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Three new species of *Pseudorhabdosynochus* (Monogenea: Diplectanidae) from Vietnamese grouper (*Epinephelus* spp.) (Perciformes: Serranidae)

Binh T. Dang[1]*, Glenn A. Bristow[2], Christoffer Schander[2] and Bjørn Berland[2]

1) Institute for Biotechnology and Environment, University of Nha Trang, Nha Trang, Vietnam
2) Department of Biology, University of Bergen, P.O. Box 7800, N-5020 Bergen, Norway

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Abstract: Three new species of monogenea are described from cultured and wild grouper from Nha Trang and Cam Ranh Bays, Vietnam. *Pseudorhabdosynochus nhatrangensis* n. sp and *P. vietnamensis* n. sp. were found on wild and cultured *Epinephelus coioides* and *E. bleekeri*, while *Pseudorhabdosynochus brunei* n. sp. parasitizes wild *E. bruneus*. *P. nhatrangensis* n. sp. is characterized by a sclerotized vagina with cup-shaped trumpet, open at the distal part, followed by a proximal tubular region, twisted to a circle at the posterior end and extending into thin branches; squamodiscs with 10 rows of rodlets with the central row being a closed circle. The tegument is scaly. *P. vietnamensis* n. sp. has a very large male copulatory organ with the tube being widened and slightly curved posteriorly. A sclerotized vagina comprised of an anterior trumpet, followed by convoluted structure which twists and divides into two distinct chambers. The central row of the squamodiscs forms a closed circle with a central core present. A heavily scaled tegument with scales on ventral and dorsal surfaces extends from the squamodiscs to the level of the ovary. *P. nhatrangensiis* n. sp. can be differentiated from other *Pseudorhabdosynochus* species by the vaginal structure. The complex vaginal structure and extremely large male copulatory organ of *P. vietnamensis* are similar to *P. pai* reported from *E. tauvina*, but these two can be distinguished by details of the vagina, and number of rows of squamodiscs (17–18 in *P. vietnamensis* and 10–11 in *P. pai*). *P. brunei* n. sp. possesses a sclerotized vagina with a tubular region and two serial chambers at the distal opening; squamodiscs have only 7 rows of rodlets. An egg of this species was found, being large and oval with a long sclerotized tail.

Key Words: Grouper, Monogenea, *Pseudorhabdosynochus,* Nha Trang Bay

(✉) binhdangthuy@gmail.com

Intordaction

Species of *Pseudorhabdosynochus* are increasingly reported parasitizing grouper (Serranidae; Epinephelinae) throughout warm and temperature waters of oceanic regions (Oliver, 1986; Young, 1969; Dyer *et al.,* 1994, 1995; Vidal-Martinez *et al.,* 1997; Vidal-Martínez and Mendoza-Franco, 1998; Bu *et al.,* 1999; Santos *et al.,* 2000; Yang *et al*., 2005a;Yang, *et al*. 2005b;Wu *et al*., 2005a; Wu *et al*., 2005b; Justine and Euzet, 2005 a,b; Hinsinger and Justine, 2006 a.b; Justine and Sigura, 2007; Zeng and Yang, 2007; Neifar and Euzet, 2007; Justine, 2007a,b and 2008; Sigura *et al.,* 2007; Justine, 2009; Justine and Vignon, 2009). They are relatively host specific, even when widely distributed (Santos *et al.,* 2000). Santos *et al*. (2000) were the first to discuss species totals within *Pseudorhabdosynochus*, listing 23 species and describing 1 new species. Species richness currently comprises over 40 species with and without nominal status according to a summary by Justine (2007a). Since 2007, a number of *Pseudorhabdosynochus* species have been described (Yang *et al*., 2005a, Yang *et al*., 2005b; Neifar and Euzet, 2007; Justine, 2007b; Zeng and Yang, 2007; Sigura *et al.,* 2007; Justine and Sigura, 2007; Justine, 2008; 2009 and Justine and Vignon, 2009). The species in this genus now number over 60. As an increasing number of grouper are examined, additional species may be described.

During a survey of monogenean fauna of *Epinephelus* spp. (Serranidae) in Vietnam (Dang *et al.,* 2010), both new and previously known species of *Pseudorhabdosynochus* were collected. Three new species are described herein.

Materials and Methods

Parasite sampling and identification

Sea cage cultured *Epinephelus coioides* (Hamilton, 1822) (n=45), *E. bleekeri* (Vaillant, 1878) (n=40) and wild *E. bruneus* (Bloch, 1793) (n=28) (all Serranidae) were collected from Nha Trang Bay (12° 15' N, 109° 21' E) and Cam Ranh Bay (11° 52' 60 N, 109° 10' E), Vietnam, from January to August 2007, and from January 2008 to July 2009. Prior to examination, fish were held in indoor aerated water tanks. Skin, fins and gills of freshly euthanized fish were subsequently examined for monogenean parasites.

