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On two blood flukes (*Trematoda*) of the family *Spirorchidae* Stunkard, 1921 from Indian marine turtles

O dwóch przywrach (*Trematoda*) z rodziny *Spirorchidae* Stunkard, 1921,
pasożytach naczyń krwionośnych morskich żółwi z Indii

Abstract

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Two species of blood flukes, *Amphiorchis indicus* Mehrotra, 1973 and *Hapalotrema pambanensis* Mehrotra, 1973 from the heart of *Eretmochelys imbricata* and *Chelone mydas*, respectively, from the Gulf of Mannar (Pamban, Tamil Nadu, India) are described in detail. The validity of each species is discussed. Keys to species of the genera *Amphiorchis* and *Hapalotrema* are proposed, generic diagnoses being emended.

This paper presents the detailed account of two blood flukes, *Amphiorchis indicus* Mehrotra, 1973 and *Hapalotrema pambanensis* Mehrotra, 1973. The diagnostic features of these trematodes based on the same material had earlier appeared in a concise form (MEHROTRA 1973).

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Material and methods

Twenty two specimens of *Amphiorchis indicus* were recovered from the teased tissue of the liver of one out of three marine turtles, *Eretmochelys imbricata* (L.), caught from the Gulf of Mannar at Pamban (Tamil Nadu), India, and dissected at Chandigarh. Named after the country, this species is the first record of the genus *Amphiorchis* Price, 1934 from India. Autopsy of two edible green turtles, *Chelone mydas* (L.), caught from the same locality, revealed the presence of twenty-three specimens of *Hapalotrema pambanensis* in the heart of its hosts. Of these two turtles, one harboured a single parasite.

The flukes were washed in saline. For whole mount preparations, after studying them alive, they were narcotized in a weak solution of alcohol, fixed in Bouin under the pressure of a cover glass, stained in borax carmine, Mayer's carmalum as modified by Gower or in Ehrlich's haematoxylin, cleared in methyl benzoate, and mounted in Canada balsam. Sections cut 5 μ m thick, were stained by double staining techniques using Ehrlich's haematoxylin and eosin and triple staining technique using Haidenhain's Azan stain.

The measurements are taken from ten flattened specimens in each case and, unless otherwise stated, are in micrometers.

Descriptions

Amphiorchis indicus Mehrotra, 1973 (Figs 1 a, 2)

(*Spirorchidae* Stunkard, 1921; *Amphiorchiinae* Yamaguti, 1958; *Amphiorchis* Price, 1934).

Body delicate, subcylindrical, attenuating towards anterior extremity, 2.69–3.517 mm in length and 0.246–0.396 mm wide, maximum breadth occurring across the posterior testis or a little behind it. Cuticle* without any striations. Oral sucker terminal, protrusible, 59–77 long by 45–69 wide. Ventral sucker also very small, 41–82 × 77–110 in size, 46–88 behind anterior extremity, in the first half of body. Oesophagus 295–426 long, slightly wavy in some specimens; gland cells surrounding the oesophagus, the characteristic of most of the reptilian blood flukes, are not distinct. Intestinal caeca terminating asymmetrically; right caecum always longer than the left, ending 196–278 and 418–639 in front of the posterior extremity of body. Caeca filled with a blackish-brown material.

Excretory vesicle Y-shaped, with a very short stem dividing into two branches, excretory pore terminal, situated at posterior extremity of body.

Testes two, faintly lobed or with irregular contour and varying shapes; anterior testis 139–278 × 155–221, somewhat rectangular or oval, lying immediately anterior to cirrus sac and vesicula seminalis externa; posterior testis 393–557 × 196–278, almost twice as long as the anterior one, disposed some distance behind ovary and separated from the latter by vitellaria. Vesicula seminalis externa somewhat oval or pear-shaped, antero-dextral to cirrus sac, 55–110 × 73–139 in size. Cirrus sac obliquely or transversely oval or club-shaped, 69–94 × 94–135 in size, enclosing vesicula seminalis interna and pars prostatica; cirrus in protruded form could not be observed. Genital pore median or submedian, ventral, preovarian and pre-equatorial.

