# Four New Lepocreadiid Digeneans (Trematoda) from Marine Fishes of Southern Japan and the Philippines

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**Abstract** Four new species of lepocreadiid digeneans (Trematoda) are described from marine fishes of southern Japan and the Philippines. They are: *Neolepidapedoides ittoudai* sp. nov. from the intestine of *Sargocentron spinosissimum* (Holocentridae) of southern Japan, *Neolepidapedoides amamiensis* sp. nov. from the intestine of *Giganthias immaculatus* (Giganthidae) of southern Japan, *Lepidapedoides miharahanadai* sp. nov. from the intestine of *Giganthias immaculatus* of southern Japan, and *Neolepocreadium ampahan* sp. nov. from the intestine of *Trachinotus blochii* (Carangidae) of the Philippines.

Key words: Digenea, Lepocreadiidae, new species, marine fish, Japan, Philippines.

# Introduction

This paper deals with four new species of the family Lepocreadiidae (Trematoda, Digenea) from marine fishes of southern Japan and the Philippines. Host fishes were obtained from the local fish markets. The digeneans collected were washed in saline, fixed in AFA under slight pressure, stained with Heidenhain's hematoxylin, cleared in creosote and mounted in Canada balsam. The specimens are deposited in the National Museum of Nature and Science, Tsukuba (NSMT). Measurements are given in millimeters unless otherwise stated, with the range followed by measurements of the holotype in parentheses.

#### Neolepidapedoides ittoudai sp. nov.

#### (Figs. 1-3)

*Type host. Sargocentron spinosissimum* (Holo-centridae).

Site. Intestine.

*Type locality*. Kushimoto, Higashimuro County, Wakayama Pref., Japan, 27-X-1979.

Specimens. Holotype and 1 paratype, NSMT-

Pl 2271.

*Etymology.* Specific name *ittoudai* is from the Japanese name of the host.

Description. Based on two specimens. Body slender, rounded at both ends, 4.28–4.43 (4.28) long by 0.78–0.83 (0.78) wide. Body width 18–19 (18)% of length. Tegument spinose. Oral sucker subterminal, 0.15  $(0.15) \times 0.16-0.17$  (0.17), mouth opening apically; prepharynx 0.03 (0.03) long; pharynx 0.13 (0.13)  $\times 0.14-0.16$  (0.16); oesophagus 0.14–0.16 (0.16) long, bifurcating midway between suckers or slightly closer to acetabulum; caeca extending almost to posterior end of body. Acetabulum 0.23–0.25 (0.25)  $\times$  0.24–0.26 (0.24). Sucker ratio 1:1.44–1.55 (1.44). Forebody 16–18 (18)% of body length.

Testes almost rounded, tandem, intercaecal, separated; anterior testis 0.32-0.37 (0.37)× 0.37-0.40 (0.40); posterior testis 0.41-0.45 (0.45)×0.42 (0.42). Posttesticular space 10-12 (10)% of body length. Vasa efferentia not joining each other. External seminal vesicle long tubular, sinuous, reaching near midlevel between posterior end of cirrus sac and ovary, surrounded by gland cells except proximal end. Cirrus sac clavi-



Figs. 1–3. Neolepidapedoides ittoudai sp. nov. — 1, Entire worm, dorsal view (holotype, NSMT-Pl 2271); 2, terminal genitalia, dorsal view; 3, ovarian complex, dorsal view. Abbreviations: A, acetabulum; C, left caecum; CS, cirrus sac; E, egg; ES, external seminal vesicle; G, genital pore; IS, internal seminal vesicle; L, Laurer's canal; M, metraterm; MG, Mehlis' gland; O, ovary; P, pars prostatica; R, seminal receptacle; U, uterus; V, common vitelline duct.

form, thick-walled, 0.35-0.39 (0.39)  $\times 0.15-0.18$  (0.15), straight or slightly arcuate, extending on left side of acetabulum to posterior to it; containing tubular, convoluted internal seminal vesicle; slender pars prostatica 0.12-0.14 (0.14)  $\times 0.05-0.07$  (0.07); cirrus 0.07-0.09 (0.09) long, its anterior half protruding into genital atrium. Genital atrium small. Genital pore enclosed in circular sphincter and gland cells, anterosinistral to ace-

tabulum, just interior to left caecum.

