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# THE ROLE OF BIOTIC AND ABIOTIC FACTORS IN CONTROLLING DISEASE-TRANSMITTING SNAILS IN KENYA: AN EXPERIMENTAL STUDY

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## 1. Executive Summary

The goal of this project was to develop low-cost, technically simple and sustainable methods of biological control of the freshwater snails that transmit schistosomes and other trematode parasites in sub-Saharan Africa. Such parasites are prevalent in Africa and inflict an unacceptable burden of suffering on both human and domestic animal populations. Our studies have revealed that the North American red swamp crayfish Procambarus clarkii is a voracious predator of disease-transmitting pulmonate snails and can reduce or eliminate target snail populations in natural habitats in Kenya. P. clarkii, originally introduced into East Africa for aquaculture purposes, is now widely distributed in Kenya, and supports a small but thriving export industry centered at Lake Naivasha. We are of the opinion that the judicious use of crayfish in schistosomiasis control operations can provide a significant new weapon to achieve sustainable snail control and thus offers the hope of breaking the cycle of transmission of this insidious disease. As crayfish attack non-target species as well, we presently advocate their use in man-made earthen dams which are ubiquitous in Kenya, function as transmission sites, and do not harbor an endangered biota. Further discussion of these issues is provided below. Also discussed below are studies of other control agents such as competitor/predator snails, the development of a geographic information system to facilitate analysis of factors influencing snail distributions, and spin-off studies pertaining to snail systematics. The results were obtained on the U.S. side through the efforts of Dr. Eric S. Loker at the University of New Mexico and two of his students, Dr. Bruce Hofkin (crayfish work) and Ms. Tracy Boyce (GIS). On the Kenyan side, Dr. Gerald Mkoji at the Kenya Medical Research Institute (KEMRI) was the major contributor. He received

substantial administrative help from KEMRI's director, Dr. Davy K. Koech, technical help from several workers at KEMRI, and considerable assistance from Dr. John Ouma and his staff at the Division of Vector Borne Disease, Nairobi. Kenyan colleagues contributed substantially to all phases of the project.

## 2. Research Objectives

The specific objectives stated in the original proposal were to:

1. Assess the ability of indigenous freshwater decapod species to reduce or eliminate populations of disease-transmitting pulmonate snails in small, man-made impoundments in Kenya. Such decapods were to be tested both singly and possibly in combination with ampullariid snails in dams in central Kenya.

2. We proposed to assess the ability of the North American crayfish Procambarus clarkii, already known to be widely distributed in Kenya, to eliminate or reduce in size snail populations in dams where it was present.

3. We also proposed to integrate data pertaining to snail distributions in Kenya into a geographic information system so that the influence of climate, geography, and geologic factors on snails distributions could be assessed. This GIS was to be used in conjunction with multivariate statistical methods to assess the relative importance of biotic and abiotic factors in controlling snail distributions.

For reasons stated in the following section, our first field trips associated with this project convinced us that an early change in course was appropriate. Our attentions turned away from indigenous decapods and more and more to crayfish because our initial surveys indicated that target pulmonate species rarely if ever were present in dams with thriving crayfish populations. Heartened by the evident impact of crayfish on snails, our major research thrust was then directed to exploring the interactions between crayfish and snails.

Also, our field trips naturally carried us into different parts of Kenya and presented unique collecting opportunities. Dr. Loker routinely collected Biomphalaria specimens during these trips and brought them to the U.S. where they were used in systematic studies of this snail genus undertaken by one of Dr. Loker's students, Ms. Susan Bandoni. The systematic study of Biomphalaria was never one of the official objectives of the proposal, but the unique access to specimens provided by this project allowed us to make substantial contributions to this field, without in any way deflecting time, money or effort from our major project-related activities.

## 3. Methods and Results

The methods employed in our subsequent investigations and our results are thoroughly described in the publications resulting from project activities; these publications appear in chronological order in the appendices. Also presented in the appendices are methods and results associated with project-related activities that have yet to be published. A brief summary of our results is presented below.

Our initial intent was to investigate indigenous decapod species for their potential as snail control agents. We discovered their systematics were very poorly known thus rendering identification virtually impossible. Also, intensive efforts to collect indigenous decapods yielded insufficient numbers for further study. Finally, our attentions were diverted away from indigenous decapods by the observation that populations of some target pulmonate species (particularly Bulinus (Physopsis) globosus) seemed to thrive in habitats where these decapods were abundant. It seemed the target species were able to avoid attack by decapods by climbing onto floating vegetation.

We also considered indigenous competitor/predator snails such as the ampullariids Lanistes carinatus and Pila ovata as potential control agents. We discovered that they consume target snails and their egg masses and would establish in natural habitats. Over the course of several years, we have not observed them to displace target species however, and for that reason consider them to be only partially successful control agents. The same comments appear to be true for Melanoides tuberculata, a thiarid snail that in Kenya coexists over long intervals with pulmonate species. Three publications (appendices 2, 3, and 7) and some unpublished information (appendix 13) further discuss interactions between ampullariid or thiarid snails and target pulmonate species.

Early in the project we examined natural habitats containing crayfish to determine if such habitats harbored snail populations. When it became clear to us that crayfish and snails were negatively associated, much of our remaining time and resources were devoted to the study of crayfish which are widely distributed in the central and western highlands of Kenya. Details are provided in resulting publications (appendices 4, 5, 6, 8 and 9) and in unpublished observations in appendices 14-17. We first documented a strong negative association between crayfish and target snails in natural habitats. Further laboratory and field experiments indicated that crayfish readily consumed target species, and were capable of eliminating snail populations even in the presence of alternative food sources. Experiments carried out in small pools in an extensive quarry near Nairobi revealed that crayfish would quickly eliminate populations of Biomphalaria pfeifferi, intermediate host of Schistosoma mansoni. Other laboratory work indicated that crayfish also have a detrimental effect on mosquito larvae (appendix 14). Updates are also provided to describe their current geographic distribution in Kenya (appendix 15), their survival in coastal Kenya under experimental conditions (appendix 16), and their survival in quarry pits in Kangundo (appendix 17).

Another goal of the project was to develop a geographic information system to incorporate Kenyan snail distribution data. Delays were experienced in adapting our snail distribution data to a GIS format and in preparing appropriate digitized maps of Kenya. This necessitated the request for a one year no-cost extension. Although we had difficulty

in obtaining high quality temperature and rainfall data for Kenya for the GIS, we have generated a GIS covering snail distributions in Kenya and further details are provided in appendix 18. Work in this appendix relates to, and derives from, appendix 10 which is an abstract for a very lengthy paper on Kenyan freshwater snails distributions which was more fully discussed in the final report of our previous PSTC proposal. The manuscript associated with this abstract is now in press.

In addition to addressing the major goals of the project, in the final stages of the project we were also able to confirm that populations of the exotic ampullariid snail Marisa cornuarietis are present in a natural habitat on a sugar plantation near Moshi, Tanzania (see brief report in appendix 19). Although we do not advocate the use of this snail in control operations anywhere in Africa, it is noteworthy that the snail is present very close to the Kenyan border.

Also, while undertaking various trips associated with the biological control aspects of the project, Dr. Loker collected Biomphalaria snails throughout Kenya and brought them to the U.S. where they were used by Dr. Susan Bandoni in systematic studies of this medically important snail genus. Appendices 1, 11, and 12 provide details regarding systematic studies undertaken with Biomphalaria snails collected during the course of this project.

Finally, as a result of the experiences provided by this project, Dr. Bruce Hofkin has been able to put together a new theoretical foundation for consideration of biological control agents to be used against disease vectors in aquatic environments. His ideas, which he presented at a recent joint meeting of the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene, are presented in appendix 20.

## 4. Impact, Relevance and Technology Transfer

Many of the comments in this section are derived from the text of appendix 9. We feel the findings of this project will be useful and provocative in many ways. The results establish the precedent that a biotic factor, namely crayfish, can have a significant impact on the distribution and abundance of trematode-transmitting snails in East Africa. The results are especially provocative since the control agent in question is an exotic species that was originally introduced into this region for reasons totally unrelated to parasite or snail control, namely for aquaculture purposes. Indeed, crayfish support a small export industry for Kenya.

As crayfish are already well-established in Kenya, and probably in Uganda as well, our results raise the possibility of deliberately using crayfish in schistosomiasis control operations in these countries. The possibility of deploying a biological control agent to control snails in sub-Saharan Africa is both exciting and daunting. Schistosomiasis control programs in this region have tended to rely heavily on treatment of the infected human population and on intermittent or focal snail control operations using molluscicides. Snail control invariably proves to be too expensive to sustain and as chemotherapy alone is insufficient to break the cycle of transmission, the control programs typically fail. The use of an inexpensive biological control agent offers the prospect of sustainable snail control thus potentially adding another dimension to schistosomiasis control operations in Africa. Of course, use of crayfish in control operations needs to be carefully considered by the proper authorities. Crayfish are voracious omnivores and are likely to have effects on non-target biota. Their use may ultimately be restricted to small, man-made impoundments that do not harbor endangered or threatened species.

As all phases of our work have been undertaken in collaboration with the Division of Vector Borne Diseases of the Ministry of Health of Kenya, the appropriate authorities in our host country are well aware of our findings. Dr. John Ouma of the DVBD has been very supportive of our work and has become convinced of the ability of crayfish to control target pulmonate snails. He has joined with us in recommending further studies in Kenya designed to determine if crayfish can actually interrupt transmission in selected habitats. This is precisely what we hope to do with the PSTC grant we were recently awarded.

Our results have also prompted schistosomiasis workers at both the DVBD and at the Kenya Medical Research Institute (KEMRI) to reevaluate certain changes in snail distributions noted in Kenya in recent years. For example, historically Schistosoma mansoni has been considered to be abundant in the Muranga district to the north of Nairobi. Recent survey trips to the region failed to report the appropriate snail species in habitats known to formerly contain snails. One factor not heretofore considered as a possible explanation for declining snail abundance in this region is the presence of crayfish which are abundant in this part of Kenya.

We feel this project will have a long-lasting impact on our colleagues in Kenya and on their future attempts to control snail-transmitted parasites. Activities associated with this project have considerably heightened awareness among the relevant cadre of schistosomiasis control workers in Kenya of the need to consider biological options for controlling schistosome-transmitting snails. Furthermore, methods used for collecting snails and

putative control agents, for documenting their interactions in both field and laboratory studies, and for considering the value of such control agents have now become routine for these workers. Our Kenyan colleagues are fully capable of undertaking these kinds of investigations on their own and making mature and informed decisions regarding the deployment of biological control agents in their country.

In addition to both laboratory and field experience obtained in country, some of Kenyan colleagues also acquired additional knowledge about medical malacology and snail-trematode interactions in Dr. Loker's laboratory. Some of the techniques taught to Kenyan colleagues during their visits to the University of New Mexico were basic histology and sectioning procedures for snails, light-level microscopy, immunohistochemistry, electrophoresis, immunoblotting, and maintenance of trematode life cycles. Further details regarding these visits are provided below.

## 5. Project Activities and Outputs

A detailed outline of our activities, by year, are presented below.

### Year One

Synopsis: During the first year of the project, we explored the feasibility of using indigenous decapods as control agents and concluded that, although they readily consume snails in the laboratory, they were not promising for further study because it was difficult to gather sufficient numbers for study. Most of our efforts were then directed towards P. clarkii, which is now widely distributed in Kenya. We noted that habitats containing crayfish were significantly less likely to contain pulmonate snails than expected by chance alone. Indeed, crayfish habitats were essentially devoid of snails of relevance of public health whereas nearby habitats without crayfish contained healthy snail populations. We also demonstrated in the laboratory that crayfish readily eat pulmonate snails. Field enclosure studies further showed that crayfish avidly consume snails under field conditions and that they can significantly block recruitment in snail populations. These results are detailed in appended manuscripts. These results provided considerable encouragement for further studies with crayfish.

Below is provided an account of the international trips associated with the project during year one.

September, 1989 - July, 1990. In early September, 1989, Dr. Bruce Hofkin arrived in Kenya to initiate project activities. The following activities were carried out with Dr. Mkoji and other Kenyan colleagues over the next ten months:

1. A study to determine if crayfish and pulmonate snails naturally co-existed in Kenyan freshwater habitats (described in appendix 4).

2. A series of enclosure experiments conducted to determine the extent to which crayfish could control B. pfeifferi snails, and egg mass deposition. Experiments were also conducted to determine if crayfish would reduce the abundance of aquatic plants with which B. pfeifferi is known to be associated. These experiments are described in appendices 4 and 5.

3. A series of laboratory experiments designed to confirm the enclosure experiments described above, as well as to investigate a number of other aspects of snail/crayfish interaction thought to be of relevance in snail control. Description of these experiments is provided in appendices 4, 5, and 6.

4. Dr. Hofkin also made several field trips over the course of the year to help further elucidate the distribution of P. clarkii in Kenya (details of this distribution study are provided in appendices 9 and 15. Additional snail sampling trips were also made, adding to the data base we know have on Kenyan freshwater snail distribution, described in appendix 10.

During the course of his stay in Kenya, Dr. Hofkin worked closely with Dr. Mkoji of KEMRI. He also consulted regularly with Dr. Ouma of the Division of Vector-Borne

Disease, and with Dr. Koech, the director of KEMRI. In January of 1990, Dr. Hofkin collaborated with Dr. Loker, during his visit described below. In March, 1990 Dr. Armand Kuris visited Dr. Hofkin for an additional assessment of project activities. On two field trips over the course of his stay, Dr. Hofkin was accompanied by Dr. Victor Barbiero of U.S.A.I.D. He maintained contact with the USAID office in Kenya during his trip.

29 Dec 1989 - 23 Jan 1990: Dr. Loker visited KEMRI. Dr. Bruce Hofkin was already present at KEMRI where he had been working since August, 1989 on project-related activities. Upon arriving I met with Mr. Hofkin and Dr. Gerald Mkoji and discussed their data at length. We then went on a field trip to Mr. Hofkin's main study site on a coffee estate at Makuyu, approximately 1 hr north by road, from Nairobi. We all worked on building cages that were built to hold crayfish and snails as part of Mr. Hofkin's main experiment. We then, with the assistance of a large field crew comprised of KEMRI and DVBD technicians, set up several cages in the small earthen dam reservoir on the estate there. This reservoir harbored Biomphalaria pfeifferi but no crayfish. However, crayfish are found in several similar ponds around this study site.

The following day I conferred with Dr. Davy Koech, the director of KEMRI and project co-PI. KEMRI and DVBD technicians and I then traveled to ponds near Tala, about 40 km east of Nairobi, where we collected additional B. pfeifferi snails for the experiment at Makuyu. We then drove to Makuyu with these snails. At the same time, Mr. Hofkin was driving to Makuyu with crayfish he had collected in Karen, near Nairobi. Snails and crayfish were added to the cages according to the experimental design.

We then took another field trip to Eldoret to collect crayfish which are particularly abundant there in a location called the Sirikwa Dam. These were then transported back to Nairobi and then to two earthen dam reservoirs in the Makuyu area to determine if they would establish self-perpetuating populations and eliminate snails in these habitats. We had also designated two other habitats in the area as control sites which had received no crayfish and which, as far as we could tell, were free of crayfish. Unfortunately, this experiment was ruined by the discovery that the control habitats also were found to contain crayfish. As previously mentioned, crayfish are abundant in this region and one of the problems we faced was finding habitats that were suitable for snail control studies that didn't already have crayfish. This experiment was also compromised by the fact that snail populations in all dams around Makuyu were low, in our opinion because crayfish were abundant in the region.

We then visited a small dam on the Mwea rice growing scheme where we surveyed the snail populations. This site has now been under investigation for nearly four years and we continue to monitor snails there because of the interesting patterns of interaction between B. pfeifferi and the putative biocontrol snail, Melanoides tuberculata. Near the end of the trip, a field trip was taken to coastal Kenya where ampullariid snails were collected to be brought back to both KEMRI in Nairobi and to Dr. Loker's laboratory in the U.S. for laboratory studies. On the way back to the U.S. from nairobi, Dr. Loker stopped in Copenhagen to confer with schistosomiasis experts at the Danish Bilharziasis Laboratory. In particular, he conferred with Dr. Henry Madsen, a recognized expert on biological control of schistosome-transmitting snails.

8 March, 1990- 26 March, 1990. Dr. Armand Kuris visited Kenya. On March 8, Dr. Kuris and Dr. Hofkin consulted on project activities and met with Dr. Ouma. On March 10 a field trip was made to the Tala quarry pits where B. pfeifferi are abundant and transmission is high. Snails were collected for subsequent enclosure experiments. On March 11, Dr. Kuris assisted Dr. Hofkin in the preparation of enclosure experiments and made several valuable comments regarding experimental design and the design of the enclosures themselves. On March 12 Dr. Kuris and Dr. Hofkin went to Lake Naivasha and surrounding areas as part of the ongoing co-occurrence study described in appendix 4. On March 20, Dr. Kuris accompanied Drs. Hofkin and Mkoji to Makindu to collect additional snails, and on March 21, to Makuyu to initiate another set of enclosure experiments. On March 22, Drs. Kuris, Hofkin and Mkoji consulted with Dr. Scott Cooper, an expert in aquatic predator-prey interaction from the University of California who was then on sabbatical at the University of Nairobi. Further important suggestions were made regarding experimental design and the idea of conducting experiments at the Tala Quarry sites was first discussed. These experiments were eventually carried out by Dr. Mkoji (details in appendix 8). On March 23, Dr. Kuris met with Dr. Koech, director of KEMRI and attended a seminar by Dr. Hofkin, presented at the Biomedical Sciences Research Centre at KEMRI. Dr. Kuris was identified as a consultant for this project because of his experience with freshwater crustaceans and his previous work with biological control of freshwater snails. His visit turned out to be extremely helpful. He was able to provide Mr. Hofkin with excellent advice regarding design of cages used in his crayfish experiments. Earlier cage designs had proven to be too cumbersome and were flawed because the metal in the cage frames was toxic to snails. He was also able to provide excellent advice regarding experimental design that greatly strengthened the results obtained. If Dr. Kuris had not been present in the field and able to consult with Mr. Hofkin directly after a first-hand view of the field site, it is likely our progress would have been delayed by months or years. The value of Dr. Kuris' input is further reflected in the fact that he served as the off-campus member of Mr. Hofkin's Ph.D. committee.

## Year Two

Synopsis: During year two of the project, Mr. Hofkin returned to the U.S. One of the major efforts there was to analyze the results of the preceding year's field studies undertaken in Kenya and to get them written up for publication. In the U.S., additional laboratory studies were initiated to explore the potential of crayfish to control snail populations. Much of this effort culminated in Bruce Hofkin receiving his Ph.D. degree at the University of New Mexico based on laboratory work with snails and crayfish performed in the U.S., and on both laboratory and field studies performed in Kenya in collaboration with our colleagues at KEMRI and the DVBD.

In Kenya, Dr. Mkoji and his colleagues concentrated on trying to improve our knowledge of the geographic distribution of P. clarkii in Kenya. Results indicated that crayfish occupy freshwater habitats in the central and western highlands of Kenya but have not colonized Lake Victoria or the coastal lowlands. Also, as survey data suggested that

crayfish were not present in the coastal tropical lowlands of Kenya, experiments were initiated to determine if this species was capable of surviving in aquaria held at KEMRI facilities at Kilifi. Crayfish were found to be capable of surviving and reproducing under these rigorous tropical conditions.

A study was also initiated near Tala, Kenya in which crayfish were placed in abandoned quarry pits containing populations of Biomphalaria pfeifferi. Crayfish rapidly eliminated snails in these small, man-made impoundments. We also continued to monitor the impact of various prosobranch snails as possible control agents for trematode-transmitting pulmonates in selected study sites.

4-29 April, 1991: Before funding of this project was finally approved, Dr. Koech requested of USAID that some of the funds be used to support training of Kenyan scientists in the U.S. As the amount of money required to support a Kenyan student for a degree program in the U.S. was far too much for the budget we had, it was decided to try to get 3 Kenyan scientists to New Mexico for intervals of 1-2 months, to learn various techniques and to interact with U.S. scientists. In consultation with Dr. Koech and Dr. Ouma, it was decided to allow Dr. Mkoji to visit the U.S., followed by Mr. Ben Mungai and Mr. Jimmy Kihara. All 3 scientists have been invaluable to our USAID-funded project in Kenya. Dr. Mkoji was the first visitor in April of 1991. During Dr. Mkoji's visit, he conferred extensively with Dr. Loker and Mr. Bruce Hofkin regarding the project and undertook analysis of some of our data using computer and graphics facilities not available to him in Kenya. Dr. Mkoji then wrote a manuscript based on this data which appears in appendix 7. Prior to Dr. Mkoji's departure, we again extensively discussed the future of our project. During his visit, Dr. Mkoji presented a seminar of his work, met with several scientists in the Biology Department, and spent a great deal of time using the library facilities at the University of New Mexico.

29 June - 24 July 1991: Dr. Loker and Dr. Hofkin visited Kenya. The trip started with long discussions with Dr. Koech, Dr. Githurie, and Dr. Mkoji of KEMRI and Dr. Ouma of the Division of Vector Borne Diseases. The first field trip taken was to the large Mwea rice-growing irrigation scheme where we searched for evidence of crayfish both in small dams within the scheme and in the Thiba River that runs through the scheme and is a major tributary of the Tana River. We were interested in determining if crayfish had invaded the scheme and were causing any problems there since they are known to be present in areas close to the irrigation scheme. No crayfish were found in the scheme and discussions with Mr. A.A. Mohdhar, the scheme manager, indicated that crayfish had not posed a problem in the irrigation scheme. We did find Biomphalaria pfeifferi in dams in the scheme, suggesting that Schistosoma mansoni transmission was probable on the scheme.

We also visited a study site near Mwea that we have been monitoring for several years. This site is of interest because it contains populations of B. pfeifferi and Melanoides tuberculata, the latter species considered a promising control agent of Biomphalaria in the Caribbean region. At this site, both species continued to coexist, suggesting displacement of African Biomphalaria with Melanoides may simply not occur.

We then traveled to Tala where we sampled at study sites that have held ampullariid

snails for approximately 2.5 years. Lanistes carinatus populations persisted in the two original introduction sites and Pila ovata persisted in its two original introduction sites. Unfortunately, pulmonates, particularly Bulinus (Physopsis) sp., persisted in all four of these habitats and were abundant in two of the habitats. It was noted that emergent aquatic macrophytes persisted in 3 of the 4 habitats suggesting that the ampullariid species in question are not able to efficiently remove these important pulmonate food sources and oviposition sites. However, the two habitats where ampullariids were most abundant had the smallest number of pulmonates. Continued monitoring of these sites is warranted.

The next field trip taken was to Lake Naivasha where we consulted with a fisheries biologist at the lake, Mr. Roderick Kundu, who informed us in considerable detail about the biology of crayfish in Lake Naivasha. Many of these comments are incorporated into a publication, attached as appendix 9, that provides an overview of the possible use of crayfish in biological control in Kenya. After this trip, Dr. Hofkin, Dr. Mkoji, and Dr. Loker all worked on two manuscripts to be presented at the AID-sponsored meeting on aquaculture and Schistosomiasis held in August, 1992, in Manila, the Philippines.

Following this, Dr. Hofkin and Dr. Loker traveled to Mombasa where we consulted with Dr. Rene Haller who is the aquaculture expert that runs the Baobab Farms aquaculture station in Mombasa. One purpose of this visit was to determine if Dr. Haller had encountered crayfish during any of his experiences along the coastal tropical lowlands of Kenya. He informed us that he had no evidence of crayfish existing in coastal Kenya. We then visited the KEMRI research station at Kilifi where Mr. Benjamin Mungai of KEMRI had set up several outdoor aquaria to determine if crayfish could survive and reproduce under the tropical conditions of Kilifi. Preliminary indications at the time were that crayfish were thriving under the conditions experienced at Kilifi. We then searched for crayfish at the Mazeras botanical Gardens, considered a likely spot because of the presence of several introduced plant species. None were found. We also conferred with Mr. J.W. Kariuki of the Department of Fisheries in Mombasa who informed us that he was unaware of the presence of crayfish anywhere in coastal Kenya. We then returned to Nairobi and continued to work on the manuscripts for the Manila meetings. I also conferred with Mr. Curtis Kariuki who had assisted us in the field previously. We discussed his proposed Ph.D. project in detail.

The final field trip was to Lake Victoria, to Kisumu and Port Victoria, again with the objective of trying to determine if crayfish have established in or around the lake. We first visited several Fisheries offices in Kisumu and numerous discussions with officials there suggested that no crayfish are present in the lake. We then traveled to Port Victoria and again conferred with fisheries officials who informed us that no crayfish were known from the lake in that specific area. We attempted to trap crayfish at two locations in Port Victoria without success and also attempted to collect in the Nzoia River but again found no crayfish. We then returned to Nairobi and conferred with Mildred Howard and Dr. Victor Barbiero of USAID. We concluded our trip with long discussions with Dr. Mkoji and Dr. Ouma and returned to the U.S.

6 - 10 August 1991: Dr. Loker and Dr Mkoji were invited to attend the joint PSTC/CDR aquaculture meeting held in Manila, the Philippines. This meeting was particularly fruitful

because it brought us in contact not only with other scientists working on biological control of snails but also with aquaculturist working in Kenya, Zimbabwe, and elsewhere. Both Dr. Loker and Dr. Mkoji presented papers related to our USAID funded work at the meeting (see appendices 8 and 9).

**Year Three**

Synopsis: During the early part of this year, survey work continued in Kenya in an attempt to further define the geographic distribution of crayfish in Kenya. The results of this survey work are summarized in appendix . Dr. Mkoji and colleagues continued to monitor ampullariid study sites near Tala as were the quarry pits at Tala. Many of the latter sites dried totally during this year.

In the U.S. progress was slowed somewhat by the fact that Dr. Loker was on sabbatical leave from January through June, 1992 at the University of Glasgow, Scotland. However, prior to Dr. Loker's departure, he received Mr. Ben Mungai from KEMRI into his lab for training. Also, efforts continued by Drs. Loker, Hofkin and Mkoji to finish writing up the results of previous field studies in Kenya. Notable among these was the completion of a large manuscript on the geographic distribution of freshwater snails in the southern half of Kenya (appendix 10). Ms. Tracy Boyce started her master's program at UNM under Dr. Loker's direction. She was encouraged to learn Geographic Information Systems (GIS) and to apply her knowledge to our Kenyan snail distribution data. She began in earnest on this project in the second half of grant year #3. Also, in the latter half of the year, with the combined efforts of Ms. Boyce and Dr. Mkoji, data were collected regarding the impact of crayfish on populations of both larvae and pupae of Anopheles gambiae, presented in Appendix 14.

18 September 1991 - 17 November, 1991: As part of the requested program to transfer technical expertise to Kenyan scientists, Dr. Koech, Dr. Ouma, and Dr. Mkoji suggested that Mr. Ben Mungai should visit Dr. Loker's laboratory in New Mexico. Mr. Mungai, a KEMRI employee, has been associated with our USAID project since we began work in Kenya in 1986 and has been an important colleague of ours from the very beginning. While in New Mexico, Mr. Mungai had two basic projects. The first was to determine if Biomphalaria pfeifferi from certain habitats in Kenya were actively resistant to infection with the Kenyan strain of Schistosoma mansoni. Mr. Mungai exposed the snails in question to this parasite prior to coming to the U.S., and then when he was here, he learned basic histological techniques so that he could examine sections of the snails to determine if the snails harbored viable parasites. Mr. Mungai sectioned many snails while in Albuquerque and the basic result to emerge from his study was that resistance of local B. pfeifferi to S. mansoni was not obvious. In other words, snails though to be resistant did not encapsulate and destroy parasites and seemed to permit normal larval development of S. mansoni. The second project undertaken by Mr. Mungai involved immunohistochemical techniques. He sectioned Biomphalaria glabrata snails infected with the digenetic trematode Echinostoma paraensei and then attempted to determine if larval stages of this trematode bound antibodies that were raised in rabbits to lectins produced by B. glabrata in response to

echinostome infection. Mr. Mungai was able to demonstrate binding of the antibodies to parasite surfaces, suggesting that the snail-produced lectin binds to parasite antigens and may thus be a relevant part of the snail's defense against parasites. Mr. Mungai was also exposed to basic microscopical and SDS-PAGE electrophoretic techniques while in the U.S.

16 June - 27 July, 1992: Ms. Tracy Boyce, one of Dr. Loker's graduate students, visited KEMRI during this interval. During her stay in Kenya, she worked closely with Dr. Gerald Mkoji and Mr. Ben Mungai of KEMRI to explore the ability of juvenile and adult crayfish to control larvae and pupae of the mosquito Anopheles gambiae. These results which were accumulated over a five-week interval are presented in appendix 14. During her stay, she also participated in a field trip to Tala to examine quarry pits containing crayfish. She also accompanied Dr. Mkoji in helping to locate habitats that might be suitable as study sites for our new USAID project. She also accompanied Mr. Mungai to Kilifi, on the coast of Kenya, where she examined his semi-field habitats used to assess crayfish survival in coastal Kenya. She also paid a visit to the United Nations Environmental Program (UNEP) center in Nairobi where she consulted with Ms. Laura Mezaros and Dr. A. Fernandez about her GIS thesis project. She attempted to obtain suitable data sets there for Kenyan climate but discovered that none were readily available. She also visited the International Laboratory for Research on Animal Disease (ILRAD) where she conferred with Mr. Russ Kruska about GIS of East Coast Fever and obtained data pertaining to soils of Kenya. Near the end of her visit, she contacted Millie Howard at USAID in Nairobi to inform her about her trip.

**Year Four**

Synopsis: We requested a no-cost extension during year three so we might have additional time to complete some of the GIS work we had defined in the original proposal. Progress on this aspect of the project had been slower than expected because of delays in lining up personnel with appropriate experience and in obtaining the necessary climactic and geographical data for the relevant parts of Kenya. However, through the efforts of Ms. Tracy Boyce, good progress was made late in 1992 and in 1993 on the GIS project, the results of which have recently been presented at a national parasitology meeting (see appendix 16). They will soon be prepared for publication. This publication will comprise the major part of Ms. Boyce's masters thesis. Also, during the early part of grant year 4, we received a third visitor from Kenya for a two month training visit (see report below). Otherwise, our level of activity in this grant year in Kenya was relatively low as most of our primary field objectives for this project had already been met. Our progress was impaired by the theft of the last subcontract payment sent to KEMRI by UNM. Details are provided in appendix #18. Official activities pertaining to the grant were more or less concluded by Dr. Loker's visit to Kenya in June. A report of that trip is detailed below. Among the interesting results of this relatively brief trip was the discovery that crayfish had successfully survived the extensive drying of the quarry pits in Kenya. This ability to survive dry periods is an important attribute of a control agent because the target snails species are often able to withstand prolonged dry periods. Also, during Dr. Loker's trip, efforts were made to contact colleagues in Tanzania because of earlier reports of the presence of the exotic

ampullariid snail Marisa cornuarietis near Arusha, in northern Tanzania. A trip was taken to the Moshi area and a population of Marisa in a natural habitat was observed. Dr. Loker also assisted a student from Cornell University, Ms. Dee Dugger, in setting up a project at KEMRI that involved examining trematode infections in the putative biocontrol snail, Melanoides tuberculata.

18 October - 10 December 1992: As part of the requested program to allow Kenyan scientists to visit Dr. Loker's laboratory in the U.S., Dr. Koech, Dr. Ouma, and Dr Mkoji suggested that Mr. Jimmy Kihara travel to New Mexico. Mr. Kihara, an employee of the Division of Vector Borne Diseases, has been involved in our USAID-sponsored work since it began in 1986; he is the third and final Kenyan scientist to visit our lab during this funding period. During Mr. Kihara's visit, he was encouraged to investigate the interactions between juvenile and adult snails of Biomphalaria glabrata and the digenetic trematode, Echinostoma paraensei. This model system is extensively studied in Dr. Loker's laboratory. Juvenile snails are susceptible and adult snails are refractory. Mr. Kihara performed several comparisons of these two groups of snails, and in the process acquired considerable practical experience in handling snails and in maintaining trematode life cycles. He also acquired experience with basic microscopy and in SDS-PAGE electrophoretic techniques. These skills will hopefully be valuable to him as he continues his work on Kenyan snails and trematodes. The work that he performed in Dr. Loker's laboratory has been very useful in demonstrating that juvenile and adult snails have comparable numbers of hemocytes per unit volume of hemolymph. However, by virtue of having about 2.5 times the hemolymph volume as juveniles, adults have a larger total circulating hemocyte population and for this reason may be less vulnerable to trematode infection. Further studies underway in Dr. Loker's laboratory suggest this is the case. These observations have relevance to transmission of schistosomiasis in Kenya because older snails may be refractory to infection.

11 June - 28 June 1993: Dr. Loker visited Kenya as the final trip associated with this project. Just prior to traveling to Kenya, Dr. Loker visited Ms. Dee Dugger, a veterinary student at Cornell University who had been awarded a scholarship to study for two months in Kenya. After hearing of our research program, it was arranged that she should work at KEMRI under Dr. Loker and Dr. Mkoji's supervision from mid-June to mid-August. Upon arriving in Kenya, Dr. Loker conferred with colleagues at KEMRI and arranged to visit field sites in the Tala area. The Tala Quarry sites were visited and it was determined that crayfish were still thriving in several of the small pools in the quarry pits. No B. pfeifferi were found in these pits whereas other pits in the area lacking crayfish had thriving snail populations. The continued presence of crayfish in many of these pools was remarkable because most had dried out completely during the preceding dry season. Crayfish were therefore able to survive in these pits in humid microhabitats under rocks at the bottom of the pits. Also visited were ampullariid introduction sites in and around Tala. Lanistes carinatus populations persisted in the two original introduction sites and Pila ovata persisted in its two original introduction sites. Unfortunately, pulmonates, particularly Bulinus (Physopsis) sp., persisted in three of these habitats and were abundant in two of the habitats.

After the field trips to Tala, Dr. Loker then helped Ms. Dugger get settled in at

KEMRI and then set up a project for her to examine different populations of the putative biocontrol snail Melanoides tuberculata derived from different parts of Kenya. The object of this study which is still underway is to determine if Kenyan populations of this species ever possess functional males. This species is generally considered to be parthenogenetic but some sexual populations are known from the Caribbean region where this species seems to be an effective control agent of Biomphalaria glabrata. Thus far no males have been found. We also sought to determine if the burden of parasitism by digenetic trematodes might favor the appearance of sexual populations of M tuberculata. Although several digenetic trematodes were found, no evidence for such a correlation has been found.

A final activity for this trip was to visit colleagues at the Tropical Pesticides Research Institute in Arusha, Tanzania. The purpose of this trip was to determine if the exotic ampullariid snail Marisa cornuarietis was still present in natural habitats in northern Tanzania (at Kisangara) following its introduction there by Dr. Ngumu in the mid 1970s. Although we did not find Marisa in its original introduction site, we did find a thriving populations in irrigation ditches at a large sugar estate, the Tanzania Planting Company, near Moshi, Tanzania. No Biomphalaria or Bulinus were found in this ditch although a few Lymnaea natalensis were found in this location. Several Marisa were collected and brought back to the laboratory at KEMRI in Nairobi where they were dissected. None were found infected with digenetic trematodes.

