# The Morphology and Emergence Patterns of the Cercaria of *Allocreadium pseudotritoni* Rankin 1937 (Trematoda: Allocreadiidae)[1,2]

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**ABSTRACT.** Cercariae of *Allocreadium pseudotritoni,* a trematode parasite of salamanders, develop in the sphaeriid clam, *Pisidium casertanum,* and have the eyespots and stylet (ophthalmoxiphidiocercaria) characteristic of this larval stage in the genus *Allocreadium. Allocreadium pseudotritoni* cercariae are further characterized by a flame cell formula 2[(4 + 4 + 4) + (4 + 4 + 4)] = 48, three pairs of penetration glands, cystogenous glands, and a slender unadorned tail. Maximum cercarial emergence occurs within three hours of the onset of light with large numbers from individual clams usually appearing in a single day.

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## INTRODUCTION

Systematics in the large, complex family Allocreadiidae (Looss 1902) Stossich 1903 and particularly the genus *Allocreadium* Looss 1900 have been problematic since their creation. This is due in part to inadequate descriptions of some species, and the fact that relatively few of the large number of species assigned to the genus have had their larval stages described. Detailed descriptions were given for the cercaria of *A. isoporum* Looss 1894 by Looss (1894) and Dollfus (1949); for *A. alloneotenicum* Wooton 1957 by Wooton (1957); for *A. fasciatusi* Kakaji 1969 by Madhavi 1978; and for *A. handiai* Pande 1937 by Madhavi 1980. A brief description of the cercaria of *A. lobatum* Wallin 1909 was provided by DeGiusti (1962); similarily, brief accounts of the cercaria of *A. ictaluri* Pearse 1924 were given by Peters (1957b) and by Peters and Self (1963) (corrections of a previous description by Seitner (1951)). Peters (1957a) also gave the cercarial type for *A. neotenicum* as an ophthalmoxiphidiocercaria, based on the presence of eyespot pigment and stylet in the adult, and established this cercarial type as characteristic of the genus (Peters 1957b).

Where intramolluscan stages are known for the above species, cercarial development is in rediae; they differ, however, in the type of intermediate host used. *Allocreadium isoporum, A. alloneotenicum,* and *A. lobatum* develop in lamellibranch pelecypods (sphaeriid clams), *A. fasciatusi* and *A. handiai* use prosobranch gastropods, and *A. ictaluri* develops in ancylid pulmonate gastropods (freshwater limpets).

Greater uniformity is seen in cercarial morphology and the molluscan hosts used by species in three other extensively studied genera of North American Allocreadiidae: *Crepidostomum, Bunodera,* and *Bunoderella.* Ophthalmoxiphidiocercaria developing in rediae in sphaeriid clams were reported for six species of *Crepidostomum* (Brown 1927, Hopkins 1933, 1934, Ameel 1937, Henderson 1938, Crawford 1943, Choquette 1954, Cheng and James 1959), for *Bunodera sacculata* VanCleave and Mueller 1932 (Cannon 1971), for *B. lucioperca* (Muller 1776) (Wisniewski 1958, Moravec 1969, Cannon 1971), and *Bunoderella metteri* Schell 1964 (Anderson et al. 1965).

The present study describes the cercaria of *A. pseudotritoni* Rankin 1937 the adult of which was prevalent in the spring salamander, *Gyrinophilus porphyriticus* (Green) from several streams in South-Central Ohio (Catalano et al. 1982). A careful search at one site, Churn Creek in Adams County, revealed that a sphaeriid clam, *Pisidium casertanum* Poe, released a single species of allocreadiid ophthalmoxiphidiocercaria. These cercariae encysted in amphipods, *Crangonyx* sp., where they developed into metacercariae that matured into adult *A. pseudotritoni* when fed to *G. porphyriticus* (Catalano 1982).

## MATERIALS AND METHODS

Infected *Pisidium casertanum* were collected from August 1979 to March 1981 from two sites in Ohio: Churn Creek in Adams County and East Fork Creek in Scioto County. The Churn Creek site provided most of the material for study, as the clams were infected with only one trematode species, and the prevalence of *Allocreadium pseudotritoni* in salamanders at this site was high (Catalano et al. 1982).

