

Preparation of specimens for morphological evaluation

Following fixation in formalin for two to three weeks, the tapeworms were transferred to 70% ethanol (for storage) and the remaining processes, i.e., staining and preparation of whole mounts, histological sections, and scanning electron microscopy (SEM), were accomplished. For permanent whole mounts, specimens were stained with Mayer's hydrochloric carmine, dehydrated in an ascending ethanol series, cleared in eugenol (clove oil) and mounted on slides using Damar gum. For histological sections, the scolex and a few portions of the strobila were dehydrated, embedded in paraplast, sectioned (at a thickness of 5–7 μm) and stained with Weigert's haematoxylin-eosin. Five scoleces, portions of the strobila and eggs were processed for SEM as follows: specimens were dehydrated through a graded ethanol series, transferred to hexamethyldisilazane (see Kuchta & Caira, 2010), dried in air, sputter-coated with gold (approximately 7–10 nm thick), and examined with a Zeiss Sigma-300 FE-SEM microscope. Eggs isolated from gravid proglottids in a Petri dish containing distilled water were measured and photographed. For observation and detailed line drawing of whole mounted specimens, an Olympus BX53F2 microscope with Nomarski interference contrast optics and a drawing attachment was used.

Scientific and common names of fish hosts follow Froese & Pauly (2024). The terminology of microtriches follows Chervy (2009). Morphometric characters of the scolex and apical disc resulted from the measurements taken from both the lateral and dorsoventral side. Abbreviations of the terms used in descriptions are as follows: n = number of measurements, L/W = length/width ratio, WA/LS = width of apical disc/length of scolex, WS/LS = width of scolex/length of scolex.

Prepared slides of the newly collected specimens were deposited in the following museum collections: Zoological Survey of India, Kolkata, India (ZSI) and Helminthological Collection of the Institute of Parasitology of the Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS) (see details in taxonomic summary under Results section).

Molecular study

Tapeworm tissue from four gravid worms, stored in 100% molecular-grade ethanol, was air-dried in 1.5 mL microcentrifuge tubes for 30–40 min to remove residual ethanol, following which genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA) and the manufacturer's protocol. The *ITS-2* region of the rRNA gene array and the cytochrome *c* oxidase subunit 1 (*COI*) gene were partially amplified by polymerase chain reaction (PCR) from one and four samples, respectively. PCR reactions were performed on an Applied Biosystems Veriti thermal cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) using 0.25–0.5 μL of Ex Taq DNA polymerase (TaKaRa Bio USA, Inc., Mountain View, CA) in a total reaction volume of 50 μL containing 31.75 μL of nuclease free water (Qiagen, Inc.), 5 μL of extracted DNA as template, 2 μL each of forward and reverse primers at a concentration of 1 pmol/ μL , 5 μL of 10X Ex Taq Buffer (Mg²⁺ plus) (TaKaRa Bio USA Inc.) and 4 μL (200 μM) of deoxynucleoside triphosphates (TaKaRa Bio USA Inc.). The following primers were used for amplification: For *ITS-2*, Proteo-1: 5'-CGGTGGATCACTCGGCTC-3' (forward) and Proteo-2: 5'-TCCTCCGCTTATTGATATGC-3' (reverse) (Škeříková *et al.*, 2004), and for *COI*, JB3 (=2575 of Bowles *et al.*, 1992): 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' (forward) and JB5 5'-TAAAGAAAGAACATAATGAAAATG-3' (reverse) (Bowles *et al.*, 1992; Derycke *et al.*, 2005). The amplification protocol consisted of an initial denaturing cycle of 5 min at 94 °C, 25–35 cycles of the following: 94 °C for 30 sec, 54 °C for 30 sec, 72 °C for 1 min, and a final elongation at 72 °C for 5 or 7 min. PCR products were purified with ExoSAP-IT Express PCR Product Cleanup (Affymetrix, Inc., Santa Clara, CA). Purified products were sent to MCLab (South San Francisco, CA), for automated Sanger sequencing. The PCR primers were used for sequencing.

The amplicon sequences were manually checked, edited for accuracy, trimmed in FinchTV (Geospiza Inc., Seattle, WA), and a consensus *ITS-2* and *COI* partial sequences assembled in MEGA X (Kumar *et al.*, 2018). The 672-bp-long partial *ITS-2* sequence was aligned with four isolates of *S. acheilognathi* (DQ866997, DQ866995, AF362431, AF362430), one of *Schyzocotyle nayarensis* (KX060598) obtained from GenBank (www.ncbi.nlm.nih.gov) using Clustal W in MEGA X. Similarly, the four partial (321 bp long) *COI* sequences were aligned with *COI* sequences of *S. acheilognathi* (OM675718, PP210023, MG968746, MG968745, MG968744, KR780792), *S. nayarensis* (KR780829), *Bothriocephalus scorpii* (Müller, 1776) (KR780788) and *B. claviceps* (= *Bothriocestus claviceps*) (Goeze, 1782) (KR780818) downloaded from GenBank.

The sequences generated in this study were deposited in GenBank with the following accession numbers: *ITS-2*: PQ134488. *COI*: PQ134520, PQ134521, PQ134522, PQ134523.

Phylogenetic analysis

There were 321 positions in the final aligned *COI* sequence dataset. Phylogenetic trees were generated from this aligned *COI* dataset using the Maximum Likelihood method based on the GTR + G + I

(General Time Reversal + G + I) model as implemented in MEGA X. GTR + G + I was determined to be the appropriate model by first analyzing the dataset for best fit using the algorithm implemented under 'Test Model' in MEGA X. Initial trees for the heuristic search were obtained using Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (four or five categories [+G]) and the rate variation model allowed for some sites to be evolutionarily invariable ([+I]). All sites were used for the analyses. Codon positions included were 1st + 2nd + 3rd + Non-coding. The resultant ML tree was rooted using the *COI* sequence of *Bothriocephalus scorpii* (KR780788) based on a previous phylogenetic analysis of bothriocephalidean tapeworms (Brabec *et al.*, 2015).

Results

Morphological evaluation

Based on the newly collected specimen the species is redescribed here.

Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008

Bothriocephalidae Blanchard, 1849

Schyzocotyle Akhmerov, 1960

Schyzocotyle nayarensis (Malhotra, 1983) Brabec, Waeschensch, Scholz, Littlewood & Kuchta, 2015 (Figs. 1–4)

Redescription (based on 30 specimens from *Raiamas bola* from West Bengal, India, including 10 specimens for SEM and five sectioned specimens; measurements in micrometres unless otherwise stated. Measurements of Malhotra (1983) are given in brackets): Strobila up to 91 mm (n = 5) long [12–27 mm] with numerous acraspedote proglottids, up to 1800 (n = 5) wide [1131]. Proglottids usually wider than long, variable in size. Immature proglottids without primordia of genital organs numerous. Mature proglottids few in number, 281–531 [208–517] long by 969–1613 [924–1631] wide, L/W = 1:1.4–5.2 (n = 15); gravid proglottids vary in number (9–140) in different worms, 333–833 [216–650] long by 700–1800 [443–1605] wide, L/W = 1:1.3–4.3 (n = 200).

Inner longitudinal musculature well developed, formed by numerous bundles of muscle fibres (Fig. 1d). Surface of first proglottids covered with capilliform filitriches (Fig. 2c). Several pairs of osmoregulatory canal (Fig. 3a); canals difficult to see in mature and gravid proglottids. Ventral canals wide, thin-walled, usually in two pairs crossing testicular fields, slightly sinuous. Dorsal canals usually in two pairs, with median canals wide, thin-walled and more lateral canals narrow, thick-walled, sinuous. One pair of thin-walled lateral canals at level of lateral-most vitelline follicles. Canals may anastomose.

Scolex lanceolate (arrowhead-shaped), with weakly developed apical disc (Figs 1a, 2a & 4a), 963–1600 [652–1250] long by 831–1540 [434–1241] wide; L/W = 1:0.82–1.06 (n = 17). Apical disc 131–194 [156–234] long by 213–388 [221–377] wide, occupies 20–30% width of scolex (n = 17) (Fig. 4c). Bothria deep, narrow, with simple non-crenulated margins, 775–1414 [403–980] long by 306–622 [91–500] deep; L/W = 1:0.35–0.57 (n = 32) (Figs 1b, 2a & 4b, d). 'Median column', structure between two bothria, uniform in width with slightly enlarged anterior end (Fig. 1a, b). Surface of scolex covered with coniform spinitriches and capilliform filitriches (Fig. 2b, d). Neck absent; width of strobila ('peduncle') immediately posterior to scolex 148–201.

