

Some helminth parasites of Nevada bullfrogs, *Rana catesbiana* Shaw

by

Bert B. Babero*

and

Katharina Golling*

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ABSTRACT: In a survey of the helminth parasites of bullfrogs in the area of Ash Meadows, Nevada, 12 species comprising 7 genera were collected. The helminths recovered are as follows: (Nematoda) *Eustrongylides wenrichi*, *Spirogonia catesbiana* and *Abbreviata* sp.; (Trematoda) *Langeonia protitellaria*, *Glypibelmis quieta*, *G. subtropica*, *G. proximus*, *G. sera*, *Haematoloechus buttensis*, *H. breviflexus*, and *H. parviflexus*; (Cestoda) *Ophiotaenia magna*. Brief discussions on each of the preceding parasites are presented. *Eustrongylides wenrichi*, in its encystment in the musculature, viscera, and egg-mass, was found to be highly pathogenic to bullfrogs.

The bullfrog is an introduced animal in Nevada, although the precise date of introduction is not known. STEJNEGER (35) does not list *R. catesbiana* among the anurans collected in his Death Valley expedition. However, according to Norman Woods, Nevada Department of Fish and Game (personal communication), a recent article in a local newspaper refers to the presence of bullfrogs in the Mormon Fort in 1910. Notwithstanding, the animal is now widely distributed throughout the state, being present in all counties. In a few instances, it is even raised for commercial purposes. In the past, the sport of "frogging" was indiscriminate; however, since 1947 the bullfrog has been on the protected list, being classified as a game animal.

In June, 1970, Dale Lockard of the Nevada Department of Fish and Game brought to the writers' laboratory for identification several helminth-infected bullfrog legs taken from animals collected at Ash Meadows (Nye County). Because of the obvious and extensive pathological damage done to the musculature of these legs, the writers were persuaded to initiate an investigation of these amphibians.

* Department of Biological Sciences, University of Nevada, Las Vegas 89154 Nevada, U.S.A.

Ash Meadows, elevation 2,175 feet, is considered to be a portion of the Death Valley System; it consists of a large valley or plain lying east of the Amargosa Range and 50 miles north of the great bend of the Amargosa River. It was so named because of a small desert ash (*Fraxinus coriacea*) which was formerly abundant (PALMER, 28). The boundary line between California and Nevada passes through Ash Meadows. The region contains numerous isolated warm springs and a few disconnected surface flows, which according to MILLER (26), are remnants of the Amargosa River system. During periods of heavy rain in spring and summer, the headwaters of this river pour their flood waters onto the great Death Valley salt flats. The numerous warm springs of the Death Valley region have temperatures that vary from 22 C to 43 C (average 23.8-24.1 C.); however, according to Miller, temperature fluctuation in individual springs is very narrow. The prevalent vegetation which covers the area is discussed in detail by BEATLEY (3).

The occurrence of bullfrogs at Ash Meadows is of long duration. LINDSALE (21) cites collections made in 1933 and 1936. From this area, a total of 95 bullfrogs, mostly of adult-size, were collected by the present writers. Collection sites included a large isolated pond, marshy meadow areas, and a drainage ditch on the Tubb's ranch; two small ponds near the Ash Meadows lodge, and a vast meadow area about a mile west of Forest Spring. From June to August, 1970, 35 animals were examined and from April to August, 1971, 50 animals. Despite several trips to the area in May and June, 1972, no frogs were collected. However, in July of this latter year we collected 10 animals. Although the spring of 1972 was exceptionally dry, it is highly possible that the population of bullfrogs in the Ash Meadows area may have been decimated due to parasitism, predation, or the drainage of marshes associated with extensive development of irrigation agriculture (DEACON and BUNNELL, 9; LITTON, 22). Most frogs were captured by hand at night and with the aid of a searchlight, but a few were taken with the use of a "frog gig".

