

STUDIES ON ASIATIC HOLOSTOMES (CLASS TREMATODA)

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(Plates XIV—XXI.)

I. AN UNUSUAL HOLOSTOME, *CLEISTOGAMIA HOLOTHURIANA* FAUST, 1924, FROM THE ANDAMAN SEA.*

This unique holostome was collected by Dr. Stanley W Kemp, late Superintendent, Zoological Survey of India, at Andaman Collecting Station No. 26, in the Andaman Sea. About fifty specimens of this fluke were obtained from the intestinal tract of a holothurian, *Actinopyga mauritiana*, and were lent to me for study and identification. A preliminary report of my findings was presented before the Helminthological Society of Washington (*Journ. Parasitol.*, XI, p. 121; 1924), at which time the form was designated as *Cleistogamia holothuriana* gen. et sp. nov.

C. holothuriana is a pyriform organism, measuring from 1.7 to 2.5 mm. in length by 1.2 to 1.6 mm. in breadth with an average measurement of 2.1 by 1.4 mm. The body is concave on the ventral aspect and convex on the dorsum. The posterior end is gracefully rounded while the anterior end projects forward from the body proper as a semi-conical protrusion. The entire body is aspinose and the integument is very thick.

The oral sucker (*os*) is a small muscular organ measuring from 0.083 to 0.1 mm. in length by 0.11 to 0.14 mm. in breadth. The acetabulum or holdfast organ is an unusually large suckorial cup. It consists of an outer muscular suckorial hood (*ohf*) and an inner semi-glandular region (*ihf*) whose function is probably that of the secretion of a viscous substance to assist in attaching the fluke to the intestinal wall of the host. Neither in the mature worm nor in the slightly immature specimens in the collection are there lateral suckorial cups or pockets such as one finds in typical tetracotyliform larvae. Suckorial appendages, which might have been present in the vicinity of the functional ventral sucker during the larval stage, are also entirely wanting in the mature worm. Likewise, the true ventral sucker appears to have entirely disappeared.

The pharynx (*ph*), which lies just inside the oral cavity, is slightly larger than the oral sucker and has the appearance of an inverted truncated cone. Posterior to it there is a very short oesophagus, which forks almost immediately behind the pharynx to form the two digestive caeca. The caeca (*c*) describe a figure like a pair of ice-tongs as they extend posteriad to the subdistal region of the body.

No details of the excretory system have been observed either in *toto* mounts or in sections.

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The genital organs are of unusual significance. The paired testes (t_1, t_2) are oval bodies situated side by side in the anterior plane of the ventral sucker. The ovary (*ov*) is a long sausage-shaped body lying in a nearly vertical plane slightly to the right of the mid-region of the worm and just anterior to the testes. It opens at its posterior extremity through a very short oviduct which proceeds to the ootype (*oo*). The vitellaria (*vit*) consist of large discrete masses of yolk-cells distributed in a fan-shaped pattern in the region dorsal to the holdfast organ. Their ducts gradually coalesce from behind forward, uniting into a common vitelline duct which enters the ootype from the posterior aspect. The seminal vesicle (*sv*) is an elongate oval organ lying for the most part in a vertical plane behind the ventral sucker. The uterus (*ut*) consists of a large thin-walled pouch, the inner opening of which is at the ootype and the confines of which are limited to the median plane between the pharynx and the anterior margin of the holdfast organ, with an out-pocketing extending at times as far posteriad as the middle of the latter organ.