Testes medullary, in two lateral fields, almost spherical, larger than vitelline follicles, 19–50 [26–79] in diameter (n = 100), 70–170 [52–78] in number per proglottid (n = 23), absent medially and near lateral margins, confluent between proglottids (Figs 1e, f & 3a, c). Cirrus-sac spherical, muscular, thick-walled, anterior to ovary, 94–144 [50–343] long by 69–94 [40–165] wide, length/width ratio 1.25–1.75 (n = 15), slightly pre-equatorial, equatorial to slightly post-equatorial; cirrus unarmed (Figs 1e, f & 3b). Internal seminal vesicle present, Vas deferens forms numerous loops anterolateral to cirrus-sac. Gonopore dorsal, median, slightly post-equatorial to almost equatorial (Figs 1f, 2e, g & 3b).

Ovary asymmetrical, median, lateral arms are formed by individual grape-like lobes, bilobed, usually at distance from posterior margin of proglottids, 131–213 [12–156] long by 219–400 [105–273] wide, representing 36–56% of length of proglottids and 22–30% of width of proglottids (n = 15) (Figs 1e, f & 3c). Vagina a straight, thick-walled tube, opens posterior to cirrus-sac into common gonopore; vaginal sphincter absent (Fig. 3b). Vitelline follicles numerous, small, mostly spherical, 16–25 [9–76 × 10–99] (n = 100) in diameter, almost circumcortical, missing medially in most uterine region, confluent between proglottids (Figs 1d–f & 3d).

Uterine duct sinuous, forms numerous tightly coiled loops, filled with eggs, enlarged in gravid proglottids. Uterine sac near anterior margin of proglottids, slightly submedian, alternating irregularly in position, thick-walled, spherical to transversely oval, enlarged in gravid proglottids to occupy large part of proglottids. Uterine pore submedian to median, thin-walled, open near anterior margin of proglottids (Figs 1e, 2f, h & 3c). Eggs operculate, unembryonated, 42–47 [10–49] long by 31–34 [10–46] wide (n = 52) (Fig. 2i–k).

Taxonomic summary

Synonyms: *Ptychobothrium nayarensis* Malhotra, 1983; *Bothriocephalus teleostei* Malhotra, 1984; *Capooria barilii* Malhotra, 1985

Unavailable names: *Ptychobothrium tetraodoni* Ghosh, 2013; *Ptychobothrium bariliusi* Ghosh, 2013

Type host: Not specified; first listed *Raiamas bola* (Hamilton, 1822), followed by *Schizothorax richardsonii* (Gray, 1832).

Additional host (tentative): *Tetraodon* (= *Leiodon*) *cutcutia* (Hamilton, 1822), *Puntius* (= *Systomus*) *sarana* (Hamilton, 1822). In addition, *Bothriocephalus teleostei*, which is considered a synonym of *S. nayarensis*, has been reported by Malhotra (1984b, 1989), Malhotra & Chauhan (1984) and Chauhan & Malhotra (1984, 1986) from other cyprinoids such as *Barilius* (= *Opsarius*) *bendelisis* (Hamilton, 1807), *Garra gotyla gotyla* (= *Garra gotyla*) (Gray, 1830), *Labeo* (= *Bangana*) *dero* (Hamilton, 1822), *Labeo rohita* (Hamilton, 1822), *Labeo dyocheilus* (McClelland, 1839), *Schizothorax plagiostomus* Heckel, 1838 and *Tor tor* (Hamilton, 1822). However, none of these reports contain morphological description of the cestodes found, and voucher specimens were never deposited.

Type locality: Nayar River (East and West), Pauri Garhwal, Uttarakhand, India.

Other localities: Fulbari Dam Lake, south of Siliguri, West Bengal, India; Bongaon, North 24-Parganas, West Bengal, India; Mynaguri, West Bengal, India; Bodh Gaya, Bihar, India.

Distribution: India (Bihar, Uttarakhand, and West Bengal). It is possible that *S. nayarensis* occurs in *Schizothorax* spp. outside India in the countries in which bothriocephalid tapeworms have been reported from snowtrout (*Schizothorax*), such as China, Pakistan, Tajikistan and Uzbekistan (see references in Kuchta *et al.*, 2018). However, none of these reports contain morphological data,

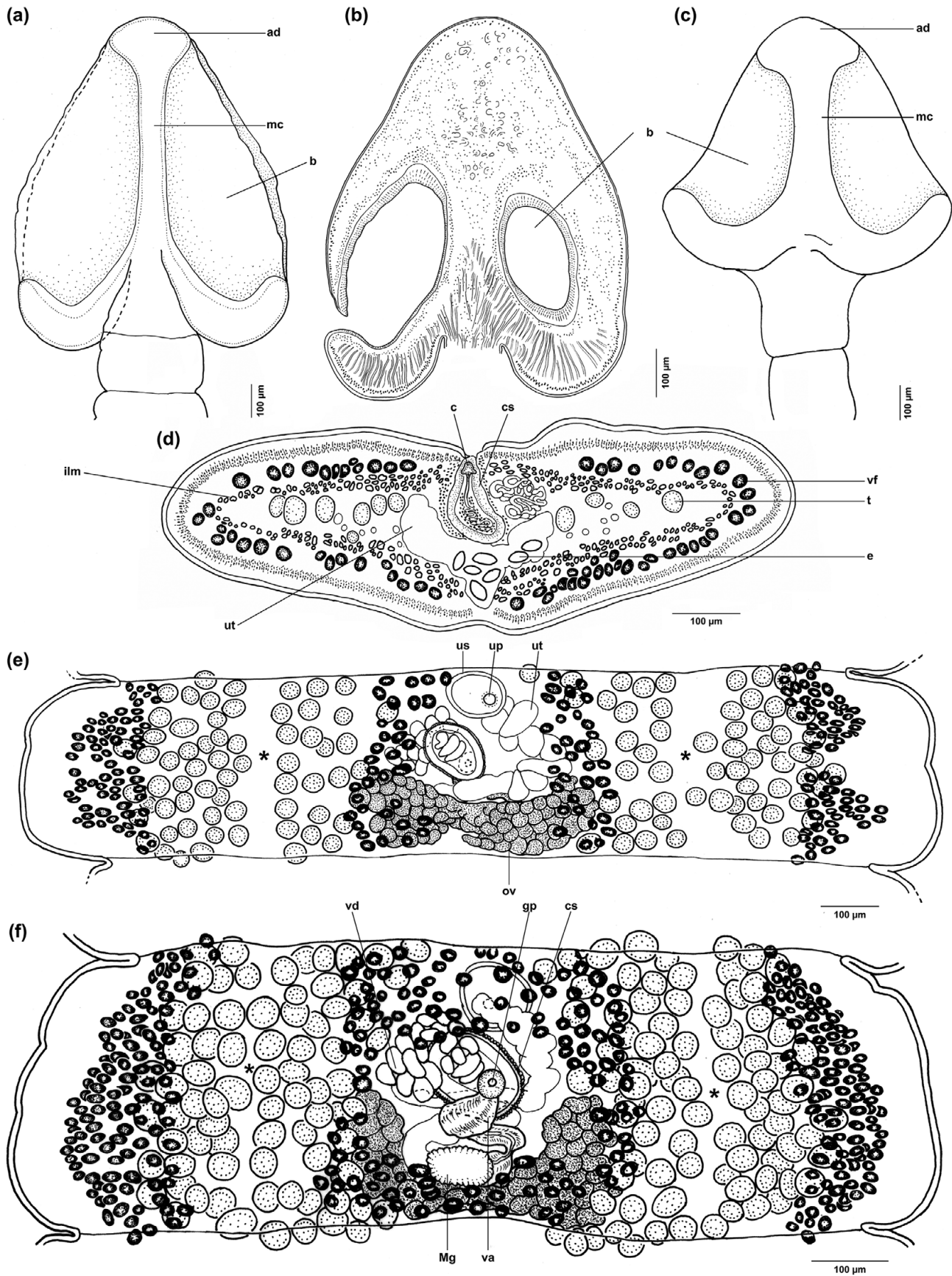


Figure 1. Line drawings of *Schyzocotyle nayarensis* from *Raiamas bola*, Fulbari, Siliguri, West Bengal, India (a–b, d–f). (a) Scolex, dorsoventral view; (b) frontal section of the scolex; (c) scolex, dorsoventral view of *Schyzocotyle acheilognathi* from *Labeobarbus kimberleyensis*, South Africa; (d) cross section of gravid proglottid, note individual muscle fibres forming small bundles; (e) mature proglottid, ventral view; (f) mature proglottid, dorsal view. *Denotes continuation of vitelline follicles. Abbreviations: ad, apical disc; b, bothria; c, cirrus; cs, cirrus-sac; e, egg; gp, gonopore; ilm, inner longitudinal musculature; mc, median column; Mg, Mehl's gland; ov, ovary; t, testes; up, uterine pore; us, uterine sac; ut, uterus; va, vagina; vd, vas deferens; vf, vitelline follicles.