Helminths were recovered from frogs by routine parasitological procedures. In many instances blood smears were made, however no helminths were observed. A total of eleven species were obtained, including three of Nematoda, seven of Trematoda, and one of Cestoda. All parasites were fixed in alcohol-acetic acid-formalin (AFA) solutions. The latter two parasite groups were stained with Semichon's Acid Carmine, while nematodes were cleared for study in lactophenol or glycerin and studied from temporary mounts. Brief discussions of each parasite collected follow:

NEMATODA

Eustrongylides Jägerskiöld, 1909

Eustrongylides wenrichi Canavan, 1929

(Figs. 1-3)

This reddish larval dioctophymid nematode was the most common helminth collected from bullfrogs, being recovered in 53 animals. The worms

were usually coiled and encysted within a thin whitish membrane. Most occurred singly but sometimes two or three worms were present within a single cyst. Cysts occurred more frequently in and on the musculature, especially of the appendages and stomach wall. However, they were observed on the peritoneum, along the mesentery of the digestive tract, and occasionally on the surface of the liver and lungs. In a few instances, cysts were seen within the egg-mass of gravid females. The maximum number of cysts obtained from a single host was 25; the average number was five. The number of viable worms decreased as the summer progressed. During the latter part of August and early September, most cysts in the musculature appeared compressed and partially resorbed by the surrounding tissue. Worms within such cysts were dead. Coiled unencysted worms were also encountered. In several hosts, such worms were found under the skin of the abdominal and thoracic regions.

CANAVAN (6) reported *E. wenrichi* from the coelomic cavity of stream pike, brook trout, calico bass, and the nine-spined sunfish. He also collected encysted worms from the mesentery, peritoneum, and liver of bullfrogs, and suggested that the primary hosts for the roundworm are fish-eating birds and that fish may be secondary hosts. The bullfrog was considered by him to be an aberrant host. Dwarf cyprinodonts, *Cyprinodon nevadensis* were also found in the streams and marshes of the Ash Meadows area; MILLER (25) offers an account of these fishes.

None of the fishes we examined harbored *E. wenrichi*; their small size, usually not more than 20 mm in length, seemingly negates infection. The large number of infected frogs collected indicates that these animals, at least in the areas studied, are true secondary hosts for *E. wenrichi*.

YAMAGUTI (43) reports 16 species as comprising the genus *Eustrongylides*, however, only two have been reported from the U.S.A.: *E. ignotus* Jägersk., 1909, and *E. wenrichi*. Morphologically, the Nevada species fits the description of the latter as presented by Canavan. The species is recognizable by the large muscular bursal cup of males, in which is a protrusile terminal cloacal orifice, another smaller bursal cup, and by the lateral rows of papillae extending the length of the body. Such papillae also surround the larger bursal cup. The lengths of the worms are variable, depending upon the age. Canavan reported males as being 106 mm long by 0.86 mm wide and females as 123 mm long by 0.96 mm wide. The Nevada specimens approximate the measurements given by Canavan, however, the length of worms obviously is dependent upon age and development.

Eustrongylides is highly pathogenic to frogs. In attaching itself to organs — i.e., liver and lungs, the parasite conceivably could interfere with vital metabolic function. Since some cysts were also observed in egg-masses within gravid females, there could be increased reproductive morbidity. Extensive damage due to encystment within the somatic musculature was the most obvious pathological, damage observed. JÄGERSKIÖLD (16) reported that *Eustrongylides* may even burrow its way through the stomach wall.

Spiroonoura Leidy, 1856*Spiroonoura catesbiana* (Walton, 1929)

(Figs. 4-7)

Species of the genus *Spiroonoura* primarily occur in fishes, amphibia and reptiles. *S. catesbiana* was described as *Falcnstra catesbiana* by WALTON (39) from the bullfrog, *Rana catesbiana*, from Illinois, Oklahoma, and Louisiana. Walton stated that the species was the most common intestinal nematode occurring in bullfrogs. He later found the species in the same host in the state of Georgia. YAMAGUTI (43) cites other hosts for the species as follows: *Rana grylio*, *R. sphenoccephala*, *Hyla gratiosa*, *H. cinera*, *Gastrophryne carolinensis*, *Pseudacris ocellaris*, and *Siren lacertina*, all of the U.S.A. Nevada constitutes a new locality record for this nematode species.

S. catesbiana was the second most common parasite recovered in this study, occurring in the large intestine of 44 animals, with a range of infection from 1 to over 100 parasites per host. Generally, the Nevada specimens fitted the original description as given by Walton, however, both male and female specimens seemed to be longer than those reported by Walton. This size difference, however, does not seem to be sufficient for justifying a different identification. Although often prevalent in large numbers in bullfrogs, pathological damage due to *S. catesbiana* was not discerned.