In the respects thus far considered *Cleistogamia holothuriana* differs very little from the genus *Cyathocotyle* (Odhner 1913, Faust 1922). There is, however, this extremely significant difference: neither seminal vesicle nor uterus opens to the outside of the body. Instead, at the posterior end of the seminal vesicle, where a genital pore or a cirrus-sac might be expected, the organ bends abruptly forward and after passing through an annular reinforcement (pl. XV, fig. 2, *a*) becomes constricted into a hollow "chitinous" capillary filament (*cf*), which is here designated as a *cirrus- or ejaculatory-filament*. This filament continues its forward course, passing over the holdfast organ, and near the anterior border of this organ is found to be fused with a long sacculate organ, the vagina (*vg*), where, again, an annular reinforcement (pl. XV, fig. 2, *j*) is seen at the junction. The vagina, in turn, opens into the left side of the uterus (*ut*). Spermatozoa have been found at both the proximal and distal ends of this filament although they have not been seen inside the capillary tubule itself. However, the filament has an internal diameter throughout its entire length sufficient to allow several spermatozoa to pass through at one time, so that it is a practical certainty that this organ functions in transferring sperm from the seminal vesicle directly into the vaginal sac, thus obviating the hazard of miscarriage through a genital atrium with an external opening. The process here involved is not merely self-fertilization; it is obligatory self-fertilization or *cleistogamy*, a process known in the plant kingdom (as for example in certain Violaceae), but apparently unrecorded in the animal kingdom as the sole means of fertilization. The monogenetic trematode, *Polystoma integerrimum*, has, to be sure, as a part of its dimorphic life-cycle (Zeller 1876, pp. 261-268), a period of development attached to the gills of the tadpole, where the customary sexual apparatus, utilized in the more usual cross-fertilization process, has become non-functional and where Zeller (*l. c.* taf. xviii, fig. 18, *g*) claimed to have found an "inner sperm duct" leading directly from the testis to the oviduct, an observation disputed by Ijima (1884). Even if such a condition obtains in

developed, and then for only one phase of the life-cycle, alternating with the condition commonly found in the trematodes. But in *Cleistogamia holothuriana* the transformation is complete, since there are no vestiges of the usual cirrus organ, genital atrium, or uterine pore.

The egg of *Cleistogamia holothuriana* is also unique for holostomes (pl. XIV, fig. 1a). It is ovoid in outline, measuring 92μ in breadth by 77μ in length, while at one end there is a polar filament from ten to twelve times as long as the body length. The filament is frequently coiled on itself like a watch spring. It seems likely that it may serve in rupturing the blind uterine sac and thus allow the dispersal of the enclosed eggs, or it may possibly serve as a means of entanglement and lodgement for the egg in its marine environment, where the chances of continued existence are extremely precarious for so small an organism. In the possession of a polar filament the egg resembles those of certain Monogenea, as well as those of *Halipegus* and certain species of *Allocreadium*.

Discussion.

The intermediate host of this species is not known but it is believed to be a marine mollusc into which the miracidium penetrates, and from which, after the usual processes of parthenogenetic development have taken place, the pharyngeal furcocercous cercaria is derived. The cercaria then infects the holothurian host, becomes metamorphosed into the tetracotyloform larva and eventually into the mature holostome.

The features which distinguish this holostome from all others of the group are undoubtedly adaptations to a marine existence. It seems probable, however, that they represent a side-line of development rather than a main tendency. This genus and species, therefore, is not to be regarded as possessing structures that have been lost but rather more recent modifications that have come about because of the difficulties encountered in propagating its kind in a marine habitat. While it is not unusual to find trematode larvae of the cercarial generation maturing precociously in invertebrates, such hosts are almost without exception regarded as larval or intermediate hosts. The writer has been unable to find any instance, except that of *Aspidogaster*, in which the fluke develops normally to adulthood in an invertebrate. Such seems to be the case in *Cleistogamia*. Furthermore, there appears to be no trematode, either larva or adult, which has been recorded from a holothurian. The host for this mature holostome is, therefore, as unique as the modified structures which distinguish the parasite.

DESIGNATION OF *Cleistogamia holothuriana*, FAUST 1924.

Genus, *Cleistogamia*. Mature holostome, oval to pyriform in shape; 2.1 by 1.4 mm., concavo-convex, with large functional ventral sucker; testes, 2, symmetrically placed in the anterior plane of ventral sucker; ovary, an elongate sac to the right and in front of the testes; seminal vesicle, elongate oval, with no external opening but with a long capillary cirrus filament extending directly to a vaginal sac, which, in turn, runs into the uterine pouch, through which channel spermatozoa appear to be transferred to the uterus; uterus a blind thin-walled sac, lying

between the pharynx and the holdfast organ; ova, 92 by 77 μ , with a long polar filament. Genus, *monotypic*. Only known species,—*C. holothuriana* with characteristics of the genus. Host, a holothurian.

