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Intraspecific variation in the caudal papillae of *Raphidascaris mundeswariensis* (Nematoda: Raphidascarididae) from gobiiform fishes in eastern India

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Abstract: The morphological variation of the nematode *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021 in four fish hosts, *Apocryptes bato* (Hamilton), *Glossogobius giuris* (Hamilton), *Taenioides cirratus* (Blyth) and *Eleotris lutea* (Day), from the Mundeswari River of West Bengal, India, was studied. Partial sequences of the 28S rRNA and COI gene were generated to verify the conspecificity of these isolates while evaluating host-related variation in these samples. Little to no genetic variation was found among isolates from the different hosts and all were identified as *R. mundeswariensis.* However, subtle differences were found in the morphology among specimens of *R. mundeswariensis* from the four different hosts, mainly the number of caudal papillae and spicule length. The difference in the nematode morphology from various fish species can be a case of host-induced morphological variation which may enhance the adaptive capabilities of these helminths to parasitise a wide range of hosts. The present study raises questions about the use of the number of caudal papillae as an important taxonomic character in this species and other related nematodes. The results from this study also highlight the importance of examining as many specimens as possible from different hosts in the same localities to cover the range of intraspecific variation.

Keywords: Morphological variations, 28S rDNA, COI, Ascaridoidea, Pisces, Mundeswari River

Morphology plays a crucial role in recognising species and as primary step for alpha-taxonomy (Dayrat 2005, Moravec and Justine 2005, 2011, Anderson et al. 2009). Morphological variation is a well-known phenomenon in terms of the presence/absence of certain characters, their type, number, as well as their positions and measurements, that are visible and commonly utilised in nematodes and in other groups of parasites such as protistans, microsporidians, platyhelminths, pentastomids and mites (González et al. 2019). However, data on the intraspecific variability of morphological characters are often lacking, thus compromising their taxonomic value (Tarte and Mai 1976). Members of the nematode order Ascaridida are no exception (Pereira and Luque 2017, González et al. 2019).

The genus *Raphidascaris* Railliet et Henry, 1915 (Nematoda: Ascaridida: Raphidascarididae) comprises a large and diverse assemblage of 32 species parasitising freshwater and marine fishes (Moravec and Nagasawa 2002, Xu et al. 2012, Patra et al. 2021). One of these species, *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021, was originally described from the mudskipper *Apocryptes bato* (Hamilton) (Gobiidae) collected in the Mundeswari River, West Bengal, India and represents the first credible documentation of a *Raphidascaris* species from the Indian subcontinent (Patra et al. 2021).

Although the number and arrangement of male caudal papillae were considered important taxonomic characters in ascaridoid taxa, including *Raphidascaris* (Fagerholm 1991, Nadler 1992, Nadler and Hudspeth 1998, 2000, Pereira and Luque 2017), variations in caudal papillae were observed only in three populations of *Raphidascaris* (*Sprentascaris*) *lanfrediae* Melo, Santos, Giese, Santos et Santos, 2011 collected in *Geophagus argyrosticus* (Kullander), *Geophagus proximus* (Castelnau) and *Satanoperca jurupari* (Heckel), all from Brazil (Melo et al. 2011, Pereira and Luque 2017).

The present work utilises an integrative taxonomy approach by using both morphometric and genetic features (where available) of freshly collected nematodes, from three different gobiiform hosts in the same locality (Mundeswari River, Hooghly, West Bengal, India), to evaluate the importance of various taxonomically important characters (mainly caudal papillae number) for species delimitation in this group of nematodes.

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MATERIALS AND METHODS

In total, 559 fishes belonging to four species, *Apocryptes bato* (Oxydercinae), *Glossogobius giuris* (Hamilton) (Gobiinae), *Taenioides cirratus* (Blyth) (Amblyopinae) (all Gobiidae) and *Eleotris lutea* (Day) (Eleotridae: Eleotrinae), were examined from the Mundeswari River at Ranjitbati, Hooghly, West Bengal, India (22.6832N, 87.8901E), during winter (December, January, February), summer (March, April, May), monsoon (June, July, August), and post-monsoon (September, October, November) season from December 2017 to November 2021. Live fish were killed by dorsal pithing.

Nematodes were found in three fish species (infection parameters follow Margolis et al. 1982): *G. giuris* – prevalence 11% (n = 297 fish), mean intensity 1.9 ± 0.7 (1–11 worms/host); *T. cirratus* – prevalence 29% (n = 38), mean intensity 3.3 ± 1.0 (1–7 worms/host); *E. lutea* – infected two fish of three examined, mean intensity 1.5 (1–2 worms/host).

Live nematodes were collected from the digestive tract, cleaned in saline, fixed in hot 4% formaldehyde solution, and subsequently preserved in 70% ethanol (Moravec 2013). Some worms from all host species were directly fixed in 99% ethanol for molecular work (Moravec et al. 2010).

In total, 121 nematode specimens (42 males and 79 females) were examined for both morphological (measurements, SEM and line drawing) and molecular study. The specimens were cleared in glycerine for light microscopical examination, using an Olympus BX53 (Olympus Corporation, Tokyo, Japan) microscope. Detailed line drawings were made using an Olympus BX53 drawing attachment. All measurements are in micrometres unless otherwise stated.

For scanning electron microscopy, specimens fixed in formalin were post-fixed in 1% osmium tetroxide, dehydrated using a graded alcohol series, infiltrated using hexamethyldisilazane, air-dried (modified from Bowen et al. 1990), mounted on stubs and coated with gold. Gold-coated specimens were examined with a Zeiss Sigma-300 FE Scanning Electron Microscope (Carl Zeiss AG, Oberkochen, Baden-Württemberg, Germany) at an accelerating voltage of 10 kV. For scanning electron microscopic study, one male nematode from *A. bato*, three male and three female nematodes from *G. giuris*, and five male and three female nematodes from *T. cirratus* were used.

