# The *Bulinus nasutus* complex (*Bulinus nasutus* (Martens, 1879) and *Bulinus productus* Mandahl-Barth, 1960) (Gastropoda: Planorbidae) in the Lake Victoria area elucidated by enzyme-profile electrophoresis and natural infections with *Schistosoma* spp. (Trematoda: Schistosomatidae)

JENS ERIK JELNES, FREDERICK W. THIONGÓ, NICHOLAS J. S. LWAMBO & JOHN H. OUMA

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Data on natural *Schistosoma* spp. infections in snails belonging to the *Bulinus nasutus* complex are provided along with enzyme-profile data for *B. nasutus* (Martens, 1879) and *B. productus* Mandahl-Barth, 1960. Natural infections with *Schistosoma* only occur in *B. productus* and this species shows an enzyme-profile strikingly different from that of *B. nasutus*. At least two of the eight enzymes studied produced enzyme bands ascribable to schistosomes. Further studies in this area are suggested, and a translation table to previous studies is given.

Keywords: Africa, Kenya, Tanzania, experimental taxonomy, freshwater snails, enzyme-profiles, *Bulinus*, *Schistosoma*

Jens Erik Jelnes, Zoological Museum, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark. E-mail: jejelnes@zmuc.ku.dk [Prior to 1984, when most of the work reported on here was carried out, JEJ was employed at the Danish Bilharziasis Laboratory, Charlottenlund, Denmark].

Frederick W. Thiongó, Division of Vector Borne Diseases, Ministry of Health, PO Box 20750, Nairobi, Kenya. E-mail: FWT50@hotmail.com

Nicholas J. S. Lwambo, National Institute for Medical Research, Mwanza Research Institute, PO Box 1462, Mwanza, Tanzania. E-mail: LwamboN@hotmail.com

John H. Ouma, Kenya Medical Research Institute, PO Box 54840, Nairobi, Kenya. E-mail: Schisto @africa-online.co.ke

## INTRODUCTION

Species of schistosome intermediate snail hosts are difficult to identify correctly, especially so if only young individuals are available. The *Bulinus nasutus* complex in the Lake Victoria region is no exception. This complex has been studied morphologically (Kristensen 1986, Kristensen et al. 1987), by isoelectric focusing of 4 enzymes (Rollinson & Southgate 1979, Wright & Rollinson 1979), by single enzyme electrophoresis (Jelnes 1979) and by DNA techniques (Raahauge & Kristensen 2000). It is well known that this complex plays a major role in transmission of *Schistosoma haematobium* (Bilharz, 1852) in the Kisumu area, Kenya (Kinoti 1971) and in the Mwanza area, Tanzania (Webbe 1962, McCullough et al. 1968, Loker 1981). The role the *Bulinus nasutus* complex plays in transmission of *Schistosoma bovis* (Sonsino, 1876) is not yet clarified, but Kinoti (1971) identified *S. bovis* infection in both *B. africanus* (Krauss, 1848) and *B. nasutus*. Southgate & Knowles (1975) isolated *S. bovis* cercariae from snails characterized as "intermediate between *B. africanus* and *B. globosus*" in one area around Kisumu and snails "with affinities for *B. africanus/B. nasutus*" in another area around Kisumu, and Southgate et al. (1980) identified one *B. africanus* infected with both male and female miracidia of *S. bovis* in the Mwanza area. Several studies have dealt with laboratory infection of bulinid snails with *S. bovis* (see Brown 1994, chapter 5 for an overview).

This study presents data that can be used to correctly identify snails of all sizes of the *Bulinus nasutus* complex to species and gives preliminary data, based on which further studies could provide a clue to the identification of schistosome infection in snails to the correct parasite species without carrying out experimental infections in rodents.

## MATERIALS AND METHODS

Snails were collected in the field and either mailed or hand carried live to the Danish Bilharziasis Laboratory (DBL), where the snails were put singly into small plastic cups containing aerated tap water under artificial light for at least 4 hours to assess survival and possible patent trematode infections.

The following material was studied:

Topotypic material of *Bulinus nasutus* (Martens, 1879) collected by JEJ and A. Mutani on 23 January 1981: Sample DBL 81/21 originating from Pimbini Pool at Bagamoyo, Tanzanian coast; sample DBL 81/17 originating from a pool 800 m E of Zanzale River, Bagamoyo area, Tanzanian coast.