Ovary ovoid, slightly dextral, 0.20-0.26(0.26)  $\times$  0.26-0.28 (0.26), lying 62 (62)% of body length from anterior end of body, separated from anterior testis by about length of testis. Oviduct arising from near center of ovary, connecting short duct from seminal receptacle, branching Laurer's canal, then running upward to receive common vitelline duct, entering Mehlis' gland. Mehlis' gland immediately anterosinistral to ovary. Laurer's canal running sinistrally, sinuous, opening on left body margin at mid- to postovarian level. Seminal receptacle ovoid, 0.20–  $0.25 (0.25) \times 0.16$ –0.22 (0.16), posterosinistral to ovary. Uterus preovarian, with sphincter just before joining metraterm; metraterm slightly shorter than cirrus sac, enclosed in gland cells. Eggs thin-shelled, partially collapsed, 64–71 × 43–48 $\mu$ m. Vitelline follicles extending from near posterior border of cirrus sac to posterior end of body, dorsal and ventral to caeca, confluent in posttesticular space. Excretory vesicle tubular, ending at level of intestinal bifurcation; pore terminal, surrounded by sphincter and gland cells.

Remarks. Bray and Gibson (1989) recognized ten species of Neolepidapedoides. Of them, two species possess vitelline follicles extending anteriorly near the posterior border of the cirrus sac, and an excretory vesicle reaching the intestinal bifurcation, they are: Neolepidapedoides hypoplectri (Nahhas and Cable, 1964) and Neolepidapedoides macrum (Overstreet, 1969). The present new species differs from N. hypoplectri in having a larger body and eggs, larger sucker ratio, and a different position of the genital pore. It differs from N. macrum in having a wider body and eggs, larger sucker ratio, and an external seminal vesicle surrounded by gland cells except the proximal end. In addition, the present species is distinguishable by possessing a mouth opening apically and a genital pore enclosed in the sphincter.

## Neolepidapedoides amamiensis sp. nov.

#### (Figs. 4-6)

*Type host. Giganthias immaculatus* (Giganthiidae).

Site. Intestine.

*Type locality.* Koniya, Ohshima County, Kagoshima Pref., Japan, 9-III-1991.

Specimens. Holotype and 9 paratypes, NSMT-Pl 4157a.

*Etymology.* The specific name *amamiensis* is from the type locality, Amami Islands.

Description. Based on 10 specimens. Body linguiform, tapering slightly toward ends, 1.83– 2.51(1.83) long by 0.53–0.65 (0.58) wide. Body width 21–32 (32)% of length. Tegument spinose, sparse posteriorly. Eyespot pigments present. Oral sucker subterminal, 0.16–0.20 (0.16)× 0.19–0.26 (0.19); prepharynx 0.05–0.13 (0.12) long; pharynx 0.09–0.11 (0.10)×0.09–0.12(0.09); oesophagus 0.09–0.30 (0.10) long, bifurcating nearer acetabulum than oral sucker; caeca extending almost posterior end of body. Acetabulum 0.13–0.18 (0.13)×0.14–0.19 (0.14). Sucker ratio 1:0.69–0.86 (0.77). Forebody 31–41 (34)% of body length.

Testes rounded to ovoid, tandem, intercaecal, contiguous; anterior testis 0.18-0.27 (0.18) × 0.20-0.32 (0.24); posterior testis 0.25-0.34  $(0.26) \times 0.21 - 0.32$  (0.21). Posttesticular space 7-14 (8)% of body length. Vasa efferentia not joining each other. External seminal vesicle tubular, two or three transverse loops, extending near ovary, completely surrounded by gland cells. Cirrus sac claviform, straight or a little arcuate, 0.35-0.50 (0.37)  $\times 0.14-0.21$  (0.15), extending midway between acetabulum and ovary; containing oval internal seminal vesicle 0.06-0.15  $(0.06) \times 0.04 - 0.09$  (0.07), pars prostatica 0.05- $0.11 (0.06) \times 0.05 - 0.10 (0.06)$ , and cirrus 0.13 - 0.11 = 0.0000.25 (0.20) long. Genital atrium small. Genital pore sinistral near antero- to midlateral border of acetabulum.

