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A METHOD FOR FIXING AND STAINING PERITRICH CILIATES¹

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HAZEN, T. C., SMITH, G. & DIMOCK, R. V., JR. 1976. A method for fixing and staining peritrich ciliates. *Trans. Amer. Micros. Soc.*, 95: 693–695. A simple technique for fixing and staining peritrich ciliates involving fixation in phosphate buffered formalin and staining with Semichon's Acetic-Carmine is presented. This procedure appears to be superior to conventional methods for achieving relaxation of zooids and for differentiation of the macronucleus.

Our ongoing investigation of the epizootiology of a disease involving the bacterium *Aeromonas hydrophila* and the peritrich ciliate *Epistylis* sp. among centrarchid fishes in a South Carolina cooling reservoir (Esch et al., 1976) necessitates fixing in the field the characteristic lesions produced by this disease. Several attempts with well established fixation methods have failed to produce sufficiently well relaxed and expanded *Epistylis* colonies to allow subsequent examination of the preserved specimens. A suitable method to achieve the desired relaxation and fixation is herein described.

Pennak (1953), Kudo (1971), and Humason (1972) suggest that either Bouin's or FAA (Formalin-Acetic Acid-Alcohol) can be used as a fixative for ciliated protozoa. However, we have found that the use of either of these fixatives resulted in such contraction of the zooids that poor stain penetration was obtained and it was impossible to observe the oral ciliature (Fig. 1). Neither heating nor cooling these fixatives prior to use significantly alleviated the contraction problem.

In contrast to the results obtained with either Bouin's or FAA, we have had considerable success in fixing several peritrichs with phosphate buffered formalin (pH 7.0) (Humason, 1972). This fixative resulted in good relaxation of the zooids which permits observation of the oral ciliature and effects better stain penetration (Fig. 2).

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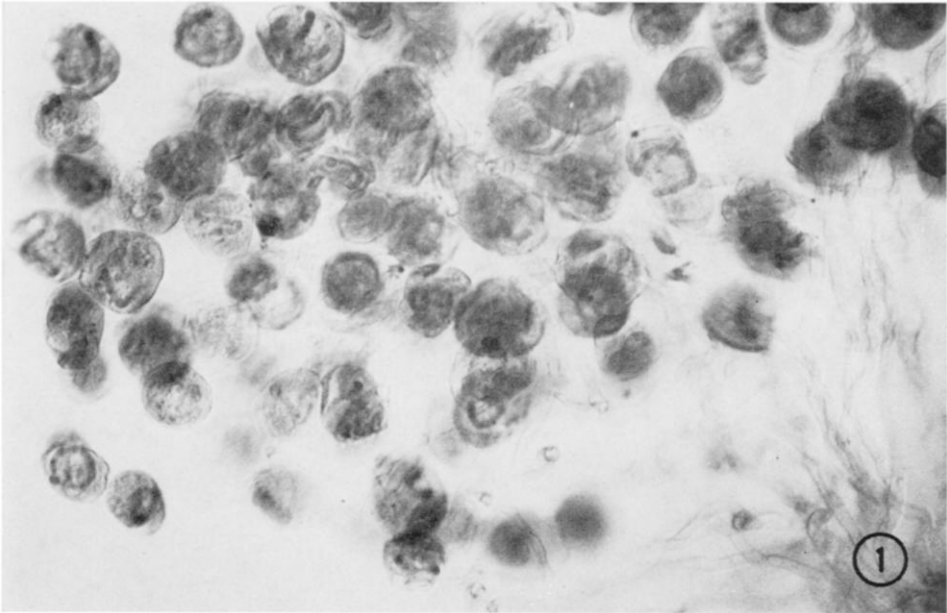


Fig. 1. Scrapings of *Micropterus salmoides* lesion. Fixed with Bouin's and stained with Semichon's Acetic-Carmine stain. $\times 500$.