In view of the differences obtaining in the genital organs of *Cleistogamia holothuriana*, which distinguish it from all described holostomes, it is necessary to create for this genus and species a new sub-family, *Cleistogamiinae*, which is characterized by a cleistogamous type of fertilization, involving a direct connection from the seminal vesicle to the uterus through an ejaculatory filament and vaginal sac. This sub-family is placed close to the sub-family *Cyathocotylineae* Muehling 1898 (family *Strigeidae* Railliet 1919). It is monogeneric.

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II. HOLOSTOMES FROM FISHES OF CENTRAL ASIA.*

In the collection of fishes made by the Zoological Survey of India from Kashmir and Seistan there were several species infested with trematode cysts. Through the kind permission of the late Dr. Nelson Annandale the writer has been enabled to examine representative specimens from these collections. Study of the cysts after sectioning and reconstruction of models of the enclosed larvae has revealed the presence of three species of holostome adolescariae new to science. In view of the fact that the fishes involved are commonly caught for food and that the flukes may possibly become parasites of man if the fishes are consumed raw or insufficiently cooked, the findings have an economic and possibly a medical significance.

***Diplostomum schizothoracis*, sp. nov.**

(Plate XVI; pl. XVII, XIX, figs. 9a—d.)

These larval flukes were found encysted in and under the skin of *Schizothorax zarudnyi* Nikolsky, collected by Drs. N. Annandale and S. W. Kemp in the pools among the reeds of the Hamun-i-Helmand, near Lab-i-Baring, Seistan, Persia. Annandale and Hora (1920, p. 173) refer to the infestation as follows: "Both large individuals (of *S. zarudnyi*)

* Contribution No. 85 from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.

from the Hamun and young ones from small pools were infested by an immature trematode, which was encysted in their skin, in the superficial muscles, in the membrane of the fins and on both the outer and inner aspect of the operculum. The cysts were of a blackish colour and resembled those shown in Herzenstein's figure of *S. altior* (*op. cit.* pl. xii, fig. 1)." In a later publication from the same laboratory (Kemp, 1921, pp. 232-233) Annandale figured *toto* mounts of the larva but was unable to decide the taxonomic position of the organism. His statement of the difficulties encountered in sectioning the cysts has been borne out by the writer's attempts to secure satisfactory material for study.

Description of the Parasite.

In preserved specimens of this host the cysts consist of spherical nodules situated in or under the skin and ectodermal membranes and giving rising to rounded elevations. They measure up to 2 mm. in diameter and appear dark brown or black to the naked eye. They are most conspicuous on the head, opercula and fins of the fish, but are also seen without difficulty in the superficial muscles, since the scales of this host are small and relatively transparent (see pl. XVII, fig. 6). On section of the cyst the pigmentation is found to be laid down in the secondary (outer) cyst-wall around the thick elastic hyaline inner or true cyst-capsule. The pigment consists of dark brown granules arranged in an arabesque pattern fitting around the true cyst-wall and interwoven on its outer aspect with the host tissue. It is thus a host production, its deposition being heaviest around the cysts in the superficial tissue where melanophores are most common.

The inner true cyst-wall is a hollow sphere, varying from 0.35 to 0.5 mm. in outer diameter, and having a thickness of 25 to 30 μ . The wall substance is non-cellular, consisting of a tough elastic hyaline or semi-opalescent material, which is extremely resistant to the microtome knife, so that in preparing sections of the larval fluke residing within the capsule it has first been necessary to dissect off the wall. This has been successful in only three or four out of hundreds of attempts made by the writer, due to the fact that the larva is much softer than the cyst-wall and almost invariably breaks in attempts to dissect it out of the capsule.