Ovary irregularly shaped, immediately behind the cirrus sac, 172–311 long by 155–196 wide. Mehlis' gland complex postovarian, dorsal to receptaculum seminis; the latter rounded or somewhat triangular, 69–118 × 49–77 in size, to the right side of yolk reservoir. Laurer's canal present. Uterus small, turning upwards and running to the left of ovary. A single egg present, 131–164 × 164–205, somewhat sickle-shaped, with a long and thick polar prolongation. Vitellaria commencing at a little behind caecal bifurcation and extending up to just in front of hind extremity, densely distributed in front of anterior testis, in the region between receptaculum seminis and posterior testis, and behind the latter, covering intestinal caeca, interrupted in the regions lateral to gonads. Yolk reservoir postovarian, slightly to left of median line, 94–172 × 49–118 in size.

Host: *Eretmochelys imbricata* (L.). Location: liver. Locality: Gulf of Mannar (Pamban, Tamil Nadu). Type-specimens: deposited in the senior writer's helminthological collection.

Discussion. The presence of two testes, one anterior to the cirrus sac and the vesicula seminalis externa and the other posterior to the ovary and the receptaculum seminis, the preovarian genital pore, and the ovary immediately posterior to the cirrus sac, allocate the present species to the genus *Amphiorchis* Price, 1934.

Only two species, namely *Amphiorchis amphiorchis* Price, 1934 from *Chelone mydas* of Washington (U.S.A.) and *A. lateralis* Oguro, 1938 from *Eretmochelys imbricata* of the Palao Islands and the South Sea Islands, have been assigned to

* The term "cuticle" has been used for "tegument" since the former is in common use.

