



A NEW SPECIES OF *Procamallanus* (NEMATODA: CAMALLANIDAE) FROM *Ailia coila* (SILURIFORMES, AILIIDAE) FROM BARAK RIVER, CACHAR, ASSAM

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author LK done sampling, data collection, analysis and drafted the manuscript. Author NM provided creative ideas and designed the studies as well as done critical revision in order to provide only the important intellectual content. Both authors read and approved the final manuscript.

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ABSTRACT

A new species of parasitic nematode, *Procamallanus* (*Spirocamallanus*) *ailiasus* n. sp. is described from the intestine of the catfish, *Ailia coila* F. Helminton, 1822 (Siluriformes, Ailiidae) from Barak river, Cachar, Assam. *Procamallanus* (*Spirocamallanus*) *ailiosus* n. sp. differs markedly from all related species of the same fish hosts, in possessing a greater number (7+2+4) of caudal papillae in the male; presence of lesser number (9-10), 2-3 of them being incomplete (not extending from one lateral margin of capsule to other) of spiral ridges; barrel shaped with unequal cuticular expansion on dorsal and ventral side in female specimens, presence of peculiar two thick step like folds just above the basal ring and other morphological features. Parasitological examinations of some freshwater fishes of Barak River, Cachar, Assam was carried out during 2018 and 2019 and the study revealed, among other helminths, nematodes of the Family Camallanidae parasitizing the intestine of the catfish, *Ailia coila*. The new species is characterized by the presence of spiral thickenings, nerve ring and excretory pore position, length of spicules, number and arrangement of caudal papillae and shape of tail end. Abundance and prevalence is observed to be 1.5 and 50% respectively.

Keywords: *Procamallanus*; nematode; *Ailia coila*; barak; Assam; helminthes.

1. INTRODUCTION

Assam is richly endowed with abundant aquatic resources including fishes which constitute major

component of diet for the people of North-East India. Despite its importance for human consumption, a majority of fishes carry heavy infection of parasites, which causes deterioration in the food value of fish

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and may even result in their mortality. Helminthes are important group of pathogens, causing infection and diseases of fish both in freshwater and marine environments, their importance being related directly to the fish that may affect the general public health. Parasites are metabolically host dependent mainly for their nutritional requirements [1].

Nematodes of the genus *Procamallanus* Baylis, 1923 [2] (Camallanida, Procamallaninae) are predominantly parasites of freshwater fish that are distributed over several zoogeographical regions [3]. Despite the importance of introduced species of fish as mechanisms of co-introduction of parasites into native populations as described by Bautista-Hernandez [4], helminthes of most of introduced species have not been studied. As part of an ongoing study of the helminth parasites of fishes of Barak Valley, Assam, samples of *Ailia coila* were collected and examined for parasites. Procamallanin nematodes were found as parasites of these fishes. Since one of the parasites could not be assigned to a known species; it has been introduced as a new species.

2. MATERIALS AND METHODS

Fishes were caught by local fishermen in the Barak River, Assam (India) and some of them were kept in captivity for few days before their examination. The fishes were transported alive and immediately examined for the presence of helminthes parasites. The recovered nematodes were washed in physiological saline and then fixed in hot 70% ethanol or 4% formaldehyde solution. Additional fishes belonging to the same genus were collected but no camallanid nematodes were found in them. For light microscopical examination, the nematodes were cleared with glycerine. Measurements and photomicrographs were prepared by using a camera mounted microscope Nikon Eclipse E200. All measurements were in micrometers unless otherwise stated. The names of fishes follow Jayaram [5]. Helminth parasites were identified on the basis of available taxonomical characters as described by Yamaguti [6,7,8].

Prevalence, Abundance, Mean density and Index of infection were determined by following the formula proposed by Margolis et al. [9].

Prevalence = Total no. of infected fishes / Total no. of fishes host examined $\times$ 100.

Abundance = Total no. of parasites recovered/ Total no. of fish hosts examined.

Mean density = Total no. of parasites recovered/ Total no. of infected host examined.

Index of infection = No. of host infected $\times$ No. of parasites collected/ Total host examined.

3. RESULTS

Order Spirurida Chitwood, 1933

Family Camallanidae Railliet & Henry, 1915

Genus *Procamallanus* Baylis, 1923

Subgenus *Spirocamallanus* Olsen, 1952

***Procamallanus (Spirocamallanus) ailiosus* n. sp.**

Type material: Holotype: Female; allotype: Male; Paratype: female

Type locality: Barak River, Cachar, Assam.

