

David A. Erasmus

The Biology of Trematodes



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PREFACE

The experimental investigation of the biology of the trematodes has always been a particularly challenging task. The establishment of a trematode species in the laboratory is dependent on a supply of parasite-free hosts and, because of the systematic diversity of the host animals sometimes required, culture and maintenance procedures become very elaborate. The use of natural infections from the field is often unsatisfactory for many purposes because of seasonal variation in availability and because the material is ill-defined in terms of age and host/parasite strains. The situation is rendered more difficult by the relatively short life of many of the larvae.

In all stages, the isolation of organ systems is not easily accomplished because of the absence of a body cavity and the intimate association of the structures with the surrounding parenchyma. Thus, trematodes do not lend themselves readily to the isolation and fractionization techniques favoured by biochemists. In spite of these difficulties, the introduction of methods for the maintenance of species such as *Schistosoma* and *Fasciola* on a large scale in the laboratory, and the application of histochemistry, autoradiography, electron microscopy and *in vitro* culture techniques has enabled considerable progress to be made in our understanding of these parasites over the last two decades.

The aim of this text is to introduce final honours and first year postgraduate students, who are already familiar with the basic characteristics of the group, to some of the more recent discoveries and ideas developed through the use of modern techniques. The choice of the title, which might concern the more pedantic reader, is justified by the following reasons. The treatment in this book is largely non-systematic and the term 'Biology' has been chosen because its inclusive nature permits the correlation of information from a wide variety of disciplines. The much criticised systematic unit 'Trematoda' has been retained because it enables me to consider both 'monogenean' and 'digenean' trematodes in a comparative fashion. In spite of the controversy concerning the systematic status of the units, both groups become involved in similar host-parasite situations and possess systems which are similar in structure. Thus both the oncomiracidium and the miracidium are free swimming ciliated stages and have similar problems of host location and attachment to overcome. Adults of both groups have an external cytoplasmic tegument which is the 'buffer' between the parasite and its external environment and is a surface which is involved in secretion and absorption. The alimentary tract and the excretory system of both groups show considerable morphological similarity. Thus in order to discuss the essentially nonsystematic but largely biological aspects of these parasites the older, and perhaps more familiar, systematic unit has been retained. A final point is that the emphasis in this book is on larval stages, which have so often been given inadequate coverage in the past.

My interest in the trematodes has been fostered and encouraged by Professor J. Brough, D.Sc., and I will always be indebted to him for the facilities which have enabled my interests to be fulfilled. This book would not have been completed but for the magnificent way in which Mrs. R. Hyde accepted the challenge of endless notes and alterations, and translated them into readable text. I am sincerely grateful to her for her considerable labour and to my colleague, Dr. R. Hammond, who read the text in its early stage and made many valuable suggestions.

Several of my postgraduate students have generously allowed me the use of their photographs and I thank Dr. A. J. Probert, Dr. J. Jenkinson, Mrs. L. Gregory and Mr. R. Robson for this. I am also grateful to Dr. R. A. Wilson who sent me his manuscript on the body wall of *Fasciola* miracidium and on whose work my text-fig. 32 is based. Finally, but by no means least I will always be grateful to my wife, Sylvia, for her understanding and encouragement, without which this book would not have been possible.

This text contains many illustrations taken from other publications and I gratefully acknowledge the permission of Professor B. Dawes and the editors of the American Journal of Tropical Medicine and Hygiene, Blackwell's Scientific Publications, Ltd., Comparative Biochemistry and Physiology, Det Kongelige Danske Videnskabernes Selskab, Experimental Parasitology, Journal of Helminthology, University of Illinois Press, Japanese Journal of Medical Science and Biology, Journal of the Marine Biological Association, U.K., Journal of Parasitology, University of Neuchatel, Oliver and Boyd, Parasitology, Proceedings of the Helminthological Society of Washington, the Royal Society of Tropical Medicine and Hygiene, Journal of Shanghai Science, Sobretire del Libro Homenge al Dr. Eduardo Caballero y Caballero, T. Cheng and W. B. Saunders Co., Transactions of the American Microscopical Society, Zeitschrift fur Parasitenkunde and the Zoological Society of London to reproduce them. Figures 49a and b are reproduced by permission of the National Research Council of Canada from the Canadian Journal of Zoology, 34, 295-386 (1956) and figure 7 from 'The Invertebrata, vol. II, Platyhelminthes and Rhynco-coela' by L. H. Hyman, copyright 1951 held by the McGraw-Hill Book Company Inc., and used with the permission of McGraw-Hill Book Company Inc. Specific author acknowledgements are made alongside each figure.

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I General Features

All trematodes are parasitic and may live on the skin, fins, gills, buccal and cloacal surfaces of aquatic hosts, in the alimentary tract and its accessory structures, or in the reproductive, excretory, respiratory, blood and nervous systems. In fact there are very few organ systems which have not become invaded by trematodes. The majority of trematodes are parasitic in chordate hosts although the wide distribution of larval stages in the invertebrate fauna results in the association of most animal taxa with the biology of the trematodes.

Attachment to the host is usually by means of suckers of one sort or another and the evidence suggests that attachment is not permanent but that the parasites are able to move around and reattach themselves within the selected environment. The selection of certain organs, e.g. the brain or eye as habitats often necessitates considerable migration within the host body by some parasite species. The life-cycle of trematode parasites may be simple in possessing a single larval stage with no multiplication so that one egg is potentially capable of giving rise to one adult. In other cycles the development is complex in that several larval stages may occur involving a succession of different intermediate hosts as well as multiplication so that a single egg is potentially capable of giving rise to hundreds or thousands of adults, and this compensates to a considerable extent for the hazards which are encountered during the developmental period.

