Faunal change over three decades to reveal Rotundocollarette capoori n. gen., n.sp., a unique anisakid from Johnius dussumieri at the Central west coast of India at Goa

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**ABSTRACT**

A unique anisakid roundworm, *Rotundocollarette capoori* n. gen., n.sp. infesting *Johnius dussumieri* from the Central West coast of India at Goa has been described. The worms are typically characterized by a cephalic collarette, a muscular collarette in post-caudal zone, intestinal caecum and a ventricular appendix. 3 pores, a part of cephalic infrastructure– a dorsal oral aperture, a dorso-laterally placed genital pore, and a sub-ventral excretory pore at the junction of the bases of the 2 ventro-lateral lips were typically unique as revealed by SEM studies. A pair of large squarish teeth flanked each of these three pores in the cephalic region, with 3-5 denticles in the median group, at the junction of the two arms of V-shaped structure, that joined 26-29 denticles on each arm of this V-shaped formation running parallel and crossed past dentigerous ridges on each lip. In addition a set of 49-50 medially organized denticles occupying the dorsal arm of V- shaped formation, in the medial space, were a conspicuous configuration, besides 40 minute triangular denticles at the junction of sub-ventral lips. A spined mucron at the tip of tail. The key provided by Moravec and Justine (2020) to segregate *Porrocaecum*-like nematode genera (*Porrocaecum, Pseudoterranova, Pulchrascaris, Euterranova, and Neoterranova*) has been revised to include the newly proposed genus, *Rotundocollarette capoori* n. gen., n.sp.

Keywords: *Rotundocollarette capoori* n. gen., n.sp., collarette, denticles, oral, excretory and genital pores.
INTRODUCTION

In recent years anisakidosis has emerged to be a pretty serious disease, that usually spreaded due to consumption of uncooked meat of fish (Timi et al., 2014). However, in Indian context noticeable advances have been made by the worms of Anisakidae where proven invasion of these worms from marine to freshwater ecosystems have been published (Jaiswal et al., 2016). Additionally, in the present investigation a more serious change in anisakid fauna from camallanid fauna to anisakid fauna over a longer period of time has been concluded. The events could thus easily have serious bioecological consequences, on which the authors have focussed over a long period of time. This study deals with the emergence of a newer anisakid fauna, whose characteristics were never observed in earlier reports on members of Anisakidae. Van Thiel (1960) was the first to publish evidence of zoonosis by a larval anisakid nematode from herring in the human gastro-intestinal tract in the Netherlands.

The undetermined configuration of cephalic apparatus, in absence of detailed SEM studies, of a large variety of species of genus Terranova and the related anisakid genera (Gibson, 1983, Deardorff, 1987, Bruce and Cannon, 1990) have opened up a newer challenge to the Taxonomists in India to validate older descriptions published since past several decades. The significance of the morphology of oesophageo-intestinal region was emphasized by Fagerholm (1991) to be used as a basis for classification of nematodes of Ascaridoidea sensu Chabaud (1965). Sprent (1983), however, desisted to assign much of a significance of taxonomic value to the presence or absence of intestinal caeca in his modified scheme of classification to establish a newer taxon beyond species level. In spite of extensive data on taxonomy of a variety of species by Sprent (1983), Gibson (1983) and Lichtenfels et al. (1978), Fagerholm (1991) emphasized the importance of numbers and distribution pattern of caudal papillae on the body of nematodes to develop clarity on validity of roundworms under Ascaridoidea. Five families namely, Anisakidae, Cossophoridae, Heterocheilidae, Acanthocheilidae and Ascarididae were accommodated under Ascaridoidea by Hartwich (1974). The current worms showed affiliation with family Anisakidae, of the above referred families, on account of presence of certain labial structures.
like specialized 3 lips with teeth and excretory pore near oral aperture, and presence of intestinal caeca with ventriculus and ventricular appendix.

