ISSN 1759-0116 (Online)

ZooNova

Occasional Papers in Zoology

Number 21, Pages 1 – 2

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Published on-line at https://zoonova.afriherp.org

Afriherp Communications, Greenford, United Kingdom

Date of publication: 11 December 2022

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A non-lethal method of blood collection from small anurans

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Abstract

This note describes a method of collecting small blood samples from living frogs that was used successfully a number of years ago but apparently has not been previously described.

Keywords

Diethyl Ether, Anaethesia, Heparinised micro capillary tubes, Blood Plasma, Isoelectric Focussing.

Introduction

During the preparation of the description of a new species of reed frog, *Hyperolius pickersgilli* Raw, 1982, it was decided to use isoelectric focussing (IEF) of blood plasma proteins in order to establish whether these were different from related species. This paper describes the non-lethal method used to collect the blood samples from the small species, some less than 25mm head and body length, used for this aspect of the study.

Materials and methods

Live specimens of several species of frogs were collected from sites on the KwaZulu-Natal coast, South Africa and in the vicinity of Pietermaritzburg. These were *Afrixalus fomasinii, Hyperolius pickersgilli, H. semidiscus, H.argus, H.pusillus, H.tuberilinguis, H. marmoratus* and *Leptopelis natalensis.*

Individual frogs were placed in a sealed container containing a wad of cotton wool to which a few drops of ether (Diethyl Ether) anaesthetic agent had been added. After a few minutes, as soon as the animal was observed to be unconscious, it was removed from the container and placed on its back to expose the ventral surface. The abdominal vein could then be seen through the skin. Using pre-sterilised fine iris scissors, a tiny incision was made through the skin and the abdominal vein nicked. A small sample of blood was then collected using heparinised glass micro-capillary tubes. As soon as sufficient blood was collected in the tube

(<50 µl) the incision was washed with water, this closed the wound and the frog was then placed in a second ventilated container to recover.

This was relatively quick and uneventful and once recovered the frog was returned to its normal vivarium. Some male *Hyperolius* were observed calling the same night following the earlier blood collection. This was taken to be a sign that the frogs were not significantly harmed during the procedure.

After sealing the ends of the micro-capillary tubes with plasticine, the samples were centrifuged to separate the red blood cells and buffy coat from the plasma.

The plasma samples were then loaded on a LKB Ampholine PAG plate (pH 3.5 - 9.5) before being subjected to IEF using a Pharmacia-LKB 2117 Multiphor flat bed electrophoresis apparatus powered by a Pharmacia ECPS 3000/150 power supply.

The results were subsequently published in Raw (1982: 125).

Discussion

While this obviously took place some forty years ago, the only other source traced regarding blood collection from anuran abdominal veins refers to a lethal method prior to specimen preservation (Swierk, Smylie & Owen 2017).

It is quite possible that improved anaesthesia agents for frogs are now readily available however this method is presented as the basis for further development.

Acknowledgements

The writer thanks Ron Berry (pers. comm. 2022) for details of the IEF process and Deon Oosthuizen for encouraging him to write this note. Drs Angelo Lambiris, Alex Morrow and Andrew Whittington kindly commented on the draft.

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Accepted 10 December 2022