HOSTS: *Erolia alpina*, *E. maritima*, *Arenaria interpres*, *Calidris canutus*.

LOCALITY: Woods Hole, Massachusetts, and Sapelo Island, Georgia.

SITE OF INFECTION: Intestinal ceca.

Holotype (No. 72000) and paratypes (Nos. 72001 and 72002) in USNM Helm. Coll., Beltsville, Maryland.

Literature Cited


Life Cycle of *Phyllodistomum bufonis* (Digenea: Gorgoderidae) from the Boreal Toad, *Bufo boreas*

JOHN E. UBELAKER* and O. WILFORD OLSEN

Department of Biology, Southern Methodist University, Dallas, Texas, and Department of Biology, Colorado State University, Fort Collins, Colorado

ABSTRACT: The life cycle of *Phyllodistomum bufonis* is described and illustrated. Hosts include fingernail clams (*Pisidium adamsii*), naiads of dragonflies (*Libidula sp.*), and toads (*Bufo b. boreas*). Experimental infections performed by feeding progenetic metacercariae to various amphibians and fish indicated that only *B. boreas* could serve as the definitive host. The formation of testes from nine primordia appears to characterize the genus *Gorgoderina* whereas the development of the testes from a single primordium is distinctive of *Phyllodistomum*.

The validity of the trematode genera *Gorgoderina* Looss, 1902, and *Phyllodistomum* Braun, 1899, has been questioned by various authors since Osborn (1903) described a transitional species, *P. americanum*, from *Ambystoma tigrinum* in North America and noted its similarity to *C. translucida* Stafford, 1902, from *Bufo lentiginosus* and *Rana virescens* (probably *B. americanus* and *R. pipiens*). Goodchild (1943) listed the various authors who have commented on the identity of these trematode genera.

Crawford (1939, 1940) published brief accounts of the life cycle of *P. americanum* based on specimens collected in Colorado from the boreal toad, *B. boreas* Baird and Girard, 1852, and the tiger salamander, *A. tigrinum* (Green, 1825). Adult flukes passed eggs containing...
mircidia which upon hatching penetrated *Pisidium* sp. Cystocercous cercariae, shed by the bivalve, encysted in the esophagus when eaten by trichopteran larvae, diving beetles, or by naiads of damselflies. Juvenile flukes in *Bufo* migrated first to the kidneys where they remained for 2 weeks before proceeding to the urinary bladder where they matured in approximately 5 weeks.

Frandsen (1957) described *P. buonis* from *B. boreas* in Utah and distinguished this species from *P. americanaus* "by its different sucker-size ratio, the significantly smaller size of its capsules, and by the fact that *P. americanaus* has a slight posterior notch." Tonn (1950, 1961) found morphological variation in *P. buonis* from *B. boreas* in Colorado sufficiently great to include all species described for the genus.

Since Crawford (loc. cit.) did not publish detailed observations on the material that he studied and may have confused two species, the present study was undertaken to determine the morphology of the larval stages and the specific identity of the *Phylodistomum* from *B. boreas* in Colorado.

**Materials and Methods**

Mature *Phylodistomum* were obtained from *Bufo boreas* collected at Trapp Lake, Larimer Co., Colorado. Fingernail clams, *Pisidium adamsi* Prime, collected at the same locality, shed cercariae of the *Phylodistomum* sp. in the toad.

Parasite-free clams were raised in stender dishes at room temperature for several weeks before being exposed to miracidia. Monomircidial races of *Phylodistomum* were established and development of the larval stages studied. Naiads of dragonflies, *Libellula* sp., were collected from small pools lacking fish or amphibia and allowed to eat cercariae from experimentally infected clams. Metacercariae obtained by dissection of the naiads were fed by stomach tube to numerous hosts: *B. boreas* from Trapp Lake; *B. woodhousei woodhousei* Girard, 1854, from gravel pits 4 miles east of Windsor Reservoir; *Rana pipiens brachycephala* Cope, 1889, from tree dump, north edge of Fort Collins; *Ambystoma tigrinum mavortium* Baird, 1850, from tree dump, north edge of Fort Collins; *B. woodhousei woodhousei* Girard, 1854, from gravel pits 4 miles east of Windsor Reservoir; and a topminnow, *Fundulus sciadicus* Cope, 1865, from the tree dump. *B. woodhousei*, *Bufo boreas*, and *F. sciadicus* were reared from eggs or fry and maintained free of trematodes. Other amphibians were examined in the laboratory for helminths before being used in experiments.