Extensive research surveys are being conducted by the author and coworkers, since past three decades, in marine as well as freshwater areas in India to analyse molecular and SEM analysis of a variety of anisakid and other nematodes, cestodes including varied helminths. The current report constitutes a part of such research under critical observation that just after the Tsunami period in December, 2004 a gradual transformation in the nematode fauna of marine fish in Arabian Sea has been noticed. A decade later, after the huge environmental upheaval of Tsunami, not only the perciform fish of family Lutjanidae, *Lutjanus malabaricus* has disappeared (extinct) at the Goan coast, the camallanid, *Paracamallanus* sp. populations also seem to have disappeared in this fish. Instead, the current worms collected from another co-existing fish, were harboured by the sciaenid fish of family Sciaenidae, *Johnius dussuumieri* that has become freshly infected. Therefore, this change of nematode fauna in fish of sciaenid fish has been significant over longer period of time, particularly because roundworms of Camallanidae were scarcely observed, and those of Anisakidae never ever occurred in Sciaenidae. Moravec and Justine (2020) emphatically argued that in the absence of details of labial characteristics, morphological details of caeca, ventriculus, ventricular appendix, vulva, caudal papillae, spicules and the associated structures, it was difficult to ascertain species characters of genus *Terranova*. A good number of studies conducted by using molecular methods have been helpful to resolve features of species significance under genera *Terranova*, *Pseudoterranova* and the related taxa, particularly when larvae were collected from a wide variety of host fishes. The adult anisakid survey from elasmobranchs of New Caledonian waters by Baylis (1931), and molecular analysis conducted by Al-Hoshani et al. (2020), and Shamsi *et al.*, (2019) to reveal identity of larvae of *Terranova* spp. have been noticeable contributions to resolve hurdles of complications arisen due to the missing morphological data on cephalic and other structures on which the critical taxonomic data could be generated to segregate species of genus *Terranova*, *Pseudoterranova*, *Pulchrascaris* (Shamsi *et al.*, 2020), *Hysterothyacium* (Shamsi *et al.*, 2015) and other related anisakid genera (Shamsi *et al.*, 2018). Till such time the incredible information available in world literature on a great variety of helminths, particularly in Indian
context, should be dealt with utmost care. It is advisable to make fresh collections from the areas where senior Helminthologists conducted studies in yesteryears, so that SEM and molecular details could be retrieved to make descriptions complete, instead of discarding the wealth of information available without re-examining the worms (Moravec and Justine, 2020), that are already on record. One such detailed investigation published on molecular analysis by Malhotra and Jaiswal (2015) has cautioned that discarding the descriptions available on record, without making fresh collections of helminths could be a dangerous precedent.

Materials and Methods

Collection and examination of fish for parasites

A total of 58 specimens were collected from the bearded sinfish croaker, *Johnius dussumieri* (n=170) at Dona Paula Beach, Goa off the Central West coast of India during 2021-2022. All fish were either already euthanized as part of other research projects or were bought from fishermen in various fish markets at Dona Paula Beach, Goa, India. Dead fish were cut open and first examined for presence of larval nematodes on the surface of the internal organs and immediately thereafter, the gastrointestinal tract was examined for the presence of nematodes. Adult nematodes were killed in lukewarm water; washed thoroughly in 0.85% normal saline, and fixed in Berland’s solution. A small piece of the mid-body of each of the 9 individual nematodes were removed with a scalpel, and preserved in 100% ethanol for molecular analysis. The remainder 22 worms (male, 12; female, 10) of these roundworms were processed for morphological examination with other worms (Malhotra et al., 2012).

SEM Analysis

Nine mature roundworms from fish of marine habitats, were fixed in Glutaraldehyde (2.5% in 0.1M phosphate buffer) for the Scanning Electron Microscopy (SEM) and processed after rehydration (Malhotra et al., 2012). SEM analysis was performed on JEOL JSM 6510LV at the University Sophisticated Instrument Facility (USIF), Aligarh Muslim University, Aligarh, India.

Drawings were made with the aid of a drawing tube and measurements were made directly with an eyepiece micrometer. Measurements of roundworms were recorded in µm and expressed as
range, followed by mean in parentheses, unless otherwise stated. Photomicrographs were taken using Motic research microscope with Biovis Image Analysis software.

Adult nematodes were identified to species based on the available keys and descriptions (Anderson et al., 1974; Deardorff & Overstreet, 1981; Gibson, 1983; Sprent, 1983; Bruce & Cannon, 1989, 1990). Specimens have been deposited in collections of the Zoological Survey of India, Jabalpur, India.

Results

Family Anisakidae
Subfamily Anisakinae
Genus Rotundocollarette n. gen.
Species capoori n.sp.
Type-host Johnius dussumieri (Actinopterygii: Sciaenidae)
Type-locality: Central west coast of India at Goa (15°19'09.56"N, 73°38'09.77"E).
Etymology: The new genus owes its name to the word “Rotunda” of ‘Latin’ origin, meaning “round and firm”. The new name to the genus has been assigned to signify sturdy muscular collar at head as well as in the post-caudal region of the newer worm. The species has been named after the eminent Parasitologist from India, Professor Vireshwar Nath Capoor Former Professor of the Department of Zoology, University of Allahabad, Prayagraj, U.P., India.
Site of infection: Pyloric caeca
Mean intensity: 4.6–9.0 (Male), 12–40(Female).
Prevalence: 14.6%-20.0% (Female), 2.5-11.1% (Male).
Specimens deposited: Holotype, Female- A/23105; Zoological Survey of India, Jabalpur, M.P.
RESULTS

*Rotundocollarette capoori* n.gen., n.sp.