Type host: *Ailia coila*, F. Helminton, 1822 (Siluriformes, Ailiidae)

Prevalence: 50%; Abundance: 1.5

Mean density: 3; Index of infection: 1.5

Deposition of specimens: Holotype on slide MUPS-P₁ (Table 1) Allotype: MUPS-P₃ (Table 1)

Paratype – MUPS-P₂ (Table 1), deposited in the Parasite collection of Parasitology Section, Department of Life sciences, Manipur University.

Etymology: The specific name is derived from the genus of the type host where it was discovered, i.e., *Ailia*.

Site of infection: Intestine.

Description: Small-sized nematodes with finely transversely striated thick cuticle (Fig. 1a,b,c; 2a,b). Body of live specimens reddish, fixed specimens light grey to brown. Buccal capsule orange-brown, peculiar barrel shaped with unequal cuticular expansion on dorsal and ventral side of female and barrel shape in male, slightly wider than length, with well-developed basal ring; narrow part of buccal capsule proper anterior to basal ring, somewhat thickened, forming conspicuous circular ledge and presence of two thick step like fold just above the basal ring. Inner surface of capsule provided with 9–10 spiral ridges in lateral view, 2–3 of them being incomplete (not extending from one lateral margin of capsule to other) (Figs. 2a,b). Trident absent. Muscular oesophagus somewhat shorter than glandular oesophagus; both parts of oesophagus long, narrow, slender, slightly

expanded near their posterior ends (Fig. 1a and b). Intestine brown, narrow and long. Tail tapering, broad, rounded with a terminal cuticular protrusion without cuticular spikes in both sexes (Figs. 1e,f and 2e,f).

Female (Two ovigerous specimen). Body with thick cuticle striated transversely. Buccal capsule with cuticular expansion; 9 spiral ridges, of which 2 being incomplete (not extending from one lateral margin of capsule to other). Length of entire oesophagus and buccal capsule representing 18.5% of body length.

Nerve ring encircle the muscular esophagus located at the anterior to mid region; excretory pore situated at the midway between nerve ring and posterior end of muscular esophagus. Vulva prominent, post equatorial with conspicuous lips at 53.48% of body length from anterior extremity (Fig. 1d, 2c). Vagina muscular, directed posteriorly from vulva. Uterus packed with numerous oval to spherical, thin walled embryonated eggs (Fig. 1d; 2c). Tail with small protrusion with smooth, rounded tips, terminal cuticular protrusion 1.8(1.9) μ m long and 2 μ m wide (Fig. 1e,f; 2f).