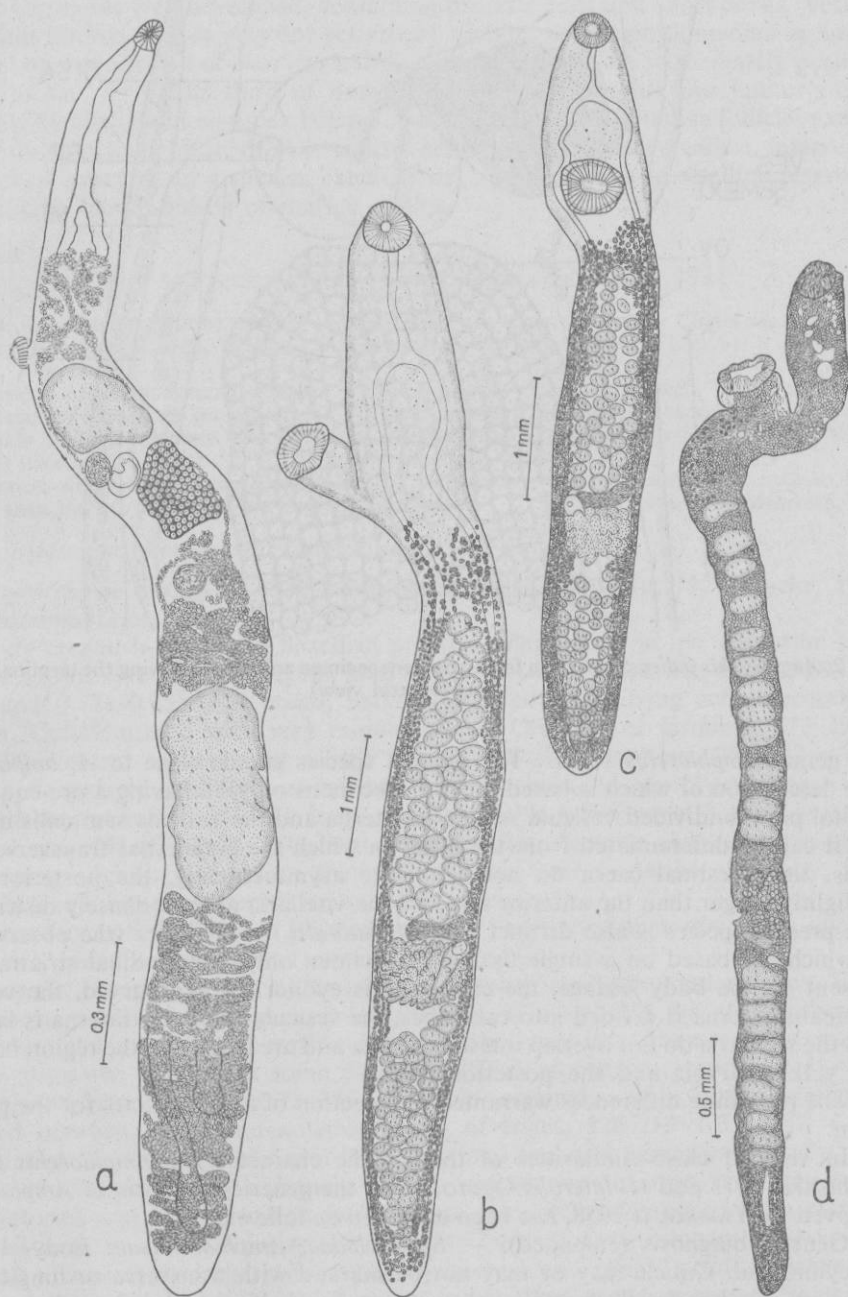


Fig. 1. a — *Amphiorchis indicus* Mehrotra, 1973, a whole mount (ventral view); b-d — *Hapalotrema pambanensis* sp.n. b — a whole mount (ventral view), c — another specimen with dorsoventrally compressed pedunculated ventral sucker (dorsal view), d — sagittal section

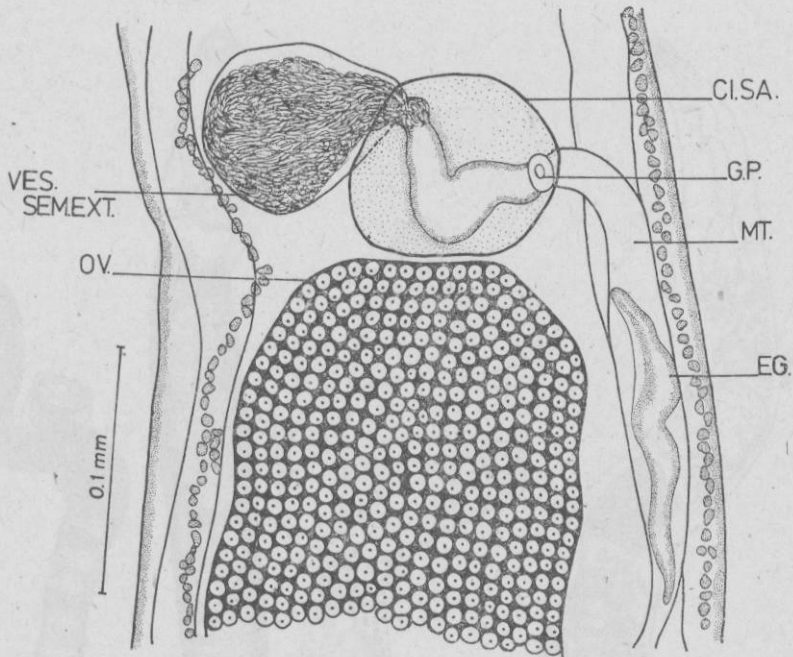


Fig. 2. *Amphiorchis indicus*, a portion from another specimen enlarged, showing the terminal genital ducts (ventral view)

the genus *Amphiorchis* so far. The present species stands close to *A. amphiorchis* (the description of which is based on two specimens only) in having a pre-equatorial genital pore, undivided vesicula seminalis externa and the vesicula seminalis interna, but it can be differentiated from the latter in which the cuticle has transverse striations, the intestinal caeca do not terminate asymmetrically, the posterior testis is slightly longer than the anterior one and the vitellaria are not densely distributed. The present species is also distinct from *A. lateralis*. In the latter (the observations of which are based on a single flattened specimen only) longitudinal striations are present on the body surface, the cirrus sac is cylindrical and curved, the vesicula seminalis externa is divided into two lobes, the vesicula seminalis interna is lacking and the vitellaria do not overlap intestinal caeca and are lacking in the region between the yolk reservoir and the posterior testis.