Ovary subglobular, slightly dextral, 0.11–0.18  $(0.11) \times 0.14$ –0.17 (0.15), in contact with or a little separated from anterior testis, lying 56–66 (59)% of body length from anterior end of body. Oviduct arising from near center of ovary, connecting short duct from seminal receptacle, giving off Laurer's canal, receiving common vitelline duct, entering Mehlis' gland. Laurer's canal running downward, opening dorsally near left margin of anterior testis at its anterior to middle level. Mehlis' gland sinistral to ovary. Seminal receptacle ovoid, 0.08–0.13 (0.09)  $\times$  0.11–0.29 (0.22), posterosinistral to ovary, slightly overlapping anterior testis. Uterus preovarian, with sphincter at junction with metraterm; metraterm



Figs. 4–6. Neolepidapedoides amamiensis sp. nov. — 4, Entire worm, ventral view (holotype, NSMT-Pl 4157a); 5, terminal genitalia, ventral view; 6, ovarian complex, ventral view. Abbreviations: A, acetabulum; CS, cirrus sac; ES, external seminal vesicle; G, genital pore; IS, internal seminal vesicle; L, Laurer's canal; M, metraterm; MG, Mehlis' gland; O, ovary; P, pars prostatica; R, seminal receptacle; T, anterior testis; U, uterus (omitted); V, common vitelline duct.

saccate, 0.18-0.30  $(0.19) \times 0.06-0.09$  (0.06), enclosed in gland cells. Eggs thin-shelled, partially collapsed,  $64-74 \times 48-51 \mu m$ . Vitelline follicles extending from intestinal bifurcation to near posterior end of body; dorsal and ventral to caeca; confluent in forebody and posttesticular space. Excretory vesicle tubular, reaching to intestinal bifurcation; pore terminal, enclosed in sphincter and gland cells.

Remarks. The present new species is most like

*Neolepidapedoides trachinoti* (Siddiqi and Cable, 1960). Both differ from all other species of *Neolepidapedoides* by possessing vitelline follicles that intrude into the forebody. The present species differs from *N. trachinoti* by having an excretory vesicle which extends to the intestinal bifurcation as compared with the posterior margin of the anterior testis, smaller sucker ratio (1:0.69 to 0.86 as compared with 1:1), larger eggs (64 to 74 by 48 to 51 $\mu$ m as compared with

## Lepidapedoides miharahanadai sp. nov.

# (Figs. 7-9)

*Type host. Giganthias immaculatus* (Giganthiidae).

Site. Intestine.

*Type locality*. Koniya, Ohshima County, Kagoshima Pref., Japan, 9-III-1991.

*Specimens*. Holotype and 10 paratypes, NSMT-PI 4157b.

*Etymology.* The specific name *miharahanadai* is from the Japanese name of the host.

Description. Based on 11 specimens. Body linguiform with rounded ends, 1.96–2.72 (1.96) long by 0.51–0.65 (0.51) wide. Body width 19–33 (26)% of length. Tegument spinose. Eye-



Figs. 7–9. Lepidapedoides miharahanadai sp. nov. — 7, Entire worm, ventral view (holotype, NSMT-Pl 4157b), 8, terminal genitalia, ventral view, 9, ovarian complex, ventral view. Abbreviations: A, acetabulum; CS, cirrus sac; EC, everted cirrus; ES, external seminal vesicle; G, genital pore; IS, internal seminal vesicle; L, Laurer's canal; M, metraterm; MG, Mehlis' gland; O, ovary; P, pars prostatica; R, seminal receptacle; T, anterior testis; V, common vitelline duct.

spot pigments present. Oral sucker subterminal, 0.15–0.21 (0.15)  $\times$  0.21–0.25 (0.21); prepharynx 0.04–0.10 (0.05) long; pharynx well-developed, 0.10–0.13 (0.11)  $\times$  0.13–0.17 (0.13); oesophagus 0.10–0.30 (0.11) long, bifurcating slightly nearer acetabulum than oral sucker; caeca extending almost posterior end of body. Acetabulum 0.15– 0.18 (0.15)  $\times$  0.15–0.19 (0.16). Sucker ratio 1 : 0.70–0.79 (0.76). Forebody 30–38 (33)% of body length.