Dr. Loker visited with Mildred Howard at the USAID mission, consulted with Dr. Ouma of the Division of Vector Borne Diseases, and had extensive conversations with Dr. Mkoji prior to his departure. Following Dr. Loker's return to the U.S., Ms. Dugger remained at KEMRI and continued to work under Dr. Mkoji's direction on the project outlined above.

**Talks Presented at Scientific Meetings Based on Project Results**

Hofkin, B.V., Koech, D.K., and Loker, E.S. 1990. Interactions between the North American crayfish Procambarus clarkii and schistosome-transmitting snails in Kenya: Field and laboratory studies. Presented at the 39th Annual Meeting of the American Society of Tropical Medicine and Hygiene, New Orleans, La., 4-8 November, 1990.

Hofkin, B.V., Koech, D.K., and Loker, E.S. 1991. Interactions between the North American crayfish, Procambarus clarkii and schistosome-transmitting snails in Kenya. Presented at the 66th Annual Meeting of the American Society of Parasitologists, Madison, Wisc., 4-8 August, 1991.

Mkoji, G.M.,, Hofkin, B.V., Mungai, B.N., Kihara, J.H., Mungai, F.K., Ouma, J.H., Koech, D.K. and Loker, E.S. 1992. Control of natural populations of schistosome-transmitting snails by the crayfish Procambarus clarkii in temporary man-made ponds in Kenya. Aquaculture and schistosomiasis. Proceedings of a network meeting held in Manila, Philippines, August 6-10, 1991. National Academy Press. Washington, D.C., pp 264-271.

Loker, E.S., Hofkin, B.V., Mkoji, G.M., Kihara, J.H., Mungai, F.K., Mungai, B.N., and Koech, D.K. 1992. Procambarus clarkii in Kenya: Does it have a role to play in the control of schistosomiasis? Aquaculture and schistosomiasis. Proceedings of a network meeting held in Manila, Philippines, August 6-10, 1991. National Academy Press. Washington, D.C., pp 272-282.

Boyce, T.G., Rizor, D.D., and Loker, E.S. 1993. The use of a geographic information system (GIS) to analyze the distribution of the human schistosome-transmitting snails in Kenya. Presented at the joint meetings of the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, 31 October - 4 November, 1993.

Hofkin, B.V. 1993. Recommendations for the selection of control agents for the biological control of aquatic vector organisms. Presented at the joint meetings of the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, 31 October - 4 November, 1993.

**Scientific Publications Attributable to the Project**

1. Bandoni, S., Mulvey, M., Koech, D.K. and Loker, E.S. 1990. Genetic structure of Kenyan populations of Biomphalaria pfeifferi. Journal of Molluscan Studies 56: 383-391.

2. Hofkin, B.V., Stryker, G.A., Ouma, J.H., Koech, D.K., and Loker, E.S. 1991. Consumption of Biomphalaria glabrata egg masses and juveniles by the ampullariid snails Pila ovata, Lanistes carinatus and Marisa cornuarietis. Acta Tropica 49: 37-44.

3. Stryker, G.A., Koech, D.K., and Loker, E.S. 1991. Growth of Biomphalaria glabrata populations in the presence of the ampullariid snails Pila ovata, Lanistes carinatus and Marisa cornuarietis. Acta Tropica 49: 137-147.

4. Hofkin B.V., Mkoji, G., Koech, D.K., and Loker, E.S. 1991. Control of schistosome-transmitting snails in Kenya by the North American crayfish Procambarus clarkii. American Journal of Tropical Medicine and Hygiene 45: 339-344.

5. Hofkin, B.V., Koech, D.K., Ouma, J.H., and Loker, E.S. 1991. The North American crayfish Procambarus clarkii and the biological control of schistosome-transmitting snails in Kenya: laboratory and field investigations. Biological Control 1: 183-187.

6. Hofkin, B.V., Hofinger, D.M., Koech, D.K., and Loker, E.S. 1992. Predation of Biomphalaria and non-target molluscs by the crayfish Procambarus clarkii: implications for the biological control of schistosomes. Annals of Tropical Medicine and Hygiene 86: 663-670.

7. Mkoji, G.M., Hofkin, B.V., Mungai, B.N., Kihara, J.H., Kageni, F.M., Koech, D.K., and

Loker, E.S. 1992. Does the snail Melanoides tuberculata have a role in biological control of Biomphalaria pfeifferi and other medically important African pulmonates? Annals of Tropical Medicine and Parasitology 86: 201-204.

8. Mkoji, G.M.,, Hofkin, B.V., Mungai, B.N., Kihara, J.H., Mungai, F.K., Ouma, J.H., Koech, D.K. and Loker, E.S. 1992. Control of natural populations of schistosome-transmitting snails by the crayfish Procambarus clarkii in temporary man-made ponds in Kenya. Aquaculture and schistosomiasis. Proceedings of a network meeting held in Manila, Philippines, August 6-10, 1991. National Academy Press. Washington, D.C., pp 264-271.

9. Loker, E.S., Hofkin, B.V., Mkoji, G.M., Kihara, J.H., Mungai, F.K., Mungai, B.N., and Koech, D.K. 1992. Procambarus clarkii in Kenya: Does it have a role to play in the control of schistosomiasis? Aquaculture and schistosomiasis. Proceedings of a network meeting held in Manila, Philippines, August 6-10, 1991. National Academy Press. Washington, D.C., pp 272-282.

10. Loker, E.S., Hofkin, B.V., Mungai, B. and Koech, D.K. 19XX. Observations on distributional patterns of freshwater snails in Kenya, and implications for biological control of schistosomiasis. Journal of Medical and Applied Malacology. In press.

11. Bandoni, S.M., Mulvey, M., and Loker, E.S. 19XX. Phylogenetic analysis of eleven species of Biomphalaria Preston. 1910 (Gastropoda: Planorbidae) based on comparison of allozymes. Biological Journal of the Linnean Society: in press.

12. Bandoni, S.M., Mulvey, M., and Loker, E.S. 19XX. Intraspecific and interspecific patterns of allozyme variation among species of Biomphalaria Preston, 1910 (Gastropoda: Pulmonata): submitted for publication.

13. Boyce, T.G., Rizor, D.D., Mkoji, G.M., and Loker, E.S. 19XX. The use of a geographic information system (GIS) to analyze the distribution of the human schistosome-transmitting snails in Kenya. In preparation.

## 6. Project Productivity

The project, in our view, was a great success because we were able to identify a biological agent that was capable of having an unmistakable effect on disease-transmitting snails in Kenya. The work accomplished during this project has enabled us to go forward to the next step, namely to try to demonstrate an actual effect of crayfish on schistosomiasis transmission in selected man-made habitats in Kenya.

There were some specific things we were unable to accomplish. Studies with indigenous decapod species did not materialize because of the difficulties mentioned above. Although annoying, we did not consider this a major setback because our impression was that decapods were probably unlikely to prevent schistosomiasis transmission in any case. Also, were unable to apply the multivariate statistical analysis to the snail distribution data we had accumulated. There were two fundamental problems: the most important was getting access to quality data pertaining to temperature, rainfall and other relevant parameters for Kenya. We contacted GIS experts at the United Nations Environmental Program and at the International Laboratory for Research on Animal Diseases, both in Nairobi, but were unable to secure usable data sets. The second problem was of insufficient funds. Based on the budget we had to work with, we felt very fortunate to be able to generate the geographic information system in the first place. We simply did not have enough money left over after all other project activities to insure completion of the multivariate analysis. It should be noted that the theft of the last subcontract payment to KEMRI (see appendix 21) did not impact the GIS study as funds for the GIS study came from the U.S. portion of the budget.

## 7. Future Work

This project has already lead to additional investigations. We are initiating a new USAID-funded project to explore the potential of crayfish to demonstrably reduce the level of schistosomiasis transmission to children living adjacent to our study sites. This project should help to resolve the issue of whether effective snail control actually translates to lowered parasite transmission and reduced parasite-induced morbidity. This study will be undertaken by the same group of collaborators and the results will be closely monitored by the Division of Vector Borne Diseases to determine if the results are sufficiently encouraging to warrant use of this control method in other habitats.

Other parting comments and suggestions for future study are listed below. Once again, these ideas are derived largely from appendix 9.

1. The potential environmental ramifications of further P. clarkii introductions that have been highlighted in the appendices underscore the need for additional study. Current concerns regarding the protection of natural ecosystems, the maintenance of biodiversity, and the protection of rare, endemic species should give pause to anyone contemplating additional introductions of this exotic species into East Africa. These very real concerns must, however, be balanced by the realization that a species such as P. clarkii offers the potential of increased economic opportunity and improved public health. Furthermore, P. clarkii is, for better or worse, already firmly established in Kenya, and may well increase its present range, irrespective of human activity. Bearing this in mind, further studies should focus on how to best manage this resource, and how to take maximum advantage of its positive attributes while keeping its environmental effects to a minimum.

2. Effects of P. clarkii on the abundance and species composition of mosquito larvae should be further investigated. It is intriguing to consider what effect crayfish, used to control schistosomiasis, might have on the transmission of mosquito-borne diseases such as malaria, filariasis, and arboviral infections. Because it has the capacity to alter aquatic habitats by consuming macrophytes, P. clarkii has been suggested as a means of controlling mosquitoes in California marshes. In tropical Africa, the effect on mosquito breeding might be positive or negative, depending on the tendency of a particular mosquito species to breed in open or shaded water.

3. Are commercially available pesticides used in rice cultivation effective in controlling the spread of P. clarkii? Crayfish are absent from major Kenyan rice growing schemes, possibly due to the use of certain pesticides such as Furadan to control insect pests. Even if P. clarkii should prove to be highly successful as a means of controlling schistosomiasis, its presence in rice fields would probably be counterproductive, owing to its ability to consume rice seedlings. Studies should be initiated to determine whether Furadan or other pesticides that are routinely used for agricultural purposes can be used to eliminate crayfish from areas where they pose economic or environmental risks.

4. The effects of P. clarkii on other components of the aquatic environments of Kenya need to investigated. Careful studies are required to ascertain how P. clarkii introductions might effect non-target species. With such information available, decisions about the use of crayfish for snail control can be better evaluated. Special care should be taken to elucidate how crayfish introductions might affect endangered aquatic species.

5. What factors control the present distribution of P. clarkii in Kenya? Is its distribution likely to expand? Procambarus clarkii is absent from large areas of Kenya, and it is unclear to what degree various biotic and abiotic factors interact to explain this distribution. A clearer understanding of these factors would insure a greater likelihood of success in snail control operations, and would make predictions about its capacity for environmental damage more valid.

# Appendix 1

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## GENETIC STRUCTURE OF KENYAN POPULATIONS OF *BIOMPHALARIA PFEIFFERI* (GASTROPODA: PLANORBIDAE)

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### ABSTRACT

*Biomphalaria pfeifferi* is widespread in Africa, tolerates a diverse array of habitats, and is highly susceptible to schistosome infection. As such, it is the most important host of *Schistosoma mansoni* in the Old World. Representatives of *Biomphalaria pfeifferi* from twelve localities in southern Kenya were examined using starch gel electrophoresis in order to describe the population structure. Ten to fifteen loci were resolved in each population. Genotypic frequencies obtained for 10 loci have been used to calculate Nei's genetic distances. Nine eastern populations were relatively similar to each other ($D \leq 0.016$), but divergent from western populations ($D \leq 0.178$). Two of three western populations were quite similar ($D = 0.001$), the third being more divergent ($D \leq 0.258$). Genotypic frequencies showed a substantial departure from Hardy-Weinberg equilibrium, due to a marked deficiency of heterozygotes. Calculation of F-statistics revealed evidence of non-random mating as well as considerable differentiation among localities. *Biomphalaria pfeifferi* differs from other *Biomphalaria* species studied to date in exhibiting evidence of non-random mating. Higher levels of inbreeding could alter the pattern of response to selection imposed by parasites such as *Schistosoma mansoni*.

### INTRODUCTION

*Biomphalaria pfeifferi* (Krauss) is the most important intermediate host for *Schistosoma mansoni* in the Old World. It is geographically widespread, being found throughout most of Africa south of the Sahara, and it occupies a great diversity of habitat types, including areas affected by human activities such as drainage and irrigation ditches and dams (Appleton, 1978; Brown, 1980; Mandahl-Barth, 1958). *B. pfeifferi* is highly susceptible to infection with *Schistosoma mansoni* (Basch, 1976 and references therein; Frandsen, 1979).

Despite the medical importance of *B. pfeifferi*, this species remains quite poorly understood taxonomically. It is morphologically heterogeneous, with many described subspecies and variants, the genetic and environmental bases of which are not well understood (Mandahl-Barth, 1958; Danish Bilharziasis Laboratory, 1987). Representatives of twelve populations of *B. pfeifferi* were collected and examined using starch gel electrophoresis in order to describe the intra-population and inter-population genetic composition of this species. A second objective of this study was to examine possible implications of the population structure for the transmission of *S. mansoni*.

### MATERIALS AND METHODS

Snails were collected during January 1987; collecting localities are illustrated in Figure 1. All collecting sites were located in areas in which schistosomiasis is endemic (Brown *et al.*, 1981). Although we did not attempt to determine whether the snails used in our study were infected with *S. mansoni*, all sites were freely accessible to the public, and therefore represent potential sites for transmission. Longitude, latitude and habitat type are as follows: (1) Matingani Seepage (1°7'S, 37°53'E, seepage area); (2) Muthamo Seepage (1°8.4'S, 38°2.7'E, seepage area); (3) Mbondoni Dam (0°57'S, 38°2.4'E, dam); (4) Kangonde Dam (1°2.1'S, 37°43'E, intermittent stream); (5) Mwea East (0°40'S, 37°30'E, roadside borrow pit); (6) Mwea West (0°40'S, 37°30'E, swamp); (7) Grogan Canal (3°36.5'S, 37°44.5'E; permanent stream); (8) Kwahoma Stream (3°23.5'S, 37°39.4'E, permanent stream); (9) Asawo Stream (0°16.3'S, 34°59.4'E, intermittent stream); (10) Martin's Drain (0°4.7'S, 34°44.5'E, intermittent stream); (11) Kamayoga

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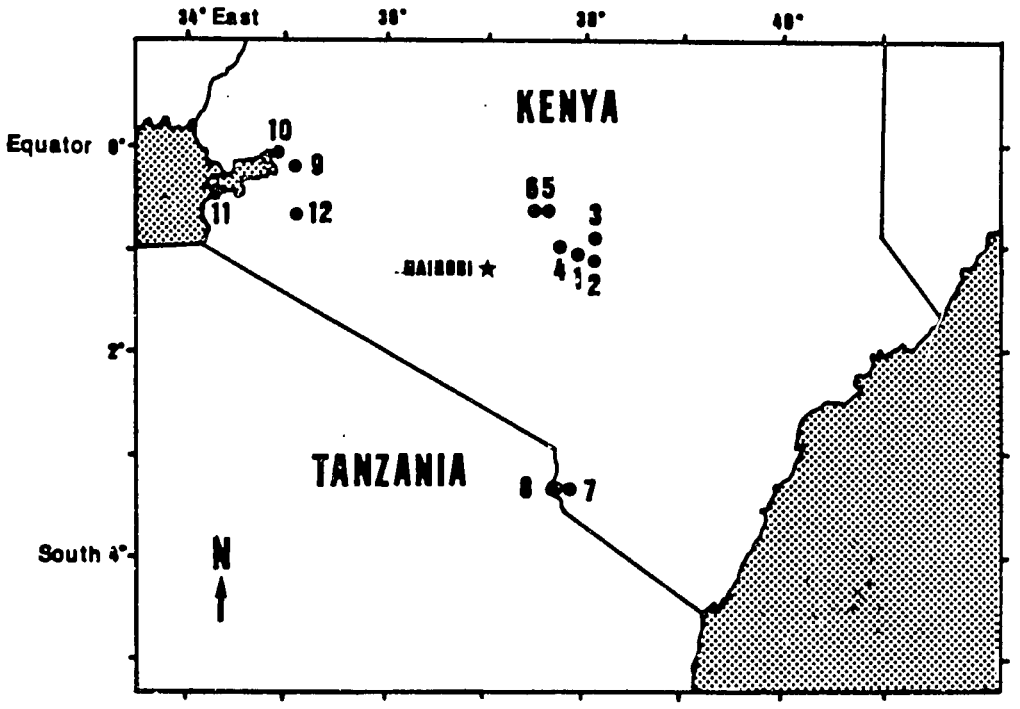


Fig. 1. Map of Kenya, showing collecting localities: (1) Matingani Seepage; (2) Muthamo Seepage; (3) Mbondoni Dam; (4) Kangonde Dam; (5) Mwea East; (6) Mwea West; (7) Grogan Canal; (8) Kwahoma Stream; (9) Asawo Stream; (10) Martin's Drain; (11) Kamayoga Stream; (12) Onsando Dam.

Stream (0°24.3'S, 34°9.8'E, intermittent stream); (12) Onsando Dam (0°42.8'S, 35°2.4'E, dam). Snails were collected by means of a dipnet, rinsed to remove mud and epiphytes, wrapped in foil, and stored in liquid nitrogen for transport. After transport, snails were stored at −70°C until used for electrophoresis. Representative shells have been deposited in the collections of the Savannah River Ecology Laboratory.

Methods for starch gel electrophoresis of *Biomphalaria* species have been described elsewhere (Mulvey *et al.*, 1988). Snails used for electrophoresis ranged from approximately 2.5 to 15 mm in diameter, and were homogenized whole. To facilitate comparison with previous studies, mobilities of alleles were determined relative to the M-line stock of *B. glabrata* (Newton, 1955). Fifteen loci were examined; at least ten loci were resolved in all populations. This subset of 10 loci resolved in all populations was used for all subsequent calculations. Enzymes, abbreviations and numbers assigned by the International Union of Biochemistry are as follows: aconitate hydratase (ACON, 4.2.1.3), alkaline phosphatase (AKP, 3.1.3.1), carboxylesterase (EST, 3.1.1.1), aspartate aminotransferase (AAT, 2.6.1.1), α-glycerol-3-phosphate dehydrogenase ($NAD^+$) (GPD, 1.1.1.8), hemoglobin (HB), isocitrate dehydrogenase ($NADP^+$) (IDH, 1.1.1.42), malate dehydrogenase (MDH, 1.1.1.37), purine nucleoside phosphorylase (NSP, 2.4.2.1), aminopeptidase (PEP, 3.4.–.–, using the substrates leucylalanine and leucylglycylglycine), 6-phosphogluconate dehydrogenase (decarboxylating) (PGD, 1.1.1.44), glucose-6-phosphate isomerase (PGI, 5.3.1.9), and phosphoglucomutase (PGM, 5.4.2.2).

Sample sizes varied considerably. Factors influencing the sample size for particular loci include the sizes of individual snails and the sensitivity of allozymes to freezing and storage. Gels were scored conservatively, with individuals exhibiting evidence of staining artifacts or tissue degradation for a particular allozyme being excluded from the sample for that locus. In our experience, additional bands of enzyme activity are found in zymograms of snails infected with digenetic trematodes. Occasionally additional bands were observed for this field collected material; however, they did not interfere with interpretation of snail-associated electrophoretic patterns.

Data were analysed using the BIOSYS program of Swofford and Selander (1981). Genotypic frequencies were calculated for each population at each locus, and these were compared with frequencies expected under Hardy-Weinberg equilibrium using a chi-square statistic. Allele frequencies were used to calculate Nei's genetic distances (Nei, 1972). A coefficient of inbreeding, $F_I$, can be calculated as (1 − observed heterozygotes/expected heterozygotes). When inbreeding is frequent, heterozygotes will be rare, and the inbreeding coefficient will approach one.

Two factors may contribute to inbreeding, finite population size and non-random mating, the latter consisting of either positive assortative or consanguineous mating. Wright (1965, 1969) and Nei (1973, 1977) developed a series of coefficients (F statistics) for assessing the relative contributions of these factors by describing the departure from Hardy-Weinberg equilibrium; a brief summary is presented below. In the model the entire region sampled is considered a population, and individual sampling localities are considered subpopulations. The inbreeding coefficient for the total population, $F_{it}$, defines the correlation between 2 alleles in an individual across localities in the large population. The inbreeding coefficient for subpopulations, $F_{is}$, represents the correlation between 2 alleles in an individual within a subpopulation, and describes the contribution of non-random mating to inbreeding. Both $F_{it}$ and $F_{is}$ can range from −1 to +1, with positive values indicating a deficiency of heterozygotes, and negative values indicating an excess of heterozygotes. The third coefficient, $F_{st}$, measures the effects of finite population size. Also called the standardized variance in allele frequency, $F_{st}$ is a measure of genetic differentiation among localities. Since $F_{st}$ is a variance, only positive values may be obtained. Values of $F_{st}$ may range from 0 to 1, with larger $F_{st}$ values corresponding to higher levels of genetic differentiation. The three inbreeding coefficients are related as follows: $(1-F_{it}) = (1-F_{is})(1-F_{st})$. For each of these coefficients, corrections are made for variation in the sizes of subpopulations.

## RESULTS

Three loci, *Pgi*, *Hb*, and *Mdh-2*, were found to be monomorphic in all sampling localities. For two additional loci, *Mdh-1* and *Got-1*, variants were rare, occurring with frequencies less than 5% in a single locality each. The remaining loci were polymorphic in one or more localities, for an overall level of polymorphism of 80.0%. The percentage of polymorphic loci ranged from zero in one population (Kangonde Dam) to 40% in Asawo Stream, with an average value of approximately 20%.

Loci, mobilities and observed genotypic frequencies are presented in Table 1. Chi square values for the fit of genotypic frequencies to Hardy-Weinberg expectations are presented in Table 2 for a subset of loci and populations. The chi square statistic is unreliable when expected frequencies are very low (Zar, 1984), so the application of the chi square test was restricted to cases where at least 2 homozygotes or 4 heterozygotes for the second allele were observed. In 6 of 7 cases, the observed genotypic frequencies deviate substantially from Hardy-Weinberg expectations, in each case because of a deficiency of heterozygotes. In the single remaining case, observed genotypic frequencies are not significantly different from expectations under Hardy-Weinberg equilibrium.

Coefficients of inbreeding were calculated from average observed and expected heterozygosities for ten loci for each population; inbreeding coefficients (fixation indices) are presented in Table 3. Eleven of twelve are large and positive, indicating a substantial deficiency of heterozygotes.

The F-coefficients calculated for the Kenyan *Biomphalaria pfeifferi* populations are presented in Table 4; these are based on the subset of ten loci for which all populations could be characterised. All values of $F_{it}$ are large and positive, indicating a substantial deficiency of heterozygotes across the entire region. Values of $F_{is}$ are also all positive and generally high, indicating a deficiency of heterozygotes within local populations. The $F_{st}$ values are more heterogeneous, but the average value is high (0.586). The calculated values of $F_{st}$ were evaluated using a chi square test according to the following formula:

$$\chi^2 = 2N\,F_{st}(k-1),$$

with degrees of freedom equal to $(k-1)(s-1)$, where $k$ is the number of alleles for the locus and $s$ is the number of populations (Workman and Niswander, 1970). All of the $F_{st}$ values were found to be significant.

The observed genetic distances (Table 5) among the eastern populations of *B. pfeifferi* are extremely small ($D < 0.02$), as is the distance between two of the western populations, Asawo Stream and Martin's Drain ($D = 0.001$). The calculated distances between the latter two populations and the third western population, Kamayoga Stream, are comparatively large ($D < 0.26$), and distances between eastern and western populations are intermediate ($D = 0.20$).

## DISCUSSION

The level of detectable electrophoretic polymorphism (80%) was higher than in three previous studies of *B. pfeifferi* (Wium-Andersen, 1974; Dogba & Jelnes, 1985; Mimpfoundi *et al.*, 1986). This is probably attributable to larger numbers of individuals sampled per population, a larger number of sampling localities, and a larger study area. The level of polymorphism observed was also higher than in other species and stocks of *Biomphalaria* previously examined (Mulvey & Vrijenhoek, 1981a,b, 1982; Graven,

**Table 1.** Genotypic frequencies for polymorphic loci for 12 populations of *Biomphalaria pfeifferi*. Only genotypes observed in one or more populations are listed. Population numbers: (1) Matingani Seepage; (2) Muthamo Seepage; (3) Mbondoni Dam; (4) Kangonde Dam; (5) Mwea East; (6) Mwea West; (7) Grogan Canal; (8) Kwahoma Stream; (9) Asawo Stream; (10) Martin's Drain; (11) Kamayoga Stream; (12) Onsando Dam. Mobilities are expressed relative to the mobilities of alleles observed in the M-line stock of *Biomphalaria glabrata*, which are designated as 100. Three alleles were observed too rarely for accurate measurement of mobilities.

| Locus | | Population 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | | | |
| *Acn-1* | | | | | | | | | | | | | |
| 107/107 | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.24 | 1.00 | — | — |
| 107/98 | | | | | | | | | | 0.76 | | | |
| 0 | (N) | 28 | 21 | 13 | 5 | 48 | 31 | 38 | 24 | 17 | 18 | | |
| *Acn-2* | | | | | | | | | | | | | |
| 196/196 | | 0.05 | | | | | | | | | | | |
| 196/<140 | | | | | | | 0.19 | | | | | | |
| 196/140 | | 0.95 | 1.00 | 1.00 | 1.00 | 1.00 | 0.81 | 1.00 | 1.00 | 1.00 | — | — | — |
| | (N) | 21 | 18 | 4 | 2 | 12 | 16 | 2 | 3 | 16 | | | |
| *Akp* | | | | | | | | | | | | | |
| 100/100 | | 0.97 | 0.95 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.13 | 0.15 | 1.00 | 0.85 |
| 100/42 | | 0.03 | 0.05 | | | | | | | | | | 0.07 |
| 42/42 | | | | | | | | | | 0.87 | 0.85 | | 0.08 |
| | (N) | 29 | 21 | 20 | 8 | 51 | 16 | 50 | 34 | 31 | 27 | 7 | 13 |
| 200/200 | | 0.97 | 0.87 | 1.00 | 1.00 | 0.90 | 1.00 | 1.00 | 1.00 | 0.94 | 1.00 | | 1.00 |
| 200/100 | | | 0.06 | | | 0.07 | | | | 0.03 | | | |
| 100/100 | | 0.03 | 0.07 | | | 0.03 | | | | 0.03 | | 1.00 | |
| | (N) | 34 | 15 | 21 | 10 | 40 | 36 | 37 | 32 | 31 | 26 | 13 | 35 |
| *Got-1* | | | | | | | | | | | | | |
| 240/240 | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.96 | 1.00 |
| 100/100 | | | | | | | | | | | | 0.04 | |
| | (N) | 32 | 18 | 25 | 11 | 37 | 41 | 48 | 33 | 27 | 19 | 22 | 32 |
| *Gpd* | | | | | | | | | | | | | |
| 100/100 | | 1.00 | 1.00 | 1.00 | — | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.86 | — | 1.000 |
| 100/<100 | | | | | | | | | | | 0.07 | | |
| <100/<100 | | | | | | | | | | | 0.07 | | |
| | (N) | 8 | 20 | 13 | | 42 | 11 | 34 | 23 | 20 | 14 | | 10 |
| *Idh* | | | | | | | | | | | | | |
| 131/131 | | 1.00 | 0.61 | 0.94 | 1.00 | 0.88 | 0.94 | 0.98 | 1.00 | 1.00 | 0.96 | 1.00 | 0.67 |
| 131/123 | | | | | | | 0.03 | | | | | | |
| 131/<123 | | | | | | | 0.03 | | | | | | |
| 123/123 | | | 0.39 | 0.06 | | 0.12 | | 0.02 | | | 0.04 | | 0.33 |
| | (N) | 36 | 18 | 17 | 7 | 50 | 36 | 48 | 31 | 18 | 24 | 7 | 9 |
| *Mdh-1* | | | | | | | | | | | | | |
| 100/100 | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.96 | 1.00 | 0.67 |
| <100/<10 | | | | | | | | | | | 0.04 | | 0.33 |
| | (N) | 48 | 29 | 23 | 10 | 57 | 29 | 58 | 34 | 49 | 27 | 23 | 31 |
| *Nsp* | | | | | | | | | | | | | |
| 129/129 | | | | | | | | | | | | 0.04 | |
| 129/122 | | | | | | | | | | 0.02 | | | |
| 122/122 | | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.96 | 0.89 | 0.962 | 1.000 |
| 100/100 | | | | | | | | | | 0.02 | 0.07 | | |
| 100/88 | | | | | | | | | | | 0.04 | | |
| | (N) | 46 | 27 | 25 | 12 | 54 | 31 | 49 | 35 | 49 | 27 | 26 | 33 |
| *Pep-2* | | | | | | | | | | | | | |
| 100/94 | | | | | | | | | | 0.02 | | | |
| 94/94 | | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98 | 1.00 | 0.25 | 1.00 |
| 94/79 | | 0.10 | | | | | | | | | | 0.25 | |
| 79/79 | | | | | | | | | | | | 0.05 | |
| | (N) | 39 | 26 | 31 | 8 | 38 | 39 | 49 | 34 | 8 | 13 | 4 | 7 |

**Table 1.** continued

| Locus | | Population 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pgd* | | | | | | | | | | | | | |
| 175/157 | | 0.09 | | | | | | | | | | | |
| 157/157 | | 0.87 | 1.00 | 1.00 | 1.00 | 1.00 | 0.94 | 1.00 | 1.00 | 0.84 | 0.92 | 1.00 | 1.00 |
| 157/79 | | | | | | | | | | 0.08 | 0.08 | | |
| 79/79 | | 0.04 | | | | | 0.06 | | | 0.08 | | | |
| | (N) | 23 | 12 | 21 | | 37 | 36 | 29 | 14 | 12 | 13 | 15 | 14 |
| *Pgm-1* | | | | | | | | | | | | | |
| 175/175 | | 0.91 | 1.00 | 1.00 | | 1.00 | 1.00 | 1.00 | 0.56 | 1.00 | 0.40 | 1.00 | 1.00 |
| 175/100 | | | | | | | | | 0.33 | | | | |
| 100/100 | | 0.09 | | | | | | | 0.11 | | 0.60 | | |
| | (N) | 11 | 26 | 4 | — | 53 | 10 | 29 | 9 | 23 | 5 | 12 | 36 |

1984; Woodruff *et al.*, 1985; Mulvey *et al.*, 1988) but is nonetheless typical for molluscs in general (Selander & Ochman 1983).

Marked deviations from expected genotypic frequencies under Hardy-Weinberg equilibrium were observed in 6 of the 7 cases appropriate for evaluation. In all 6 cases a deficiency of heterozygotes was observed, suggesting non-random mating. Other loci support this conclusion also, but were not included in the table because of low expected frequencies. However, if deviations from Hardy-Weinberg predictions were due to the problems in the chi square analysis alone, it would follow that the discrepancies would be randomly distributed between heterozygote excess and heterozygote

**Table 2.** Chi square analysis.

| Population | Locus | Observed and Expected Frequencies | Chi square | P-value |
|---|---|---|---|---|
| Matingani | *Pep-2* | | | |
| | 94/94 | 35 (35.08) | 0.084 | 0.771 |
| | 94/79 | 4 (3.84) | | |
| | 79/79 | 0 (0.08) | | |
| Muthamo | *Idh* | | | |
| | 131/131 | 11 (6.60) | 19.179 | 0.000 |
| | 131/123 | 0 (8.80) | | |
| | 123/123 | 7 (2.60) | | |
| Mwea East | *Idh* | | | |
| | 131/131 | 44 (38.67) | 54.069 | 0.000 |
| | 131/123 | 0 (10.67) | | |
| | 123/123 | 6 (0.67) | | |
| Mwea West | *Acn-2* | | | |
| | 107/107 | 3 (0.48) | 18.720 | 0.000 |
| | 107/98 | 0 (5.03) | | |
| | 98/98 | 13 (10.49) | | |
| Asawo Stream | *Acn-2* | | | |
| | 107/107 | 4 (0.85) | 19.017 | 0.000 |
| | 107/98 | 0 (6.30) | | |
| | 98/98 | 13 (9.05) | | |
| | *Akp* | | | |
| | 100/100 | 4 (0.46) | 34.933 | 0.000 |
| | 100/42 | 0 (7.08) | | |
| | 42/42 | 27 (23.46) | | |
| Martin's Drain | *Akp* | | | |
| | 100/100 | 4 (0.53) | 30.375 | 0.000 |
| | 100/42 | 0 (6.94) | | |
| | 42/42 | 23 (19.53) | | |

**Table 3.** Fixation Indices.

| Population | Mean Heterozygosity Observed (Expected) | Fixation Index |
|---|---|---|
| Matingani Seepage | 0.01 (0.02) | 0.26 |
| Muthamo Seepage | 0.01 (0.07) | 0.85 |
| Mbendoni Dam | 0.00 (0.01) | 1.00 |
| Kangonde Dam | 0.00 (0.00) | 1.00 |
| Mwea East | 0.01 (0.03) | 0.79 |
| Mwea West | 0.01 (0.01) | 0.00 |
| Grogan Canal | 0.00 (0.00) | 1.00 |
| Kwahoma Stream | 0.00 (0.00) | 1.00 |
| Asawo Stream | 0.01 (0.04) | 0.83 |
| Martin's Drain | 0.00 (0.05) | 0.93 |
| Kamayoga Stream | 0.03 (0.08) | 0.68 |
| Onsando Dam | 0.01 (0.07) | 0.88 |

**Table 4.** Summary of F-Statistics of all loci. All chi squares of $F_{st}$ are significant at $p < 0.001$.

| Locus | $F_{is}$ | $F_{it}$ | $F_{st}$ | N | $\chi^2$ |
|---|---|---|---|---|---|
| *Akp* | 0.791 | 0.950 | 0.760 | 307 | 933.28 |
| *Est-2* | 0.610 | 0.922 | 0.799 | 330 | 527.34 |
| *Got-1* | 1.000 | 1.000 | 0.042 | 345 | 57.96 |
| *Idh* | 0.961 | 0.969 | 0.222 | 317 | 281.50 |
| *Mdh-1* | 1.000 | 1.000 | 0.040 | 418 | 33.44 |
| *Nsp* | 0.828 | 0.839 | 0.061 | 414 | 151.52 |
| *Pep-2* | 0.364 | 0.712 | 0.547 | 336 | 1102.75 |
| Average | 0.780 | 0.909 | 0.589 | | |

deficiency. The absence of any cases of heterozygote excess among the 6 cases in combination with the preponderance of cases of the absence of heterozygotes evident in Table 2 suggests that deviations from Hardy-Weinberg expectations may be real, and stem from non-random mating.