The preceding differences warranted the erection of a new species for the present form.

In view of close similarities of the specific characters of *Amphiorchis indicus* Mehrotra, 1973 and *A. lateralis* Oguro, 1938, the generic diagnosis of *Amphiorchis*, as given by YAMAGUTI 1958, has been emended as follows:

Generic diagnosis (emended). — *Spirorchidae*, *Amphiorchiinae*: Body slender, subcylindrical. Cuticle may or may not be marked with transverse or longitudinal striations. Sucker small, ventral sucker in anterior half of body. Oesophagus long. Intestinal caeca terminating symmetrically or asymmetrically blindly a short distance from posterior extremity. Excretory vesicle Y- or V-shaped with short stem. Testes two, one anterior to male terminal genitalia, the other posterior to ovarian complex. Vesicula seminalis externa intercalated between cirrus sac and anterior

testis. Cirrus sac well developed, containing prostate cells and short cirrus. Vesicula seminalis interna may or may not be present. Genital pore ventral, median or lateral, at level of anterior end of ovary or a little in front of it. Ovary immediately posterior to cirrus sac, in middle third of body. Receptaculum seminis and Laurer's canal present. Mehlis' gland complex behind ovary. Uterus short. Vitelline follicles extending from intestinal bifurcation or a little behind it to excretory vesicle, interrupted at level of ovary or to a greater extent (from anterior testis to vitelline reservoir). Parasitic in blood vessels of marine turtles.

Key to species of the genus *Amphiorchis* Price, 1934

1. Vesicula seminalis interna absent, vesicula seminalis externa bilobed. Cirrus sac cylindrical. Genital pore postequatorial. Vitellaria not covering the intestinal caeca *A. lateralis* Oguro, 1938

Vesicula seminalis interna present, vesicula seminalis externa not lobed. Cirrus sac oval or club-shaped. Genital pore pre-equatorial. Vitellaria covering the intestinal caeca 2

2. Cuticle marked with transverse striations. Intestinal caeca terminating symmetrically. Vitellaria sparsely distributed *A. amphiorchis* Price, 1934

Cuticle without striations. Intestinal caeca terminating asymmetrically, right caecum being longer than the left one. Vitellaria densely distributed *A. indicus* Mehrotra, 1973

Hapalotrema pambanensis Mehrotra, 1973 (Figs 1b-d, 3)

(*Spirorchidae* Stunkard, 1921; *Hapalotrematinae* (Stunkard, 1921) Poche, 1926; *Hapalotrema* Looss, 1899).

Body creamish-white and flexed in preacetabular region in live condition so as to give a hook-like shape, elongate, hind extremity slightly tapering, 6.669–9.694 mm long and 0.721–0.955 mm broad, maximum breadth occurring across acetabular region. Cuticle armed with very minute spines. Oral sucker terminal, 273–390 × 302–390. Ventral sucker lying a little behind caecal bifurcation, 273–487 × 409–546, pedunculated and with somewhat wavy or infolded periphery, larger than oral sucker; peduncle 331–741 long, not observable when pressed dorsoventrally. Oesophagus 448–877 long. Intestinal caeca slender, running along body margins, converging slightly just before their termination near posterior extremity, filled with blackish-brown material in live condition.

Excretory pore terminal at hind end of body; excretory stem very short, dividing into two branches just posterior to caecal termination.

