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Source: Journal of Wildlife Diseases, 24(4) : 642-646

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-24.4.642>

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## AMPHILEPTUS BRANCHIARUM (PROTOZOA: AMPHILEPTIDAE) IN POND REARED FISH IN ARKANSAS

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**ABSTRACT:** *Amphileptus branchiarum* is a protozoan parasite of fish in North America, Europe and Asia. In North America (Arkansas) it usually occurs on the gills of cyprinids from July to October. It seldom causes disease epizootics but may be responsible for epithelial hyperplasia and cell displacement in branchial tissue.

**Key words:** *Amphileptus branchiarum*, *Hemiophrys branchiarum*, Ciliophora, *Notemigonus crysoleucas*, *Ictalurus punctatus*, *Pimephales promelas*, *Carassius auratus*, protozoan infection, pathology.

### INTRODUCTION

*Amphileptus branchiarum* is a ciliated protozoan parasite of the external body and gills of certain freshwater fishes and frogs in North America, Asia and Europe. It has a ventral slitlike cytostome, a ciliated dorsal surface, a naked ventral surface, two macronuclei surrounding the micronucleus, numerous contractile vacuoles and a row of trichocysts along the posterior margin of the body (Bykhovskaya-Pavlovskaya et al., 1962; Hoffman, 1978). It occurs in three forms (Fig. 1): free-living (hastate), attached and embedded (spherical) (Ergens and Lom, 1970; Wenrich, 1924). Because the forms differ, the dimensions and shapes vary. Live specimens range from 29  $\mu\text{m}$  (A. J. Mitchell, pers. obs.) to 135  $\mu\text{m}$  (Wenrich, 1924) in length and from 22  $\mu\text{m}$  to 77  $\mu\text{m}$  (A. J. Mitchell, pers. obs.) in width. The organism often has been referred to as *Hemiophrys branchiarum* (Kahl, 1931). However, in 1960 *Hemiophrys* was synonymized with *Amphileptus*; therefore, *Amphileptus branchiarum* is now the generally accepted name (Foissner, 1983).

In order to expand the knowledge on this little known ciliate, we have extracted information from diagnostic records. In this paper we present information on the seasonal occurrence, host and tissue specificity, geographic distribution and histopathology of *A. branchiarum* and evaluate its importance as a pathogen.

### MATERIALS AND METHODS

The major source of information about host site specificity, geographic distribution and seasonal occurrence was the disease diagnostic records of the U.S. Fish and Wildlife Service, Fish Farming Experimental Station (Stuttgart, Arkansas 72160, USA). Additional information was obtained from fish health laboratories concerned with warmwater fish culture in the United States and from the published literature. The fish health laboratories included Southeastern Cooperative Fish Disease Laboratory (Department of Fisheries, Auburn University, Alabama 36849, USA), Extension and Wildlife Fisheries (P.O. Box 5405, Starkville, Mississippi 39762, USA), Aquatic Animal Diagnostic Laboratory (School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA), Department of Medicine (School of Veterinary Medicine, University of California, Davis, California 95616, USA), Department of Wildlife and Fisheries (Room 202, Nagal Hall, Texas A&M University, College Station, Texas 77843, USA), and Athens Diagnostic Laboratory (College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, USA).

Gill tissue from golden shiners (*Notemigonus crysoleucas*) infected with a high number of *A. branchiarum* was preserved in Bouin's fixative (Humason, 1962), embedded in paraffin, sectioned and stained with hematoxylin and eosin. Similar stained sections from gills of channel catfish (*Ictalurus punctatus*) that were lightly infected with the parasite were provided by Dr. Thomas Bucci (The National Center for Toxicological Research, Jefferson, Arkansas 72079, USA).

