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of Nebraska at Lincoln; Smithsonian Institution, United States National Museum, Washington, D. C.; Museum of Zoology, University of Wisconsin at Madison; Museum of Zoology, Coe College, Cedar Rapids, Iowa.

The remainder of the specimens remain in the author's collection.

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Observations on the Morphology and Life History of *Oswaldocruzia sp.* in Frogs[1]

B. T. Ridgeway[2]

Abstract. A series of experiments were instituted in order to clarify the life history of *Oswaldocruzia sp.*, a nematode of frogs. Suitable hosts were collected and examined for the nematode. Artificial infection of laboratory reared frog tadpoles and young frogs by use of incubated juvenile stages of the parasite was undertaken with negative results. Several reasons are suggested to explain failure of infection attempts. The likelihood that juvenile *Oswaldocruzia* used experimentally were not infective stages of the parasite is suggested. Morphological characters of the worms support this idea. The possibility that tadpoles and young frogs are refractory to infection, and involvement of an intermediate is discussed.

Morphology of adult and juvenile *Oswaldocruzia* is outlined, and comparisons to recorded descriptions are made.

The nematode, *Oswaldocruzia* Travasos, 1917, an intestinal parasite of amphibians, is frequently found in frogs of Emmet and Cheboygan counties, Michigan. Little information is available concerning the life history of nematodes of this genus save that concerning *O. filiformis* (Maupas and Seurat, 1913), a European species.

During the summer of 1962 adult *Oswaldocruzia* were obtained from frogs collected in the vicinity of Douglas Lake, Michigan, and attempts were made to clarify the life history of these parasites. Morphological studies of both adult and juvenile *Oswaldocruzia* were also carried out.

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Materials and Methods

Of 98 frogs collected and examined 68 were found to harbor adult *Oswaldocruzia*. Of the three species of frogs *Rana clamitans*, *R. catesbeiana* and *R. pipiens*, the last appeared to be most heavily parasitized.

Nematodes were removed from the intestinal tracts of parasitized frogs and transferred to saline. Adult *Oswaldocruzia* remained active for as long as four weeks when stored in refrigerated saline.

Morphological studies were carried out by observing living adults and juveniles as well as preserved specimens either as whole mounts or teased tissues. Measurements of worms was accomplished either by use of an ocular micrometer or calibrated camera lucida drawings.

Lacto-phenol was discovered to be the most effective medium in which to critically examine the worms. It was possible to transfer nematodes from water or alcohol directly to lacto-phenol. Clearing was accomplished within five minutes and the treated worms were rendered pliable so that they could be rotated to any desired position. After examination, specimens were washed in 70% alcohol containing a trace of lithium carbonate and then stored in 70% alcohol.

