Improved method for staining skeletal components of tilapias and some other fish in Alizarin Red S

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ABSTRACT

The use of morphometrical characteristics in the identification of freshwater fish species require certain specialized techniques. The staining of vertebrae and other skeletal components proved to be a tedious and timeconsuming affair in the past. The method that will be presented allows the staining of certain fish skeletons to an extent that it is possible to identify individual bone segments within a period of two days at the most. It is also less expensive than previous methods.

1 INTRODUCTION

The current taxonomic criteria for the determination of tilapias are still mainly based on classical morphometric analysis (Avtalion *et al.*, 1976). Vertebrae and intermuscular bones, as well as ray and spine counts on fins, have greatly assisted to accomplish the taxonomic differentiation between species (Boulenger, 1899, 1915; Dannevig, 1932; 1950; Trewavas, 1966; 1983; Jubb, 1967; 1974). This is especially usefull in areas, such as the Eastern Caprivi, where several closely related species inhabit the same water bodies. To facilitate classical morphometric analysis, it was necessary to find a method whereby skeletal features could be rapidly and reliably distinguished.

Some of the most generally used techniques include the following: Russell's (1973) improved method (a modification of the method described by Davies and Gore, 1936); include a fixation period for at least one week, digestion for up to four days, staining for 12 hr., destaining and clearing for up to four weeks. Sedra's (1950) method on the other hand, takes about two weeks and Humason's (1962; 1967) methods six days to complete. Taylor (1967) and Dingerkus & Uhler (1977) proposed enzyme methods for the clearing and staining of small vertebrates. These methods, although very effective, are far too expensive for everyday use.

The present method differs from those of the abovementioned authors in that less fixation is required and less chemicals are thus used accordingly. It is mainly the elimination of fixation that is responsible for the time gain by this method. At the most, it will take about two days to complete this technique. It will thus enable taxonomists and morphologists who are interested in the counts of vertebrae, intermuscular bones, spines and even rays to differentiate more rapidly between species.

2 MATERIAL AND METHODS

The following procedure is a modification of the standard methods and can be used for staining the vertebrae and bones of Osteichthyes.

(i) Kill the fish by transferring it into 70% ethanol and remove it from the ethanol when dead.

(ii) The fish is skinned, dissected and enviscerated so that only a thin layer of muscle and connective tissue is left to keep the skeleton intact.

(iii) Immerse into staining solution for some time depending on the size of the specimen. (About three hours for a 10 cm fish and longer for bigger fish).

Staining solution: 0,5 g Alizarin Red S
500 ml (4%) Potassium hydroxide

(vi) Destain overnight, or until the muscles are transparent, in three parts of a 4% solution KOH and one part (100%) glycerine.

(v) Counts, photographs, and morphometrical characteristics of the skeleton can now be analysed and the specimen can be stored in one part (4%) KOH and three parts (100%) glycerine.

It is recommended that small fish (7-30 cm) are used as specimens because less chemicals are required and the handling of small fish is much easier.

3 RESULTS

4 DISCUSSION

This is a simple and effective method that helps skeletal evaluation in fishes. The tissue is partially digested in KOH, stained with alizarin and cleared by adding glycerine to the muscles of the fish. When completed, there is a brilliant red skeletal structure in the transparent muscle and connective tissue due to the faster destaining of these tissues in comparison with bone. The specimen can then be stored in 100% glycerine or embedded in plastic. The use of less fixation did not cause putrefaction of the speciemens; seeing that fish are being stored, at present, for more than a year and a half with no signs of decay.

This method was tested on the following species: *Tilapia rendalli, T. sparrmanii, Serranochromis robustus jallae, S. macrocephalus, S. angusticeps, S. thunbergi, Sargochromis giardi, S. carlottae, S. codringtoni, Oreochromis mossambicus, O. andersonii, O. macrochir, Hemichromis elongatus, Chetia flaviventris, Labeo capensis, L. umbratus, Barbus holubi* and *B. aeneus* and was proved to be successful in all cases.

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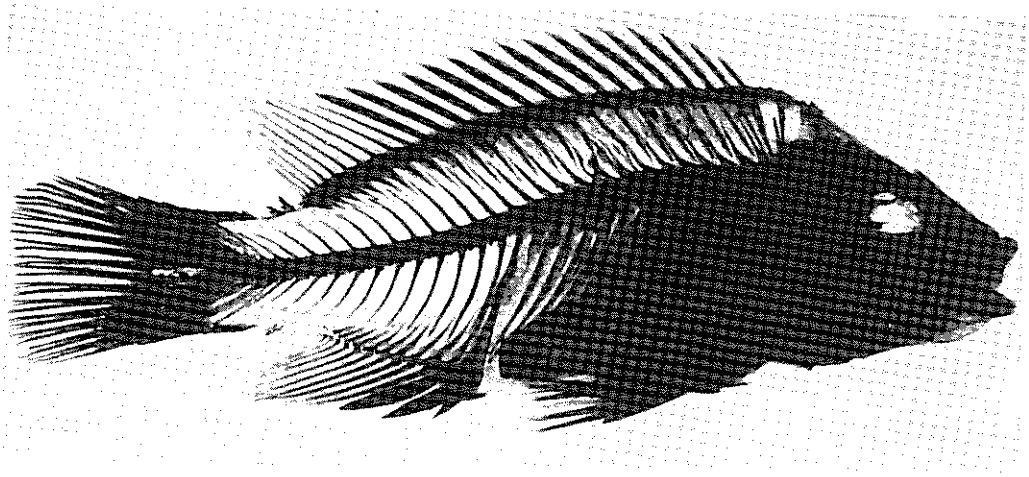

PLATE 1: *Oreochromis mossambicus* stained with Alizarin Red S.
It is evident that individual skeletal components such as vertebrae, pterygiophores, fin rays and spines can be clearly distinguished.