Scientific and common names of fishes are according to Froese and Pauly (2024). Examined specimens were deposited in the Zoological Survey of India, Kolkata, India (ZSI /WN 3114/2 and ZSI/WN 3114/3), and the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska, USA (HWML 118817–118821).

DNA was extracted from nematodes as described in Patra et al. (2021). Worms used for extraction in this study were as follows: 2 worms (male and female) from *A. bato*, 5 worms (4 females, 1 male) from *G. giuris*, 3 worms (2 females, 1 male) from *T. cirratus*, and 2 worms (both females) from *E. lutea*. The 28S rRNA gene was partially amplified and sequences obtained following the methods described in Patra et al. (2021). The cytochrome *c* oxidase subunit 1 (COI) gene was partially amplified by PCR using the primers 507 (forward) 5’AGTTCTAATCATAARGATATYGG 3’ (Nadler et al. 2006) and HCO 2198 (reverse): 5’ TAAACTTCAGGGTGACCAAAAAATCA 3’ (Folmer et al. 1994). These molecular markers were chosen because of their established application in nematode systematics (De Ley et al. 2005, Prosser et al. 2013, Gonçalves et al. 2021).

PCR reactions were performed on an Applied Biosystems Veriti thermal cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts, USA) in a total reaction volume of 50 µl containing 25 µl of Phusion® Hot Start Flex 2X Master Mix (New England BioLabs Inc.), 5 µl of extracted DNA as template, 2.5 µl each of forward and reverse primers at a concentration of 10 µmol/µl, and 15 µl of nuclease free water. The amplification protocol consisted of a touchdown protocol as follows: an initial step of 30 s. at 98°C, followed by 1.5 min at 94°C, 3 cycles of 40 s. at 94°C, 40 s. at 51°C (annealing) and 1 min at 72°C (elongation), 1 cycle of 40 s. at 94°C, 40 s. at 50°C (annealing) and 1 min at 72°C (elongation), and 30 cycles of 40 s. at 94°C, 40 s. at 48°C (annealing) and 1 min at 72°C (elongation), followed by a final extension of 7 min at 72°C.

PCR products were purified using ExoSAP-IT Express PCR Product Cleanup (Affymetrix Inc., Santa Clara, California). Purified products were sent to MCLab (South San Francisco, California), for automated Sanger sequencing. Contigs were manually checked, edited for accuracy and trimmed using FinchTV (Geospiza Inc., Seattle, Washington), and assembled in MEGA X (Kumar et al. 2018). Sequences were aligned using Clustal W and pairwise differences analysed in MEGA X.

Sequences generated in this study were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/) with the following accession numbers: 28S rRNA gene: PP034299–PP034300; COI: PP025438–PP025440. Hologenophores and paragenophores (Pleijel et al. 2008) are deposited in the HWML collection with the following accession numbers: HWML 118817–118821.

RESULTS

Raphidascaris mundeswariensis Patra, Choudhury, Thorn et Ash, 2021

Morphological characterisation

All measurements were taken from adult specimens and are listed in Table 1 with host data (due to the paucity of specimens, a comparable detailed morphological study of the nematodes from *Eleotris lutea* could not be accomplished and those limited data are not provided here).

General description (based on specimens from *Apocryptes bato*, *Glossogobius giuris*, and *Taenioides cirratus*).

Anterior end with three well-developed lips, nearly equal in size (Figs. 1C, 2C, 3A–C).

Males. Body 3.35–6.41 mm long, maximum width 91–288. Dorsal lip 19–43 long, 22–42 wide. Left ventrolateral lip 22–55 long, 20–41 wide. Right ventrolateral lip 22–52 long, 21–44 wide. Oesophagus 363–594 long, 53–144 in maximum width, representing 9–12% of body length. Nerve ring encircling oesophagus 27–40% from anterior end of oesophagus. Nerve ring 122–225 and excretory pore 194–463 from anterior extremity (Figs. 1A, 2B). Ventriculus 31–53 long, 53–113 wide. Ventricular appendix 194–388 long, 29–69 wide. Intestine 2.78–4.73 mm long, 43–144 wide. Distance from anterior end to single testes loop 571–1,069. Seminal vesicle 559–1,419 long, 59–216 wide. Ejaculatory duct 421–844 long, 31–138

Table 1. Morphological measurements of *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021 parasitic in various gobiiform fish hosts from Mundeswari River, West Bengal, India. Measurements in micrometres unless otherwise stated. Main characteristic differences of nematodes from different gobiiform fish are in bold.

