A NEW SPECIES OF CŒLOMIC TREMATODE OF THE GENUS STAPHYLORCHIS FROM THE TIGER SHARK GALEOCERDO TIGRINUS FROM INDIAN WATERS*

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THE trematodes of the sub-family Anaporrhutinæ Looss, comprise a number of genera, namely, Anaporrhutum Ofenheim, 1900, Probolitrema Looss, 1901. Plesiochorus Looss, 1901, Petalodistomum Johnston, 1913, Staphylorchis Travassos, 1920, and Nagmia Nagaty, 1930, which are known to infest the pericardial and coelomic cavities of Sharks and Rays and the urinary bladder of marine turtles. In view of the extreme rarity of colomic trematodes and the fragmentary knowledge concerning those of Indian fishes, it seems proper to record certain observations on a new species of one of the largest colomic trematodes ever known. Possibly the earliest contribution on this group of trematodes in India is of Shipley and Hornell (1905) who recorded a number of specimens of Distomum richiardii, Lopez, from the colom of Rhinodon typicus. Later Max Luhe (1906) described the trematode Anaporrhutum largum collected by Professor Herdman from the coelom of Rhinoptera javanica. Southwell (1913) recorded Anaporrhutum largum, Luhe from the body cavity of Chiloscyllium indicum, Ginglymostoma concolor, and Aetobatis narinari obtained from Ceylon and Stegostoma tigrinum caught off Orissa Coast and Chaudhuri (1916) whose work was based on parasites belonging to the same species A. largum, Luhe, added an additional host, Hypolophus sephen from the Chilka lake.

Nagaty (1930) has reviewed the sub-family Anaporrhutinæ and the genus Staphylorchis, supplementing previous descriptions of the known species Anaporrhutum largum, Luhe. A new Anaporrhutine trematode Nagmia yorkei was described by Nagaty who also gave a tabular description of all known genera of the sub-family Anaporrhutinæ. A critical review of the trematode parasites of elasmobranchs was published by

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Dolfus (1937). References concerning the genera may be obtained from the above papers which contain reviews of the earlier work.

Johnston (1913) created the genus *Petalodistomum* with *P. polycladum* as the type species and in it he included *P. cymatodes* from the coelomic cavity of the leopard ray, *Dasybatus kuhlii* from Australian waters. Travassos (1920) formed a new genus *Staphylorchis* to accommodate *P. cymatodes* Johnston (1913). The new form described by Luhe (1906) as *Anaporrhutum largum* from the coelomic cavity of the Ray *Rhinoptera javanica*, from Kalpitiya, Ceylon, has since been referred by Baylis (1927) to the genus *Staphylorchis*. Nagaty (1930) redescribed the specimens of *A. largum* from *Ginglymostoma concolor* from pearl banks, Ceylon and confirmed its position in *Staphylorchis* as indicated by Baylis.

GENERIC DIAGNOSIS OF Staphylorchis TRAVASSOS, 1920

The genus Staphylorchis clearly distinguishable by the large or above medium size, broad oval petal-like shape, smooth cuticle, and fleshy muscular body. Ventral and oral sucker more or less of the same size. Intestinal cæca sinuous and each diverticulum in the form of a continuous letter 'S' extending to the posterior margin of the body. Excretory pore median, sub-terminal and posterior. The genital pore median, preacetabular and behind the bifurcation of the gut. Testes 40 to 52 on each side of the body, spherical to oval, external to the intestinal crura. Ovary lobed or mulberry shaped, posterior to the acetabulum, right or left of the median line. Receptaculum seminis large, lateral to the ovary and post-acetabular. Vitellaria disposed in two lateral groups of branching follicles or tubules, post-acetabular and internal to the digestive cæca.

Staphylorchis gigas Spec. Nov.

Habitat.-Parasitic in the cœlom of Galeocerdo tigrinus.

Locality.-Madras and Nagapattinam, Bay of Bengal.

Diagnosis.—With the characters of the genus Staphylorchis. Large size, petal-like in shape with the posterior half of the body almost circular. Pharynx cup-shaped. Cirrus sac absent; vesicula seminalis comparatively long with the terminal portion dilated into a simple elongated oval sac. Ovary lobed, uterine coils filling space between the intestinal folds and may extend beyond terminal ends of the digestive cæca. Vitellaria in two compact group of branching finger-shaped follicles. Excretory vesicle tubular, elongate, reaching almost to the level of the posterior border of the ovary before bifurcation into lateral tubules.