**Description:** Worms small to medium-sized, the largest being 14.38, with creamish-white coloration and cuticular striations evident. The morphometric measurements of 22 worms (male, 12; female, 10), out of the total 58 roundworms collected from *J. dussumieri* have been compiled in Table 1. Body widest at the level of oesophageo-intestinal junction to mid-body. The typical cuticularized striations (Fig. 3) were an essential part of the structural organization of the newer nematodes being described here. Three distinct pores as part of the cephalic apparatus have been observed.- i. Oral aperture guarded by two prominent oral papillae located on elevated muscular lobes (Fig. 1a, 1b), ii. A ventral Excretory pore guarded by two prominent teeth (Fig. 7b), and iii. A dorso-laterally placed genital pore, that was clearly visible in SEM photograph, and was also flanked by two prominent squarish teeth (Figs. 5,6,7a,7b,8,9a,9b). Two sub-ventral double papillae in lower one third part of the dorso-ventral lip with one single pair of papillae adjacent to the sub-ventral double papillae. One pair sub-dorsal double papillae near the upper rim of dorsal lip accompanied by 3 single papillae in the same region. One double papilla on either side of the oral aperture. Another double papilla at the basal third of the collarette on the mid-ventral side. One cervical papilla has been located on the dorso-ventral side of collarette. Four double papillae occupied, two on the dorso-lateral, and the other on the ventro-lateral positions at the basal periphery of anterior collarette. Three large single papillated structures on the dorsal lip, were entangled in the midst of the radial muscles (Fig. 10b) that encircled the muscular cushion in the enface view providing support to the two large oral papillae, each of whom comprised lobular base with a papilla atop each of these. These single papillae also were seen in clusters dorso-laterally and ventro-laterally to the buccal cavity (Figs. 10b, 10c).

Another set of three minute papillae (Fig. 7b) were observed at the elevated base of centrally placed twin enlarged oral papillae (Figs. 7a, 7b.). A heavily muscular transverse band attached (Fig. 1a) further downward to another border transverse muscular band marked the supportive apparatus on each dorso-lateral as well as ventro-lateral position at the base of the two oral papillae mounted atop the elevated muscular base. A strong muscular frill of the muscular collarette emerged at the base of 3 lips
(Fig. 2a). This continued as the main collarette in the form of a wider circular band around the anterior end of body to cover the base of 3 lips (Fig. 2a) for quite a distance. Thereafter the terminating skirting frill (Fig. 2a) emanated at the end of muscular collarette.

The array of radial linear bands as well as longitudinal folds has been amply magnified in Fig. 10b. The radial striae matched the appearance of alternating linear bands of high and low attenuation in radial pattern extending through the cortico-medullary layers of the kidney on Iodine-based intravenous contrast enhanced imaging. But in view of Tian et al. (2018) and Luo et al. (2020) and the striated appearance might have not been associated with pathologic effect. The striations resulted from stasis and concentration of contrast material in edematous or increased tubules. At best, that demonstrated increasing attenuation over time (Tian et al., 2018).

Head, gives appearance of predominantly occupied by lobulated structures atop (Figs. 8b, 10a). Lips 3, equisized, armed with prominent squarish teeth, with rounded margins that had 2 distinct teeth laterally mounted on a median bulging. The presence of excretory pore (Fig. 7a, 7b) on the ventral side of cephalic armature at the junction of the bases of the two sub-ventral lips was typically unique of the members of Anisakidae. The enface view of the oral end revealed that all the three apertures, viz., excretory pore, oral and the genital pores were located linearly across the ventro-dorsal plane (Figs. 7a, 7b).

The internal median lobes on the dorsal cephalic lip equipped with an armature of paired comb-like dentigerous ridges, in a specific distribution of 49-50 denticles adjacent to the medio-dorsal wall of the V-shaped formation that ran parallel to each other longitudinally towards posterior end of the worm, as revealed by SEM (Fig. 12). This extended well past comb-like dentigerous ridges, with sparse distribution on the dorso-lateral side, in the region around the two comb-like ridges. An additional set of 70 denticles were linearly distributed mid-ventrally extending from the excretory pore (Fig. 13) down to the anterior end level of the anterior collarette. The constriction at the base of three lips was supported by a papilla each on each lateral side of the collarette, anteriorly as well as posteriorly (Fig. 1a). Collarette extended like a broad muscular plate to a short distance bearing a smaller extra muscular flap along its circular rim (Fig. 2).
The anteriormost cephalic apparatus comprised two prominent larger papillae located on centrally placed protuberances (Fig. 10b). The cephalic apparatus comprised a total of six teeth - 2 distinctly dorsal in the vicinity of the 2 larger cephalic papillae, from where the V–shaped formation of denticles started (Fig. 12). A pair of ventral teeth were located apposed each other around the ventral excretory pore, while the third pair of dorso-ventrally placed squarish teeth was also present adjacent to the 2 large cephalic papillae surrounding oral aperture. The intestinal caecum about one and a half times the length of ventricular appendix. Cervical and lateral alae prominent as well as cephalic alae (Fig. 2, 3) and caudal alae (Fig. 4) distinct in (Fig. 7b) both the sexes.