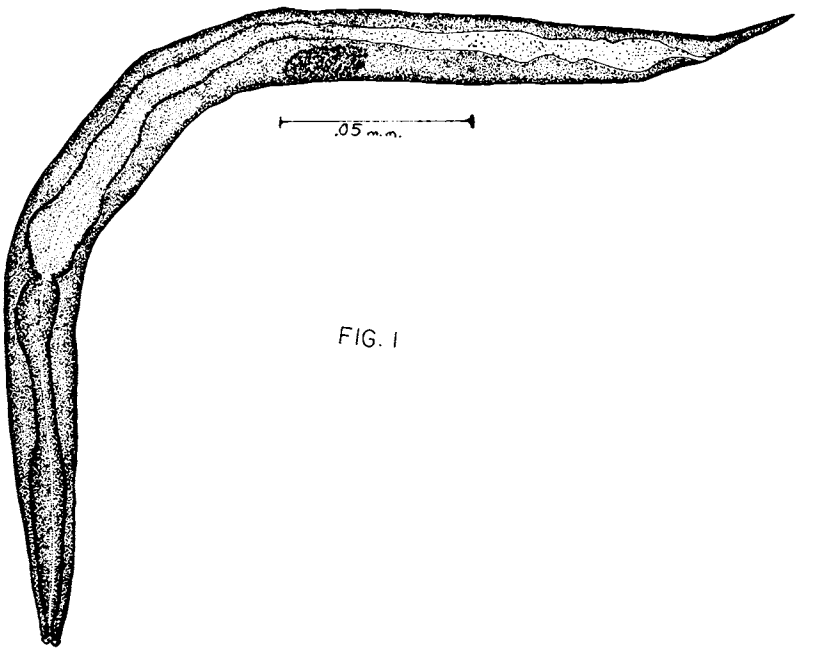


FIG. 1

Fixation was accomplished by immersing worms in hot 70% alcohol or A.F.A. Worms killed in these fixatives were found to undergo only slight shrinkage and to maintain a relatively straight position.

Nematodes to be mounted in glycerine jelly were placed in a mixture of alcohol-glycerine, subsequently dessicated in a warm (37° C) oven, and finally mounted in glycerine jelly.

Individual methods and their applications associated with life cycle studies will be considered where applicable.

Results

Descriptions are based upon observation of 15 adult *Oswaldocruzia* of each sex selected at random from those available for study.

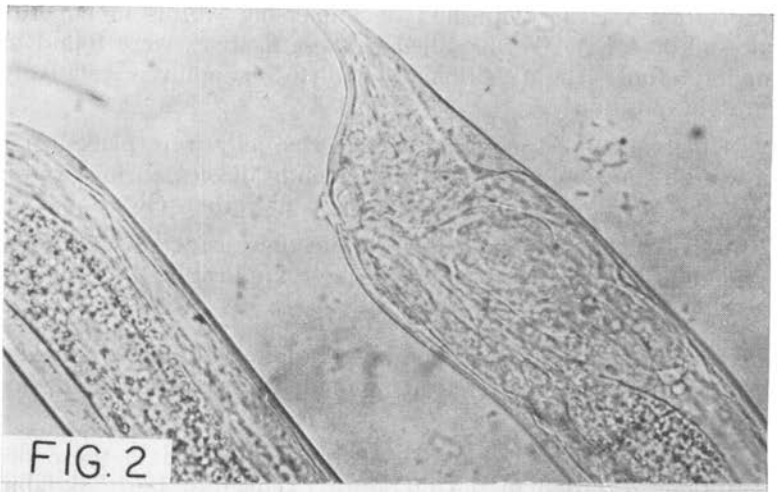
In general male and female worms conformed to those descriptions recorded in the literature (Yamaguti, 1961; Skrjabin et al., 1954), being slender, filiform, colorless, semi-transparent and somewhat narrowed anteriorly.

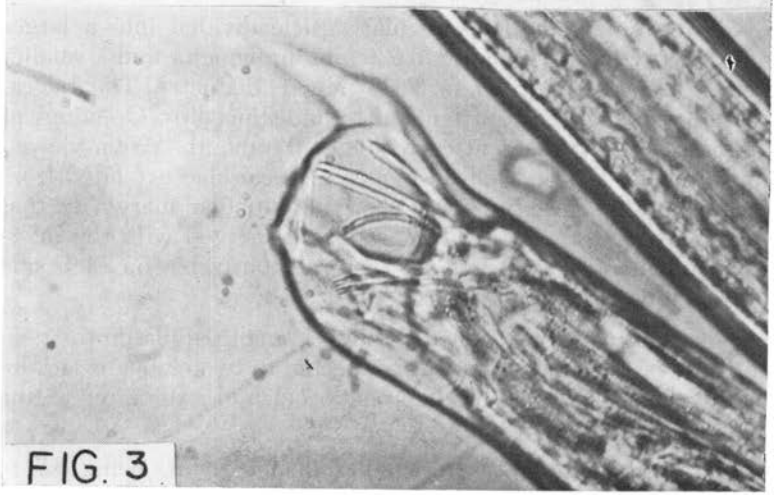
The head possessed a cuticular vesicle divided into a larger anterior portion 0.046 mm to 0.036 mm in diameter and a smaller posterior part 0.042 mm to 0.033 mm in diameter. The buccal cavity was small and surrounded by indistinct lips. Openings of amphids could be seen at each side of the mouth. When viewed in the dorsal aspect, well developed cervical alae extended from just below the posterior margin or the cuticular margin to that region where the club-shaped esophagus merged with the intestine. A pair of elongate glands were demonstrable on each side of the esophagus.