Freshly excised gills were kept in separate Petri dishes filled with filtered sea water and examined under a dissecting microscope. Monogeneans were removed and examined either alive or in 75% ethanol. *Pseudorhabdosynochus* species identification was based primarily on the morphology of the haptoral sclerites and the reproductive organs using various keys and species descriptions (Beverley-Burton and Suriano, 1981; Kritsky and Beverley-Burton, 1986; Bu *et al.,* 1999; Justine

and Euzet, 2005a; Yang *et al*., 2005b; Justine and Euzet, 2005b; Hinsinger and Justine, 2006a,b; Justine and Sigura, 2007; Justine, 2007a, b, Zeng and Yang, 2007; Justine and Vignon, 2009). For morphological analysis, whole mounts were prepared following Berland (2005). Various diagnostic characters were studied using differential interference contrast microscopy (Olympus BX51) and Cell* digital image analysis software (Olympus Soft Imaging Solutions GmbH, Münster, Germany). Body width was measured at mid-body or vagina level. All measurements are given in µm and presented as mean (range). The characters measured and the terminologies used are in accordance with Justine (2007a). Drawings of the haptoral hardparts and copulatory complexes were prepared with aid of a camera lucida. The images were digitally processed in Adobe Photoshop Elements®, version 5.0.

Holotype and paratypes specimens were deposited in the Natural History collection, Bergen museum (ZMBN), University of Bergen, Bergen, Norway.

Description

Pseudorhabdosynochus nhatrangensis n. sp.

(Figures: 1, 2 A–J)

Diagnostic: Body slightly dorsoventrally flattened, widest at level of ovary, length 458 (370–570, n=10), width 155 (120–185, n=10). Tegument armed with scales in region of haptoral peduncle. Anterior region with 3 pairs of head organs, 2 pair of eye-spots with posterior pair larger and more closely situated than anterior. Distance between outer margins of anterior eye-spot pair 18 (15– 20, n=5), posterior eye-spot pair 12 (10–14, n=5).

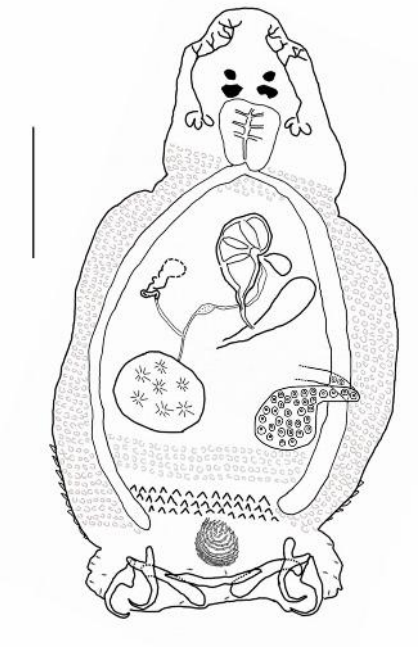

Fig. 1: *Pseudorhabdosynochus nhatrangensis* n. sp. from *Epinephelus coioides* and *E. bleekeri* in Nha Trang Bay, Vietnam. Dorsal view, composite drawing from holotype, paratypes and observation of live specimens. Scale bar: 100 µm.

Haptor differentiated from rest of body, almost as wide as body, width 157 (130–188, n=7), provided with 2 similar squamodiscs, 2 pairs of lateral hamuli, 3 bars and 14 marginal hooklets. Squamodiscs round in shape, made

up of rows of rodlets which tend to link with neighbors of same row; central rows forming closed circles; rodlets slightly thinner from centre to periphery; ventral and dorsal squamodiscs similar; length 48 (45–50, n=6), width 44 (38–55, n=6), always 10 (n=6) rows of rodlets and 1 closed circle; ventral hamuli with elongate root and broad shaft, outer length 48 (40–58, n=10), inner length 38 (33–40, n=10); dorsal hamuli with reduce root, outer length 38 (33–43, n=10), inner length 24 (23–25, n=10); ventral bars elongate and taper in two extremities; length 95 (90–103, n=10), maximum width 11 (9–14, n=10); groove not visible on its ventral side. Dorsal bars elongate, slightly broadened in mid-body, length 61 (55–68, n=10), maximum width 12 (11–14, n=10) (Fig. 2 G–L).

Mouth ventrally subterminal; pharynx subspherical, length 37 (30–45, n=6), width 36 (30–45, n=6). Oesophagus apparently absent, such that intestinal bifurcation immediately follows pharynx. Caeca simple, terminates blindly at level of posterior margin of vitelline field.