Abbreviata Travassos, 1919*Abbreviata* sp.

This genus was encountered only once. A single female worm was found in the large intestine wherein it was detected partially extending through the serosa of the intestinal wall. *In situ*, the worm appeared reddish, however, this color disappeared upon fixation. Members of *Abbreviata* normally are found in the stomach rather than the intestine, so it is possible that the worm may have migrated to the intestine.

TREMATODA

Langeronia Caballero & Bravo-Hollis, 1949*Langeronia provitellaria* Sacks, 1952

(Fig 8)

Langeronia provitellaria was described by SACKS (31) from the small intestine of eight leopard frogs, *Rana pipiens sphenoccephala*, collected in Florida. A total of only three specimens from two bullfrogs were recovered in the present study. The Nevada specimens were slightly smaller with proportionately

smaller organs than those reported by Sacks. Notwithstanding, they are considered to be of this species. *Langeronia provitellaria* is not a common parasite of bullfrogs, as evidenced by the few times it has been collected.

Glyptelmins Stafford, 1905

There has been much controversy concerning the validity of species assigned to the genus *Glyptelmins* and numerous investigators have attempted to straighten out the systematics of this group. (MILLER, 24; FREITAS, 10; RANKIN, 29; RUIZ, 30; BYRD, 4; BABERO, 1; CHENG, 7; BYRD and MAPLES, 5; NASIR, 27; TRAVASSOS *et al.*, 38; YAMAGUTI, 44). Thus, members of *Glyptelmins* have been variously assigned to different genera — *Margeana* Cort, 1919; *Microderma* Mehra, 1931; *Choledocystus* Pereira and Choccola, 1941; *Rauschiella* Babero, 1951; *Reynoldstrema* Cheng, 1959; *Repandum* Byrd and Maples, 1963.

BABERO (2) suggested that the genus *Glyptelmins* could be divisible into two groups: "a.) species with peripharyngeal glands and without uterine coils developed in the pretesticular zone; b.) species without peripharyngeal glands and with uterine coils developed in the pretesticular zone." CHENG (7) pointed out that since *Margeana* was described as a new genus because of the absence of peripharyngeal glands that the erection of another genus as suggested was not necessary. He, therefore, proposed the reestablishment of *Margeana* as a valid genus. MILLER (24) synonymized this genus with *Glyptelmins*. Subsequently, eleven species previously assigned to *Glyptelmins* were removed to *Margeana*, and *G. quieta* (Stafford, 1900), *G. subtropica* Harwood, 1932, and *G. festina* Cordero, 1944, were retained. *G. palmipedis* (Lutz, 1928) was considered a synonym of *M. linguatula* (Rud., 1819).

BABERO (2) created the genus *Rauschiella* for a trematode species from a Mexican frog. Because of certain morphological similarities with the genotype *R. tineri*, *G. repandum* (Rud., 1819) was transferred to *Rauschiella*. In assigning the latter species to *Margeana*, Cheng was unaware of the erection of *Rauschiella* and the new position of *repandum*. Also, apparently unaware of the erection of *Rauschiella* and the new position of *repandum*, BYRD and MAPLES (5) established *Repandum* n.g., with *Repandum repandum* as genotype. These investigators also rejected the separation of *Glyptelmins* into *Glyptelmins* and *Margeana* for species described as lacking peripharyngeal glands, stating that these glands seem to depend heavily on the method used in handling and staining whole mounted worms. Nasir studied both whole mounts and sectioned specimens, and observed deeply stained structures in the peripharyngeal region. However, he felt that this character was too difficult to be worked out with certainty and, therefore, stated that it did not provide a sound basis for generic separation. TRAVASSOS *et al.* (38) without discussion or reference to Cheng's division, retained most species of *Glyptelmins* previously assigned to the genus. *Glyptelmins elegans* Travassos, 1926 was transferred to the genus *Choledocystus*. YAMAGUTI (44) listed twenty-three species as having been assigned to *Glypt-*

belmins and the genera *Margeana*, *Choledocystus* and *Repandum* were considered by him to be synonyms of *Glypthelmins*. *Choledocystus*, however, is considered by numerous investigators as a valid genus. *Glypthelmins ramitesticularis* Nasir, 1966 obviously does not belong in this genus. The highly branched testes and the extensive extracecal disposition of the uterus are characters not consistent with other members of the genus. While it is true that sometimes the peripharyngeal glands are not distinctly stained, their presence usually is indicated by their faint appearance. However, in view of the general consensus concerning the presence or absence of peripharyngeal glands as a character for generic separation, the writers will employ the name *Glypthelmins* for the following flukes obtained from the intestine of bullfrogs.