Material of the *Bulinus nasutus* complex from the Lake Victoria area: Samples DBL 81/235, 235A, 245 collected in Tiengre Stream, 8 km W of Kisumu, western Kenya by FWT in November 1981. These samples were transported to DBL by Division of Vector Borne Diseases (DVBD) students. Sample DBL 79/166 collected by JEJ in November 1979 in Bandani pools, Kano Plain, Western Kenya. Sample DBL 78/39 collected in Kisat Stream, Kisumu by JEJ in August 1978. Samples from the Mwanza area included: Samples DBL 79/118 and DBL 79/119 collected by JEJ and D. B. Matovu on 23 June 1979 at Mbela, Missungwi and Missungwi streams, respectively, and samples DBL 83/101 and DBL 83/102, which were brought live to the DBL by NJSL in August 1983. These two samples originate from residual pools, Mwanza, Ihale and Missungwi, Nyang'homango, Usagara, respectively.

Mice were infected with schistosome cercariae from single infected snails using standard laboratory methods. The mice were maintained for 60 days, then anesthetized with Nembutal® and perfused to recover the adult worms. Thereafter the mice were sacrificed and their livers and intestines were digested in 10% sodium hydroxide at 37°C overnight. The next day samples of the digested material were studied under the microscope to determine the type of schistosome eggs contained in individual mice.

Enzymes from the living snails were analyzed electrophoretically by freezing the snails on a piece of filter paper. After thawing, the soft parts were removed from the shell and part of the digestive gland and gonads were placed in a micro centrifuge tube for maceration. The remaining soft parts and the shell of individual snails were preserved in 70% alcohol in small vials marked with date and electrophoresis number, thus making a morphological and anatomical study possible on material used for electrophoresis.

Determination of chromosome number and enzyme-profile electrophoresis on individual snails were carried out as described by Jelnes (1984), using *Bulinus truncatus* (Audouin, 1827) from Dezful, Iran as a reference. Enzyme electrophoresis of cercariae and adult schistosomes was attempted in the same electrophoretic system on some of the material.

## RESULTS

### *Initial parasitological survey*

The results of screening the snails of samples 79/166, and 81/235 on arrival at DBL revealed the following: 79/166: 44 live, 5 dead, 8 infected with schistosomes; 81/235: 182 live, 52 dead, 16 infected with schistosomes. No other cercarial type was observed. No data are recorded for infections in samples 83/101 and 83/102.

*Enzyme-profile results*

All in all more than 200 individuals were used for enzyme-profile electrophoresis. The results obtained are given in Table 1. As snails from some samples were analyzed electrophoretically days or even months apart, it was possible to calculate the standard deviation for those enzymes that differed in relative mobility from the reference material. The standard deviation ranged from 0.09 to 0.003, mostly in the range 0.02–0.04 based on 2–4 electrophoretic runs. As no comparisons were possible between material collected in Kisumu area (79/166, 81/235 and 81/245) plus Bagamoyo area (81/17 and 81/21) versus material collected in the Mwanza area (83/101 and 83/102), the electrophoretic mobilities of the latter samples are given as the actual (mean) value for the samples.

Table 1 gives the sample numbers, species, locality, number of individuals analyzed and the enzyme-profiles observed. In case of polymorphic enzymes the mobilities observed are given with the calculated gene frequency in brackets.

The data in Table 1 show that in the Lake Victoria area the enzyme mobilities of phosphoglucose isomerase (PGI) (1.07 versus 1.4), beta-hydroxybutyrate dehydrogenase (HBDH) (0.98 versus 1.30) and xanthine oxidase (XO) (missing sharp band versus 0.95) can be used to safely differentiate between *Bulinus nasutus* and *B. productus*. Further differences in electrophoretic mobility are found in the enzymes alpha-glycerophosphate dehydrogenase (GPDH) and isocitrate dehydrogenase (IDH), but here the differences are less pronounced and thus not recommended for identification purposes. In phosphoglucomutase (PGM 1, PGM 2) and in glutamate-oxaloacetate transaminase (GOT) no clear differences in enzyme mobility could be found. In the area of the type locality of *B. nasutus* (Bagamoyo on the Tanzanian Coast) a polymorphism occurs in HBDH. Polymorphisms in phosphoglucose isomerase were found in *B. nasatus* (Kisumu area) and in *B. productus* (Mwanza area). Further material of *B. nasutus* from Kisumu area has been studied electrophoretically for PGI and HBDH in the years 1974–76. These data (see Jelnes 1979 for PGI) confirm the observations given above,