Testis subspherical, intercaecal, length 54 (53–63, n=4), width 38 (30–45, n=4). Vas deferens emerges from antero-sinistral part of testis, enlarges into small seminal vesicle; seminal vesicle in mid-region of body, transforms into duct; duct form bends then transform into small bulb followed by duct; duct enlarges to form vesicle, then connects to a copulatory organ. Prostatic reservoir conspicuous, connects with male copulatory organ (MCO) (Fig. 1).

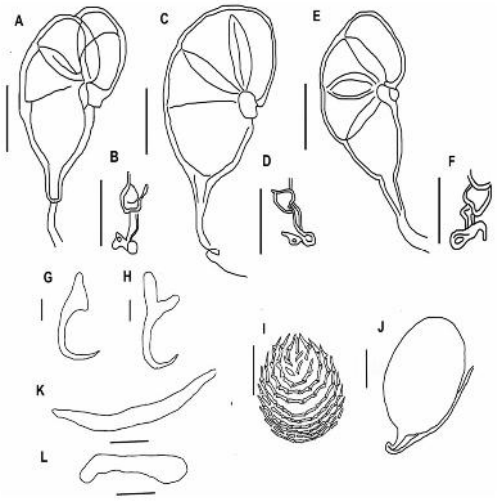

Fig. 2: *Pseudorhabdosynochus nhatrangensis* n. sp. A –F: Male copulatory organ and corresponding sclerotized vagina in three individuals; G, H: Ventral and dorsal hamuli; I: Squamodisc; J. The egg; K, L: Transverse and dorsal bars. Scale bare: 20µm for MCO, transverse, and dorsal bars; 10 µm for vagina, dorsal, ventral hamuli, egg and, squamodisc.

MCO elongate, slightly curved, divides into four chambers with third and fourth (posterior) chambers more sclerotized than 2 anterior chambers; fourth chamber ends in very sclerotized cone; cone prolonged by sclerotized tube; filaments are sometimes observed. Inner length 58 (52–65, n=10); cone length 14 (12–15, n=10); tube length 21 (18–25, n=10) (Fig. 2 A, C, E).Ovary subequatorial, intercaecal, pre-

testicular, encircles right caecum. Ovary length 52 (48–55, n=4); width 31 (25–40, n=4). Oviduct pass medially to form ootype, surrounded by Mehlis' gland; ootype short, opens into uterus. Uterus dextral. Duct from sclerotized vagina to ootype visible only in live specimens. Vitelline fields extend posteriorly from posterior to pharyngeal level in 2 lateral bands, confluent in post-testicular region, terminate anterior to peduncle. Bilateral connections from vitelline fields to ootype inconspicuous. Mature egg oval–shaped with long filament, length 45, 66, n=2 width 35, 50, n=2 (Fig. 2 L). Egg observed in uterus oval or ellipse–shaped with thick wall. Vagina comprised of unsclerotized region, inconspicuous, visible only in living animal, followed by sclerotized region. Sclerotized vagina sinistral, a complex sclerotized structure; length 29 (25–33, n=10) (Fig. 2 C, D, F). Sclerotized vagina comprises cup–shaped trumpet with opening at distal end, followed by proximal tubular region. Tube twisted in circle at posterior end, extending into thin branches. Trumpet and tube more sclerotized with thick wall.

Taxonomic summary

Type-host: Sea cage cultured *Epinephelus coioides* (Hamilton, 1822) and *E. bleekeri* (Vaillant, 1878) (Serranidae).

Type-locality: Nha Trang Bay (12° 15' N, 109° 21' E), Vietnam.

Site: Between secondary gill lamellae.

Type-material: Holotype ZMBN collection N0: 84826, collected on 18 January 2008, Nha Trang Bay, Vietnam on *E. coioides*. 3 paratypes (ZMBN collection N0: 84827-84829), all in ammonium picrate.

Material examined: 15 specimens, including 5 in ammonium picrate, 5 cleared with lactophenol and 5 live specimens. Measurements on 10 specimens.

Prevalence: 27% (12/45) on *E. coioides* and 10% (4/40) on *E. bleeckeri*

Etymology: The name of this species refers to Nha Trang Bay, where the species was found.

Remarks

Pseudorhabdosynochus nhatrangensis n. sp. has the characteristic features of *Pseudorhabdosynochus*, possessing an MCO of reniform, sclerotized structure with four characteristic compartments. With squamodiscs of 10 rows of rodlets, *P. nhatrangensis* can be easily separated from *Pseudorhabdosynochus* species possessing more than 14 rows (*P. riouxi* (Oliver, 1986), *P. monaensis* Dyer *et al.*, 1994, *P. epinepheli* (Yamaguti, 1938), *P. americanus* (Price, 1937) (synonymous with *P. hargisi* (Oliver and Paperna, 1984), *P. coioidesis* Bu *et al.*, 1999, *P. amplidiscatum* (Bravo-Hollis, 1954), *P. chinensis* Zhang *et al.*, 2001, *P. sulamericanus* Santos *et al.* 2000, *P. bouaini*

Neufar and Euzet, 2007, *P. enitsuji* Neufar and Euzet, 2007).

