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A New Species of *Chloromyxum* (Myxozoa: Chloromyxidae) from the Gall Bladder of *Eurycea* spp. (Caudata: Plethodontidae) in North America

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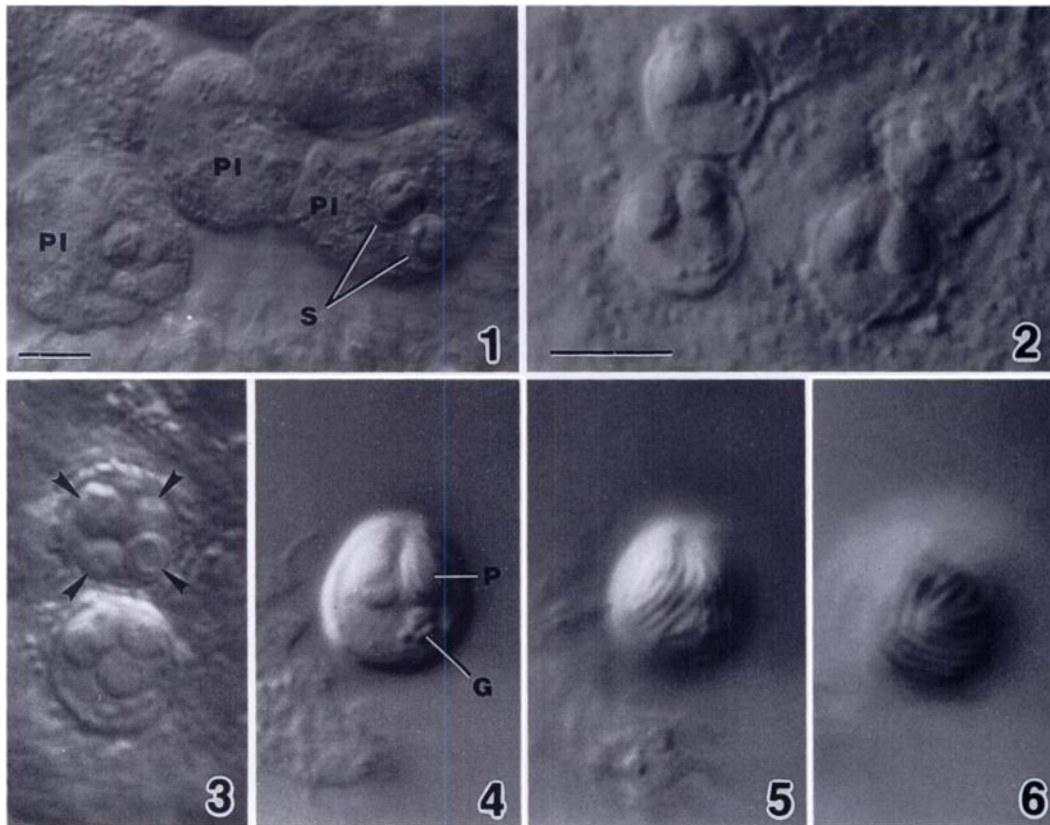
ABSTRACT: A previously undescribed species of *Chloromyxum* (Myxozoa: Chloromyxidae) was found in plasmodia adhering to the epithelium of the gall bladders in salamanders of the genus *Eurycea* (Caudata: Plethodontidae) from Arkansas and Texas (USA) in November, December, and January, 1987 to 1994. Bivalved spores of *Chloromyxum salamandrae* sp. n. from *Eurycea multiplicata griseogaster* (type host) were subspherical, with a mean size \pm SD of $8.3 \pm 0.3 \times 7.7 \pm 0.4$ (7.8 to 8.8×7.0 to 8.2) μm ($n = 20$), and had a shape index (length/width) of 1.07 ± 0.03 (1.02 to 1.14). The valves measured 0.8 to $1.0 \mu\text{m}$ thick and had 10 to 12 external striations each. Each of the four polar capsules were piriform, with a mean size \pm SD of $4.0 \pm 0.1 \times 2.6 \pm 0.1$ (3.8 to 4.2×2.4 to 2.8) μm ($n = 20$), and there appeared to be about four coils of each polar filament. The sporoplasm was irregular in shape and appeared to be binucleate. Adherent plasmodia observed in winter months were small, with a mean size \pm SD of $31.5 \pm 6.3 \times 24.9 \pm 2.6$ (20 to 40×20 to 30) μm ($n = 20$), and contained zero to eight disporoblastic spores each. The myxozoan occurred in nine of 14 *E. multiplicata griseogaster*, three of eight *E. multiplicata multiplicata*, and two of 12 *E. neotenes*. This represents the first report of a *Chloromyxum* sp. from Amphibia in the Western hemisphere.

