Henneguya sp. (Myxosporea: Bivalvulida: Myxobolidae) from the Black Bullhead, *Ameiurus melas* (Siluriformes: Ictaluridae), from Southeastern Oklahoma

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Stanley E. Trauth
Department of Biological Sciences, Arkansas State University, State University, AR 72467

Myxosporeans of the genus *Henneguya* Thélohan are one of the most speciose of myxozoans and includes at least 204 described species infecting freshwater, estuarine and marine fishes (Lom and Dyková 2006). In the catfish family Ictaluridae, 20 species have been reported from commercially-raised Blue Catfish (*Ictalurus furcatus*) and Channel Catfish (*Ictalurus punctatus*), hybrid crosses of *I. punctatus* and *I. furcatus*, and wild Black Bullhead (*Ameiurus melas*) and Brown Bullhead (*Ameiurus nebulosus*) (Bosworth et al. 2003; Iwanowicz et al. 2008; Pote et al. 2012). Of the species from ictalurids, five (25%), including *H. adiposa, H. exilis, H. gurleyi, H. ictaluri*, and *H. sutherlandi* have been identified using molecular techniques via 18S rDNA sequence (see Griffin et al. 2009). In addition, only three species (*H. ameiurensis*, *H. exilis*, *H. gurleyi*) are known to infect bullheads (*Ameiurus* spp.) and, despite frequent examination of these fishes, especially of their skin abnormalities, cutaneous species of *Henneguya* are not commonly observed in ameiurids (Iwanowicz et al. 2008; Griffin et al. 2009).

As far as we know, there are only two reports of *Henneguya* in Oklahoma fishes as follows: (1) the report by Parker et al. (1971) of *H. gambusi* from skin cysts of 5 of 143 (3%) Western Mosquitofish, *Gambusia affinis* from Payne County, and (2) a "mandibular form" of a *Henneguya* sp. from a wild *I. punctatus*, also from Payne County (McCraren et al. 1975). Nothing else has been published about any other *Henneguya* infecting fish in the state, particularly those in the genus *Ameiurus*. Here, we report, for the first time, a species of *Henneguya* from *A. melas* from southeastern Oklahoma, and include light and scanning electron microscopy of the plasmodia and myxospores.

Between May 2013 and July 2015, 17 juvenile and adult *A. melas* (mean ± 1SD total length [TL] = 143.0 ± 43.8, range 92–200 mm) were collected by seine or backpack electroshocker from Yashau Creek, McCurtain County (34.018852°N, 94.756761°W). Fish were placed in aerated creek water and taken to the laboratory for necropsy. They were killed by immersion in a concentrated chloretone solution, measured for TL, and their integument, gills, fins, peritoneal cavity, liver and kidney were examined for myxozoan plasmodia using a stereomicroscope. When plasmodia were seen, they were photographed *in situ*, excised by sharp dissection and their contents placed on a microscopic slide with a drop of 0.85% saline for microscopic examination. Additional samples were placed directly into 10% neutral buffered

formalin (NBF) for examination via scanning electron microscopy (SEM) or in 95% DNA grade ethanol for future molecular sequencing. For SEM, we transferred liquid suspensions in 10% NBF containing free myxospores 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