The Trematoda represents a somewhat heterogeneous group of parasites which, because of the nature of their life-cycle, exhibit a strong and persistent association with aquatic habitats. The appearance and survival of these parasites is therefore influenced by the general biotic factors associated with aquatic environments as well as by the more intimate physiological and immunological interchanges present in any host-parasite relationship. In this way the survival of both adult and larval trematodes is particularly susceptible to climatic and seasonal changes in the environment and to fluctuations in the free-living fauna also resulting from such changes. The freshwater habitat for example is considerably affected by seasonal changes so that the trematode fauna associated with such environments is in a continual state of flux.

The complexity of the life-cycle, particularly in the case of the digeneans, is reflected in the involvement in it of many representatives, both permanent and temporary, of the free-living fauna. Thus, trematode parasites can be regarded as a component of the fauna associated with aquatic environments although this aspect is generally overlooked until epidemic disease makes the association inescapable. Water is essential to all animals and many essentially terrestrial animals have to maintain permanent or temporary contact with natural bodies of water. The evolution of the parasites of these terrestrial hosts has occurred in such a way that this period of contact with water permits the establishment of parasitic stages in host animals which lead a largely terrestrial existence.

The association of adult trematodes with clinical disease is relatively rare in natural populations although several genera (e.g. *Fasciola*, *Schistosoma*, *Clonorchis* and *Paragonimus*) are notorious in producing severe disease and mortality in Man and domestic animals.

ADULT MORPHOLOGY

The majority of trematodes fall into the range of 2–15 mm but some may reach a length of 80 mm (*Fasciola gigantica* and *Fasciolopsis buski*). NOBLE (1967) records a forty-foot fluke! They are usually cream or white in colour although the presence of large numbers of eggs in the uterus, or host blood or other tissues in the gut, may give a brown or reddish tinge to the body. Larval stages may possess pigment, which is intrinsic or derived from host food, so that these stages may be green, yellow or brown in colour, but the majority are translucent. The body usually has the form of an elongated oval in outline although many of the strigeoid trematodes possess a transverse constriction dividing the body into anterior and posterior regions. The conspicuous posterior attachment organs of the monogeneans may be correlated also with a posterior demarkation of the body. The body is dorso-ventrally flattened but variations on this may result in spherical, cup-shaped or cylindrical forms appearing. The absence of a rigid skeleton and the presence of an extensive musculature allow continual changes of body shape to occur, much to the dismay of many systematists. It is now realized that the trematodes (data not available yet on the Aspidobothria) are bounded externally by a highly modified epidermis in the form of a cytoplasmic tegument. Associated with the tegument are hooks and spines of various types and these are usually distributed in a manner constant and characteristic for the species. Below the tegument lies the basement layer which has, as its major component, a fibrous layer and it is this which serves as a skeleton for the attachment of muscles as well as limiting the extent by which the body changes shape.

Immediately below the basement layer lie the muscles arranged with circular muscles outermost and longitudinal innermost. The muscles (as seen in the strigeoid genera *Apatemon*, *Diplostomum* and *Cyathocotyle*) (Plate 1-1) resemble very closely those described by MORITA (1965) from the planarian *Dugesia dorotocephala*. The sarcoplasm encloses two types of myofilaments—a central one approximately $0.02\ \mu$ in diameter and this is surrounded by a ring of smaller myofilaments approximately $70\ \text{\AA}$ in diameter. The sarcoplasmic extension of the muscle contains the nucleus and patches of dense endoplasmic reticulum and mitochondria. Each muscle cell is enclosed in a sheath of connective tissue. The characteristic features of the muscle seem to be the lack of bands or striations, the parallel arrangement of the fibres (not helical as in the molluscs) and the presence of two types of myofilaments. The fibres of the basement layer merge into the interstitial fibre material (GRESSON and THREADGOLD, 1964; THREADGOLD and GALLAGHER, 1966) and these fibres become inserted into conical depressions in the connective tissue coat of the muscle so that the muscle becomes attached to the fibrous layers. At the point of insertion dense material is usually apparent. This arrangement is illustrated by the electron micrographs of the oral sucker of *Diplostomum* (Plate 1-2). Within the suckers the muscles are arranged in a radial fashion.

The basic connective tissue of the trematodes is the parenchyma and the ultrastructure of this tissue has been studied in *Fasciola hepatica* (THREADGOLD and GALLAGHER, 1966) as well as in several turbellarians. It appears that the connective tissue consists of parenchymal cells which lie in contact with the cells of other tissues and organs or may be separated by fibrous, interstitial material. A similar pattern exists in certain strigeoid trematodes studied by the author and there seems little evidence to support the concept of large fluid filled spaces described by earlier workers. The parenchymal cell is large and polymorphic with a finely granular cytoplasm containing a nucleus, numerous mitochondria and relatively sparse endoplasmic reticulum.

The cytoplasm is generally rich in glycogen but rarely contains lipid droplets (THREADGOLD and GALLAGHER, 1966).

Extending between the parenchymal cells are several, fairly elaborate, organ systems. The alimentary tract is well developed and possesses a mouth, pharynx and two intestinal caeca although the system is variously modified in different trematodes. The excretory system is basically a protonephridial one consisting of flame-cells, capillaries, large collecting ducts, a bladder and an excretory pore. The pore may be double and anterior or single and posterior. The system is extensively modified in different trematodes, and the reader is referred to DAWES (1946 and 1953), HYMAN (1951) and GRASSÉ (1961) for further details. In some digenean trematodes a lymphatic system exists containing fluid and cells and recent descriptions of this system in Paramphistomes have been made by TANDON (1960a,b) and LOWE (1966).