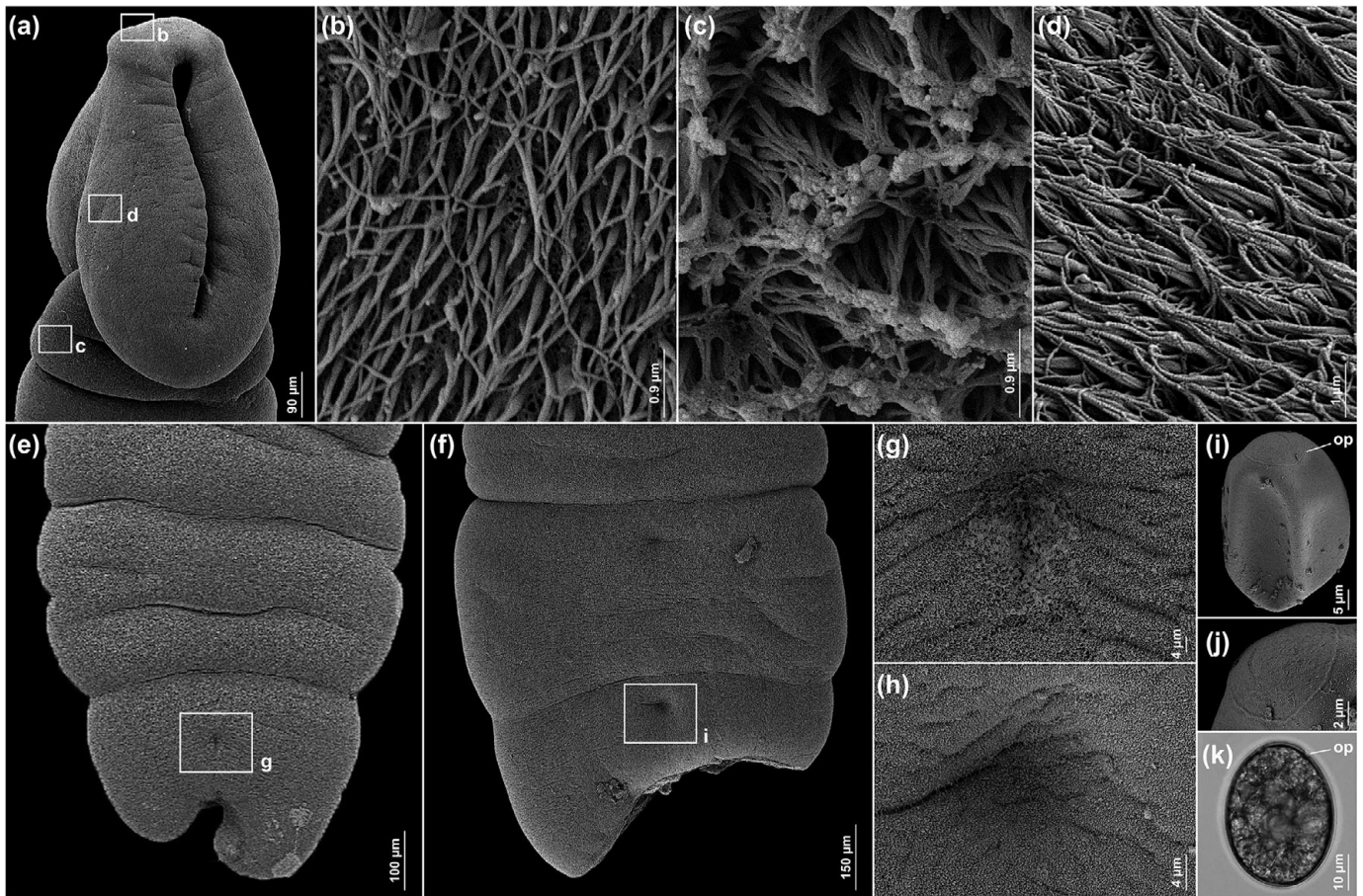


Figure 2. *Schyzocotyle nayarensis* from *Raiamas bola*, Fulbari, Siliguri, West Bengal, India. SEM (a–j); light microscopy (k). (a) sublateral view of scolex; (b) detail of capilliform filitriches on the scolex; (c) detail of capilliform filitriches on first proglottid; (d) detail of coniform spinitriches on the scolex; (e) gravid proglottid, dorsal view, note gonopore; (f) gravid proglottid, ventral view, note uterine pore; (g) detail of gonopore; (h) detail of uterine pore; (i) egg, note operculum; (j) detail of operculum; (k) egg liberated to water, note no embryo. Abbreviation: op, operculum.

including illustrations of the tapeworms found, that would allow reliable identification of bothriocephalid tapeworms in these fish. Therefore, we only considered those specimens for which morphological descriptions were available.

Type material: Allegedly deposited in Parasitology Laboratory, Department of Zoology, University of Garhwal, Srinagar (Garhwal) 246 174 (slide no. PCLS 041/81 according to Malhotra, 1983). This type specimen could not be located and almost certainly does not exist. To facilitate further comparative taxonomic studies and to avoid confusion, a complete specimen from *Raiamas bola* collected from the Fulbari (Mahananda River basin) in Siliguri, West Bengal, India (Field No. NBF-19-397c) is designated as a neotype (ZSI/W11621/1).

Deposition of new specimens: ZSI (ZSI/W11621/1–ZSI/W11625/1), IPCAS (IPCAS C-695).

Prevalence and intensity of infection: A total of 13 *Raiamas bola* were examined; among them 11 fish were found infected with 125 *S. nayarensis*, overall prevalence 85% and the mean intensity of infection 11.4 (4–27 worms/host) (see Table 1 for data on infection rate).

DNA sequences: The 672-bp partial *ITS-2* sequence and four partial (321-bp long) *COI* sequences generated in this study is being deposited in GenBank with the following accession numbers: *ITS-2*: PQ134488. *COI*: PQ134520, PQ134521, PQ134522, PQ134523.

Molecular study

The 672-bp long partial *ITS-2* sequence generated in this study is identical to the *ITS-2* sequence of *S. nayarensis* (KX060598),

previously obtained by Brabec *et al.* (2016). The ML tree generated from the phylogenetic analysis of the *COI* dataset shows all four isolates of *S. nayarensis* used in this study forming a strongly supported clade with the previously sequenced isolate of *S. nayarensis* (KR780829), distinct from *S. acheilognathi* (Fig. 5).

Remarks

Malhotra (1983) described *Ptychobothrium nayarensis* from Pauni-Garhwal, Uttarakhand, India. The original description was based on specimens that appeared contracted and deformed due to fixation under pressure, as obvious from figures 1–4 in Malhotra (1983). Despite limited similarities to *P. belones* (Dujardin, 1845), the type species of the genus, Malhotra (1983) placed his taxon within *Ptychobothrium*, a genus of marine cestodes (Kuchta *et al.*, 2008a; Kuchta & Scholz, 2017). Subsequently, Kuchta & Scholz (2007) synonymized *P. nayarensis* along with 13 other taxa described from freshwater fishes, with *Bothriocephalus acheilognathi* (= *Schyzocotyle acheilognathi*) based on their overall resemblance. However, these studies did not include molecular data or examination of specimens, which were never available on request to the authors of individual species.

Brabec *et al.* (2015) resurrected the genus *Schyzocotyle* Akhmerov, 1960 to accommodate *Bothriocephalus acheilognathi* based on molecular data. Additionally, they transferred *Ptychobothrium nayarensis* to *Schyzocotyle* but this decision was mainly based on molecular analysis of fresh samples collected from *Barilius*