Testes rounded to ovoid, tandem, intercaecal, contiguous; anterior testis 0.17-0.24 (0.20) × 0.22-0.28 (0.23); posterior testis 0.20-0.28  $(0.23) \times 0.23 - 0.28$  (0.23). Posttesticular space 12-17 (13)% of body length. Vasa efferentia not joining each other. External seminal vesicle tubular, sinuous, surrounded by gland cell mass which is delimited by membranous sac, extending to near ovary. Cirrus sac claviform, straight or slightly arcuate, more or less thick-walled, 0.24-0.48 (0.32)  $\times 0.13-0.20$  (0.15), extending midway between acetabulum and ovary; containing oval internal seminal vesicle 0.07-0.16  $(0.11) \times 0.07 - 0.14$  (0.10), flask-shaped pars prostatica 0.08-0.22 (0.09)  $\times$  0.07-0.18 (0.08) and cirrus. Cirrus often everted out of genital pore. Pars prostatica stretching to 0.50 long when the cirrus is everted. Genital atrium small. Genital pore sinistral near antero- to midlateral border of acetabulum.

Ovary globular, slightly dextral, 0.10-0.13  $(0.10) \times 0.11 - 0.15$  (0.12), a little separated from or in contact with anterior testis, lying 53-64 (62)% of body length from anterior end of body. Oviduct arising from anterior to middle portion of ovary, running to the left, receiving short duct from seminal receptacle at the same point branching Laurer's canal, running upward to connect common vitelline duct, entering Mehlis' gland. Laurer's canal opening dorsally on left margin of anterior testis at its anterior to middle level. Mehlis' gland sinistral to ovary. Seminal receptacle  $0.10-0.19 (0.18) \times 0.13 - 0.29 (0.13)$ , posterosinistral to ovary, partially overlapping anterior testis. Uterus preovarian, with sphincter at junction with metraterm; metraterm saccate,

enclosed in gland cells, 0.27-0.39 (0.28) × 0.10-0.14 (0.12), along left side of cirrus sac. Eggs thin-shelled, partially collapsed,  $68-74 \times 41-46 \mu m$ . Vitelline follicles extending from oesophageal level to posterior end of body. Excretory vesicle tubular, terminating near intestinal bifurcation; pore terminal, enclosed in sphincter and gland cells.

*Remarks.* The genus *Lepidapedoides* is most closely related to *Neolepidapedoides*, differing by possessing gland cells around the external seminal vesicle delimited by membrane as compared with those scattered in parenchyma (Bray, 2005).

The present new species was found with the above-mentioned *N. amamiensis* from the same host individual and resembles it, but differs from it by having an external seminal vesicle which is surrounded by gland cells enclosed in a membranous sac, a slightly thicker-walled cirrus sac and a larger pharynx and metraterm. The membranous sac is sometimes not clear.

There are three species of *Lepidapedoides* that possess vitelline follicles and an excretory vesicle extending to the intestinal bifurcation; they are *Lepidapedoides dollfusi* (Durio and Manter, 1968), *Lepidapedoides manteri* (Hafeezullah, 1970) and *Lepidapedoides angustus* Bray, Cribb and Barker, 1996. Bray *et al.* (1996) redescribed *L. dollfusi*. The present new species differs from all three by having a larger pharynx, tandem testes, a cirrus sac which extends midway between the acetabulum and ovary, and a shorter posttesticular space. It further differs from *L. dollfusi* in body shape and egg size; from *L. manteri* in sucker ratio and position of the genital pore; and from *L. angustus* in sucker ratio and egg size.

## Neolepocreadium ampahan sp. nov.

#### (Figs. 10-12)

*Type host. Trachinotus blochii* (Carangidae). *Site.* Intestine.

*Type locality*. Puerto Princesa, Palawan, the Philippines, 10-XI-1988.