Glypthelmins quieta (Stafford 1900)
(Fig. 9)

Glypthelmins quieta was the most prevalent trematode collected in this study, being recovered from the small intestines of 32 bullfrogs. Infections ranged from 1 to 97 specimens per host. Both mature and immature worms were frequently collected from the same host. The morphology of the species fitted the redescription as presented by MILLER (24) and CHENG (7) *Distomum quietum* Stafford, 1900 was originally described by STAFFORD (33); he later (34) removed the species to *Glypthelmins*, stating that it occurs in frogs — *Rana catesbiana*, *R. virescens*, and *Hyla pickeeringii*. *G. quieta* has since been found to be a common trematode of frogs and has been reported numerous times from the bullfrog, including reports by MANTER (23), RANKIN (29) and WALTON (40). The life cycle of the species has been reported by RANKIN (29) and LEIGH (19, 20).

Glypthelmins subtropica Harwood, 1932

This species was also a common trematode of bullfrogs. It is easily confused with *G. quieta*, since both have peripharyngeal glands. In fact MANTER (23), RANKIN (29) and NASIR (27) considered the species to be synonymous with *G. quieta*. However, Cheng felt "that the distinct pattern of the vitellaria of *G. subtropica*, plus its reduction to approximately half the size of *G. quieta*, are of sufficient significance to separate it from the genotype." Employing the character cited by Cheng and by HARWOOD (12) relative to the vitellaria, species of *G. subtropica* were uncertainly identified. Eight frogs harbored this parasite, with the number per host ranging from 2 to 12.

Glypthelmins proximus Freitas, 1941

Five slides containing specimens of this species were identified by Dr. Pir Nasir, Oriente University, Venezuela. This species lacks peripharyngeal glands and uterine coils developed in the pretesticular zone and, therefore, was

placed by Cheng in the genus *Margeana*. However, the present writers for the reason already explained, are regarding the species as a member of *Glypthelmins*.

Glypthelmins sera Cordero, 1944

According to Cheng's redescription, *M. sera* (= *G. sera*) is primarily identifiable by its oval-elongated body, intestinal ceca reaching to one-third of the body and by its extracecal vitellaria, which consist of grouped follicles extending from the level of the ovary to near the tip of the ceca. It also has testes which are obliquely arranged. Nasir (personal communication) identified as *G. sera* worms on a single slide sent to him by the writers. Apparently, these specimens were the only ones collected. In making his identification, apparently Nasir no longer feels *G. sera* to be a synonym of *G. linguatula* as previously stated (27).

Haematoloechus LOOSS, 1899

Members of the genus *Haematoloechus* are not uncommon parasites in the lungs of bullfrogs. Accounts of the life cycle of several members of this group are presented by KRULL (18). YAMAGUTI (41) lists five species of *Haematoloechus* as occurring in *Rana catesbiana* — *H. variegatus* (Rud., 1819), *H. breviplexus* Stafford, 1902, *H. complexus* (Seeley, 1906), *H. floedae* Harwood, 1932 and *H. longiplexus* Stafford, 1902. Additionally, WALTON (40) lists *H. parviplexus* (Irwin, 1929). In the present study, members of the genus comprising three species have been collected nine times, with the number of specimens in a single host ranging from 1 to 17.

Haematoloechus buttensis Ingles, 1936

(Fig. 10)

Flukes assigned to this species were collected four times, both mature and immature specimens being recovered. *H. buttensis* was originally described from *R. boyli* in California by INGLES (14). The Nevada specimens, in addition to showing resemblance to *H. buttensis*, also showed similarities to *H. kernensis* and *H. oxyorchis* as described by INGLES (13). However, organ sizes did not concur with either of these latter species. The Nevada specimens also resembled to some extent *H. floedae* as described by HARWOOD (12), however, they differed from this fluke by the presence of a "distinct visible collar" that was described by INGLES (13) for the species. Although spines appeared to be absent from the Nevada specimens and the average sizes of organs did not quite agree with those given by Ingles for *H. buttensis*, organ size did fall within the ranges given. Nevada bullfrogs, therefore, constitute a new locality and host record for *H. buttensis*.