The excretory pore opened through a small papilla on the ventral side of the worm, and was connected to two elongate sacs located along the sides of the pseudocoel and extending to the middle third of the body.

Males of the genus were smaller than females, being 6.7 to 4.2 mm long and 0.091 to 0.063 mm wide at the middle of the body. The excretory pore was 0.180 to 0.24 mm from the anterior end. The bursa and supporting muscular rays were similar to those descriptions recorded for the genus. The spicules were 0.16 mm in length, complex in shape, and terminated in three processes united by a hyaline membrane.

Juvenile males exhibiting two developmental stages of the bursa were found in several frogs. As seen in what was considered to be the younger stage, the bursa was observed as a pair of ventrolateral swellings on each side of the anus (Fig. 2). These swellings seemed to include both the epidermis and the underly-





ing muscle layer. Since an enveloping sheath was present, these were considered to represent fourth-stage, post-infection larvae. An apparent fifth-stage larva (Lucker, 1938), still ensheathed but having a bursa with a muscular ray pattern characteristic of the genus, is illustrated in Figure 3. The hosts of these particular juveniles were very young *Rana pipiens*.

Females of *Oswaldocruzia* were 16.9 to 10.9 mm long, and 0.191 to 0.144 mm wide in the area of the vulva with the vulva located in the posterior half of the body, 9.8 to 6.8 mm from the anterior end. The excretory pore opened 0.34 to 0.28 mm from the tip of

the head, and the nerve ring, when seen, was 0.225 to 0.202 mm from the tip. The female reproductive system was amphidelphyic. Eighteen to 25 eggs in various stages of development could be seen in each uterus except in those females in which hatched juveniles in the uterus were observed. No seminal receptacle was present, but sperm were concentrated at the end of the uterus most distal to the ovijector.

Life History Observations

Attempts to elucidate the life history of *Oswaldocruzia* in volved the following:

1. Incubation and hatching of eggs.
2. Experimental infection of laboratory reared tadpoles and young frogs using recently hatched juveniles.
3. Examination of these tadpoles and frogs for developing worms.

Adult female *Oswaldocruzia* were placed in stender dishes of filtered lake water. Following egg deposition, the worms were removed to saline. Microscopic examination of gravid females revealed that blastomeric development began in the distal end of the uterus, close to the oviduct. The eggshell was thin and lacked an operculum, with hyaline areas being clearly defined at each pole. The lengths of fifteen eggs of different females averaged 0.081 mm with a range from 0.079 to 0.084 mm. The width of these eggs averaged .043 mm, ranging from 0.040 to 0.046 mm. Generally, eggs were deposited in the eight- to sixteen-cell stage and in groups of five or six. At times eggs were deposited with worms already in the tadpole stage. Also, hatched juveniles were observed within the uterus of some females.

Eggs deposited by a single female were examined every two hours from the time of deposition (in the 16-cell stage) to hatching. Progression of development was noted so that the length of the incubation at room temperature could be determined.

The stages of development were: late gastrula at four hours, late tadpole at eight hours with embryonic movement. Hatching had begun by the fourteenth hour. Hatching continued for the following eight hours with some eggs failing to hatch. Several eggs failed to develop further than initial segmentation. Interestingly, eggs deposited on the same day by different females hatched at different times, even though maintained under identical conditions.