The nervous system (Fig. 7) consists of cerebral ganglia in the vicinity of the pharynx and from this extends a number of longitudinal trunks joined in some cases by transverse commissures. From this bilaterally symmetrical system arise branches which innervate the suckers and the organ systems. One of the best descriptions is still that by BETTENDORF (1897) although recent accounts have been published by REISINGER and GRAAK (1962). Cholinesterases have been recorded from the nervous system of adult *Fasciola hepatica* by HALTON (1967c). The ultrastructure of the nervous system has been described for the cercarial stage of *F. hepatica* (DIXON and MERCER, 1965) and these authors, as well as UDE (1962) and ROHDE (1965), suggest the presence of a neurosecretory system in the digeneans. The publication by MORITA and BEST (1965) and that of other authors reviewed by them indicate the presence of a neurosecretory system in the turbellarians and it seems likely that many of their comments will apply to the trematodes. Several sense organs have been described in ultrastructural studies and these are considered in more detail in chapter 9. There seems no justification for perpetuating the idea implying a degeneration of sensory and nervous systems in the trematodes.

The majority of trematodes are hermaphrodite and possess a fairly elaborate reproductive system (Fig. 1). The ovary is usually single and there may be one to many testes. As in all platyhelminthes the yolk is produced by a separate structure the vitelline gland. The uterus is long and accommodates a large number of eggs. The female system may possess a single or a double copulatory canal and the male system terminates in a cirrus. The female system possesses a characteristic chamber—the ootype which has associated with it a distinct gland, usually referred to as Mehlis' gland, and the entire structure has considerable significance in the assemblage of the ovum and yolk cells and also in the formation of the egg shell. In some trematodes e.g. *Schistosoma* and *Didymozoidae* the sexes are separate or exhibit protandry. The variation in the structure of the reproductive system is considerable and for further details the reader is referred to BYCHOWSKY (1957) and to the general texts mentioned earlier. Ultrastructural observations on the flagellum of the spermatozoa of the digeneans *Haematoloechus* (SHAPIRO *et al*, 1961), *Fasciola* (GRESSON *et al*, 1961), and *Gorgodera amplicava* (HERSHENOV *et al*, 1966) have shown that it consists of a double filament in its central and proximal regions and that each filament contains nine peripheral fibres encircling a central single one. The pattern of the axial filament complex is therefore nine plus one. In addition to the axial filament, a number of single micro-tubules lining the sheath are present. This arrangement has also been observed in *Cyathocotyle bushiensis* (see Plate 2-3). The nature of the digenean spermatozoa and the process of spermatogenesis has been reviewed by FRANZEN (1956) and HENDELBERG (1962) and the similarities existing between these structures and processes of the Digenea and Cestoda has been commented on by RYBICKA (1966). In certain monogeneans (LLEWELLYN and EUZET (1964);