Gonads occupying most of the intercaecal space in postacetabular region. Testes numerous, present in two groups one in front of ovary and the other behind it; oval, rounded or pyriform, with entire contour, lying into close contact with or overlapping one another at some places, 42–50 in preovarian group and 35–50 in the postovarian, 78–195 × 78–234. Vesicula seminalis transversely elongated, located between ovary and anterior group of testes, 126–219 × 331–487. Cirrus sac ventral to ovary, broad towards its proximal end and narrowing distally, 343–455 × 85–112, proceeding obliquely inwards in posterior direction, enclosing ductus ejaculatorius and small cirrus, a few prostate gland cells also observed in sections. Genital pore in anterior region of posterior third of body, sinistral and ventral. Genital atrium fairly large, surrounded by circular and genital atrial radial muscle fibres that give the appearance of genital sucker but not demarcated from body parenchyma by a limiting membrane (Fig. 3d).

Ovary multilobed, in posterior third of body, contacting and/or overlapping intestinal caeca at places, 507–702 × 390–721. Oviduct arising from submedian dorsal side of ovary, running obliquely towards posterior end and then taking an

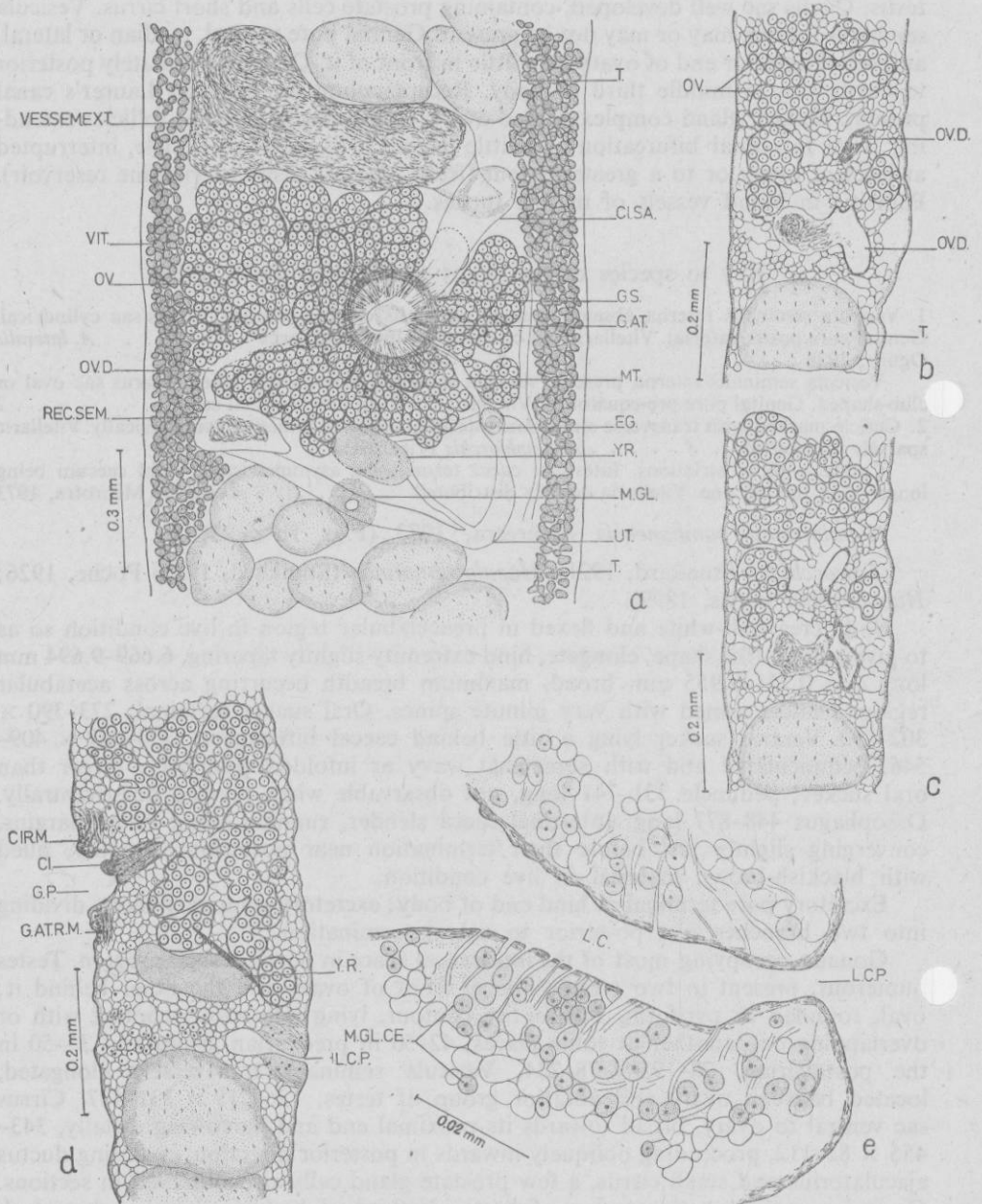


Fig. 3. *Hapalotrema pambanensis* sp.n. a — magnified portion showing genital ducts and gonads of specimen c from Fig. 1 (ventral view); b and c — receptaculum seminis and oviduct in sagittal sections; d — a magnified portion of the same showing the genital opening and Laurer's canal pore; e — an enlarged portion of sagittal section showing some of the ducts of the Mehlis' gland cells opening into the lumen of the Laurer's canal

inward turn where it is joined by a sac-like structure filled with a mass of sperm. MONTICELLI 1896 in his work stated that the oviduct widened to form a structure which he called a receptaculum seminis. LOOSS 1899 and TAKEUTI 1942 observed a similar broadening of the oviduct filled with spermatozoa in their species and referred to this broadened part as "fertilization space". Considering the observations on the serial sections (Fig. 3b, c) of the present form, the writers feel that this sac-like structure is, in fact, the receptaculum seminis and not the widened part