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Miscellanea

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Isoelectric Focusing in the Taxonomy of Bulinid Snails*

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The identification of Bulinid snails is still difficult and their taxonomy is not always well established (SOUTHGATE & KNOWLES, 1975). Field workers who are not familiar with morphological taxonomy are frequently confronted with this problem. This was the case in Madagascar, where it was very difficult to distinguish *Bulinus obtusispira*, the intermediate host of *Schistosoma haematobium*, from *B. liratus* (DEGRÉMONT, 1973).

Several attempts to develop serological and biochemical taxonomy have been made (WRIGHT, 1966) using different methods such as electrophoresis on cellulose acetate membranes (WRIGHT & ROSS, 1967), enzyme electrophoresis (COLES, 1970), gel diffusion (BURCH & LINDSAY, 1970) and disc electrophoresis (DAVIS & LINDSAY, 1967). We have already studied the abilities of all the methods mentioned above to distinguish between Bulinid snails from Madagascar (WEISS et al., 1974). Recently, the method of isoelectric focusing was used in bacterial (MATTHEW & HARRIS, 1975) and bird taxonomy (FRÉLIN et al., 1973).

The concept of isoelectric focusing in gels (IEF) was developed by SVENSSON (1961). We used the method described by RIGHETTI & DRYSDALE (1974) employing the Multiphor (LKB 2117) apparatus and Ampholine^R polyacrylamide gel plates with the pH-range from 3.5 to 9.5 (PAG plate Nr. 1804-101 by LKB). The d.c. power supply was a PS 10A multistab capable of delivering 1000 V.

The snails studied were: *Bulinus obtusispira* and *Bulinus liratus* (Tanandava, Madagascar). We compared their patterns with those of snails of three Bulinid groups, namely: *Bulinus tropicus*¹ (Zambia), *Bulinus cernicus*¹ (Mauritius) and *Bulinus nasutus* (Ifakara, Tanzania). For the experiment an egg-clutch was simply crushed on a filter-paper, which was laid upon the PAG plate. The number of eggs per clutch should be between three and ten to get the main bands and between ten and twenty to get the minor bands. Up to 48 samples could be examined on one plate. The egg clutches can be preserved in tap water at 4 °C, in glycerol or in physiological salt solution at room temperature to inhibit the development of the snail embryos.

The patterns obtained are presented in figures 1 and 2. With the IEF-method we found at least twice the number of separated proteins as with disc-electrophoresis. With the procedure described above the reproducibility was very good. Although the general pattern is very complex, some of the most characteristic bands are easily identified. In agreement with the systematics of the Bulinid group *B. liratus* and *B. tropicus* are showing very similar patterns. The pattern of *B. obtusispira* is completely different from that of *B. (Physopsis) nasutus*. The distinction between *B. obtusispira* and *B. liratus* can be made accurately with only one egg-clutch. *B. cernicus* shows the pattern of a species belonging to the *forskalii* group.

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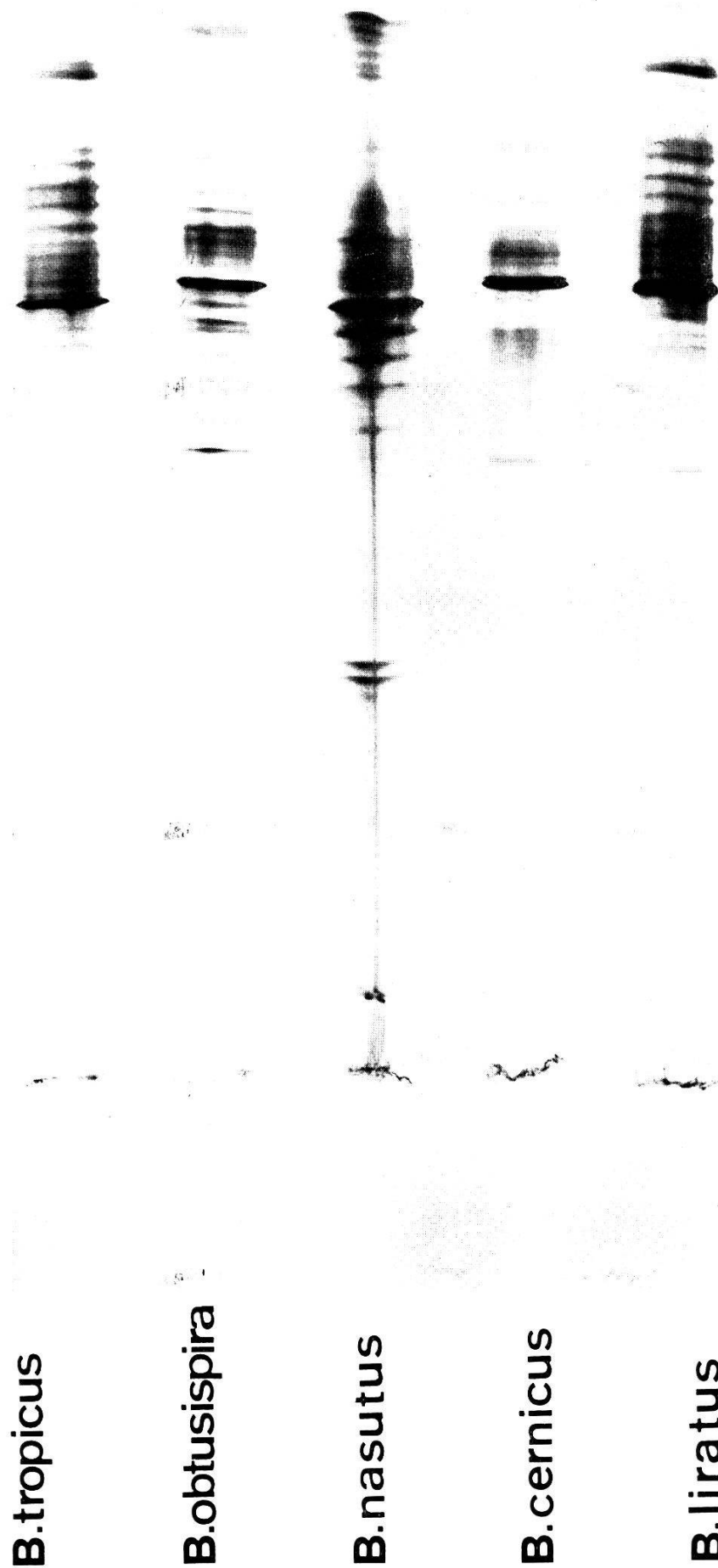