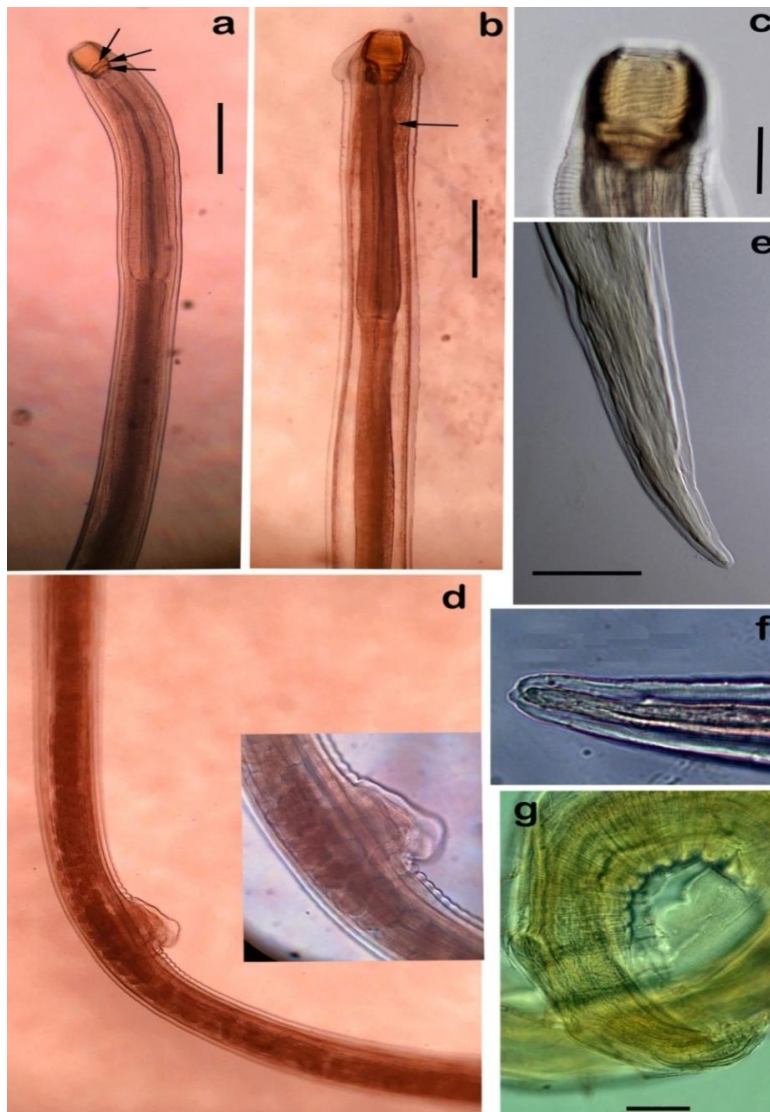


Fig. 1. Photomicrograph of *Procammallanus*(*Spirocamallanus*) *ailius* n. sp. a & b-anterior end of male (1st and 2nd arrowhead showing two step like folds and basal ring by 3rd arrowhead); b) female anterior end showing basal ring(arrowhead); c) anterior end of male showing the spiral ridges and steplike folds(enlarge view); d) vulval region of female worms with widely distributed eggs; e & f- posterior end of female; g) posterior end of male. Bars: a&b=0.2 mm, c=50 μ m, g=0.1 mm

Male. (Measurement of one allotype)

Buccal capsule barrel shaped. Presence of 10 Spiral ridges, 3 of which are incomplete (Fig. 2b). Length of entire oesophagus and buccal capsule representing 21.20% of body length. Nerve ring encircle the muscular oesophagus located at the anterior to mid

region; excretory pore posterior to nerve ring. Posterior end of body ventrally bent, provided with wide, vesiculated caudal alae supported by pedunculate papillae; anterior alae interconnected by a mound, and posteriorly reaching to caudal terminal cuticular protrusions (Fig. 2e).