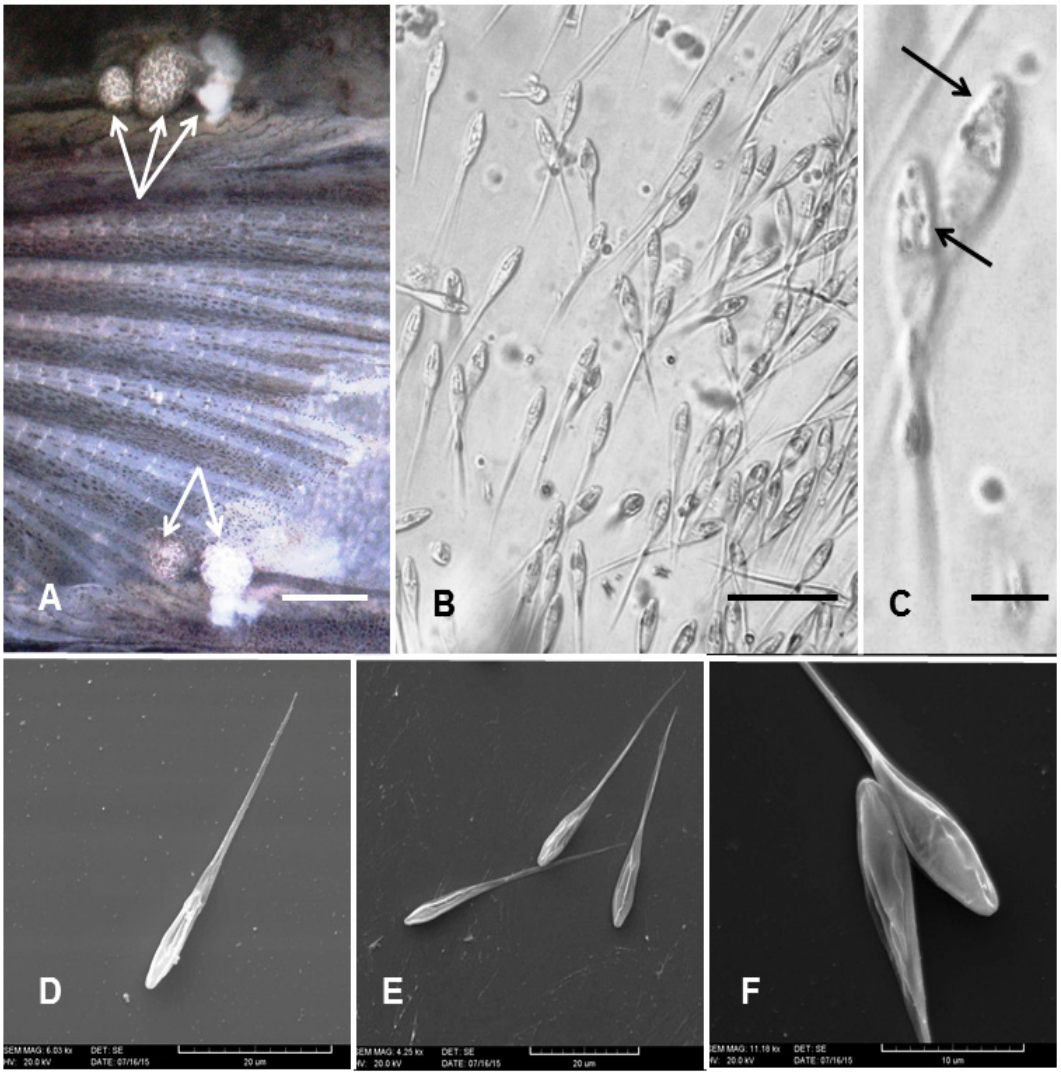

Figure 1. *Henneguya* sp. from *Ameiurus melas* from Oklahoma. A. In situ view showing plasmodia (arrows) on the pectoral fin. Scale bar = 2 mm. B. Numerous myxospores from wet mount preparation. Scale bar = 50 µm. C. Wet mount preparation of unstained *Henneguya* sp. myxospores in sutural view showing the coiled polar filaments (arrows) within the polar capsule. Scale bar = 10 µm. D. Scanning electron micrograph (SEM) of single myxospore. E. SEM of group of three myxospores showing variation in morphology. F. Higher magnification SEM showing morphology of spore body of myxospore.

× 5 mm) with double-sided tape and mounted onto sticky-tabbed 12 mm aluminum pin stub mounts. Copper boats and stubs were then coated with gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK). Specimens were examined with a Vega TS 5136XM digital scanning electron microscope (Tescan USA Inc., Cranberry Township, PA) at 19.5 kV. A host voucher specimen was deposited in the Henderson State University Collection (HSU), Arkadelphia, Arkansas; a parasite photovoucher was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska.

One of 17 (6%) *A. melas* (juvenile, 102 mm TL) collected on 25 June 2015 was found to possess multiple white, ovoidal plasmodial pseudocysts measuring 1–2 mm in diameter on its dorsal and both pectoral fins (Fig. 1A). There were no pseudocysts on the gills or any other part of the body (barbels), including the peritoneal cavity, liver and kidney. Rupture and subsequent removal of the exudate for wet mount preparation revealed numerous lanceolate myxospores containing two polar granules with an elongate caudate process (Figs. 1B-C), fitting the description of *Henneguya* (see Hoffman 1999). Examination of wet mount material revealed myxospores with an elongate, lanceolate spore body, flattened parallel to the suture line and measuring 16.3 (range 15–19) long × 3.8 (3.7–4.0) µm wide in sutural view; total spore length (TSL) was 45.5 (range 42–50) µm. There were two valves and two polar capsules which were pyriform and of unequal size, oriented in plane with the sutural ridge.

In addition, 230 other fishes from Yashau Creek, including four *Aphredoderus sayanus*, four *Ameiurus natalis*, 14 *Campostoma spadiceum*, 10 *Cyprinella venusta*, 25 *C. whipplei*, seven *Esox americanus*, 101 *Fundulus notatus*, 10 *Gambusia affinis*, 13 *Labidesthes sicculus*, two *Lepomis cyanellus*, three *L. gulosus*, one *L. megalotis*, 26 *Luxilus chrysocephalus*, six *Lythrurus umbratilus*, and eight *Notemigonus crysoleucus*, were not found to be infected with *Henneguya* plasmodia.

The Black Bullhead has been previously reported to harbor three *Henneguya* spp. Kudo (1920) reported *H. gurleyi* from the dorsal fin of bullheads from Iowa, Kudo (1929) documented *H. exilis* from the gills of *A. melas* from Illinois, Guilford (1965) reported *H. limatula* from the gall bladder of *A. melas* from Wisconsin, and Iwanowicz et al. (2008) found *H. gurleyi* on fish from North Carolina. The measurements of myxospores of our specimens of *Henneguya* sp. from dermal plasmodia on fins most closely matches that of *H. diversis* Minchew from pectoral fins of *I. punctatus* from Mississippi (Minchew 1977); all *Henneguya* spp. previously reported from *A. melas* are larger in TSL compared to our specimens. However, we understand that myxospores can be indistinguishable from others based solely on morphological comparisons. Host- and tissue-specificity is also necessary for accurate identification in the absence of molecular analyses. Indeed, the most sensitive approach at defining species of myxozoans is utilization of small-subunit ribosomal RNA (SSU rDNA) gene sequences (Iwanowicz et al. 2008). Future studies are designed to obtain these sequences from our material (C. Whipps, *pers. comm.*) and examine additional ictalurids for this parasite.

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