High positive values of $F_{it}$ indicate a deficiency of heterozygotes across the region. These values are much higher than those obtained in an earlier study of *B. glabrata* (Mulvey & Vrijenhoek, 1982). The deficiency of heterozygotes across localities is probably partly attributable to a deficiency within localities. High positive values of $F_{is}$ indicate a deficiency of heterozygotes within localities. The high value of $F_{is}$ is also indicative of non-random mating. High $F_{st}$ values suggest strong genetic differentiation among subpopulations. Indeed, the most prevalent allele shifts among localities for some loci. Both non-random mating and population subdivision are needed to explain the high levels of $F_{it}$ observed.

Pulmonate gastropods exhibit a variety of breeding systems, from obligate selfing to obligate outcrossing (see Selander & Ochman,

**Table 5.** Matrix of Nei's genetic distances.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Matingani | | | | | | | | | | | |
| 2 Muthamo | 0.016 | | | | | | | | | | |
| 3 Mbondoni | 0.001 | 0.012 | | | | | | | | | |
| 4 Kangonde | 0.000 | 0.016 | 0.000 | | | | | | | | |
| 5 Mwea East | 0.002 | 0.008 | 0.001 | 0.002 | | | | | | | |
| 6 Mwea West | 0.000 | 0.015 | 0.000 | 0.000 | 0.001 | | | | | | |
| 7 Grogan | 0.000 | 0.0[illegible] | 0.000 | 0.000 | 0.001 | 0.000 | | | | | |
| 8 Kwahoma | 0.000 | 0.016 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | | | | |
| 9 Asawo | 0.078 | 0.097 | 0.082 | 0.081 | 0.084 | 0.0[illegible] | 0.081 | 0.081 | | | |
| 10 Martin's Drain | 0.077 | 0.092 | 0.079 | 0.078 | 0.081 | 0.079 | 0.079 | 0.078 | 0.001 | | |
| 11 Kamayoga | 0.143 | 0.158 | 0.157 | 0.156 | 0.146 | 0.156 | 0.156 | 0.156 | 0.245 | 0.258 | |
| 12 Onsando | 0.013 | 0.002 | 0.009 | 0.012 | 0.006 | 0.011 | 0.011 | 0.012 | 0.075 | 0.070 | 0.178 |

1983). Populations of the land snail *Rumina decollata* (Selander *et al.*, 1973; Selander & Kaufman, 1973; Selander & Hudson, 1976) and some arionid and limacid slugs (Foltz *et al.*, 1982; Foltz *et al.*, 1984) are known to consist of a number of monotypic clones, with or without the potential to interbreed. Members of the genus *Biomphalaria* are generally assumed to be self-compatible hermaphrodites. Despite the capacity for selfing, however, populations of *B. glabrata*, *B. obstructa*, *B. straminea*, and *B. alexandrina* all appear to be primarily outcrossing (Paraense, 1956; Mulvey & Vrijenhoek, 1981a, 1982; Graven, 1984; Woodruff *et al.*, 1985). A previous study of West African *B. pfeifferi* populations reported little or no variation within populations or among very close localities, but considerable variation among distant localities (Mimpfoundi *et al.*, 1986). These authors interpret their data as indicating a higher reliance on selfing in this species. Mimpfoundi and coworkers (1986) did not consider the role of genetic drift in explaining low observed levels of genetic variation. Our results confirm those of Mimpfoundi *et al.*, (1986) and provide stronger evidence of both genetic drift and non-random mating, most probably selfing. Clearly, additional research on the mating behaviour of *B. pfeifferi* is warranted in order to determine the degree to which this species is reliant on selfing.

The calculated $F_{st}$ values suggest that there is little gene flow among the localities studied. The migration frequency that would be needed to obtain such differentiation among populations can be estimated as follows:

$$F_{st} = \frac{1}{4N_e m + 1}$$

where $N_e$ is the effective population size, and $m$ is the migration frequency per generation (Cavalli-Sforza & Bodmer, 1971). These two terms are difficult to estimate independently, but their product, $N_e m$, can be interpreted as the effective number of migrants per generation. This estimate assumes demographic stability, genetic equilibrium, random dispersal, and adaptive neutrality, and so is perhaps unrealistic as an estimate of gene flow, but it can nonetheless serve to allow us to compare our findings with other studies. When the average value of $F_{st}$ is used, $N_e m$ equals 0.176, which is equivalent to one migrant every 5 to 6 generations. This estimate of migration frequency is much lower than that reported for *B. glabrata* in Puerto Rico (Mulvey & Vrijenhoek, 1982), a species of similar vagility and habitat preference. The much lower estimate of migration frequency obtained in our study can probably be attributed to the larger geographic scale of our study (about 500 km between most distant localities as compared with 40 km for the Puerto Rican study of Mulvey & Vrijenhoek (1982)).

Geographic patterns are evident in the variation among sampling localities. Genetic distances among the sampling localities are well within the range described for local populations within a species in *Drosophila* (D < 0.23; Ayala, 1983). Considerable caution should be used in interpreting results of genetic distance analyses when small numbers of loci are used, as a single locus may drive the analysis (Wright, 1978). The genetic distances should therefore be viewed as an indication of the degree of resemblance of these populations across the 10 loci, and not as an indication of relationship.

All of the eastern samples are extremely similar. Two of three western samples also closely resemble each other. The third western locality, Kamayoga Stream, is comparatively divergent from the other western samples as well as from the eastern samples. Sample sizes for this last locality are unfortunately extremely small for some loci (N = 4–20). Thus, some of the apparent differentiation of this sample may be an artifact of small sample size. However, it should be noted that Kamayoga Stream is located on Rusinga Island in Lake Victoria, and may be somewhat isolated from other western localities. These snails were a little larger and flatter than the other two western samples, resembling the eastern samples more closely, so the differentiation could perhaps be real. Mandahl-Barth (1958) described at least two subspecies of *B. pfeifferi* from Kenya on the basis of conchological variation. In addition, two other species of *Biomphalaria*, *B. choanomphala* and *B. sudanica* inhabit Lake Victoria and adjacent swamps, respectively. All of the East African species and subspecies of *Biomphalaria* are defined on the basis of conchological characteristics, and although the organisms included in this study fall within the range of morphological variation described for *B. pfeifferi*, the possibility remains that conchological features may be a poor indicator of genetic relationship. Further investigation of this possibility is clearly warranted.

Although we did not examine snails for evidence of infection with *S. mansoni*, our collecting sites were located in areas in which schistosome transmission is known to occur. What implications might the population structure of *B. pfeifferi* have for the transmission of

*S. mansoni*? Susceptibility to schistosome infection is under genetic control, although the mechanism and the number of loci involved remain unknown (Richards, 1976). Based on their observations of population structure in *B. glabrata* in Puerto Rico, Mulvey & Vrijenhoek (1982) concluded that genetic drift might explain the patchy distribution of susceptibility to *S. mansoni* infection in *B. glabrata*. In *B. pfeifferi* we have observed evidence of both genetic drift and non-random mating, most probably attributable to low vagility and self-fertilization, respectively. Non-random mating does not result in a reduction in the number of alleles present, but rather in a reduction in the number of genotypes represented. As a result of the reduction in the number of genotypes, inbred populations can respond more rapidly to selection than outcrossed populations. Together, genetic drift and non-random mating act to produce highly localized gene combinations, as we are reporting for *B. pfeifferi*. The production of highly localized gene combinations may extend to all traits under genetic control, including susceptibility to schistosome infection. Clearly, the occurrence of both genetic drift and non-random mating could complicate the intrapopulation and interpopulation patterns of susceptibility. Studies of the susceptibility of *B. pfeifferi* (Basch, 1976 and references therein; Frandsen, 1979) have compared populations from very different areas (e.g. East vs. West or South Africa), and have concluded that this species exhibits a more uniform level of susceptibility to schistosome infection than do other species investigated. However, the geographic scale of these studies was very broad, and additional studies on a smaller biogeographic scale are needed to assess the variation in susceptibilty of *B. pfeifferi* to *S. mansoni* and its relationship to the population structure of the molluscan host.

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# Consumption of *Biomphalaria glabrata* egg masses and juveniles by the ampullariid snails *Pila ovata*, *Lanistes carinatus* and *Marisa cornuarietis*

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*Lanistes carinatus* and *Pila ovata* from Kenya and *Marisa cornuarietis* from the Dominican Republic were examined for their ability to consume egg masses and juveniles of *Biomphalaria glabrata* (M line strain) under laboratory conditions. Adults of all three ampullariid species consumed all egg masses presented to them over a five day period of observation. Juvenile *P. ovata* consumed significantly more egg masses than juveniles of *M. cornuarietis* or *L. carinatus*. Both adult and juvenile ampullariids generally ate more egg masses when maintained under a temperature regime (25–32°C) approximating conditions in coastal Kenya than at 13–25°C which approximates the temperature regime near Nairobi, Kenya. Egg masses attached to floating 'refugia' were not attacked, apparently reflecting the difficulty experienced by ampullariids in reaching free-floating objects. Adults of each ampullariid species ate approximately 25% of all $1.5 \pm 0.5$ mm *B. glabrata* juvenile snails presented to them. *P. ovata* adults consumed significantly more $3.0 \pm 0.5$ mm juveniles than adults of the other two species. *B. glabrata* egg masses and juveniles were consumed even though lettuce was continually present in experimental aquaria. Implications of these results for biological control studies in East Africa are discussed.

Key words: Ampullariid snails; Biological control; Schistosomiasis; Snail control; Trematode

## Introduction

Biological control of schistosome-transmitting snails achieved through the use of competitor or predator snails may, in certain kinds of habitats, offer a relatively simple, potentially self-renewing and inexpensive alternative to traditional methods of schistosomiasis control (McCullough, 1981; Pointier and McCullough, 1989). The neotropical ampullariid snail *Marisa cornuarietis* has attracted considerable attention in this context (Ferguson, 1977; Jobin et al., 1977, 1984; Combes and Cheng, 1986). *M. cornuarietis* consumes egg masses, juveniles, and, in some cases, even adults of schistosome-transmitting snails (e.g. Demian and Lutfy, 1965a, b, 1966) and is a voracious consumer of aquatic macrophytes (Seaman and Porterfield, 1964) that serve as food sources and oviposition sites for such snails. Introductions of this

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gastropod into Egypt (Demian and Kamel, 1973), Tanzania (Nguma et al., 1982), and the Sudan (Haridi et al., 1985; Karoum, 1988) suggest that *M. cornuarietis* may have control potential outside of the neotropics.

Species within the family Ampullariidae are widely distributed across both the old and new world tropics (Michelson, 1961). Although evidence exists that ampullariids other than *Marisa* may have value as control agents (e.g. Pointier et al., 1988), most species have not been adequately studied in this context. Before the introduction of exotic species such as *M. cornuarietis* into tropical Africa or elsewhere can be condoned, the potential of indigenous ampullariid species to exert comparable predatory or competitive effects should be examined. Indigenous species may have certain advantages such as increased availability and better adaptation to local conditions than exotic species. Furthermore, the introduction of any non-native species poses certain health, economic, and environmental risks (Nishimura et al., 1986) that might be avoided through the use of indigenous control agents.

Two African ampullariid species, *Pila ovata* and *Lanistes carinatus*, are common throughout large areas of Africa (Brown, 1980), but their potential as biological control agents, if any, remains largely unexplored. In this paper, we document the capacity of these two African ampullariid species to consume egg masses and juveniles of a planorbid snail under laboratory conditions; *M. cornuarietis* was also studied under identical conditions to provide a basis of comparison. *B. glabrata* was selected as the target species because large numbers of egg masses and juveniles were available for use in these experiments.

## Materials and Methods

*Pila ovata* was obtained in Kisumu, Kenya, *Lanistes carinatus* in Malindi, Kenya, and *Marisa cornuarietis* in Jarabacoa, Dominican Republic. All snails were maintained in the laboratory for at least one month prior to use in experiments. A large laboratory colony of *Biomphalaria glabrata* snails (M-line strain) provided the numerous egg masses and juveniles required for the experiments described below. Unless otherwise indicated, all experiments were performed at $25 \pm 1$°C, aeration was provided continuously, crushed oyster shell was used as a substrate in aquaria, and fresh leaf lettuce was supplied ad libitum. Lettuce was held on the bottom of aquaria with stones to facilitate access by ampullariids. A 12 h light, 12 h dark photoperiod was maintained for all experiments. Juvenile ampullariids used in these experiments ranged from 8–12 mm in size (shell diameter for *Lanistes* and *Marisa*, shell height for *Pila*) and adults were 30–40 mm in size. Juvenile or adult status of snails was assessed by examination of the reproductive organs of dissected snails of comparable size. Data were transformed to arc-sine values when necessary and were analyzed using Student's *t*-test or by ANOVA using a $p = 0.05$ level of significance. Following ANOVA, Tukey's test was used to assess differences between pairs of means.

### *Experiment 1. Consumption of Biomphalaria glabrata egg masses by ampullariids*

Adult *B. glabrata* were allowed to deposit egg masses on the sides of four litre aquaria. After 15–20 egg masses had been deposited, the *B. glabrata* adults were removed, and the water changed. Two ampullariids of a particular species, either

both adults or both juveniles, were then introduced and the number of egg masses totally consumed was monitored daily for five days. For each ampullariid species, six replicates were performed for both juvenile and adult specimens and the mean percentage of egg masses consumed was calculated.

*Experiment 2. Effect of different temperature regimes on egg mass consumption*

The ability of ampullariids to consume egg masses under two different fluctuating temperature regimes was assessed. These two regimes approximated temperatures encountered in natural habitats in two distinct regions of Kenya, as estimated using air temperature data obtained from the Kenya Meteorological Department (1984). A high temperature cycle, ranging from 25 to 32°C, and similar to the mean diurnal air temperature fluctuations in January at Mombasa, on the coast of Kenya, was established. A low temperature cycle ranging from 13 to 25°C, and similar to diurnal fluctuations during January in Nairobi was initiated. The temperature regimes were established by placing aquaria in rooms with different ambient temperatures; 75 W aquarium heaters were used to elevate temperatures appropriately. After the maximum temperature was reached, the water temperature was allowed to fall naturally to ambient levels.

These experiments were conducted in four litre aquaria as described above for experiment 1, except that the *B. glabrata* egg masses were presented to the ampullariids on plastic petri dishes which were placed in the bottom of each aquarium. The number of egg masses consumed over a three day interval was noted. Adults of all three species, as well as juvenile *P. ovata* and *M. cornuarietis* were tested. The experiment was repeated six times for each species and age group under each temperature regime. The mean percentage of egg masses consumed was compared statistically between species and between temperature regimes.

*Experiment 3. Consumption of egg masses attached to floating objects*

The ability of the three ampullariid species to find and consume eggs located on floating objects was compared. Two adult ampullariids of a particular species were introduced into a four litre aquarium. Five styrofoam squares (2 × 2 cm), each containing a single *B. glabrata* egg mass, were floated at the surface. The squares were secured so that they would not move laterally and they were positioned at least 2 cm from the sides of the aquarium. After three days, eggs were checked for evidence of consumption. Six replicates were performed with each ampullariid species. This experiment was also repeated six times under similar conditions except that lettuce was withheld from aquaria during the three day observation period.

*Experiment 4. Consumption of B. glabrata juveniles*

Two adult ampullariids of a given species were introduced into a four litre aquarium containing twenty *B. glabrata* juveniles (1.5 ± 0.5 mm). To facilitate observation of juvenile *B. glabrata*, no oyster shell was present in these aquaria. The aquaria were covered with tight-fitting lids to prevent the escape of juvenile snails. The number of juveniles present in the aquaria was carefully checked at two day intervals; at day eight, total consumption of juveniles was recorded. This procedure was repeated six

times for each ampullariid species, the mean percentage of juvenile *B. glabrata* that were consumed was calculated, and data were analyzed as described above. *B. glabrata* juveniles of 3.0±0.5 mm were also used as prey items; only 10 juveniles were used per replicate in this case.

## Results

*Experiment 1. Consumption of Biomphalaria glabrata egg masses by ampullariids*

Adults and juveniles of all three ampullariid species consumed *B. glabrata* egg masses (Table 1). Adults consumed all eggs presented to them within five days. Juveniles consumed fewer egg masses than adults, although juveniles of *P. ovata* did not differ significantly from adults in this regard. Juvenile *P. ovata* consumed significantly more egg masses than juveniles of the other two species.

*Experiment 2. Effect of different temperature regimes on egg mass consumption*

For both adult and juvenile ampullariids, egg masses were consumed at both temperature regimes. Fewer egg masses were consumed at the low temperature cycle, but owing to variability between replicates, the difference between regimes was significant only for juvenile and adult *P. ovata*, and for juvenile *M. cornuarietis*. At both temperature regimes, adult *L. carinatus* ate fewer egg masses than adults of the other species,

TABLE 1

The percentage (±S.E.) of *B. glabrata* egg masses or juveniles consumed by adults or juveniles of *Pila ovata*, *Lanistes carinatus*, or *Marisa cornuarietis*. Each experiment was replicated six times

| Experiment[d] | *Pila ovata* | *Lanistes carinatus* | *Marisa cornuarietis* |
|---|---|---|---|
| *Experiment 1* | | | |
| Adults | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 |
| Juveniles | 85.7±14.3[a] | 23.9±11.9 | 4.0±2.0 |
| *Experiment 2* | | | |
| Adults | | | |
| low temp. | 27.8±8.5 | 8.3±3.1 | 29.1±8.2 |
| high temp. | 70.8±10.2[b,c] | 23.6±11.1 | 43.1±10.0 |
| Juveniles | | | |
| low temp. | 8.4±6.9 | N.D. | 9.7±3.4 |
| high temp. | 69.7±6.9[a,c] | N.D. | 29.1±8.2[c] |
| *Experiment 4* | | | |
| 1.5±0.5 mm juveniles | 25.8±11.6 | 26.7±5.3 | 25.8±10.5 |
| 3.0±0.5 mm juveniles | 50.0±14.4[a] | 1.7±1.7 | 1.7±1.7 |

[a] Significantly greater than other similar-sized ampullariids, $p<0.05$.
[b] Significantly greater than *L. carinatus*, $p<0.05$.
[c] Significantly greater than corresponding group at low temperature regime, $p<0.05$.
[d] Experiment 3 is not represented as no *B. glabrata* egg masses or juveniles were consumed.
N.D., not done.

but results of individual trials were variable and the mean for *L. carinatus* was significantly lower only at the high temperature regime. The highest mean levels of egg mass consumption were achieved by *P. ovata* juveniles and adults at the high temperature regime; *P. ovata* juveniles consumed significantly more egg masses than juvenile *M. cornuarietis* at this temperature regime. Overall, adults ate relatively fewer egg masses in this experiment as compared to experiment 1 although the results are not strictly comparable because the two experiments differed in both temperature and duration.

*Experiment 3. Consumption of egg masses attached to floating objects*

None of the *B. glabrata* egg masses deposited on floating styrofoam 'refugia' were eaten by the ampullariids. In no case were ampullariids observed to be in contact with the styrofoam, suggesting they were unable to reach free-floating objects in the aquaria. The same results were obtained in replicates in which lettuce or other alternative food sources were not available.

*Experiment 4. Consumption of B. glabrata juveniles*

For each ampullariid species examined, approximately 25% of all $1.5 \pm 0.5$ mm juvenile *B. glabrata* snails disappeared over the course of the eight-day observation period. As the juveniles were unable to crawl out of the aquaria, these losses were attributed to predation by the ampullariids. This conclusion was reinforced by direct observations of attacks on juveniles by ampullariids and by finding fragments of *B. glabrata* shells in several aquaria. When $3.0 \pm 0.5$ mm *B. glabrata* juveniles were used as prey items, *P. ovata* consumed significantly more juveniles than the other two species.

## Discussion

Numerous published reports (Chernin et al., 1956; Oliver-Gonzales et al., 1956, Demian and Lutfy, 1965a,b, 1966; Cedeno-Leon and Thomas, 1983) indicate that both juvenile and adult *M. cornuarietis* can consume *Biomphalaria* egg masses and juveniles under laboratory conditions. Our study confirms these results for *Marisa* and demonstrates a similar capability for both *P. ovata* and *L. carinatus* from Kenya. Adults of all three ampullariid species consumed all *B. glabrata* egg masses they were given in experiment 1, even though lettuce was continually available to them. Under similar conditions, juvenile *P. ovata* consumed significantly more *B. glabrata* egg masses than juveniles of the other two ampullariid species. Although we have only limited numbers of the African planorbids *Biomphalaria sudanica* and *Bulinus globosus* available for study, egg masses of both species are eaten by both *P. ovata* and *L. carinatus* under similar conditions (unpublished observations). Thomas and Tait (1984) concluded that it was likely that *Lanistes libycus* from Nigeria consumed *B. pfeifferi* eggs during their laboratory experiments, and *L. carinatus* has been implicated as an egg predator of *B. pfeifferi* in laboratory experiments in the Sudan (Blue Nile Health Project, cited by Karoum, 1988). The west African ampullariid *Saulea*

*vitrea* eats egg masses and also adults of schistosome-transmitting snails in the laboratory (White, personal observations, cited in White et al., 1989).

The two different temperature regimes used in experiment 2 were selected because they approximated conditions in the warm, coastal regions of eastern Kenya and in the high, relatively cool regions around Nairobi. In Kenya, both *L. carinatus* and *P. ovata* are abundant in the coastal region and are essentially absent above 1200 m elevation (Brown, 1980; personal observations). It was of interest to determine if ampullariids would consume eggs under the low temperature regime, particularly because schistosome-transmitting pulmonates are common in Kenya at locations that experience these approximate temperatures. Our results indicate that the rate of egg mass consumption was consistently lower, but not eliminated, at the low temperature regime. The results also suggest that under the conditions of this experiment and *L. carinatus* was less likely to eat pulmonate egg masses than the other two species. Both the natural absence of ampullariids at higher altitudes in Kenya, and the diminished rates of egg consumption noted at the lower temperature regime suggest that any deliberate introductions of ampullariids as control agents in Kenya may have greater success at lower, warmer altitudes.

Egg masses placed on floating pieces of styrofoam were not attacked, even if alternative food sources were unavailable for the three day duration of the experiment. Owing to their large size, ampullariids have difficulty moving along the surface film of water. Also, aeration disturbs the water surface and seems to interfere further with the ability of ampullariids to move to floating objects. In no case did we observe ampullariids attached to the styrofoam blocks even though the blocks were prevented from moving laterally. These results suggest that in natural habitats, refugia for pulmonates and their egg masses may be found on floating objects; it may be particularly difficult for ampullariids to attach to small, free-floating objects such as leaves. Depositions of egg masses in spaces too small to be reached by ampullariids may also prevent attack.

All three ampullariid species consumed 1.5 mm juveniles of *B. glabrata*, even when lettuce was continually available in the aquaria. Only *P. ovata* consumed appreciable numbers of larger juveniles in the 2.6–3.5 mm size range. Demian and Lutfy (1965a,b, 1966) noted that *M. cornuarietis* attacked and killed even adults of *Bulinus truncatus* and *Lymnaea caillaudi*, but were unable to consume *Biomphalaria alexandrina* with shell diameters larger than 5 mm.

Although results of these laboratory experiments should be interpreted with caution, they indicate that *P. ovata* performed at least as well as *Marisa* under the described conditions. They further suggest that before wide-scale introductions of *Marisa* into sub-Saharan Africa are contemplated, the impact of native ampullariids on target species populations should be carefully studied under laboratory and especially field conditions.

Enthusiasm for African ampullariids must, however, be tempered by the fact that they frequently co-exist with pulmonates in natural habitats. For example, Thomas and Tait (1984) and Ndifon and Ukoli (1989) both found *Lanistes libycus* to have a significant positive association with *Bulinus globosus* in Nigeria, and Madsen et al. (1988) found a positive correlation between the presence of *L. carinatus* and *B. pfeifferi* in natural habitats in the Sudan. Karoum (1988) introduced *L. carinatus* into three canals in the Gezira Agricultural Scheme and, although populations of this snail did become established, their presence did not result in a dramatic reduction

of pulmonates. These observations coupled with our present results suggest that future investigation should focus on *P. ovata*. White et al. (1989) also noted that the ampullariid *Saulea vitrea* does not coexist with schistosome-transmitting pulmonates in Sierra Leone.

Finally, it remains to be seen if African ampullariids serve as resource competitors with pulmonate snails. Other ampullariids such as *Ampullaria glauca* have been shown to displace pulmonates in natural habitats by eliminating floating macrophytes (Pointier et al., 1988). Little is known regarding the ability of *P. ovata* and *L. carinatus* to consume macrophytes. Karoum (1988), however, found that *L. carinatus* had little effect on macrophyte density in Sudanese irrigation canals. *Pila globosa* is known as a voracious consumer of macrophytes in India, and has been proposed as a means of controlling aquatic weeds (Thomas, 1975). In this connection, as has been stressed elsewhere (WHO, 1984), it is evident that further studies relevant to the possible effects of native and exotic ampullariids on rice cultivation in Africa (see also Haridi et al., 1985) are required before their use in biological control operations can be condoned without reserve.

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## Appendix 3

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# Growth of *Biomphalaria glabrata* populations in the presence of the ampullariid snails *Pila ovata*, *Lanistes carinatus* and *Marisa cornuarietis*

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Adults of three ampullariid species were examined for their ability to affect the population growth of *Biomphalaria glabrata* (M line strain) under laboratory conditions in which food (lettuce) was supplied ad libitum over an eleven week interval. *Lanistes carinatus* and *Pila ovata* were obtained from Kenya, and *Marisa cornuarietis* originated from the Dominican Republic. The presence of either 3 or 9 *L. carinatus* or 3 *M. cornuarietis* per 40 l aquarium did not reduce the population size of *B. glabrata* below levels attained in control aquaria lacking ampullariids The presence of 9 *M. cornuarietis* adults significantly reduced *B. glabrata* populations by week 10 of the experiment. Presence of 3 or 9 *P. ovata* per aquarium significantly reduced *B. glabrata* numbers below control levels starting at week four of the experiment. Aquaria with 9 *P. ovata* were observed to have fewer *B. glabrata* egg masses than control aquaria, but the number of egg masses observed was not significantly reduced in aquaria with 3 *P. ovata* or with 3 or 9 *L. carinatus* or *M. cornuarietis*. However, the presence of 3 or 9 adults of each ampullariid species resulted in a significant increase in the percentage of such egg masses that disappeared prior to expected hatching date and that were presumed to have been consumed. The results of this laboratory study suggest that further investigation of the role of ampullariids as biological control agents for pulmonate snails in sub-Saharan Africa should focus on *P. ovata*.

Key words: Ampullariid snails; Biological control; Schistosomiasis; Snail control; Trematode

## Introduction

Schistosomes and other snail-transmitted parasites continue to exert a significant negative effect on the health of humans and domestic animals in sub-Saharan Africa. Although considerable improvements in chemotherapy have alleviated some of this burden and hope remains high for the development of effective vaccines, these control measures alone are unlikely to break the cycle of transmission; they do not directly address the role of the molluscan host in the life cycle. Ideally, simultaneous application of measures to eliminate both adult and larval trematodes could directly reduce morbidity and mortality, minimize the immediate risk of reinfection and, over the long-term, minimize or eliminate transmission.

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Control of snails and larval trematodes is widely recognized as a very difficult endeavor, largely because of the potentially vast volumes of water available for colonization by snails. The shortcomings of chemical methods of snail control are now well-appreciated (Klumpp and Chu, 1987) and for most epidemiological situations, new approaches are required if any hope is to be offered for controlling snails. Biological methods of snail control are frequently discussed (Ferguson, 1977; McCullough, 1981; Thomas and Tait, 1984; Combes and Cheng, 1986; Pointier and McCullough, 1989; Madsen, 1990) and have considerable appeal because of their relative low cost and potential sustainability, both of which are important considerations for the developing countries affected.

Although much more research is required to identify promising control agents, particularly viruses or bacteria, for aquatic molluscs the approach most commonly cited for biological control of medically important snails is the use of competitor or predator snails (WHO, 1984). *Marisa cornuarietis*, an ampullariid snail originating from South America, has attracted considerable attention as a biological control agent as it consumes egg masses, juveniles, and adults of schistosome-transmitting snails, and is known to avidly consume aquatic macrophytes required as food and oviposition sites for such snails (Ferguson, 1977; Jobin et al., 1977, 1984). This snail has already been introduced into Africa for the purpose of controlling the intermediate hosts of schistosomes (Demian and Kamel, 1973; Nguma et al., 1982; Haridi et al., 1985; Karoum, 1988).

Several ampullariid species are indigenous to Africa (Brown, 1980) and their role as potential biological control agents of disease-transmitting pulmonates has never been adequately studied. Although it could be argued that such species have no potential value in control given the ubiquity of schistosomiasis, fascioliasis, and amphistomiasis in Africa, this view overlooks the possibility that native ampullariids may exert some measure of natural control which has never been properly measured or appreciated. Different groups of freshwater snails may have different distributional patterns such that pulmonates often avoid negative competitive or predatory interactions with ampullariids. Attributes of African ampullariids relevant to biological control should be documented and compared directly to those exhibited by *M. cornuarietis* before further introductions of the latter species into African waters are contemplated.

In a prior study, it was documented that *Lanistes carinatus* and *Pila ovata*, both widely distributed African ampullariids, consume egg masses and juveniles of *Biomphalaria glabrata* at rates comparable to, or in some cases greater than, those exhibited by *M. cornuarietis* (Hofkin et al., 1991). In the present study, we compare the ability of the same three ampullariid species to affect the population growth of *B. glabrata* in laboratory aquaria under conditions of food excess. *B. glabrata* was again selected for study because of the requirement for a target pulmonate species that predictably establishes fluorishing populations in laboratory aquaria.

## Materials and Methods

### *Snails*

*Marisa cornuarietis* were derived from a laboratory colony established in 1986 from snails originating from Jarabacoa, the Dominican Republic. Both *Lanistes carinatus*

(from Malindi, Kenya) and *Pila ovata* (from Kisumu, Kenya) were collected in the field as juveniles in 1989, and were grown to maturity in the laboratory. All experiments used adult ampullariid snails ranging from 30–40 mm in size (shell diameter for *Lanistes* and *Marisa*, shell height for *Pila*). *Biomphalaria glabrata* (M line strain) were derived from a laboratory colony.

*Experimental conditions*

Aquaria (each 40 liters) were used for all experiments. Aeration was provided continuously, crushed oyster shell was used as a substrate in each aquarium, and fresh leaf lettuce was supplied ad libitum. Lettuce was held on the bottom of each aquarium with stones to facilitate access by ampullariids. All experiments were performed at 25±2°C; 75 W heaters were placed in each aquarium to help stabilize the temperature.

Ten adult *B. glabrata* were placed in each of 30 aquaria. For each of the three ampullariid species, a total of eight of these aquaria were used; four received three adult ampullariids each and four received nine adult ampullariids each. The six remaining aquaria served as controls and received no ampullariids. At weekly intervals thereafter, without disrupting the sediments, the number of *B. glabrata* of <2 mm, 2–9 mm, and >9 mm shell diameter in each aquarium was determined. All aquaria were maintained for 11 weeks. Water in the aquaria was changed monthly. Any ampullariid snail that died was replaced immediately; ampullariid egg masses were removed from the aquaria.

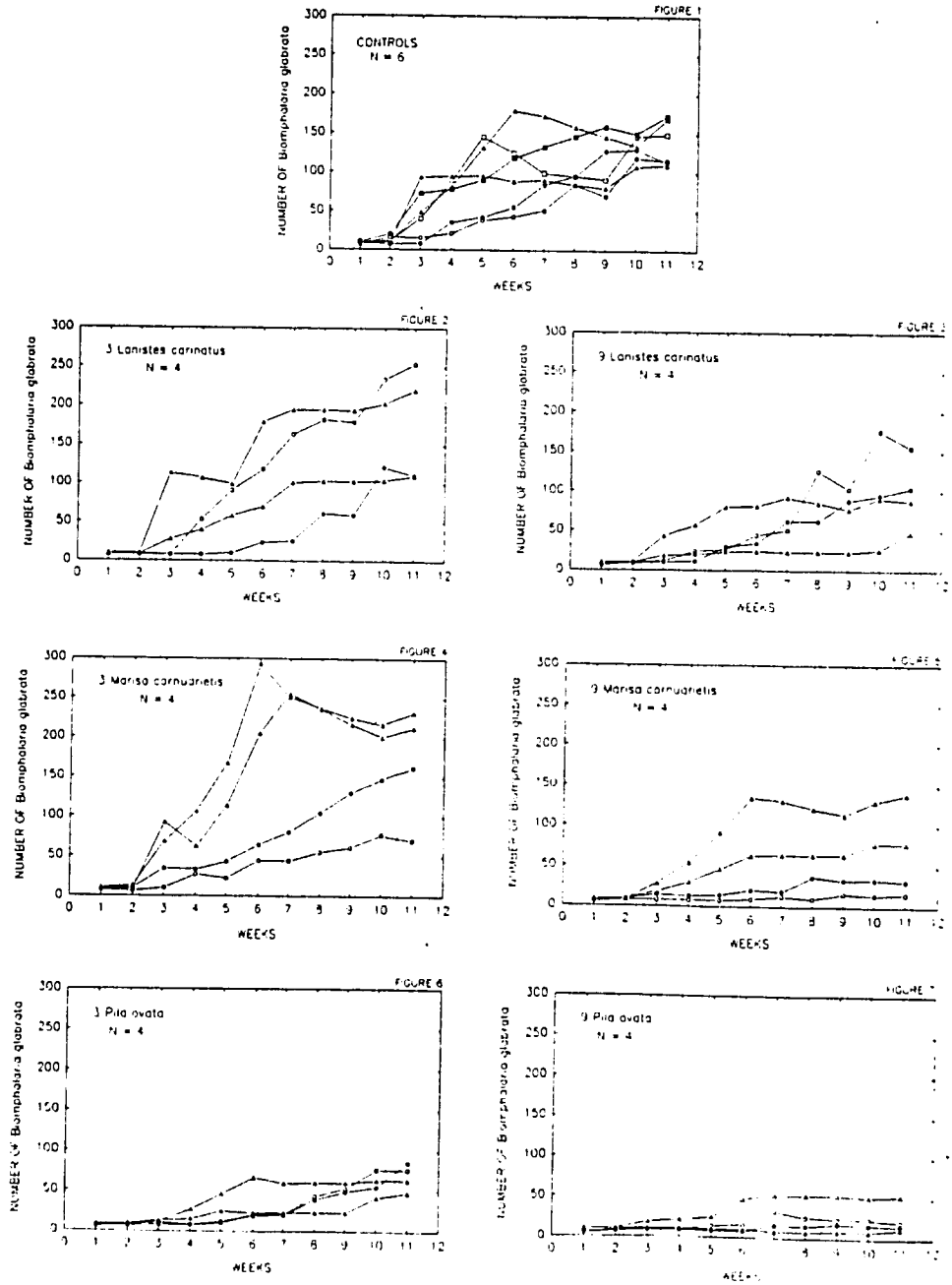
All *B. glabrata* egg masses deposited on one wall of each aquarium were observed three times weekly. The date of initial appearance and position of each egg mass was noted. It was determined if the egg mass disappeared prior to the expected hatching date or if the eggs it contained completed normal development and hatched. The minimum development time was conservatively considered to be five days. Egg masses that disappeared prior to their expected hatching date were assumed to have been eaten.