Clams were maintained in Talawanda spring water (SW) at 14° to 18°C. Living cercariae were obtained by dissection of the clam host or from natural emergence. Neutral red was used as an intravitam stain.

Cercariae were fixed in either hot neutral buffered formalin (NBF) or hot AFA. The preserved specimens were stained with Semichon's carmine or a one-step Mallory-Heidenhain stain (Cason 1950, Catalano 1982). To reveal details of the nervous system, the bromindoxyl acetate (BiAc) method for esterase was used (Halton and Jennings 1964). Live cercariae were placed in cooled substrate diluted 10:1 with SW; when sufficient blue color had developed, they were washed in SW and fixed in NBF.

Stained cercariae were dehydrated in an ethanol series, cleared in an absolute ethanol (AE)–methyl salicylate (MS) (oil of wintergreen) series (AE, 2:1, 1:1, 1:2, MS), and mounted in gum damar or DPX (Raymond A. Lamb Co., London). Measurements were made with a calibrated ocular micrometer.

To study cercarial emergence patterns, newly collected clams isolated in watch glasses or shell vials of SW were placed in an environmental chamber (Percival model PT 80 or model I 35 L) in which a 10L:14D photoperiod was established. In the PT 80, containers were 92 cm from eight fluorescent bulbs (F72T12D-HO) with a light intensity of 1250 foot-candles; in the I 35 L chamber the containers were 50 cm from one fluorescent bulb (F20T12CW) and illumination was 170 foot-candles. Initially, clams were transferred to new containers and the used container was examined for cercariae every six hours to determine general emergence patterns. Later intervals of 30 to 60 minutes were used to establish emergence peaks.

## RESULTS

Measurements are given in micrometers in the following description. Where appropriate, sizes are given as ranges followed by the mean ($\bar{x}$) ±standard deviation (SD) and sample size (N) in parentheses.

*Allocreadium pseudotritoni* cercariae developed in rediae in the sphaeriid clam *Pisidium casertanum.* Cercariae pos-

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sess paired eyespots and a stylet typical of the genus *Allocreadium* (Figs. 1, 2). Bodies of 10 live emerged cercariae measured 140-270 (220.4 ± 40.7) by 75-130 (110.0 ± 18.4). The tegument is aspinous; the slender unadorned tail nearly equals body length, 120-300 (212.3 ± 65.6). The oral sucker, 30-70 (48.6 ± 13.8), is slightly larger in diameter than the ventral sucker, 32-50 (42.5 ± 6.7). The bifurcate digestive tract is confined to the preacetabular region: prepharynx long; pharynx, 10-18 (15.5 ± 3.5) diam., located midway between suckers; ceca very short. Stylets are 12-20 (16.6 ± 3.8, N = 3) long and up to 5 in width. Fifty-five preserved cercarial bodies were 165-290 (199.7 ± 29.2) by 70-112 (86.8 ± 11.7); tails, 157-298 (213.8 ± 34.9) by 17-30 (22.8 ± 4.3); oral sucker 32-55 (41.4 ± 8.3) diam., ventral sucker 30-47 (38.3 ± 4.6) diam.; pharynx 12-17 (14.6 ± 1.8) diam.; eyespot 8-13 (11.1 ± 1.3) diam. Thirty stylets, 11-21 (16.3 ± 2.7) long by 5-8 (6.6 ± 1.1) maximum width, and 2-5 (3.8 ± 1.0) minimum width, terminal spine 2-4 (2.9 ± 0.6); from a side view, maximum depth 4-5 (4.9 ± 0.7) and minimum depth 2-4 (2.9 ± 0.6).

The oral and ventral suckers of *A. pseudotritoni* cercariae possess numerous slender papillae, larger and more densely arranged on the latter. Three pairs of penetration glands lie lateral to the ventral sucker; their paired ducts, two lateral and one medial to the eyespots, lead to openings beside the stylet (Figs. 1, 2). Cystogenous glands stain yellow to orange with neutral red and are most abundant dorsally and posterior to the acetabulum. The densely staining germ cell mass is located just posterior and dorsal to the ventral sucker. The flame cell formula is 2[(4 + 4 + 4) + (4 + 4 + 4)] = 48 (Fig. 1). Bromindoxyl acetate staining revealed a pair of cephalic ganglia connected by a broad commissure and giving rise to paired nerve trunks (Figs. 1, 2).