The shape and number of the row of rodlets, which form complete circles or concentric rings (the so-called lamellosquamodisc in "*P. cupatus* group" (Hinsinger and Justine, 2006b) are also important taxonomic features. With only central row forming a complete circle, *P. nhatrangensis* n. sp. can be differentiated from *P. cupatus* Young, 1968, *P. kritskyi* Dyer *et al*. 1995, *P. capurroi* Vidal-Martinez and Mendoza-Franco, 1998, *P. melanesiensis* Laird, 1958, *P. beverleyburtonae* (Oliver, 1984), *P. buitoe* Justine, 2007, *P. cuitoe* Justine, 2007, and, *P. duitoe* Justine, 2007, *P. fuitoe* Justine, 2007, and *P. guitoe* Justine, 2007, all having at least the two innermost rows forming complete circles or rings.

According to Justine (2007a), the sclerotized vagina is likely the key structure for *Pseudorhabdosynochus* identification. *P. nhatrangensis* n. sp. differentiates from the remaining species, which have a similar number of rows in the squamodisc, by the shape of the sclerotized part of the vagina. *P. nhatrangensis* n. sp. is characterized by a short and thin tubular vagina with a cup-shape trumpet opening anteriorly. It is somewhat similar to that of *P. serrani* Yamaguti, 1953, in that the proximal tubular region twists in a round circle before extending into thin chambers, but the later species possesses an oval, saccular distal part (trumpet), while it is cup-shaped in the former. The sclerotized vagina is smaller in *P. nhatrangensis* n. sp. with a total length of 25–33 vs 34.5–43.5 in *P. serrani*. *P. shenzhenensis* Yang *et al*., 2005 is differentiated from the new species by possessing a vagina with a flask-shaped distal section and hook-shaped tube.

Pseudorhabdosynochus vietnamensis n. sp.

(Figure 3, 4 A–I)

Diagnostic: Body elongate, length 1040 (840–1240, n=6), width 213 (190–250, n=6). Tegument scaly; scales on ventral and dorsal faces from level of ovary to squamodiscs. Anterior region with 3 pairs of head organs and 2 pairs of eye-spots; distance between outer margins of anterior eye-spot pair 24 (19–26, n=5), of posterior eye-spot pair 19 (15–22, n=5).

Haptor distinctly differentiated from rest of body, narrower than body, width 80 (75–85, n=4); length 22 (20–25, n=4), provided with 2 similar squamodiscs, 2 pairs of lateral hamuli, 3 bars and 14 marginal hooklets. Squamodiscs round in shape, made up of rows of rodlets; central rows forming closed circles and possessing an inner core; rodlets progressively thinner from centre to periphery; last row with very thin rodlets; ventral and dorsal squamodiscs similar; length 82 (75–90, n=6),

width 83 (73–95, n=6), with 17–18 (n=6) rows of rodlets; ventral hamuli with shaft straight and broad root, outer length 53 (18–58, n=6), inner length 41 (38–45, n=6); dorsal hamuli with reduced root, outer length 45 (43–50, n=6), inner length 32 (30–35, n=6); ventral bar lip-like shaped with elongate extremities; length 87 (70–100, n=6), maximum width 15 (13–20, n=6); groove visible on its ventral side. Dorsal bar broadened in mid-body, length 59 (53–70, n=6), maximum width 18 (13–20, n=6) (Fig. 4 E–H).

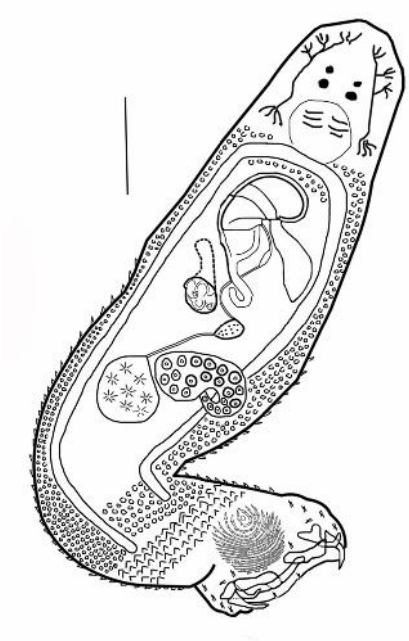

Figure 3: *Pseudorhabdosynochus vietnamensis* n. sp. from *Epinephelus coioides* in Nha Trang Bay, Vietnam. Dorsal view, composite drawing from holotype, paratype and observation of live specimens. Scale bar: 100 µm.