Statistical comparisons between individual experimental treatments and controls were made using Student's *t* test, with the level of significance selected as 0.05.

## Results

For the six control aquaria, the total number of *B. glabrata* counted during each week of the experiment is presented in Fig. 1. For each experimental treatment, similar counts are provided in Figs. 2–7. The mean weekly counts for control aquaria and for each experimental treatment are presented in Fig. 8. Fig. 9 indicates the mean total number of egg masses observed per tank, and the mean total number of egg masses that disappeared and were presumed to have been eaten. Fig. 10 summarizes the mean percentage of egg masses that were assumed to have been consumed for each experimental group and for control aquaria. Indicated in Fig. 11 is the mean number of *B. glabrata* in each recognized size category at the end of the experiment. The percentage of all snails in each size category at the end of the experiment is presented in Fig. 12.

For the control aquaria, the number of *B. glabrata* generally rose throughout the




Figs. 1–7. In Fig. 1 is indicated the total number of *Biomphalaria glabrata* counted in each of the six control aquaria during each week of the experiment. Each aquarium is represented by a separate line. Weekly counts of *B. glabrata* are also provided for each of the six experimental treatments (see Figs. 2–7). For example, Fig. 2 indicates the number of *B. glabrata* recovered each week from each aquarium containing 3 *Lanistes carinatus*. Each of the four aquaria used for this experimental treatment is indicated by a separate line.

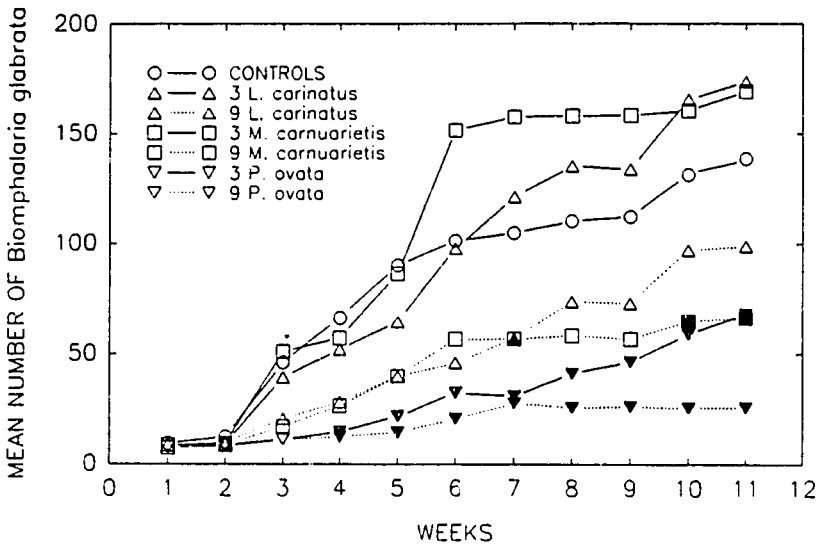



Fig. 8. Indicated for control aquaria and for the six different groups of experimental aquaria is the mean number of *Biomphalaria glabrata* recovered during each of the eleven weeks of the experiment. Solid symbols indicate times and treatments that differ significantly from control values.

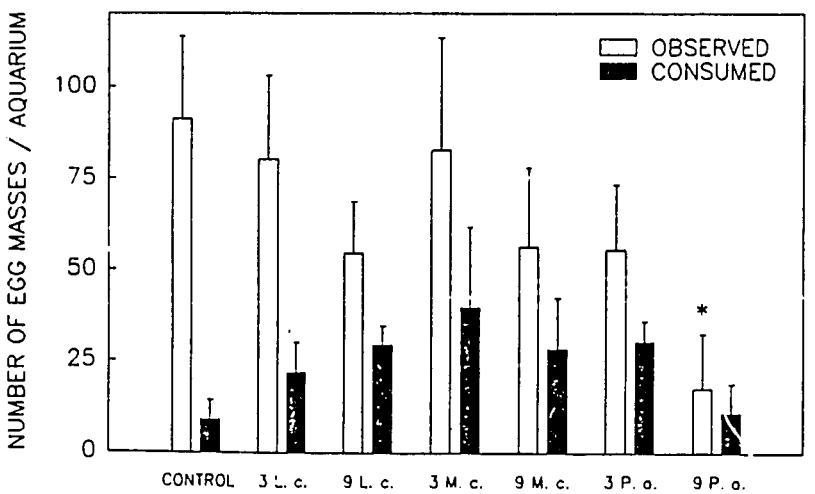



Fig. 9. Indicated for control aquaria and for the six different groups of experimental aquaria is the mean total number of different egg masses observed on one side of each aquarium throughout the eleven week experiment. Also indicated is the mean total number of these egg masses that were observed to have disappeared prior to their expected hatching date and are presumed to have been consumed. An asterisk indicates a significant reduction in total number of egg masses observed.

course of the experiment such that final counts were on average about 13-fold greater than the starting numbers. Relatively large numbers of egg masses were observed in control aquaria and most hatched normally; about 7% of the egg masses in control aquaria disappeared prior to the expected hatching date. As evidence that successful recruitment was taking place, at the end of the experiment, control aquaria contained relatively large numbers of <2 mm snails as compared to experimental aquaria. They also contained many 2–9 mm snails.

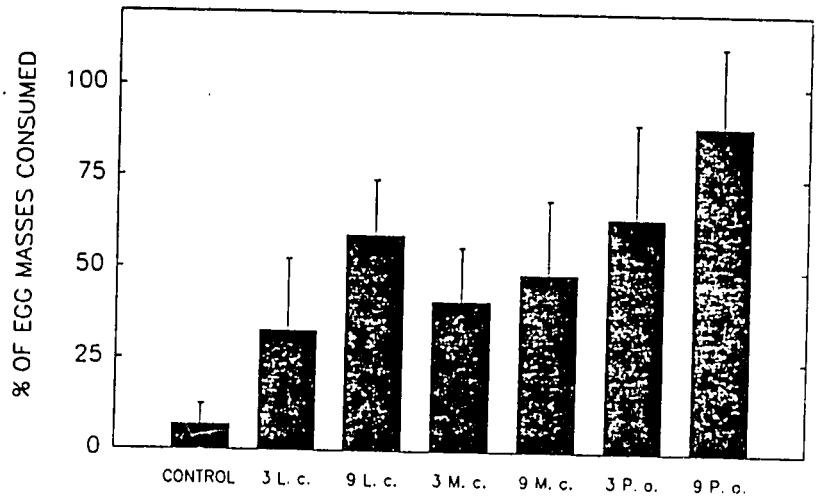



Fig. 10. The mean percentage of all egg masses that were observed and subsequently disappeared is indicated for control aquaria and for each of the six experimental groups. The value for each experimental group is significantly higher than the control value.

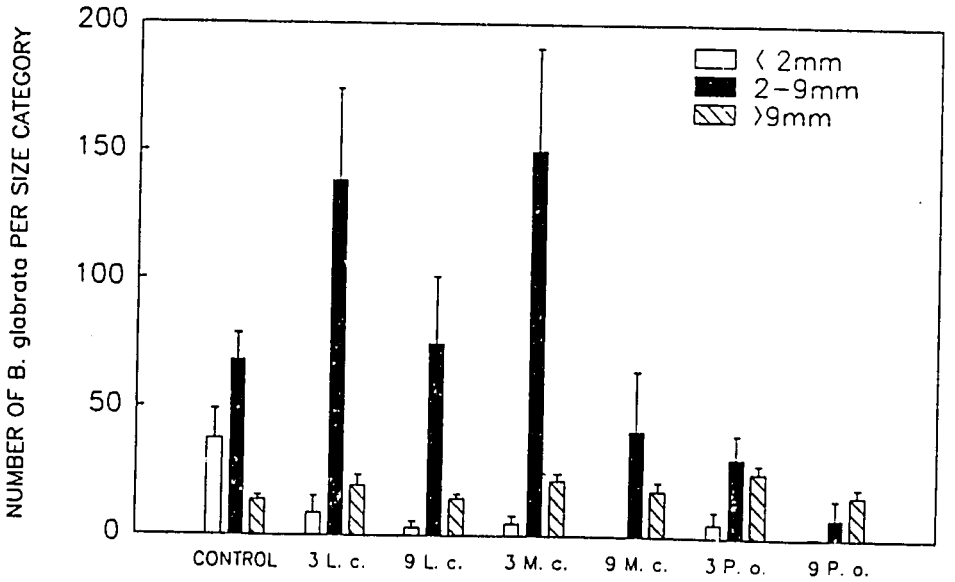



Fig. 11. The mean number of *Biomphalaria glabrata* of <2 mm, 2-9 mm, and >9 mm present in each aquarium at the end of the experiment is indicated for control aquaria and for each experimental group of aquaria.

Experimental aquaria containing 3 *L. carinatus* on average produced more *B. glabrata* than did aquaria of any other group, including controls. However, total *B. glabrata* counts did not differ significantly from controls at any time. The number of *B. glabrata* egg masses observed in such tanks did not differ significantly from controls, but the percentage of such egg masses that disappeared prior to expected hatching date (33%) was significantly higher than in control aquaria. Relatively

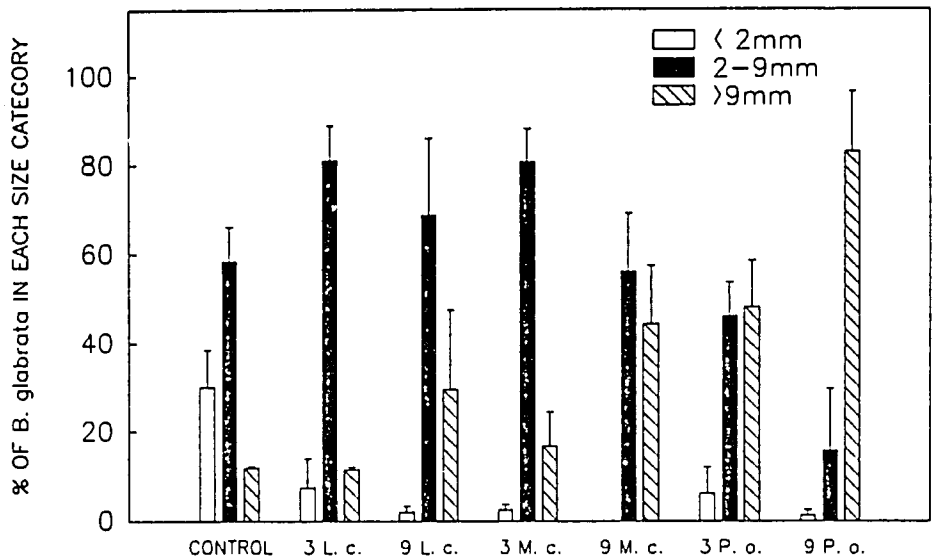



Fig. 12. The mean percentage of all *Biomphalaria glabrata* that were >2 mm, 2–9 mm, or >9 mm at the end of the experiment is indicated for control aquaria and for each experimental group of aquaria.

large numbers of 2–9 mm snails were found in such aquaria by the end of the experiment, indicating generally favourable conditions for growth.

Aquaria containing 9 *L. carinatus* had, on average, fewer *B. glabrata* than control aquaria, but the difference was not significant. Fewer total egg masses were observed in such aquaria, but this difference was not significant. A large percentage (59%) of these egg masses disappeared before hatching, significantly more than in control aquaria. By the end of the experiment, such aquaria contained many fewer <2 mm snails than controls although the presence of several 2–9 mm snails suggested that conditions were generally favorable for growth of *B. glabrata*.

The results obtained with *M. cornuarietis* were in most respects similar to those obtained with *L. carinatus*. The numbers of *B. glabrata* recovered in aquaria receiving 3 *M. cornuarietis* were higher than control levels whereas aquaria receiving 9 adults of this species had lower final mean counts than control aquaria. By weeks 10 and 11, the number of *B. glabrata* in aquaria with 9 *M. cornuarietis* was significantly lower than in controls. Fewer egg masses were observed in aquaria with *M. cornuarietis*, but the difference from control levels was not significant for either the 3 or 9 snail treatment group. The mean percentage of egg masses that disappeared prior to hatching in aquaria receiving 3 or 9 *M. cornuarietis* was 41% and 48.5%, respectively, both figures being significantly higher than control values.

Of the three ampullariid species examined, *P. ovata* had the greatest impact on *B. glabrata* populations. Starting at week four and lasting until the end of the experiment, aquaria receiving either 3 or 9 adults of this species had significantly lower *B. glabrata* counts than control aquaria. Aquaria receiving 9 *P. ovata* on average supported only a 2.5-fold increase in *B. glabrata* counts. In aquaria containing 3 *P. ovata*, the number of egg masses observed was reduced, but the difference with control aquaria was not significant; 64% of these egg masses disappeared before hatching, a significantly higher rate of loss than controls. In aquaria containing 9

*P. ovata*, the number of egg masses observed was reduced significantly, to about 20% of control levels. Of these, 89% disappeared before they had sufficient time to hatch, the highest rate of egg mass disappearance noted in this study. The strong effect of *P. ovata* in blocking recruitment is also reflected by the small number of <2 mm and 2–9 mm snails observed in such aquaria.

## Discussion

In all 30 aquaria, *B. glabrata* populations persisted throughout the eleven week period of study and in most they increased several-fold beyond the starting population size of ten adults. The presence of either 3 or 9 adults of *L. carinatus* or of 3 adults of *M. cornuarietis* was insufficient to prevent *B. glabrata* populations from attaining sizes comparable to those observed in control aquaria. Addition of 9 adults of *M. cornuarietis* or of 3 or 9 *P. ovata* had the effect of reducing *B. glabrata* populations below control levels by the end of the experiment. For each ampullariid species examined, 9 adults achieved better control than 3 adults. *P. ovata* achieved substantially better control than *M. cornuarietis* which in turn was marginally better than *L. carinatus*.

The mean total number of egg masses per aquarium observed throughout this experiment was highest, and the percentage of these egg masses that disappeared was lowest, in control aquaria. Approximately 7% of the egg masses disappeared in control aquaria, indicating a low level of egg cannabalism by adult *B. glabrata*. The number of egg masses observed was generally lower in aquaria containing ampullariids, especially when 9 ampullariids were present, but except for the 9 *P. ovata* treatment, these differences were not significant. Our data do not allow us to determine if this decline was due to reduced production of egg masses by *B. glabrata* in the presence of ampullariids, or to rapid consumption of new egg masses by ampullariids in the 2–3 day interval between observations.

Decreased production of *B. glabrata* egg masses might result from competition for resources which has been reported in other laboratory studies of interactions between *M. cornuarietis* and *B. glabrata* (Cedeno-Leon, 1975). Similarly, in a natural habitat in Guadeloupe, the disappearance of *B. glabrata* has been attributed to the consumption of its preferred food by the ampullariid *Ampullaria glauca* (Pointier et al., 1988). In the present case, lettuce was supplied ad libitum. Furthermore, no obvious form of interference competition was observed between *B. glabrata* and ampullariids and they were observed to feed side-by-side, but this does not rule out the possibility that subtle interference effects occurred, as has been observed by Madsen (1986) with *Helisoma duryi* and *Bulinus truncatus*.

Alternatively, decreased *B. glabrata* egg production might result from the presence of inhibitory chemicals produced by ampullariids. Other studies have documented the production of such compounds by freshwater snails (see discussion by Thomas et al., 1975 and Chaudhry and Morgan, 1986), and Cedeno-Leon (1975) noted that conditioning of media by *M. cornuarietis* did impair growth and reproduction by *B. glabrata*. Although this possibility was not specifically addressed in the present study, we think it is unlikely to account for the small *B. glabrata* populations observed in some ampullariid tanks, for the following reason. At the end of the experiment, in two of the tanks that had contained 9 *P. ovata*, the ampullariids were removed and

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# Appendix 4

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## CONTROL OF SCHISTOSOME-TRANSMITTING SNAILS IN KENYA BY THE NORTH AMERICAN CRAYFISH *PROCAMBARUS CLARKII*

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*Abstract.* Snail-transmitted trematode parasites such as schistosomes and liver flukes assume considerable medical and veterinary significance in tropical Africa. We have observed a strong negative association between the presence of medically important pulmonate snails and the crayfish *Procambarus clarkii* in freshwater habitats in Kenya. This crayfish, introduced into Kenya around 1970, readily consumes these snails in the laboratory. Field enclosure experiments indicate that crayfish exert a significant negative impact on the abundance of *Biomphalaria pfeifferi*, the intermediate host of the human blood fluke *Schistosoma mansoni*. It is likely that *P. clarkii* will continue to spread naturally in Kenya and that schistosome-transmitting snails will be excluded or reduced in numbers where crayfish are present. *Procambarus clarkii* may represent an alternative, biological means of snail control in East Africa.

The North American crayfish, *Procambarus clarkii*, introduced into East Africa for aquacultural purposes in about 1970,[1,2] has since dispersed into all major drainage systems in Kenya. It is particularly abundant in ponds in central Kenya.[3] Numerous studies outside Africa indicate that crayfish can significantly reduce populations of freshwater snails,[4–6] and that predation by such macroinvertebrates plays a dominant role in structuring invertebrate communities in certain habitat types.[7,8] Occasional inferences also suggest that crayfish may be suitable for the control of snail-borne diseases.[9,10] Among the snails affected or potentially affected by this decapod in Kenya are *Biomphalaria pfeifferi* and *B. sudanica*, intermediate hosts of the human bloodfluke *Schistosoma mansoni*, *Bulinus globosus*, which transmits the related human bloodfluke *S. haematobium*, and *Lymnaea natalensis*, intermediate host of the liver fluke *Fasciola gigantica*.[11]

In a related study (unpublished data), we demonstrate that *P. clarkii* is able to control laboratory populations of *B. pfeifferi*. In this report, that possibility is examined in natural foci of disease transmission. An analysis of the extent to which medically important snails and *P. clarkii* naturally coexist, as well as results with experimental field enclosures, indicate what may be a fortuitous and unexpected consequence of the introduction of this crayfish into Kenya. *Procambarus clarkii* appears able to eliminate otherwise abundant pulmonate snails that serve as obligatory hosts for several important trematodes of medical or veterinary significance. It may offer an alternative, biological means of snail control that is compatible with economic and logistic realities in the developing world.

### MATERIALS AND METHODS

#### *Natural coexistence of* P. clarkii *and pulmonate snails*

To determine whether established populations of *P. clarkii* naturally coexist with vector snails, a survey of 53 freshwater habitats in Kenya was conducted. These sites were all within the known distribution range of both *P. clarkii* and medically important pulmonates,[3,11] and were considered a priori to be suitable pulmonate snail and crayfish habitats. Unsuitable habitats, such as polluted ponds, ephemeral waterbodies, or fast-flowing streams were not included in this analysis.

A systematic search for snails along the shoreline of each site was conducted using long-handled snail scoops. The presence of *P. clarkii* was assessed using meat-baited crab traps. Fisher's exact test was used to detect positive or negative associations between the presence of crayfish and vector snails.





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*Laboratory consumption of medically important pulmonate snails*

The capacity of *P. clarkii* to consume selected Kenyan pulmonates was subsequently examined in the laboratory. Five adult snails of each species (either *B. pfeifferi*, *B. globosus*, or *L. natalensis*, with a maximum shell dimension of 5mm) were placed in a 10-liter aquarium with either a single adult (carapace length > 3 cm) or juvenile (1.2–2.6 cm carapace length) crayfish. Snail consumption was recorded after 48 hr. No alternative food was provided for *P. clarkii*. The experiment with adult crayfish was repeated 20 times for *B. pfeifferi* and *L. natalensis* and 14 times for *B. globosus*. Juvenile trials were repeated 10 times for *B. pfeifferi* and *L. natalensis* and seven times for *B. globosus*. Crayfish size classes specified here were also used in subsequent experiments to categorize adult and juvenile *P. clarkii*.

*Enclosure experiments*

Enclosure experiments were conducted in a small man-made reservoir in central Kenya near Makuyu, Maranga District, which already contained an established population of *B. pfeifferi*. Plastic basins, 52 cm in diameter, 24 cm in depth, and perforated with 5-mm diameter holes were used as enclosures. A layer of soil from the dam, approximately 8 cm in depth, was added to provide a natural substrate. In each basin, a single waterlily (*Nymphaea caerula*) was provided as snail refuge and food and as an alternative food for crayfish. The presence of this aquatic plant has been positively correlated with that of *Biomphalaria* snails.[12] *Nymphaea caerula* is also readily consumed by *P. clarkii*.[2, 13] Basins were submerged in the dam to within about 2 cm of their rims and covered with nylon mesh (2mm$^2$ mesh size). All enclosures received 30 adult *B. pfeifferi* (> 5mm in diameter) and half received a single *P. clarkii* collected near Nairobi. After five days, snails were counted in all enclosures, as were snail egg masses deposited on *N. caerula*. The experiment was repeated 12 times with adult *P. clarkii* and 12 times with juveniles. Paired *t*-tests were used to assess statistical differences between experimental and control treatment values of snail survival and egg mass deposition.

These same enclosures were used to determine the average daily consumption of snails by both adult and juvenile crayfish. Enclosures received a single crayfish and an excess number of snails (80 *B. pfeifferi* for adults and 30 for juveniles). *Nymphaea caerula* again served as an alternative food for crayfish. After five days, snail consumption was determined for each crayfish, and the average daily consumption was calculated. The experiment was repeated 12 times for both adult and juvenile *P. clarkii*.

An additional set of enclosure experiments was conducted, in which caged *B. pfeifferi* were placed into a snail-free reservoir near Nairobi. A population of *P. clarkii* was already present in this reservoir. Snails were introduced in either protected plastic cages (15 × 15 × 20 cm), in which snails were safe from predation, or in similar exposed cages, from which one side (15 × 15 cm) had been removed, thus allowing entry of potential predators. Snails in these exposed cages were tethered, using nontoxic rubber cement, to 14-cm strands of monofilament that were tied to the cage bottom to minimize escape. Five snails per cage were placed underwater at a depth of 0.5 m for five days, at which time cages were recovered and snail survival was tabulated. These two treatments were duplicated in a predator-free control habitat. Each treatment was repeated 15 times. Analysis of variance was used to compare mean snail survival in the four treatments.

## RESULTS

*Natural coexistence of* P. clarkii *and pulmonate snails*

Of the 53 sites surveyed in this study, 19 had pulmonate snails and no crayfish, 21 contained crayfish only, four had both snails and crayfish, and nine contained neither snails nor crayfish. This distribution indicates a highly significant negative association between snails and *P. clarkii* ($P < 0.001$, by Fisher's exact test). None of the sites with crayfish contained snails capable of transmitting human schistosomes, including sites in Lake Naivasha and at Nairobi Dam, from which *Biomphalaria* snails have previously been reported.[11, 14] *Physa acuta*, a pulmonate snail introduced from North America, was collected in all four habitats where coexistence was observed. Small numbers (< 5) of native pulmonates were found in two of these habitats, but the species found do not transmit human schistosomiasis.

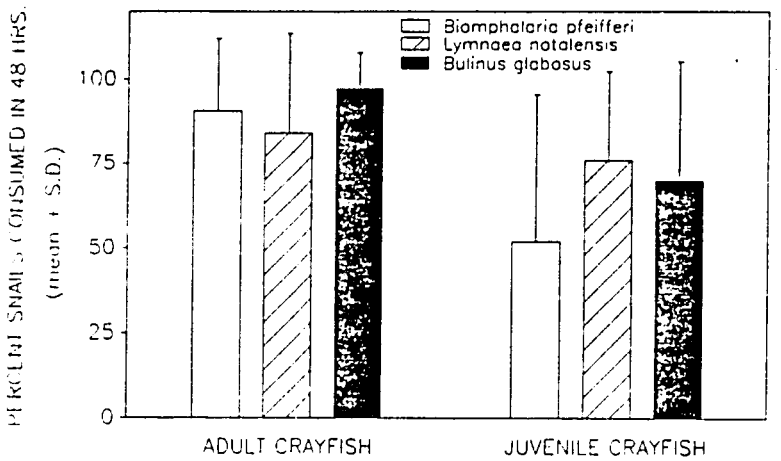


FIGURE 1. Mean ± SD laboratory consumption of medically important pulmonate snails by adult and juvenile *Procambarus clarkii* after 48 hr.

Snail counts in habitats where crayfish were absent ranged from fewer than 10 to several hundred individuals.

*Laboratory consumption of medically important pulmonate snails*

Results of laboratory experiments to document the capacity of *P. clarkii* to consume medically important snails are provided in Figure 1. All three snail species presented to crayfish were readily consumed by both adults and juveniles.

*Enclosure experiments*

Within the enclosures, both adult and juvenile *P. clarkii* significantly reduced *B. pfeifferi* abundance relative to controls within the five-day experimental period ($P < 0.001$; Figure 2). In six of the replicates with adult crayfish and one with juveniles, *B. pfeifferi* was completely eliminated within five days. Total snail recovery (living snails plus empty shells) averaged 98.3% in enclosures without *P. clarkii*, indicating that few, if any, snails escaped from the enclosures.

*Biomphalaria pfeifferi* egg mass deposition was

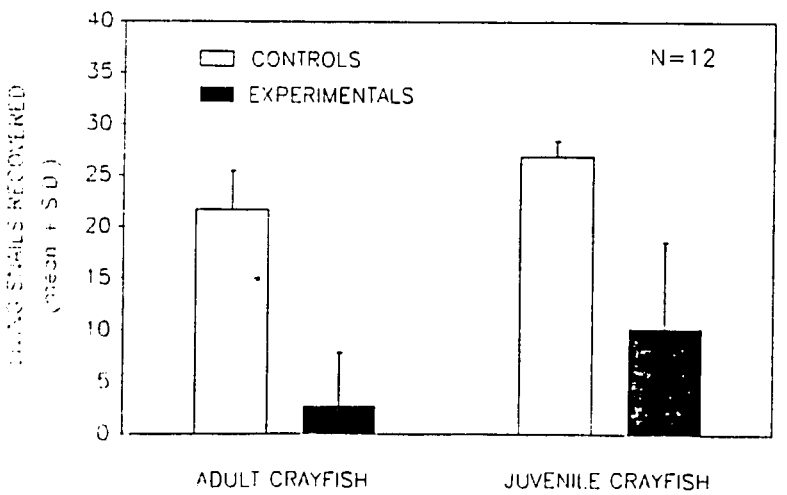


FIGURE 2. Mean ± SD recovery of living *Biomphalaria pfeifferi* after five days in experimental enclosures with *Procambarus clarkii* and control enclosures without *P. clarkii*.

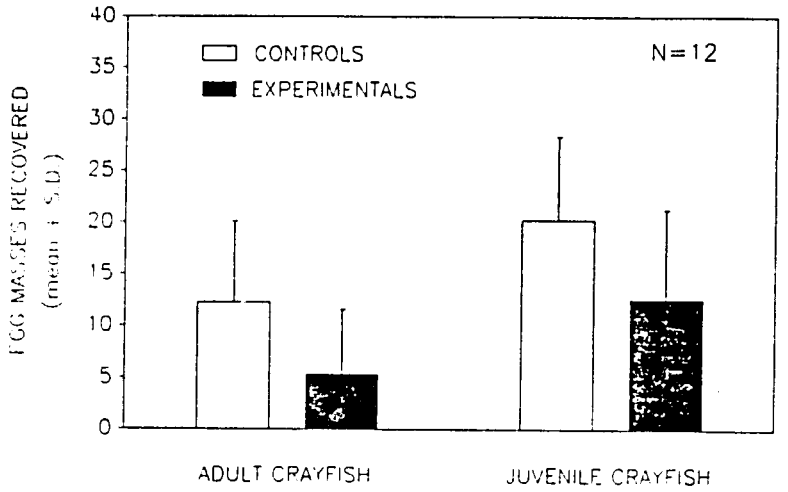


FIGURE 3. Mean ± SD recovery of *Biomphalaria pfeifferi* egg masses deposited on *Nymphaea caerula* after five days in experimental enclosures with *Procambarus clarkii* and control enclosures without *P. clarkii*.

also significantly lower in enclosures containing *P. clarkii* ($P < 0.05$; Figure 3). In subsequent experiments using these enclosures, where crayfish were provided with an excess number of snails, it was estimated that adults consumed an average of 14.9 ± 1.9 (mean ± SD) snails per day, while juveniles averaged 3.6 ± 1.8 snails per day.

Mean snail survival in both protected and exposed enclosure cages is presented in Figure 4. No living snails were recovered from exposed experimental cages. Significantly fewer living snails were recovered from exposed control cages compared with experimental protected cages ($P < 0.05$). At the conclusion of this experiment, two minnow traps, each baited with 10 live *B. pfeifferi*, were placed into the dam for 24 hr. In both cases, crayfish were found inside the traps, and *B. pfeifferi* had been consumed, indicating that crayfish, rather than some other predator, had consumed snails in exposed experimental cages.

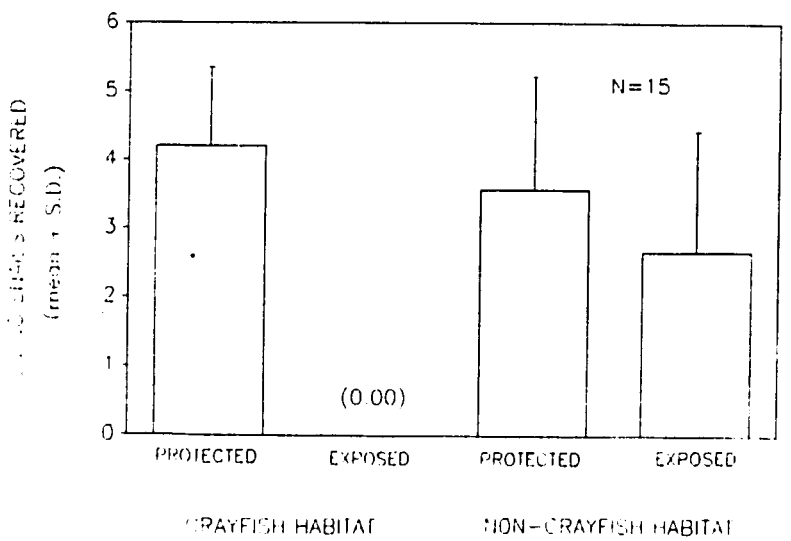


FIGURE 4. Mean ± SD recovery of living *Biomphalaria pfeifferi* in protected and exposed cages. Experimental treatments were conducted in a habitat in which crayfish were present, while control treatments were conducted in a habitat that did not contain crayfish.

## DISCUSSION

The results of this coexistence study suggest that pulmonate snails may be controlled and/or eliminated from natural habitats in Kenya as a consequence of crayfish predation. Laboratory experiments indicated that *P. clarkii* taken from Kenyan habitats readily consume these snails.

Enclosure experiments confirmed the significant impact that *P. clarkii* can have on the abundance of *B. pfeifferi*. Crayfish consumed large numbers of these snails, even when alternative foods such as aquatic macrophytes, algae, detritus, and small aquatic invertebrates were available. Although the results were somewhat less dramatic, it appears that crayfish can also limit snail egg mass deposition and therefore produce an additional negative impact on the snail population.

In the experiment using exposed and protected cages, the high survival observed in protected experimental cages indicates that crayfish habitats are not necessarily unsuitable for snails. The uniformly zero survival rate in exposed experimental cages may be attributable, in part, to snail escape. In exposed control cages, total recovery (living plus dead snails) averaged 80%, meaning that 20% of these snails disappeared and can be presumed to have escaped. This escape may explain the significantly lower survival of exposed control snails compared with that observed in protected experimental trials. Similar escape is also likely to have occurred in exposed experimental cages. However, even allowing for such escape, the significant difference in snail survival between control and experimental exposed trials indicates a strong predation effect, which suggests that snails that disperse into a crayfish habitat will be rapidly controlled and/or eliminated.

These results suggest that *P. clarkii* should receive serious consideration as a control agent of snail-borne parasitic diseases such as schistosomiasis. Although crayfish do not meet many of the criteria traditionally used for the selection of biological control agents,[15-19] the value of attributes such as host specificity and short generation time in aquatic systems has at times been questioned.[20-22] Because they are relatively long-lived food generalists, they are typical aquatic predators.[23] Such predators often drive their preferred prey to extinction,[8, 22] and then switch to alternative foods. In such a situation, predator density will not track that of the prey, and should the prey species re-invade, predators will already be present in sufficient numbers and can begin impacting on the prey at once. Such "top down" asymmetrical interactions are well known for snails and crayfish.[24]

Furthermore, an exotic species such as *P. clarkii* may be especially suited for the control of African snail species.[25] At least some snails that have co-evolved with crayfish appear to have developed anti-predator adaptations rendering crayfish a less effective control agent.[26] However, since Africa lacks an indigenous crayfish fauna,[27] African gastropods may, at present, lack such defense mechanisms. It remains to be seen if over time, African snails develop the ability to respond to crayfish in a manner similar to that observed in co-evolved snail species. It is of interest that the only snail found co-occurring naturally with *P. clarkii* with any regularity in our study was the North American exotic *P. acuta*. An additional impetus for the use of *P. clarkii* in control operations stems from its value in aquaculture. Kenya exports 500 metric tons of *P. clarkii* annually, making it a significant source of income.[28]

The deliberate use of *P. clarkii* for snail control is not without potential drawbacks. *Procambarus clarkii* can destroy rice seedlings, negatively impact freshwater fisheries, and damage earthen dams, as a result of its burrowing activity.[2, 28, 29] Yet any debate regarding the deliberate introduction of *P. clarkii* for the biological control of medically important pulmonate snails may be academic if this crayfish continues to rapidly expand its range, either naturally or as a consequence of human activity.

Bearing in mind these environmental limitations, *P. clarkii* may represent a cost-effective means to aid in the control of human schistosomiasis. It may be especially valuable when used in tandem with other control strategies such as chemotherapy, which by itself cannot prevent reinfection.

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## Appendix 5

# The North American Crayfish *Procambarus clarkii* and the Biological Control of Schistosome-Transmitting Snails in Kenya: Laboratory and Field Investigations

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**The Louisiana red swamp crayfish, *Procambarus clarkii* (Girard), was examined for its ability to serve as a biological control agent of *Biomphalaria pfeifferi* (Krauss), a molluscan intermediate host of human schistosomiasis in Kenya. Either two or four *P. clarkii* in large (100 × 60 × 60 cm) outdoor aquaria were able to significantly reduce *B. pfeifferi* abundance relative to control aquaria lacking crayfish, in experiments lasting 5, 15, or 30 days. *B. pfeifferi* was completely eliminated from aquaria containing four crayfish for 30 days. The ability of *P. clarkii* to alter the abundance of *Nymphaea caerulea* Savigny, a common aquatic plant with which *B. pfeifferi* is known to be positively associated, was also assessed. Crayfish were able to significantly reduce *N. caerula* abundance in both the above-described aquaria as well as in field enclosure cages (75 × 75 × 45 cm), but only when four crayfish were present for 30 days. Our results imply that *P. clarkii* may have potential both as a predator of and as a resource competitor with schistosome-transmitting snails in East Africa.** © 1991 Academic Press, Inc.