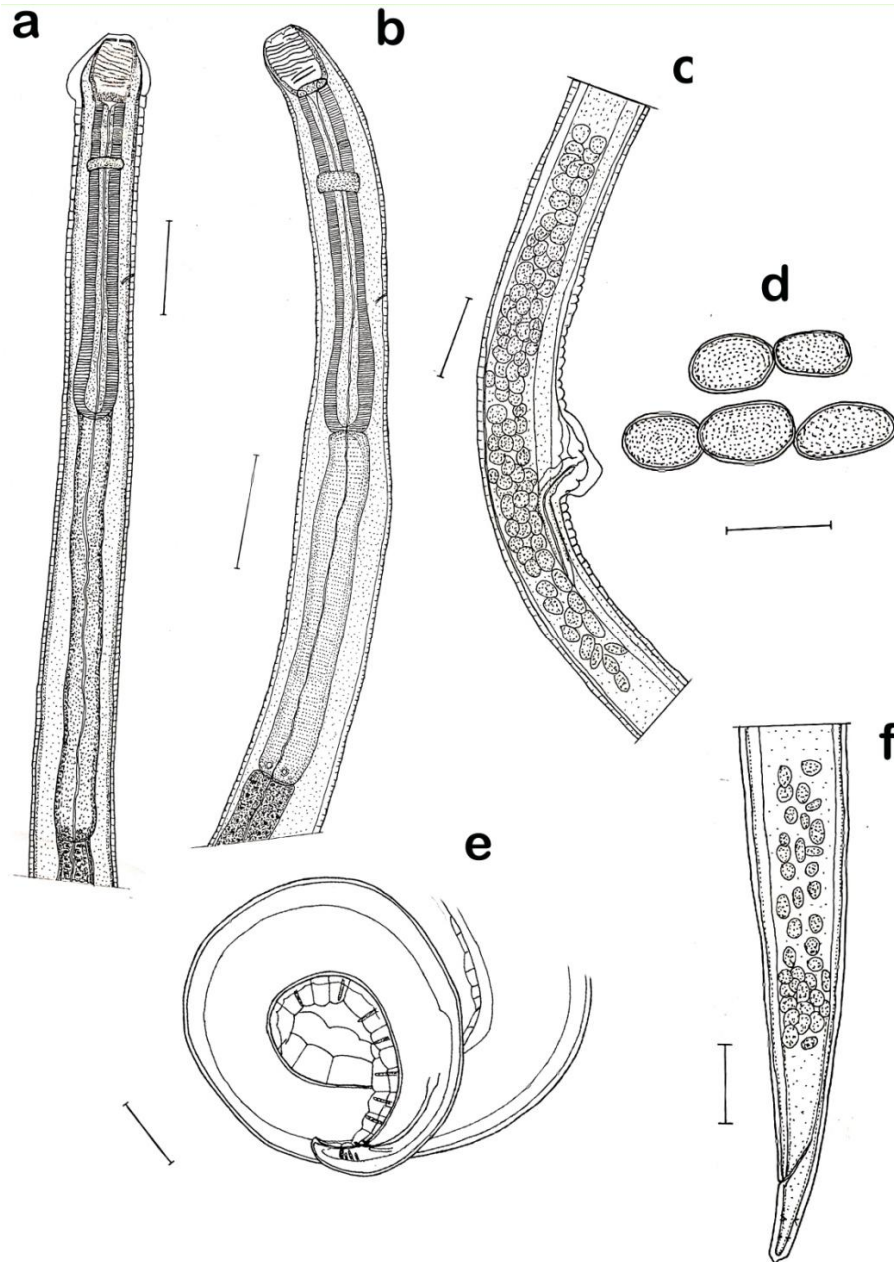


Fig. 2. Line drawings of *Procammallanus (Spirocammallanus) ailiosus* n. sp. a&b- anterior end of female and respectively; c) vulval region of female with full of eggs; d) eggs enlarged view; e&f posterior end of male(showing well developed allae and caudal papillae) and female respectively. Bars: a,b & f=0.2 mm; - c&d=0.1 mm, e=0.3 mm

Table 1. Morphometric measurements of gravid male and females of *Procamallanus* (*Spirocamallanus*) *ailiosus* n. sp

Characters	Holotype	Allotype	Paratype
	♀	♂	♀
Total body length	8.75 mm	6.13 mm	8.99 mm
Body width	100.02	102.11	103.32
Buccal capsule length	125.12	88.77	127.91
Buccal capsule width	135.63	75.44	139.3
Width/length ratio of buccal capsule	1:1.08	1:1.18	1:09
Cephalic ring length	20.19	14.11	23.89
Cephalic ring width	66.84	45.84	74.23
Muscular esophagus total length	690.46	581.76	720.46
Glandular esophagus total length	740.78	627.35	752.66
Muscular/glandular esophagus ratio	1:1.07	1:1.07	1:1.04
Nerve ring – anterior end	418.79	352.49	421.79
Vulva from anterior end	4.68 mm		4.72 mm
% of vulva distance from anterior end	53.48%		52.50%
Spicule		155.7	
Length of tail	202.06	201.36	204.68

Preanal papillae, seven which are subventral pedunculated papillae, of which sixth and seventh pair are closer to each other than other pairs; two adanal papillae surrounding cloacal opening; ifour pairs of pedunculated postanal papillae; additional two pairs of small sessile ventral papillae (Fig. 2e). Spicule single, with sharply pointed distal ends (right). Gubernaculum present. Tail conical, with small terminal cuticular protrusion, 3µm long, without cuticular spikes (Fig. 2e).

4. DISCUSSION

A large number of species are placed in *Procamallanus* Baylis, 1923 [2] which are mostly parasites of freshwater and marine fishes [10,11,12,13,14,15]. The new species herein described belongs to the subgenus *Spirocamallanus* Olsen, 1952, as both males and females have spiral thickenings (ridges) in the buccal capsule and the base of the male capsule without teeth [16]. Although, authors like Andrade–Salas consider *Spirocamallanus* Olsen, 1952 as a distinct genus, Moravec and Sey [17] consider *Spirocamallanus* a subgenus of *Procamallanus* to accommodate the species where both males and females have the buccal capsule with spiral ridges. The present finding is the first report of a parasite (Nematode) recovered from the fish species *Ailia coila*, F. Helmhinton, 1822 (Siluriformes, Ailiidae) in Barak River, Cachar, Assam. Based on the comparative morphometric analysis, type host and type locality, the present species is identified and proposed as new to science and given the name *Procamallanus* (*Spirocamallanus*) *ailiosus* n. sp.