Pharynx subspherical, length 44 (30–52, n=5), width 42 (30–55, n=5). Oesophagus apparently absent, such that intestinal bifurcation immediately follows pharynx. Caeca simple, terminate blindly at level of posterior margin of vitelline field.

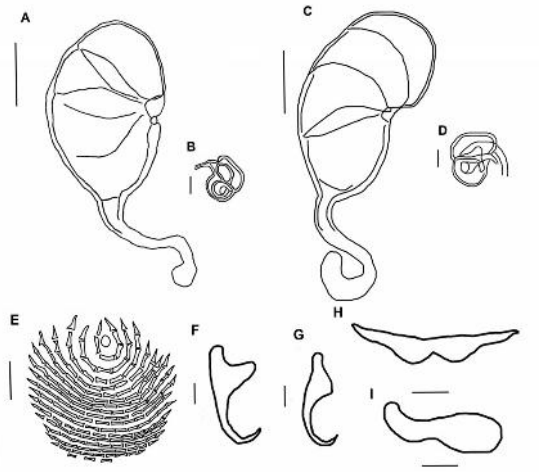

Fig. 4: *Pseudorhabdosynochus vietnamensis* n. sp. A-D: Male copulatory organ and corresponding sclerotized vagina in two individuals. E, F: Ventral and dorsal hamuli; G, H Transverse and dorsal bars: I: Squamodisc; Scale bare: 20µm for MCO, transverse, and, dorsal bars; 10 µm for vagina, dorsal, ventral hamuli and, squamodisc.

Testis subspherical, intercaecal. Vas deferens emerges from antero-sinistral part of testis, enlarges into small seminal vesicle; seminal vesicle in middle region of body, transforms into duct; duct forms bends, then transform into small bulb followed by duct; duct enlarges to form vesicle, then connects to male copulatory organ (MCO). Prostatic reservoir conspicuous, elongate, connects with male copulatory organ (MCO) (Fig. 3B).

MCO very large, divided into four chambers with fourth chamber more sclerotized than the 3 anterior chambers; first chamber with very thin anterior wall; fourth chamber ends in very sclerotized cone; characteristic thickening of wall present inside fourth chamber at base of cone; cone prolonged by elongate sclerotized tube, usually widened and slightly curved posteriorly, filaments not observed. Inner length 108 (102–115, n=6); cone length 18 (15–20, n=6); tube length 46 (40–55, n=6) (Fig. 4 A, C).

Ovary subequatorial, intercaecal, pre-testicular, encircles right caecum. Oviduct passes medially to form ootype, surrounded by Mehlis' gland; ootype short, uterus not observed. Duct from sclerotized vagina to ootype conspicuous. Vitelline fields extend posteriorly from posterior to pharygeal level in 2 lateral bands, confluent in post-testicular region, terminate anterior to peduncle (Fig 3 A,B). Egg not seen.

Vagina comprised of unsclerotized region, visible only in living animal, followed by sclerotized region. Sclerotized vagina sinistral in a complex structure; length 31 (30–32, n=6), width 24 (23–26, n=6). Sclerotized vagina comprises small anterior trumpet, which is continuous with unsclerotized vagina (Fig. 3). Trumpet is followed by convoluted structure, twisted and divided in to two distinct chambers, which lay over each other (Fig. 4 B, D).

Taxonomic summary

Type-host: *Epinephelus coioides* (Hamilton, 1872*)*.

Type-locality: Nha Trang Bay (12° 15' N, 109° 21' E), Vietnam.

Site: Between secondary gill lamellae.

Type-material: Holotype ZMBL collection N0: 84830, collected on 20 July 2007, Nha Trang Bay, Vietnam in ammonium picrate. 3 paratypes (ZMBN collection N0: 84831-84833) all cleared with lactophenol.

Material examined: 10 specimens, including 3 in ammonium picrate, 3 cleared with lactophenol and 4 live specimens. Measurements from 6 specimens.

Prevalence: 7 % (3/45).

Etymology: The name of this species refers the country Vietnam, where the species was found.