KEY WORDS: ***Biomphalaria pfeifferi; Procambarus clarkii; Nymphaea caerulea;*** **decapod crustaceans; macrophytes; schistosomiasis; snail control.**

## INTRODUCTION

Schistosomiasis continues to inflict an unacceptable burden on public health in tropical Africa. In Kenya, over 40% of the population is at risk for contracting either intestinal or urinary schistosomiasis (Iarotski and Davis, 1981). The pathology that occurs as a consequence of infection can be severe, especially in children (Wilkins, 1987). Since significant progress has been made in controlling this disease through the development and use of safe and effective anti-schistosomal drugs (Mott, 1987), emphasis has shifted from the control of the molluscan intermediate hosts of schistosomes to the therapeutic treatment of infected individuals (Madsen, 1990). However, successfully treated patients often rapidly become reinfected (Wilkins, 1987; Sturrock *et al.*, 1990; Zwingenberger *et al.*, 1990), and a control program based only on drug treatment cannot break the cycle of disease transmission.

Snail control is difficult and the implementation of large-scale molluscicide campaigns has generally proven unsatisfactory as a means of reducing schistosomiasis (Madsen, 1990). Biological control, frequently discussed as an appealing alternative snail management strategy because of its low cost, technological simplicity, and potential for self renewal (e.g., Ferguson, 1977; Jobin *et al.*, 1984; Combes and Cheng, 1986), has provided only limited success (Madsen, 1990). Competitor and predator snails have thus far shown the most promise in this regard (Ferguson, 1977; Jobin *et al.*, 1977; WHO, 1984; Pointier and McCullough, 1989; Pointier *et al.*, 1989).

Although decapod crustaceans have received relatively little attention as biological control agents, they are effective snail predators and some studies have indicated their potential utility in snail control operations. The freshwater prawn *Macrobrachium rosenbergii* (De-Man) has demonstrated promise in laboratory studies (Roberts and Kuris, 1990), and earlier investigators have suggested that freshwater crayfish may also be suitable for the control of snail-borne diseases (Deschiens and Lamy, 1954; Ritchie, 1955; Huner, 1977).

The Louisiana red swamp crayfish, *Procambarus clarkii* (Girard), was introduced into Kenya from Uganda in approximately 1965 and was subsequently introduced into Lake Naivasha in 1970 for aquaculture purposes (Parker, 1974; Olouch, 1990). This crayfish has rapidly expanded its range and is now found in almost all drainage basins in the country (Lowrey and Mendes, 1977a).

*P. clarkii* is omnivorous and is known to consume large numbers of freshwater snails (Huner and Barr, 1983). This crayfish also is a voracious consumer of aquatic macrophytes (Feminella and Resh, 1989).



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Lowrey and Mendes (1977b) noted, in particular, a dramatic decline in the abundance of the water lily *Nymphaea caerulea* Savigny in areas of Lake Naivasha where crayfish were present. The presence of *Biomphalaria* and *Bulinus* snails, both of which transmit human schistosomiasis in Kenya, has been positively associated with that of *N. caerulea* in Africa (Woolhouse and Chandiwana, 1989).

In this study, we investigated the capacity of *P. clarkii* to control *B. pfeifferi* (Krauss) under laboratory conditions. Furthermore, *P. clarkii* might serve as a snail competitor as well as a predator if it can reduce or eliminate aquatic macrophytes with which target snails are associated. Therefore, we also investigated the capacity of *P. clarkii* to alter the abundance of *N. caerulea*, a common and widely distributed water lily in Kenya, in both the laboratory and the field.

## MATERIALS AND METHODS

Crayfish used in all experiments were collected from a small reservoir near Nairobi. *B. pfeifferi* and *N. caerulea* were collected from habitats near the towns of Makindu, 180 km southeast of Nairobi, and Makuyu, 70 km north of Nairobi, respectively.

### *Effect of Crayfish Predation on Snail Abundance*

Experiments were conducted in large (100 × 60 × 60 cm) plastic tanks at the Kenya Medical Research Institute, Nairobi, that were maintained in a partially shaded, open air location, subject to ambient temperatures and daylength. The bottom of each tank received a layer of soil 10 cm deep to provide a natural substrate. Three or four *N. caerulea* plants were rooted into the soil to provide food and refuge for snails and to serve as an alternative food for crayfish. Water level in the tanks was maintained at 30 cm. Fifty adult *B. pfeifferi* were added to each tank.

Four experiments, utilizing different numbers of adult crayfish (carapace length > 3 cm) for different periods of time, were conducted. Equal numbers of experimental replicates with, and control replicates without, crayfish were carried out for each experiment. An experiment using four crayfish was ended after 30 days. Experiments with two crayfish were of 5, 15, and 30 days duration. At the completion of each experiment, tanks were drained and the number of living snails was determined. All experiments were repeated six times, except for that utilizing two crayfish for 30 days, which was repeated five times. The Mann–Whitney test ($\alpha = 0.05$) was used to compare the mean number of living snails recovered from tanks with and without crayfish for each of the four experiments.

### *Effect of Crayfish on N. caerula*

In each of the tanks described above, a "plant cover index" was taken prior to the introduction of both snails and crayfish. This was achieved by placing a grid of 60 10-cm$^2$ squares over each tank and counting the number of squares under which viable plant leaf tissue was observed. Dead leaves, or those detached from their stems, were not considered. Since the leaves of water lilies naturally flatten out and float at the surface, this was a relatively easy and reliable means of estimating plant cover. This same procedure was repeated at the conclusion of each experiment. The number of positive squares for each of these estimates was compared and, by assigning the initial estimate of leaf area a value of 100%, the change in percentage plant cover over the course of the experiment was calculated. Values greater than 100% represented increases over the initial estimate, while those less than 100% represented declines. The number of replicates for each of the four experimental treatments was identical to that described in the previous experiment.

Field experiments involved the use of enclosure cages placed in a small reservoir near Makuyu, Maranga District. These enclosures, constructed of galvanized steel hardware cloth (mesh size, 5 cm$^2$), measured 75 × 75 × 45 cm. Five water lily plants were secured with nylon string to the bottom of each enclosure. Approximately 10 cm of soil was added and the enclosures were placed in the reservoir at a depth of about 30 cm. Experimental enclosures received either two or four crayfish. No crayfish were present in control enclosures. Equal numbers of experimental and control replicates were conducted for each of four experiments. Experiments with two crayfish were of 5 and 15 days duration. These experiments were each repeated 8 times. Experiments with four crayfish, lasting either 15 or 30 days, were repeated 14 and 6 times, respectively. Prior to and at the conclusion of each experiment, the plant cover index was determined for each enclosure in the manner described above, except that the grid consisted of 100 7.5-cm$^2$ squares.

In both laboratory tanks and field enclosures the mean change in percentage macrophyte cover was compared for treatments with and without crayfish for each experiment. All comparisons were made using the Mann–Whitney test with $\alpha = 0.05$.

## RESULTS

### *Effect of Crayfish Predation on Snail Abundance*

Significantly fewer *B. pfeifferi* were recovered from tanks containing *P. clarkii* than from tanks lacking crayfish ($P < 0.05$; Fig. 1). The mean number of living snails recovered from experimental tanks decreased as the number of crayfish or length of experiment in-

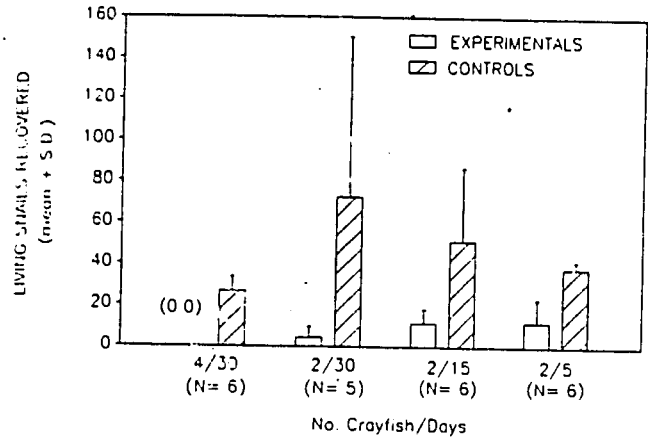


FIG. 1. Mean number of living *B. pfeifferi* recovered from experimental tanks with, and control tanks without, crayfish. Each treatment began with 50 *B. pfeifferi*. ($N$ = the number of replicates.)

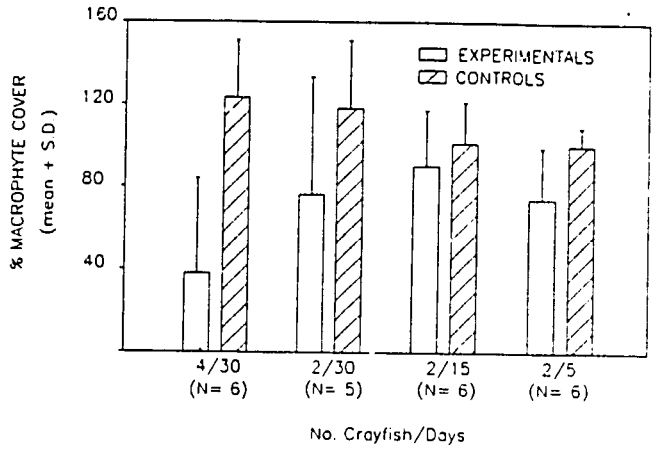


FIG. 2. Mean percentage change in *N. caerula* leaf area in experimental tanks with, and control tanks without, crayfish. Means represent a percentage increase or decrease in leaf area relative to an initial value of 100%. ($N$ = the number of replicates.)

creased. No living snails were recovered from tanks containing four crayfish for 30 days. Likewise, no snails were recovered from two of five 30-day replicates with two crayfish. The mean number of snails recovered from the 5- and 15-day treatments lacking crayfish was more than 3.0- and 4.5-fold greater, respectively, than it was in experimental treatments with two crayfish. In two experiments (two crayfish for 15 or 30 days), the number of *B. pfeifferi* recovered from control tanks averaged higher than the 50 snails originally introduced. In all control tanks from which more than 50 snails were recovered (two of six 15-day and three of five 30-day control treatments), >50% of the snails were juveniles, less than 5 mm in shell diameter. In the majority of tanks containing crayfish, no juvenile snails were observed. In the few tanks with crayfish where such juveniles were recovered (two of six 15-day and two of five 30-day experimental tanks), the number of snails recovered was small (≤10).

### *Effect of Crayfish on N. caerula*

Although plant cover was lower in most laboratory tanks or field enclosures with *P. clarkii*, there was much variation between replicates and there were no significant differences between experimental and control treatments for most experiments (Figs. 2 and 3). For both laboratory tanks and field enclosures, only when four crayfish were present for 30 days, was plant cover significantly reduced relative to treatments in which no crayfish were present ($P < 0.05$). *N. caerulea* was completely eliminated in three of six tanks and in 2 of 14 enclosures containing four crayfish after 30 days. Little change in mean plant cover over the 5-, 15-, or 30-day time periods was observed in tanks and enclosures from which crayfish were absent.

## DISCUSSION

Either two or four *P. clarkii* significantly reduced *B. pfeifferi* abundance in laboratory tanks in as few as 5 days. Furthermore, tanks with crayfish contained substantially fewer juvenile *B. pfeifferi* relative to crayfish-free tanks, despite the presence of food alternatives such as macrophytes, algae, detritus, and other aquatic invertebrates, all of which are readily consumed by crayfish (Goddard, 1988). These results are in agreement with those of Covich (1977) who showed that molluscs remain a preferred food of crayfish even in the presence of food alternatives. Additionally, pulmonate snails and crayfish coexisted in only 4 of 53 Kenyan freshwater habitats considered to be environmentally suitable for either *P. clarkii* or medically important

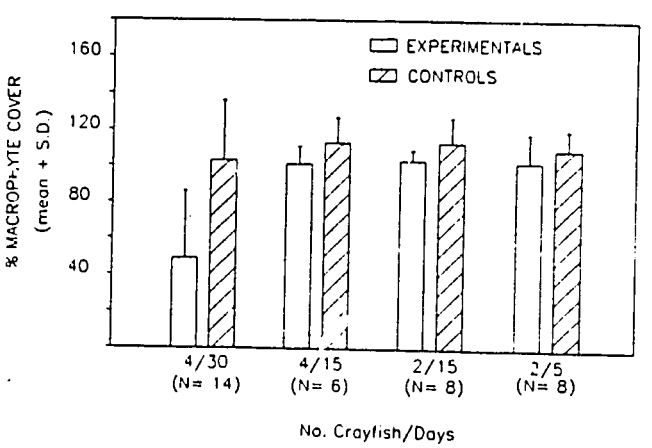


FIG. 3. Mean percentage change in *N. caerula* cover in experimental enclosures with, and control enclosures without, crayfish. ($N$ = the number of replicates.)

snails (Hofkin *et al.*, 1991). None of these four habitats contained *B. pfeifferi* or other snail species capable of transmitting human schistosomiasis. The results presented here lend support to the possibility that the observed lack of co-occurrence between crayfish and schistosome-transmitting snails is a consequence of predation.

Only when four crayfish were present for 30 days were significant decreases in the abundance of *N. caerulea* observed. Thus it appears that any effect that crayfish might exert on *N. caerulea* may require longer to occur than that on target snails themselves. In Kenya, however, aquatic habitats containing *P. clarkii* are often devoid of aquatic plants (Lowrey and Mendes, 1977b). Although *N. caerulea* is common and widespread in Kenya, it was not observed in any of the more than 25 habitats from which *P. clarkii* was collected (B. V. Hofkin, unpublished observation).

Our results support those of other investigations which have shown that crayfish can exert a substantial impact on the abundance of both gastropods and their associated aquatic plants (e.g., Covich, 1981; Lodge and Lorman, 1987; Weber and Lodge, 1990; Chambers *et al.*, 1990; Hanson *et al.*, 1990). Furthermore, our results indicate that in East Africa, *P. clarkii* could assume practical importance as a biological control agent of schistosome-transmitting snails.

Emerging theory regarding the use of biological control in aquatic systems suggests that crayfish may be ideal in this context. Long-lived generalist predators such as crayfish often drive their prey to extinction, especially in permanent stationary waterbodies (Lodge *et al.*, 1987; Murdoch and Bence, 1987). When such an extinction occurs, the predator will switch to alternative foods, and consequently, its population will remain unaffected (Weber and Lodge, 1990). Should the target organism reinvade, it can be rapidly eliminated owing to the substantial numbers of predators already present. Indeed, few agents have thus far proven to be as effective in aquatic biological control as *Gambusia* and *Notonecta*, relatively long-lived generalist predators that are able to control mosquito larvae (Murdoch *et al.*, 1985).

If *P. clarkii* can eliminate *N. caerulea* and other plants used by medically important snails as food, refuge, and oviposition sites, it might effectively function as both a predator and a competitor of these gastropods. The importance of such macrophyte reduction has been highlighted in Guadeloupe, where the disappearance of *Biomphalaria glabrata* (Say) was attributed to the consumption of its preferred food by ampullariid snails (Pointier *et al.*, 1988). Use of the snail *Marisa cornuarietis* (L.) for the biological control of schistosome-transmitting snails has been encouraged because of its ability to exert both predatory and competitive effects on target species (Ferguson, 1977). It is also intriguing to consider the effect, if any, that alteration of aquatic macrophyte abundance by *P. clarkii* might have on mosquito breeding, and consequently on mosquito-borne diseases such as malaria. Feminella and Resh (1986), for example, suggested that in California, crayfish grazing may benefit mosquito control programs by reducing the amount of habitat for larval mosquitoes. However, certain other species of aquatic plants in Kenya, such as *Salvinia molesta* Mitchell, are apparently unaffected by *P. clarkii* and it remains to be seen if such plants can serve as snail refugia in the absence of *N. caerulea*.

Before the widespread use of *P. clarkii* for snail control can be condoned, several additional economic and environmental concerns should be considered. For example, Kenya harvests and exports approximately 500 metric tons of crayfish annually, making it an important source of income (Hobbs *et al.*, 1989). Drawbacks to the use of *P. clarkii* include its ability to damage rice seedlings (Miltner and Avault, 1980) and its capacity to damage irrigation canals as a consequence of its burrowing activity (Sommer and Goldman, 1983). In Lake Naivasha, it may disrupt the breeding of certain fish species and has created problems for fisherman by consuming fish taken in gill nets (Lowrey and Mendes, 1977b).

*P. clarkii* is already widespread in Kenya and it will most likely continue to spread naturally. Consequently, the overall impact of *P. clarkii* on Kenyan freshwater habitats, its capacity to control schistosome-transmitting snails, and its role in interrupting schistosome transmission deserve additional study.

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## Appendix 6

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# Predation of *Biomphalaria* and non-target molluscs by the crayfish *Procambarus clarkii*: implications for the biological control of schistosomiasis

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The North American crayfish *Procambarus clarkii* was examined under laboratory conditions for its ability to prey on *Biomphalaria pfeifferi* and *B. glabrata*, molluscan intermediate hosts of human schistosomiasis, and other, non-target gastropod species. Both male and female adult crayfish significantly reduced survival of neonate snails, even though alternative animal and plant foods were both available. In subsequent experiments, no differences in snail consumption were detected, for either adult or juvenile crayfish, in the presence or absence of a plant food alternative. Both adult and juvenile crayfish were able to consume small (2·5 mm) and large (17·5 mm) *B. glabrata*, suggesting that no size refuge from predation exists. Both adult and juvenile crayfish consumed *Biomphalaria* egg masses, although this consumption was significantly greater for juveniles. *Procambarus clarkii* adults were unable to consume substantial numbers of the relatively thick-shelled prosobranch snails *Pila ovata* and *Lanistes carinatus*. Crayfish did consume a third prosobranch, *Melanoides tuberculata*, and the pulmonate snail *Physa acuta*, but at a lower rate relative to consumption of *Biomphalaria*. *Physa acuta*, itself of North American origin, responded to the presence of crayfish by rapidly leaving the water and thereby avoided predation. Implications of these results for the biological control of schistosome-transmitting snails in East Africa are discussed.

The elimination of molluscan intermediate hosts can play an important role in schistosomiasis control programmes. Snail control can interrupt parasite transmission but, because only a few infected snails are needed to re-establish the infection cycle, such control must be maintained over a considerable period of time. Molluscicides have been used extensively, but their high cost, possible undesirable long-term effects, and the need for frequent reapplication have rendered them a less attractive option (Brown, 1981; Combes & Cheng, 1986; Madsen,

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1990). Biological control may offer an alternative that is long-lasting and relatively inexpensive (e.g. Kuris, 1973; Jobin *et al.*, 1984), but in spite of the large number of potential control agents which have been proposed (see reviews by Ferguson, 1977; Jordan *et al.*, 1980; McCullough, 1981; Combes and Cheng, 1986; Madsen, 1990), only a limited number of putative competitor or predator snail species, such as *Marisa cornuarietis* and *Helisoma duryi*, have been subjected to extensive laboratory and field studies advocated by the WHO (1984).

The North American crayfish, *Procambarus clarkii*, was introduced into Lake Naivasha in Kenya *c.* 1970 as a potential aquaculture species (Lowrey and Mendes, 1977*a*). It has since spread over a wide part of the country and is now found in most important drainage basins (Lowrey and Mendes, 1977*b*; unpubl. obs.). In an earlier study (Hofkin *et al.*, 1991*a*), we documented that this crayfish can exert a negative impact on *Biomphalaria pfeifferi* populations under laboratory conditions. Subsequently, we reported that a negative association exists between this and other schistosome-transmitting snail species and *Pro. clarkii* in freshwater habitats in Kenya, and that crayfish can significantly reduce *B. pfeifferi* abundance in field enclosures (Hofkin *et al.*, 1991*b*). Here we investigate, under laboratory conditions, several other aspects of crayfish predation that have some bearing in assessing the ultimate utility of *Pro. clarkii* as a biological control agent.

The ability of *Pro. clarkii* to limit survival of juvenile *Biomphalaria* is examined, as is the capacity of both adult and juvenile crayfish to prey upon various life history stages of this target organism. We additionally assess crayfish predation of snails in the presence of alternative food. Finally, because *Physa acuta*, an introduced mollusc of North American origin, is the only pulmonate snail to regularly co-occur with *Pro. clarkii* in Kenya (Hofkin *et al.*, 1991*b*), we investigated the ability of *Pro. clarkii* to consume both this and other selected non-target molluscs. These observations are discussed in relation to the potential use of *Pro. clarkii* to control schistosome-transmitting snails in East Africa.

## MATERIALS AND METHODS

Experiments were conducted both at the University of New Mexico and at the Kenya Medical Research Institute. In New Mexico, *Biomphalaria glabrata*, a neotropical species, was used in all experiments, as this is the only *Biomphalaria* species available to us that will predictably establish flourishing laboratory populations. In Kenya, *B. pfeifferi*, collected from natural habits near the town of Makindu, were employed in all experiments requiring *Biomphalaria* snails. *Physa acuta* and *Melanoides tuberculata* were collected from Lake Naivasha and a man-made pond near the town of Makindu, respectively. Both *Pila ovata* and *Lanistes carinatus* were collected from a natural habitat near the town of Mazeras. *Procambarus clarkii* were obtained from Carolina Biologicals (North Carolina, U.S.A.), and were maintained in stock tanks at the University of New Mexico, or they were collected from a small reservoir near Nairobi, Kenya.

### Experiment 1. Restriction of Juvenile *Biomphalaria* Survival

Mature *B. glabrata* were placed into 20-litre aquaria filled with water to a depth of approximately 20 cm, and allowed to oviposit on the aquarium walls. After several days, snails were removed and the number of egg masses in each aquarium was adjusted to 30 by removing egg masses in excess of that number. Aquarium water was changed, crushed oystershell was added to provide calcium, and an assortment of rocks was added to provide refuge for newly-hatched snails. A single adult (carapace length > 3 cm) crayfish was introduced into each of 12 aquaria. Twelve crayfish-free aquaria served as controls. All aquaria were aerated, maintained at 25°C, and subjected to a 12 hour:12 hour light–dark cycle. Fresh lettuce leaf was added *ad libitum* to all aquaria to provide food for both crayfish and hatchling snails. Aquaria containing crayfish also received approximately 0·5 g of raw beef, three times a week as an additional alternative food. Any uneaten beef was removed after 48 hours. After 30 days, living snails from all aquaria were removed, counted, and measured to the nearest mm (shell diameter).

The experiment, using paired experimental and control aquaria, was repeated six times with male crayfish, and six times with females. Because of large differences between replicates, the Mann–Whitney test was used to compare mean number of surviving snails in experimental and control aquaria. Student's *t*-tests were used to compare mean snail size in treatments with and without *Pro. clarkii* and to compare results with male and female crayfish.

### Experiment 2. Consumption of *Biomphalaria* Egg Masses

Mature *B. glabrata* were introduced into 10-litre aquaria filled with water to a depth of 5 cm. Egg masses were allowed to accumulate on the aquarium walls for approximately 48 hours, at which time snails were removed and the number of egg masses was adjusted to 10. A single adult or juvenile (carapace length 1·5–2·6 cm) crayfish was then introduced into each aquarium for 72 hours, after which time the number of remaining egg masses was recorded. No alternative food was provided for crayfish. Water depth was maintained at this shallow level to insure that all egg masses were easily accessible to crayfish. The experiment was repeated 10 times each with adult and juvenile crayfish. Six identical, crayfish-free replicates served as controls. A Student's *t*-test was used to assess any differential egg mass consumption between adult and juvenile *Pro. clarkii*.

### Experiment 3. Size-Specific Predation

To determine if *Pro. clarkii* predation is restricted to snails of specific sizes, individual crayfish were placed in 10-litre aquaria, filled to a depth of 5 cm. Five small (2·5 ± 0·5 mm) *B. glabrata* were introduced into each aquarium and after 96 hours, any consumption of these snails was recorded. If no snails were consumed at this time, 0·5 g of raw beef was added. If the crayfish consumed the beef, we assumed that the crayfish was healthy and feeding, but that 2·5 mm snails were safe from predation. If the beef was not consumed within 24 hours, we assumed the crayfish was not feeding and the replicate was excluded from analysis. If 2·5-mm snails were not consumed and the crayfish was considered to be healthy, snails of the next size class (3·5 ± 0·5 mm) were added. This process was repeated until snails were consumed and a minimum prey size for the crayfish could be determined. Subsequently, this entire procedure was repeated, starting with a single *B. glabrata* of the largest size class employed (17·5 ± 0·5 mm). If the crayfish was unable to consume a snail of this size, a smaller snail (16·5 ± 0·5 mm) was introduced. This process was repeated until the maximum prey size for each crayfish was determined. The experiment was repeated 15 times with adult *Pro. clarkii* and 10 times with juveniles.

### Experiment 4. Snail Predation in the Presence of a Food Alternative

Leaves and stems of the waterlily *Nymphaea caerula*, a common aquatic plant that is readily consumed by *Pro. clarkii* (Lowrey and Mendes, 1977*a*; Hofkin *et al.*, 1991*a*), was used as the alternative food. A single crayfish and 10 *B. pfeifferi* (shell diameter > 5 mm) were placed in each 10-litre aquarium filled with water to a depth of 5 cm. Half the aquaria received an *N. caerula* leaf and its attached stem. After 24 hours, crayfish consumption of snails in aquaria with and without plant material was tabulated. The experiment was repeated 10 times each for adult and juvenile crayfish. Student's *t*-tests were used to compare mean snail consumption for adult and juvenile trials with and without the alternative food.

### Experiment 5. Consumption of Non-Target Molluscs

Individual adult *Pro. clarkii* were placed in 10-litre aquaria filled with water to a depth of 5 cm. At that point, either five *Physa acuta* or *Melanoides tuberculata* adults (> 5 mm shell length) or two *Pila ovata* or *Lanistes carinatus* juveniles (4–7 mm maximum shell dimension) were introduced. Snail consumption was recorded after 48 hours. No alternative food was provided for crayfish. The experiment was repeated 22 times for *Phy. acuta*, 15 times for *M. tuberculata*, and 17 times for both *Pil. ovata* and *L. carinatus*.

Furthermore, because *Phy. acuta* showed a strong tendency to crawl quickly above the waterline when placed in the presence of *Pro. clarkii*,

TABLE
*Predation of snails by* Procambarus clarkii*

| | *Experimental* | *Control* |
|---|---|---|
| EXPERIMENT 1 | | |
| No. of *B. glabrata* recovered | 38·7 ± 37·2 (*N* = 12) | 365·8 ± 261·6† (*N* = 12) |
| | *Adult* | *Juvenile* |
| EXPERIMENT 2 | | |
| Egg masses consumed (%) | 26·0 ± 31·3 (*N* = 10) | 86·7 ± 29·0‡ (*N* = 10) |
| EXPERIMENT 3 | | |
| Minimum size of snail consumed (mm) | 2·5 ± 0·0 (*N* = 15) | 2·5 ± 0·0 (*N* = 10) |
| Maximum size of snail consumed (mm) | 16·9 ± 2·1 (*N* = 15) | 17·1 ± 1·0 (*N* = 10) |
| EXPERIMENT 4 | | |
| *B. pfeifferi* consumed (%) | | |
| No alternative food | 66·0 ± 39·8 (*N* = 10) | 58·0 ± 29·8 (*N* = 10) |
| Alternative food | 68·0 ± 36·8 (*N* = 10) | 48·0 ± 29·8 (*N* = 10) |

*Values are means ± S. D. *N*, Number of replicates.
†Significantly greater than experimental aquaria; $P < 0{\cdot}001$.
‡Significantly greater than for adults; $P < 0{\cdot}001$.

this apparent capacity to escape predation was investigated experimentally. Individual *Phy. acuta* were subjected to one of three treatments, in 10-litre aquaria filled to a depth of 5 cm. Aquaria contained either unconditioned water, water that had previously been conditioned by *Pro. clarkii* for 48 hours, or unconditioned water and a single adult *Pro. clarkii*. After 2·5 hours, the location of the snail (either above or below the waterline) was recorded for all three treatments. The experiment was repeated 16 times.

## RESULTS

### Experiment 1. Restriction of Juvenile *Biomphalaria* Survival

A comparison of the mean number of *B. glabrata* recovered from experimental aquaria with, and control aquaria without, crayfish is presented in the Table. Because no significant difference was detected between treatments with male and female crayfish ($P = 0{\cdot}46$), these data were pooled for subsequent analysis. When the 12 experimental replicates were compared with control aquaria, large and significant differences in mean snail recovery were apparent ($P < 0{\cdot}001$). Snail recovery in control aquaria averaged approximately 10 times that in experimental aquaria. The majority of snails recovered from either experimental or control aquaria were small (< 5 mm), and no significant differences were detected between control and experimental aquaria in terms of average snail size.

### Experiment 2. Consumption of *Biomphalaria* Egg Masses

Both adult and juvenile *Pro. clarkii* consumed *B. glabrata* egg masses (Table). All egg masses in control aquaria were accounted for, indicating that egg masses missing in experimental aquaria had indeed been consumed. Juveniles consumed 87% of those egg masses presented to them, significantly more than the 26% consumption average of adults ($P < 0{\cdot}001$).

### Experiment 3. Size-specific Predation

Snails of both the smallest (2·5 mm) and largest (17·5 mm) size classes used were consumed by

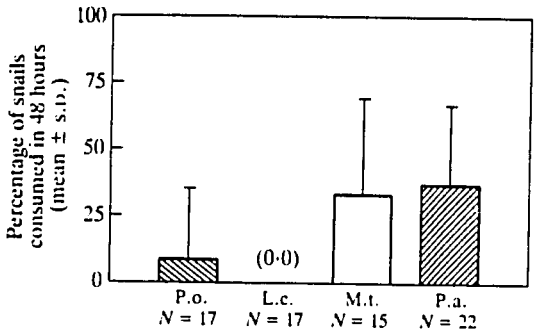


**Fig.** Percentage (mean ± S.D.) of *Pila ovata* (P.o.), *Lanistes carinatus* (L.c.), *Melanoides tuberculata* (M.t.), and *Physa acuta* (P.a.) consumed by adult *Procambarus clarkii* after 48 hours. *N*, Number of replicates.

both adult and juvenile *Pro. clarkii* (Table). All crayfish examined consumed all 2·5 mm snails presented to them within 96 hours. The largest snails were consumed by all but two adult and two juvenile crayfish.

### Experiment 4. Snail Consumption in the Presence of a Food Alternative

Neither adult nor juvenile crayfish significantly altered their snail consumption when *N. caerula* was available as a food alternative ($P = 0{\cdot}89$ and 0·48 for adults and juveniles, respectively; see the Table). Of those crayfish provided with plant material, four of 10 juveniles and seven of 10 adults consumed at least a portion of this alternative food.

### Experiment 5. Consumption of Non-target Molluscs

Crayfish displayed little tendency to consume either *Pil. ovata* or *L. carinatus* juvenile snails. Only two of 17 crayfish consumed at least one *Pil. ovata*. No *L. carinatus* were consumed. Crayfish consumed 33·3% of all *M. tuberculata*, and 37·3% of *Phy. acuta* presented to them within 48 hours (Fig.).

In the experiment designed to assess the ability of *Phy. acuta* to escape crayfish predation by crawling above the waterline, 13 of 16 (81·3%) of those snails placed in unconditioned water were found below the waterline after 2·5 hours. Similarly, 15 of 16 (93·8%) *Phy. acuta* placed in crayfish-conditioned water remained below the waterline. When *Phy. acuta* was placed in the presence of an adult crayfish, however, only one of 16 (6·3%) remained in the water. Thirteen snails had crawled above the waterline a distance of at least 1·5 cm, while two individuals were consumed within the 2·5-hour time limit.

## DISCUSSION

Individual adult *Pro. clarkii* were able to significantly reduce juvenile *Biomphalaria* survival in experimental aquaria, even though both animal and plant food alternatives were available for the crayfish. Likewise, we found that snail consumption did not decline when *N. caerula* was offered as a food alternative. These results are in agreement with those of Covich (1977), who showed that molluscs remain a preferred food of crayfish even in the presence of alternatives.

Subsequent experiments indicated that the effect of adult crayfish on juvenile *Biomphalaria* survival was largely due to consumption of the juvenile snails rather than of the egg masses. Both adult and juvenile crayfish consume these egg masses, but this tendency was considerably more pronounced in juveniles. *Procambarus clarkii* of all sizes were able to consume both the smallest and largest *B. glabrata* to which they were exposed, suggesting that no 'size refuge' exists for these snails. *Biomphalaria glabrata* often grows larger than 17·5 mm, the maximum size used in this experiment, but *B. pfeifferi*, a likely target organism in an East African snail control programme, rarely exceeds this size. Covich *et al.* (1980) found that *Pro. clarkii* could

consume large numbers of the freshwater bivalve *Corbicula*, but only when they were less than 6 mm in shell length. This probably reflects the relative ease with which crayfish can manipulate relatively thin-shelled, planorbid snails compared with thicker-shelled bivalves.

Likewise, the relative inability of *Pro. clarkii* to consume *Pil. ovata* and *L. carinatus* is probably due to the thick protective shell and operculum that is characteristic of prosobranch snails. Crayfish are apparently able to consume the smaller prosobranch, *M. tuberculata*, but the rate of consumption was considerably lower for this snail than it was for medically-important pulmonate species such as *B. pfeifferi* and *Lymnaea natalensis* under identical experimental conditions (Hofkin *et al.*, 1991*b*). The relative ease with which *Pro. clarkii* consumes pulmonates compared with prosobranch snails may be of relevance, as all three prosobranch species tested here have themselves been considered as possible control agents of *Biomphalaria* snails (Pointier *et al.*, 1989; Pointier and McCullough, 1989; Stryker *et al.*, 1991).

The relative inability of *Pro. clarkii* to consume *Phy. acuta* compared with other pulmonates, and the apparent escape behaviour of this snail are of particular interest. Both *Pro. clarkii* and *Phy. acuta* are of North American origin (Penn, 1943; Brown, 1981). Crowl and Covich (1990) demonstrated that physid snails alter both their growth and reproductive patterns in the presence of crayfish, and Hofkin *et al.* (1991*b*) found *Phy. acuta* to be the only pulmonate snail that co-occurs with *Pro. clarkii* in Kenya with any regularity. These results indicate that at least some snails that have co-evolved with crayfish appear to have developed anti-predator adaptations, rendering crayfish a less effective control agent. As Africa lacks an indigenous crayfish fauna (Hobbs, 1988), African gastropods may lack such specific anti-crayfish defence mechanisms. Neither *B. pfeifferi* nor *Bulinus globosus* demonstrated escape behaviour of the type documented for *Phy. acuta* (unpubl. obs.).

This suggests that an exotic species like *Pro. clarkii* may be especially appropriate for the control of African snail species. Hokkanen and Pimentel (1984) argue that in contrast to 'classical' biological control (Huffaker *et al.*, 1971; Waage and Greathead, 1988), the ideal control agent will be one that has not co-existed with the target in the past, as there will have been no opportunity for the involved organisms to evolve tolerance toward each other. Our data suggest that, at least in aquatic systems utilizing a predator as the control agent, this may indeed be the case. However, several species of crabs and shrimps inhabit African freshwater habitats, and it remains to be seen if associated gastropods have developed specific escape behaviour in response to these decapods.