### RESULTS AND DISCUSSION

*Amphileptus branchiarum* infections were not reported by any of the fish health

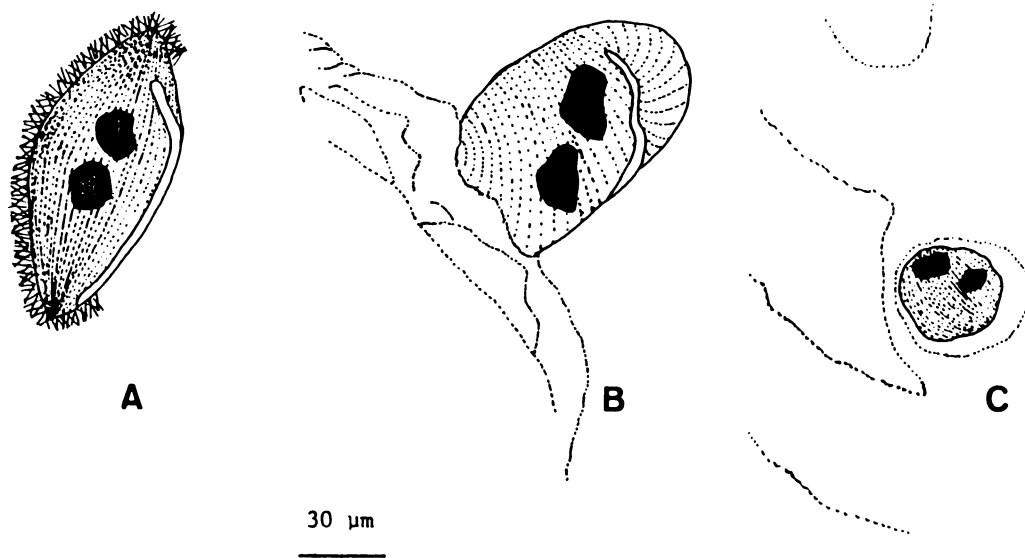


FIGURE 1. *Amphileptus branchiarum*: A. free-living (hastate) form; B. attached form on gill filament; C. embedded (spherical) form in branchial tissue.

laboratories that we contacted. The parasite was reported in Arkansas by Dr. Fred P. Meyer from the gills of golden shiners on 15 May 1972 (Fish Farming Experimental Station records). From 15 May 1972 to 31 December 1986, *A. branchiarum* was recorded in 110 of 3,161 fish disease cases processed from fish farms in 15 states. The majority (>90%) of the 3,161 cases came from Arkansas and all of the 110 cases of *A. branchiarum* were from Arkansas fish farms. Approximately 50% of the 3,161 cases involved channel catfish and other ictalurids. The rest of the cases involved cyprinids and a few centrarchids, tropical fish, cichlids and percichthyids. The 110 cases of *A. branchiarum* involved four host species: golden shiners (72 cases), goldfish (*Carassius auratus*, 27 cases), channel catfish (9 cases), and fathead minnows (*Pimephales promelas*, 2 cases).

*Amphileptus branchiarum* was first reported on the gills of tadpoles collected near Philadelphia, Pennsylvania (USA) (Wenrich, 1924). A similar and possibly identical species, *A. vorax*, was found on three fish from the Mississippi River at Fairport, Iowa (USA) and a minnow from

Kearneysville, West Virginia (USA) (Davis, 1947; Chen, 1956). *Amphileptus branchiarum* also has been found in Europe (Ergens and Lom, 1970) and Asia (Bykhovskaya-Pavlovskaya et al., 1962). Two other *Amphileptus* spp., reported as *Hemiophrys macrostoma* and *H. disciformis*, were described by Chen (1955, 1956) from four cyprinid fishes in China. *Amphileptus* spp. have been reported from 17 host fish species worldwide. Of these hosts, 12 were cyprinids, 2 were suckers (Cato-stomidae), 2 were sticklebacks (Gasterosteidae) and 1 was the channel catfish (Table 1). We agree with Ergens and Lom (1970) and Bykhovskaya-Pavlovskaya et al. (1962) that many other fish species probably could serve as hosts for this parasite.