Remarks

Pseudorhabdosynochus vietnamensis n. sp. possesses a characteristic vagina, trumpet shaped anteriorly, followed by a convoluted structure, twisted and divided into two distinct chambers. The morphology of vagina serves as a diagnostic character for *P. vietnamensis* n. sp., easily differentiating it from *Pseudorhabd-osynochu*s species with squamodisc having more than 14 rows of rodlets (listed above). *Pseudorhabdosynochus fuitoe* Justine, 2007 from *Epinephelus maculates* (Bloch, 1790), *P. sinediscus* Neifar and Euzet, 2007, on *E. costae*

(Steindachner, 1878) and *P. pai* Justine and Vignon, 2009 on *E. tauvina* (Forsskål, 1775) (all Serranidae) are characterized by sclerotized vagina with anterior trumpet followed by a primary canal, primary chamber, secondary canal and secondary chamber. These species share with *P. vietnamensis* n. sp. a similar vagina with a trumpet anteriorly and a complicated structure with several canals and chambers. However, the above species all possess squamodiscs with less than 14 rows of rodlets, and *P. sinediscus* is differentiated from other species by the absence of squamodiscs. *P. vietnamensis* n. sp. and *P. pai* both possess extremely developed male cupulatory organs (inner length: 108 and 118, Core length: 18 and 17; Tube length: 46 and 54; respectively) (Measurement of *P. pai* specimens stored in ammonium picrate (Justine and Vignon, 2009), but differ in the number of rows in the squamodisc (17–18 vs 11–13), and in the detail in the structure of the vagina.

Pseudorhabdosynochus brunei n. sp.

(Figure 5, 6 A–H, 7)

Diagnostic: Body slightly dorsoventrally flattened, widest at level of ovary, length 245 (100–320, n=4), width 102 (90–150, n=4).Tegument smooth. Anterior region with 3 pairs of head organs and 2 pairs of eye-spots; distance between outer margins of anterior eye-spot pair 13 (11–15, n=5, of posterior eye-spot pair 7 (5–10, n=5).

Haptor differentiated from rest of body, narrow than body, width 85 (80–90, n=3); length 36 (34–38, n=3), provided with 2 similar squamodiscs, 2 pairs of lateral hamuli, 3 bars and 14 marginal hooklets. Squamodiscs round in shape, made up of rows of rodlets; central row not forming closed circles; rodlets progressively thinner from centre to periphery; last row with very thin rodlets; ventral and dorsal squamodiscs similar; length 20 (19–21, n=5), width 26 (25–28, n=5), with 7 (n=5) rows of rodlets (Fig. 6H); Ventral hamuli with shaft elongate and broad root, outer length 34 (30–36, n=5), inner length 29 (26–31, n=5); Dorsal hamuli with reduced root and curved shaft, outer length 32 (30–39, n=3), inner length 21 (20–22, n=3); Ventral bar massive, slightly constricted at medial part, tapering at extremities; length 62 (60–66, n=5); groove not visible on ventral side. Dorsal bar broadens in mid-body, length 54 (50–58, n=5) (Fig. 6 D–G).

Pharynx subspherical, length 33 (25–40, n=4), width 33 (28–42, n=4). Oesophagus apparently absent, such that intestinal bifurcation immediately follows pharynx. Caeca simple, terminates blindly at level of posterior margin of vitelline field.

Testes subspherical, intercaecal. Vas deferens emerge from antero-sinistral part of testis, enlarge into small seminal vesicle;

seminal vesicle in middle region of body, transform into duct; duct form bends, then transform into small bulb followed by duct; duct enlarge to form vesicle, then connect to male copulatory organ (MCO).

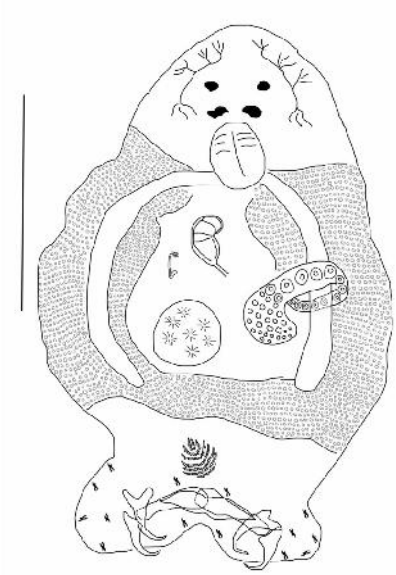

Fig. 5: *Pseudorhabdosynochus brunei* n. sp. from *Epinephelus bruneus* in Nha Trang Bay, Vietnam. Dorsal view, composite drawing from holotype, paratype and observation of live specimens. Scale bar: 100 µm.

MCO slightly curved, divided into four chambers with fourth chamber more sclerotized than 3 anterior chambers, which have similar thin walls; fourth chamber ends in very sclerotized cone; characteristic thicken of wall present inside fourth chamber at base of cone; cone prolonged by elongate sclerotized tube, filaments not observed. Length 28 (21–40, n=5); cone and tube length 12 (8–15, n=4) (Fig. 6 A).