Trematodes belonging to the genus Staphylorchis now recorded for the first time as occurring in Indian waters was found in the coelomic cavity of the tiger shark, Galeocerdo tigrinus caught off Madras in May 1948, and Nagapattinam in June 1949. In the adult stage this species parasitizes the commercially important tiger shark. Of over a dozen specimens of tiger sharks examined, two harboured about a dozen of these parasites. These trematodes were found on opening the body cavity of the infested host and their resemblance to the description of Petalodistomum cymatodes (Johnston, 1913) indicated that the two were closely related. Further more, their morphological agreement with P. cymatodes suggested that they might belong to the same genus. The meagre account of the sexually mature worm of P. cymatodes given by Johnston is limited to the morphology of a single specimen and very little is known of the anatomical details. Since the specimens of the present study differ with the account of Johnston (1913) in certain specific characters as the general shape, greater size, structure of the male genital end organ, the shape of the ovary and vitelline follicles and the structure of the excretory system, and is also from a different host and different locality, their specific identity with those of Johnston's cannot be assumed.

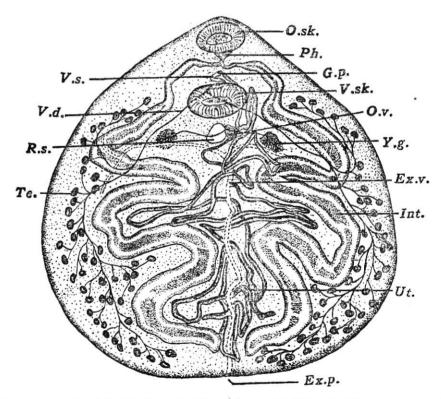
The parasites were first expanded by spreading a few drops of rectified spirit over the surface of the body before fixing in 5% formalin. In sea water they are relatively sluggish and tend to remain in a contracted condition. The fixed specimens were dehydrated, stained in paracarmine and cleared in cedar wood oil for the study of internal anatomy. The measurements were taken from killed and formalin preserved material.

External Characters.—The parasites are large, chocolate brown in colour and conspicuous on the opened abdominal cavity of the host. They occur scattered throughout the length of the cœlomic cavity, although the majority are located on the surface of the liver; often they are deep between the lobes of the liver and may adhere tenaciously with the powerful acetabular sucker.

The shape of the body is portrayed in Photograph I and Fig. 1. The body is muscular, fleshy and smooth and does not bear any scales or spines. The cuticle is thick and smooth. In fixed specimens the contraction of the circular and longitudinal muscles gives the surface of the body at times, a ringed appearance. The shape of the body varies with the degree of extension. The worms are spherical to oval, the anterior extremity being produced into a conical projection while the posterior margin is broadly circular. The preacetabular region is more active and, when extended, it tends to bend ventrally forming a shallow ventral concavity.

Gravid specimens measure from 25 mm. to 27 mm. in length and 20 mm. to 25 mm. in breadth. The oral sucker (o. sk.) is large, sub-terminal, spherical to ovoid in shape and with a longitudinal diameter of 1.8 mm. to 2 mm. and transverse diameter of 2.2 mm. to 2.6 mm. The acetabulum (v. sk.) lies within the anterior third of the body and measures from 1.8 mm. to 2.25 mm. by 2.25 mm. to 2.8 mm. in diameter. The apertures of the suckers are transverse and Johnston (1913) made no mention of the muscular development which determine the shape. The opening of the sucker is provided with a powerful spinchter which, when contracted, forms thickened muscular masses anterior and posterior to the apertures. These masses may protrude into the lumen giving the opening of the sucker a narrow slit-like shape.