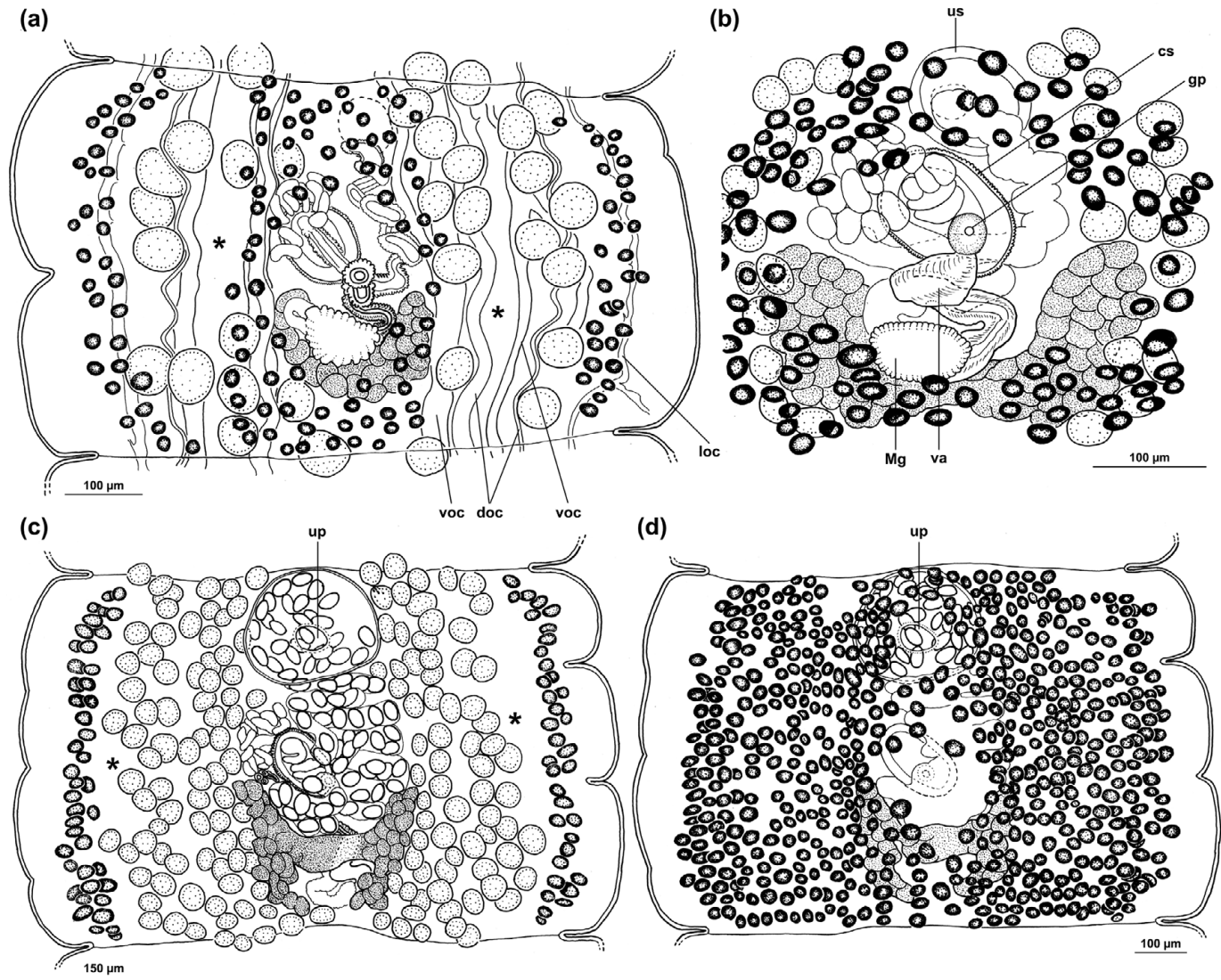


Figure 3. Line drawings of *Schyzocotyle nayarensis* from *Raiamas bola*, Fulbari, Siliguri, West Bengal, India. (a) Immature proglottid, dorsal view, with distribution of osmoregulatory canals; (b) detail of genitalia; (c, d) gravid proglottid, ventral view, (c) without distribution of vitelline follicles, (d) with distribution of vitelline follicles. *Denotes continuation of vitelline follicles. Abbreviations: cs, cirrus-sac; doc, dorsal osmoregulatory canal; gp, gonopore; loc, lateral osmoregulatory canal; Mg, Mehlis' gland; up, uterine pore; us, uterine sac; va, vagina; voc, ventral osmoregulatory canal.

sp. (in fact *Raiamas bola*) in Fulbari, West Bengal, India. The taxonomic change was reiterated by Kuchta & Scholz (2017). However, it is worth noting that Brabec et al. (2015) did not discuss morphological similarities between their specimen and Malhotra's (1983) *Ptychobothrium nayarensis*, nor did they propose morphological characteristics to distinguish between *S. acheilognathi* and *S. nayarensis*.

It is important to mention that although *S. acheilognathi* has been reported globally, including in India (Chaudhary et al., 2015), *S. nayarensis* has only been identified in two Indian cyprinoids (with potentially two additional hosts pending further investigation). Apart from variations in host range and geographical distribution, several morphological features distinguish these two species. A comprehensive differential diagnosis between these two valid species of the genus *Schyzocotyle* is outlined below: (i) arrow-shaped scolex in *S. nayarensis* vs heart-shaped scolex in *S. acheilognathi*; WA/LS = 0.22–0.29, WS/LS = 0.82–1.04 in *S. nayarensis* vs WA/LS = 0.36–0.39, WS/LS = 0.88–1.10 in

S. acheilognathi (Fig. 4a vs Fig. 4e & Fig. 1a vs Fig. 1c); (ii) 'median column' uniform in width with slightly enlarged anterior portion in *S. nayarensis* vs median 'column' narrowest in the middle, widening distally and proximally with the more widened anterior portion in *S. acheilognathi* (Fig. 1a vs Fig. 1c); (iii) comparatively narrower apical disc in *S. nayarensis* (20–30% of scolex width) than that of *S. acheilognathi* (30–40% of scolex width) (Fig. 4a vs 4e, Fig. 4b vs Fig. 4f, Fig. 4c vs Fig. 4g & Fig. 4d vs Fig. 4h); (iv) more testes per proglottid in *S. nayarensis* (70–170, n = 23) compared to *S. acheilognathi* (26–86, n = 23) (see Table 2 for detailed comparison of *S. acheilognathi* and *S. nayarensis*).

The molecular characterization in this study demonstrates that the studied worms are the same species as the specimen studied by Brabec et al. (2015, 2016) from Fulbari, India. Regrettably, it seems that the type material of *Ptychobothrium nayarensis* may no longer be available, and attempts to obtain it through written or personal communication with the author have been unsuccessful. Furthermore, in addition to sharing the same host species, significant

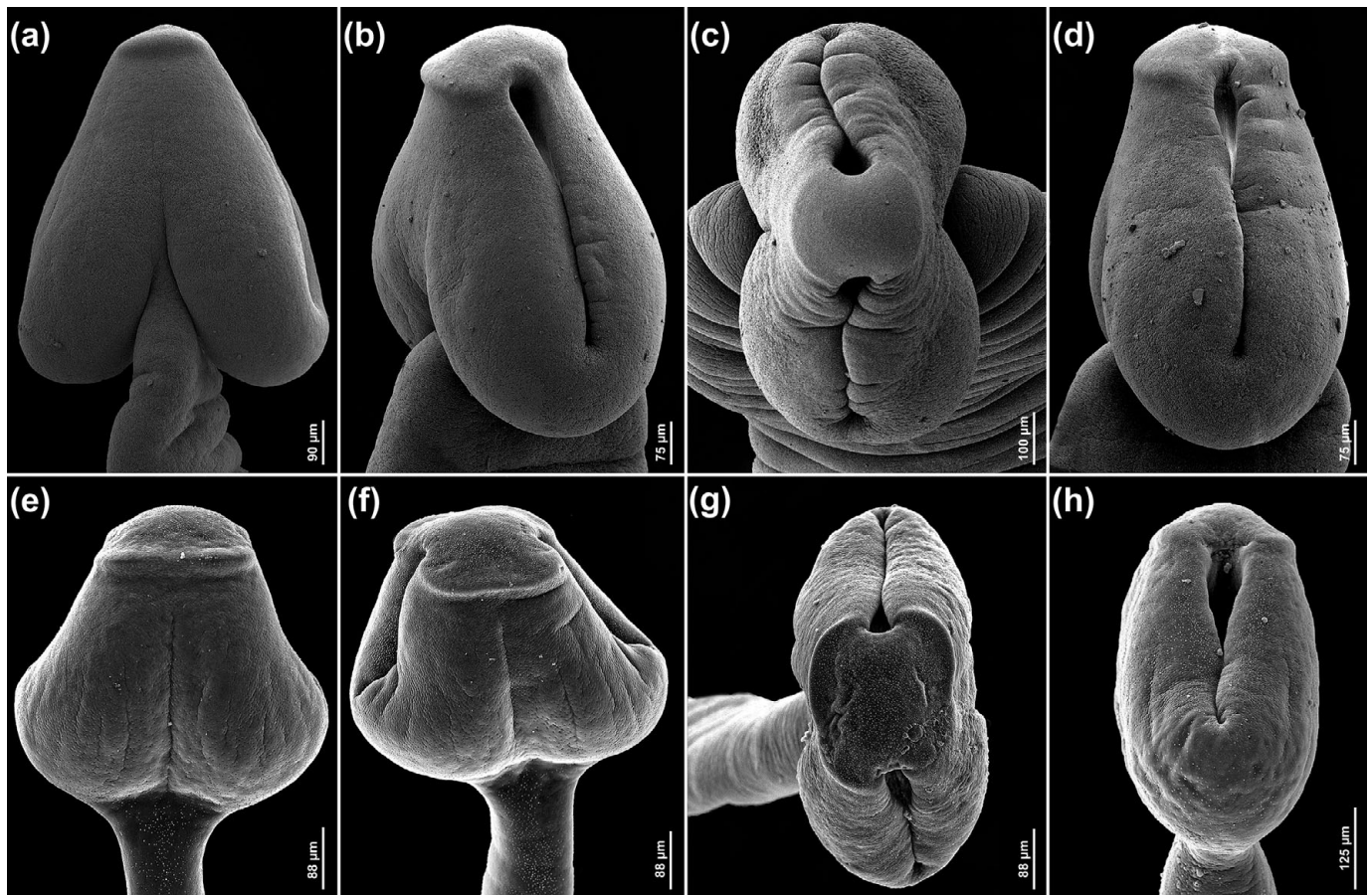


Figure 4. Scanning electron micrographs. (a–d) different views of scoleces of *Schyzocotyle nayarensis*, collected from *Raiamas bola* in Fulbari (Mahananda River basin), Siliguri, West Bengal, India, fixed with hot 4% formaldehyde solution; (e–h) different views of scoleces of *Schyzocotyle acheilognathi*, collected from *Gila cypha* (e, f, h) and *Cyprinus carpio* (g) in the Little Colorado River, Grand Canyon, Arizona, fixed with hot 4% formaldehyde solution.