Haematoloechus breviflexus Stafford, 1902
(Fig. 11)

H. breviflexus is one of the largest lung flukes occurring in North American frogs. From Nevada frogs it was collected five times. The morphology of the specimens fitted the description as presented by CORT (8). The worms were readily identified by their large size and distinctly lobed ovary and testes. They showed some resemblance to *H. longiflexus* but could be separated from it by the more pronounced lobations of the ovary and testes, as well as by the contour of the longitudinal folds of the uterus. The life history of *H. breviflexus* is given by SCHELL (32), who states that dragonflies (*Aeshno multicolor*) pass the infections on to frogs.

Haematoloechus parviflexus (Irwin, 1939)
(Fig. 12)

A single specimen of this species was collected from a lung of a bullfrog. The specimen was not readily identified and was initially thought to be *H. buttensis*, however, the lobed nature of the ovary showed the fluke to be different, and as to morphology, measurements, and disposition of organs it agrees closely with the original description and diagram as given by IRWIN (15). Apparently, there are no other reports of this species in bullfrogs. A detailed description of the life cycle of *H. parviflexus* is given by KRULL (18).

CESTODA

Ophiotaenia LaRue, 1911
Ophiotaenia magna Hannum, 1925
(Figs. 13-15)

The genus *Ophiotaenia* infects fishes, amphibians and reptiles. YAMAGUTI (42) lists only two species as infecting bullfrogs, *O. magna* and *O. gracilis* Jones, Cheng and Gillespie, 1958. Six Nevada bullfrogs harbored adult cestodes in their small intestines, with a range of infection per host of 1 to 3 worms. Morphological study of these tapeworms showed that they fitted the description of *O. magna* as presented by HANNUM (11).

THOMAS (37) in his discussion of *O. perspicua* LaRue, 1911 stated that the first intermediate host for the species is *Cyclops vulgaris*, although other copepods, such as *Cyclops viridis*, *C. prasinus* and *Mesocyclops obsoletus* will take the infection. In these latter hosts, however, growth, according to Thomas, is much slower. Thomas also states that tadpoles, frogs and mudminnows (*Umbra limi*) may also serve as the second intermediate host, passing the infections on to snakes (*Thamnophis sirtalis* and *Natrix sipedon*). He states that the plerocercoids in tadpoles continue to grow with the metamorphosis of

the animals into frogs and may break out into the coelom, and immature segments may form. In the case of *O. saphena* Osler, 1931, THOMAS (36) observed Copepoda, Cladocera, and aquatic beetles, as well as snails, in the intestinal debris of frogs and suggested that these amphibia can become directly infected by the accidental eating of infected *Cyclops*. Although no copepods were examined from the Ash Meadows area from which bullfrogs were collected, they were observed to be present. The life-cycle of *O. magna* parallels that of *O. saphena* in that bullfrogs may become directly infected by the consumption of copepods.

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RESUMEN

En un estudio de los parásitos de las ranas, llevado a cabo en Ash Meadows, Estado de Nevada, EE.UU., colectamos doce especies de helmintos comprendidos en siete géneros, como sigue: (Nematoda) - *Eustrongylides wenrichi*, *Spironoura catesbeianae* y *Abbreviata* sp.; (Trematoda) - *Langeronia provitellaria*, *Glythelmins quieta*, *G. subtropica*, *G. proximus*, *G. sera*, *Haemaloechus buttensis*, *H. breviplexus* y *H. parviplexus*; (Cestoda) - *Ophiotaenia magna*. Se hace un comentario breve de cada uno de estos parásitos. *Eustrongylides wenrichi* es el helminto que se encontró con más frecuencia en ranas en el área de estudio, y enquistado en los músculos, vísceras y huevos causa alteraciones patogénicas graves.