Host	***Apocryptes bato*** (type species)	***Glossogobius giuris***	***Taenioides cirratus***
Source	Patra et al. 2021; present study	present study	present study
Male	n = 7	n = 9	n = 9
Total length (mm)	3.73–5.44	3.35–6.41	3.36–3.37
Total width (end of oesophagus)	106–202	116–288	91–103
Dorsal lip length	35–38	28–43	19–25
Dorsal lip width	30–42	23–39	22–23
Left ventrolateral lip length	37–55	23–45	22–23
Left ventrolateral lip width	23–33	25–41	20–23
Right ventrolateral lip length	39–52	31–44	22–23
Right ventrolateral lip width	21–30	26–44	22–26
Oesophagus length	419–562	384–594	363–367
Oesophagus width	73–125	75–144	53–70
Oesophagus/body length ratio (%)	8.9–11.1	8.7–12.4	9.2–9.3
Nerve ring from anterior end	144–221	146–225	122–123
Nerve ring encircling oesophagus from the anterior end of oesophagus (%)	26.9–30.4	30.3–40.4	27.6–27.7
Excretory pore from anterior end	306–381	256–463	194–294
Ventriculus length	42–49	50–53	31–44
Ventriculus width	81–99	63–113	53–64
Ventricular appendix length	219–339	200–388	194–214
Ventricular appendix width	29–63	31–69	31–39
Intestinal length (mm)	3.21–4.73	2.78–4.49	2.81–2.83
Intestinal width	78–121	66–144	43–66
Seminal vesicle length	706–1,287	663–1,419	559–572
Seminal vesicle width	79–148	69–216	59–60
Ejaculatory duct length	494–844	421–840	419–425
Ejaculatory duct width	31–93	34–138	41–44
Spicule length	**214–255**	**181–288**	**147–175**
Spicule length/body length (%)	17.1–22.2	18.5–33.6	19.2–22.9
Testes loop to anterior end	571–913	731–1069	611–700
Caudal papillae (pairs)	**21/25**	**21/23**	**23**
Preanal papillae (pairs)	**14/17**	**15**	**16**
Adanal papillae (pairs)	**1**	**1**	**1**
Postanal papillae (pairs)	**6/7**	**5/7**	**6**
Tail length	69–104	72–91	64–75
Tail width (at anus)	69–106	44–88	47–59
Caudal mucron	15–19	16–19	8–19
Female	n = 7	n = 12	n = 12
Total length (mm)	7.06–11.92	4.40–8.86	4.30–5.64
Total width (end of oesophagus)	155–454	121–369	116–720
Dorsal lip length	39–76	31–42	34–59
Dorsal lip width	48–62	25–48	25–42
Left ventrolateral lip length	52–73	31–50	34–69
Left ventrolateral lip width	34–48	25–53	27–56
Right ventrolateral lip length	48–80	31–45	33–72
Right ventrolateral lip width	30–53	27–56	30–66
Oesophagus length	513–947	396–881	372–797
Oesophagus width	94–228	69–213	72–191
Oesophagus/body length ratio (%)	9.0–13.0	10.0–12.6	9.5–11.6
Nerve ring from anterior end	175–288	144–256	169–331
Nerve ring encircling oesophagus from the anterior end of oesophagus (%)	26.4–36.9	24.1–38.0	24.9–38.7
Excretory pore from anterior end	248–424	294–631	272–453
Ventriculus length	44–94	41–69	41–78
Ventriculus width	78–157	66–109	72–153
Ventricular appendix length	288–415	237–475	203–434
Ventricular appendix width	44–106	25–100	28–69
Intestinal length (mm)	6.41–10.57	3.69–7.23	1.40–4.70
Intestinal width	68–181	59–159	66–128
Vulva from anterior end	956–1,777	778–1,613	763–1,481
Vulva from anterior end/ body length ratio (%)	5.92–6.75	5.27–7.45	3.89–5.75
Vagina length	300–438	425–768	694–1,037
Egg diameter	19–29	19–31	19–25
Anterior ovary loop to oesophagus	556–994	378–1,900	694–1,834
Posterior ovary loop to anus	495–619	272–1,097	422–941
Tail length	181–352	100–269	178–331
Tail width (at anus)	106–138	75–259	72–128