The young encysted fluke is bent upon its ventral side and its anterior end is frequently folded to one side to fit the spherical prison house. Annandale's sketches (Text-fig. 2 A, B, C, in Kemp, 1921) give a fair idea of the external appearance of the partially extended fluke, although he figures the worm up-side-down. In order to get a correct idea of the relationship of the various organs the writer has supplemented his study of transverse sections of the larva with a wax-model reconstruction and on the basis of the two has sketched the ventral and lateral aspects of the extended fluke (pl. XVI, figs. 5, 5a). It appears as a broadly oval or slightly pyriform object, 0.65 mm. long by 0.47 mm. wide, and with a maximum thickness of 0.25 mm. from the ventral margin of the outer cup wall to the dorsum. It is broader anteriorly than posteriorly. On ventral view it reminds one of a low thick-walled bowl with incurved

margin notched at one end (the posterior end of the organism). The ventral cavity thus produced constitutes the inclusive suckorial cup of the fluke. The worm is completely aspinose.

The anterior sucker (*os*) is situated just in front of the suckorial cup or just at its anterior margin. It measures about 50μ in greater diameter. A pharynx (*ph*) lies immediately behind the oral sucker; it measures 40μ in cross section. A very short oesophagus leads into two long caeca which extend into the posterior fifth of the body. Along the mid-line within the concavity of the inclusive suckorial cup, are two acetabula, a small one, measuring 60μ in transverse diameter, at the junction of the anterior and middle thirds of the body, and a much larger one, 150μ in transverse diameter, situated somewhat behind the middle of the body. The anterior one (*vs*) is apparently the ventral sucker, while the posterior one is probably the accessory holdfast organ (*hf*). The genital anlagen (*ga*) are still undifferentiated; they consist of three club-shaped elements, one lying to the right of the holdfast organ, one immediately behind it and the third situated some distance posteriorly and extending dextrad above the right digestive caecum. The former two elements are attached proximally to the inner portion of the holdfast organ (see pl. XVI, figs. 5, 5*a*, also pl. XIX, figs. 9*a*—9*d*).

Although this organism is immature its relationship to the genus *Prohemistomum* Odhner 1913 is fairly certain. If this assumption is correct, the position of the two anterior genital anlagen with respect to the holdfast organ is indicative of the earlier distomate history of this genus, while the posterior element (possibly the anlage of the cirrus pouch) is suggestive of the later holostomate modification.

Strigea annandalei, sp. nov.

(Plate XVIII, fig. 7; pl. XIX, fig. 10*a*—*c*; pl. XX, figs. 12, 13.)

This larval strigeid was obtained from a small loach, *Nemachilus rupiculus* McClelland, obtained by the Kashmir Survey Party of the Zoological Survey of India from Sonamarg Nullah and from the hill streams of Kashmir. The hosts examined by the writer were studded with black nodular elevations (pl. XX, figs. 12, 13), marking the location of the encysted parasites. The latter were imbedded in the subcutaneous and muscular layers, elevating the outermost tissues in the manner described. The true cyst was found to be surrounded by a layer of fibrous connective tissue with extensive melanoid pigmentation. The gross diameter of the cyst, with its outer pigmented wall laid down by the host, was about 0.45 mm., while the diameter of the true cyst capsule was about 0.34 mm. The capsule, which was secreted by the fluke at the time of its encystment, consisted of a thick-walled hollow sphere, about 20μ in section and having a semi-translucent appearance.

Description of the Parasite.

In order to study the encysted larva it was necessary to section the cysts and make a wax-model reconstruction. Although sectioning was possible without dissecting the organism out of the cyst capsule, considerable difficulty was experienced in interpreting the reconstructed

model, due to the fact that it was tightly twisted and folded upon itself. It was found, however, that the fluke consisted of an anterior hollow suckorial cup and a posterior fleshy portion, the two parts being connected with one another by a pipe-stem middle region. Thus in sections of the worm (pl. XIX, figs. 10a—10c) cut sagittally through the anterior end, the posterior portion appears in oblique or nearly transverse section. The sketch (pl. XVIII, fig. 7) represents the entire worm as extended after dissection out of the cyst capsule. It resembles a large-bowled, thin-stemmed pipe with an inflated mouth end. The entire organism is delicately spinose.