**Male (12 specimens):**

Worms medium-sized, the largest being 9.408 long. Head with a prominent muscular collarette that distinctly enveloped most part of head (Fig. 9a, b). Buccal cavity small. Anterior part of oesophagus almost equal in size to the posterior part of oesophagus. Worm body width maximum at mid-body level. Intestinal width at the level of ventricular appendix, 0.12-0.20 (0.192). Intestine, 0.01-0.015 (0.014) in diameter. The ventral post-cloacal plectanes were present (Fig. 7b, 18c). Spicules two, subequal (Fig. 16). The pattern of distribution of caudal papillae was unique (Fig. 19b), inasmuch as 39-60 pre-caudal; 54-56 post-caudal; and 15 pairs in the area around anal region. One isolated sunflower papilla was observed using SEM, in the vicinity of plectanes with 6 plates in the post-caudal area (Fig. 17, 19c). A mucron (Figs. 16, 20b) located at the posterior extremity of the tail possessed 8 rows of minute spine with 6 spine in each row. A sunflower papilla (Fig. 18) was recorded in the post-caudal region near plectanes in male worm.

A single papilla present closer to the tail tip. One unpaired amphid was also recorded near the tail tip. Gubernaculum absent.

**Female (10 specimens):**

Worms medium to large-sized, the largest being 15.6 long. A deirid was noticed (Fig. 10d) post nerve ring level. Oesophagus attained moderate length. Ventriculus small, elongated to heart-shaped, at the junction of posterior end of oesophagus and the intestine (Figs. 14, 15). A finger-like elongate intestinal caecum, longer than ventricular appendix was distinct (Fig. 1a). Vulva post-equatorial (Fig. 11). Tail extremity gradually terminating with a small mucron (Figs. 16, 20a-d) at the tip, bearing
minute spines all over. The mucron (Fig. 19a, 20a) was basally attached with the terminal end of body by 5-8 digitated smaller muscular blocks, that supported mucron (Fig. 20d). The posterior collarette (Fig. 8d) typically extended in the post-caudal region upto base of the mucron. However, the proximal extremity displayed the skirting frill (Fig. 9d) as similar and sturdy to that observed in anterior collarette.

**DISCUSSION**

**Differential Diagnosis**

A distinct muscular collarette around head as well as tail terminating into a skirting frill posteriorly. Cephalic and caudal alae prominent. Interlabia absent. Ventriculus present with ventricular appendix. Intestinal caeca present. The ratio of caeca to ventricular appendix upto 1:1.5. Length ratio of ventriculus : ceca, male, 1:0.74; female, 1:0.52. Cephalic apparatus comprised 3 pairs of large, prominent squarish teeth-one pair each around oral aperture, sub-ventral excretory pore and dorso-lateral genital pore; typical distribution of eight double papillae, 6 paired simple papillae along with three pairs of minute conical papillae at the base of large oral papillae guarding the oral aperture, with four large cephalic double papillae at the base of collarette at 4 corners. Additionally, one pair of large cervical papillae near the nerve ring, and a deirid closer to the cervical papilla. Spicules 2, small to medium-sized, sub-equal. A unique sunflower papilla with 6 plated plectane post-cloacal. Numerous linearly arranged 39-60 pairs pre-cloacal, 15 pairs para-cloacal and 54-56 pairs post-cloacal distinct. Tail tapers in both sexes into a terminal wider mucron that bear minute spines. Parasitic in marine teleosts.

In earlier years, the genera *Terranova* and *Porrocaecum* were considered as synonym to *Phocanema* by Myers (1960). *Phocanema decipiens* (Myers, 1959a) has been an ascaridoid placed under subfamily Anisakinae Railliet and Henry, 1912 (Myers, 1959a,b). The mystifying character combinations of members of Anisakidae contributed to severe taxonomic impediments that created difficulties to have these worms coherently organized under the taxonomic framework. *Ascaris decipiens* was the first to be described from seals by Krebbe (1878), as cited by Myers (1959a,b). Later the nematode, *Terranova antarctica*, a worm found closer to *A. decipiens*, parasitizing a shark in New
Zealand, was described by Leiper and Atkinson (1914), followed by the discovery of *Ascaris simplex* (von Linstow, 1888), by Baylis (1916), bearing identical features of oesophagus to *Terranova antarctica*, but on account of caeca synonymized it with *Porrocaecum*. Simultaneously Myers (1959a,b) cited Johnson and Mawson (1945), on an interesting observation of interlabia being present in the latter but absent in *Terranova*, the nematodes parasitizing avian hosts be assigned to *Porrocaecum*, while those encountered in fish, elasmobranchs and seals be transferred to *Terranova*. Conclusively, Myers (1959a,b) acknowledged peculiar differences in the morphological make-up of the cephalic and tail of male specimens of *Terranova* from elasmobranchs and fishes vis-à-vis *P. decipiens* that emanated from seals. Therefore, the outcome was a new generic name, *Phocanema* to suggest *Phocanema decipiens* (Krabbe, 1878) that emerged as the lone species, different from *Porrocaecum*, devoid of interlabia, and that did not parasitize avian hosts.