Although *A. branchiarum* occurs throughout the year, it has been observed most often in summer to early fall in Arkansas. The occurrence of the parasite peaked from July to October; 71 of the 110 cases appeared during this period (Fig. 2). Gill filaments and gill lamellae are the primary target tissues. The tissue sites in the 110 cases were gills only (99 cases),

TABLE 1. A listing of hosts infected with *Amphileptus* spp.

Host species	Citation
<b>Catostomidae</b>	
<i>Carpiodes carpio</i>	Davis, 1947
<i>Ictiobus bubalus</i>	Davis, 1947
<b>Cyprinidae</b>	
<i>Alburnus alburnus</i> *	Bykhovskaya-Pavlovskaya et al., 1962
<i>Aristichthys nobilis</i>	Chen, 1956
<i>Carassius auratus</i>	A. J. Mitchell, pers. obs.
<i>Ctenopharyngodon idellus</i>	Chen, 1955
<i>Cyprinus carpio</i>	Ergens and Lom, 1970
<i>Gobio gobio</i> *	Bykhovskaya-Pavlovskaya et al., 1962
<i>Hypophthalmichthys molitrix</i>	Chen, 1956
<i>Mylopharyngodon piceus</i>	Chen, 1955
<i>Notemigonus crysoleucas</i>	A. J. Mitchell, pers. obs.
<i>Pimephales promelas</i>	A. J. Mitchell, pers. obs.
<i>Rutilus rutilus</i>	Ergens and Lom, 1970
<i>Semotilus (Margariscus) margarita</i>	Davis, 1947
<b>Gasterosteidae</b>	
<i>Gasterosteus aculeatus</i> *	Bykhovskaya-Pavlovskaya et al., 1962
<i>Pungitius pungitius</i> *	Bykhovskaya-Pavlovskaya et al., 1962
<b>Ictaluridae</b>	
<i>Ictalurus punctatus</i>	Davis, 1947
<i>Ictalurus punctatus</i>	A. J. Mitchell, pers. obs.
<b>Ranidae</b>	
<i>Rana catesbeiana</i>	Wenrich, 1924
<i>R. clamitans</i>	Wenrich, 1924
<i>R. palustris</i>	Wenrich, 1924
Tadpoles (not identified)	Bykhovskaya-Pavlovskaya et al., 1962

\* Scientific names derived from common names given in text.

body only (2 cases) and gills and body (9 cases). The embedded spherical form is the form that was most often observed.

Reports about the importance of *Amphileptus* spp. as pathogens vary. Chen (1955, 1956) reported that embedding by the parasite destroys host cells. Wenrich (1924) observed engulfment of host gill tissue. We have observed the ingestion of *Trichodina* sp. but not of host tissue by *A. branchiarum*. Davis (1947) did not observe tissue ingestion but acknowledged that some damage may accompany embedding in tissue and stated that the benefit derived from the consumption of other parasites was probably greater than any damage from cell displacement.

The importance of *A. branchiarum* as a pathogen is suggested by the diagnostic records of the Fish Farming Experimental Station over a 16-yr period. Only in 2 of 3,161 diagnostic cases was *A. branchiarum* considered to be the primary cause of death. Thus the case records indicate that *A. branchiarum* seldom causes mortality in cultured fishes; it has never been reported as a problem in feral fish populations. Histopathological examination of the gills of heavily infected golden shiners indicated epithelial hyperplasia and some fusion of lamellae at the host-parasite interface (Fig. 3). Although bacteria and other parasites were not present, the effect of nutritional deficiencies and water quality