Specimens. Holotype and 19 paratypes,



Figs. 10–12. Neolepocreadium ampahan sp. nov. — 10, Entire worm, ventral view (holotype, NSMT-Pl 3546); 11, terminal genitalia, ventral view; 12, ovarian complex, dorsal view. Abbreviations: A, acetabulum; CS, cirrus sac; E, egg; ES, external seminal vesicle; G, genital pore; GC, crescent-shaped gland cells; IS, internal seminal vesicle; L, Laurer's canal; M, metraterm; MG, Mehlis' gland; O, ovary; P, pars prostatica; R, seminal receptacle; V, common vitelline duct.

# NSMT-Pl 3546.

*Etymology*. Specific name *ampahan* is from the Philippine local name of the host.

*Description.* Based on 20 specimens. Body elongated, tapering anteriorly and rounded posteriorly, 2.14–3.96 (3.96) long by 0.50–0.72 (0.56) wide at ovarian level. Tegument spinose, sparse posteriorly. Eyespot pigments scattered in pharyngeal-oesophageal region and numerous gland

cells without granules also scattered in forebody. Oral sucker subterminal, sometimes nearly funnel-shaped, 0.09-0.12 (0.11)  $\times$  0.07-0.14 (0.12); prepharynx unrecognizable; pharynx 0.05-0.08 (0.06)  $\times$  0.05-0.08 (0.07); oesophagus 0.07-0.19 (0.19) long, bifurcating midway between suckers; caeca extending near posterior end of body. Acetabulum 0.14-0.20 (0.20)  $\times$  0.16-0.23 (0.21). Sucker ratio 1:1.48-2.23 (1.69). Forebody 12-27 (20)% of body length.

Testes globular, tandem, separated, approximately in middle third of hindbody; anterior testis  $0.23-0.36 (0.35) \times 0.29-0.43 (0.34)$ ; posterior testis 0.24-0.42 (0.37) × 0.28-0.43 (0.34). Posttesticular space 25-43 (30)% of body length. Vasa efferentia not joining each other. External seminal vesicle swollen tubular, winding, 0.32- $0.91 (0.68) \times 0.06 - 0.14 (0.13)$ , extending anterior 1/3 between acetabulum and ovary. No gland cells surrounding external seminal vesicle. Cirrus sac semicircular, 0.16-0.32  $(0.31) \times 0.09-0.14$ (0.13), usually anterosinistral to acetabulum; containing oval internal seminal vesicle 0.16- $0.21 (0.21) \times 0.04 - 0.13 (0.13)$ ; internal seminal vesicle connected with pars prostatica by short duct; pars prostatica 15–25 (25) $\mu$ m long with prostatic cells; cirrus conical, 45-65 (65)µm long. Genital atrium shallow, with four small gland cells on anterior edge of genital atrium. The gland cells lined side by side in the shape of a crescent, each of the cells opening into genital atrium. Genital pore anterior to acetabulum.

Ovary with two to four lobes, 0.10-0.18  $(0.13) \times 0.14 - 0.21$  (0.16), lying 25-43 (38)% of body length from anterior end. Oviduct arising from anterosinistral margin of ovary, provided with sphincter just before junction with seminal receptacle, branching Laurer's canal, receiving common vitelline duct, entering Mehlis' gland. Mehlis' gland anterosinistral to ovary. Laurer's canal short, opening dorsally sinistral or anterosinistral to ovary. Seminal receptacle claviform, 0.33-0.72 (0.34) × 0.10-0.20 (0.11), usually immediately posterosinistral to ovary. Uterus between ovary and acetabulum; metraterm narrow, about half as long as cirrus sac. Eggs  $61-68 \times 33-40 \,\mu\text{m}$ . Vitelline follicles relatively large, extending from preovarian level to near posterior end of body, overlapping caeca dorsally and ventrally, confluent posterior to testes and between gonads. Excretory vesicle tubular, terminating in ovarian zone; pore terminal.