General morphological features are shown in Fig. 1. A conspicuous feature of the worms is the two rami of the gut running on either side of the



Text-fig. 1. Staphylorchis gigas, Sp. Nov. Ex. v., excretory vessel; Ex. p., excretory pore; G.p., genital pore; Int., intestinal limb; O.sk., oral sucker; Ov., ovary; Ph., pharynx; R.s., receptaculum seminis; Te., testis; Ut., uterus; V.d., vas deferens; V.s., vesicula seminalis; V.sk., ventral sucker; Y.g., yolk gland.

middle third of the body prominently as a pair of highly sinuous undulating tubes from the anterior end to the posterior end where they end blindly. The large number of testes occupying the whole length of the lateral margin of the worm external to the cæcum and the vitelline glands conspicuous as darkish aggregate of follicles lateral to the ovary and internal to the intestinal diverticulum are striking characters.

Digestive System.—The mouth opening is subterminal and is situated at the base of the oral sucker. A muscular pharynx (Ph.) leads directly out of the oral sucker and is followed by an œsophagus. The prepharynx is absent. The cup-shaped pharynx is 1 mm. anterio-posteriorly and 0.8 mm. transversely. The anterior half of the pharynx is hidden under the posterior lip of the oral sucker. The esophagus is as long as or a little longer than the pharynx but the measurements could not be taken with certainty as the œsophagus was contracted in its longitudinal axis. The anterior portion of the œsophagus immediately behind the pharynx is narrow and tubular but the posterior end gradually widens and the intestinal cæca arise from the enlargement. The bifurcation of the digestive tract occurring anterior to the median genital pore is well in front of the ventral sucker. The intestinal diverticula (Int.) which are wide and unbranched as in P. cymatodes (Johnston, 1913) show a number of deep wavy undulations in the form of a continuous letter 'S' and extend to the posterior border of the worm. The terminal portions curve inwards towards its fellow and then somewhat posteriorly and end blindly. There are ordinarily about three folds on either side, but this, however, may not be a constant number. diameter of the cæcum ranges from 0.6 to 1 mm. The folds of the intestinal cæcum extend between the lateral border of the worm and more than half way to the mid-longitudinal axis and occupy a considerable part of the middle $\frac{2}{3}$ of the length and breadth of the fluke. The intestinal contents stain deeply and, as observed by Looss (1901), Ofenheim (1900) and Nagaty (1930) in other distomes, there is always a clear space between the wall and the contents.

Excretory System.—The excretory pore (Ex. p.) is median and subterminal and lies on the dorsal surface near the posterior end. It leads into a long narrow tubular vesicle (Ex. v.) which extends along the mid-longitudinal axis of the worm to almost the level of the posterior border of the ovary where it bifurcates into two undulating branches of narrower calibre. These branches pass obliquely towards the lateral regions of the body, reaching a position between the testicles and the intestinal folds where they divide again. In addition to the usual prolongation of the limbs towards the anterior ends of the body, there is a backward prolongation towards the posterior margins

of the body. The structure of the excretory system is noteworthy as it is different in its disposition from that of the type species *P. cymatodes*.

Reproductive System.—The large size of the worm is favourable for observing the reproductive system, the details of which can be completely studied in fixed and cleared specimens. The genital pore (G.P.) is median and lies on the ventral surface a short distance behind the bifurcation of the digestive tract and neither sinus nor papillæ are present. The male and female openings are close together, the male opening disposed slightly anterior to the female.

Male Genitalia.—The testes (Te.) consist of about 40 to 52 compact testicles on either side varying in form from oval to spherical, oval ones averaging 0.8 mm. by 0.6 mm. while the spherical ones are 0.4 mm. to 0.8 mm. in diameter. They lie dispersed or in groups of two or three between the lateral margin of the body and the intestinal folds. The majority of them lies completely outside the intestinal diverticula but isolated testicles are to be found within the space bounded by the intestinal folds. testicles extend anteriorly to the level of the acetabulum and posteriorly to the terminals of the intestinal ramus. Vasa efferentia arise from the mediar faces of the testicles and the ducts from the separate testicles join up in groups and converge towards the inner side of the area occupied by the testes and enter a longitudinal vas deferens (V.d.) on either side. The vasa deferentia cross the intestinal cæcum from the outer to the inner side ventrally, unite at the level of the anterior border of the acetabulum between the two intestinal cæca and pass forwards to a fairly long narrow slightly coiled vesicula seminalis (V.S.), the terminal portion of which dilates into a simple, elongated oval sac. The latter opens in the middle line approximately midway between the pharynx and the anterior border of the acetabulum and slightly anterior to the level of the female genital opening. This is different in structure from the vesicula seminalis as noted by Johnston (1913) in the type species P. cymatodes where he describes the vesicula seminalis as short. There is no cirrus or cirrus sac as described by Johnston (1913) for P. cymatodes. The testes are of the same type as in P. cymatodes but here they vary in shape from spherical to oval and at times lie in groups of two and three.