The extended reconstruction measures one millimeter in length. The cross diameter of the cup is 0.28 mm. and its depth 0.38 mm. The neck is about 0.34 mm. long by 40μ in cross section. The posterior fleshy portion has a length of 0.5 mm. and a transverse diameter of 0.26 mm. The oral sucker (*os*) is very minute (15μ in section) and is situated just within the posterior rim of the cup. The ventral sucker (80μ in greatest diameter) lies well down within the posterior wall of the cup. There is no evidence of any holdfast or accessory suckorial apparatus. Within the oral opening are a short prepharynx, a pharynx (*ph*), 24μ in transverse diameter, and a long oesophagus, the last organ bifurcating just anterior to the plane of the ventral sucker. The caeca extend into the subdistal region of the posterior fleshy portion of the body.

The genital anlagen are well developed. They are all contained within the posterior portion of the body. By comparison of sections (pl. XIX, figs. 10a—10c) with the reconstructed drawing (pl. XVIII, fig. 7) the following organs can be made out: (1) an ovary (*ov*), lying well forward in the fleshy posterior region, and connected by a coiled duct, the uterus (*ut*), with the posterior genital atrium; (2) two relatively large testes (t_1, t_2) in tandem arrangement behind the ovary, with efferent ducts joining to form the vas deferens (*vd*), which, in turn, leads into (3) the seminal vesicle (*sv*). This latter organ opens into a canal (the cirrus-sac), which empties along with the uterus into the genital atrium. The latter organ has the usual genital pore (*g.p.*). The various ducts in question are not solid cords of cells but hollow tubules, while the seminal vesicle is also a hollow sac (see pl. XIX, fig. 10c), indicating that the genital organs are far along in their development. In sections (pl. XIX, fig. 10c) elements of the vitellaria (*vit*) have also been observed, although the full distribution of these bodies has not been worked out. They appear to be confined to the posterior part of the larva.

The general shape of the organism together with the early differentiation of the genital organs make it clear that the organism should be placed in the genus *Strigeu*.

***Neodiplostomum kashmirianum*, sp. nov.**

(Plate XVIII, fig. 8; Pl. XIX, figs. 11a—b; Pl. XX, fig. 14.)

Numerous fishes belonging to the species *Schizothorax curvifrons* Heckel, *S. niger* Heckel and *Crossochilus latia* (Ham. Buch.) Day, collected by the Kashmir Survey Party of the Zoological Survey of India, were observed to have splotchy black nodular elevations of the skin, which the writer has found on examination to be holostome cysts, all

of the same species. In the case of the two *Schizothorax* host-species the collections were made from the Jhelum River and from Anchar Lake (Sindh River), Kashmir. In the case of *Crossochilus latia* the specimens were secured from Anchar Lake and Wular Lake, Kashmir. On account of the thicker, tougher skin of the *Schizothorax* specimen infested with this fluke, the elevations at the points of infestation are slight and the pigmented areas more or less confluent one with the other, but in specimens of *Crossochilus* (pl. XX, fig. 14) the pigmented elevations are prominent and discrete. In all of the infested hosts the cysts lie in the subcutaneous or connective tissue layers superficial to the muscles. They are oval-elongated in shape with their long axes parallel to that of the host. The encapsulated cyst measures about 0.55 mm. in lesser diameter and 1.1 mm. in greater diameter. The outer (false) cyst capsule is densely impregnated with melanoid pigment, being drawn out as a whole into more or less pointed ends. It measures 60 μ or more in thickness in transverse diameter and up to 230 μ from end to end. The true cyst capsule within is thick and tough, frequently resisting all attempts at sectioning. For this reason good sections were obtained only after the larvae had been dissected out of their cysts. The study of this species was made from sections cut from cysts removed from all three species of hosts taken from six different habitats. A wax-model reconstruction was made from sections of the cysts removed from *Crossochilus latia* obtained from the Jhelum River at Srinagar, so that this fish is to be regarded as the type host-species.