Moravec and Justine (2020) emphasized the synonymy of genus *Terranova* under *Porrocaecum* as its junior synonym, upholding the assertion by Johnston and Mawson (1945), notwithstanding the earlier assertion of synonymy of Terranova under *Porrocaecum* by Baylis (1916), for the judicious reason that both of these genera possessed caeca.

Myers (1959) cited that Krabbe (1878) initially described *Ascaris decipiens* that parasitized seals, and another closely related specimen described by Leiper and Atkinson (1914) which parasitized a NewZealand shark was assigned a new name, *Terranova antarctica*, to his specimen. Soon, Baylis (1916) also came across another nematode with closer characteristics from the stomach of seals, and reported it to be *Anisakis simplex* (von Linstow, 1888). However, the structure of oesophagus was at the centre stage due to which all the three nematodes were clubbed closely with *Terranova antarctica*. In my considered opinion, the credit of first author be given to Krabbe (1878) as cited by Myers (1959), instead of Leiper and Atkinson (1914) following the Principle of Priority as per Rules of Zoological Nomenclature. Therefore, the name of this species be correctly mentioned henceforth, as:-

*Terranova antarctica* Leiper and Atkinson, 1914 (Krabbe, 1878 *Ascaris decipiens*)

The taxonomic status of genus *Pseudoterranova* was recognized (Moravec and Justine, 2020) to have been established by Mozgovoy (1953). Moravec and Justine (2020) also endorsed the validity of genus *Pulchrascaris* like Deardorff (1987), Bruce and Cannon (1990), Shamsi, Barton and Zhu
In addition, two new genera, namely, *Euterranova* and *Neoterranova* were raised recently by Moravec and Justine (2020), on account of presence of lips with comb-like ridges – narrower in *Euterranova*, & formed by 2 prominent lateral teeth and several medial denticles; but broader in *Neoterranova* formed by several even-sized denticles, and absence of prominent teeth. The specimens of newer genus described herein, *Rotundocollarette capoori* n.gen., n.sp. were in striking contrast from the former two, as they too possessed comb-like ridges, with denticles more skewed dorsally, on lips; prominent lateral teeth as well as several medial uneven-sized denticles. An additional set of 70 denticles were linearly distributed mid-ventrally extending from the excretory pore (Fig. 13) down to the anterior end level of the anterior collarette. In addition, the cephalic apparatus armed with thickened muscular collarette, a post-equatorial vulva, 3 distinct pores, *viz.*, dorsal oral aperture, and genital pore, and a sub-ventral excretory pore, each guarded by a pair of squarish teeth around, number and distribution of caudal papillae, a sunflower papilla and a spiny mucron at the tail tip were the other differentiating characteristics. Moravec and Justine (2020) considered all the 16 species of *Porrocaecum* from India as poorly described and illustrated, and hence species dubiae as well as incertae sedis. These authors included *Porrocaecum*, *Pseudoterranova*, *Pulchrascaris*, *Euterranova* and *Neoterranova* in the key that they published. A revised key is proposed herewith to accommodate the newer genus *Rotundocollarette capoori* n.gen., n.sp., and given as below:-

1. Anisakidae. Worms with cephalic and caudal collarette......................Rotundocollarette capoori n.gen., n.sp.

Rest of the description in the key published by Moravec and Justine (2020) are proposed to be retained.

Moravec and Justine (2020) mentioned that Olsen (1952) first published *Euterranova* (as *Terranova*) *ginglymostomae* parasitizing *Ginglymostomacirratum* (Bonnaterre) (Ginglystomatidae, Orectolobiformes) in the northern West Atlantic (off Florida, USA). Bruce and Cannon (1990) recorded *T. (=E.) galeocerdonis* harboured by *Orectolobus maculatus* (Bonnaterre) (Orectolobidae, Orectolobiformes) at the coastal areas of southeastern Queensland, Australia.
The significance of the morphology of oesophageo-intestinal region was emphasized by Fagerholm (1991) to be used as a basis for classification of nematodes of Ascaridoidea sensu Chabaud (1965). Sprent (1983), however, desisted to assign much of a significance of taxonomic value to the presence or absence of intestinal caeca in his modified scheme of classification. In spite of extensive data on taxonomy of a variety of species by Sprent (1983), Gibson (1983) and Lichtenfels et al. (1978), Fagerholm (1991) emphasized the importance of numbers and distribution pattern of caudal papillae on the body of nematodes to develop clarity on validity of roundworms under Ascaridoidea. Fagerholm (1991) split the Anisakids into two families: Anisakidae (Subfamilies: Anisakinae and Contracaecinae) and Raphidascarididae. Luque et al., 2011) summarized in recent years that Family Anisakidae, contained Subfamilies Raphidascaridinae, Anisakinae, Goeziinae. The genus Goezia was assigned to subfamily Goeziinae and simultaneously Raphidascaridinae comprised Raphidascaris and Hysterothylacium.