Cercarial emergence was observed in the laboratory year round; in clams collected in August (1979, 1980) and December (1980) (dry conditions), emergence began within 48 hours after immersion in spring water. The heaviest emergence and largest numbers of patent infections were observed in November. Cercarial emergence from individual clams was sporadic, with large numbers usually appearing on one day (Fig. 3). The number released daily by single clams varied from one to over 300 with few cercariae emerging in the dark. Peak emergence occurred within three hours of the onset of light (Fig. 4). No differences in this pattern were seen with different intensities of light in the two environmental chambers.

After emerging, *A. pseudotritoni* cercariae swim for several hours, first moving to the surface and later accumulating near the bottom of the container. Cercariae swim tail first, with the body arching back and forth while the tail describes an S-curve. After three to four hours of continuous swimming, they settle to the bottom where they use the oral and ventral suckers to crawl about. Cercariae remain active for up to 12 hours at room temperature.

## DISCUSSION

The present description of the cercaria of *Allocreadium pseudotritoni* supports Rankin's (1937) allocation of this species to the genus *Allocreadium*. Yamaguti (1958) transferred *A. pseudotritoni* to the genus *Cainocreadium* Nicoll 1909, then included in the Allocreadiidae, and later (1971) moved *Cainocreadium* to the family Opecoeliidae Ozaki 1925. *Allocreadium pseudotritoni* was included in this

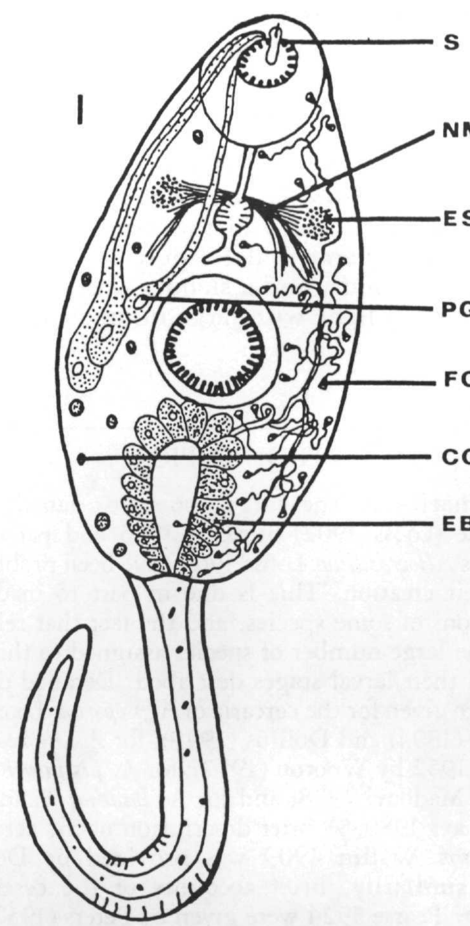


FIGURE 1. A composite drawing (from live and preserved specimens) of the cercaria of *Allocreadium pseudotritoni*. Bar equals 10 micrometers. Cystogenous glands (CG), eyespot (ES), excretory bladder epithelium (EBE), flame cell (FC), neural mass (NM), penetration gland (PG), stylet (S).

move (perhaps as an oversight) despite Rankin's report of remnants of eyespot pigment in adults, a characteristic absent in the opecoelids. Other authors (Seitner 1951, Peters 1957a, Wooton 1957, Durio and Manter 1968, Saoud et al. 1974, Catalano et al. 1982) recognized

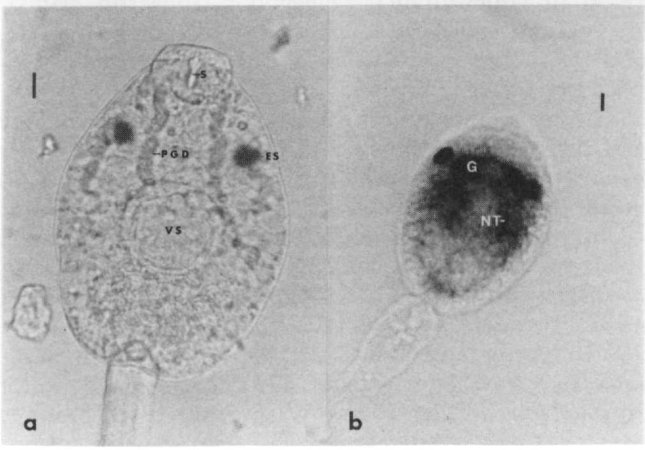


FIGURE 2. Ophthalmoxiphidiocercaria of *Allocreadium pseudotritoni*. Bar equals 10 micrometers.