Table 1. Enzyme-profile for material of *Bulinus nasutus* and *B. productus* from the Lake Victoria region and the type locality of *B. nasatus.*

| Sample no | Locality | No of individuals | Enzyme-profile | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | PGI | HBDH | XO | PGM1 | PGM2 | GPDH | IDH | GOT |
| | | | ***Bulinus nasutus*** | | | | | | | |
| 81/17 | Bagamoyo, Tanzania (Type locality) | 17 | 1.08 | 0.93 (0.91)<br>1.14 (0.09) | Missing | 0.84 | 1.03 | 1.59 | 0.94 | 1.00 |
| 81/21 | Bagamoyo, Tanzania (Type locality) | 42 | 1.08 | 0.93 (0.39)<br>1.14 (0.61) | Missing | 0.84 | 1.03 | 1.59 | 0.94 | 1.00 |
| 79/118 | Mwanza, Tanzania | 28 | 1.08 | 0.93 | Missing | 0.84 | 1.03 | 1.59 | 0.94 | 1.00 |
| 83/102 | Mwanza, Tanzania | 20 | 1.08 | 0.93 | Missing | 0.84 | 1.04 | 1.59 | 0.94 | 1.00 |
| 81/235A | Kisumu, Kenya | 5 | 1.08 (0.90)<br>0.93 (0.10) | 0.93 | Missing | 0.84 | 1.03 | 1.59 | 0.94 | 1.00 |
| | | | ***Bulinus productus*** | | | | | | | |
| 79/119 | Mwanza, Tanzania | 10 | 1.20 (0.50)<br>1.40 (0.50) | 1.26 | 0.93 | 0.83 | 1.03 | 1.57 | 0.84 | 1.00 |
| 83/101 | Mwanza, Tanzania | 20 | 1.22 (0.92)<br>1.40 (0.08) | 1.29 | 0.95 | 0.84 | 1.04 | 1.58 | 0.85 | 1.00 |
| 78/39 | Kisumu, Kenya | 3 | 1.36 | 1.29 | 0.96 | 0.82 | 1.00 | 1.51 | 0.82 | 1.00 |
| 79/166 | Kisumu, Kenya | 27 | 1.36 | 1.29 | 0.94 | 0.87 | 1.05 | 1.48 | 0.84 | 1.00 |
| 81/235 | Kisumu, Kenya | 70 | 1.37 | 1.31 | 0.95 | 0.84 | 1.05 | 1.61 | 0.85 | 1.00 |
| 81/245 | Kisumu, Kenya | 16 | 1.40 | 1.30 | 0.96 | 0.81 | 1.00 | 1.55 | 0.84 | 1.00 |

and in no individuals were parasite bands observed in PGI.

When the electrophoretic plates were stained and left for a sufficiently long time, additional bands turned up in PGI and alpha-glycerophosphate dehydrogenase (GPDH). The bands were not systematically searched for, but combining the different band positions observed in samples 79/166 and 81/235 resulted in the following parasite enzyme bands originating from schistosomes:

PGI-(-0.12); 0.00; 0.12; 0.24; 0.36, 0.48, and

GPDH-0.90 (only observed in two runs and only in individuals that also showed parasite PGI bands).

Both PGI and GPDH parasite bands were only observed in *Bulinus productus*.

The schistosome eggs observed after digestion of mice livers and intestines gave the following preliminary results: some mice contained *Schistosoma bovis*, some *S. haematobium*, and one mouse contained both *S. bovis* and *S. haematobium* eggs. The latter indicates infection with at least four miracidia in that snail.

Chromosome numbers in the egg masses studied of the two species *Bulinus* of revealed a diploid number of 2n = 36.

## DISCUSSION

From the data presented in Table 1 differences in electrophoretic mobility were recorded for five of the seven enzymes reported on. As the material either is from the same body of water or from a limited geographic area (Mwanza samples) sampled at times within intervals of a few days, there can be no doubt that in this case we are dealing with two distinct species and not two subspecies as suggested by Brown (1994). This was also the conclusion of Kristensen (1986) and Kristensen et al. (1987) using morphometric analysis on, among other material, shells of the Kisumu and Bagamoyo area samples reported on here.

In the period 1950–1975 it was generally the opinion that one waterhole could not contain more than one species belonging to the same species group. However, Jelnes (1979 & 1980) found up to three different species of a species group in one single body of water. As one of us (JEJ) has firsthand knowledge of the tradition of identification by the persons who identified snail material from the different epidemiological studies, the interpretation of the snail material with respect to distinction between *B. nasutus* and *B. productus* is indicated in Table 2.