Adult trematodes were killed in warm AFA, stained in Grenacher's alum carmine, cleared in beechwood creosote, and mounted in Piccolyte before measuring. Measurements are based on five specimens from each of 10 toads. All measurements are in millimeters unless otherwise indicated. Drawings were made with the aid of a camera lucida, microprojector, and by tracing projected photomicrographs.

**Results**

**Egg and miracidia (Figs. 1, 2)**

Eggs freshly deposited, 24.0 to 35.3 μ long by 18.4 to 23.1 μ wide, ovoid, nonoperculated, increasing in size while in utero to accommodate developing miracidia; eggs in metratrem often with dark shell and resistant to immediate hatching in water.

Miracidia fully developed in eggs when laid. Hatching occurs within minutes after eggs are placed in water. Body mucrocuneate in shape, often assumes pyriform shape when swimming, 0.053-0.067 long by 0.030-0.042 wide. Fifteen ciliated epidermal plates present, arranged in three transverse rows with six plates in each of the first two rows and three in the last row. Apical gland in anterior region of body, 0.012 to 0.010 long by 0.029 to 0.021 wide, opens in a small apical pore. Large asymmetrical gland of Goodchild (1943) opens in space at anterior end of body; smaller homologue gland of Goodchild present in miracidia younger than 12 hr, opens similar to asymmetrical gland. Two flame cells present near middle of body, ducts open on lateral margins just anterior to last row of epidermal plates. Four germ cells present in encapsulated miracidia.

Wotton and Peters (1957) listed 15 epidermal plates for miracidia of *Gorgoderina attenuata*, *P. superbum*, *P. staffordi*, and *P. undulans*, all parasites of fishes as adults except *G. attenuata*. Goodchild (1943) reported that 16 plates were present on miracidia of *P. solidum*.

---

*Post and Potts (1966) report that *Rana pipiens* of Fort Collins may differ from those in southern Colorado.*
and Schell (1967) found 18 plates on *P. staffordi*. The morphology of miracidia of *P. bufonis* are similar to those of other gorgoderids having a 6, 6, 3 pattern of epidermal plates.

**Mother sporocyst (Fig. 3)**

Development of the mother sporocyst occurs within the gill lamella of *Pisidium adamsi* as described by Goodchild (1943) for *P. solidum* and Schell (1957) for *P. staffordi*. After 36 hr, the central cavity can be differentiated from the wall of the sporocyst and by 70 hr the cells with granular cytoplasm appear (Fig. 3). Embryos of daughter sporocysts are present as early as 80 hr after infection. Sixteen days after infection, the sporocyst moves to the inner surfaces of the gill lamellae. Twenty-one days after infection, mother sporocysts 0.45 long split, releasing the daughter sporocysts.

**Daughter sporocysts (Fig. 4)**

Daughter sporocysts are located in the gills anchored by the end with the birth pore with the opposite end extending into the interlamellar gill space. Sporocyst body tubular, 1.00 to 1.96 long by 0.55 to 0.70 wide; sporocyst wall generally wrinkled, thinner than wall of mother sporocyst, and contains similar granules. Birth pore subterminal on anterior end. Body cavity with 10 to 12 developing cercariae. Cercariae shed 38 to 48 days after initial exposure to miracidia.

**Cercariae (Figs. 5–7)**

Cercariae of *P. bufonis* are macrocercous, tail 1.46 to 1.93 by 0.14 to 0.19; cercarial chamber 0.14 to 0.16 by 0.11 to 0.13, neck 0.01 to 0.04 in length; anterior portion of tail as wide as chamber, diminishes gradually in width posteriorly, ending bluntly.