a. Typical living cercaria showing eyespots (ES), penetration gland ducts (PGD), stylet (S), and ventral sucker (VS).
b. Nervous system, bromindoxy acetate reaction. Bar equals 10 micrometers. Dorsal view showing ganglion (G), nerve trunk (NT).

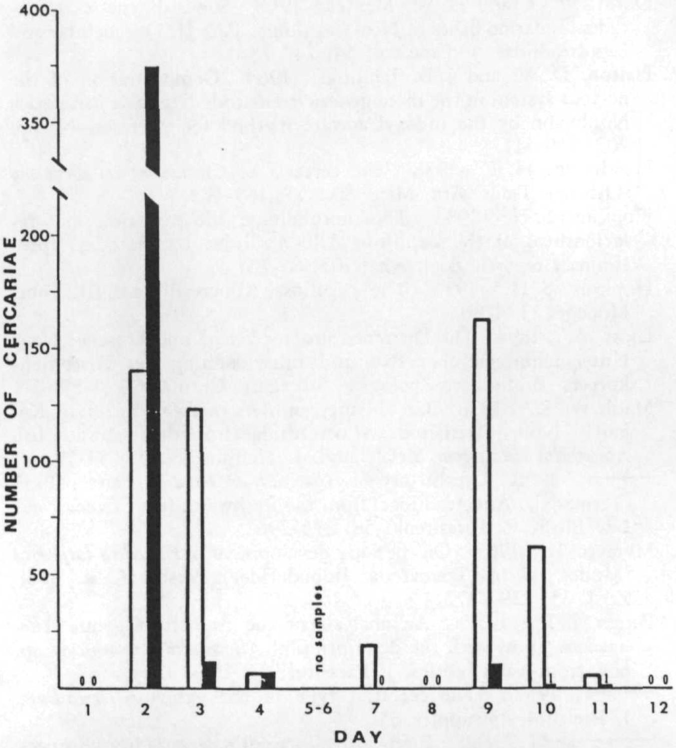


FIGURE 3. Total daily output of *Allocreadium pseudotritoni* cercariae from each of two clams, *Pisidium casertanum*, over a 12 day period.

*A. pseudotritoni* as a valid allocreadid species. The presence of eyespot pigment and general morphology of the cercaria confirm this allocation.

Development of opthalmoxiphidiocercariae in rediae, the use of a sphaeriid clam as the molluscan host, and morphology of the cercariae further establish close relationships between *A. pseudotritoni*, *A. isoporum*, *A. lobatum*, *A. alloneotenicum*, and *A. neotenicum*. These findings also support the views of Dollfus (1949), Hopkins (1934), Wooton (1957), and Cannon (1971) that the genera *Allocreadium*, *Crepidostomum*, and *Bunodera* are closely related. They are also consistent with the familial and subfamilial scheme of Yamaguti (1971).

Among the species of *Allocreadium* for which life cycles are known, *A. pseudotritoni* differs most from *A. ictaluri*, in which intramolluscan stages develop in freshwater limpets (pulmonate gastropods), and *A. handiai* and *A. fasciatusi* which develop in prosobranch gastropods. It also differs from the latter two species in stylet and tail morphology.