Ovary subequatorial, intercaecal, pre-testicular, encircles right caecum. Oviduct passes medially to form ootype, surrounded by Mehlis' gland; ootype short, uterus not observed. Duct from sclerotized vagina to ootype inconspicuous. Vitelline fields extend posteriorly from posterior to pharyngeal level in 2 lateral bands, confluent in post-testicular region and terminate anterior to peduncle. Egg large, oval, with long sclerotized tail, length 115, tail length 50 (in 1 live specimen) (Fig. 6 C). The laying egg process of *P. brunei* is presented in Fig. 7.

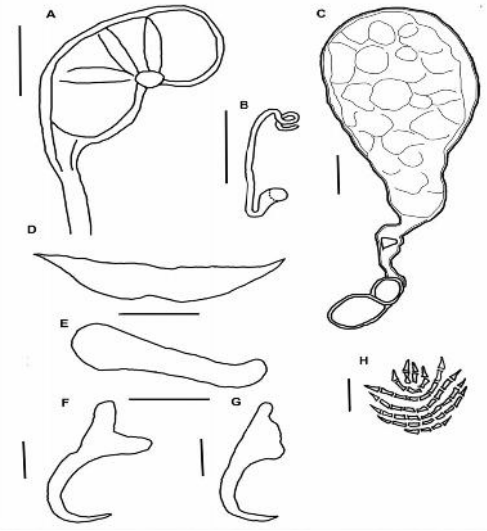

Fig. 6: *Pseudorhabdosynochus brunei* n. sp. A: Male copulatory organ B: Sclerotized vagina; C: Sclerotized egg; D, E: dorsal and transverse bars; F, G: Ventral and dorsal hamuli; H: Squamodisc. Scale bare: 10 µm for MCO, vagina, dorsal, ventral hamuli and, squamodisc, 20 µm for transverse and dorsal bars and the egg.

Sclerotized vagina, length 18 (15–20, n=4); very difficult to observe, even in live specimens. Sclerotized vagina with long sclerotized tube extending close to distal opening. Distal region make up of two small sequenced chambers. Posteriorly, tube divides into broad branch (Fig. 6 B).

Taxonomic summary

Type-host: *Epinephelus bruneus* (Bloch, 1793*)*.

Type-locality: Cam Ranh Bay (11° 52' 60 N, 109° 10' E), Vietnam

Site: Between secondary gill lamellae.

Type-material: Holotype ZMBN collection N0: 84834, collected on 25 July 2007, Nha Trang Bay, Vietnam; 3 paratypes (ZMBN 84835-84837), all in ammonium picrate.

Material examined: 6 specimens, including 4 in ammonium picrate, 2 cleared with lactophenol and 2 live specimens.

Prevalence: 32 % (9/28)

Etymology: The specific name refers to the scientific name of the host *E. bruneus*

Remarks

P. brunei n. sp. is the only species found on *E. bruneus* in Nha Trang Bay, Vietnam. It is the smallest *Pseudorhabdosynochus* so far found on grouper in Vietnam. With all characteristics of the genus, *Pseudorhabdosynochus brunei* possesses a vagina which shares features with *Pseudorhabdosynochus summanoides* Yang *et al*., 2005 in having a sclerotized tube extending close to the distal opening, and the distal region being made up of sequenced chambers, but the size is much smaller (18 (15–29) vs 29–56 (45.8)), and the distal region possesses two sequential chambers, while it winds into a small irregular convolution in *P. summonoides.* In addition, it differs from *P. summanoides* in that the posterior end of its tube does not form a loop (twisted in round circle). The smaller size of vagina and its distal region being made up of sequential chambers separate *P. brunei* from *P. lantauensis* Beverley-Burton and Suriano, 1981 which was originally reported from *E. bruneus*. *P. brunei* n. sp. can easily be differentiated from other *Pseudorhabdosynochus* species by the squamodisc having only 7 rows of rodlets, specific haptoral hadparts, and characteristics of the egg. The egg laying process, presented in Fig. 7, shows the sclerotized structure with the tail. The eggs are also larger than eggs found in other species of the genus.

Discussion

In Vietnam, both *E. coioides* and *E. bleekeri* are intensively cultured in sea cages, along with *E. fuscoguttatus* (Forsskål, 1775) and *Plectroponus leopardus* (Lacepède, 1802), and in some cases with snapper (*Lutjanus argentimaculatus* (Forsskål, 1775) and other finfish. Arthur and Te (2006) reported *Pseudorhabdosynochus harigisi* Oliver and

Paperna, 1984*, P. cupatus, P. epinepheli* and *Haliotrema* sp. from *Epinephelus bruneus, E. sexfasciatus* (Valenciennes, 1828) and *E. tauvina* from Gulf of Tonkin. The current study is the first report of new *Pseudorhabdosynochus* species from Vietnamese grouper.