Body of cercaria fusiform, emerges readily from chamber under slight pressure, 0.21 to 0.25 by 0.09 to 0.11. Cuticle with fine striations and grooves. Stylet robust, 0.018 to 0.025 by 0.003 to 0.004. Oral sucker 0.040 to 0.055 by 0.054 to 0.060; acetabulum 0.041 to 0.070 by 0.062 to 0.082. Twelve unicellular penetration glands present, six per side, located dorsolateral to acetabulum, open in two pores on each side of stylet. Esophagus bifurcates just anterior to acetabulum into oeca which extend to posterior margin of the body. Excretory pore terminal; bladder extends to posterior margin of acetabulum, receives two main collecting ducts anteriad (subterminally), ducts extend to near posterior border of oral sucker before dividing. Flame cell pattern 2 [(4 + 4) + (4 + 4 + 4 + 4)] = 48; bladder surrounded by cystogenous cells. Genital primordium immediately posterior to acetabulum and ventral to the anterior end of excretory bladder, 0.008 to 0.012 by 0.03 to 0.04.

**Remarks**

Cercariae of *P. bufonis* leave via the birth pore after rupturing the thin host membrane surrounding the sporocyst are free in the interlamellar space and epibranchial cavity eventually to emerge through the excurrent siphon during day or night. The cercariae and sporocysts appear to do little physical damage to the host. Sporocysts, however, inhibit reproduction. Infected clams from experimental or natural infections never contained young clams.

The cercaria resembles other gorgoderid cercariae that are macrocercous, possess stylets, and have 12 penetration glands. The wide anterior portion of the tail is similar to *Cercaria conica* Goodchild, 1939; however, the remainder of the tail is not set off as distinctly and the stylet shape is different.

**Metacercaria (Fig. 8)**

Macrocercous cercariae liberated from the clam adhere to the substratum where they contract and extend vigorously for several hours. Naiads of dragonflies, *Libellula* spp., readily ate the cercariae which penetrated the intestinal wall and encysted in the hemocoel by means of secretions from the cystogenous glands as described by Sinitsin (1905), Goodchild (1943), and Thomas (1958). Metacercariae were usually found in the hemocoel near the posterior end of the body. Two-day-old meta-

---

cercariae measure 0.16 to 0.20 in diameter. Older ones are slightly larger. Genital organs are differentiated. In the oldest metacercariae eggs are already present in the uterus.

This is the first report of a progenetic Phyllodistomum from the United States. Rai (1964) reported progenetic metacercariae of *P. sriwesta*ca occurred in *Macrobrachium dayanus*, a freshwater shrimp in India.

**Adult trematode (Figs. 9, 10)**

The adult trematode used in this study resembles *P. americanum* and *P. bufonis* with minor exceptions. Measurements given by Osborn (1903) and by Fransen (1957) reveal that *P. americanum* is smaller in size, the acetabulum is located more anteriorly, and the excretory bladder extends only to the posterior testis. Flame cell formula 2 \( (4 + 4) + (4 + 4 + 4 + 4) \) = 48 in *P. bufonis*.

Crawford (1940) reported that both *Bufo* and *Ambystoma* were infected with *P. americanum*. Tonn (1950) noted that *Rana p. piens*, *Ambystoma* spp., and *Pseudacris triseriata* collected with *B. boreas* were never infected with *Phyllodistomum*. To determine if various amphibians and fish could serve as experimental hosts, 10 metacercariae were fed to each of several hosts (Table 1). Only *B. boreas* became infected, whereas *Ambystoma* spp., *R. piens*, *B. woodhousei*, and *F. sciaticus* did not.