Description of the parasite.

The worm within the cyst capsule is bent upon itself, with two main lines of curvature, one just in front of the middle and one just behind the middle of the body. The figure illustrating the reconstructed specimen (pl. XVIII, fig. 8) has been stretched out to represent an excysted living specimen. The worm as a whole has a rounded anterior end, deeply scooped out on the ventral side, and a rounded fleshy posterior end. The integument is aspinose. The dorsal surface-measurement over all is about 1.8 mm., and the ventral 1.1 mm. The hollowed-out anterior portion constitutes about four-fifths of the entire organism. The lateral margins of the anterior two-fifths of the worm fold outward as in adult worms of the genus *Neodiplostomum*. The ventral sucker lies slightly back of the middle of the body. It measures 66 μ in diameter. The large muscular holdfast organ, with its deep suckorial pocket, is situated a short distance behind the ventral sucker. It is 140 μ long and 160 μ deep.

The oral sucker (*os*) is a comparatively minute structure some 20 μ in diameter. Immediately behind it lies the pharynx (*ph*), about 35 μ in diameter. The oesophagus is very short, branching almost immediately behind the pharynx to form the long slender caeca (*c*) which extend to the subdistal region of the fluke.

The genital anlagen are differentiated in so far as organic masses are concerned, but cellular differentiation has not yet occurred. Thus from their relative positions and sizes (see pl. XVIII, fig. 8 and pl. XIX, fig. 11b) the ovary (*ov*), anterior and posterior testes (*t*₁, *t*₂), vitellaria

(*vit*), seminal vesicle (*sv*) and cirrus-pouch (*cp*) are distinguishable, although the uterus and the efferent and deferent sperm ducts are still undifferentiated. The cirrus-pouch is particularly well advanced in its development and possesses muscular elements.

In certain respects this species resembles *Neodiplostomum spathula* (Diesing), but in view of the relatively narrower and longer anterior end, the more distal position of the ventral sucker and holdfast organ and the closer association of these latter organs one with the other, *Neodiplostomum kashmirianum* differs from *N. spathula*, and must be regarded as a distinct species.

Discussion.

The three larvae, representing three different subfamilies of the family Strigeidae, which have been described in this paper have the common characteristic of residing in the subcutaneous and superficial muscle-tissues of fresh-water fishes. Except for Herzenstein's figure (1888) showing a cystic infestation of the fins and opercula of a specimen of *Schizothorax altior* from Lob-Nor, collected in 1885, and for Pawlowsky and Anitschkow's description (1923) of *Tetracotyle sogdiana*, obtained from *Schizothorax intermedius* from the Zerabshan River, Samarkand (1908), the holostome parasites from fishes of Central Asia have not been studied. In the case of *T. sogdiana*, moreover, the larvae were found encysted in abdominal adhesions and not in the superficial layers of the body.

The recent investigations on the life-history of the Strigeidae by Ruzskowski (1922) and Szidat (1924) indicate that the tetracotyle stage of this fluke is preceded by a cercaria, characterized by the possession of a pharynx and a bifid tail. Cort (1918) and La Rue (1926) have shown by analogies of the excretory system that this group is related to the schistosomes, which also have a fork-tailed cercaria. This cercaria develops in molluscs and upon maturing may either encyst in the same mollusc or may migrate out of this host and invade other molluscs or other host-species, including the lower vertebrates, where it discards its caudal organ and encysts. The species herein described from fishes may be considered as having passed the parthenitic phase of their life-cycle in fresh-water molluscs, after which the mature cercariae had escaped and invaded the superficial tissues of the fish species in question, in which they encysted and were awaiting passive transmission to the next host, probably a mammal or a bird. In view of the relatively advanced condition of the genital anlagen of these encysted flukes considerable development of the larvae must have taken place after encystment. The change in shape from the tetracotyliform larva to the diversified features characteristic of the three respective subfamilies and genera to which the three species belong is also indicative of growth and differentiation. This modification is most striking when the inclusive suckorial cup and the accessory acetabula of the ventral surface are compared with those of the cercariae of this group. While Szidat (*l. c.*) is probably correct in his analogies of these accessory acetabula in the case of his "Cercaria A" and *Tetracotyle typica* Dies., it seems entirely unwise in the light of our present knowledge to jump at the conclusion that this analogy