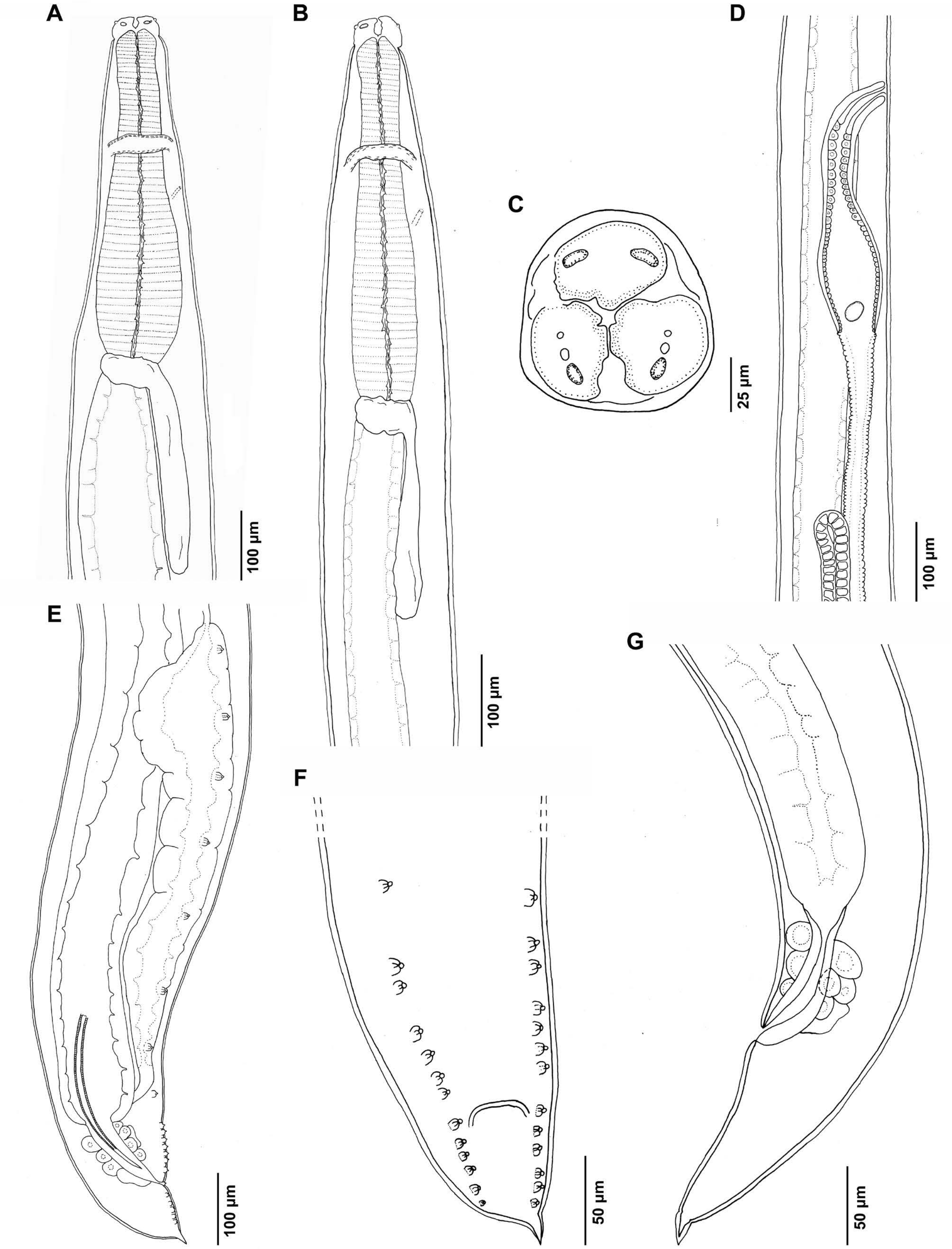

Fig. 1. *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021 from *Glossogobius giuris* (Hamilton). **A** – anterior end of male, sublateral view; **B** – anterior end of female, lateral view; **C** – cephalic end of female, apical view; **D** – vulval region, lateral view; **E** – caudal region of male, lateral view; **F** – posterior end of male, subventral view; **G** – posterior end of female, lateral view.

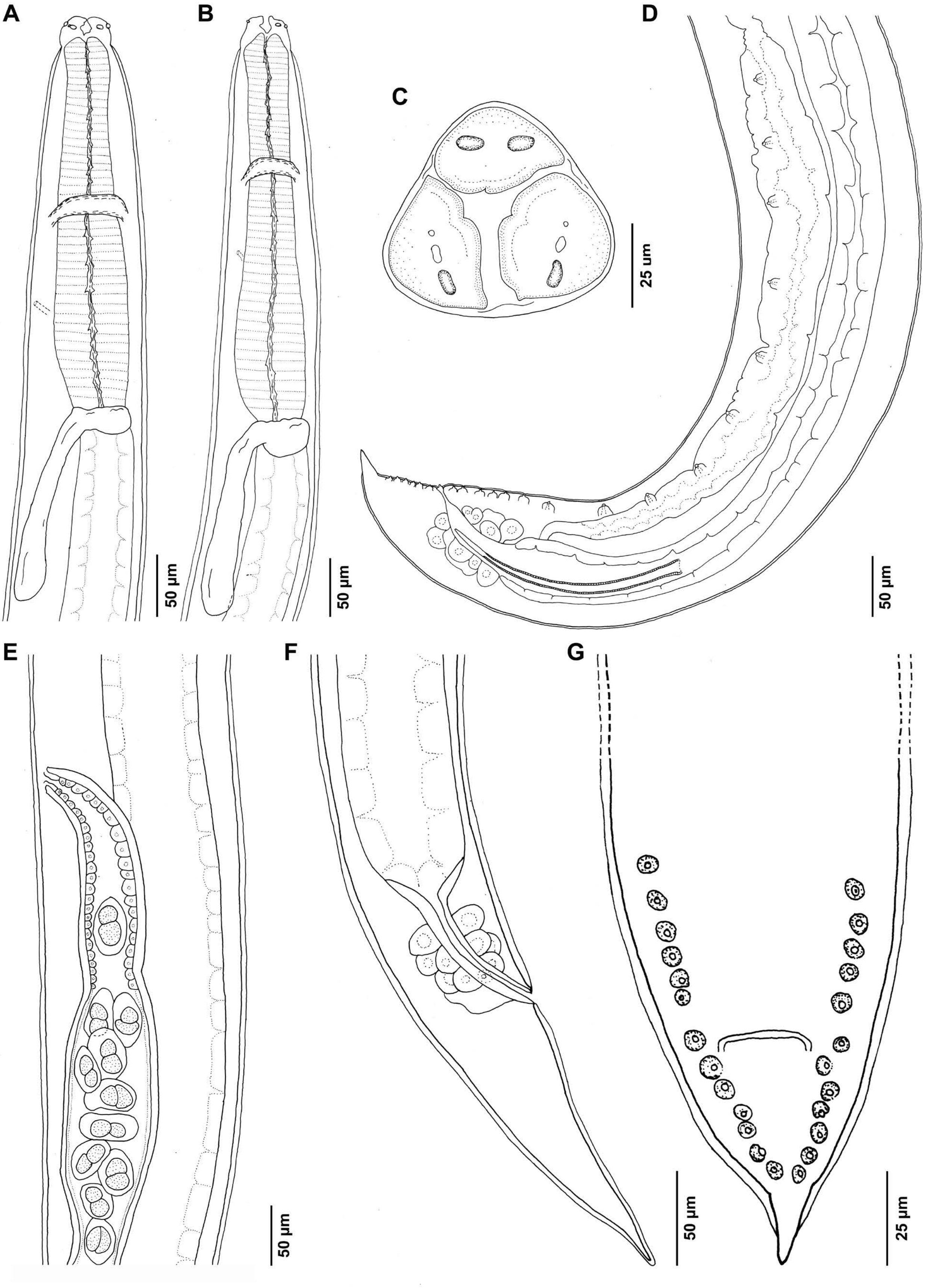

Fig. 2. *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021 from *Taenioides cirratus* (Blyth). **A** – anterior region of female, sublateral view; **B** – anterior end of male, lateral view; **C** – cephalic end of female, apical view; **D** – caudal region of male, lateral view; **E** – vulva region, lateral view; **F** – posterior end of female, lateral view; **G** – posterior end of male, ventral view.