The authors agree with the assertion by Moravec and Justine (2020) that the constituents of cephalic apparatus in nematodes, particularly Anisakidae, constitute characteristics of taxonomic significance. Moravec and Justine (2020) also emphasized that those anisakid worms that possessed a cylindrical ventriculus and intestinal caecum besides being parasitized in poikilothermic hosts have currently been considered, as anisakid genera, under genera Terranova and Pulchrascaris Vicente et dos Santos, 1972 (Anderson et al., 2009; Deardorff, 1987; Gibbons, 2010).

The present worms occupied a place of distinction on characters of anterior and posterior collarette, that enveloped the configuration of three pores (oral, excretory and genital) with 6 large, prominent teeth in the cephalic infrastructure, in addition to triangular denticles of varied sizes on sub-ventral lip as well, post-equatorial vulva and ventricular appendix as part of oesophageo-intestinal complex. These newer worms further stand out distinct from Pulchrascaris, Porrocaecum, Terranova (though this has been assigned as species inquerenda) and Pseudoterranova specifically on the characteristics of collarette as well as those pertinent ones, described above. However, the authors are examining molecular characteristics based on 4 genes, viz. 18S rDNA, ITS1, ITS2 and mit-coi genes so that the appropriate status could be assigned to the newly proposed genus. Till that time its taxonomic status as new genus is proposed.
CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS, AND/OR ANIMALS

No human participants were involved in the research. Marine fishes were involved in current research that need no permission separately.

INFORMED CONSENT

All the three authors give consent for having no objection to the consideration of manuscript for publication.

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**EXPLANATION OF FIGURES**

_Rotundocollarette capoori*_ n.gen., n.sp.

**Fig. 1a-b.** *Rotundocollarette capoori* n.gen., n.sp. a. Schematic plan of enface view of male worm to show distribution of cephalic apertures and papillae with denticles (Not to scale); b. Anterior end of female to show cephalic lateral papillated folds, intestinal caeca and ventricular appendix (x 240).

**Figs. 2-3.** *Rotundocollarette capoori* n.gen., n.sp. 2. Light microscopic image of anterior end of worm showing cephalic alae with anterior collarette comprised of skirting frill at its distal end. (x 100); 3. Light microscopic image of anterior end of worm showing prominent lateral and cephalic alae (x 100).

**Fig. 4.** *Rotundocollarette capoori* n.gen., n.sp. Posterior end of tail of male worm to show caudal alae. (x 100; Scale: 0.10mm).
Fig. 5. *Rotundocollarette capoori* n.gen., n.sp. Enface view of female to show 3 pores-i. Oral aperture flanked by cephalic papillae mounted atop elevated muscular base; ii. Excretory pore flanked by 2 teeth, and iii. Genital pore flanked by 2 additional teeth. (Scale: 10µm)

Fig. 6. *Rotundocollarette capoori* n.gen., n.sp. Anterior end of male worm to show linear distribution of minute denticles at the base of sub-ventral lips with 3 pores on cephalic infrastructure. (Scale: 10µm)

Figs. 7a-b. *Rotundocollarette capoori* n.gen., n.sp. a. 3 pores on cephalic infrastructure; b. cephalic papillae and minute papillae at the base of elevated cephalic papillae. (Not to scale)

Fig. 8a-d. *Rotundocollarette capoori* n.gen., n.sp. a. Anterior end of worm to show 3 pores with teeth on enface view. (Scale: 10µm); b. Anterior end of worm to show double papillae at the base of anterior collarette with cervical papillae. (Scale: 50µm); c. Posterior end of male worm to show distribution of pre-caudal, ad-anal and post-caudal papillae. (Scale: 20µm); d. Posterior end of female worm to show posterior collarette on tail. (Scale: 100µm).

Fig. 9. *Rotundocollarette capoori* n.gen., n.sp. a. Anterior end of female to show cephalic papillae, teeth, muscular papillae on head with collarette. (Scale: 10µm); b. Anterior end of male worms showing cephalic papillae, teeth and anterior collarette comprised of skirting frill. (Scale: 10µm); c. Anterior end of female worms to show cervical papillae at the level of nerve ring. (Scale: 50µm); d. Posterior end of female to show posterior collarette extending upto skirting frill at its terminal end. (Scale: 50µm).