of the oviduct, as interpreted by the earlier workers. Laurer's canal joining oviduct a short distance after receptaculum seminis does so, opening on dorsal surface of body by a conspicuous pore visible even in whole mounts; characteristically surrounded by Mehlis' gland cells, the ducts of which open into its lumen (Fig. 3d; Plate I: 1-3). No definite ootype present. Uterus short, running upwards on right side of median line, containing a single egg. Egg thin-shelled and elongate, with tapering ends, approximately 220×294 in one specimen. Vitellaria commencing at a level little behind ventral sucker, extending laterally up to posterior extremity of body, follicles of the two sides coalescing medially in front of anterior group of testes and also behind posterior group of them. Transverse vitelline ducts from both sides meeting medially to form yolk reservoir; the latter lies either behind or ventral to posterior lobes of ovary. Common vitelline duct running obliquely and posteriorly, joining the oviduct some distance after the Laurer's canal meets the latter.

Host: *Chelone mydas* (L.). Location: heart. Locality: Gulf of Mannar (Pamban, Tamil Nadu). Type-specimens: deposited in the senior writer's helminthological collection.

Discussion: On account of the features like the presence of numerous testes divided into two groups, one preovarian and the other postovarian, vesicula seminalis between the anterior group of the testes and the ovary, the latter lobed, the cirrus sac without processes, the vitellaria occupying lateral fields behind the ventral sucker the present form fits the genus *Hapalotrema* Looss, 1899.

The genus *Hapalotrema* comprises the following known* species: *H. loossi* Price, 1934 (syn. *H. constrictum* (Leared, 1862) Looss, 1899** nec *Distomum constrictum* Leared, 1862) from *Thalassochelys corticata* in Egypt, *Caretta caretta* and *Chelone mydas* in the Nile Valley; *H. mistroides* (Monticelli, 1896) Stiles et Hassall, 1908 (syn. *Mesogonimus constrictus* (Leared, 1862) Monticelli, 1896 nec *Distomum constrictum* Leared, 1862) from *Thalassochelys caretta* of Italy***; *H. synorchis* Luhman, 1935 from *Caretta caretta* in Florida (U.S.A.), and *H. orientalis* Takeuti, 1942 from *Eretmochelys squamosa* from Itoyan, Loochoo Islands, Japan.