The ability of crayfish to influence strongly the abundance and distribution of aquatic gastropods is well known (e.g. Covich, 1981; Weber and Lodge, 1990; Hanson *et al.* 1990). Results from this study suggest that the significant impact that *Pro. clarkii* can exert on *B. pfeifferi*, both in the laboratory (Hofkin *et al.*, 1991*a*) and in the field (Hofkin *et al.*, 1991*b*), is due to both consumption of adult and juvenile snails. The consumption of egg masses may play a lesser role, especially during those times of year when *Pro. clarkii* juveniles are relatively scarce. These results indicate that *Pro. clarkii* is similar to freshwater shrimps, such as *Macrobrachium*, that have also demonstrated their potential to control laboratory populations of *Biomphalaria* snails (Barnish and Prentice, 1982). Roberts and Kuris (1990) also found that *M. rosenbergii* was able to control *Biomphalaria* snails but, unlike crayfish, the adult shrimps also consumed substantial numbers of egg masses.

That crayfish prey on snails of all sizes and in various stages of their life history may have important consequences for snail control and the control of trematode transmission. Our results suggest that crayfish may be capable of blocking the recruitment of juvenile *Biomphalaria* snails into an established population. The removal of young, pre-reproductive individuals from a population is considered to have a larger impact on prey population than removal of older individuals that may have already exhausted a portion of their reproductive potential (Pianka, 1988). Alternatively, adult snails are more likely than juveniles to harbour patent schistosome infections (Sturrock,

1973; Woolhouse and Chandiwana, 1989) and therefore their role in transmission is disproportionally high. The ability of crayfish to prey on these larger snails therefore assumes relatively greater importance.

These results suggest that *Pro. clarkii* should receive further serious consideration as a possible control agent of schistosome-transmitting snails. In particular, controlled field introductions are essential to determine ultimately the ability of this crayfish to control vector snails and reduce disease transmission.

The introduction of any organism into new habitats carries with it potential risks that must be carefully weighed. *Procambarus clarkii*, for example, is known to consume rice seedlings and in Lake Naivasha it has disrupted local fisheries (Lowrey and Mendes, 1977*a*; Sommer and Goldman, 1983). Should the eventual introduction of *Pro. clarkii* into additional habitats in Kenya prove warranted, however, small man-made impoundments, used by local inhabitants for domestic needs and to water livestock, may be appropriate sites for field trials because of their relative isolation from other water bodies. Such impoundments are very numerous and often serve as important transmission sites for human schistosomiasis (Berrie, 1965; Heyneman, 1979; Jewsbury and Imevbore, 1988). *Procambarus clarkii* is known to be adapted to such lentic habitats (Penn, 1943; Huner, 1988). It is noteworthy that *Pro. clarkii* is already widespread in Kenya and will most likely continue to spread either naturally or as a consequence of human activity.

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## Appendix 7

Loker

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# Does the snail *Melanoides tuberculata* have a role in biological control of *Biomphalaria pfeifferi* and other medically important African pulmonates?

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Annals of Tropical Medicine and Parasitology, Vol. 86, No. 2, 201–204 (1992)

# Does the snail *Melanoides tuberculata* have a role in biological control of *Biomphalaria pfeifferi* and other medically important African pulmonates?

The thiarid prosobranch *Melanoides* (= *Thiara*) *tuberculata* and its close relative *Thiara granifera* have, in recent years, been shown to have promise in controlling schistosome-transmitting snails in freshwater habitats in the Caribbean region. Both species, although not indigenous to the Western Hemisphere, have become common in this region and in other areas in the Neotropics (Pointier and McCullough, 1989). They have been implicated in the competitive displacement of the pulmonate species *Biomphalaria glabrata* (the intermediate host of the human parasite *Schistosoma mansoni* in this region) in several locations, including Puerto Rico, St. Lucia, Martinique and Guadeloupe (Butlers *et al.*, 1980; Prentice, 1983; Pointier *et al.*, 1989; Pointier and McCullough, 1989).

*Melanoides tuberculata* is a natural component of the snail fauna in freshwater habitats in sub-Saharan Africa. In Kenya, it is the most commonly collected prosobranch species (Brown, 1980; Loker *et al.*, unpubl.) It is frequently found in the same habitats as pulmonate snails known to transmit parasites of medical and veterinary significance, including *Biomphalaria pfeifferi*, an important transmitter of *S. mansoni*, and *Lymnaea natalensis*, the intermediate host of the liver fluke *Fasciola gigantica*. In view of the reports from the Caribbean area regarding the possible role of thiarid snails in the control of the pulmonate snail *Biomph. glabrata*, further studies on *M. tuberculata*, particularly in Africa, are warranted.

Thus far, the potential role of this species in the biological control of pulmonate snails in Africa remains unclear. A study conducted by Thomas and Tait (1984) in Nigeria indicated that this species may be useful for the control of pulmonates in lotic environments, but the observed interaction of *M. tuberculata* with pulmonates was less dramatic than that observed in the Neotropics. Other studies, conducted in the Sudan and Nigeria, indicated a positive association between *M. tuberculata* and *Biomph. pfeifferi* (Karoum, 1988; Madsen *et al.*, 1988; Ndifon and Ukoli, 1989). Although *M. tuberculata* apparently co-exists with pulmonate snails of medical or veterinary significance in many habitats in Kenya, the possibility that this species exerts a negative effect on pulmonate populations under such circumstances has not been investigated.

In this paper we report on observations made on co-existent populations of *M. tuberculata* and three pulmonate snails (*Biomph. pfeifferi*, *L. natalensis* and *Bulinus tropicus*) in four habitats located in south-central Kenya. These studies were conducted as part of an on-going effort to identify locally-occurring agents with potential for the biological control of schistosomiasis and other snail-borne parasites of humans and domestic animals. Three of the four habitats selected for study are located in the Kitui area and consist of two man-made impoundments (Kangonde, 1°2′S, 37°43′E and Mbondoni, 0°57′S, 38°2′E) and a stream (Matingani, 1°7′S, 37°53′E). The fourth habitat is located in the Mwea area and is a man-made pond (Mwea, 0°4′S, 37°30′E). Kangonde and Mbondoni cover about 1·0 and 3·5 hectares in this area, respectively, and Mwea is about 0·5 hectare. Matingani Stream is about 6 m wide. All four habitats are permanent waterbodies and are subject to large fluctuations in volume. In general, they have shallow littoral regions of 1–2 m in width, which often contain emergent macrophytes. All are used as sources of water for animals and for domestic use.

Observations at the study sites began in January 1987. At each study site six regular sampling stations were established. The sites were visited repeatedly over a course of three years or more, and on each visit a systematic 10-minute search for snails was conducted at each sampling station, by the same team of three experienced collectors. All snails encountered were collected, identified, enumerated and returned to the habitat. The number of snails of each species was summed across the six sampling stations, thus providing a measure of the overall relative abundance of different snail species in each of the four study habitats.

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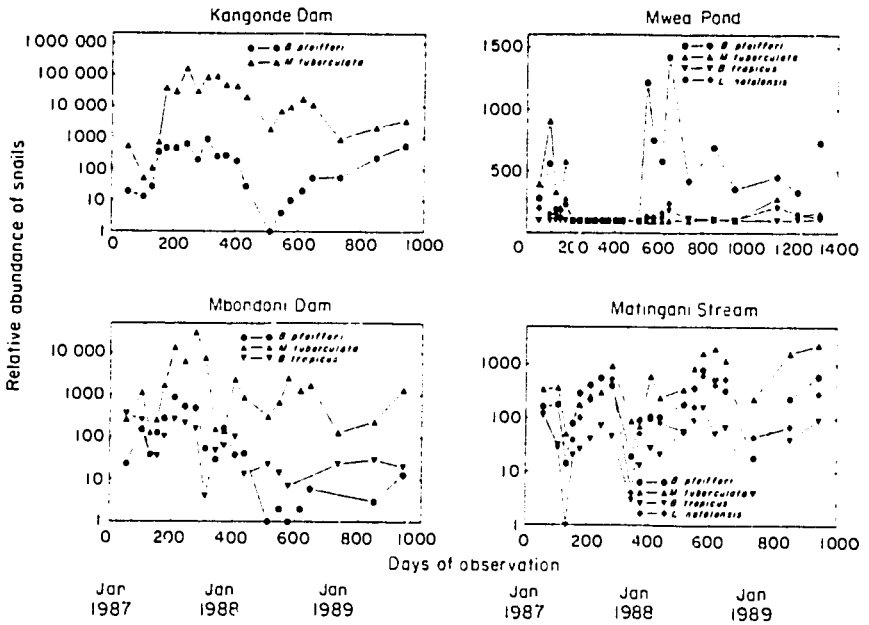


**Fig.** Relative abundance of populations of *Melanoides tuberculata* and pulmonate snails in four study sites located in south central Kenya.

The results of these observations are presented in the Fig. In Kangonde, the only two species present were *M. tuberculata* and *Biomph. pfeifferi*. Both species persisted throughout the study period (January 1987 to August 1989), with *M. tuberculata* being consistently more abundant than *Biomph. pfeifferi*. On many occasions there was a 100- to 1000-fold difference in relative abundance. Occasional visits to this habitat since 1988 have confirmed that the two species continue to co-exist there. In Mwea, *M. tuberculata*, *Biomph. pfeifferi*, *L. natalensis* and *Bul. tropicus* were present. Until mid-1987, *M. tuberculata* was the most abundant snail present but *Biomph. pfeifferi* was also relatively common. *Lymnaea natalensis* and *Bul. tropicus* were present in relatively low numbers. In mid-1987 an interesting situation arose. Without our knowledge or participation, this habitat was treated with molluscicide by the local health officials. After molluscicide application, all four snail species became scarce for almost a year. By mid-1988, however, *Biomph. pfeifferi* populations had recovered and achieved levels of relative abundance considerably higher than in the study period prior to molluscicide application. In contrast, *M. tuberculata* populations had still not recovered to pre-molluscicide levels. The situation here is still being investigated, and as of August 1990, *Biomph. pfeifferi* remained abundant while *M. tuberculata* had still not achieved its former abundance. In Mbondoni, *M. tuberculata*, *Biomph. pfeifferi* and *Bul. tropicus* were present. In this habitat also, all three species persisted throughout the study period, but once again *M. tuberculata* was numerically dominant. During most of 1988, *Biomph. pfeifferi* and *Bul. tropicus* showed an overall decline in abundance, but more recent visits to this location confirmed that all three species were still present, and that the pulmonates were persisting, albeit at low levels. In Matingani stream, *M. tuberculata*, *Biomph. pfeifferi*, *L. natalensis* and *Bul. tropicus* were present. As in the three impoundments, *M. tuberculata* was the most abundant snail present in this habitat, but its dominance was relatively less. The three pulmonate species

persisted throughout the study period and showed no signs of being eliminated by the presence of *M. tuberculata*. These results indicate that appreciable numbers of the pulmonate species *Biomph. pfeifferi*, *L. natalensis* and *Bul. tropicus* co-existed with *M. tuberculata* over long intervals of time in Kangonde, Mbondoni and Matingani, and no obvious trend towards total displacement of the pulmonate species was observed. Even when *M. tuberculata* achieved extremely high abundance, as in the case for Kangonde, the pulmonate populations persisted. Nevertheless, the pattern observed in Mwea before and after molluscicide treatment of the site raises a number of interesting points. First, it may suggest that *M. tuberculata* does exert an inhibitory effect on *Biomph. pfeifferi* populations, such that when the thiarid is absent, *Biomph. pfeifferi* is able to achieve a larger population size. Second, it is interesting to speculate that if *M. tuberculata* is exerting a negative impact on *Biomph. pfeifferi* populations, mollusciciding may inadvertly favour schistosomiasis transmission, because it seems to have a longer-term impact on *M. tuberculata* than on the pulmonate snails. Although thiarid snails are capable of maintaining very high population densities and live much longer than the pulmonate species, they have a much lower reproductive rate than do the pulmonates (Pointier *et al.*, 1989), and therefore they probably take much longer to recover when their populations drastically decline. Third, under circumstances where *M. tuberculata* attains large populations, it can limit *Biomph. pfeifferi* population size, and consequently it may have the effect of reducing the rate of schistosomiasis transmission. It will, however, be interesting to see if *Biomph. pfeifferi* populations decline in Mwea, and if the *M. tuberculata* population attains its pre-molluscicide levels.

Our observations in Kenya, and other observations elsewhere in Africa, indicate that *M. tuberculata* is not capable of totally displacing populations of *Biomph. pfeifferi* or other pulmonate species. These observations differ somewhat from those made in the Caribbean area, where *M. tuberculata* has successfully eliminated *Biomph. glabrata* in several locations, through competitive displacement (Pointier *et al.*, 1989; Pointier and McCullough, 1989). One possible explanation for this difference is that, in Africa, *M. tuberculata* and the indigenous pulmonates have lived together for long periods of time and have evolved towards co-existence and equilibrium. In contrast, the thiarid species were more recently introduced into the Neotropics and may therefore have a greater negative impact on the pulmonate species than they have in Africa. It is also possible that the habitats we sampled, although representative of freshwater snail habitats in central Kenya, may not have been conducive for total displacement of pulmonates by *M. tuberculata*. Pontier and McCullough (1989) suggested that thiarid snails, as competitors of pulmonates, are favoured by the presence of permanent, shallow and stable habitats. The habitats we studied, although permanent, experience large annual fluctuations in volume, and are only shallow at the narrow littoral zones. The mechanisms thiarids use to reduce or displace populations of *Biomphalaria* are not yet fully understood. Prentice (1983) suggested that prolonged pressure for space and competition for food may be involved. Recently, Gomez *et al.* (1989) observed that *T. granifera* probably produces a chemical factor(s) that reduces the fecundity of *Biomph. glabrata*. To summarize, *M. tuberculata* co-exists with *Biomph. pfeifferi* and other pulmonates in Kenyan freshwater habitats, and possibly acts to regulate pulmonate populations, but it is not able to displace them completely.

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Appendix 8

# CONTROL OF NATURAL POPULATIONS OF SCHISTOSOME-TRANSMITTING SNAILS BY THE CRAYFISH, *PROCAMBARUS CLARKII* IN TEMPORARY MAN-MADE PONDS IN KENYA

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## ABSTRACT

A study was conducted to assess the ability of the Louisiana red swamp crayfish, *Procambarus clarkii*, to control the schistosome-transmitting snail *Biomphalaria pfeifferi* in small, rain-filled quarry pits near Kangundo, Machakos, Kenya. This area is within the known geographic range of crayfish in Kenya and these man-made habitats are far removed from other water bodies. In six control sites where crayfish were not introduced, populations of *B. pfeifferi*, *Bulinus* (*Physopsis*) or *Bul. forskali* persisted and in three of the sites, *B. pfeifferi* populations remained abundant. Crayfish were introduced into four experimental sites that initially had snail populations. In two of these sites, snails disappeared within one month and have not reappeared, and crayfish populations have persisted. In the remaining two sites, snails also quickly disappeared following crayfish introduction, but the habitats subsequently dried, interfering with long-term establishment of crayfish. In one additional site originally selected as a control habitat, a sharp reduction in snail abundance was correlated temporally with the unexpected appearance of crayfish. The results are in agreement with earlier studies implicating *P. clarkii* as a potential biological control agent for schistosome-transmitting snails in selected habitats in Kenya and indicate that additional study of this possibility is warranted.

## INTRODUCTION

Freshwater habitats play an integral role in the life cycle of the parasitic flatworms responsible for causing schistosomiasis, one of the most common infectious diseases of humanity (Walsh and Warren 1979). Infections are acquired in freshwater when free-swimming schistosome cercariae penetrate human skin. Following the development of adult schistosomes in humans, their eggs are

passed in feces or urine. If deposited in water, the eggs will hatch and release miracidia, which penetrate and initiate larval development in certain species of freshwater pulmonate snails. This culminates in the production and release of cercariae into water. These parasites, therefore, take advantage of the need for people to contact water and are also favored by the contamination of water bodies by human wastes. (For a review of schistosome biology, see Rollinson and Simpson 1987.)

Within major endemic regions, it is very difficult to reduce morbidity and mortality to acceptable levels over sustained intervals of time; except in a few unique circumstances, eradication is not a feasible goal (Jordan 1977, WHO 1985, Mott 1987). The task of schistosomiasis control is rendered more difficult by water-development projects such as dams, irrigation schemes, and aquaculture projects that create new, and often favorable, habitats for snails, and that offer new opportunities for human water contact (Heyneman 1979).

Various strategies for control include chemotherapy to treat infected people, improved sanitation, public health education programs, and snail control. Each of these approaches have advantages and drawbacks (e.g., Combes and Cheng 1986, Mott 1987, Madsen 1990) and it is generally acknowledged that a combination of methods is required to achieve success.

The focus of our work is to investigate alternative methods of snail control that offer increased hope for sustained control and that deemphasize dependence on the use of relatively nonspecific, expensive molluscicides. We have concentrated on elucidating the potential of other freshwater invertebrates to control the pulmonate snails in Kenya that transmit not only the human schistosomes *Schistosoma mansoni* and *S. haematobium*, but also schistosomes and liver flukes of domestic animals.

In the early stages of our investigations, our efforts concentrated on species of ampullariid snails indigenous to Kenya that might function as effective competitors and/or predators of disease-transmitting pulmonates (Hofkin et al. 1991a, Stryker et al. 1991). While defining the geographic ranges and patterns of coexistence of ampullariids and pulmonates, we encountered several habitats that contained the North American crayfish *Procambarus clarkii* (see Loker et al. in these proceedings). Further study indicated that habitats containing crayfish were significantly less likely than crayfish-free habitats to support pulmonate species (Hofkin et al. 1991b). A possible explanation for this phenomenon was provided by laboratory experiments, which showed that crayfish readily consumed pulmonate snails, and by field experiments demonstrating that crayfish were capable of eliminating pulmonates in enclosures and that snails protected by cages were able to survive in crayfish habitats (Hofkin et al. 1991b). Further field and laboratory studies showed that crayfish could significantly reduce the abundance of macrophytes used by snails for food and oviposition sites (Hofkin et al. 1991c). It was also documented that crayfish could effectively block the build-up of pulmonate populations in laboratory aquaria (Hofkin et al., unpublished observations).

Given the encouraging results obtained in both laboratory and field experiments, we were interested in determining if *P. clarkii* could displace natural populations of pulmonate snails. This represents an important step in determining the potential utility of *P. clarkii* as a possible biological control agent of schistosomiasis in Kenya. In conjunction with the Division of Vector Borne Diseases of the Kenya Ministry of Health, small, isolated, man-made habitats containing target snail species were selected for a preliminary trial of this possibility. Although *P. clarkii* is an introduced species in Kenya, it is now widely distributed in the country and is present in areas immediately adjacent to the sites selected for study. The results of this pilot study are outlined below.

## MATERIALS AND METHODS

### Study Sites

This study was conducted near Kangundo, Machakos District, in Kenya. The study sites consisted of man-made pits excavated to obtain stone blocks used in building construction. During the rainy season, these pits fill with water and, at maximum water volume, measure about 60-70 $m^2$ in area and 1-1.5 m in depth. Water volumes fluctuate seasonally, and many of the pits dry completely during the dry season. In spite of such fluctuations, many of these sites support thriving populations of pulmonate snails including *Biomphalaria pfeifferi* and *Bulinus* (*Physopsis*) spp., important schistosome-transmitting species in Kenya. The pits contained clear water, had rocky substrates, and some emergent vegetation was found near their margins.

Crayfish were not known to occur here, but the sites are within the known geographic range of crayfish in Kenya, and are far removed (at least 10 km) from other waterbodies. Eleven sites were selected for the present study, seven of which served as controls and received no crayfish, and four of which were designated as experimental sites and received *P. clarkii*. These particular sites were chosen because they contained populations of pulmonate snails and their shorelines were easily accessible for sampling. In July 1990, just prior to crayfish introductions, snail searches on the shorelines of the sites were conducted (see below), and snails were identified and enumerated. Three control and three experimental habitats contained relatively small numbers of snails (< 20 snails per 5 minutes searching) and were designated as "low-density" habitats; four control and one experimental site typically contained >50 snails per 5 minutes searching and were designated as "high density" sites.

### Crayfish Introductions

*Procambarus clarkii* (carapace size 3.5-4.5 cm) were obtained from a dam near Thika, 35 km away, and were transferred the same day into the sites selected to receive crayfish. Each experimental site received 80 crayfish.

**Sampling of the Sites** Regular sampling was initiated at the time of crayfish introductions. During sampling, a systematic five-minute search for snails was conducted along the shoreline of each site. As the water was relatively shallow and clear, the relative abundance of snails on the surfaces of submerged rocks was enumerated directly, and disturbance to the habitat resulting from the use of other sampling procedures was avoided. Presence and relative abundance of crayfish was assessed by counting crayfish caught in meat-baited minnow traps held in each site overnight.

## RESULTS

The three pulmonate species recorded in the 11 study sites were *B. pfeifferi*, *Bulinus (Physopsis)* spp., and *Bul. forskali*, with the first species comprising > 95% of all collected snails. To simplify presentation, the counts for all three species are combined in the results. Relative abundance of pulmonates in the three low-density control sites are shown in Figure 1. Snails were found in low numbers in each site, with the number recovered per site varying considerably between sampling dates. In some months no snails were found in some of these habitats; however, snails were found in each control site for up to at least seven months after initiation of sampling. These sites dried by 12 months.

In the first low density experimental site, crayfish clearly became established and exhibited a pattern of increasing abundance for the 11 months of study (Figure 1). Snails were not found after crayfish had been placed in the habitat. In the two remaining low density experimental sites, snails quickly disappeared following the introduction of crayfish. However, crayfish did not become established and small numbers of snails later appeared. Snails could be found in these sites for up to eight months (data not shown).

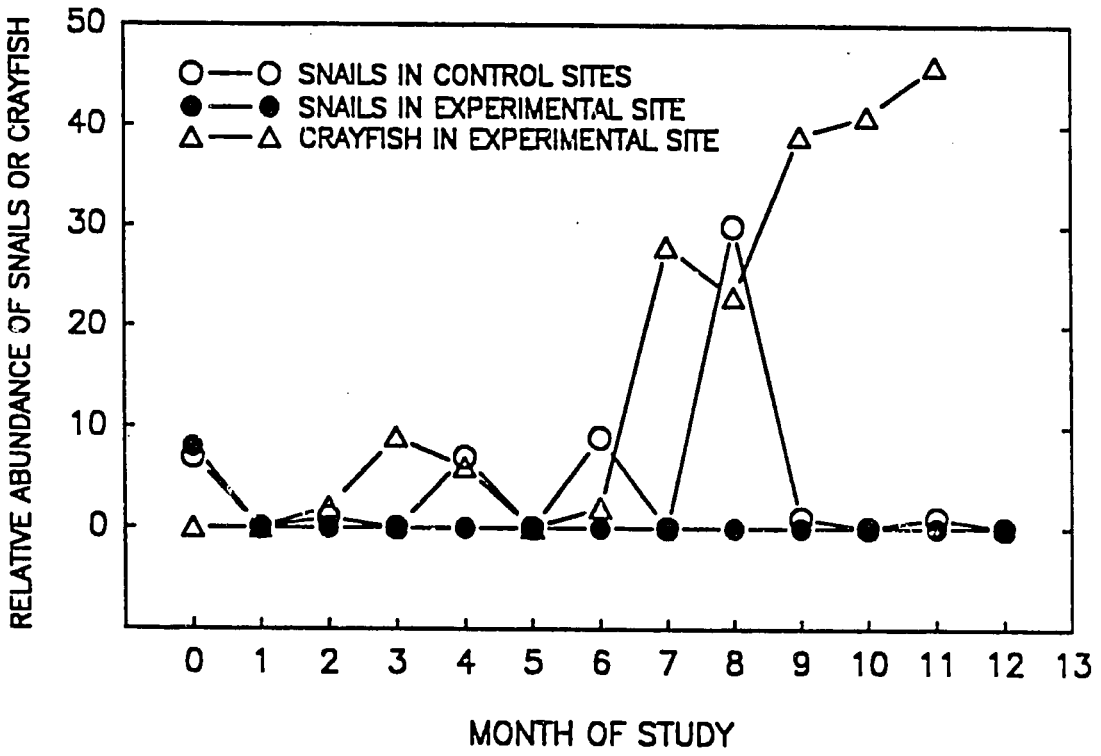


FIGURE 1 Mean relative abundance of pulmonate snails (mostly *Biomphalaria pfeifferi*) for the three low density control sites is indicated by the open circles. Solid circles indicate relative snail abundance, and the triangles the relative abundance of crayfish, in the single low density experimental site.

The three high density control sites supported vigorous snail populations throughout the five-month period of observation (Figure 2), and although sampling was discontinued after this time, these habitats continued to support viable snail populations. In contrast, in the one experimental site, no snails were ever found after crayfish had been introduced (Figure 2). Crayfish persisted in this location and increased in abundance after six months.

An additional high density control site was of particular interest because crayfish appeared there unexpectedly (Figure 3). During the first five months of study, the snail population was

abundant, but by month six had started to decline, and by month nine had disappeared. This decline was correlated with the appearance of crayfish, which were first detected by month six. Crayfish subsequently disappeared, also by month nine of the study.

## DISCUSSION

Schistosome-transmitting snails were apparently eliminated within one month from both experimental quarry pits in which crayfish became well established. Snail surveys, conducted monthly at these sites for up to 12 months, were consistently negative, suggesting that snails were unable to recolonize these sites. Snails also rapidly disappeared in the two experimental sites where crayfish did not persist, but subsequently reappeared following the disappearance of crayfish. Snails were also eliminated within four months of the unexpected appearance of crayfish in a control site. In the three sites where crayfish persisted, their numbers began to increase after about 5-6 months, indicating they were capable of successful reproduction in the quarry pits. In the remaining control sites, snail populations persisted for at least four months after the disappearance of snails from crayfish introduction sites.

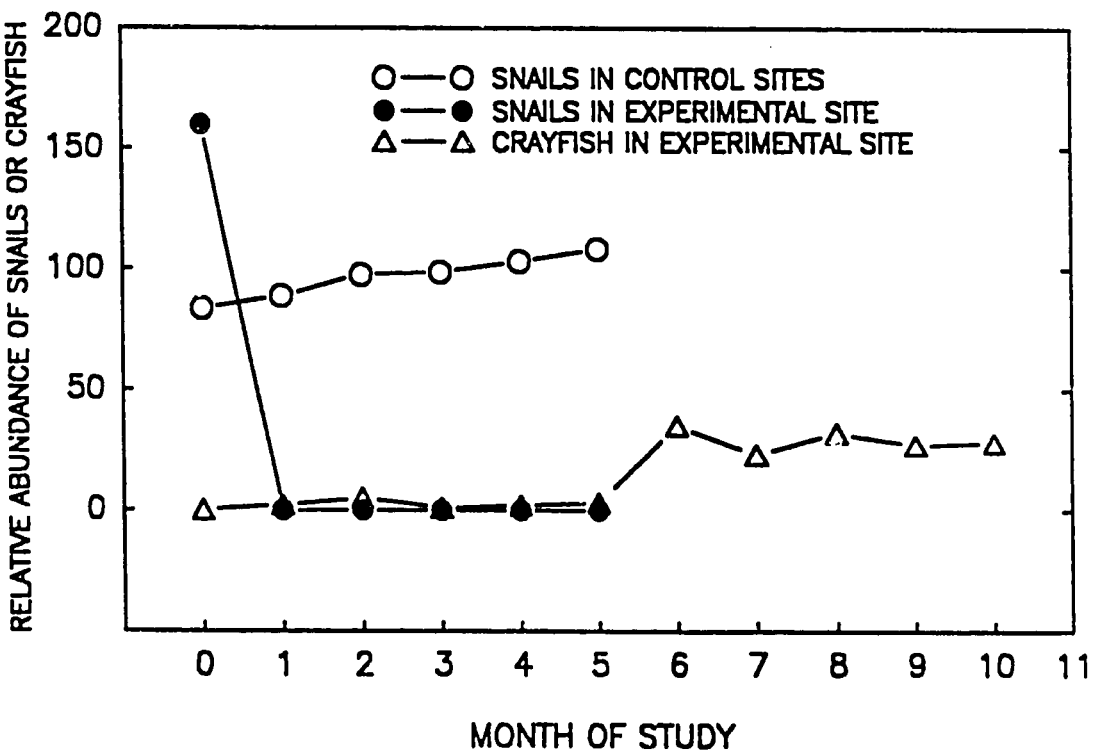


FIGURE 2 Mean relative abundance of pulmonate snails (mostly *Biomphalaria pfeifferi*) for the three high density control sites is indicated by the open circles. Solid circles indicate relative snail abundance, and the triangles indicate relative abundance of crayfish, in the single high density experimental site.

The unexpected arrival of *P. clarkii* in one control site, apparently after having migrated from an experimental site approximately 12 miles away, illustrates the dispersal ability of this crayfish species. In conjunction with earlier results (Hofkin et al. 1991b), the results indicate that *P. clarkii* can rapidly eliminate and/or control *B. pfeifferi* and other snails of public health significance, at least in certain habitat types.

The Kangundo quarry pits are small and man-made. They are completely dependent on rain water and they experience large seasonal fluctuations in volume. Many of the pits dry completely for up to three months of each year. Because of these considerations, it is unclear if the results obtained here can be extrapolated to larger, more permanent water bodies, or to lotic habitats that serve as important transmission foci. Nevertheless, the results obtained in the quarry pits are encouraging as well as relevant to public health because these sites are important transmission foci. Contact by local people with these sites is frequent, and human feces is commonly observed near the pits. We have also observed large numbers of snails recovered from the pits to be shedding schistosome cercariae (unpublished observations).

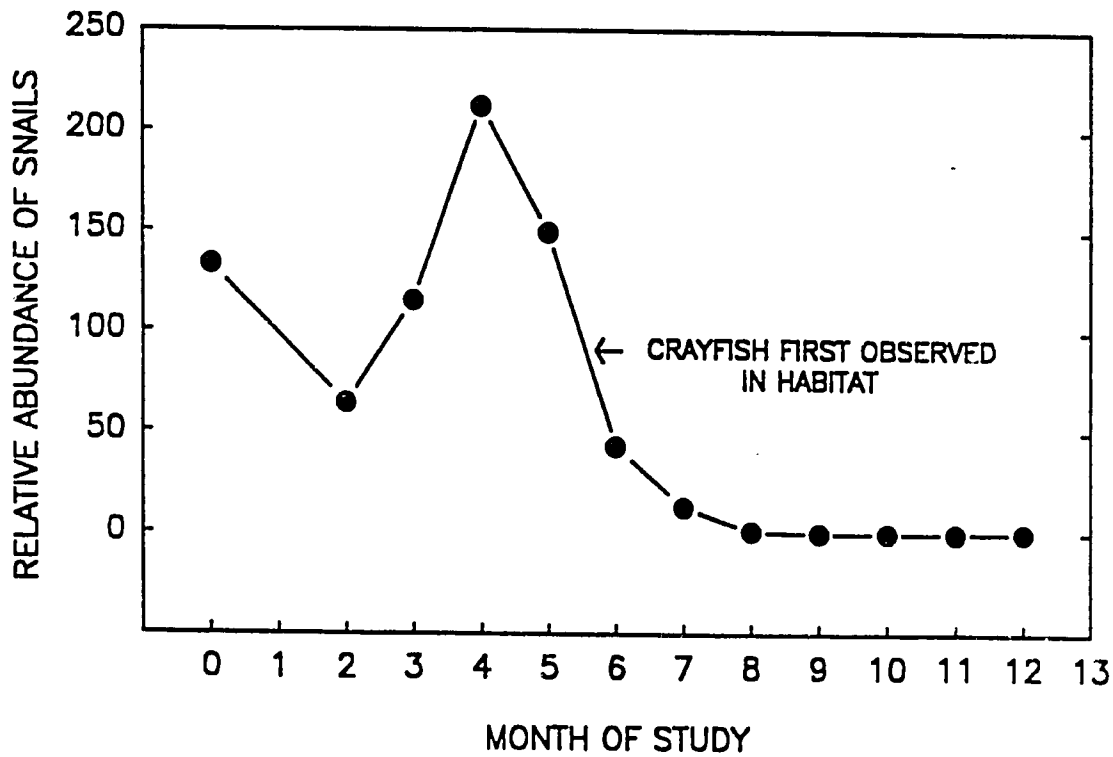


FIGURE 3 The relative abundance of pulmonate snails (mostly *Biomphalaria pfeifferi*) for the one control site in which crayfish unexpectedly appeared is indicated by the solid circles. The arrow indicates the point in time when crayfish were first recovered for the habitat.

It will be of particular interest to learn if crayfish will persist through the dry season in Kangundo, and whether snails will recolonize the introduction sites. *Procambarus clarkii* is capable of surviving dry periods by constructing burrows and is well adapted to temporary habitats (Huner 1988). Whether it can survive in quarry pits with limited opportunities for burrowing requires further investigation. Also, *B. pfeifferi* is capable of rapid population growth when conditions become favorable (Teesdale 1962, Woolhouse and Chandiwana 1989), and it remains to be seen if surviving crayfish are able to prevent such population expansion.

The ultimate goal of snail control is to reduce morbidity and mortality in the human population. Should *P. clarkii* prove capable of long-term snail control, studies should be initiated to determine if the presence of crayfish can be associated with lower disease incidence. The Kangundo quarry pits may be especially suitable for such an epidemiological study. The quarry pits are appropriately isolated and schistosomiasis is an important public health concern in this area. For many local inhabitants, these sites represent the only available source of water. Should such a study indicate that the presence of *P. clarkii* can be associated with lower disease incidence and prevalence, further study of its potential for achieving control in other habitat types would be warranted.

