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Running Title: *Epistylis* sp. on *Lepomis cyanellus* in Arkansas

Ciliates of the genus *Epistylus* Ehrenberg, 1830 are sessile peritrichous organisms often present as a branching colony with a short oral disc and collar, and non-contractile rigid stalk (Dias et al. 2006). There are about 120 described species that generally live in freshwater environments (Lynn 2008). Epistylids are not considered true parasites but are common on crayfishes (Vogelbein and Thune 1988), the carapace of turtles (Bishop and Jahn 1941, Bovee 1976) and on the eggs, skin and gills of fishes where they may contribute to mortality from partial smothering or secondary infection (Fischthal 1949, Reichenbach-Klinke 1973, Esch et al. 1976, Miller and Chapman 1976, Crites 1977, Hazen et al. 1978, Hoffman 1999). However, some historically considered these ciliates epibionts, which colonize the surface of live substrates and serve a commensal ecological role (Wahl 1989, Fernandez-Leborans et al. 2006). Lewis et al. (1978) reported *Epistylis* on 16 of 32 fishes examined in 2 North Carolina reservoirs, mainly from centrarchids, ictalurids, and moronids. Ictalurids and salmonids seem to be especially susceptible to infestation (Hubert and Warner 1975, Hoffman 1999). Epistylids use the host as an attachment substrate, so it can feed on bacteria and suspended particles in water.

Little is known about these ciliates on Arkansas fishes. Foissner et al. (1985) reported an epizootic of *Heteropolaria colisarum* Foissner and Shubert, 1977 on the scales and fins of cultured Green Sunfish (*Lepomis cyanellus*) from the Fish Farming Experimental Station, Stuttgart, Arkansas County. Interestingly, this infection was also shown in photos on the front cover of the second edition of Hoffman's (1999) classic, *Parasites of North American Freshwater Fishes*. Lom and Dykovà (1992) have synonymized *H. colisarum* with *Epistylis*. However, to our knowledge, nothing has been published on this ciliate in native fishes from natural waters in the state. Here, we present a case of *Epistylis* sp. on *L. cyanellus* with light microscopy and scanning electron microscopy (SEM) of the infestation.

On 14 October 2015, an adult (195 mm total length) *L. cyanellus* was collected with a backpack electroshocker from the South Fork of Fourche La Fave River at Hollis, Perry County (34.8706°N, 93.109458°W). As this specimen was noted to be unusual in possessing scales and spines on the dorsal fin with some unidentified whitish growth (Fig. 1), the fish was photographed alive. No growth was noted on the gills. The specimen was immediately overdosed by immersion in tricaine methanesulfonate and several scales were removed and placed in individual vials containing 70–95% (v/v) DNA grade ethanol and 10% neutral buffered formalin (NBF). The fish was preserved in 10% formalin and later transferred to 70% (v/v) ethanol. On return to the laboratory, scrapings were taken with fine forceps from scales originally placed in ethanol. Specimens were stained with Gomori trichrome, dehydrated in 95–100% (v/v) ethanol, cleared with xylene, mounted with Canada balsam and examined using light microscopy. Photomicrographs of stereoscopic samples were taken with Canon Powershot S3IS camera fitted with a Martin Microscope adaptor (Martin Microscope, Easley, SC). Photomicrographs for light microscopy were taken with a Swift M10 Series microscope fitted with a digital camera mount. For SEM, we transferred scales and liquid suspensions in 10% NBF through a graded series of increasing ethanol solutions (70–100%). Specimens were then extracted from vials with a pipette and placed onto segments of glass coverslips (18 × 4 mm) previously coated with poly-L-lysine. An Autosamdri®–815 critical point drier (Tousimis Research Corporation, Rockville, MD; 31°C, 1072 psi, ventilation rate ~100 psi/min) was used to remove excess ethanol from cells. Dehydrated specimens on coverslips were then adhered to rectangular copper transfer boats (25 × 5 mm) with double-sided tape and

Figure 1. *Lepomis cyanellus* with whitish growth on dorsal fin and scales. Scale bar = 50 mm. Photo by Uland Thomas.

mounted onto sticky-tabbed 12 mm aluminum pin stubs. Copper boats and stubs were then coated with gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK). Specimens examined for SEM were generated at the Arkansas Nano & Bio Materials Characterization Facility (UA-Fayetteville). A host voucher specimen was deposited in the Henderson State University Collection (HSU), Arkadelphia, Arkansas; a voucher slide of the infestation was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska as HWML 101962.

The unusual growth on scales of *L. cyanellus* (Fig. 1) was identified as an *Epistylis* sp. Examination of colonies using stereoscopic and light microscopy (Fig. 2) revealed colonies comprising a various number of individuals, with a branched and smooth, noncontractile stalk and fully expanded zooids. When examined by SEM (Fig. 3), apical views of contracted zooids were prominent, and cilia could be seen atop them. The macronucleus is typically horseshoe shaped and transversely oriented in the middle-adoral region of the cell.