similarities in key morphological features have been observed between our specimen and *P. nayarensis* (Malhotra, 1983). These include the uniform width of the median column (refer to Fig. 1a of present study and Fig. 1 of Malhotra, 1983), similar apical disc (which notably differs from those found in recognized species of *Ptychobothrium*; compare Fig. 1a of present study and Fig. 1 of Malhotra, 1983 with Fig. A of Deshmukh & Shinde, 1975, and

Fig. 1B of Châari & Neifar, 2022), and consistent cross-sectional profile (see Fig. 1d of present study and Fig. 5 of Malhotra, 1983). These resemblances lead us to conclude that the worms studied by Brabec *et al.* (2015, 2016) and by us (this study) are the same as Malhotra's (1983) *P. nayarensis* (= *S. nayarensis*).

Table 1. Prevalence and intensity of infection in Fulbari (Mahananda River basin), Siliguri, West Bengal, India

Month/year	No. of fish examined	No. of fish infected	Prevalence	Intensity (mean)
March 2009	1	1	100%	27 (27)
March 2011	1	1	100%	20 (20)
September 2018	2	2	100%	6 (6)
November 2018	2	1	50%	5 (5)
February 2019	1	1	100%	19 (19)
March 2019	3	2	67%	6–13 (10)
February 2021	3	3	100%	4–12 (8)

Malhotra's (1983) description of the scolex structure lacked detailed mention of the apical disc and bothria. However, SEM observations in this study reveal a weakly developed apical disc and narrow (slit-like), deep bothria with non-crenulate margins. Detailed characterization of bothria is crucial for accurate generic diagnosis and serves as a key distinguishing feature between the two genera, *Ptychobothrium* and *Schyzocotyle* (see Kuchta *et al.*, 2008a; Brabec *et al.*, 2015 for more information). An intriguing discovery in our study is the presence of circumcortical vitelline follicles surrounding the medullary testes from both sides, a feature not explicitly mentioned in Malhotra's (1983) description, which only referred to cortical vitelline follicles. However, in bothriocephalids, "cortical" might also imply circumcortical. Additionally, we provide for the first time for this species details of the egg structure through both light and scanning electron microscopy and confirm the presence of unembryonated eggs, which do not match the description provided by Malhotra (1983) (embryonated with measurement of oncosphere).

The following morphological characteristics of *Schyzocotyle* distinguish it from *Ptychobothrium*: i) heart- or arrow-shaped scolex (as opposed to sagittiform to fan-shaped in *Ptychobothrium*),

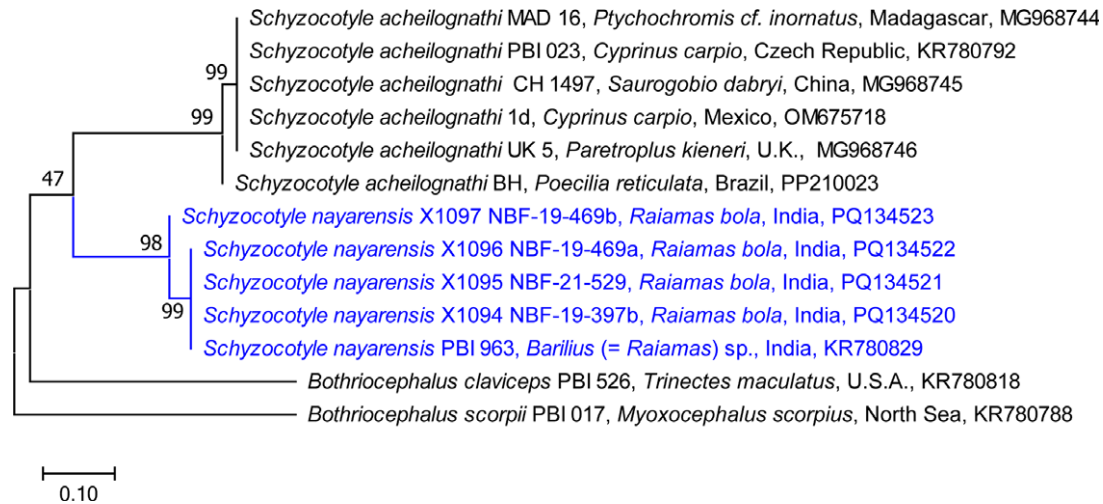


Figure 5. Phylogenetic tree from the ML analysis of the COI sequence data, based on the GTR+G+I model. The bootstrap nodal support (1000 replicates) is shown next to the branch. Branches with nodal support values <40 have been collapsed. The taxon name is followed by the isolate code, fish host, region/country of origin and the GenBank accession number.

Table 2. Differences between the species of *Schyzocotyle* Akhmerov, 1960

Species	<i>Schyzocotyle acheilognathi</i> (Yamaguti, 1934)*	<i>Schyzocotyle nayarensis</i> (Malhotra, 1983) [†]
Host	Broad spectrum of freshwater fish, especially cyprinoids (see Kuchta et al., 2018)	<i>Raiamas bola</i> (Hamilton, 1822), <i>Schizothorax richardsonii</i> (Gray, 1832), probably <i>Leiodon cutcutia</i> (Hamilton, 1822), <i>Systomus sarana</i> (Hamilton, 1822)
Total length (mm)	Up to 1000	Up to 91 (n = 5)
Maximum width (mm)	Up to 4.3	Up to 1.8 (n = 5)
Mature proglottids (L)	110–508	281–531 (n = 15)
Mature proglottids (W)	576–1,320	969–1,613 (n = 15)
Length/width ratio	1: 1.6–8.0	1: 1.4–5.2 (n = 15)
Gravid proglottids (L)	140–1,015	333–833 (n = 200)
Gravid proglottids (W)	480–3,600	700–1,800 (n = 200)
Length/width ratio	1: 0.45–4.5	1: 1.3–4.3 (n = 200)
Scolex (L)	400–1,600	963–1,600 (n = 17)
Scolex (W)	320–1,800	831–1,540 (n = 17)
Length/width ratio	1: 0.88–1.10	1: 0.82–1.06
Scolex shape	Heart-shaped	Arrow-shaped
Shape of median 'column'	Narrowest in middle, widening distally and proximally with more widened anterior end	Uniform in width with slightly enlarged anterior end
Width of apical disc to width of scolex (%)	30–40%	20–30% (n = 17)
Bothria (L)	473–733	775–1414
Bothria (W)	253–387	306–622
Length/width ratio	1: 0.48–0.63	1: 0.35–0.57

(Continued)

Table 2. (Continued)

Species	<i>Schyzocotyle acheilognathi</i> (Yamaguti, 1934)*	<i>Schyzocotyle nayarensis</i> (Malhotra, 1983) [†]
Number of testes	33–100	70–170 (n = 23)
Testes (L)	23–115	19–50 (n = 100)
Testes (W)	19–101	19–50 (n = 100)
Cirrus-sac (L)	35–180	94–144 (n = 15)
Cirrus-sac (W)	39–160	69–94 (n = 15)
Ovary (L)	260–750	131–213 (n = 15)
Ovary (W)	70–300	219–400 (n = 15)
Distribution of vitelline follicles	Confluent	Confluent
Vitelline follicles (L)	19–83	16–25 (n = 100)
Vitelline follicles (W)	13–69	16–25 (n = 100)
Eggs (L)	44–66	42–47 (n = 52)
Eggs (W)	22–46	31–34 (n = 52)

*Measurements of *S. acheilognathi* are adapted from Scholz (1997).

[†]Measurements of *S. nayarensis* from *Raiamas bola* are portrayed in the present study. Differential features in bold. Abbreviations: L, Length; W, Width.

ii) bothria exhibiting non-crenulated margins (as opposed to slightly crenulated internal margins in *Ptychobothrium*), iii) circumcortical vitelline follicles (contrasting with the medullary arrangement in *Ptychobothrium*) [Malhotra, 1983 referred to cortical vitelline follicles; see Fig. 1d of the present study], iv) operculate eggs, unembryonated (versus non-operculate, embryonated eggs in *Ptychobothrium*) [Malhotra, 1983 mentioned operculate eggs; see Fig 2i–k of the present study] (see Kuchta et al., 2008a; Brabec et al., 2015 for further details).