## ACKNOWLEDGMENTS

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Appendix 9

# *PROCAMBARUS CLARKII* IN KENYA: DOES IT HAVE A ROLE TO PLAY IN THE CONTROL OF SCHISTOSOMIASIS?

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## ABSTRACT

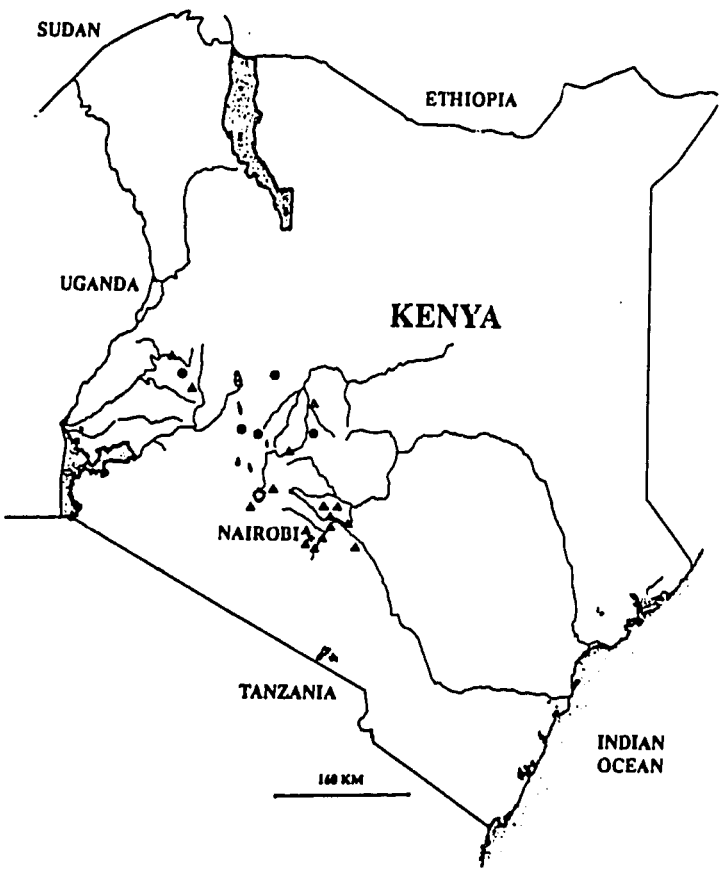
The Louisiana red swamp crayfish, *Procambarus clarkii*, introduced into Lake Naivasha, Kenya, about 1970, has become well established and now supports a small but thriving aquaculture industry. This crayfish has since dispersed over a wide area in Kenya, giving rise to concerns about its ability to disrupt natural ecosystems both in Lake Naivasha and elsewhere. In addition, *P. clarkii* may adversely affect fin fisheries in Lake Naivasha. and has been implicated as a pest in rice cultivation schemes outside of Africa. In contrast, our investigations indicate that *P. clarkii* can effectively control or eliminate certain freshwater snail species, including those involved in the transmission of human schistosomiasis. As such, *P. clarkii* appears to offer hope as a biological control agent of snail-transmitted diseases in selected habitats in East Africa. In this paper, we present details regarding the current distribution of *P. clarkii* in Kenya and discuss possible approaches to the use of crayfish in snail control operations. Furthermore, concerns regarding the impact of *P. clarkii* on non-target species and on Kenyan freshwater habitats are discussed, and areas in need of additional investigation are highlighted.

## INTRODUCTION

The Louisiana red swamp crayfish, *Procambarus clarkii*, is the most widely distributed of all crayfish species, having been introduced into 24 countries on five continents (Hobbs et al. 1989). In the United States, *P. clarkii* supports a thriving aquaculture industry. Elsewhere, this species is now harvested commercially in countries as diverse as Spain, Turkey, and Kenya (Hobbs et al. 1989).

Africa represents a unique situation regarding such introductions in that it lacks an indigenous crayfish fauna (Hobbs 1988). *Procambarus clarkii* is now present in the Sudan, Kenya, Uganda, Zambia, Zimbabwe, and South Africa (Hobbs et al. 1989). Details regarding the original introduction of *P. clarkii* into Kenya remain unclear, but the first individuals were probably imported from Uganda into small ponds in the Rift Valley in the mid-1960s (Oluoch 1990). In 1970, *P. clarkii* was

introduced into Lake Naivasha, apparently in an attempt to develop a fishery for this species (Lowery and Mendes 1977a, Parker 1974, Oluoch 1990). Actually, there is an unconfirmed report that *P. clarkii* may have been introduced into the lake as early as 1952 for the purpose of leech control, but this claim needs verification. Since its original introduction, *P. clarkii* has become well established in Lake Naivasha and has supported a viable aquaculture industry. Crayfish are now considered to be one of four important commercial species harvested from the lake, with an annual average catch of approximately 60,000 kg (live wet weight) over the past 10 years. More than 80% of this catch is exported, primarily to western European countries (Kenya Fisheries Department, personal




KNOWN DISTRIBUTION OF *P. clarkii* IN KENYA

FIGURE 1 Localities confirmed to contain *Procambarus clarkii* in southern Kenya. Triangles represent collections of *Procambarus clarkii* reported by Lowery and Mendes (1977b). Circles represent localities where *Procambarus clarkii* has been found by the authors.

communication). The remainder is consumed locally, mostly in restaurants in Nairobi; crayfish have not been readily adopted by most Kenyans as a preferred food item. Other large freshwater habitats in Kenya, for example, the Masinga dam complex to the south and east of Mt. Kenya, may have potential for crayfish aquaculture. In Kenya, crayfish are not harvested commercially from the numerous small dams and ponds they occupy at present, and it seems unlikely that this will change in the future, owing to excessive transportation costs.

## CURRENT DISTRIBUTION OF *PROCAMBARUS CLARKII*

In the estimated 20-30 years since its first introduction into Kenya, *P. clarkii* has expanded its range and its known distribution now includes a large portion of the country (Figure 1). Besides Lake Naivasha, its presence in the Rift Valley has been confirmed in freshwater habitats near Lake Nakuru (Lowery and Mendes 1977b) and from streams and ponds in the western highlands (near the towns of Eldoret and Kitale), which form part of the Lake Victoria drainage basin. *Procambarus clarkii* is especially abundant in the Athi River basin in and around Nairobi. It extends through the Muranga District in the central highlands and has been collected as far north as Nanyuki. Its presence has also been confirmed in the upper basin of Kenya's largest river, the Tana. We have also recovered *P. clarkii* in Machakos District, near the town of Tala. It has apparently not colonized habitats on the tropical coastal plain. The town of Matuu, approximately 300 km from the Indian Ocean, represents the easternmost site from which we have collected this species. Reports of its presence in Lake Victoria (Lowery and Mendes 1977b) have recently been discounted by local fisheries experts, and our own recent efforts to locate crayfish in Lake Victoria and in inflowing streams have not been successful. Of considerable interest are possible future extensions of its range in East Africa, either through natural or human-assisted dispersal.

## SNAIL-TRANSMITTED PARASITES OF MEDICAL AND VETERINARY SIGNIFICANCE

Our interest in *P. clarkii* stems from our investigations into the biological control of schistosomes and other snail-transmitted parasites in Kenya. Schistosomiasis, a debilitating, chronic infection of the tropics and subtropics, is caused by blood flukes of the genus *Schistosoma*. It is estimated that between one and two million Kenyans are infected with either the intestinal or urinary forms of the disease, or both (Highton 1974, Diesfeld and Hecklau 1978, Iarotski and Davis 1981, Butterworth et al. 1984, Ouma et al. 1985, King et al. 1988). Pathology, especially in children, can be severe (Wilkins 1987). The life cycle of schistosomes is complex and involves humans (and potentially other mammals) and particular species of freshwater snails as hosts for adult and larval worms, respectively (see review by Rollinson and Simpson 1987). Humans are exposed to infection by entering bodies of water containing infected snails. Infective stages, termed cercariae, released from the snails penetrate human skin to initiate infection. Infected humans facilitate transmission by urinating or defecating in water, thereby ensuring passage of parasite eggs into the environment of the snail intermediate hosts.

Snails of the genus *Biomphalaria* serve as intermediate hosts for *Schistosoma mansoni*, the causative agent of intestinal schistosomiasis. Snails within the genus *Bulinus* fulfill this function for *S. haematobium*, the parasite responsible for urinary schistosomiasis. Other snail-transmitted trematode

parasites commonly infect domestic ruminants in Kenya and include the blood flukes *S. bovis* and *S. mattheei*, the common liver fluke *Fasciola gigantica*, and paramphistome flukes (see Brown 1980 for a comprehensive review of freshwater snails and associated trematode parasites in Africa).

The distribution of human schistosomes in Kenya is widespread (Figure 2) and is dictated ultimately by the distributions of the associated snail species (Brown et al. 1981, Loker et al. unpublished data). It is noteworthy that the present geographic ranges of the relevant snails and of *P. clarkii* are broadly, but not completely, overlapping (Figures 1 and 2). Owing to a rapidly increasing human population, unabated pollution of freshwater habitats by human excrement, construction of dams and irrigation schemes that favor the growth of snail populations, and the lack of a comprehensive national control program, it is likely that the prevalence of human schistosomiasis will remain high, or even increase. Although effective chemotherapeutic agents are available and are capable of reducing morbidity and mortality resulting from infection, they are expensive, and unless repeatedly applied to infected individuals are unlikely to diminish transmission significantly. A schistosome vaccine is a laudable goal, but remains elusive (Sher et al. 1989). Snail control, in conjunction with other control

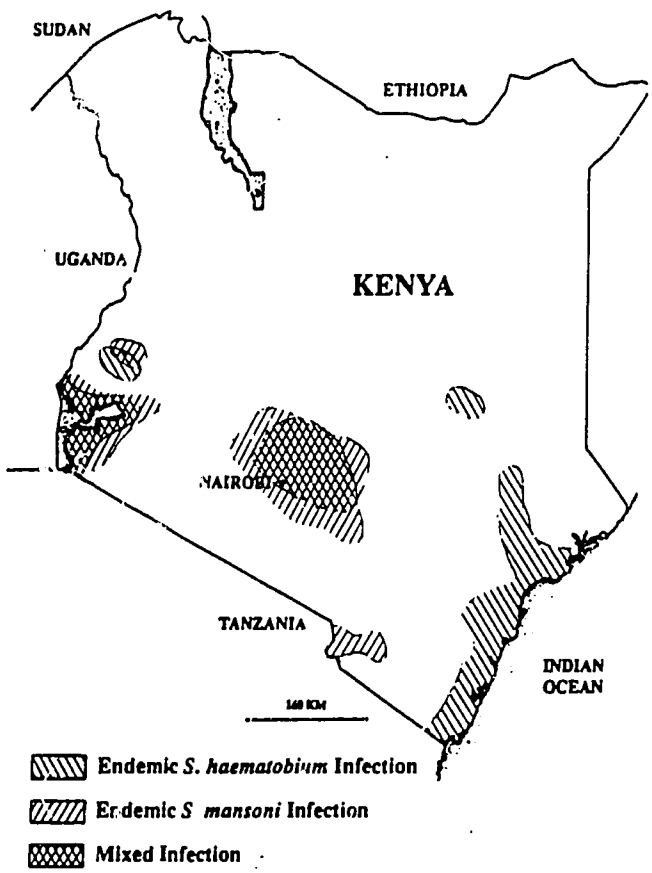



FIGURE 2 Distribution of human schistosomiasis in Kenya (modified from Highton 1974, Brown et al. 1981, Diesfeld and Hecklau 1978).

measures, offers hope for interrupting the transmission cycle, but traditional methods for controlling snails have primarily relied on molluscicidal chemicals that are also expensive. Moreover, because of their lack of selectivity, molluscicides pose environmental concerns and can only be condoned for use in focal applications (Klumpp and Chu 1987). New approaches to the control of schistosomes are needed (Mott 1987, Madsen 1990).

## CRAYFISH-SNAIL INTERACTIONS

In the course of snail survey studies of Kenyan freshwater habitats, it was observed that schistosome-transmitting snail species rarely, if ever, were found in habitats containing *P. clarkii*. A more formal analysis of the extent to which crayfish and medically important gastropods occurred at the same time revealed a highly significant ($p < 0.001$) negative association between the presence of snails and *P. clarkii* (Hofkin et al. 1991). Of the 53 sites examined, snail-crayfish co-occurrence was recorded in only four; the snail species recovered from these four sites do not transmit human schistosomes. All 53 sites were within the known distribution of both medically important snails and crayfish and were considered, *a priori*, as suitable snail and crayfish habitats.

Subsequent experiments indicated that both juvenile and adult *P. clarkii* were able to significantly reduce *Biomphalaria* abundance in both laboratory aquaria and field enclosures in as few as five days (Hofkin et al., unpublished data). Crayfish consumed large numbers of snails even when alternative foods were available and frequently eradicated *Biomphalaria* populations within the five-day experimental period.

Furthermore, *P. clarkii* was able to significantly reduce the abundance of the aquatic plant, *Nymphaea caerulea*, in experimental aquaria and field enclosure cages (Hofkin et al., unpublished data). The presence of this common water lily has been positively correlated with the presence of both *Biomphalaria* and *Bulinus* snails (Woolhouse and Chandiwana 1989), which use them for food, oviposition sites, and refuge. By feeding on, and reducing the abundance of such plants, *P. clarkii* may also serve as a competitor of snails of medical and veterinary significance.

These research results have led us to believe that *P. clarkii* is effective in controlling and/or eliminating certain snails of relevance to human public health. As such, this crayfish may represent a viable option as a biological control agent of schistosomes and other snail-borne diseases. Some of the potential advantages offered by *P. clarkii* as a control agent are: 1) its impact on target species is likely to be long-lasting and, unlike chemically based methods of control, would not have to be repeatedly applied; 2) the expense involved in its use would be minimal; 3) it could simultaneously reduce transmission of all snail-transmitted parasites, including species relevant to both humans and domestic animals; and 4) because *P. clarkii* is able to withstand habitat drying, it could be used in both permanent and seasonal transmission sites. Enthusiasm for the use of *P. clarkii* in snail control operations must, however, be tempered by the realization that biological control, in and of itself, is probably only rarely sufficient for bringing about a complete cessation of transmission. Moreover, the use of *P. clarkii* in particular poses certain environmental risks (see below) that must be carefully considered before their widespread use as putative control agents can be advocated.

## ENVIRONMENTAL CONCERNS

Perhaps the most immediate concern regarding the presence of *P. clarkii* in Kenya is its impact on fin fisheries in Lake Naivasha and, potentially, in other waterbodies with commercially viable fisheries. Because of its voracious feeding habits, *P. clarkii* is thought to pose a risk to nest-breeding, commercially important species such as *Tilapia* (Lowery and Mendes 1977a). For example, crayfish might interfere with the breeding of *Tilapia zillii* (itself an introduced species in Lake Naivasha) either by consuming its eggs or by disturbing its nest sites. Also, because of its omnivorous nature, *P. clarkii* can reduce the abundance of aquatic plants (Lowery and Mendes 1977a, Feminella and Resh 1989, Hofkin et al., unpublished) and therefore might restrict the available nursery areas for young fish. For example, crayfish are thought to be at least partially responsible for the dramatic decline of indigenous water plants in Lake Naivasha. *Tilapia zillii* catches have declined in the lake since the introduction of *P. clarkii*, to the point that fewer than 3,100 kg of this species have been harvested since 1980 (Kenya Fisheries Department, personal communication).

Crayfish might adversely affect commercial fisheries in Lake Naivasha in other ways. Fish taken in gill nets are often attacked and partially consumed by crayfish before the nets are recovered, resulting in a lower market price for the damaged catch. Crayfish also become entangled in the nets, necessitating both their removal and repair of the nets. It is interesting to note, however, that fishermen in Lake Naivasha are legally required to place their nets at least 100 m from the lakeshore. The vast majority of *P. clarkii* in the lake are found in shallow water near the shore, meaning that most net fishermen who suffer crayfish-related damage are actually fishing illegally (Kenya Fisheries Department, personal communications).

The exact role that crayfish may have played in the decline of lake fisheries is complex and unclear, owing in part to simultaneous man-made habitat modifications, natural climatic fluctuations, and introductions of several other exotic species. For example, a section of Lake Naivasha known as "Small Lake" was cut off from the main lake basin when a causeway was constructed in 1982. This now isolated part of the lake was regarded as a prime breeding area of *T. zillii*, so the decline in catches for this species may, in part, reflect this loss of breeding habitat. In addition, fish populations in the lake tend to fluctuate naturally as increasing or decreasing water levels either add to, or subtract from, the amount of suitable inshore breeding habitat. Lake Naivasha has experienced relatively dry conditions in recent years, which partially explains reduced *T. zillii* catches (Kenya Fisheries Department, personal communications).

The effect of *P. clarkii* on the other two important fisheries species in Lake Naivasha, largemouth bass (*Micropterus salmoides*) and a second tilapia species (*Oreochromis leucostictus*), seems more likely to be neutral or even beneficial. *Oreochromis leucostictus* is a mouth breeder and is therefore not subject to egg predation by crayfish. Largemouth bass nest along rocky shorelines where crayfish are uncommon. Additionally, *P. clarkii* comprise more than 70% of the largemouth bass diet, which, in turn, relieves predation pressure by bass on other potential prey, including *Tilapia* (Kenya Fisheries Department, personal communications).

A second concern regarding the further introduction of *P. clarkii* in Kenya involves its potential as a pest in rice cultivation schemes. Rice is grown in Kenya both for domestic consumption and for export. *Procambarus clarkii* is known to consume rice seedlings and to damage dikes as a consequence of its burrowing activities (Miltner and Avault 1990, Huner 1988). To date, *P. clarkii* has not been

considered a problem for Kenyan rice farmers (Kenya National Irrigation Board, personal communication). In all major rice growing schemes in Kenya, seedlings are cultivated in nurseries for one month prior to transplantation into the paddies. At the time of transplantation, Furadan®, a carbamate insecticide and nematicide, is applied to the paddies at a concentration of 4 kg/acre. The effects of Furadan®, as used in Kenya, on crayfish are not known, but its use may explain the apparent absence of *P. clarkii* from rice schemes. For example, crayfish are absent from the Mwea scheme but are present in the Makuyu area, approximately 15 km away.

Thus, the potential impact of *P. clarkii* on rice cultivation remains unclear and deserves additional consideration. If crayfish are indeed excluded from rice fields through the routine use of Furadan®, it is encouraging that a widely used, commercially available pesticide may have value in preventing the spread of crayfish into habitats where their use in snail control might be contraindicated.

Another concern pertaining to *P. clarkii* that deserves consideration relates to its possible involvement in transmission of parasites of medical significance. Crayfish can serve as second intermediate hosts for lung flukes of the genus *Paragonimus*, including species known to infect humans (Sogandares-Bernal 1965). *Paragonimus uterobilateralis* and *P. africanus* are known from West Africa but have not been reported in Kenya or other parts of East Africa, presumably because the appropriate species of snail first intermediate host is absent (Brown 1980). In any case, it seems unlikely that lung fluke transmission in Africa would be substantially altered by the introduction of *P. clarkii* as several species of freshwater decapods are already present and are probably as likely as *P. clarkii* to serve as efficient second intermediate hosts. Because *P. clarkii* are sometimes frozen and usually cooked thoroughly before consumption, it is unlikely that they would serve as a significant source of parasitic infection for humans.

In addition to its possible impact on species of direct relevance to human commerce and health, the effect of *P. clarkii* on other non-target organisms and on natural ecosystems in general should be considered as well. Crayfish are voracious omnivores and are often able to significantly alter the structure of natural aquatic communities (Carpenter and Lodge 1986, Lodge and Lorman 1986). This might be achieved by direct depredation of indigenous aquatic invertebrates or vertebrates, or by vigorous consumption of macrophytes and detritus used by other organisms as food sources or as cover from predators. Its escape into large natural habitats such as Lake Victoria, or the other African great lakes, with their large numbers of endemic animal species, might have particularly drastic consequences.

## POSSIBLE SITES APPROPRIATE FOR USE OF *P. CLARKII* IN BIOLOGICAL CONTROL

Thousands of small man-made impoundments or rain-filled pools exist throughout rural Kenya and are heavily used as a source of water for domestic purposes and for watering livestock. These habitats are often heavily polluted by human or domestic animal wastes, and are frequently populated with disease-transmitting snails and/or mosquitoes (Ripert and Raccurt 1987, Jewsberry and Imbevore 1988). Insofar as such habitats are already extensively modified by intense usage, often in ways that promote disease transmission, they may be ideal candidates for receiving crayfish in schistosomiasis control operations. Another category of habitat that should be considered as possible introduction sites are polluted streams that are located within drainage basins in which crayfish are already abundant (for example, the upper basin of the Athi River).

Additional research addressing the points raised below should be undertaken before any introductions are contemplated, with both appropriate local and national authorities involved in the decision-making process. In general, experience should be gathered regarding the desirability of crayfish introductions in candidate habitats in regions within Kenya in which crayfish already are present. Particular attention should be given to the effects of crayfish activities such as burrowing on the structural integrity of dams and on the suitability of water for human consumption. Based on these experiences, decisions should then be made regarding possible introduction in suitable habitats within areas such as the coastal lowlands where crayfish are not known to be present. We presently do not advocate the introduction of crayfish for snail control into countries where they are not already found.

## CONCLUSION AND PRIORITIES FOR FUTURE STUDY

The potential environmental ramifications of further *P. clarkii* introductions that have been highlighted here underscore the need for additional study. Current concerns regarding the protection of natural ecosystems, the maintenance of biodiversity, and the protection of rare endemic species should give pause to anyone contemplating additional introductions of this exotic species into East Africa. These very real concerns must, however, be balanced by the realization that a species such as *P. clarkii* offers the potential of increased economic opportunity and improved public health. Furthermore, *P. clarkii* is, for better or worse, already firmly established in Kenya and may well increase its present range, irrespective of human activity. Bearing this in mind, further studies should focus on how to best manage this resource, and how to take maximum advantage of its positive attributes while keeping its environmental effects to a minimum.

### Potential Avenues For Further Study

1. The ability of *P. clarkii* to reduce actual schistosome transmission. Although *P. clarkii* can control schistosome-transmitting snails in the laboratory and in field enclosures, this by no means ensures that it can actually reduce schistosomiasis transmission in complex natural foci of infection. That *P. clarkii* can actually reduce incidence and prevalence of both *S. mansoni* and *S. haematobium* in human populations in an endemic area should be clearly demonstrated before any decision to use *P. clarkii* in large-scale control programs. Such field trials might best be conducted in small man-made impoundments or quarry pits, for reasons specified above.

2. Effects of *P. clarkii* on the abundance and species composition of mosquito larvae. It is intriguing to consider what effect crayfish, used to control schistosomes, might have on the transmission of mosquito-borne diseases such as malaria, filariasis, and arboviral infections. Because it has the capacity to alter aquatic habitats by consuming macrophytes, *P. clarkii* has been suggested as a means of controlling mosquitoes in California marshes (Feminella and Resh 1986). In tropical Africa, the effect on mosquito breeding might be positive or negative, depending on the tendency of a particular mosquito species to breed in open or shaded water.

3. Are commercially available pesticides used in rice cultivation effective in controlling the spread of *P. clarkii*? Crayfish are absent from major Kenyan rice growing schemes, possibly due to the use of certain pesticides such as Furadan® to control insect pests. Even if *P. clarkii* should prove highly successful as a means of controlling schistosomes, its presence in rice fields would probably be counterproductive, owing to its ability to consume rice seedlings. Studies should be initiated to

determine whether Furadan® or other pesticides that are routinely used for agricultural purposes can be used to eliminate crayfish from areas where they pose economic or environmental risks.

4. Assessment of the effects of *P. clarkii* on other components of the aquatic environment. Careful studies are required to ascertain how *P. clarkii* introductions might effect non-target species. With such information available, decisions about the use of crayfish for snail control can be better evaluated. Special care should be taken to elucidate how crayfish introductions might affect endangered aquatic species.

5. What factors control the present distribution of *P. clarkii* in Kenya? Is its distribution likely to expand? *Procambarus clarkii* is absent from large areas of Kenya, and it is unclear to what degree various biotic and abiotic factors interact to explain this distribution. A clearer understanding of these factors would ensure a greater likelihood of success in snail control operations and would make predictions about its capacity for environmental damage more valid.

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Appendix 10
In press: Journal of Medical and Applied Malacology

# DISTRIBUTIONS OF FRESHWATER SNAILS IN SOUTHERN KENYA WITH IMPLICATIONS FOR THE BIOLOGICAL CONTROL OF SCHISTOSOMIASIS AND OTHER SNAIL-TRANSMITTED PARASITES

BY

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RUNNING HEAD: Freshwater Snails in Kenya

ABSTRACT

From 1987-1990, freshwater snails were collected from 210 natural and 8 artificial habitats in the southern half of Kenya. From these habitats 19 pulmonate and 15 prosobranch taxa were collected. Of these habitats, 68.8% contained snails capable of transmitting human schistosomiasis and/or fascioliasis. The distributions of most prominent snail taxa were confined to characteristic altitudinal ranges in Kenya, suggesting that temperature plays an important direct or indirect role in controlling distributions. Prosobranch taxa dominated in habitats below 300 meters whereas habitats above this elevation contained more pulmonate taxa. Most taxa occupied a diversity of different habitats, suggesting they are not strict habitat specialists. Apart from Lake Victoria which supports a relatively diverse fauna, typical freshwater habitats in this region contained between two and four gastropod species. Coefficients of association were calculated for pairs of prominent taxa, most of which were not significant. Of particular interest were species considered to have some potential as biological control agents of pulmonate hosts of medically significant trematode parasites. Included among such putative control agents in Kenya are Helisoma sp., Melanoides tuberculata (Muller 1774), and the ampullariid snails Pila ovata (Olivier 1804), Lanistes carinatus (Olivier 1804), and Lanistes ovum Peters 1845. Helisoma sp. was found only in artificial habitats, and M. tuberculata and L. ovum had no significant negative associations with parasite-transmitting pulmonates, whereas both L. carinatus and P. ovata did. The role of competitor/predator snails in biological control of pulmonate snails in Kenya is discussed.

# Appendix 11
# In press: Biological Journal of the Linnean Society

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TITLE: Phylogenetic analysis of eleven species of Biomphalaria Preston, 1910 (Gastropoda: Planorbidae) based on comparisons of allozymes.

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SUBMITTED TO: Biological Journal of the Linnean Society

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HIERARCHY OF HEADINGS:

INTRODUCTION

MATERIALS AND METHODS

- Specimens
- Electrophoresis
- Analysis

RESULTS

DISCUSSION

- Phylogenetic analysis
- Coevolution with *S. mansoni*
- Biogeography

Phylogenetic analysis of eleven species of Biomphalaria Preston, 1910 (Gastropoda: Planorbidae) based on comparisons of allozymes.

by

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RUNNING HEAD: PHYLOGENETIC ANALYSIS OF BIOMPHALARIA



ABSTRACT

Freshwater snails in the genus Biomphalaria transmit Schistosoma mansoni in Africa, South America, and the Caribbean region. Although considerable attention has been given to the identification of species, little is known of evolutionary relationships among the species. A phylogenetic analysis of 25 populations representing 11 species was performed on 25 enzyme loci examined using starch gel electrophoresis. A phylogenetic analysis of the individual populations produced 60 trees of equal length. The 60 trees have a consistency index value of 75.9%, a retention index value of 76.5%, and a homoplasy slope ratio value of 5%. The phylogenetic analysis provided strong support for the monophyly of Biomphalaria with either 14 or 15 synapomorphies uniting all of the species included and separating them from the outgroup, two species of Helisoma. Four nominal species represented by multiple populations formed monophyletic groups. Populations of B. sudanica, B. choanomphala, and B. alexandrina were interspersed. Ten arrangements were obtained for the populations of these three species. A variety of ingroup taxa were used to root the tree, and all provided support for the use of Helisoma species as an outgroup. In all of the trees obtained, the African species together formed a monophyletic group. In none of the trees obtained did the neotropical species form a monophyletic group. A constrained analysis requiring the monophyly of the neotopical species as well as the African species resulted in 90 trees just two steps longer than the shortest trees. Analysis of the species from either hemisphere alone resulted in decreased resolution, as measured by an increase in the number of trees obtained. This finding suggests that further comparisons of species from the two hemispheres will be of considerable value. Finally, two species which are resistant to infection with S. mansoni were included among the eleven studied. Neither of these species formed the sister group to the other species included, indicating that susceptibility is the plesiomorphic state, and that resistance is

derived. Similarly, in none of the trees obtained did the two resistant species fall out as sister taxa, indicating that resistance arose independently twice.





Freshwater snails belonging to the genus Biomphalaria transmit Schistosoma mansoni in Africa and the neotropical region (Malek, 1980). Because not all species of Biomphalaria play an equal role in the transmission of S. mansoni (Malek, 1985), considerable attention has been given to the systematic relationships among the species. The emphasis of such efforts has been on the recognition of species, determination of susceptibility, and mapping of the geographic distributions of susceptible species (Mandahl-Barth, 1958; Barbosa et al., 1968; Paraense, 1975; Brown, 1980; Malek, 1985). Comparatively little attention has been given to evolutionary relationships, coevolution with digenetic trematodes, or historical biogeography.

Members of the genus Biomphalaria are widely distributed in South America and the Carihbean region, and also occur in Central America and the southern U.S. (Malek, 1985). Species of Biomphalaria are also found in most of sub-Saharan Africa as well as northeastern Africa, Madagascar and parts of the Middle East (Brown, 1980). The distribution of Biomphalaria is generally thought to reflect an origin in the Cretaceous or earlier (Davis, 1980; Meier-Brook, 1984; Van Damme, 1984).

Because of the broad geographic distribution of the genus Biomphalaria, taxonomic research on these snails has generally been regional in focus. For example, most of the neotropical species have been described by Paraense (e.g., 1975) or Malek (1985), and most of the African species have been studied by Mandahl-Barth (1958) and Brown (1980). In general, the African species are identified on the basis of conchological features, and the neotropical species on the basis of the soft anatomy and reproductive compatibility. However, the approach taken by each of these authors is unique. The consequence of this regional specialization is that the classifications produced are not comparable. The formulation of a hypothesis of evolutionary relationship for species belonging to the genus Biomphalaria will require the development of a common database.

The research reported herein represents the most comprehensive study to date of species from the two hemispheres. Few studies to date have compared species from different regions, and none have placed their findings in a phylogenetic context. Little is known at present of the evolutionary relationships among species of Biomphalaria. Previous studies of species of Biomphalaria employing allozymes (Mulvey & Vrijenhoek 1981a, 1981b, 1982; Woodruff et al., 1985; Mimpfoundi et al., 1986; Jelnes, 1987; Mulvey et al., 1988; Bandoni et al., 1990; Mimpfoundi & Greer, 1990a, 1990b) suggested that the use of allozymes would represent an effective and efficient means of developing a comparable database for species from the different regions.

The choice of starch gel electrophoresis was based on widespread acceptance of the technique in systematics (Richardson et al., 1986; Hillis & Moritz, 1990), in spite of some well documented limitations. Strengths of the technique include the speed and ease with which information can be gathered, the relatively large amount of genetic information that can be sampled, the large number of individuals that can be studied, and the very large number of studies to which our data can be compared, including studies of other freshwater molluscs. Potential weaknesses include the problem of hidden heterogeneity, the limited span of evolutionary time over which the technique is effective, and the indirect nature of the comparison of the molecules.

Eleven species of the genus Biomphalaria have been examined using starch gel electrophoresis. Seven neotropical and four African species were studied, including the most important hosts for S. mansoni from both hemispheres. Comparisons of intraspecific and interspecific patterns of variation are presented elsewhere (Bandoni, 1991; Bandoni et al., in preparation). Presented herein is a phylogenetic analysis of the electrophoretic data. In this initial attempt to reconstruct the phylogenetic relationships among species of Biomphalaria, the following issues were investigated. (1) Do species of Biomphalaria form a monophyletic group? (2) How are species of Biomphalaria related? (3) Do the African and neotropical species form monophyletic groups? (4) Do the resistant and susceptible species form monophyletic groups? Because so little is known of the evolutionary relationships among species of Biomphalaria, the present study should be viewed as providing a framework for further studies of the genus.

## MATERIALS AND METHODS

Specimens:

Specimens were either taken from aquarium stocks or collected in the field. Sources are listed in Table 1. Aquarium stocks and field populations do not differ markedly in levels of genetic variation, thereby justifying the use of both in a single study (Mulvey & Vrijenhoek, 1981b, 1982). Specimens were collected using a dip net, rinsed free of vegetation, and either wrapped in foil and frozen in liquid nitrogen, or transported back to the laboratory and maintained in aquaria until prepared for shipping. Snails were either shipped live wrapped in damp gauze or paper towels, or frozen with dry ice.

Representatives of two species of Helisoma were included as an outgroup in the phylogenetic analysis. In addition, a laboratory stock of Drepanotrema cimex Moricand, 1839 and Bulinus tropicus/truncatus from Kenya were also used for comparison.

Voucher specimens for all but one of the field populations of species of Biomphalaria and Helisoma have been prepared and deposited in the collections of the Academy of Natural Sciences of Philadelphia. Specimens of B. obstructa were obtained from a site where no other species of Biomphalaria are known to occur, and were identified by Dr. Emile Malek of Tulane University, who has published the most complete descriptions of this species (Malek, 1969, 1985).

Electrophoresis:

Electrophoretic protocols were similar to those described by Mulvey et al., (1988) and Murphy et al., (1990). Enzymes examined and gel conditions are listed in Table 2. Whole snails were homogenized either with or without the shell, and centrifuged for 20 seconds. Extensive studies of allozyme variation in six species of Biomphalaria enabled recognition of bands attributable to other organisms such as digenetic trematodes. Such additional bands did not interfere with scoring of the gels. A relatively small number of enzymes could be analyzed because of the small size of many of the specimens available. Loci that were shown to be either invariant or highly variable in our previous studies of Biomphalaria were not used (Mulvey & Vrijenhoek, 1981a, 1981b, 1982; Woodruff et al., 1985; Mimpfoundi et al., 1986; Bandoni et al., 1990). Twenty-five loci could be scored reliably in all or nearly all populations of each of the eleven species.

Individuals of many Biomphalaria species are quite small, and contain relatively low levels of enzyme activity, necessitating some modifications of the design of the study from that usually employed in electrophoretic comparisons of different species (Richardson et al., 1986). Several comparative combinations of taxa were run per gel, as some loci could not be scored reliably in samples refrozen and rerun a second day. Larger sample sizes per population were therefore needed in order to make these comparisons. Our sample sizes per population were therefore in the range recommended by Archie et al., (1989), and larger than the minimum sample sizes suggested by Gorman and Renzi (1979). Sample sizes per locus vary from 2 to 57 with an average of 17.7, because of variation in the sizes of the animals, the quality of preservation of the tissue, and the difficulties of interpreting particular loci.

Samples of the Salvadoran and the M-line (Newton, 1955) stocks of B. glabrata were run as marker populations. Three to five individuals of one or both stocks were



run per gel to ensure that variation within the marker stocks did not interfere with the interpretation of the gel. All gels except those stained for carbonate dehydratase, a fluorescent stain, were photographed, and scores were confirmed from black and white prints.

In synonymizing alleles, the following conventions were adopted. If populations 1 and 2 shared their most common allele on one gel, and populations 1 and 3 shared their most common allele on another, then populations 2 and 3 were assumed to also share the same allele. Alphabetic designations were assigned beginning with the most anodal allele. The mobility of each allele was measured relative to the marker stocks of B. glabrata. Relative mobility ranges overlapped for some alleles for a few loci, so the order was taken directly from their relative order on the gels whenever possible. Although the protocol used allowed most alleles to be observed together on one or more occasions, some very rare alleles of similar relative mobility were not seen side by side or with a common intermediate. These low frequency alleles were given unique designations for each species, so as to reflect their uncertain relationship to other alleles. Although this procedure would elevate estimates of divergence among the species, the effect on the phylogenetic analysis should be small in comparison with the potential effects of lumping alleles that are not the same.