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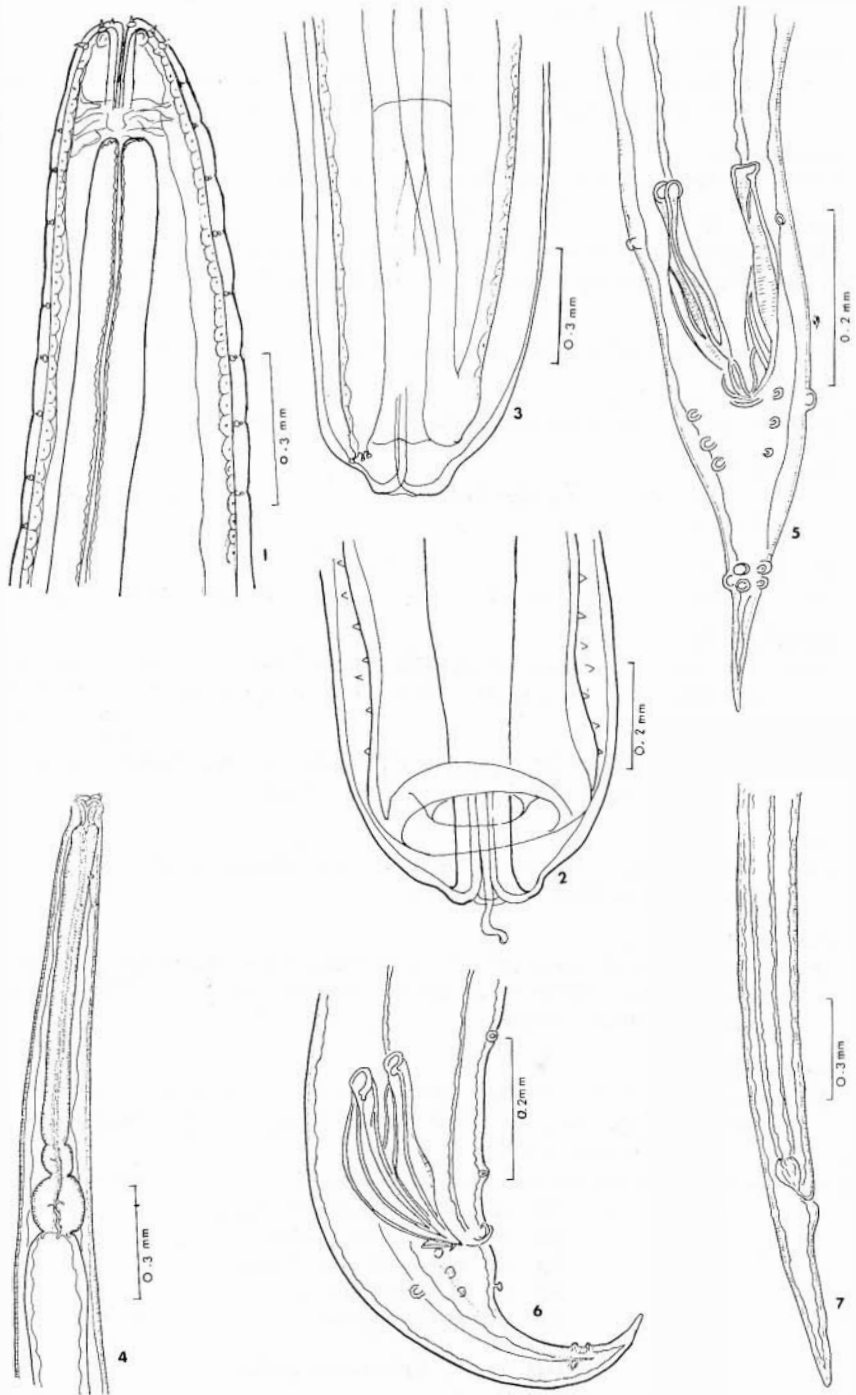
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Figs. 1 - 3. *Eustrongylides wenrichi*

- Fig. 1. Anterior end (lateral view, male)
- Fig. 2. Posterior end (lateral view, male)
- Fig. 3. Posterior end (lateral view, female)