Lönnerberg (1889) established the genus *Ptychobothrium* within the family Ptychobothriidae Lühe, 1902, in the order Pseudophyllidea, to redefine *Bothriocephalus belones* Dujardin, 1845 as *Ptychobothrium belones* (Dujardin, 1845), originally found in garfish, *Belone belone* (Linnaeus, 1761). Currently, the genus

Table 3. List of bothriocephalidean cestodes considered morphologically indistinguishable from the species of the genus *Schyzocotyle* Akhmerov, 1960. Species are listed chronologically according to genera (newly assigned or changed taxonomic designations resulting from the present study are marked in bold).

Species	Locality, country	Host	Taxonomic status
<i>Bothriocephalus acheilognathi</i> Yamaguti, 1934	Lake Ogura, Japan	<i>Acheilognathus rhombea</i> (Temminck & Schlegel, 1846)	Synonym of <i>Schyzocotyle acheilognathi</i> (Yamaguti, 1934) [#]
<i>Bothriocephalus opsariichthydis</i> Yamaguti, 1934	Lake Biwa, Japan	<i>Opsariichthys uncirostris</i> (Temminck & Schlegel, 1846)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Bothriocephalus fluviatilis</i> Yamaguti, 1952	River Yodo, Kyoto Prefecture, Japan	<i>Hymenophysa curta</i> (= <i>Parabotia curta</i>) (Temminck & Schlegel, 1846)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Bothriocephalus gowkongensis</i> Yeh, 1955	Gowkong near Guangzhou China	<i>Ctenopharyngodon idella</i> (Valenciennes, 1844)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Bothriocephalus kivuensis</i> Baer & Fain, 1958	Lake Kivu Democratic Republic of Congo	<i>Barbus</i> (= <i>Labeobarbus</i>) <i>altianalis</i> (Boulenger, 1900)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Bothriocephalus phoxini</i> Molnár, 1968	Lake Balaton, Hungary	<i>Phoxinus phoxinus</i> (Linnaeus, 1758)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Bothriocephalus aegyptiacus</i> Ryšavý & Moravec, 1975	River Nile near Cairo, Egypt	<i>Barbus</i> (= <i>Labeobarbus</i>) <i>bynni</i> (Fabricius, 1775)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Bothriocephalus barbus</i> Fahmy, Mandour & El-Naffar, 1978	River Nile in Assiut, Egypt	<i>Barbus</i> (= <i>Labeobarbus</i>) <i>bynni</i>	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Bothriocephalus teleostei</i> Malhotra, 1984	River East and West Nayar, Pauri Garhwal district, India	<i>Barilius</i> (= <i>Raiamas</i>) <i>bola</i> (Hamilton, 1822), <i>Schizothorax richardsonii</i> (Gray, 1832)	Synonym of <i>Schyzocotyle nayarensis</i> (Malhotra, 1983)
<i>Capoeria barilii</i> Malhotra, 1985	River East and West Nayar, Pauri Garhwal district, India	<i>Barilius</i> (= <i>Raiamas</i>) <i>bola</i>	Synonym of <i>Schyzocotyle nayarensis</i>
<i>Coelobothrium monodi</i> Dollfus, 1970	Nasratabad, Iran	<i>Varicorhinus damascinus umbla</i> (= <i>Capoeta damascina</i>) (Valenciennes, 1842)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Coelobothrium oitense</i> Kugi & Matsuo, 1990	River Chikugo, Kami-tsue Village, Oita Prefecture, Japan	<i>Tribolodon</i> (= <i>Pseudaspius</i>) <i>hakonensis</i> (Günther, 1877)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Coelobothrium gambusiense</i> Yang, Wang, Peng, Zhou & Liu, 2005	Fujian Province, China	<i>Gambusia affinis</i> (Baird & Girard, 1853)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Ptychobothrium chelai</i> Shinde & Deshmukh, 1976	Aurangabad, Maharashtra, India	<i>Chela clupeioides</i> (= <i>Salmostoma balookee</i>) (Sykes, 1839)	Nomen nudum ^{a6*}
<i>Ptychobothrium khami</i> Shinde & Deshmukh, 1975	Kham River, Aurangabad, Maharashtra, India	<i>Nemacheilus</i> (= <i>Acanthocobitis</i>) <i>botia</i> (Hamilton, 1822) ⁵ , <i>Chela</i> (= <i>Salmostoma</i>) <i>phulo</i> (Hamilton, 1822)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Ptychobothrium phuloi</i> Shinde & Deshmukh, 1975	Kham River, Aurangabad, Maharashtra, India	<i>Chela</i> (= <i>Salmostoma</i>) <i>phulo</i>	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Ptychobothrium clupeioidesii</i> Chincholikar, Shinde, Deshmukh & Jadhav, 1976	Aurangabad, Maharashtra, India	<i>Chela clupeioides</i> (= <i>Salmophasia balookee</i>)	Synonym of <i>Schyzocotyle acheilognathi</i> [#]
<i>Ptychobothrium nayarensis</i> Malhotra, 1983	River East and West Nayar, Pauri Garhwal district, India	<i>Barilius</i> (= <i>Raiamas</i>) <i>bola</i> , <i>Schizothorax richardsonii</i>	Synonym of <i>Schyzocotyle nayarensis</i> [#]
<i>Ptychobothrium pangaonensis</i> Phad, 1983	Rena River at Pangaon, Taluka Ambajogai (Beed), Maharashtra, India	<i>Nemacheilus</i> (= <i>Acanthocobitis</i>) <i>botia</i>	Unavailable name^a
<i>Ptychobothrium bilaspurensis</i> Wadhawan, 1985	Gobind Sagar Lake, Bilaspur, Punjab, India	<i>Eutropiichthys vacha</i> (Hamilton, 1822)	Unavailable name^a
<i>Ptychobothrium maesae</i> Wongsawad, 1992	Maesa Stream, Chiang Mai, Thailand	" <i>Gambusia striatus</i> " [apparently mistake; most likely <i>Channa striata</i> (Bloch, 1793)]	Nomen nudum ^{a*}
<i>Ptychobothrium osmanabadensis</i> Kadam, 1993	Jakekur, Taluka Omagra, District Osmanabad, Maharashtra, India	<i>Chela</i> (= <i>Salmostoma</i>) <i>phulo</i>	Unavailable name^a
<i>Ptychobothrium discusae</i> Wongsawad, Kumchoo & Pachanawan, 1998	Maesa Stream, Chiang Mai, Thailand	<i>Mystacoleucus marginatus</i> (Valenciennes, 1842)	Synonym (tentative) of <i>Schyzocotyle acheilognathi</i> [#]
<i>Ptychobothrium mystacoleucysi</i> Wongsawad, 1998	Maesa Stream, Chiang Mai, Thailand	<i>Mystacoleucus marginatus</i>	Synonym (tentative) of <i>Schyzocotyle acheilognathi</i> [#]

(Continued)

Table 3. (Continued)

Species	Locality, country	Host	Taxonomic status
<i>Ptychobothrium rojanapaibuli</i> Wongsawad, 1998	Maesa Stream, Chiang Mai, Thailand	<i>Mystacoleucus marginatus</i>	Synonym (tentative) of <i>Schyzocotyle acheilognathi</i> ^{5#}
<i>Ptychobothrium bariliusi</i> Ghosh, 2013	Bodh Gaya, Bihar, India	<i>Puntius</i> (= <i>Systemus</i>) <i>sarana</i> (Hamilton, 1822), <i>Barilius</i> (= <i>Raiamas</i>) <i>bola</i> ⁵	Unavailable name⁵
<i>Ptychobothrium tetraodoni</i> Ghosh, 2013	Bongaon, North 24-Parganas, West Bengal, India and Mynaguri, West Bengal, India	<i>Tetraodon</i> (= <i>Leiodon</i>) <i>cutcutia</i> ⁵ (Hamilton, 1822), <i>Barilius</i> (= <i>Raiamas</i>) <i>bola</i>	Unavailable name⁵
<i>Schyzocotyle fluviatilis</i> Akhmerov, 1960	Petropavlovsk Lake, Far East of Russia	<i>Pseudaspis leptocephalus</i> (Pallas, 1776)	Synonym of <i>Schyzocotyle acheilognathi</i> ⁷

⁵Morphologically indistinguishable from *Schyzocotyle acheilognathi*

⁶Morphologically indistinguishable from *Schyzocotyle nayarensis*

⁷Type host (if more than one host documented and type host mentioned in the original description)

[#]Brabec et al. (2015)

¹Yeh (1955)

²Pool & Chubb (1985)

³Körting (1975)

⁴Pool (1987)

⁵Molnár (1977)

⁶Kuchta & Scholz (2007)

^{*}Caira et al. (2024)

⁷Dubinina (1982)

Ptychobothrium comprises two valid species, *P. belones* (Dujardin, 1845) and *P. ratnagiriensis* Deshmukh & Shinde, 1975, exclusively found in marine fishes (Deshmukh & Shinde, 1975; Kuchta et al., 2008a; Châari & Neifar, 2022).