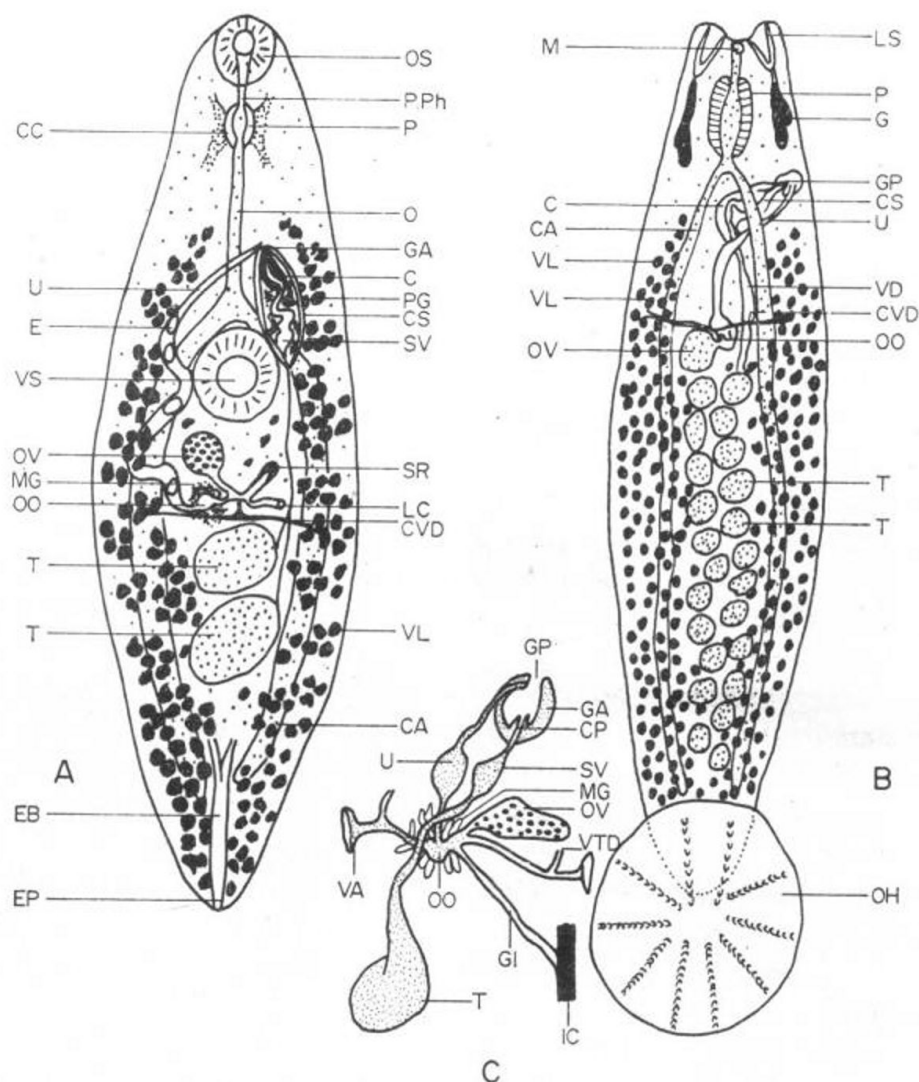


FIG. 1 The generalized structure of monogenean and digenean trematodes. **A** Digenean adult; **B** Monogenean adult; **C** schematized representation of the monogenean reproductive system. (C: cirrus; CA: caecum; CC: cerebral complex; CP: cirrus papilla; CS: cirrus sac; CVD: common vitelline duct; E: eggs; EB: excretory bladder; EP: excretory pore; G: glands; GA: genital atrium; GI: gastrointestinal canal; GP: genital pore; IC: intestinal caecum; LC: Laurer's canal; LS: lateral suckers; M: mouth; MG: Mehlis' Gland; O: oesophagus; OH: opisthaptor; OO: ootype; OS: oral sucker; OV: ovary; P: pharynx; PG: prostate glands; P.Ph: prepharynx; SR: seminal receptacle; SV seminal vesicle; T: testes; U: uterus; VA: vagina; VD: vas deferens; VL: vitelline lobes; VTD: duct from vitelline gland; VS: ventral sucker.) All figures original.

PALING (1966)) a specialization in the form of spermatophore production occurs. The gross morphology of the ovary in *F. hepatica* has been studied frequently but a reappraisal of its structure was published by BJÖRKMAN and THORSELL (1964b). The ovary is surrounded by an outer capsule containing muscles. Within this is the basement membrane 80–130 mμ thick, and on the inner side occurs a continuous layer of peripheral cells. The germ cells adjacent to this layer are in intimate contact with it, with processes from the germ cells entering the peripheral layer. The authors suggested that the layer may function as a zone of nurse cells. The cytoplasm of these cells was dense, contained granular endoplasmic reticulum, mitochondria and a nucleus. The germ cells adjacent to this nurse layer were small (oogonia) and were roughly ovoid in outline. The larger germ cells (oocytes) in the centre of the ovary were polyhedral in outline and possessed microvilli facing the intercellular spaces. The cytoplasm of these germ cells was rich in ribosomes

although most of them were free and not bound to a membrane system. The cytoplasm also contained dense spherical granules the nature and function of which was not apparent. The process of oogenesis as it occurs in the digeneans has been reviewed by GRESSON (1964).

CLASSIFICATION

The system of classification used in this text is the conventional one as it appears in DAWES (1946; 1953) and YAMAGUTI (1958; 1963). In this system the Trematoda constitutes a Class of the Phylum Platyhelminthes and contains three orders, the Monogenea, the Aspidogastrea and the Digenea. The distinctive features of these systematic units are given below:

Phylum platyhelminthes

Basically bilaterally symmetrical and usually dorsoventrally flattened animals. The body is acoelomate and without anus, or distinct respiratory and circulatory systems. There is a characteristic connective tissue referred to as parenchyma and a protonephridial flame-cell system. Hermaphrodite.

CLASS TURBELLARIA

Usually free-living. The body is covered with a simple cellular or syncytial epidermis and generally ciliated to some extent. The epidermis contains rhabdites which can be extruded to the exterior. The body is undivided and with intestinal caeca except in the Acoela. The life-cycles are simple and species are usually free-living in an aquatic environment although some are commensal and parasitic in their habit. Hermaphrodite.

CLASS CESTODA

Endoparasitic platyhelminthes, divided into segments and without an alimentary tract. The body is covered with a specialized epidermis in the form of a cytoplasmic tegument possessing microtriches. Anterior end specialized for attachment. Life-cycle complex involving a hooked embryo, a variety of larval stages and two or more hosts. Adult stage usually in the intestine of vertebrates. Hermaphrodite.

CLASS TREMATODA

Ecto- or endoparasitic platyhelminthes with an undivided body possessing a mouth, pharynx and intestinal caeca. The body is covered with a specialized epidermis in the form of a cytoplasmic tegument bounded on the outside by a plasma membrane not elevated into microtriches. Life-cycles simple, or complex involving one or more larval stages. Attachment structures in the form of suckers. Hermaphrodite (Fig. 1).

Order 1. Monogenea Main attachment organ at the posterior end of the body, usually consisting of adhesive structures and hooks and termed the opisthaptor (Plates 4-2 to 4-5). Oral sucker poorly developed or absent but the anterior end possessing some type of attachment