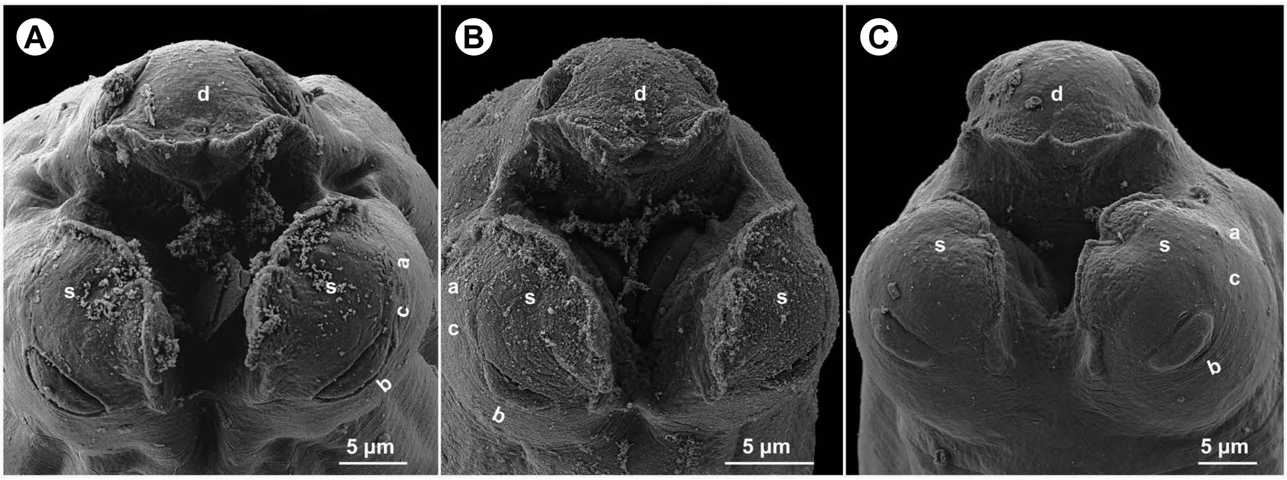

Fig. 3. SEM micrograph of *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021 from different hosts. Apical view of cephalic ends of females from *Apocryptes bato* (Hamilton) (A), *Taenioides cirratus* (Blyth) (B) and *Glossogobius giuris* (Hamilton) (C). *Abbreviations*: a – amphid; b – double papilla, c – single papilla; d – dorsal lip; s – subventral lip.

wide. Spicules slender, almost equal in length, 147–288 long, representing 17–34% of body length. Caudal papillae 21–25 pairs, arranged as follows: 14–17 pairs of preanal papillae, 1 pair of adanal papillae, 5–7 pairs of postanal papillae. Tail 64–104 long, 44–106 wide at anus. Caudal mucron 8–19 long.

Males collected from *A. bato* possess 14 or 17 pairs of preanal papillae, of which 6–7 pairs are smaller and closer to anus, one pair of adanal papillae, and 6–7 pairs of postanal papillae (Figs. 4C, 5A). Males collected from *G. giuris* possess 15 pairs of preanal papillae, of which 6 pairs are smaller and closer to anus, one pair of adanal papillae, and 5 or 7 pairs of postanal papillae (Figs. 1E,F, 4A, 5C,D). Males collected from *T. cirratus*, possess 16 pairs of preanal papillae, of which 7 pairs are smaller and closer to anus, one pair of adanal papillae and six pairs of postanal papillae. Furthermore, spicules much shorter than in worms from other hosts (Figs. 2D,G, 4B, 5B).

Females. Body 4.30–11.92 mm long, maximum width 116–720. Dorsal lip 31–76 long, 25–62 wide. Left ventrolateral lip 31–73 long, 25–56 wide. Right ventrolateral lip 31–80 long, 27–66 wide. Oesophagus 372–947 long, 69–228 in maximum width, representing 9–13% of body length. Nerve ring encircling oesophagus 24–39% from anterior end of oesophagus. Nerve ring and excretory pore 144–331 and 248–631, respectively, from anterior extremity (Figs. 1B, 2A). Ventriculus 41–94 long, 66–157 wide. Ventricular appendix 203–475 long, 25–106 wide. Intestine 1.4–10.6 mm long, 59–181 wide. Vulva pre-equatorial, 763–1,777 from anterior extremity, 3.9–7.5% of body length. Vagina 300–1,037 long (Figs. 1D, 2E). Anterior ovarian loop 378–1,900 posterior to oesophagus and posterior ovarian loop 272–1,097 anterior to anus. Eggs nearly rounded, 19–31 long. Tail 100–352 long, 72–259 wide at anus (Figs. 1G, 2F).

Molecular characterisation

Partial sequences (996–1,001 bp) of the 28S rRNA gene were successfully generated for 1–2 specimens of *Raphidascaris* from both *T. cirratus,* and *E. lutea*, which are identical to that of *R. mundeswariensis* from *A. bato*. 28S rRNA gene sequences of worms from *G. giuris* could not be reliably assembled because of the poor quality of several contigs. Partial sequences (501–587 nucleotides) of the COI gene were successfully generated for 1–2 specimens of *Raphidascaris* from *T. cirratus* and *G. giuris*, and *R. mundeswariensis* from *A. bato*.

The COI sequence for worms from *A. bato* and *T. cirratus* were based on clean sequences generated from the reverse primer (HCO 2198) only, whereas the COI sequence of worms from *G. giuris* was assembled using sequences generated from both forward (507) and reverse (HCO 2198) primers. The COI sequences (aligned and trimmed to 496 nucleotide) differed by 0–2 base pairs among the three different isolates. The isolates from *A. bato* differ in 1 base pair (0.2% divergence) from isolates from *G. giuris* and *T. cirratus*. The isolates from *G. giuris* differ in 2 base pairs (0.4% divergence) from isolates from *T. cirratus*. The combined information from the 28S rDNA and COI sequences shows that the isolates from these four hosts are conspecific and belong to *R. mundeswariensis*.

Representative DNA sequences (GenBank accession number in parentheses): 28S rDNA (partial) sequence of nematodes from *T. cirratus* (PP034299) and *E. lutea* (PP034300); COI gene (partial) sequences of nematodes from *A. bato* (PP025439), *G. giuris* (PP025438) and *T. cirratus* (PP025440).