Key words: *Chloromyxum salamandrae* sp. n.; Myxozoa; Chloromyxidae; salamander; Caudata; *Eurycea* spp.

Although commonly reported from fish, the finding of chloromyxids in caudate Amphibia are rare. *Caudomyxum caudatum* (Thélohan, 1894) comb. n. (syn. *Chloromyxum caudatum* Thélohan, 1894) infects the gall bladder of the crested newt (*Triturus cristatus*) in France (Thélohan, 1894). A second species, *Chloromyxum protei* Joseph, 1905, occurs in the renal tubules of *Proteus anguineus* near Vienna (Joseph, 1905, 1907). We are aware of no

other reports of chloromyxids infecting other species of Caudata.

Thirty-four salamanders (*Eurycea* spp.) were collected by hand in November, December, or January, 1987 to 1994, and consisted of 14 graybelly salamanders (*Eurycea multiplicata griseogaster* Moore and Hughes, 1941), eight many-ribbed salamanders (*Eurycea multiplicata multiplicata* (Cope, 1869)), and 12 Texas salamanders (*Eurycea neotenes* Bishop and Wright, 1937) from six locations in Arkansas and Texas, USA. In Arkansas, these counties were Conway ($35^{\circ}9'N$, $92^{\circ}45'W$), Independence ($35^{\circ}49'N$, $92^{\circ}5'W$), Pulaski ($34^{\circ}44'N$, $92^{\circ}15'W$), Saline ($34^{\circ}34'N$, $92^{\circ}35'W$), and Van Buren ($35^{\circ}36'N$, $92^{\circ}28'W$). In Texas, a single site in Real County ($29^{\circ}43'N$, $99^{\circ}45'W$) was selected. Salamanders were killed by prolonged immersion in a 1.5 liter bath of 0.5% (v/v) aqueous tricaine methanesulfonate (Sigma Chemical Co., St. Louis, Missouri, USA). Salamanders were checked for parasites and blood smears obtained as reported by McAllister and Upton (1987). Whole gall bladders were removed, placed in 10% neutral buffered formalin (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and shipped to Kansas State University, Manhattan, Kansas (USA). Voucher specimens are deposited in the Arkansas State Museum of Zoology as ASUMZ 19223 to 19238 and 19242 to 19253. Gall bladders were compressed between microscope slide and coverslip and examined as wet mount preparations using Nomarski interference-contrast optics, as reported for coccidia (Upton and McAllister, 1988). Measurements of parasites were made using an



FIGURES 1 to 6. Nomarski interference-contrast photomicrographs of *Chloromyxum salamandrae* sp. n. from *Eurycea multiplicata griseogaster*. Fig. 1. Low magnification of spores developing in plasmodia. Scale bar = 10 μ m. Figs. 2 to 6. Higher magnification of spores. Scale bar in Fig. 2 = 10 μ m and represents same magnification for Figs. 2 to 6. Abbreviations: G, granular mass in sporoplasm; P, polar capsule; Pl, plasmodium adhering to gall bladder epithelium. Arrowheads in Fig. 3 are used to point out the four polar capsules seen in cross section in the spore.

Olympus BH-S photomicroscope (Olympus Corp., Tokyo, Japan) equipped with a calibrated ocular micrometer and are reported as micrometers (μ m), followed by the standard deviation, and then ranges in parentheses.

We found the gall bladders of eight of 14 *E. multiplicata griseogaster* infected: four of four from Van Buren County, four of seven from Conway County, and none of three from Independence. Two of seven *E. multiplicata multiplicata* from Saline County and one of one from Pulaski County were infected, as were two of 12 *E. neotenes* from Real County, Texas. Because of the higher prevalence and more numerous parasite stages in *E. multipli-*

cata griseogaster, this species was designated as the type host and all measurements taken from the parasites they harbored. No extra-histozoic forms were noted grossly in the tissues, nor were any stages found in blood smears.