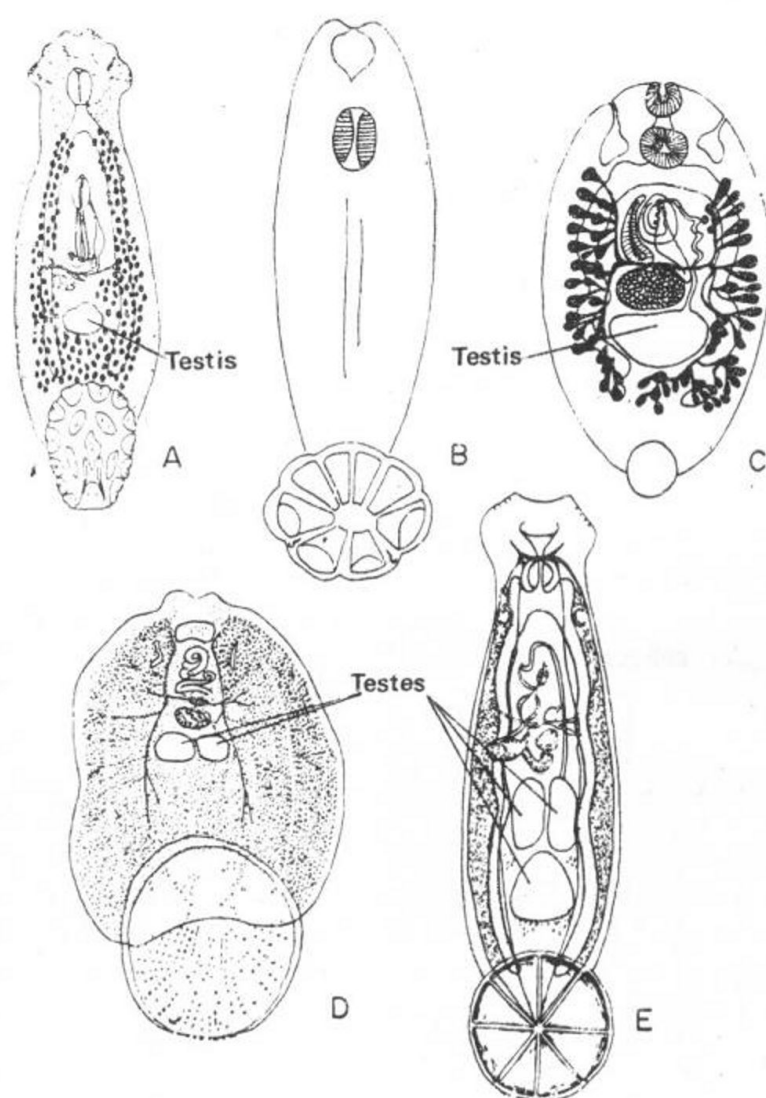


FIG. 2 Examples of monogenetic trematodes, viewed from the ventral surface. **A** *Thaumatacotyle dasybatis*; **B** *Heterocotyle pastinacae*; **C** *Leptocotyle minor*; **D** *Entobdella hippoglossi*; **E** *Monocotyle* sp. Note the relatively simple form of the opisthaptor in these Monopisthocotyleans. Redrawn from various sources from CHENG, 1964.

structure. The pores of the protonephridial system are paired, anterior and dorsal. Endo- or ectoparasitic and usually with a simple life cycle with no alternation of hosts. Hermaphrodite with one ovary and one to many testes. Uterus short, containing relatively few eggs which may possess filaments and may or may not have an operculum. Usually oviparous but sometimes viviparous. The larva is ciliated and possesses a posterior attachment disc and is described as an 'oncomiracidium'. Monogenea are usually parasitic on or in cold blooded vertebrates, sometimes on aquatic mammals, cephalopods and occasionally on parasitic crustacea from fish. (Figs. 2 and 3).

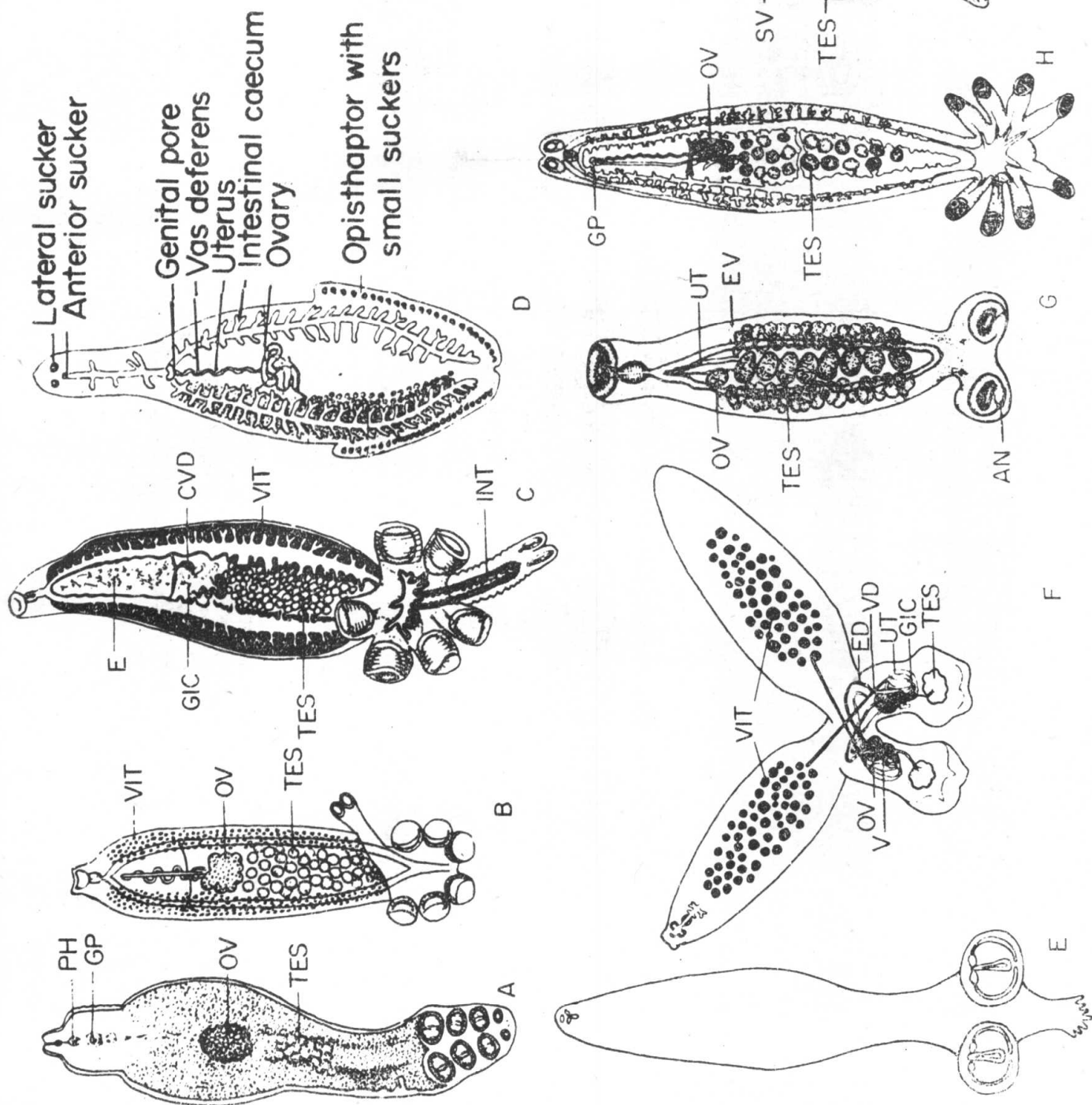
Generally divided into two suborders: 1. Monopisthocotylea . . . with the opisthaptor as a single structure. Genito-intestinal canal absent. 2. Polyopisthocotylea . . . with a complex opisthaptor consisting of several suckers or clamps. Gastro-intestinal canal present.