Additional measurements on Japanese specimens. Two specimens of this species were obtained from the intestine of *Trachinotus*  *blochii* from Nago, Okinawa Pref., Japan on 11-III-1996 (NSMT-Pl 4891). They are too macerated to examine the morphological details: Body  $10.42-10.75 \times 0.63-0.73$ . Oral sucker  $0.09-0.12 \times 0.13-0.16$ . Pharynx  $0.09-0.12 \times 0.09-0.12$ . Oesophagus 0.33-0.41 long. Acetabulum  $0.30-0.35 \times 0.29-0.35$ . Sucker ratio 1:2.2-2.3. Forebody 18–19% of body length. Anterior testis  $0.60-0.77 \times 0.42$ . Posterior testis  $0.60-0.77 \times 0.42$ . Posterior testis  $0.60-0.77 \times 0.42$ . Posterior testis 0.60-0.12. Internal seminal vesicle  $0.23-0.26 \times 0.16-0.19$ . External seminal vesicle  $1.42-1.91 \times 0.23-0.25$ . Ovary  $0.25-0.29 \times 0.23-0.24$ . Seminal receptacle  $0.70-0.77 \times 0.20-0.29$ . Eggs  $61-68 \times 33-40 \mu$ m.

*Remarks*. Two other species of *Neolepocreadium* have been described, both from fishes of the genus *Trachinotus*: *Neolepocreadium caballeroi* Thomas, 1960 (type species) from *T. goreensis* and *T. glancus* of Ghana and *Neolepocreadium trachinoti* Madhavi, Narasimhulu and Shameem, 1986 from *T. blochii* of the Bay of Bengal, India. The present new species is very similar to *N. caballeroi*, differing in having a distinctive pars prostatica and a thin cirrus without muscular wall. The most distinctive feature of the present species is the genital atrium provided anteriorly with crescent-shaped gland cells. These gland cells have not been described for the two other species of *Neolepocreadium*.

#### References

- Bray, R. A. 2005. Family Lepocreadiidae Odhner, 1905. In Jones, A., R. A. Bray and D. I. Gibson (eds.): Key to the Trematoda, Vol. 2, pp. 545–602. CAB International and Natural History Museum, London.
- Bray, R. A., T. H. Cribb and S. C. Barker 1996. Four species of *Lepidapedoides* Yamaguti, 1970 (Digenea: Lepocreadiidae) from fishes of the southern Great Barrier Reef, with a tabulation of host-parasite data on the group. Systematic Parasitology, 34: 179–195.
- Bray, R. A. and D. I. Gibson 1989. The Lepocreadiidae (Digenea) of fishes from north-east Atlantic: review of the genus *Neolepidapedon* Manter, 1954, with a description of *N. smithi* n. sp. Systematic Parasitology, 13: 11–23.
- Durio, W. O. and H. W. Manter 1968. Some digenetic trematodes of marine fishes of New Caledonia. Part II.

Opecoelidae and Lepocreadiidae. Journal of Parasitology, 54: 747–756. fishes from Biscayne Bay, Florida. Tulane Studies in Zoology and Botany, 15: 119–176.

- Hafeezullah, M. 1970. Lepocreadid trematodes of marine fishes of India. Parasitology, 61: 345–356.
- Madhavi, R., S. V. Narasimhulu and U. Shameen 1986. Digenetic trematodes from marine fishes of Kalingapatnam coast, Bay of Bengal. Families Lepocreadiidae, Deropristiidae and Schistorchiidae. Rivista di Parassitologia, 3: 111–119.
- Nahhas, F. M. and R. M. Cable 1964. Digenetic and aspidogastrid trematodes from marine fishes of Curaçao and Jamaica. Tulane Studies in Zoology, 11: 169–228.

Overstreet, R. M. 1969. Digenetic trematodes of marine

- Siddiqi, A. H. and R. M. Cable 1960. Digenetic trematodes of marine fishes of Puerto Rico. Scientific Survey of Porto Rico and the Virgin Islands, 17: 257–369.
- Thomas, J. D. 1960. Trematodes of Ghanaian sub-littoral fishes. II. The family Lepocreadiidae Nicoll, 1935, *sensu* Cable and Hunninen, 1942. In Bravo-Hollis, M., C. Zerecero, L. Flores-Barroeta and H. A. Winter (eds.): Libro Homenaje al Dr. Eduardo Caballero y Caballero, Jubileo 1930–1960, pp. 321–328. Secretaria de Educacion Publica, Mexico City.