Figs. 4 - 7. *Spironoma catesbiana*

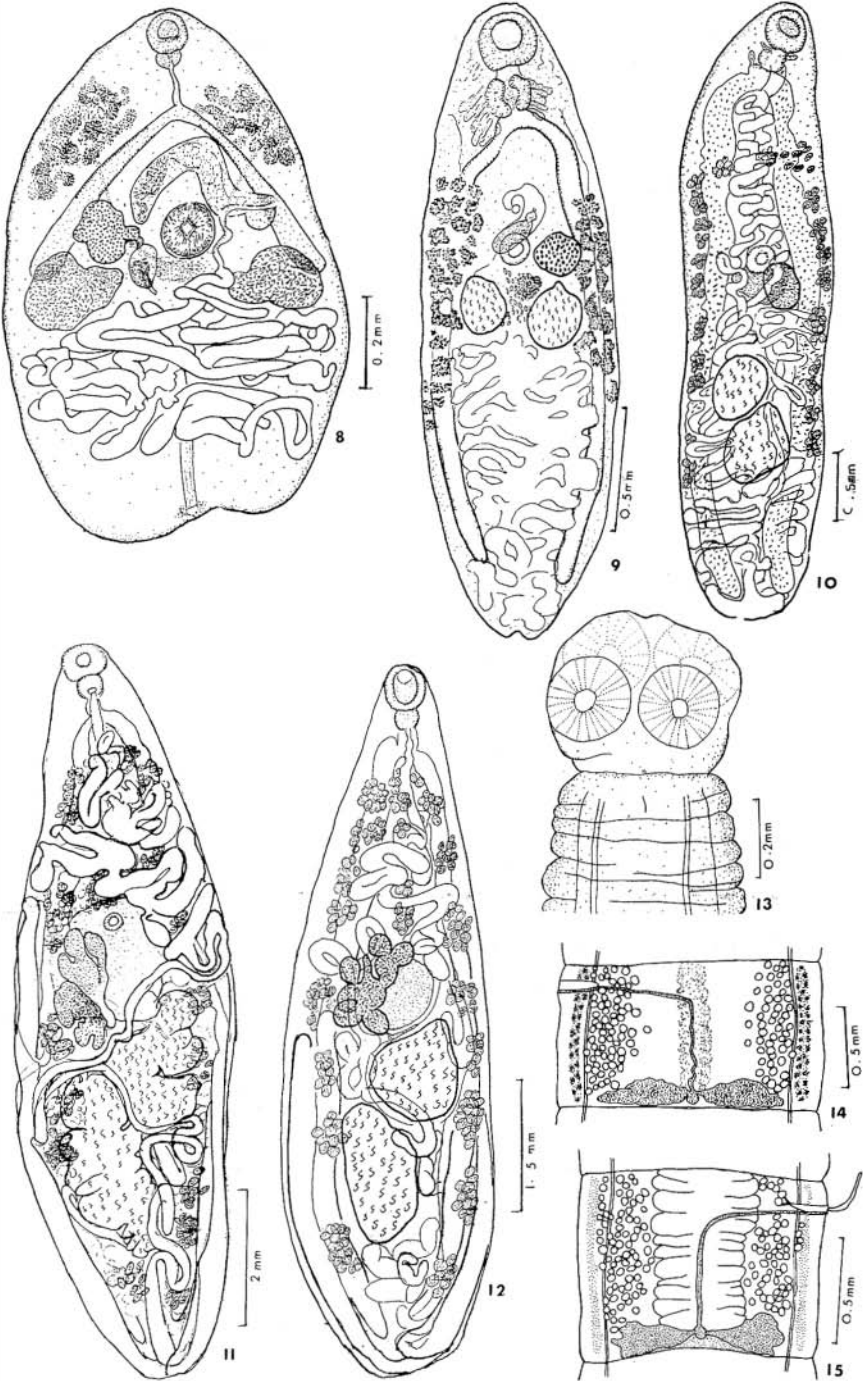
- Fig. 4. Anterior end (lateral view, male)
- Fig. 5. Posterior end (ventral view, male)
- Fig. 6. Posterior end (lateral view, male)
- Fig. 7. Posterior end (lateral view, female)



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Fig. 8. *Langeronia provitellaria*
 Fig. 9. *Glyptelminis quieta*
 Fig. 10. *Haematoloechus buttensis*
 Fig. 11. *H. breviplexus*
 Fig. 12. *H. parviplexus*

Figs. 13-15 *Ophiotaeonia magna*
 Fig. 13. Scolex
 Fig. 14. Mature proglottid
 Fig. 15. Gravid proglottid



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