Order 2. Aspidogastrea With a distinctive attachment organ consisting of a large ventral sucker subdivided into loculi or of a row of suckers. Protonephridial system with a single posterior, terminal or dorsoterminal pore. Life-cycle simple with a larva possessing suckers and

FIG. 3 Examples of monogenetic trematodes.

- A *Hexastoma extensicaudum*
 B *Erpocotyle* sp.
 C *Rajonchocotylodes emarginata*
 D *Microcotyle* sp.
 E *Anthocotyle merlucii*
 F *Diplozoon paradoxum*
 G *Sphyranyra* sp.
 H *Octodactylus minor*
 I *Diclidophora merlangi*

(AN: anchor; CVD: common vitelline duct; E: Eggs; ED: ejaculatory duct; EV: excretory vesicle; G IC: gastrointestinal canal; GP: genital pore; INT: intestine; OV: ovary; PH: pharynx; SV: seminal vesicle; TES: testis; UT: uterus; V: vagina; VD: vitelline duct; VIT: vitellaria.)
 Redrawn from various sources from CHENG, 1964.
 Note the relatively complex form of the opisthaptor in the Polyopisthocotyleans.



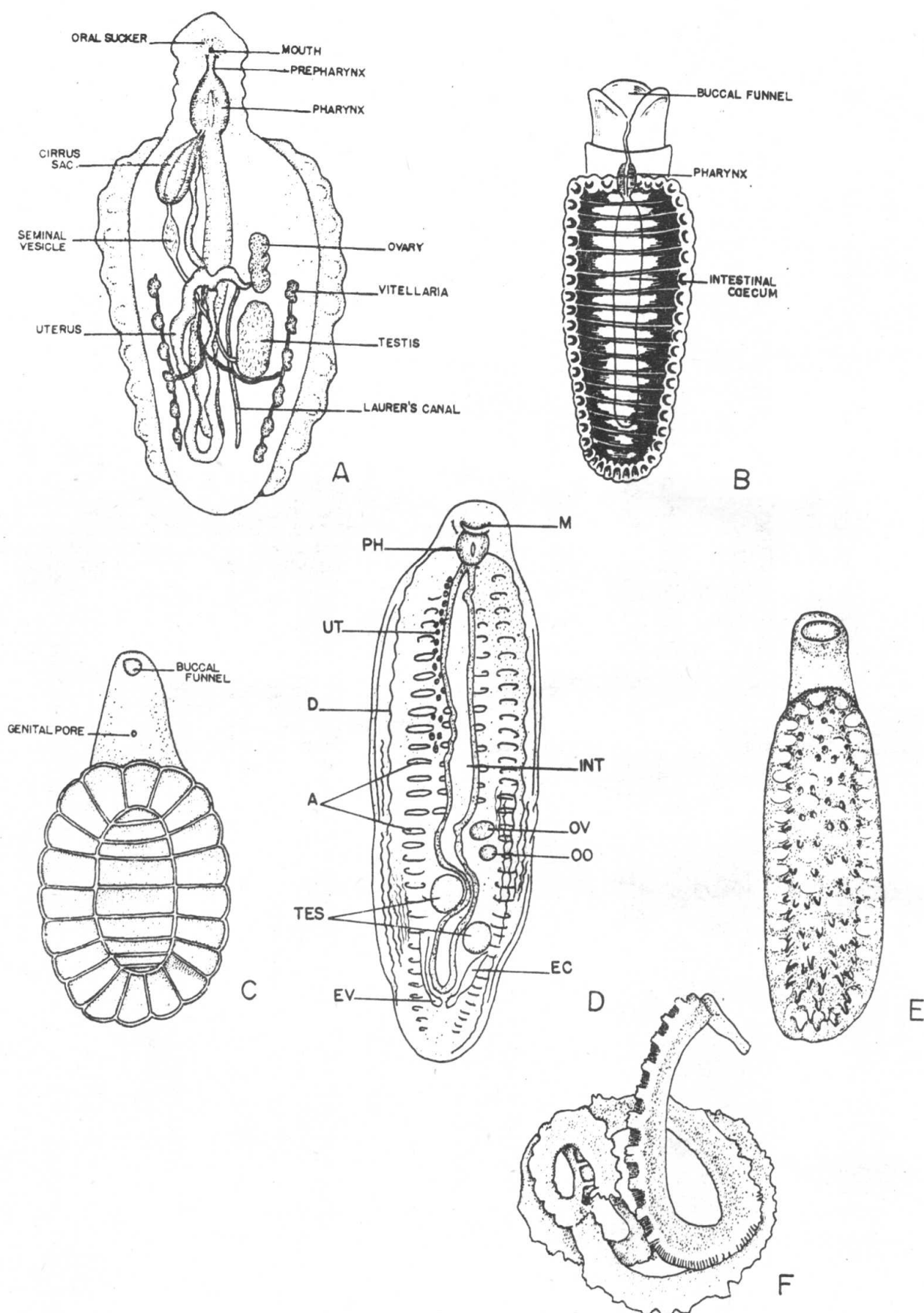


FIG. 4 Examples of Aspidogastreans. **A** *Aspidogaster conchicola*; **B** *Cotylogaster* sp.; **C** *Cotylaspis* sp.; **D** *Multicotyle purvisi* (A: Alveoli; D: Adhesive disc; EC: excretory canal; EV: excretory vesicle; INT: intestine; M: mouth; OO: ootype; OV: ovary; PH: pharynx; TES: testis; UT: uterus) **E** *Lophotaspis* sp.; **F** *Stichocotyle cristata*. Redrawn from various sources from CHENG, 1964.

with or without cilia. Described as a 'cotylocidium' (wootton, 1966a). Generally endoparasitic in molluscs and cold blooded vertebrates (Fig. 4).

Order 3. Digenea Usually with two suckers—oral and ventral. Protonephridial pore single and posterior. Uterus long and containing many eggs, usually without filaments and operculate. Complex life-cycle with several larval stages and an alternation of hosts. Eggs hatch to produce, in most cases a ciliated larva 'miracidium' (Figs. 5 and 6).