Female Genitalia.—The ovary (Ov.) is dorsal, situated immediately posterior to the acetabulum either median or lateral, right or left. It is slightly lobate, usually broader than long and in gravid specimens measures $1.7 \, \text{mm}$. by $0.9 \, \text{mm}$. The oviduct arises at the median side and the initial portion of the oviduct passes forwards into the ootype which receives the median vitelline duct and a duct from the receptaculum seminis and then

enlarges to form the uterus. The uterus (Ut.) passes backwards and is thrown into a series of close convolutions and the loops of the uterus follow a winding course which is irregular and difficult to follow. Unlike what has been described for P. cymatodes, the uterus is voluminous and fills the region between the intestinal diverticula and extends to the space bounded by the intestinal folds. The uterine coils reach almost to the posterior end of the body beyond the blind ends of the intestinal rami, return to the zone of the dispersive cases, was backwards a second time to the region of the overy and at the level of the vitelline duct run forwards as a more or less straight tube nearly to the level of the acetabulum and then folding on itself run back to the level of the vitelline gland and once again turn forwards forming a second loop before finally reaching the genital pore. In front of the ovary the uterus extends anteriorly as a moderately sinuous tube and joins the metraterm. The metraterm is almost straight and passes between the intestinal diverticulum and the terminal portion of the vesicula seminalis to the genital pore. The uterine coils are filled with numerous large oval-shaped, operculated eggs which measure 0.06 mm. by 0.03 mm.

The receptaculum seminis (R.S.) is spherical and prominent lying lateral to the ovary, slightly to the left or right of the middle line. It is larger than the ovary and its average diameter is 2.8 mm.

The vitellaria have the structure and disposition characteristic of the sub-family Anaporrhutinæ. The vitelline glands (y.g.) are paired and conspicuous and each gland consists of a cluster of branching follicles which resemble clumsy finger-like projections with rounded extremities. The vitellaria are ventral in position, post-acetabular and lateral to the ovary and receptaculum seminis and lie internally to the intestinal cæcum of its side. Their ducts have the usual disposition, a number of follicles unite to form a stem and a number of such stems unite forming the main transverse vitelline duct; the transverse ducts on either side unite between the ovary and the receptaculum seminis and join the oviduct on the right or left of the median line.

DISCUSSION

The genus Staphylorchis was erected by Travassos (1920) to accommodate Petalodistomum cymatodes (Johnston, 1913) as the type and only known representative of the genus. Johnston (1913) described the adult stage of Petalodistomum cymatodes and distinguished between this species and Petalodistomum polycladum, the type of the new genus Petalodistomum. Travassos (1920) considered that P. cymatodes, although having many features in common with P. polycladum, could not be included in the genus

because of its possession of certain characters of generic value such as a highly sinuous but unbranched intestinal cæca and the structure, number and disposition of the testes.

The only other record of species of Staphylorchis is S. largum (Luhe, 1906; Baylis, 1927) from Ginglymostoma concolor by Nagaty (1930) but the generic identity of this species is greatly disputed. The species Anaporrhutum largum has been first described by Max Luhe (1906) after a specimen collected from the cœlomic cavity of Rhinoptera javanica, at Kalpitiya, Ceylon. Travassos (1922) in revising the family Gorgoderidæ, has included in the genus Anaporrhutum, Luhe's species largum. According to Baylis (1927) Luhe's species largum belongs to the genus Staphylorchis rather than to Anaporrhutum. Baylis's inclusion of this species in the genus Staphylorchis is based only on a single character of the position of the testes. Even the statement of Baylis that the testes in Staphylorchis "are situated entirely laterally to the intestinal branches" cannot be accepted since in P. cymatodes and the new species the testicles are distributed laterally between the level of the acetabulum and the posterior terminals of the cæcum and extend towards the median line, characters which do not appear in the species described by Luhe. The disposition and shape of the digestive cæca, the number and position of testes, disposition of the vitellaria and the position of the genital pore appear to be sufficient for including it in the genus Anaporrhutum as less difference exist between Anaporrhutum and Luhe's species A. largum than Staphylorchis. It is significant that the worms studied by Luhe (1906) and Nagaty (1930) came from the same locality, as Kalpitiya is very near Periya paar from where Nagaty obtained his specimens and in all probability Nagaty's species is identical with that of Luhe's.