applies to the entire family Strigeidae. The structural modifications noted by Odhner (1913) in adult forms and by Faust (1919) in the transitional stage, the tetracotyliform larva can not be dismissed as irrelevant to the phylogenetic history of the group. While the cercarial stage and maritae of the Strigeata may be strikingly alike, various modifications and adaptations may have been utilised within the family Strigeidae in the transfer of a preacetabular to a posteriorly disposed genital atrium and pore. If such is the case then the suckorial organs in the mid-ventral line of adult holostomes may not be analogous one to the other. A study of the transitional larval forms of the various subfamilies is most likely to help in elucidating this interesting problem.

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III. NOTES ON THE LIFE CYCLE OF *PHARYNGOSTOMUM CORDATUM* (DIES).**Occurrence of the adult worm in China.*

During a survey of the trematode parasites of man and domestic animals in the vicinity of Shaohsing, Chekiang Province, China, in March

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1923, the writer autopsied several cats, one of which contained more than one hundred specimens of a fluke firmly attached to the proximal portion of the ileum. Examination of several *toto* mounts as well as serial sections indicated that the worm was a holostome, belonging to the species *Pharyngostomum cordatum* (Diesing). Some months later the writer received from Dr. Ke-fang Yao three specimens of the same species of worm obtained by him from the intestine of a cat at Changsha, Hunan Province. These two collections of this species are the first known to have been made from Asia, and indicate that the range of this species extends through Asia as well as Europe.

The larval holostome in fishes.

In considering the fluke infections of man and domestic mammals incurred through consumption of raw fish at Shaohsing, two fresh-water fishes, taken from the canals, were found to contain cysts of a holostomate fluke. The species of fish involved were the Chinese bitterling, *Rhodeus sinensis* (Fam. Cyprinidae), and the crab-eating goby, *Eleotris potamoplilia* (Fam. Gobiidae). These holostome cysts were easily distinguished from the *Clonorchis*, *Metagonimus* and *Echinochasmus* cysts found in the same fish, since the larvae had neither pigmentation nor oral armature, but were provided with an exceedingly thick hyaline inner cyst capsule and a brownish outer capsule (pl. XXI, figs. 15, 16, 17). The latter being drawn out at opposite poles into finger-like processes. The cyst was readily removed from the outer capsule (*oc*) by slight pressure of the cover glass (pl. XXI, fig. 16). The inner capsule (*ic*), however, was extremely resistant to pressure and attempts to remove it without rupturing the larval holostome residing within were almost always unsuccessful. The cyst with its outer covering measured about 0.38 mm. in length by 0.26 mm. in lesser diameter, while the outside diameter of the spherical inner cyst capsule measured 0.24 mm.

Description of the larva.

On removal of the outer capsule the encysted worm (pl. XXI, fig. 17) was found to be coiled and somewhat twisted upon itself, while the cavity between the larva and the inner wall of the capsule was filled with excretory concretions (*ec*). The integument was spinose. An oral (*os*) and a ventral (*hf*) acetabulum were present, as well as a Y-shaped excretory bladder (*eb*) and cornua. The larva on dissection out of the inner cyst capsule (pl. XXI, figs. 18, 19) measured 0.27 mm. in length by 0.176 mm. in greatest breadth. Its body was found to have a definite division into anterior and posterior portions, the division being more conspicuous from the ventral (pl. XXI, fig. 18) than from the lateral view (pl. XXI, fig. 19). The oral sucker (*os*) measured 40 μ in diameter and projected slightly in front of the body. Behind the oral sucker a small pharynx (*ph*), 16 μ in diameter, was observed, while a short oesophagus with intestinal caeca diverging posteriorly from it was faintly visible. The posterior portion of the body was approximately hemispherical, measuring 0.176 mm. in diameter and 0.08 mm. in depth, with a cupped-out ventral side having an incurved margin. Protruding