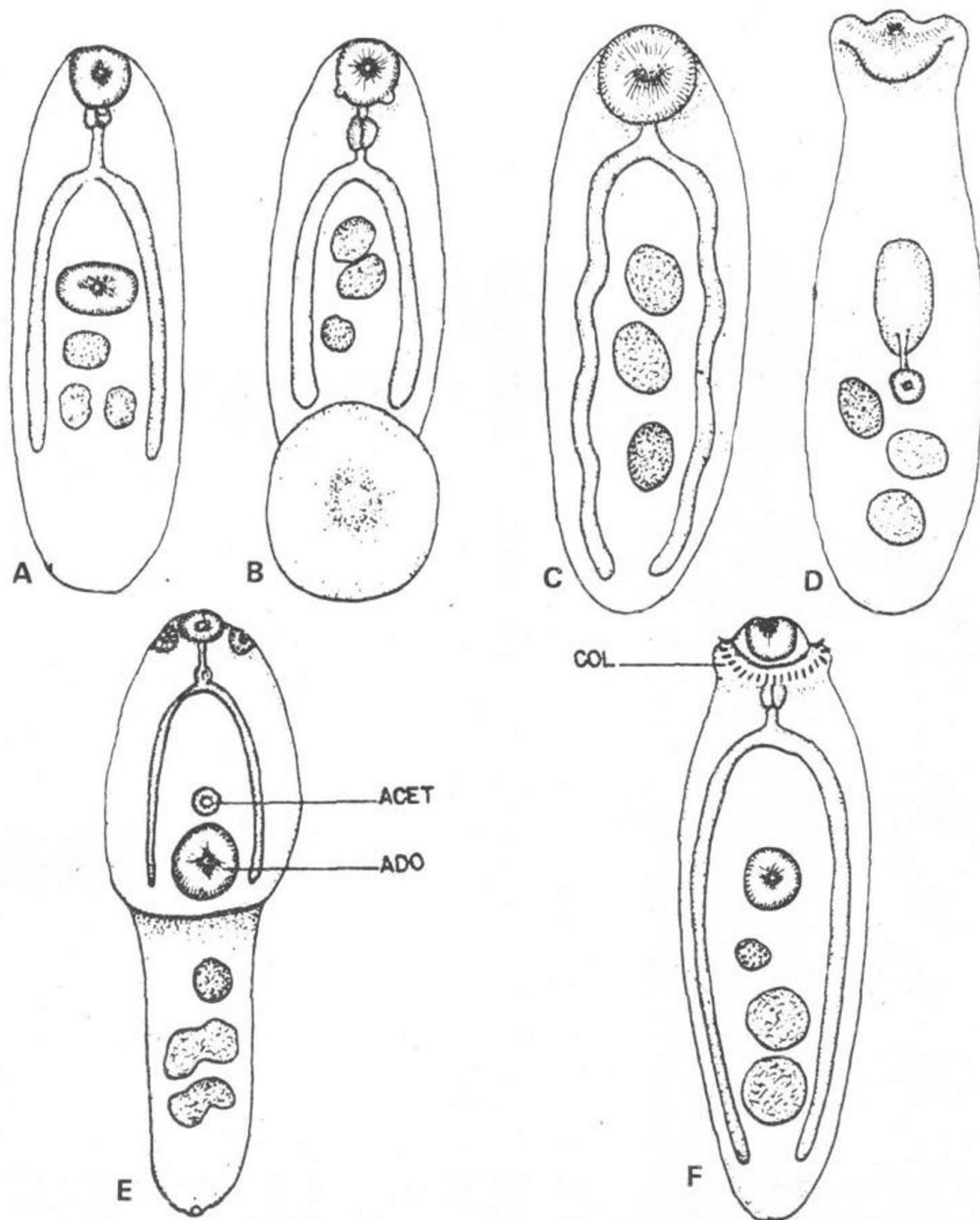


FIG. 5 Examples of the basic gross variations in body form within the digenea. **A** Distome; **B** Amphistome; **C** Monostome; **D** Gasterostome; **E** Holostome; **F** Echinostome. (Acet: acetabulum; ADO: adhesive organ; Col: collar of spines.) From CHENG, 1964.

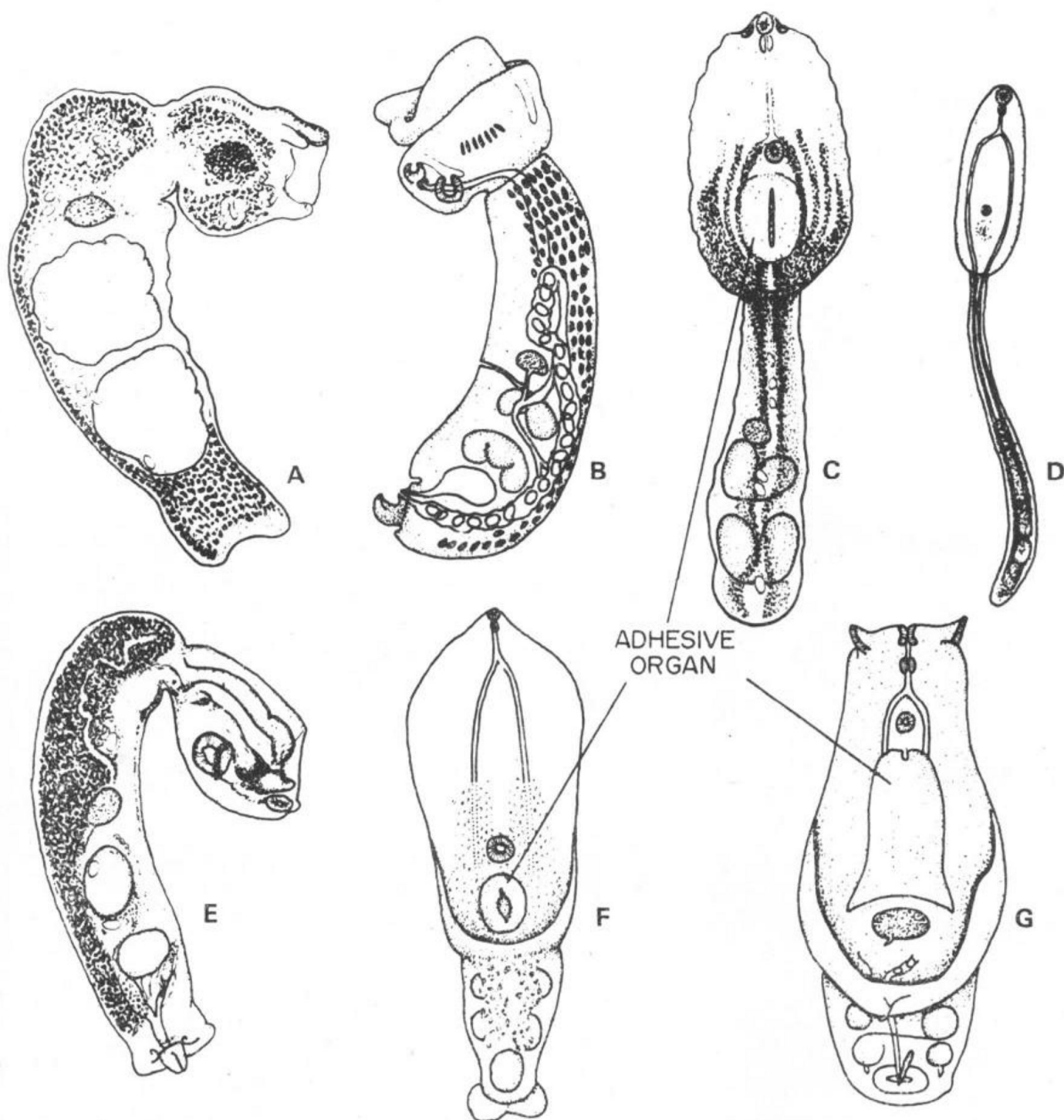


FIG. 6 Examples of strigeoid trematodes. **A** *Strigea strigis*; **B** *Cotylurus erraticus*; **C** *Diplostomum spathaceum*; **D** *Uvulifer gracilis*; **E** *Apatemon gracilis*; **F** *Neodiplostomum ochilongum*. **G** *Alaria alata*. Redrawn from various sources from CHENG, 1964.

The taxonomic treatment of the Trematoda has been frequently reassessed as new information becomes available, particularly that concerned with life-cycles and larval stages. The scheme outlined above is in general usage at present but is unsatisfactory in several ways and new approaches have been discussed and reviewed by LLEWELLYN (1965), STUNKARD (1946, 1963), LA RUE (1938, 1957), and BYCHOWSKY (1957). The main bases for disagreement are (a) the status of the Monogenea; (b) the status and position of the Aspidogastrea; (c) the basis