Procamallanus (*Spirocamallanus*) *ailiosus* n. sp. is mainly characterized by barrel shaped buccal capsule with unequal cuticular expansion on dorsal and ventral side in female, 9-10 spiral ridges in the inner surface, the presence of wide caudal alae, 13 pairs of caudal papillae, single right spicules (155.7 µm long) and by the broad, rounded tail with a terminal cuticular protrusion without cuticular spikes.

The new species is found very close similar with *P.(S.) nainitalensis* Arya, 1978 and *P. gontii* Sood, 1967 (Table 2). In spite of many similarities the present species differs from *P.(S.) nainitalensis* and *P. gontii* in many aspects like, in its barrel shaped buccal capsule with unequal cuticular expansion on dorsal and ventral side but there is absence of cuticular expansion of buccal capsule in *P.(S.) nainitalensis* and *P. gontii*. The present species has 9-10 spiral ridges in the inner surface of buccal capsule while *P.(S.) nainitalensis* and *P. gontii* have 15-16 discontinuous ridges with knob like swelling and basal part thickened to form a rim respectively, *P.(S.) nainitalensis* possess 11 pairs and *P. gontii* with 10 pairs of caudal papillae in the posterior end whereas the present species has 13 pairs, tail tip is bifid in both *P.(S.) nainitalensis* and *P. gontii*, which is not the case in the present species.

The present species resembles *Procamallanus bagar* in presence of 13 caudal papillae and posteriad vulva position; however, it differs with the present species in the presence of three forwardly directed cuticular fold like projections at the bottom of capsule, arrangement of pre, ad and post anal papillae and tail shape.

Table 2. Morphometric comparison of the present species with its closest relatives

Characters	<i>P.(S.) nainitalensis</i> Arya, 1978 <i>Barilius vagra</i> Ham, Nainital	<i>P.gomtii</i> Sood, 1967 <i>Eutropiichthys vacha</i> Ham, Lucknow	<i>P.(S.) ailius n. sp.</i> <i>Ailia coila</i> , F. Helmlinton, Assam. (Present study)
Location	Intestine	Intestine	Intestine
Buccal capsule	Bowl shape with 5-16 discontinuous and horizontal thickenings with knob like swelling left lateral wall	Barrel shaped. Spiral ridges not mentioned Basal part thickened to form a rim.	Barrel shaped with unequal cuticular expansion on dorsal and ventral side (in female), presence of two thick step like fold just above the basal ring
Caudal papillae	Preanal:7; adanal: Not reported; post anal: 4	Preanal:6; adanal: Not reported; post anal:4	Preanal:7; adanal: 2; post anal:4
Spicule	0.130-0.135 (right)	Single (right) 0.184 long	Single (right) 0.156 long
Gubernaculum	Slender and stout	At 0.043	Slender
Eggs	Spherical, thin walled and embryonated	Not mentioned	Oval to spherical, thin walled and embryonated
Tail tip	Blunt in male; Bifid in female	Broadly rounded in male; Bifid in female	Terminal protrusion without cuticular spikes in both sexes

Procamallanus (Spirocamallanus) ailius n. sp. is also found similar with *P.pereirai*, in the number of spiral ridges in the buccal capsule (8-10 in male, 11-13 in female); but differs from the present specimen in their body measurements, maximum width/length ratio of buccal capsule (1:1.08-1.94 vs 1:1.18), presence of two spicule. Also they differ in the number (11 vs 13) and arrangement of caudal papillae (3+2+6 vs 7+2+4) in the posterior end of male.

P. singhi differs from the present species in the absence of adanal caudal papillae, presence of two spicule, and in the position of nerve ring and excretory pore although both have equal number of preanal (7) and post anal papillae (4) and the presence of prominent vulval lips in female.

By the presence of caudal alae in males, the new species resembles *P. (S.) rarus*. However, *Procamallanus (Spirocamallanus) ailius n.sp.* has post-equatorial vulva while in *P. (S.) rarus* it is equatorial, and the spicule is single & simple in *P.(S.) ailius sp. n.* while *P.(S.) rarus* has two unequal and dissimilar spicules, the large spicule with a small ventral outgrowth and the small spicule simple.