On comparison the present form has been found different from all the above listed species of the genus. *H. loossi* and *H. mistroides* differ from the present species in having ovary and genital pore at the equatorial level of the body and a little in front of it respectively, and considerably fewer testes. The present species stands

* BYRD 1939 transferred *Spirhapalum polesianum* Ejsmont, 1927 to the genus *Hapalotrema* synonymizing the genus *Spirhapalum* Ejsmont, 1927 with the latter. In agreement with YAMAGUTI 1971 the writers also consider *Spirhapalum* a valid genus, since in it the testes are linearly arranged, the posterior group of testes comprising only one or two of them and the vitellaria extend along both sides of the intestinal caeca, anteriorly reaching up to the level of the caecal bifurcation.

** YAMAGUTI 1958 listed *H. constrictum* (Leared, 1862) Looss, 1899 as the type-species, but at the same time he also accepted the name *H. loossi* Price, 1934, for *H. constrictum* of Looss nec MONTICELLI. However, YAMAGUTI 1971 is in agreement with this renaming of *H. constrictum* of Looss by PRICE 1934.

*** Referred to by SMITH 1972.

close to *H. orientalis* and *H. synorchis* in both of which the genital pore is post-equatorial lying at the level of the one-third body-length from the hind extremity, but they also differ from it in other characters. *H. orientalis* stands apart from the new species by having the vitellaria of the two sides not coalescing medially in front of the anterior group of testes, the latter being lobed, and in the absence of the genital sucker. (According to TAKEUTI 1942, "Around the genital pore dermal muscular fibres are fairly well developed, but there are not found radial muscular fibres and a limiting membrane to receive them. The genital sucker ... is not developed in this species").

The present species can also be distinguished from *H. synorchis* in which the testes are transversely elongated and not distinct from one another, but form compact masses.

The position of the Mehlis' gland cells surrounding the Laurer's canal in the present form is quite unusual. The present species has been named *Hapalotrema pambanensis* after the island from where its host was caught.

The writers propose to emend the generic diagnosis of *Hapalotrema*, as given by YAMAGUTI 1958, 1971 in the light of the observations on *H. pambanensis* and descriptions of other known species of the genus.

Generic diagnosis (emended). — *Spirorchidae*, *Hapalotrematinae*: Body elongate, spinulate. Ventral sucker discoid, may be pendunculate, larger than or equal to oral sucker, in anterior half of body. Oesophagus long, wide or not. No pharynx. Intestinal caeca simple, reaching to the posterior extremity. Excretory vesicle Y-shaped with short stem. Testes numerous and divided into two groups, one of which lies between ventral sucker and vesicula seminalis, and the other behind the ovary. Vesicula seminalis more or less transversely elongated or pyriform, between anterior group of testes and ovary. Cirrus sac containing ductus ejaculatorius and cirrus. Pars prostatica not differentiable. Genital pore sinistral and/or ventral to ovary. Ovary lobed, intertesticular, in the middle third or anterior region of posterior third of body. Receptaculum seminis present, uterus proper lacking or present. Eggs with tapering poles or polar filaments. Vitellaria occupying whole lateral fields of hind body. Parasitic in blood vessels and heart of marine turtles.

Key to the species of the genus *Hapalotrema* Looss, 1899

1. Genital pore equatorial or preequatorial 2
 Genital pore postequatorial (in anterior region of posterior third of body) 3
2. Oral sucker equal to ventral sucker. Vitellaria not coalescing mesially in pretesticular region
 *H. mistroides* (Monticelli, 1896) Stiles et Hassall, 1908
 Oral sucker smaller than ventral sucker. Vitellaria coalescing mesially in pretesticular region
 *H. lossii* Price, 1934
3. Genital sucker-like structure absent. Vitellaria of the two sides not meeting mesially in pretesticular region *H. orientalis* Takeuti, 1942
 Genital sucker-like structure present. Vitellaria of the two sides meeting mesially in pretesticular region 4
4. Testes of each group compactly massed *H. synorchis* Luhman, 1935
 Testes of each group entire and distinct (not compactly massed) *H. pambanensis* Mehrotra, 1973

Abbreviations used in Figures and Plates

CI. — cirrus; CLSA — cirrus sac; CIR.M. — circular muscles; EG. — egg; G.AT. — genital atrium; G.AT.R.M. — genital atrial radial muscles; G.P. — genital pore; G.S. — genital sucker; L.C. — Laurer's canal; L.C.P. — Laurer's canal pore; M.GL. — Mehlis' gland; M. GL.CE. — Mehlis'

gland cells; MT. — metraterm; OV. — ovary; OV.-D. — oviduct; REC.SEM — receptaculum seminis; T. — testicles; UT. — uterus; VES. SEM. EXT. — vesicula seminalis externa; VIT. — vitellaria; Y.R. — yolk reservoir.