The cercaria of *A. pseudotritoni* most closely resembles *A. allonetenicum*, differing from it and *Bunodera lucioperca* in having cystogenous glands. *Allocreadium lobatum* has the same flame cell pattern and presumably cystogenous glands; however, DeGiusti's (1962) description is too brief to make further comparison. The number of penetration glands and flame cell pattern differentiate *A. pseudotritoni* from *A. icataluri* (L. E. Peters, pers. comm.), *A. handiai*, *A. fasciatusi*, *B. lucioperca*, and *Bunoderella metteri*. Flame cell groupings also differentiate *A. pseudotritoni* from *Bunodera sacculata* and *Crepidostomum* spp. In terms of general cercarial morphology and molluscan hosts, *A. pseudotritoni*, *A. alloneotenicum*, *A. isoporum*, *and A. lobatum* are more similar to one another and to the cercariae of both *Bunodera* species and *Bunoderella metteri* than to *A. fasciatusi* and *A. handiai*. Demonstration of additional *Allocreadium* life cycles will help to clarify the generic position of these species.

Emergence of *A. pseudotritoni* cercariae correlated with light cycling. Whether this was the result of cercarial perception of light (via photoreceptors), an innate rhythm of the parasite, a change in activity of the clam, or a combination of these has not been tested.

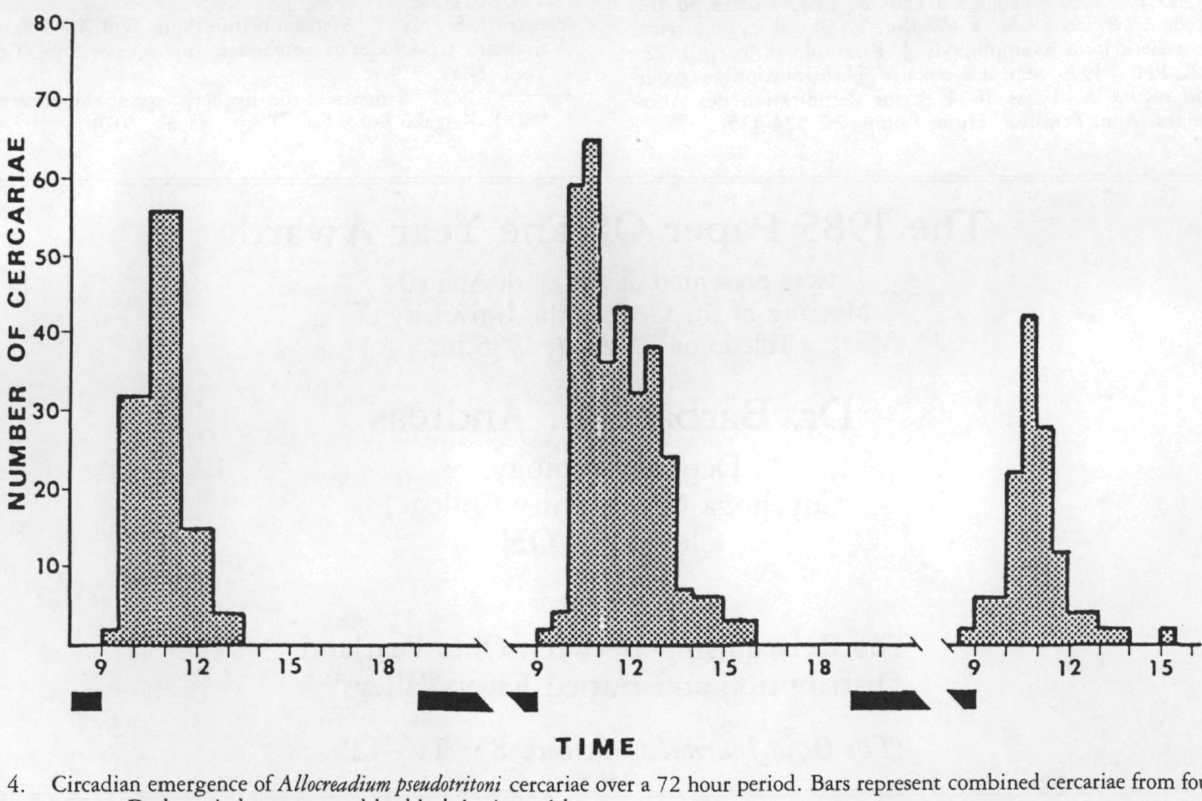


FIGURE 4. Circadian emergence of *Allocreadium pseudotritoni* cercariae over a 72 hour period. Bars represent combined cercariae from four clams, *Pisidium casertanum*. Dark periods represented by black horizontal bar.

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# The 1985 Paper Of The Year Award

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