Fig. 10. *Rotundocollarette capoori* n.gen., n.sp. a. Anterior end of female worm to show anterior collarette and cephalic infrastructure. (Scale: 20µm) b. Anterior end of male worm (magnified) to show distribution of single papillae among radial and longitudinal muscles and cephalic papillae on elevated muscular blocks (Scale: 10µm); c. Anterior end of male worm showing single papillae distributed between radial and longitudinal muscles around cephalic papillae and collarette at the base of head (Scale: 10µm). d. Deirid closer to cervical papilla on anterior end of female worm. (Scale: 10µm).

Fig. 11. *Rotundocollarette capoori* n.gen., n.sp. Post-equatorial vulva and vagina (x 60).

Fig. 12. *Rotundocollarette capoori* n.gen., n.sp. Schematic plan of distribution of denticles over medial denticular ridges in V-shaped formation on the cephalic lips. (Not to scale).
Fig. 13. *Rotundocollarette capoori* n.gen., n.sp. Schematic plan of distribution of minute conical denticles extending from sub-ventral lips backwards. (Not to scale).

Fig. 14. *Rotundocollarette capoori* n.gen., n.sp. Ventriculus-intestinal caeca-ventricular appendix complex. (x 240).

Fig. 15. *Rotundocollarette capoori* n.gen., n.sp. Ventriculus between posterior end of posterior oesophagus and intestine (Arrows mark the margins of heart-shaped ventriculus). (Not to scale)

Fig. 16. *Rotundocollarette capoori* n.gen., n.sp. Posterior end of body of male worm to show prominent caudal alae, spicules and mucron. (x 240).

Fig. 17. *Rotundocollarette capoori* n.gen., n.sp. 6-plated post-caudal plectanes on the body of male worms. (x 240).

Fig. 18. *Rotundocollarette capoori* n.gen., n.sp. Sunflower papilla in the vicinity of plectane in the post-caudal region of male worm. (x 240).

Fig. 19a-c. *Rotundocollarette capoori* n.gen., n.sp. a. Posterior end of female to show mucron at the tail tip (x 240). b. SEM image of post-caudal distribution of papillae arranged linearly. (Not to scale). c. SEM image of sun-flower papilla and post-caudal plectane in male (Not to scale).

Fig. 20a-d. *Rotundocollarette capoori* n.gen., n.sp. a-d. SEM image of Posterior part of (a) female (Scale: 10µm) (b) male (Scale: 20µm), (c) female to show mucron (Scale: 10µm), and (d) male showing basal muscular blocks to support mucron and post-caudal papillae. (Arrow shows digitized muscular block supporting mucron). (Scale: 10µm).