No particular point in the egg shell was detected through which the juveniles emerged, and a well defined mechanism for exit was lacking. Sheaths were not seen in these recently hatched *juveniles* although Hyman (1951) stated that larvae of *Oswaldo-*

cruzia hatch as a third stage infective larvae. Although morphological details are not always clearly defined at high magnifications, both mouth and anus appeared functional. The shape of the esophagus differed from that generally found in infective filariform larvae (Figure 1). This structure is slightly more than a third of the intestinal length and possesses a definite posterior bulb with an elongate anterior enlargement, although not as well defined. A well defined buccal capsule was observed. In several juveniles a group of cells were observed in the posterior third of the body, ventral to the gut .These cells were considered to be genital primordia. Measurements of 15 recently hatched juveniles were made and recorded: length, 0.292 to 0.304 mm; width at midbody, 0.018 to 0.022 mm; length of esophagus, 0.097 to 0.112 mm; length of buccal capsule, 0.009 to 0.012 mm; anus to tip of tail, 0.052 mm.

To follow the development of worms in frog tadpoles, 40 (each) recently hatched juvenile parasites were placed in stender dishes containing filtered lake water. A laboratory-reared frog tadpole was placed in one of the stender dishes and observed to ingest the larva present. A total of 10 tadpoles were so treated. On the sixteenth day following ingestion and each day thereafter, one animal was killed and examined for developing *Oswaldocruzia*. Gut tissues were pressed between microscope slides and scanned for stages of the parasite that might have been imbedded in the mucosa. No larva or developing worms were observed. The experiment was repeated by use of a group of 10 frog tadpoles fed the parasite orally by pipette and to another group given a suspension of parasitic larvae by subcutaneous injection. In both instances the results were negative.

Ten young frogs (*R. pipiens*) were infected *per os* with 20 larvae each and examined for worms at the end of a five-day period. One frog was sacrificed each day. No larvae or adult parasites were recovered. The frogs, while not laboratory-reared, were taken from a group of 30, 10 of which had been autopsied previously and found free of *Oswaldocruzia*. The 10 remaining frogs served as controls, and, when examined at the completion of the experiment, had no parasites. Attempts to induce larvae to penetrate the skin of the host were not successful.

Discussion

Several reasons may be suggested to explain failure of juvenile *Oswaldocruzia* to infect tadpoles and young frogs. Possibly, the juveniles used to infect the frogs and tadpoles were not the infective stages of the parasite. This possibility is supported by morphological characters already outlined. The rhabditiform (strongyliform) esophagus, functional gut, and lack of a sheath

suggest that the juveniles were either first- or second-stage larvae rather than infective third-stage larvae. The failure to observe further development of nematodes maintained in the laboratory may have been due to the absence of some factor normally present in the extra-host environment. A second consideration may be that the tadpoles and frogs used in this study were not receptive to invasion of the parasite. It is possible that the hosts only become infected during a very limited period of their lives. It is unlikely that the life cycle of *Oswaldocruzia* includes an intermediate host since the known life histories of the family Trichostrongylidae are most often direct.

In order to substantiate the suggestion that tadpoles and young frogs are not receptive to infection, 50 tadpoles were collected from an area where frogs were known to harbor heavy infections of *Oswaldocruzia.* These were divided into two groups, one of which was examined at once for parasites. The other group was placed in an aquarium and allowed to complete metamorphosis. The initial group was free of *Oswaldocruzia* infection. Additional tadpoles collected from the same site were autopsied with negative results. It was apparent that infection occurred at some time after metamorphosis. This idea was further strengthened following examination of 15 frogs that completed development in the laboratory. Not one was positive for the parasite; on the other hand, frogs of the same apparent age collected from the site in question were infected.

Summary

Frogs of the genus *Rana* were collected and examined for the nematode parasite *Oswaldocruzia sp.* The morphology of recovered worms was compared with descriptions in the literature. An attempt was made to clarify the life history of the parasite by artificial infection of laboratory-reared frog tadpoles and young frogs with juvenile stages of the parasite. All attempts at infection were negative, and it is assumed that the juvenile stage used is not the infective stage, or that tadpoles and young frogs are refractory to infection.

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