List of taxa with questionable taxonomic status

Bothriocephalus teleostei Malhotra, 1984, originally described from *Barilius* (= *Raiamas*) *bola* and *Schizothorax richardsonii*, as well as *Capooria barilii* Malhotra, 1985, described from the same fish host (*Raiamas bola*), are now considered synonyms of *S. nayarensis* because of their striking similarity. A comprehensive analysis of their status is provided later. Furthermore, the taxonomic status of several other bothriocephalidean cestodes, initially proposed as synonyms of *Bothriocephalus* (= *Schyzocotyle*) *acheilognathi* by Kuchta & Scholz (2007), has been re-evaluated. This re-evaluation involves a critical comparison of their diagnostic features with those of *S. acheilognathi* and *S. nayarensis*. The updated taxonomic designations of these taxa are summarized in Table 3.

Several other species of *Ptychobothrium*, such as *Ptychobothrium pangaonensis* Phad, 1983; *P. bilaspurensis* Wadhawan, 1985; *P. osmanabadensis* Kadam, 1993; *P. tetraodoni* Ghosh, 2013; *P. bariliusi* Ghosh, 2013; and *P. ovalum* Gaikwad, 2019, were originally described in unpublished PhD theses, rendering these specific names unavailable according to the Articles 8, 10, 11 of the International Code of Zoological Nomenclature (1999, 2012). Majority of these descriptions suffer from various deficiencies, including improperly fixed specimens, inadequate line drawings, and a lack of type materials, which are significant issues (for further details, see Phad, 1983; Wadhawan, 1985; Kadam, 1993; Ghosh, 2013; and Gaikwad, 2019).

Furthermore, *Ptychobothrium pangaonensis*, described from 15 specimens collected from *Nemacheilus* (= *Acanthocobitis*) *botia* (Hamilton, 1822) in the Rena River at Pangaon, Taluka Ambajogai (Beed), Maharashtra, India, shares numerous morphological characters with *S. acheilognathi*, such as the shape and size of the scolex, the size of mature and gravid proglottids, and the size of the testes. Similarly, *P. bilaspurensis* found in the intestine of *Eutropiichthys vacha* (Hamilton, 1822) from a tributary of Gobind Sagar Lake,

Bilaspur, Punjab, India, also exhibit morphological similarities with *S. acheilognathi*, including the shape and width of the scolex, the size of mature and gravid proglottids, the number and size of testes, and the size of eggs. Likewise, *P. osmanabadensis* (described based on four specimens) collected from *Chela* (= *Salmostoma*) *phulo* (Hamilton, 1822) at Jakekur, Taluka Omega, District Osmanabad, Maharashtra, India, again demonstrates striking similarities with *S. acheilognathi* in scolex shape, scolex length, neck width, and the size and number of testes. Consequently, all of these species are considered conspecific with *S. acheilognathi* (see Table 3).

Similarly, *Ptychobothrium tetraodoni*, described from *Tetraodon* (= *Leiodon*) *cutcutia* and *Barilius* (= *Raiamas*) *bola* from Bongaon, North 24-Parganas, West Bengal, India, and Mynaguri, West Bengal, India, respectively, and *P. bariliusi* from *Puntius* (= *Systemus*) *sarana* and *Barilius* (= *Raiamas*) *bola* from Bodh Gaya, Bihar, India, were documented by Ghosh (2013) in her unpublished PhD thesis. Various inconsistencies are observed in these descriptions, including immature proglottids exhibiting a greater width compared to mature ones, possibly due to inadequate fixation, as immature proglottids possess a higher density of muscle fibres (see description of *P. tetraodoni* by Ghosh, 2013). Similarly, discrepancies in the number of testes between the description and figures are also noted (see Ghosh, 2013). Additionally, apart from sharing the same host *Raiamas bola*, *P. tetraodoni* and *P. bariliusi* share several other characters with *Schyzocotyle nayarensis*, including shape of the scolex and median column (see Figs. 7a and 8a in Ghosh, 2013), number of testes (see Figs. 7c and 8a in Ghosh, 2013), and their distribution. Based on these observations, they are also considered conspecific with *S. nayarensis* (see Table 3).

In contrast, *Ptychobothrium ovalum*, described from *Mystus* (= *Sperata*) *seenghala* (Sykes, 1839) in Dharmabad, Umari District Nanded, Maharashtra, India, exhibits notable resemblance in morphological traits, particularly in the arrangement of vitelline follicles, with proteocephalid tapeworms, in particular species of *Gangesia* Woodland, 1924 (see Ash et al., 2012, 2015). *Ptychobothrium elongata* Deshmukh, Nanware & Bhure, 2016, described from five worms from *Mystus* (= *Sperata*) *seenghala* in Dharmabad, District Nanded (Maharashtra), India, is actually a bothriocephalid because of the median, sacciform uterus (Fig. 2 in Deshmukh et al.,

Table 4. List of bothriocephalidean cestodes of the genus *Ptychobothrium* Lönnberg, 1889, morphologically indistinguishable from species of different genera other than *Schyzocotyle* Akhmerov, 1960

Species	Locality, country	Host	Taxonomic status
<i>Ptychobothrium vitellaris</i> Deshmukh, Nanware & Bhure, 2015	Mahur, District Nanded (M.S.), India	<i>Mastacembelus armatus</i> (Lacepede, 1800)	Probably species of <i>Senga</i> Dollfus, 1934
<i>Ptychobothrium elongata</i> Deshmukh, Nanware & Bhure, 2016	Dharmabad, District Nanded (M.S.), India	<i>Mystus</i> (= <i>Sperata</i>) <i>seenghala</i> (Sykes, 1839)	A bothriocephalidean cestode, confirmation of generic assignment is not possible
<i>Ptychobothrium punctatum</i> Barshe, 2018	Ausa, District Latur M.S., India	<i>Channa punctata</i> (Bloch, 1793)	Unavailable name ^a
<i>Ptychobothrium follicularis</i> Gaikwad, 2019	Mahur, Hadgaon, district Nanded (M.S.), India	<i>Channa punctata</i>	Unavailable name ^a
<i>Ptychobothrium follicularis</i> Nanware, Gaikwad & Bhure, 2019	Mahur, Hadgaon, district Nanded (M.S.), India	<i>Channa punctata</i>	Probably species of <i>Senga</i>
<i>Ptychobothrium ovalum</i> Gaikwad, 2019	Dharmabad, Umari District Nanded (M.S.), India	<i>Mystus</i> (= <i>Sperata</i>) <i>seenghala</i>	Unavailable name ^b
<i>Ptychobothrium punctatum</i> Bhure, Barshe & Nanware, 2019	Ausa, District Latur M.S., India	<i>Channa punctata</i>	Probably species of <i>Senga</i>

^aMorphologically indistinguishable from *Senga* sp.

^bMorphologically indistinguishable from *Gangesia* sp.

All taxonomic designations mentioned in this table are the result of the present study.

2016). Because no scolex was illustrated (“scolex” on Figs. 2 and 3 are actually immature proglottids), it is not possible to confirm generic assignment. The specimens appear to have been poorly fixed and improperly stained. *Ptychobothrium vitellaris* Deshmukh, Nanware & Bhure, 2015, based on seven specimens from *Mastacembelus armatus* (Lacepede, 1800) from Mahur, District Nanded (M.S.), India, shares significant resemblance with species of *Senga* Dollfus, 1934, as evidenced by illustrations, photomicrographs, and morphological descriptions, including scolex morphology (for more details, see Figs. 2 and 3 of Deshmukh *et al.*, 2015). Similarly, *P. follicularis* Nanware, Gaikwad & Bhure, 2019, described from *Channa punctata* (Bloch, 1793) from Mahur, Hadgaon, district Nanded, Maharashtra, India (also described in an unpublished PhD thesis as *P. follicularis* Gaikwad, 2019), and *P. punctatum* Bhure, Barshe & Nanware, 2019 (initially described in an unpublished PhD thesis [Barshe, 2018] as *P. punctatum* Barshe, 2018), described based on four specimens parasitizing *Channa punctata* from Ausa, District Latur, Maharashtra, India, show similarity in hosts and other morphological features, including scolex morphology (refer to Fig. 1 of Nanware *et al.*, 2019; Bhure *et al.*, 2019), which suggests that they are possibly species of *Senga*. Further investigation is necessary to confirm the taxonomic designation of the five aforementioned *Ptychobothrium* species (see Table 4).