forward out of the anterior face of this concavity was a triangular lappet (*la*), behind which was a mushroom-like acetabulum, the holdfast organ (*hf*), measuring 140μ in width by 70μ in length. On the anterior face this acetabulum was partially fused with the lappet. A ventral sucker, if present, must have lain under the flap, between this structure and the anterior wall of the worm.

The genital anlagen were found to be still immature; they consisted of one posteriorly disposed muscular organ, probably the cirrus-pouch (*cp*), and three spherical masses of undifferentiated cells (*ga*).

Experimental data.

Portions of the flesh of *Rhodeus sinensis*, heavily infested with these holostome cysts were taken to Peking a few days after their discovery in Shaohsing. The material was fed to a laboratory cat known to be negative for holostomes by repeated faecal examination extending over several months. Two weeks later the cat was autopsied, at which time six hundred and eighty adult specimens of *Pharyngostomum cordatum* were found attached to the wall of the proximal region of the ileum. This experiment, therefore, confirmed the belief which the writer had entertained when he found the adult flukes in cats and the closely related larvae encysted in small fresh-water fishes commonly fed to cats in the vicinity of Shaohsing, namely, that the larvae and adults were one and the same species, and that the cats incurred their holostome infection from consumption of infested fish. Incidentally it is of interest to note that these same species of fish were also a source of infection for *Clonorchis sinensis*, *Metagonimus yokogawai* and *Echinochasmus perfoliatus*, as determined by controlled laboratory experiments in cats and dogs.

A comparison of the larvae (pl. XXI, figs. 18, 19) removed from cysts in the tissues of the infested fish and of the adult worms (pl. XXI, figs. 20, 21) indicates that the two stages of this fluke have many structures in common, although considerable modification has attended the metamorphosis from the larval to the adult form. Examination reveals the fact that the anterior end of the adult comprises a very small and inconspicuous part of the body, being confined to the region in the immediate vicinity of the oral sucker. On the other hand, the ventral sucking cup (*usc*) of the larva has become even more conspicuous than it was in the larva. The posterior cone of the adult (*pc*) represents new growth to accommodate the genital organs. The holdfast organ (*hf*) has become greatly modified and is completely fused to the lappet (*la*). In the relaxed worm (pl. XXI, fig. 20) this organ lies almost entirely under the lateral foliaceous folds (*f*) which have developed as an enveloping structure within the surface of the inclusive ventral cup; in the extended worm (pl. XXI, fig. 21) these lateral folds are completely withdrawn against the outer rim of the inclusive ventral sucking cup and the holdfast organ appears as a trilobate structure, with its own suctorial depression. The combined inner and outer suctorial organs thus provide an apparatus for attachment to the intestinal wall of the host so effective that the attached worms appear as cystic excrescences from the host tissue, both in fresh and preserved material. Although the worms can not be removed from their place of attachment without considerable

effort, the function of the sucking apparatus appears to be only for mechanical (*e.g.*, adhesive) purposes, since the mucosa of the ileum within the region of attachment is uneroded. The acetabulum of this species is very small and inconspicuous as La Rue (1926) has shown and can not be seen in surface view. In sections it may be found as a small, almost entirely non-muscular organ on the anterior face of the small pocket formed by the holdfast organ with the anterior end of the worm.

The entire integument of the larva is provided with spines. Such is the case even in immature specimens of the worm removed from the cat. In the adult, however, the integumentary spines are confined to the anterior end and in and around the suckorial apparatus, while the posterior cone is free from spines.

The internal organs of the worm (pl. XXI, fig. 21) correspond to those described by La Rue (*l. c.*) for this species.

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