Further records of natural infection with *S. haematobium* refer to *Bulinus africanus* (Mc Clelland 1956, Teesdale & Nelson 1958, Southgate et al. 1980), *B. globosus* (Morelet, 1866) (Cridland 1955), and *B. nasutus* (Dowdeswell 1938, Southgate et al. 1980), and infection with *S. bovis* is ascribed to *B. africanus* by McClelland (1956), Teesdale & Nelson (1958) and Southgate et al. (1980), and to *B. nasutus* by Dowdeswell (1938).

As can be seen, great confusion generally exists regarding which species transmit *S. haematobium*. Only the study by Webbe (1962) presents results which agree with those presented here, namely that *B. productus* is the only intermediate snail host in the area. In the section on "Biology and cercarial infection of the principal molluscan host" he states in the first line of "Observations" that "All data hereafter refer to *Bulinus (P.) nasutus productus.*". Some support for the observation that *B. nasutus* is not an intermediate host for *S. haematobium* in the Lake

Table 2. Table showing the record of intermediate snail hosts in different field studies on the occurrence and/or epidemiology of *Schistosoma haematobium*. Except for the two marked with *, all were indicated as intermediate hosts.

| Reference | *Bulinus nasutus* | *Bulinus productus* |
|---|---|---|
| This paper | *Bulinus nasutus** | *Bulinus productus* |
| Loker et al. 1981 | *B. africanus, B. nasutus* | *B. nasutus* |
| Kinoti 1971 | *B. africanus* | *B. africanus, B. nasutus* |
| McCullough 1968 | *B. africanus, B. nasutus* | *B. nasutus* |
| Webbe 1962 | *B. nasutus nasutus** | *B. nasutus productus* |

Table 3. Names used for *Bulinus nasutus* and *B. productus* in various recent taxonomic studies on material from the Lake Victoria area.

| **Reference** | ***Bulinus nasutus*** | ***Bulinus productus*** | **Remarks** |
|---|---|---|---|
| This paper | *Bulinus nasutus* | *Bulinus productus* | Enzymes, parasitology |
| Brown 1994 | *B. nasutus nasutus*, photo p. 212, fig. 103c | *B. nasutus* form *productus*, photo p. 212, fig. 103b | Morphology |
| Jelnes 1979 | *B. nasutus* PGI 1.04 | *B. africanus* PGI 1.44 | Enzymes |
| Wright & Rollinson 1979 | *B. africanus* GPI type 2? | *B. africanus* GPI type 3? | Enzymes |
| Kristensen 1986 | *B. nasutus* | *B. productus* | Morphometrics |
| Kristensen et al. 1987 | *B. nasutus* | *B. productus* | Morphometrics |
| Raahauge & Kristensen 2000 | *B. nasutus* | *B. nasutus productus* | DNA |

Victoria area can be found in observations from Zanzibar Island (Stothard et al. 1997).

As the results presented in this contribution on the intermediate host spectrum refer only to the Kisumu area, there is a need for further in-depth studies to verify the hypothesis that *B. productus* is the only intermediate host of the *B. nasutus* complex for *S. haematobium* and *S. bovis* in that area. Such a study should be based on enzymic identification of the bulinid snails. Further possibilities are *B. globosus*, which is known to be an intermediate host for these schistosomes over wide areas of Africa and *B. africanus* known as an intermediate host in Eastern and Southern Africa (Brown 1994).

Table 3 presents a translation key to the most important taxonomic studies which have appeared since 1979. It serves the purpose of correlating, to the best of our knowledge, the names used in various taxonomic studies.

Wium-Andersen & Simonsen (1974) were the first to record PGI parasite bands in electrophoresis of infected planorbid snails; indeed, they could detect the bands prior to the shedding of cercariae. In this study the same observation was made for *B. productus*, but no systematic recording of the bands was carried out. The occurrence of the parasite bands in GPDH is here recorded for the first time; it may be that this, too, can be detected in prepatent snails. In order to clarify this, and the potential of using these parasite bands as a means of separating infections with *S. bovis* from infections with *S. haematobium*, further studies based on a larger material than that studied here is required. Such a study could be conducted in the Lake Victoria area, as the electrophoretic technique used in this study has in fact been carried out in a hotel room in Kisumu.

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