Fig. 1. IEF patterns of egg proteins from different Bulinid snails.

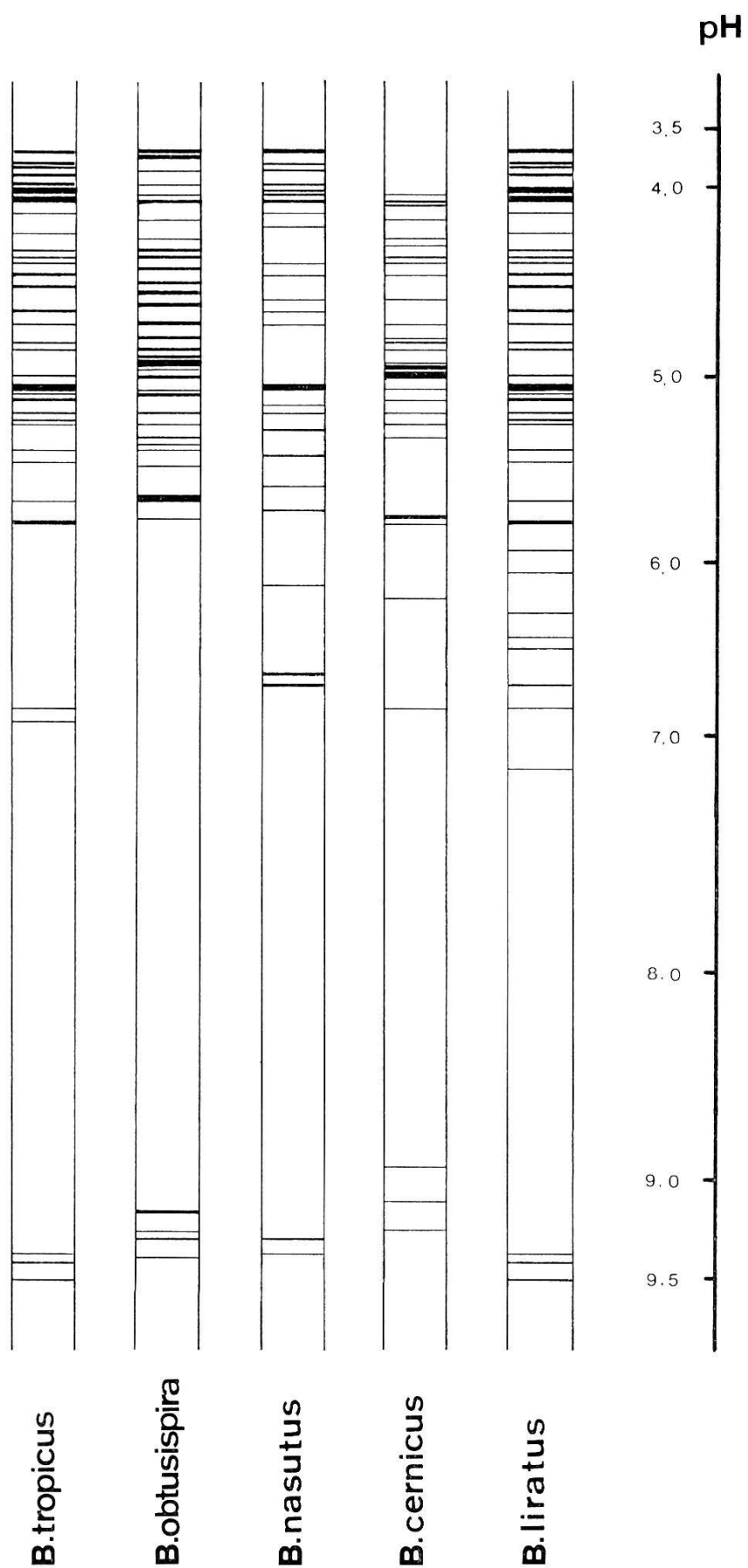


Fig. 2. Schematic representation of IEF shown in fig. 1.

With eggs stored for two weeks the results were not modified as long as the embryogenesis had been blocked.

In conclusion, isoelectric focusing appears to be an accurate and reliable technique for snail taxonomy. It has the advantage that a large number of specimens can be tested simultaneously and in a short period of time. Although this method is rather sophisticated, the facts that commercial plates are available and that eggs or living snails can be stored or posted, make it especially useful for taxonomical research and epidemiological work at large scale.

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