**ABBREVIATIONS**

A - Cloacal aperture

CP - Cephalic papilla

Cl - Collarette

COP - Posterior collarette

Cr - Cervical papilla

CSP - Cluster of single papillae

DP - Double papilla
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>EP</td>
<td>Excretory pore</td>
</tr>
<tr>
<td>F</td>
<td>Sunflower papilla</td>
</tr>
<tr>
<td>GP</td>
<td>Genital pore</td>
</tr>
<tr>
<td>H</td>
<td>Head</td>
</tr>
<tr>
<td>I</td>
<td>Intestine</td>
</tr>
<tr>
<td>M</td>
<td>Mucron</td>
</tr>
<tr>
<td>MP</td>
<td>Muscular papilla</td>
</tr>
<tr>
<td>Op</td>
<td>Posterior end of posterior part of oesophagus</td>
</tr>
<tr>
<td>OP</td>
<td>Oral aperture</td>
</tr>
<tr>
<td>P</td>
<td>Plectane</td>
</tr>
<tr>
<td>PH</td>
<td>Phasmid</td>
</tr>
<tr>
<td>Pi</td>
<td>Minute single papilla</td>
</tr>
<tr>
<td>Pl</td>
<td>Linearly arranged post-caudal papillae</td>
</tr>
<tr>
<td>R</td>
<td>Basal muscular ring at the base of head</td>
</tr>
<tr>
<td>SF</td>
<td>Skirting frill</td>
</tr>
<tr>
<td>SP</td>
<td>Single papilla</td>
</tr>
<tr>
<td>T</td>
<td>Tooth</td>
</tr>
<tr>
<td>V</td>
<td>Ventriculus</td>
</tr>
</tbody>
</table>
Table 1. Morphometric measurements of various organs on the body of *Rotundocollarette capoori* n.gen., n.sp.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Measurements (mm unless otherwise stated)</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Body</td>
<td>6.29-8.47 (6.94)</td>
</tr>
<tr>
<td>Body Wat:-</td>
<td></td>
</tr>
<tr>
<td>i.</td>
<td></td>
</tr>
<tr>
<td>oesophagus</td>
<td>0.068-0.25 (0.137)</td>
</tr>
<tr>
<td>ii.</td>
<td>0.198-0.348 (0.226)</td>
</tr>
<tr>
<td>iii.</td>
<td>0.168-0.272 (0.176)</td>
</tr>
<tr>
<td>iv.</td>
<td>0.08-0.135 (0.124)</td>
</tr>
<tr>
<td>Anterior Collarette</td>
<td>0.14-0.176 (0.169) x 0.17-0.225 (0.204)</td>
</tr>
<tr>
<td>Cuticular fold at antero-lateral margin of collarette</td>
<td>0.10-0.12 (0.11) x 0.027-0.032</td>
</tr>
<tr>
<td>Head</td>
<td>0.09-0.20 (0.12) x 0.11-0.26 (0.14)</td>
</tr>
<tr>
<td>Cicutcular Papillated Fold</td>
<td>L: 0.03-0.12 (0.08)</td>
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<tr>
<td>Buccal cavity Deep:</td>
<td>0.028-0.036 (0.032)</td>
</tr>
<tr>
<td>W:</td>
<td>0.016-0.036 (0.026)</td>
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<tr>
<td>Oesophagus. Anterior</td>
<td>0.30-0.43 (0.34) x 0.076-0.109 (0.08)</td>
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<tr>
<td>ii.</td>
<td>0.20-1.08 (0.54) x 0.056-0.198 (0.19)</td>
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<tr>
<td>Intestine W:</td>
<td>0.014-0.091 (0.037)</td>
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<tr>
<td>Intestinal caecum</td>
<td>0.12-0.216 (0.154) x 0.016-0.04 (0.034)</td>
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<tr>
<td>Ventriculus</td>
<td>0.08-0.16 (0.114) x 0.07-0.14 (0.10)</td>
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<tr>
<td>Ventricular appendix</td>
<td>0.11-0.16 (0.15) x 0.12-0.028 (0.02)</td>
</tr>
<tr>
<td>Vagina</td>
<td>-</td>
</tr>
<tr>
<td>Distance of vulva from anterior end</td>
<td>-</td>
</tr>
<tr>
<td>Distance of vulva from posterior end</td>
<td>-</td>
</tr>
<tr>
<td>Distance of anus from tail tip</td>
<td>0.184-0.192 (0.190)</td>
</tr>
<tr>
<td>Mucron</td>
<td>0.02-0.04 (0.028) x 0.005-0.033 (0.024)</td>
</tr>
<tr>
<td>Distance of Mucron from tail tip</td>
<td>-</td>
</tr>
<tr>
<td>Spicules</td>
<td>2</td>
</tr>
<tr>
<td>Size:</td>
<td>0.067-0.098 (0.07) x 0.08-0.10 (0.07)</td>
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<tr>
<td>Plectanes</td>
<td>With 6 plates; post-cloacal</td>
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<tr>
<td>Caudal papillae</td>
<td>Pre-anal: 39-60</td>
</tr>
<tr>
<td></td>
<td>Post-anal: 54-56pairs</td>
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<tr>
<td>Ratio of Caeca : Ventricular Appendix</td>
<td>1:1.02</td>
</tr>
<tr>
<td>Width/Length ratio of ventriculus</td>
<td>1 : 0.63</td>
</tr>
<tr>
<td>Length ratio of ventriculus : cece</td>
<td>1 : 0.74</td>
</tr>
<tr>
<td>Length ratio of spicule :body</td>
<td>1 : 0.10</td>
</tr>
</tbody>
</table>
Figures

1a-b. Rotundocollarette capoori n.gen., n.sp. a. Schematic plan of enface view of male worm to show distribution of cephalic apertures and papillae with denticles (Not to scale); b. Anterior end of female to show cephalic lateral papillated folds, intestinal caeca and ventricular appendix (x 240).

Figure 2
Rotundocollarette capoori n.gen., n.sp. 2. Light microscopic image of anterior end of worm showing cephalic alae with anterior collarette comprised of skirting frill at its distal end. (x 100);

Figure 3

Rotundocollarette capoori n.gen., n.sp. 3. Light microscopic image of anterior end of worm showing prominent lateral and cephalic alae (x 100).

Figure 4
Rotundocollarette capoori n.gen., n.sp. Posterior end of tail of male worm to show caudal alae. (x 100; Scale: 0.10mm).

Figure 5

Rotundocollarette capoori n.gen., n.sp. Enface view of female to show 3 pores–i. Oral aperture flanked by cephalic papillae mounted atop elevated muscular base; ii. Excretory pore flanked by 2 teeth, and iii. Genital pore flanked by 2 additional teeth. (Scale: 10μm)

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