Fischthal (1949) reported an *Epistylis* sp. from a darter in a Wisconsin stream, and Rogers (1971) found it on pond fishes in the southeastern United States. Cloutman (1975) reported an *Epistylis* sp. on White Bass (*Morone chrysops*) and Striped Bass (*Morone saxatilis*) in North and South Carolina. In addition, Crites (1977) found *Epistylis niagarae* Kellicott on Smallmouth Bass, *Micropterus dolomieu*, Rock Bass, *Ambloplites rupestris* and Freshwater Drum, *Aplodinotus grunniens* from Ohio.

Other fishes (*n* = 28) collected on the same date and locality did not possess *Epistylis* as follows: 5 Greenside Darters (*Etheostoma blennioides*), 1 Western Creek Chubsucker (*Erimyzon claviformis*), 8 Slender Madtoms (*Noturus exilis*), 1 Logperch (*Percina caprodes*), 6 Longnose Darters (*Percina nasuta*), 4 Blackspotted Topminnows (*Fundulus olivaceus*), and 3 Wedgespot Shiners (*Notropis greenei*). In addition, we have examined over 100 *L. cyanellus* from various watersheds in Arkansas over the last decade and never have noticed any with this growth.

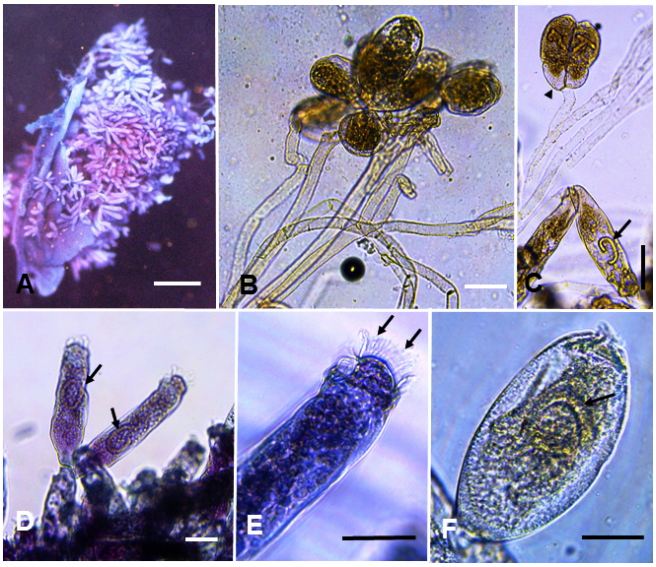

Figure 2. Light microscopy of *Epistylis* sp. from *Lepomis cyanellus*. A. Colonies from skin scraping, stereoscopic view; trichrome stain. B. Branched colonies showing group of zooids on noncontractile stalks; unstained. C. Zooids showing macronucleus (arrow) and daughter cells from binary fission (arrowhead); unstained. D. Two zooids showing macronuclei (arrows); trichrome stain. E. Single elongate zooid showing cilia (arrows); trichrome stain. F. Single vase-shaped zooid showing macronucleus (arrow); unstained. Scale bars: A = 200 µm; B–F = 50 µm.

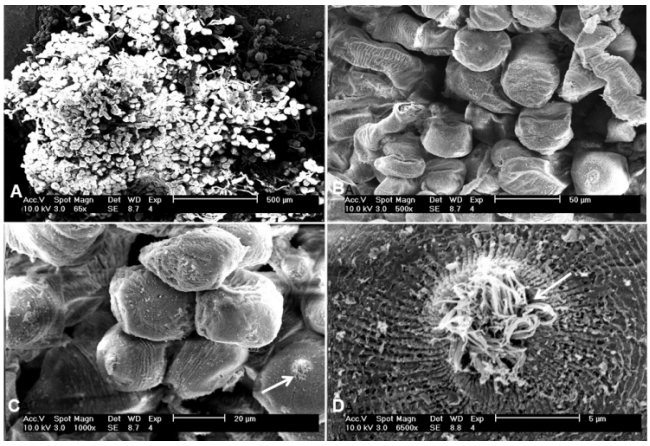

Figure 3. Scanning electron micrographs of *Epistylis* sp. on scales of *Lepomis cyanellus*. A. Low magnification showing colonies on single scale. Bar = 500 µm. B. Higher magnification of apical view of contracted zooids. Bar = 50 µm. C. Another apical view showing cilia on one colony (arrow). Bar = 20 µm. D. Close-up showing cilia (arrow). Bar = 5 µm.

In summary, we provide the initial report of an *Epistylis* sp. from a non-cultured *L. cyanellus* and the

first time SEM photomicrographs of this form have been documented. Future studies should include molecular analyses (18S rRNA sequences) to help further provide an identity of this species.

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