In a critical review of the trematode parasites of Elasmobranchs Dollfus (1937) criticizes Baylis's allocation of Anaporrhutum largum Luhe, to the genus Staphylorchis and holds the view that Anaporrhutum largum is intermediate between Anaporrhutum, Staphylorchis and even Petalodistomum and as such it will be more justifiable to consider it as a type of a particular sub-genus than to attribute it to the genus Staphylorchis. Hence there is only one known species of Staphylorchis from which it remains to differentiate the species above described.

No difficulty arises in assigning the newly described species to its systematic position, the nature of the intestinal cæca and the number and disposition of the testes excluding all genera of the sub-family Anaporrhutunæ except one and this species falls naturally into the genus *Staphylorchis* Travassos. The new species in all probability is generically identical with

the type species S. cymatodes (Johnston, 1913; Travassos, 1920). It agrees with the type species in the sucker ratio, the form of the digestive cæca, the number and disposition of the testes. In addition it agrees in the large size of the receptaculum seminis and the position of the genital pore.

Although the species shows resemblance to P. cymatodes, a comparison of the present form with the description of the type as given by Johnston (1913) shows such definite differences that they cannot be included in the same species. They differ both in habitat and locality. The present material is collected from the colom of the tiger shark, G. tigrinus from the Indian Johnston (1913) found the parasite in the body cavity of its host, the leopard ray, Dasybatus kuhlii, from Australian waters. The proposed new species Staphylorchis gigas differs substantially from P. cymatodes in the general shape and greater size. The size given for a mature specimen of P. cymatodes is 10.5 mm. in length and 8 mm. in breadth whereas the new species is more than twice as large as its congener measuring 25 mm. to 27 mm, in length and 20 mm, to 25 mm, in breadth. Differences, undoubtedly specific, which distinguished the species just described from P. cymatodes are to be found in the greater length of the vesicula seminalis, the structure of the male genital end organ and the absence of the cirrus sac. The new species differs from P. cymatodes in the shape of the vitellaria which are in the form of clumsy finger-like follicles in contrast to the "much branched tubes" of the type and the close proximity of the individual follicles of the new species gives to the vitellaria a rather compact appearance which is a specific character. In addition it differs from the type in the lobed shape of the ovary and the topographical relation of the uterine coils to the intestinal folds. A further important point of difference of specific significance is to be found in the excretory system, where the main excretory vesicle is long and tubular extending to below the level immediately posterior to the ovary before bifurcation to lateral branches, whereas in P. cymatodes the median excretory vesicle is very short and bifurcates at the level of the end of the digestive cæca in that figured by Johnston (1913). Any of these differences occurring separately may not have justified the erection of a separate species. but since a number of peculiarities occur in one species, it is considered that the specimens of Staphylorchis parasitic in the coelom of Rays and sharks from Australian and Indian seas respectively are specifically distinct.

SUMMARY

A new species of *Staphylorchis*, *S. gigas*, has been studied and its anatomical features are described. It is the only trematode parasite which has been found to infest the colomic cavity of the tiger shark, *Galeocerdo tigrinus*.

Sexually mature specimens are generically identical with the type species S. cymatodes (Johnston, 1913; Travassos, 1920) but since there are important specific differences such as the greater size, the form of the excretory system, shape of the ovary and vitellaria and the nature of the male genital end organ, they cannot be referred to the type species and hence described as a separate species. The discovery of this trematode from an Indian shark affords supplementary data concerning the little known genus Staphylorchis, previously found only in an Australian Ray and adds a second species to the genus which supports the validity of the genus.

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Photograph 1