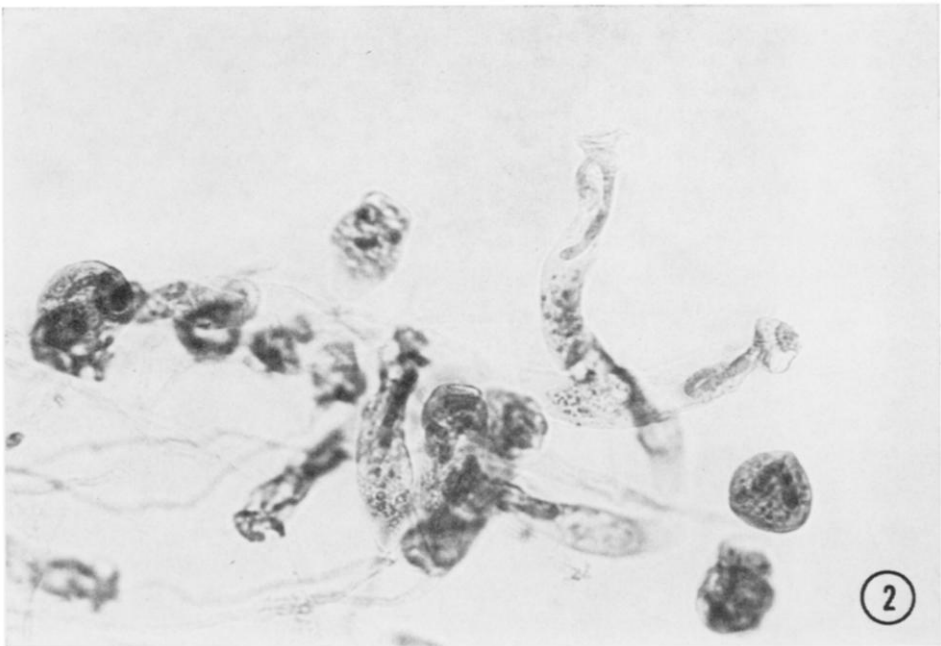


Fig. 2. Scrapings of *Micropterus salmoides* lesion. Fixed with phosphate buffered formalin and stained with Semichon's Acetic-Carmine stain. $\times 500$.

Delafield's hematoxylin and several vital stains (e.g., neutral red, methylene blue, toluidine blue) have been recommended for staining various protozoa (Humason, 1972; Kudo, 1971; Pennak, 1953). However, we have found that these stains resulted in a limited ability to differentiate the macronucleus from the cytoplasm of the peritrichs we have examined. We have achieved much better resolution of the macronucleus of *Epistylis* by staining with Semichon's Acetic-Carmine (Meyer & Olsen, 1971) followed by destaining in acid alcohol, dehydration, and clearing in xylene (Fig. 2).

This combination of techniques, involving fixing and relaxation with phosphate buffered formalin and subsequent staining with Semichon's Acetic-Carmine, has proven to be effective in our investigation of the lesions typical of the disease involving the *Aeromonas-Epistylis* complex. The techniques should have broader application in the study of other ciliates, since we have achieved similar results with *Vorticella* spp. and *Zoothamnium* sp.

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REVISION AND CHECKLIST OF THE SPECIES OF THE ASEPTATE GREGARINE GENUS *LECUDINA*¹

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LEVINE, N. D. 1976. Revision and checklist of the species of the aseptate gregarine genus *Lecudina*. *Trans. Amer. Micros. Soc.*, 95: 695-702. A checklist is given of the 42 accepted named species of gregarines of the genus *Lecudina* (phylum Apicomplexa, class Sporozoa, subclass Gregarina, order Eugregarinida, suborder Aseptatina, family Lecudinidae) together with their synonyms, the names of their hosts, their locations in their hosts, their known geographic distribution, and key references. The following taxonomic-nomenclatural innovations are introduced: *Lecudina bhatiai* n. nom., *L. bogolepovae* (Levine, 1971) n. comb., *L. criodrilii* (Sciacchitano, 1931) n. comb., *L. eunicae* (Lankester, 1866) n. comb., *L. nereicola* (Bogolepova, 1953) n. comb., *L. staurocephali* (Mingazzini, 1891) n. comb.

Gregarines are protozoa belonging to the phylum Apicomplexa, class Sporozoa, subclass Gregarina. There are almost 1,400 named species in 37 families. They live in the digestive tract or body cavity of invertebrates. There are three orders, of which by far the largest is Eugregarinida Léger, 1900. In this order,

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