Goodchild (1943) was unable to infect *Triturus viridesans*, *R. p. piens*, *R. palustris*, *R. catesbeiana*, *R. clamitans*, *Micropterus dolomieu*, *Eupomotis gibbosus*, *Carassius auratus*, and *Cyprinus* sp. with metacercariae of *Phyllodistomum solidum* from *Desmognathus fuscus*.

**Table 1. Results of feeding amphibians and fish with 10 metacercariae of Phyllodistomum bufonis.**

<table>
<thead>
<tr>
<th>Hosts</th>
<th>No. specimens</th>
<th>Percentage infected after 48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambystoma tigrinum utahense</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Ambystoma tigrinum macortium</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td><em>Rana piens</em> (northern variety)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><em>Bufo woodhousei</em></td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td><em>Bufo boreas</em></td>
<td>34</td>
<td>97</td>
</tr>
<tr>
<td><em>Fundulus sciaticus</em></td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Rai (1964) reported that metacercariae of *P. sriwesta*ca fed to *Heteropneustus fossilis*, *Mystus cassis*, and *Rana limnocha*is excysted only in the first host.

Several *Phyllodistomum* spp., on the other hand, are reported to have many definitive hosts. Dawes (1956) believed that many of the species will become synonyms of *P. foliun* when additional knowledge is gained on the range, variability, and specificity of this group. Based on experimental evidence adult *P. bufonis* appear to be host-specific.

The validity of *Gorgoderina* and *Phyllodistomum* have been discussed by various authors. Dollfus (1958) discussed the systematics of the phyllodistomes and concluded that the shape of the body and the class of the host should serve to distinguish between *Gorgoderina* and *Phyllodistomum*.

The phyllodistomes have undergone evolutionary radiation in fish and the gorgoderids in amphibia. A few species of phyllodistomes are reported to occur in amphibia, including the transitional species *P. americanum* and *P. bufonis*. We believe the development of the testes to be a more reliable character, especially for transitional species, on the genetic level. In the genus *Gorgoderina*, testes form from a fusion of nine primordia or "anlagen" (Rankin, 1939). The anlagen each form distinct testes in the *Gorgoderina* but in *Phyllodistomum* only a single primordium is present (Goodchild, 1943; Rai, 1964). We can find no exception to this characteristic and propose its use particularly when other characters are in doubt. *P. bufonis* shows formation of testes from a single anlagen and is properly placed in the correct genus. We cannot agree with Pandé (1937), Kaw (1950), or Fransen (1957) who consider *Gorgoderina* to be a synonym of *Phyllodistomum* until species assigned to *Gorgoderina* are examined more critically and life cycles are elucidated.

Goodchild (1943) suggested that *G. schistorchis* Steelman, 1938, and *G. tenua* Rankin, 1937, should be included in the genus *Phyllodistomum* since they possess prominent uterine coils between the vitelline complex and the acetabulum. Since *P. bufonis* does possess prominent uterine coils between the vitelline complex and the acetabulum which are more highly developed in older and larger worms but
has testes developing in a phyllodistome fashion, we consider G. tenua and G. schistorchis as belonging to the genus Gorgoderina until additional information is available concerning testicular development.

Crawford (1939, 1940) reported that P. americanum was present in both salamanders and toads and that miracidia were obtained from flukes in these two hosts for life cycle studies; however, our failure to infect salamanders with metacercariae, presumably P. americanum, originating from toads indicates that perhaps Crawford was dealing with two species of flukes, the species in toads being P. bufonis, the species in salamanders being P. americanum. Since it is not known experimentally whether P. americanum can infect toads, no definite conclusions may be drawn concerning the exact identity of Crawford's material.

In the same manner, the experimental work presented herein does substantiate Frandsen's decision to name those flukes from B. boreas as a distinct species.

Literature Cited


United States National Museum Helminthological Collection

Dr. J. Ralph Lichtenfels has been appointed curator of the USNM Helminthological Collection. All correspondence relative to the collection should be addressed: Dr. J. Ralph Lichtenfels, National Animal Parasite Laboratory, Veterinary Sciences Research Division, USDA-ARS, Beltsville, Maryland 20705.