As pointed out by Petter [18] and Rigby [19], the shape and structure of the female tail appears to be constant within a species of *Procamallanus (Spirocamallanus)*. Based on the presence of female

tail and the absence of any terminal spikes *P. (S.) ailius n. sp.* resembles *P.(S.)sinespinis* [1] from *Pomadasy argenteus*, *P.(S.)macaensis* from several species of marine fishes of Brazil, *P.(S.) mexicanus* (Moravec et al. 2000) from the freshwater fish *Cichlasoma geddesi* in Mexico and *P.(S.) murrayensis* (Johnston and Mawson, 1940) from the freshwater fishes *Pseudaphritis urvill*, *Macquaria colonorum* and *M. ambigua* in Australia. Moreover, *P.(S.)sinespinis* and *P.(S.) mexicanus* are found similar with the new species in the number of spiral thickenings of buccal capsule.

However, in contrast to the present *P.(S.) ailius n. sp.*, *P.(S.)sinespinis*, *P.(S.) mexicanus*, *P.(S.) murrayensis* possess two spicule that are similar and unequal, presence of lesser number of preanal papillae and arrangement of caudal papillae is different. Tail tip of *P.(S.)sinespinis* possess of a knob-like structure in the male. Also, type host of *P.(S.)sinespinis* is the marine fish and *P.(S.) mexicanus* differs in the absence of gubernaculum.

Based on the position of the vulval region of female worm, *P(S) chauhani* from *Mystus cavasius*, *P. gomtii* from *Eutrop chthys vacha*, *P.vittatusi* from *Mystus vittatus*, *P. alii* from *Johnius dussumieri*, *P.(S)ompoci* from *Ompok bimaculatus* and *P.sparus* from *Argyrops(=sparus) spinifer* are found similar with the *P.(S.) ailius n. sp.*

But *P. vittatusi* differs in having a pair of lateral finger-shaped thickenings, tail bifurcated, presence of discontinuous ridges; *P. alii* possess 11 pairs of caudal papillae vs 13pairs, double spicule vs single and spiral ridges not mentioned vs 9-10 in the new species. *P. ompoci* also found similar with the new species in the presence of single spicule as well as 9 spiral ridges in male but 14-16 in female worm instead of 9-10 ridges. *P. sparus* also differs from the new nematode in having unequal number of spiral ridges in male (10) and female (11-15) worms. Caudal allae well developed but differ in having lesser number of pre anal (3pair), lesser number of caudal papillae (10) and absence of gubernaculum.

Procamallanus spiralis Baylis, 1923, *P.(S.) parachannae* Moravec, 2015; *P.(S.) similis* Moravec, 2011; *P.(S.) gobiomori* Moravec, 2000 resembles the present species in having 10, 10 (3 incomplete) and 10-12(1-3 incomplete) spiral thickening in the inner surface of buccal capsule.

In contrast, *P. spiralis* can be distinguished in having pre equatorial vulva as against the post equatorial position in the new species and in the presence of 4 teeth like structures at the anterior end of buccal capsule instead of two step like folds in the new species. *P.(s) parachannae* also differs from the present species in having 1:1.9-2.0 as against 1:1.04-1.25 length ratio of muscular and glandular esophagus in the new species. Length of entire esophagus and buccal capsule representing 7% of total body length of the worm as against 18.5-22.8% in the new species. There is a huge difference in them and in having 3preanal, 4+2 post anal papillae and empty uterus. Also *P.(S.) similis* Moravec, 2011; *P.(S.) gobiomori* Moravec, 2000 can be differentiated in the length of worms, width/length ratio of buccal capsule (1:1.3 in *P.(S.) similis* and 1:1.15-1.3, length of entire esophagus and buccal capsule representing 6-10% in *P.(S.) gobiomori*), number and arrangement of caudal papillae in the posterior end (3 preanal, 6postanal and 2pairs sessile papillae around cloaca in *P.(S.) similis*).

5. CONCLUSION

Based on the comparative morphometric analysis, type host and type locality, *Procamallanus (Spirocamallanus) ailius* n.sp. is the first species of the genus *Procamallanus* reported from the fish *Ailia coila*, F. Helminton, 1822 (Siluriformes, Ailiidae) from Barak River, Cachar, Assam. As the present species could not be assigned to a known species; it has been introduced as a new species. Abundance and prevalence of the parasitic infection

in the present host fish is observed to be 1.5 and 50% respectively.

ETHICAL APPROVAL

According to Institutional Animal Ethics Committee, Manipur University, there is no need of approval for experimentation on the fish specimens.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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