Discussion

Kuchta *et al.* (2008b) established the order Bothriocephalida by reorganizing the previously paraphyletic order Pseudophyllida van Beneden in Carus, 1863 into two distinct monophyletic clades: Diphylobothriidea Kuchta, Scholz, Brabec & Bray, 2008, and Bothriocephalida. This classification was based on a combination of unique biological traits (primarily life cycle characteristics and host range), morphological observations, and molecular analyses (Brabec *et al.*, 2006; Kuchta *et al.*, 2008b). The order Bothriocephalida was initially divided into four families: Bothriocephalidae Blanchard, 1849; Echinophallidae Schumacher, 1914; Philobythiidae Campbell, 1977; and Trienophoridae Lönnberg,

1889 (see Bray *et al.*, 1994; Kuchta *et al.*, 2008b), with distinctions based on the position of the gonopore (median, sublateral, or lateral). However, Kuchta & Scholz (2017) later suppressed the family Philobythiidae (Brabec *et al.*, 2015; Kuchta & Scholz, 2017).

Our specimens exhibit all the diagnostic traits of Bothriocephalida as described by Kuchta *et al.* (2008a) and correspond to *Schyzocotyle* as characterized by Brabec *et al.* (2015).

The present study allows us to amend the generic diagnosis of *Schyzocotyle*, which is characterized by the following features not mentioned explicitly in Brabec *et al.* (2015): (i) heart-shaped or arrow-shaped scolex; (ii) weakly developed apical disc; (iii) narrow, deep bothria without crenulated margins; all other generic characteristics remain consistent with those outlined in Brabec *et al.* (2015).

Schyzocotyle nayarensis has only been documented in two cyprinoids in India, namely *Raiamas bola* and *Schizothorax richardsonii*, with *Leiodon cutcutia* and *Systomus sarana* potentially identified as additional hosts pending further study. *Raiamas bola*, also known as trout barb or Indian trout, exhibits a potamodromous distribution across India, Bangladesh, Myanmar, Nepal, Bhutan, and Thailand (Froese & Pauly, 2024). So far, other than *S. nayarensis*, one *Diplozoon* species, namely *Diplozoon dasashwamedhai* Agarwal & Kumar, 1989 and two nematodes, namely *Contracaecum* sp. and *Camallanus barilii* Gupta & Duggal, 1988 have been reported from this fish (Gibson *et al.*, 2005).

Another host, *Schizothorax richardsonii*, is a demersal, freshwater, potamodromous fish found in various Asian countries, including the Himalayan region of India, Bhutan, Sikkim, Nepal, Pakistan, and Afghanistan (Froese & Pauly, 2024). In addition to *S. nayarensis*, the following parasites have been reported from this little cyprinid: one ciliate protist *Ichthyophthirius multifiliis* Fouquet, 1876; one cestode, *Guptaia garhwalensis* Malhotra, 1985 (*species incertae sedis*; see Caira *et al.*, 2024); one polyopisthocotylan, *Diplozoon poochensis* Gupta, Gupta, Anjum & Gupta, 2014; several nematodes, *Camallanus khalili* Arya, 1989; *Procamallanus gupta* Arya, 1978, *Spinitectus* sp., *Paracucullanellus schizothoraxi* (Arya, 1983), *Rhabdochona* (*Filochona*) *teleostei* (Singh & Malik, 1992), *Rhabdochona* (*Filochona*) *nayari* Malhotra, Banerjee &

Chaubey, 1990, *Rhabdochona (Rhabdochona) himalayii* (Singh & Malhotra, 1989); and two trematodes, *Diplostomum tetrai* Chopra, Kumar & Singh, 1983, *Neascus vetestai* Kaw, 1950 (Gibson *et al.*, 2005; Gupta *et al.*, 2014; Mallik *et al.*, 2015; Singh & Panwar, 2020).

Malhotra (1983, 1984a, 1985) described three new cestode taxa, namely *Ptychobothrium nayarensis*, *Bothriocephalus teleostei*, and *Capooria barilii*, from the same fish host (*Raiamas bola*) and geographical area. Despite morphological similarity of all species, they were placed in three different families, i.e., Ptychobothriidae, Bothriocephalidae, and Diphylobothriidae. Bray *et al.* (1994) considered *Capooria* described as a diphylobothriid as a genus *inquirendum* because of its resemblance to members of the Bothriocephalidae. Kuchta & Scholz (2007) later synonymized *P. nayarensis*, *B. teleostei*, and *C. barilii*, and several *Ptychobothrium* species, with *Bothriocephalus acheilognathi*, based solely on morphological similarities. This action rendered *Capooria* a junior synonym of *Bothriocephalus*, a conclusion upheld by Kuchta *et al.* (2008a).

The present study focused on collecting fresh specimens of tapeworms from *R. bola*, the common host of *P. nayarensis*, *B. teleostei*, and *C. barilii*, as suggested by Malhotra *et al.* (2015). Although the rivers where the specimens were collected differ between our study and those referenced by Malhotra (1983, 1984a, 1985), both rivers originate in the Himalayan foothills, and show some similarities (Singh *et al.*, 2023). In line with Malhotra *et al.*'s (2015) emphasis on certain salient features to confirm taxonomic status, our study includes SEM micrographs to highlight morphological details and transverse histological sections to illustrate the positioning of organs like the vitellarium. The circumcortical vitelline follicles observed in our specimen bear resemblance to those described in Malhotra's work (1983, 1984a, 1985). While the scoleces of *P. nayarensis*, *B. teleostei*, and *C. barilii* appear different in Malhotra's descriptions, the consistent width of the median column in all three aligns with a key diagnostic feature of *S. nayarensis*. Additionally, our study benefits from uniformly fixed samples using hot 4% formalin, in contrast to the fixation method used by Malhotra (1983, 1984a, 1985) (aqueous Bouin's solution), which may result in unnatural variability (Pool & Chubb, 1985). Efforts to procure the type materials of *P. nayarensis*, *B. teleostei*, and *C. barilii* through written or verbal communication with the author have proven unsuccessful. Sampling in the original locations of those taxa was, logistically, not possible at this time. However, comparing our newly acquired material with the descriptions of the three tapeworms described by Malhotra, while taking into account the artifacts of fixation, we conclude that these all three taxa are conspecific and belong to *S. nayarensis*.

Conclusion

Kuchta *et al.* (2008b) highlighted several significant challenges in the study of bothriocephalideans, which have historically caused confusion in the study of these tapeworms. These obstacles include: low prevalence of bothriocephalidean cestodes in hosts, superficial morphological similarities among species, inadequate fixation of fresh biological samples, difficulty in observing the internal anatomy due to strobilar thickness, challenges in evaluating minute morphological details, paucity of deposited type material in museums, and absence of hologenophores. Among these issues, one major limitation of Malhotra's (1983) study was the use of aqueous Bouin's solution as a fixative, which has been demonstrated to cause

anomalies in taxonomic descriptions in an experimental study by Pool & Chubb (1985). They showed how scoleces of the same species can appear distinctly different with the application of different fixation techniques (see also Chervy, 2024). The morphology of the scolex stands out as a critical aspect among bothriocephalidean cestodes, playing a pivotal role in genus identification (Kuchta & Scholz, 2017). At times, distinguishing between the scoleces of bothriocephalideans can be challenging due to their striking resemblance. However, even slight variations in scolex morphology, if not accurately discerned, can lead to significant confusion regarding their taxonomy and systematics, as evidenced by past studies of bothriocephalidean cestodes (Yamaguti, 1934; Akhmerov, 1960; Molnar, 1977; Dubinina, 1982; Scholz, 1997; Kuchta & Scholz, 2007; Kuchta *et al.*, 2008a; Brabec *et al.*, 2015; Kuchta & Scholz, 2017; Choudhury & Scholz, 2020), and exemplified by *Schyzocotyle acheilognathi* and *S. nayarensis*. We have addressed these challenges and attempted to rectify the gaps by redescribing *S. nayarensis* as accurately as possible. Furthermore, the discovery of several new cestode species such as *Lobulovarium longiovatum* Oros, Ash, Brabec, Kar & Scholz, 2012 in cypriniform fishes (*Puntius* spp.) in India and Bangladesh; *Mystocetus anindoi* Scholz, Biswas, Patra & Ash, 2022 from small bagrid catfishes (*Mystus* spp.) in West Bengal and Maharashtra; and *Gangesia mukutmanipurensis* Marick, Brabec, Choudhury, Scholz & Ash, 2023 from a silurid catfish, *Ompok bimaculatus* (Bloch, 1794), in West Bengal, indicates the importance of regularly examining different unexplored fish hosts using an integrative taxonomic approach. Such examinations may reveal many interesting parasites, including new representatives of the genus *Schyzocotyle*.

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