Remarks

Patra et al. (2021) indicated that *R. mundeswariensis* is closely related to *R. gigi* Fujita, 1928, *R. lophii* (Wu, 1949) Hartwich, 1975 and *R. longispicula* Li, Liu et Zhang, 2012, although it can be distinguished by having 14–17 pairs of preanal, 1 pair of adanal and 5–7 pairs of postanal papillae in contrast to 26–30 preanal, 2–3 adanal and 9 postanal papillae of *R. gigi*, 26–32 pairs of preanal, 3–4 adanal and 8–11 postanal papillae of *R. lophii*, and 25–28 preanal,

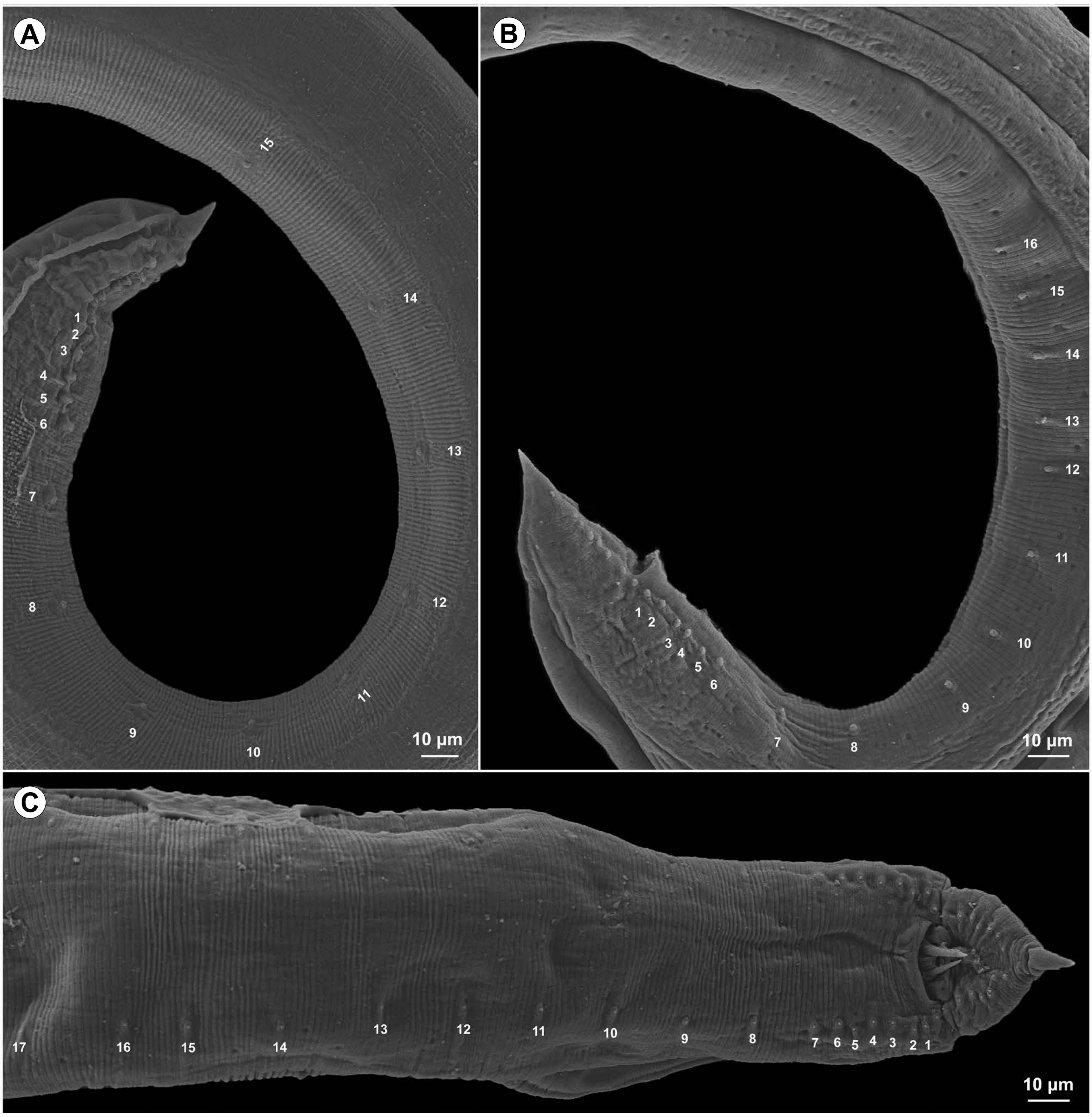

Fig. 4. SEM micrographs of *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021 from different hosts. Posterior region of male from *Glossogobius giuris* (Hamilton) (A), *Taenioides cirratus* (Blyth) (B) and *Apocryptes bato* (Hamilton) (C). Numbers show preanal papillae.

1–2 adanal, and 6–8 pairs of postanal papillae of *R. longispicula* (Moravec and Nagasawa 2002, Li et al. 2012, Xu et al. 2012). Although the number of postanal papillae of *R. mundeswariensis* and *R. longispicula* overlap, the spicule lengths of *R. mundeswariensis* (147–288 µm) and *R. longispicula* (1.13–1.32 mm) are notably different. Similarly, larger spicules are found in *R. gigi* (354–476 µm) and *R. lophii* (490–882 µm) than in *R. mundeswariensis*.

DISCUSSION

Specimens of *Raphidascaris* from gobiid and eleotrid hosts in this study were collected from the same locality of the Mundeswari River as those of *Raphidascaris mundeswariensis* from *Apocryptes bato* by Patra et al. (2021). The genetic data suggest that worms from all four gobiiform fishes are the same species. New observations on *R. mundeswariensis* indicate morphological variation in the number of caudal papillae, with up to 17 pairs of preanal papillae and 7 pairs of postanal papillae, 1 pair of adanal papillae. Due to a lack of good-quality conspecific specimens from *Eleotris lutea*, a detailed morphological study could not be performed.