REFERENCES

- BYRD E. E. 1939. Studies on the blood flukes of the family *Spirorchidae*. Part II. Revision of the family and description of new species. *J. Tenn. Acad. Sci.*, 14, 116-161.
- EJSMONT L. 1927. *Spirhapalum polesianum* n.g., n.sp. trématode du sang d'*Emys orbicularis* L. *Annls Parasit. hum. comp.*, 5, 220-235.
- LEARED A. 1862. Description of a new parasite found in the heart of the edible turtle. *Q. Jl microsc. Sci.*, 2, 168-170.
- LOOSS A. 1899. Weitere Beiträge zur Kenntnis der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus *Distoma* Retzius. *Zool. Jb. Syst.*, 12, 521-784.
- LUHMAN M. 1935. Two new trematodes from the loggerhead turtle (*Caretta caretta*). *J. Parasit.*, 21, 274-276.
- MEHROTRA V. 1973. *Digenea* from some reptilian hosts in India. Part I. *Proc. 60th Ind. Sci. Cong. Assoc. Part IV*.
- MONTICELLI F. S. 1896. Di un ematozoo della *Thalassochelys caretta* Linn. *Int. Mschr. Anat. Physiol.*, 13, 141-172.
- OGURO Y. 1938. A new blood fluke, *Amphiorchis lateralis* nov. sp. (*Spirorchidae*) found in a marine turtle in Japan. *J. Sci. Hiroshima Univ., Ser. B, Div. 1*, 6, 1-4.
- POCHE F. 1926. Das System der Platyodaria. *Arch. Naturgesch.*, A, 91, 1-240.
- PRICE E. W. 1934. New genera and species of blood flukes from a marine turtle, with a key to the genera of the family *Spirorchidae*. *J. Wash. Acad. Sci.*, 24, 132-141.
- SMITH J. W. 1972. The blood flukes (*Digenea: Sanguinicolidae* and *Spirorchidae*) of cold-blooded vertebrates and some comparison with the schistosomes. *Helm. Abstr., Ser. A*, 41, 161-204.
- STUNKARD H. W. 1921. Notes on North American blood flukes. *Am. Mus. Novit.*, 12, 1-5.
- TAKEUTI E. 1942. New blood-flukes of the family *Spirorchidae* from Japanese fresh-water tortoise and marine turtles. *Jap. J. med. Sci. Trans. Abstr.*, VI, 2, 161-174.
- YAMAGUTI S. 1958. *Systema helminthum*. I. The digenetic trematodes of vertebrates. Inter-science Publishers Inc. New York, London.
- YAMAGUTI S. 1971. Synopsis of digenetic trematodes of vertebrates. Vol. I, II. Keigaku Publishing Co., Japan.

STRESZCZENIE

Autorzy podają szczegółowy opis dwóch gatunków przywr z żółwi morskich, pochodzących z Zatoki Mannar (Pamban, Tamil Nadu, Indie): *Amphiorchis indicus* Mehrotra, 1973 z naczyń krwionośnych wątroby *Eretmochelys imbricata* (L.) oraz *Apalotrema pambanensis* Mehrotra, 1973 z serca *Chelone mydas* (L.). Nazwy oraz diagnozy różnicowe obu gatunków zostały opublikowane wcześniej. Obecnie autorzy podają wiele szczegółów budowy tych płazińców, a także proponują zmieniony opis diagnostyczny obu rodzajów oraz klucze do oznaczania gatunków.