No gross pathology was found associated with infected gall bladders, and histopathology was impossible as all gall bladders were examined as squash preparations. Plasmodia were adherent to the epithelium of the gall bladder and were small, ellipsoidal or subspherical, $31.5 \pm 6.3 \times 24.9 \pm 2.6 \mu$ m (20 to 40 \times 20 to 30 μ m) ($n = 20$) (Fig. 1). Most plasmodia failed to harbor mature spores, and the maximum number observed within those that did was

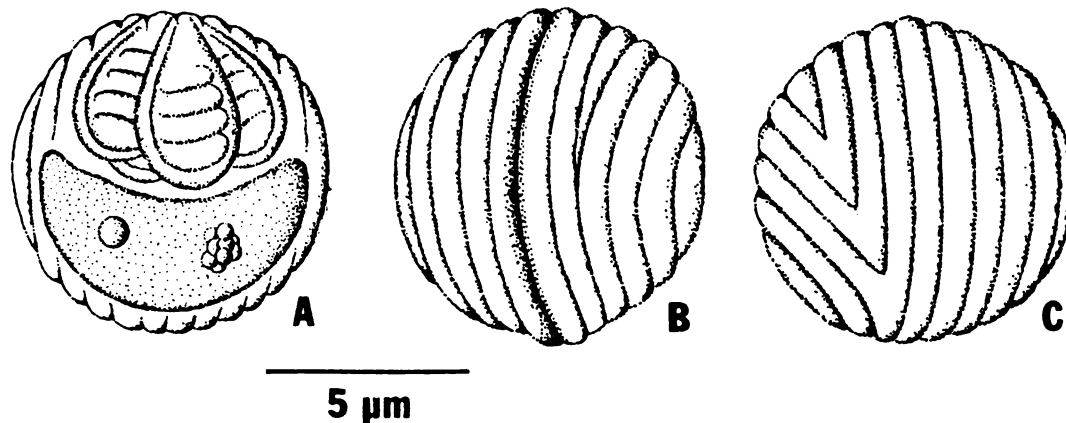


FIGURE 7. Composite line drawings of mature spores of *Chloromyxum salamandrae* sp. n. Fig. 7a. Individual spore showing internal details. Figs. 7b and 7c. Individual spores demonstrating outer wall structure.

eight. Spore development was disporoblastic (Figs. 1 to 3); mature spores were subspherical, $8.3 \pm 0.3 \times 7.7 \pm 0.4 \mu\text{m}$ (7.8 to 8.8×7.0 to $8.2 \mu\text{m}$) ($n = 20$) (Figs. 1 to 7), and had a shape index (length/width) of 1.07 ± 0.03 (1.02 to 1.14). Valves measured 0.8 to $1.0 \mu\text{m}$ thick and possessed 10 to 12 external striations each (Figs. 5 to 7). Four polar capsules were observed in each spore (Fig. 3), and each was piriform, $4.0 \pm 0.1 \times 2.6 \pm 0.1 \mu\text{m}$ (3.8 to 4.2×2.4 to $2.8 \mu\text{m}$) ($n = 20$). There appeared to be about four coils of the polar filaments. The sporoplasm was irregular in shape, and often contained a granular mass (Fig. 4). Occasionally an indistinct spherical mass could be seen in addition to the granular mass.

The chloromyxid reported by Thélohan (1894) from *Triturus cristatus* had caudal appendages; thus, it is unlike the species reported herein and its correct taxonomic placement should actually be in the genus *Caudomyxum* Bauer, 1948. Spores of *Chloromyxum protei* measured from histological sections of *Proteus anguineus* are larger, 10 to $13 \mu\text{m}$ in diameter (Joseph, 1907). Therefore, the *Chloromyxum* sp. reported herein is unlike any reported previously from amphibians, and we assign the name *Chloromyxum salamandrae* sp. n. to the parasite. Phototypes of this new spe-

cies have been deposited in the U.S. National Museum in Beltsville, Maryland (USA) as USNM No. 84161. Gall bladders containing plasmodia and spores in 10% neutral buffered formalin have also been deposited as syntypes as USNM No. 84162.

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