The coevolution of parasites and their hosts can be understood by studying closely related parasites that exhibit high host specificity and can be differentiated by their morphological characters (Downes 1990). However, intraspe-

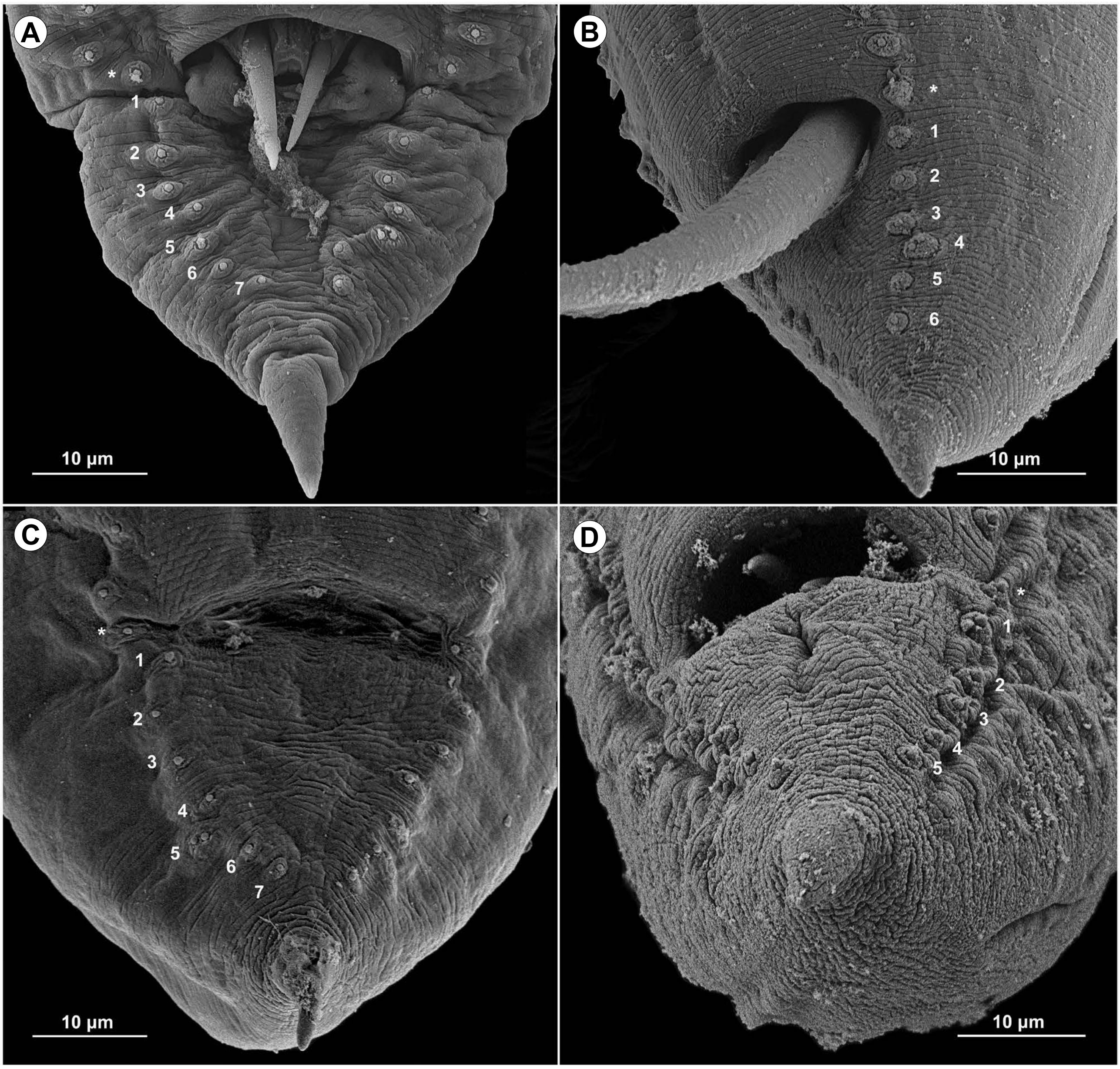

Fig. 5. SEM micrograph of *Raphidascaris mundeswariensis* Patra, Choudhury, Thorn et Ash, 2021 from different hosts. Posterior region of male from *Apocryptes bato* (Hamilton) (A), *Taenioides cirratus* (Blyth) (B) and *Glossogobius giuris* (Hamilton) (C, D). Numbers show postanal papillae. *Abbreviations*: * adanal papillae.

cific morphological variation can change gradually within a population across an ecological or landscape gradient or cline, often resulting into two extreme variable forms (Downes 1990). Clinal and geographic variation are not factors in the intraspecific variation observed in this study.

Additionally, we were able to use molecular data to show that the worms from the different gobiiform hosts are conspecific. While genetic markers are undoubtedly a powerful tool to quantify and evaluate intraspecific variation in several taxa, including helminths, morphology still plays a major role in species descriptions (Pérez-Ponce de León and Nadler 2010, Nadler and Pérez-Ponce de León 2011). Consequently, morphological variation in characters that are important in taxonomy require special attention.

Morphological variation in nematodes may reflect either phenotypic plasticity within the same host species or host-induced variation among individuals from different host species (Kirillova et al. 2021), as indicated in the present case. Conversely, our increased awareness of cryptic species (Pérez-Ponce de León and Nadler 2010) requires that genetic, morphological and biological information be brought to bear synergistically to distinguish between host-induced variation and diversification due to host-shifting (Bush 1969, 1975, Howard and Berlocher 1998, Choudhury et al. 2002, McCoy 2003). In this study, we initially considered the possibility that similar worms showing these variations in the different gobiiform hosts may represent a species complex. However, the molecular data compelled us to consider these worms as belonging to the same species, *R. mundeswariensis*, and differences in the morphology as intraspecific variation.