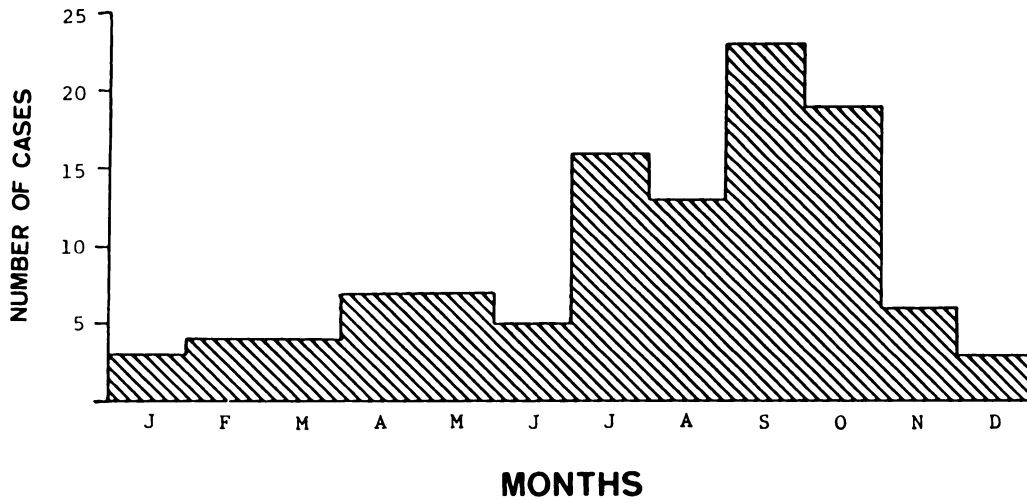


FIGURE 2. Monthly prevalence of *Amphileptus branchiarum* in 3,161 disease cases at the Fish Farming Experimental Station, 1972 to 1986.

problems on the branchial tissue was not examined. Embedded parasites could be seen readily in microscopic examination of wet mount preparations. Histological preparations of the embedded ciliates show displacement of cells in branchial tissue (Fig. 4).

More than one species of the genus *Amphileptus* may have been present in our diagnostic cases, since two species have

been described in the United States; *A. branchiarum* (Wenrich, 1924) and *A. voracicus* (Davis, 1947). However, we do not believe it is possible to identify species other than *A. branchiarum* nor to describe new species on the basis of criteria given in the literature. This is because the parasite takes three different forms that yield highly variable measurements and shapes within a single population (Wenrich, 1924; Bykhovskaya-Pavlovskaya et al., 1962; A. J. Mitchell, pers. obs.), yet most of the distinctions between species that are listed in the literature involve morphometrics.

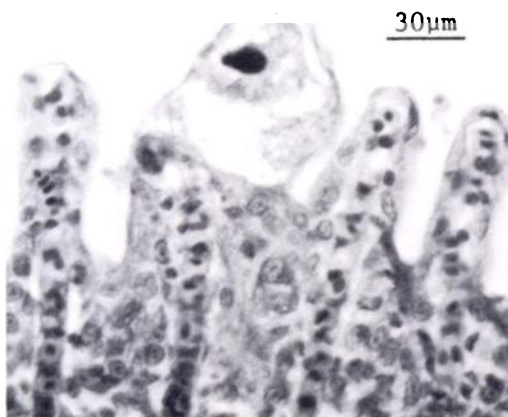


FIGURE 3. Apparent hyperplastic response of the epithelium of gill lamellae of a golden shiner to *Amphileptus branchiarum*. H&E.

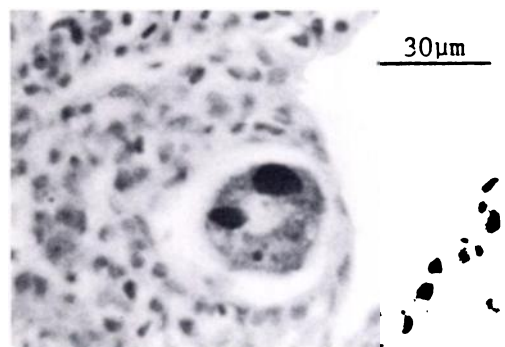


FIGURE 4. Displacement of gill epithelial cells by embedded *Amphileptus branchiarum* in a channel catfish. H&E.

In addition, form, dimensions and some structural characters used in the published species descriptions are distorted by fixation (Wenrich, 1924; Chen, 1956). The description of *A. voracus* by Davis (1947) was based on a few fixed specimens. Therefore, we have chosen to identify the parasite as *A. branchiarum* on the basis of the original description by Wenrich (1924).

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Received for publication 20 August 1987.