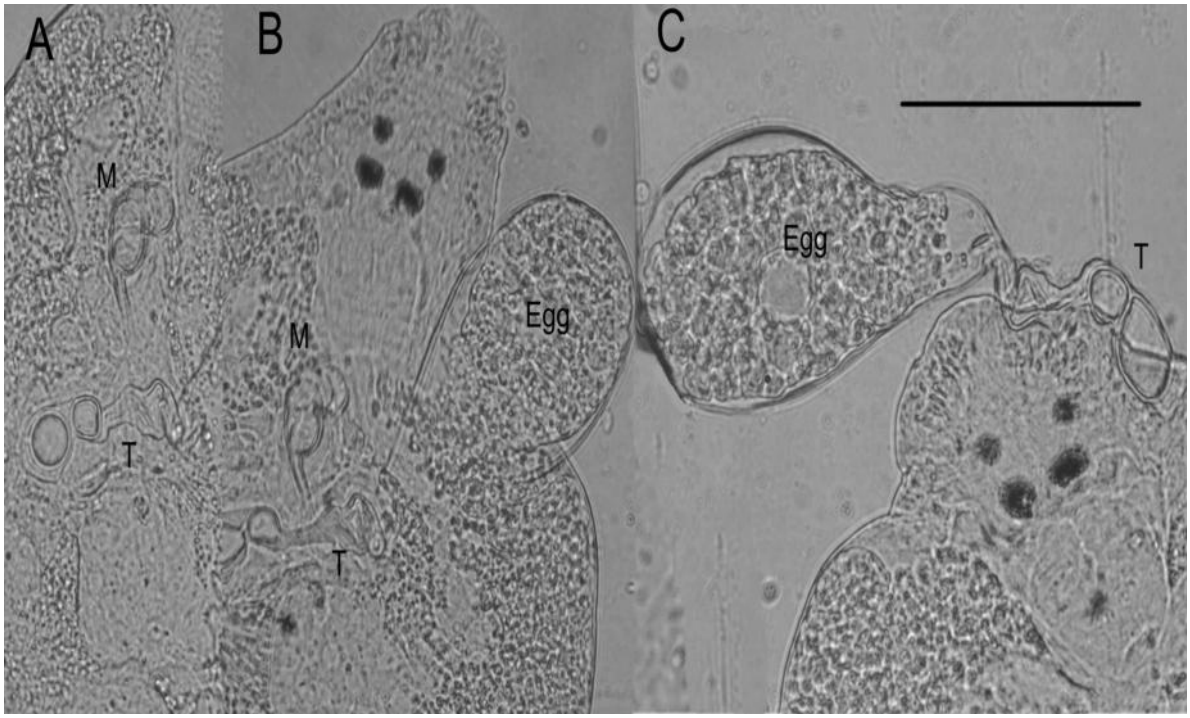

Fig. 7: Egg laying process in *P. brunei* n. sp. A: tail of the egg inside body at vaginal level; B: egg partially extruded; C: egg completely extruded. M: male copulatory organ, T: tail of egg. Scale bar. 50µm

Epinephelus coioides is presently a common species for grouper aquaculture in Southeast Asia and China. Yang *et al*. (2005a) reported that *E. coioides* harbored a considerably great number (six) of congeneric monogenean parasites. These species (*P. lantauensis*, *P. coioidesis*, *P. serrani*, *P. shenzhenensis, P. summanae* Young, 1969 and *P. summanoides)* possess a tubular vagina varying from each other by considerable length variations, and the distal parts in two extremes, except *P. coioidesis*. *P. nhatrangensis* shares similar character of tubular vagina, however, the tube is short and opening anteriorly, while they are usually long and have distal parts in the other species. The large MCO and convoluted vagina separated *P. vietnamensis* from the rest of co-generic parasites infecting *E. coioides*.

Two *Pseudorhabdosynochus* species (*P. epinepheli* and *P. lantauensis*) have so far been reported infecting *E. bruneus*. Due to possible misidentification of *E. bruneus* and *E. akaara* (Temminck and Schlegel, 1842) on small specimens, Justine (2009) suggested that

specimens of *E. bruneus* examined by Beverley-Burton and Suriano (1981) were actually *E. akaara*. In the current study, *P. brunei*, characterized by small number of rows in the squamodisc and large sclerotized egg, has been found as the only species abundant on *E. bruneus* juveniles.

Leong (1997) considered parasitic infections as the major contributing factor to disease outbreaks. The increasing number of monogenean parasites reported on cultured fish (but not observed in the wild fish of the same species) may be a result of poly-cultured systems (as host switching may be taking place between mixed culturing species), and bad environmental management. Three new species reported here increase current knowledge of the diversity of *Pseudorhabdosynochus* species, both in the wild and in the aquaculture condition.

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