The possible reasons behind host-induced variations are parasite intensity, previous parasitic exposures, involvement of other parasites, age and diet of host (González et al. 2019). Of these factors, heavy nematode infections were recorded in the host fish during the winter season as compared to summer, monsoon and post monsoon seasons, but the role of other factors listed by González et al. (2019) are unknown. Different instances of host-induced variations are found among various nematodes. *Aplectana hylambatis* (Baylis, 1927) reported from five different anuran hosts showed variations in the arrangement and number of mamelon-like protuberances and caudal papillae (González et al. 2019), whereas variations in the size and shape of the lateral alae, cephalic vesicle and male caudal bursa were found in *Oswaldocruzia filiformis* (Goeze, 1782) parasitising ten amphibian species (Kirillova et al. 2021). Pereira and Luque (2017) observed similar variation in male caudal papillae in *Raphidascaris lanfrediae*. These variations can be probably a result of gene-environment interactions as indicated in *Raphidascaroides brasiliensis* Moravec et Thatcher, 1997 (Pereira and Luque 2017). Perhaps similar interactions are at play in the case of *R. mundeswariensis*.

During our survey, fishes were collected from the same locality of the Mundeswari River, all belonging to the order Gobiiformes, namely *Apocryptes bato*, *Glossogobius giuris*, *Taenioides cirratus* and *Eleotris lutea*. *Apocryptes bato*, commonly known as chengo in West Bengal, is found in Indian Ocean drainages ranging from eastern India to Myanmar (Talwar and Jhingran 1991, Barman et al. 2000, Parenti and Jaafar 2017) and is encountered in the rivers of the central as well as the southern parts of West Bengal, most commonly in intertidal mudflats of estuaries. It is a facultative air-breather and amphibious in nature (Shukla et al. 2014, Froese and Pauly 2024).

Glossogobius giuris, locally known as bele in West Bengal, ranges from the Red Sea, across East Africa and most inland freshwater bodies over the Indian Ocean and Western Pacific. These fishes are also distributed in coastal and estuarine waters from austral Africa, Madagascar to India and southern China. It is carnivorous and also exhibits cannibalism (Froese and Pauly 2024). On the other hand, *T. cirratus*, locally known as lal chengo in West Bengal, is common in the Indo-Pacific region with a wide distribution in offshore islands of East Africa, India, and Australia and also reported from Japan, South Korea, Bangladesh, New Guinea and New Caledonia. This mud-dwelling fish is capable of living out of the water for a while (Froese and Pauly 2024).

Finally, *E. lutea*, known locally as kalo bhola in West Bengal, is distributed in India and Myanmar and also reported from Bangladesh (Froese and Pauly 2024). Although these gobiiform fishes share the same habitat, their different feeding habits and other niche differences promote ecological segregation (Hora 1935, Chatterjee et al. 2013). Whether there is any relationship between this ecological segregation and the structural variations in the nematodes parasitising these fishes remains to be explored.

Reports of a particular *Raphidascaris* species from different fish hosts is not unusual, as in the case of the type species of the genus, *R. acus* (Bloch, 1779) (see Introduction). Another *Raphidascaris* species, *R. arii* Yooyen, Moravec et Wongsawad, 2011, was described from marine siluriform fishes like *Arius maculatus* (Thunberg) and *Plotosus lineatus* (Thunberg) from the coastal region of the Gulf of Thailand, Thailand (Yooyen et al. 2011). *Raphidascaris etelidis* Moravec et Justine, 2012 was reported from the intestine of *Pristipomoides filamentosus* (Valenciennes) and *Etelis coruscans* (Valenciennes) from the southwestern coast of New Caledonia (Moravec and Justine 2012).

Raphidascaris gigi was also reported from different fish hosts such as *Pelteobagrus nudiceps* (Sauvage) in Lake Biwa, Japan, and Masu salmon *Oncorhynchus masou* (Brevoort) in the Kinu River, Tochigi Prefecture, Central Japan (Moravec and Nagasawa 2002). In addition, Yamaguti (1961) reported another host, *Sarcocheilichthys variegatus* (Temminck et Schlegel), in his book (Moravec and Nagasawa 2002).

Raphidascaris lophii was found in five marine fish hosts from three different orders namely, *Lophius litulon* (Jordan), *Lophiomus setigerus* (Vahl), *Antennarius hispidus* (Bloch et Schneider), *Zeus faber* Linnaeus, and *Ostichthys japonicus* (Cuvier) in the East and South China Sea. *Raphidascaris lophii* from these hosts showed no intraspecific nucleotide variation but notable morphological variation (Xu et al. 2012).

Raphidascaris mahnerti (Petter et Cassone, 1984) was reported from a wide range of catfish hosts namely, *Loricariichthys brunneus* (Hancock), *Loricariichthys liabialis* (Boulenger), *Loricariichthys platymetopon* Isbrücker et Nijssen and *Pseudohemiodon laticeps* (Regan) from various localities of South America (Malta et al. 2020). Morphological variations (mainly in the male caudal papillae) were found in three populations of *R. lanfrediae* from *Geophagus proximus* (Castelnau) in Amapá, Brazil, and *Satanoperca jurupari* and *Geophagus argyrostictus* Kullander in the State of Pará, Brazil (Melo et al. 2011, Pereira and Luque 2017).

It is difficult to disentangle geographical variation and host-induced variation in some of the afore-mentioned studies because hosts and their worms were collected from different localities. The importance of the current study lies in the fact that intraspecific morphological variations were found in *R. mundeswariensis* collected from different fish hosts in the same locality, Mundeswari River in West Bengal, India. The biological significance of this morphological variation remains unclear, but ecological and anatomical differences between these hosts may suggest the earliest stages of diversification. The marked diversity in pattern and number of distal papillae in aquatic ascaridoid parasites could be an indication that the aquatic parasites can exploit greater genetic potential due to selection pressures to adapt to new host groups (Fagerholm 1991).

The number and arrangement of caudal papillae have been considered as important characters in the taxonomy and classification of many ascaridoid taxa (Nadler 1992, Nadler and Hudspeth 1998, 2000, Pereira and Luque 2017) but our study suggests that the number of caudal papillae can be different in a single species in the same or different hosts. As a result, variation in such characters should be considered carefully when describing and differentiating species.

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