Analysis:

Phylogenetic analyses were performed using PAUP (versions 2.4.1 and 3.0; Swofford, 1984, 1990). Because of the large size of the data set, heuristic methods were used for tree construction rather than an exhaustive search. Branch lengths with maximum lengths of zero were collapsed. Trees were rooted using a composite outgroup encompassing two species of Helisoma.

Phylogenetic analyses were carried out using 'locus as character' coding as described by Mickevich & Mitter (1983). Each locus was considered a homologous

series, and each distinct allele profile (consisting of one or more alleles) was considered a character state and assigned a unique code. Electrophoretic profiles were identified using the qualitative approach of Mickevich & Mitter (1983). All alleles present in two or more copies were included in the electrophoretic profiles. For the average sample size of 17.7 individuals, this criterion corresponds to a frequency of 0.05, as is used in many phylogenetic studies (Mickevich & Mitter, 1981, 1983). Allele profiles for each population are presented in Table 3, and coded allele profiles are presented in Table 4.

After considering several proposed methods of ordering characters (Mickevich & Mitter, 1983), we have chosen the conservative approach advocated by Miyamoto (1986). All loci were treated as unordered homologous series, i.e., each character state was assumed to be potentially equally related to each (or all) of the others. Although the derived states were unordered, nine of 25 characters were polarized by the addition of an outgroup. The resulting trees will therefore represent the suites of relationships with which the majority of homologous series are consistent. This approach makes the fewest assumptions about the nature of evolution of allele profiles. In addition, this method is least likely to produce clades supported by false synonymies because the profiles of uncertain affinity are generally assigned unique code.

The goodness of fit of the data to the phylogenetic trees was measured using the consistency index (Kluge & Farris, 1969), and the retention index (Farris, 1989). The homoplasy slope ratio (Meier et al., 1991) was used to evaluate the level of homoplasy present in the data set relative to levels present in randomly generated data matrices of comparable size.

## RESULTS

The phylogenetic analysis produced 60 equally parsimonious trees, each with a consistency index of 75.9%, a retention index of 76.5%, and a homoplasy slope ratio of 0.05. A Nelson consensus tree for the sixty trees obtained is presented in Figure 1. The sixty trees all represent distinct topologies, i.e., all internal branches have maximum lengths greater than zero. There were ten arrangements of the populations of only three species, B. alexandrina, B. choanomphala and B. sudanica, and six arrangements of all of the remaining taxa yielding a total of 60 trees. The six major arrangements of the taxa are depicted in Figure 2, with the relationships among B. alexandrina, B. choanomphala and B. sudanica held constant. The six major arrangements result from two resolutions of the basal polytomy including B. peregrina and B. tenagophila, and three resolutions of the polytomy including B. intermedia, B. straminea and B. amazonica.

The present analysis supported the monophyly of the genus Biomphalaria, with either 14 or 15 synapomorphies uniting the eleven species included. Samples of Drepanotrema and Bulinus included exhibited radically different electrophoretic profiles, differing from Biomphalaria in either the number of loci obtained or the direction of migration (i.e., anodal versus cathodal) for some enzyme systems. The two species of Helisoma included exhibited allele profiles similar to those of the Biomphalaria species and so were used as an outgroup. Even for H. duryi and H. trivolvis, some enzyme systems could not be resolved, most probably because the buffer conditions required for activity were markedly different from those required by the species of Biomphalaria.

Because the outgroup employed was quite different from the ingroup, the trees were also rooted with ingroup taxa, including B. glabrata, B. peregrina, and B. tenagophila. These analyses produced a minimum of 80 topologies, indicating a decrease in resolution.

Six species included were represented by multiple populations. In four of these, B. glabrata, B. pfeifferi, B. straminea and B. tenagophila, the individual populations representing each nominal species formed clades. The patterns of relationships observed for the populations within these four species were also constant in all of the trees obtained. The two populations of B. choanomphala included also formed a monophyletic group, but these populations were nested within the populations of B. sudanica, which did not form a monophyletic group. Similarly, the sole population of B. alexandrina included also fell out among the populations of B. sudanica. In all of the trees obtained, the African species formed a clade but the neotropical species were paraphyletic.

Analysis of the neotropical species alone produced 57 distinct trees of equal length, each with a consistency index of 81.9% and a retention index value of 70.0%. Analysis of the African species produced 180 equally parsimonious trees, with consistency indices of 88.6% and retention indices of 82.2%. Analysis of the species from either hemisphere alone therefore resulted in decreased resolution. A constrained analysis in which the neotropical species were required to form a monophyletic group produced 90 equally parsimonious trees that were two steps longer than the minimum length trees (CI = 75.0, RI = 75.4).

## DISCUSSION

### Phylogenetic Analysis:

The phylogenetic analysis produced sixty equally parsimonious trees. In all of the trees obtained, the African species formed a monophyletic group, but the neotropical species did not. However, support for this pattern of relationships was only slightly better than for trees in which the neotropical species do form a monophyletic group. Inclusion of two species of Helisoma (a nearctic genus) as a outgroup contributed significantly to the resolution obtained, and supported the



monophyly of the group of eleven species of Biomphalaria studied. Separate analyses of African and neotropical species resulted in considerably less resolved phylogenetic relationships, indicating that the species from the two regions can be studied more profitably together than apart.

The sixty phylogenetic trees obtained are presented in Figure 1. Some caution should be used in interpeting the consensus tree, as consensus trees contain less information than do the original trees from which they were constructed (Miyamoto, 1985). The consensus tree may also be consistent with additional trees not supported by the data (Miyamoto, 1985).

The consistency index value for the 60 trees obtained was 75.9%, indicating a fairly low level of homoplasy (convergence, parallelisms or reversals) overall. The relatively high value of the retention index (76.5%) indicated a low level of homoplasy relative to the maximum amount of homoplasy possible for this data set. The homoplasy slope ratio (6%) indicates that the data set contains only a small fraction of the homoplasy present in random data matrices of comparable size.

The total of sixty trees obtained resulted from the combination of ten arrangements of the populations of B. alexandrina, B. choanomphala, and B. sudanica and six arrangements of the remaining eight species. There are a number of stable elements in the pattern of relationships in spite of the apparent profusion of trees. Some subsets of taxa (e.g., populations of tenagophila, straminea, glabrata, and pfeifferi), if considered alone, exhibit the same pattern of relationship among all of the 60 trees. Although the relationships among the populations of B. alexandrina, B. choanomphala, and B. sudanica are quite variable, these species collectively form a monophyletic group in all of the trees obtained (Figs. 1, 2). Finally, in all of the trees obtained, the African species included formed a monophyletic group, but the neotropical species did not. Thus, certain patterns of relationship among the taxa are constant and well supported.

Character state distributions were examined in detail for the six phylogenetic trees presented in Figure 2. Some internal branches grouping together populations of the same nominal species were supported by homoplasious character states alone. Internal branches grouping together populations of two or more species were all supported by non-homoplasious character states. Thus, the patterns of relationships among species are more strongly supported than are the relationships among populations within the nominal species.

Relatively few characteristics unambiguously unite the species of Biomphalaria, and still fewer can be unambiguously interpreted as synapomorphies. The present analysis provided strong support for the monophyly of the genus Biomphalaria, as either 14 or 15 synapomorphies unite the species of Biomphalaria and separate them from the outgroup.

Because outgroups are used to provide a context for the evaluation of the direction of transformation of a particular characteristic, the selection of an appropriate outgroup is an important step in a phylogenetic analysis. The choice of outgroups was based on the revisions of Hubendick (1955, 1978), who suggested first that Biomphalaria and Helisoma were closely related, and later that Biomphalaria and Drepanotrema were closely related instead. The genus Bulinus was not considered to be a close relative of Biomphalaria by Hubendick (1955, 1978). However, species of Bulinus serve as intermediate hosts for Schistosoma haematobium, another schistosome infecting humans, suggesting an affinity based on parasitological evidence.

The present study suggests that the genera Helisoma and Biomphalaria are more closely related to each other than to either Bulinus or Drepanotrema. However, the number of loci for which character states are shared by the species of Biomphalaria and Helisoma is quite small (9 of 25 loci), and the choice of outgroups therefore must be considered provisional.

Ideally, two or more outgroups which are paraphyletic in relation both to each other and to the study group should be included in a phylogenetic analysis (Madison et al., 1984). Our intention in choosing representatives of three other genera to include in our anlaysis was to provide a paraphyletic outgroup. The need for side by side comparison of alleles in starch electrophoresis makes the addition of new taxa difficult. Hence, additional outgroups could not be examined after the completion of the study. Thus, the present analysis is rooted with a single monophyletic outgroup.

Because the two species of Helisoma included shared relatively few alleles with the ingroup taxa, a variety of rooting options were employed in order to evaluate the contributions of the outgroup to the outcome of the phylogenetic analysis. Three ingroup taxa were used to root the tree: B. glabrata, B. peregrina, and B. tenagophila. The latter two species were chosen because they appear as sister taxa to the remaining species in the trees obtained here (Figs. 1, 2). Although the present analysis suggests that B. glabrata is a fairly derived lineage, this species possesses some morphological attributes, such as the renal ridge, which appear to be plesiomorphic. In all three cases, the number of trees obtained increased. The increase in the number of trees obtained with ingroup rooting indicates that there is a substantial decrease in the resolution in the absence of the two species of Helisoma. However, because the two species of Helisoma included shared relatively few alleles with the study group, the possibility remains that another planorbid taxon might be preferable. Further phylogenetic studies of the Planorbidae are needed in order to ascertain the position of Biomphalaria among the other genera and to identify suitable outgroup taxa.

All of the African species included in the analysis form a monophyletic group. The particular species included were chosen as representatives of the four species groups recognized by Mandahl-Barth (1958). If the four species groups are truly representative of the diversity among the African species, then this would suggest

that these form a monophyletic group. Further work will be required to establish the validity of Mandahl-Barth's species group.

Based on the geographic distribution and fossil record for Biomphalaria, Van Damme (1984) proposed that the diversification within the genus was tied to two groups of tectonic events, the breakup of Gondwana (and perhaps also Pangaea), and the formation of the East African rifts. The rift lakes are espcially well known for the large numbers of endemic molluscan taxa, but additional endemics are known from other lakes as well as other freshwater habitats of the region (Van Damme, 1984). Often, the closest relatives outside of the rift valley region are found in the Nile (Van Damme, 1984). The pattern observed here for Biomphalaria is generally consistent with these observations, as B. choanomphala and B. sudanica, both endemic to the rift valley region, together with B. alexandrina, a nilotic species, represent a highly derived lineage within the genus.

Several factors may be contributing to the variation in the patterns of relationship obtained for the populations of B. alexandrina, B. choanomphala, and B. sudanica. First, problems with the species boundaries may be present, and one or more of these species, particularly B. sudanica, may not be monophyletic. These three species are delineated exclusively on the basis of conchological variation. Variation in the form of the shell does not seem to be related in a simple way to genetic variation in many species of pulmonate snails, including Cerion (Woodruff & Gould, 1980), Samoana (Johnson et al., 1981a), Partula (Johnson et al., 1981b), and Lymnaea peregra (Evans, 1989).

The poor resolution obtained for the relationships among B. alexandrina, B. choanomphala, and B. sudanica could also result from unrecognized homoplasies (i.e., character states presently interpreted as synapomorphies that are actually poor indicators of phylogenetic relationships). Of particular concern here would be identical electrophoretic profiles arising through the independent loss of alleles. For



example, the profiles 'AB' and 'BC' could both give rise to the profile 'B,' but there would be little hope of recognizing this.

An additional possibility is that the poorer resolution obtained or the African species is an artifact of the sampling design. A greater proportion of the African material was derived from field populations, and for the East African species, a smaller geographic scale is represented. No differences in the levels of heterozygosity or polymorphism were detected for laboratory versus field populations, indicating that bottlenecks in the histories of lab stocks are not creating artificially low levels of variation and thus comparatively higher resolution (Bandoni, 1991; Bandoni et al., in preparation). The effects of more intensive sampling are more difficult to identify, and must be investigated empirically.

Relationships among four of the seven neotropical species (B. glabrata, B. intermedia, B. straminea, and B. tenagophila) were constant. Positions of the three remaining species varied, resulting in the six major arrangements (Figure 2). All of the species that varied in position were represented by single populations, whereas four of five of the constant species were represented by multiple populations. Although B. sudanica is apparently an exception, this observation suggests that the inclusion of multiple populations of each species has a stabilizing effect on the analysis, perhaps because the range of variation within a species is more adequately reflected.

In all three of the neotropical species represented by multiple populations, the populations constituting each nominal species formed monophyletic groups. These species are currently recognized on the basis of characteristics of the soft anatomy, and patterns of reproductive compatibility. The monophyly of these species in the present analysis suggests greater congruence of the electrophoretic data with the soft anatomy of the neotropical species than with the shells of the African species.

Two of the neotropical species which vary in position exhibit anomalous patterns of variation which may contribute to their uncertain placement in the phylogenetic tree. Specimens of B. peregrina included exhibited high levels of genetic polymorphism, a large number of unique alleles, and additional peptidase loci. Biomphalaria intermedia, which is of uncertain relationship to B. straminea (see Fig. 2), is so named because of its morphological intermediacy between B. straminea and B. peregrina (Paraense & DesLandes, 1966). This species exhibits considerable plasticity in form, for example possessing either a vaginal pouch or vaginal corrugations or both. Hybridization (reticulate evolution) would be one possible explanation for such morphological and electrophoretic anomalies, and would also represent a possible explanation for the instability and or uncertainty of the placement of particular taxa in the phylogenetic tree (Bremer & Wanntorp, 1979; Woodruff, 1989).

The core patterns of relationships obtained (Figure 2) suggest that the neotropical species included diverged earlier than did the African species included. It is somewhat unlikely that each of the neotropical species represents a single undivided lineage. The present analysis may instead have provided too few characters to resolve the relationships among the neotropical species, perhaps because these species fall into an age range at which starch gel electrophoresis provides more limited data for phylogenetic reconstruction. Support for this assertion comes from the fact that the earliest and latest divergences appear least stable.

The phylogenetic relationships were also estimated separately for the African and neotropical species. A phylogenetic analysis of just the neotropical species produced 57 trees of equal length. Analysis of the African species alone produced 180 trees of equal length. Consistency and retention indices obtained in these analyses were higher than those of the primary analysis, presented in Figures 1 and 2. Higher consistency and retention indices reflect a loss of resolution rather than an improved fit. This results from the interpretation of larger numbers of character states as being uniquely derived from the plesiomorphic state. Resulting character transformations are are likely to be uninformative with respect to relationships, yielding in poorly resolved trees, but completely consistent.

The number of possible trees in a phylogenetic analysis increases as a function of the number of taxa; a decrease in the number of taxa therefore implies a decrease in the number of trees possible. The efforts to estimate the phylogenetic relationships among species of Biomphalaria were clearly more successful when species from both hemispheres were included, based on the smaller number of trees with which the data are consistent. These findings suggest that the African and neotropical members of the genus may be more profitably studied together than in isolation.

The results obtained when the analysis was constrained so as to require the monophyly of the neotropical species indicate that there is only slightly more support for the trees summarized in Figure 1 than for a tree in which the neotropical species form a monophyletic group. Additional data could therefore easily shift the balance in favor of the latter hypothesis of relationship. However, the morphological observations of Mello (1972) on the same African species as were studied here suggest that these four African species are relatively conservative in comparison with the morphological diversity seen in the neotropical region. It is possible that the African species included could represent a single, comparatively recent lineage. Other African species, particularly those of western Africa, are very poorly known in comparison with those included here; these may prove to play a pivotal role in understanding the phylogenetic relationships within the genus.

Biogeography:

Two biogeographic scenarios emphasizing vicariant events can be envisioned for the genus Biomphalaria. First, the splitting of South America and Africa could have divided the genus into two major clades, which then underwent subsequent divergence. Under this scenario, the closest relatives of any given species would then be found on the same continent. The alternative scenario is that some divergence could already have taken place within the genus prior to the separation of South America and Africa, in which case the closest relatives of a given species may not occur on the same continent. Finally, Davis (1982) has suggested that vicariance may play only a small role in the origins of the geographic distributions of freshwater pulmonate snails, with chance long distance dispersal playing a major role. Dispersal via human activities has also been suggested (Bequaert, & Lucena, 1951). The investigation of the relative contributions of vicariance and dispersal will require the construction of a well supported phylogenetic tree including more species (Wiley, 1988).

In the present analysis, the African species included appear to form a monophyletic group more recent in origin than many of the South American species. The South American species are also separated by larger genetic distances than are the African species, suggesting a greater degree of anagenetic divergence (Bandoni, 1991). As noted above, it would seem unlikely that each of the neotropical species forms a single undivided lineage. Explanations for the pattern obtained would include the possibility that other members of each of these clades were not included in the analysis. There is presently little evidence with which to rule out the possibility that some African species are more related to South American species than to other African species. Similarly, the results of the constrained analysis suggest that additional characters may shift the balance in favor of neotropical and African clades. Clearly, additional characters, including both comparative anatomy and



molecular studies, will be needed to provide a stronger hypothesis of relationship within the genus.

Coevolution with Schistosoma mansoni:

Two of the species included in the phylogenetic analysis, B. intermedia and B. obstructa, have never been found infected with S. mansoni in the field, and have not been successfully infected in the laboratory (Malek, 1985). In none of the major arrangements obtained (Fig. 2) do either of these species form the sister group to the remaining species. Thus, susceptibility to infection with S. mansoni appears to be the plesiomorphic state for the genus. Similarly, in none of the six major arrangements (Fig. 3) are B. intermedia and B. obstructa placed together as sister taxa. The available evidence thus points to the independent origin of resistance/insusceptibility in these two species.

Not all species of Biomphalaria transmit S. mansoni, and a well supported phylogeny will be needed to examine historical patterns in the association of Biomphalaria species with schistosomes. Phylogenetic analyses increasingly are being used to examine the direction of character transformation as a means of evaluating hypotheses concerning particular evolutionary processes (Ridley, 1983; Coddington, 1988; Donoghue, 1989). The evolution of susceptibility and resistance to infection with schistosomes among species of Biomphalaria may be examined using a phylogenetic framework. It will be possible to investigate whether species of Biomphalaria are primitively susceptible or unsuitable for infection with S. mansoni, whether susceptible and resistant species each form monophyletic groups, and what is the minimum number of transitions from susceptibility to resistance and vice versa that may have occurred.

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Table 1. List of specimens, abbreviations used, sources, and original localities. Institutional affiliations of individuals providing s are listed in the acknowledgements. Localities listed are collection sites for field populations, and sites of the original field sourc laboratory stocks.

| Species | Abbreviation | Source | Lab/Field | Locality |
|---|---|---|---|---|
| B. alexandrina (Ehrenberg, 1831) | ALEX | Bruce | L | Egypt, orginal locality unknown |
| B. amazonica Paraense, 1966 | AMAZ | Paraense | L | Barao de Melgaço, Brazil (16°13'S, 55°58'W) |
| B. choanomphala (Martens, 1879) | CHOA1 | Loker | F | Rusinga Island, Kenya (0°25'S, 34°12'E) |
| B. choanomphala (Martens, 1879) | CHOA2 | Loker | F | Karungu Bay, Kenya (0°50'S, 34°10'E) |
| B. glabrata (Say, 1818) | GLAB1 | Lewis | L | Belo Horizonte, Brazil (19°54'S, 34°54'W) |
| B. glabrata (Say, 1818) | GLAB2 | Dominguez | F | Jurubocoa, Dominican Republic (19°9N, 70°39'W) |
| B. glabrata (Say, 1818) | GLAB3 | Paraense | L | Salvador, Brazil (12°58'S, 38°29'W) |
| B. intermedia (Paraense & Deslandes, 1962) | INTE | Paraense | L | Parapuã, Brazil (21°47'S, 50°50'W) |
| B. obstructa (Morelet, 1849) | OBST | Malek | F | New Orleans, USA (30°0'N, 90°3'W) |
| B. peregrina (Orbigny, 1835) | PERE | Paraense | L | Salto, Uruguay (31°23'S, 57°58'W) |
| B. pfeifferi (Krauss, 1848) | PFEI1 | Mimpfoundi | F | Yaounde, Cameroon (3°51'N, 11°31'E) |
| B. pfeifferi (Krauss, 1848) | PFEI2 | Loker | F | Mwea, Kenya (0°40'S, 37°10'E) |
| B. pfeifferi (Krauss, 1848) | PFEI3 | Loker | F | Churo Pond, Kenya (0°47'S, 37°10'E) |
| B. pfeifferi (Krauss, 1848) | PFEI4 | Loker | F | Chebwonyo Dam, Kenya (0°58'S, 35°11'E) |
| B. straminea (Dunker, 1848) | STRA1 | Paraense | L | Belem, Brazil (1°27'S, 38°54'W) |
| B. straminea (Dunker, 1848) | STRA2 | Paraense | L | Cajazeira, Brazil (6°54'S, 35°11'E) |
| B. straminea (Dunker, 1848) | STRA3 | Paraense | L | Paracatu, Brazil (17°13'S, 38°54'W) |
| B. sudanica (Martens, 1870) | SUDA1 | Loker | F | ADC Farm, Kenya (0°20'S, 34°39'E) |
| B. sudanica (Martens, 1870) | SUDA2 | Loker | F | Kendu Bay, Kenya (0°20'S, 35°11'E) |
| B. sudanica (Martens, 1870) | SUDA3 | Loker | F | Rusinga Island, Kenya (0°50'S, 34°12'E) |
| B. sudanica (Martens, 1870) | SUDA4 | Loker | F | Chebwonyo Dam, Kenya (0°58'S, 35°11'E) |
| B. sudanica (Martens, 1870) | SUDA5 | Loker | F | Karungu Bay, Kenya (0°50'S, 34°10'E) |
| B. tenagophila (Orbigny, 1834) | TENA1 | Paraense | L | Asunción, Paraguay (26°26'S, 57°40'W) |
| B. tenagophila (Orbigny, 1834) | TENA2 | Bandoni | F | Niteroi, Brazil (22°54'S, 43°06'W) |
| B. tenagophila (Orbigny, 1834) | TENA3 | Bandoni | F | Rio de Janeiro, Brazil (25°55'S, 43°30'W) |
| Helisoma duryi (Wetherby, 1879) | · | Loker | F | Bamburi, Kenya (3°59'S, 39°43'E) |
| Helisoma trivolvis (Say, 1817) | · | Bandoni | F | Par Pond, SC, USA (33°14'N, 81°32'W) |

Table 2. Enzymes examined. List of enzymes examined, with Enzyme Commission numbers, number of loci scored, and gel conditions. In some cases, the number of loci listed is the number scored, not the number obtained. Buffers are as follows: (1) Amino propylmorpholine citrate, pH 6.0 (Clayton & Tretiak, 1972), (2) Tris citrate EDTA, pH 7.0-7.1 (Ayala et al, 1972), (3) Tris-EDTA-borate, (Selander et al., 1971), (4) Lithium hydroxide, pH 8.2/8.4 (Selander et al, 1971), and (5) Poulik discontinuous tris-citrate/borate, pH 8.2/8.7 (Selander et al., 1971).

| Enzyme | Abbreviation | E. C. number | No. of loci | Gel type |
|---|---|---|---|---|
| Aconitate hydratase | ACON | 4.2.1.3 | 2 | 1 |
| Alkaline phosphatase | AKP | 3.1.3.1 | 1 | 2 |
| Aspartate amino transferase | AAT | 2.6.1.1. | 2 | 3 |
| Carbonate dehydratase | CA | 4.2.1.1 | 1 | 3 |
| Catalase | CAT | 1.11.1.6 | 1 | 2 |
| Creatine kinase | CK | 2.7.3.2 | 1 | 1 |
| Carboxylesterase (α-naphthyl acetate) | EST | 3.1.1.- | 1 | 3,4 |
| Glycerol-3-phosphate dehydrogenase | GPD | 1.1.1.8 | 1 | 1 |
| Glucose-6-phosphate dehydrogenase | PGD | 1.1.1.44 | 1 | 1 |
| Glutathione reductase (NAD(P)H) | GR | 1.6.4.2 | 1 | 5 |
| 3-Hydroxybutyrate dehydrogenase | HBDH | 1.1.1.30 | 1 | 5 |
| Isocitrate dehydrogenase | IDH | 1.1.1.42 | 1 | 2 |
| L-Lactate dehydrogenase | LDH | 1.1.1.27 | 1 | 2 |
| Malate dehydrogenase | MDH | 1.1.1.37 | 2 | 1 |
| Purine nucleoside phosphorylase | NSP | 2.4.2.1 | 1 | 3 |
| Peptidase | PEP | 3.4.-.- | | |
| (glycylleucine) | PEP-GL | | 1 | 4 |
| (leucylglycylglycine) | PEP-LGG | | 1 | 4 |
| (phenylalanylproline) | PEP-PP | | 1 | 4 |
| Phosphoglucoseisomerase | PGI | 5.3.1.9 | 1 | 5 |
| Phosphoglucomutase | PGM | 5.4.2.2. | 1 | 1 |
| Xanthine dehydrogenase | XDH | 1.1.1.204 | 1 | 3 |

Table 3. Electrophoretic profiles for all 25 loci and all 25 populations of Biomphalaria, and the composite outgroup. Asterisks indicate alleles present in only one copy.

| | Aat-1 | Aat-2 | Acon-1 | Acon-2 | Akp | CA | Cat | CK | Est-2 | Gapdh | Gpd | GR | Hbdh |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ALEX | B | D | E | BC | B | D | C | E | B | D | A | B | E |
| AMAZ | B | A | D | B | B | A | BC | BC | B | C | A | C | C |
| CHOA1 | B | D | EF | CD | BC | D | BC | DE | B | D | A | ABD | E |
| CHOA2 | B | D | EF | C | BC | D | BC | DE | B | D | A | B | E |
| GLAB1 | E | C | D | C | B | D | BC | BC | B | D | A | A | CD |
| GLAB2 | E | C | F | C | B | - | C | C | C | - | A | C | A |
| GLAB3 | E | C | DEF | CD | B | DF | BC | BC | C | F | A | C | CD |
| INTE | B | C | EF | C | B | B | BC | BC | B*C | C | A | C | E |
| OBST | C | C | E | C | B | C | BC | C | BC | C | A | F | E |
| PERE | BE | C | E | B | B | C | BC | G | C | G | B | E | EF |
| PFEI1 | E | C | E | B | C | F | B*D | D | B | D | A | B | E |
| PFEI2 | B | C | E | B | B | F | BC | D | B | D | A | D | E |
| PFEI3 | B | C | E | C | B | F | BC | D | B | D | A | DF* | E |
| PFEI4 | BC | A | EG | C | C | F | BC | A | B | D | D | E | B |
| STRA1 | BE | C | EF | B | B | AB | ABC | BC | C | F | A | BC* | F |
| STRA2 | BE | C | EF | B | B | AB | ABC | BC | C | F | A | BC | EF |
| STRA3 | BE | C | F | B | B | ABE | BC | BC | BC | F | A | BC | F |
| SUDA1 | B | D | E | C | B | DF* | BC | DEF | B | D | A | B | E |
| SUDA2 | B | D | E | CD | B | DF* | BC | DEF | B | D | A | BD | E |
| SUDA3 | B | D | E | B*CD | BC | DF | BC | DEF | B | DE | A | BDG* | E |
| SUDA4 | B | D | E | C | B | D | BC | DE | B | D | A | B | E |
| SUDA5 | B | D | E | C | BC | D | BC | DE | B | D | A | B | E |
| TENA1 | B | C | EF | BC | AB | B | BC | BC | BC | C*D | C | B | DE |
| TENA2 | B | C | E | B | AB | B | C | C | C | D | C | B | DF* |
| TENA3 | B | C | EF | BC | AB | B | C | BC | C | D | C | B | D |
| HELI | E | B | ABC | AB | B | C | A | .. | AB | AB | B | C | A |

TABLE 3. Continued.

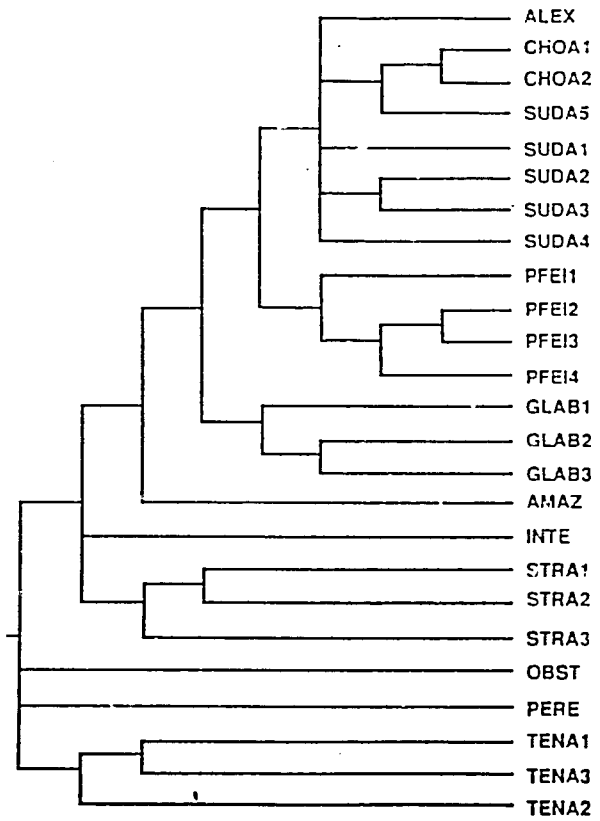
| | Idh | Ldh | Mdh-1 | Mdh-2 | Nsp | Pep-gl | Pep-lgg | Pep-pp | Pgd | Pgi | Pgm-1 | Xdh |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ALEX | E | D | E | C | CD | BC | C | B | FI | C | F | GI |
| AMAZ | EF* | D | E | B | D | B | C | A*B | F | B | G | G |
| CHOA1 | EG | BD | CE | CE* | CDI | BC | CDF* | B | FI | C | A*EH | G |
| CHOA2 | EG | D | E | C | D | BC | CD | B | FI | C | EH | G |
| GLAB1 | E | D | E | C | BC | B | C | BC | F*I | AB | D | CE |
| GLAB2 | C | D | E | C | D | B | C | B | F | B | D | CE |
| GLAB3 | E | D | E | C | D | B | CD* | BC* | F | ABC | F | CE |
| INTE | F | D | E | B | D | B | C*E | B | FH | B | D | F |
| OBST | E | B | B | B | D | BC | C | B | EF | B | CF | E |
| PERE | DF | F | G | D | D | C | B | DE | C*DFG | BC* | BDG | C*EF* |
| PFEI1 | E | B | E | C | C | D | D | B | F | C | A | G |
| PFEI2 | B | D | E | C | C | C | D | B | F | C | AE | I |
| PFEI3 | B | D | E | C | C | C | D | B | F | C | AE | I |
| PFEI4 | D | D | E | C | C | C | C*D | B | F | C | AE | G |
| STRA1 | H | D | E | B | D | B | C | B | FG | AB | G | E |
| STRA2 | H | D | BCE | B | D | B | C | B | F | B | G | EG |
| STRA3 | H | D | E | B | D | B | C | B | F | A*B | G | G |
| SUDA1 | E | D | E | C | DE | BCE | D | B | FI | C | AEH | D*G |
| SUDA2 | E | B | E | C | DE | BC | CD | B | I | C | AEH | G |
| SUDA3 | EG* | D | E | C | C*DE | BC | CD | B | F*I | C | EH* | G |
| SUDA4 | EG* | D | E | C | E | BC | D | B | I | C | E | D |
| SUDA5 | EG | D | E | C | D | BC | CD | B | FI | C | AE | G |
| TENA1 | A | C | B | B | D | B | ACE* | A | D | C | F | CE |
| TENA2 | A | C | B | B | DG | B | AC | AB | D | C | F | C*E |
| TENA3 | A | C | B | B | D | B | ACE | AB | D | C | F | CE |
| HELI | IJ | .. | D | A | A | DF | A | ABD | D | C | | BG |

Table 4. Coded electrophoretic profiles. Question marks indicate missing data. Character states are labelled 0 through 9 plus A for an eleventh character state in Pgm-1. The order of the loci is the same as in Table 3.

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ALEX | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 7 | 1 | 1 | 2 | 1 | 3 | 3 | 1 | 2 | 2 | 2 | 1 | 5 | 2 | 1 | 0 | 1 | 6 |
| AMAZ | 1 | 3 | 6 | 0 | 0 | 4 | 2 | 0 | 1 | 2 | 2 | 0 | 8 | 3 | 1 | 2 | 1 | 1 | 0 | 5 | 2 | 3 | 2 | 5 | 0 |
| CHOA1 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 7 | 3 | 2 | 4 | 4 | 2 | 7 | 1 | 3 | 2 | 1 | 0 | 2 | 0 |
| CHOA2 | 1 | 2• | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 3 | 2 | 1 | 2 | 2 | 1 | 1 | 3 | 2 | 1 | 0 | 2 | 0 |
| GLAB1 | 0 | 1 | 6 | 2 | 0 | 1 | 2 | 0 | 1 | 1 | 2 | 0 | 6 | 3 | 1 | 2 | 2 | 8 | 0 | 5 | 3 | 2 | 3 | 6 | 2 |
| GLAB2 | 0 | 1 | 4 | 2 | 0 | ? | 1 | 1 | 2 | ? | 2 | 0 | 7 | 7 | 1 | 2 | 2 | 1 | 0 | 5 | 2 | 3 | 2 | 6 | 2 |
| GLAB3 | 0 | 1 | 3 | 3 | 0 | 2 | 2 | 0 | 2 | 4 | 2 | 0 | 6 | 3 | 1 | 2 | 2 | 1 | 0 | 5 | 2 | 3 | 1 | 0 | 2 |
| INTE | 1 | 1 | 2 | 0 | 0 | 7 | 2 | 0 | 2 | 2 | 2 | 0 | 3 | 8 | 1 | 2 | 1 | 1 | 0 | 6 | 2 | 7 | 2 | 6 | 7 |
| OBST | 3 | 1 | 1 | 0 | 0 | 0 | 2 | 1 | 3 | 2 | 2 | 6 | 3 | 3 | 2 | 1 | 1 | 1 | 1 | 5 | 2 | 6 | 2 | 7 | 1 |
| PERE | 2 | 1 | 1 | 0 | 0 | 0 | 2 | 6 | 2 | 5 | 0 | 5 | 5 | 6 | 3 | 5 | 3 | 1 | 2 | 7 | 5 | 5 | 2 | 8 | 1 |
| PFEI1 | 1 | 1 | 1 | 2 | 2 | 3 | 4 | 5 | 1 | 1 | 2 | 1 | 3 | 3 | 2 | 2 | 2 | 6 | 3 | 4 | 2 | 3 | 0 | 9 | 0 |
| PFEI2 | 1 | 1 | 1 | 3 | 0 | 3 | 2 | 5 | 1 | 1 | 2 | 4 | 3 | 5 | 1 | 2 | 2 | 6 | 2 | 4 | 2 | 3 | 0 | 1 | 5 |
| PFEI3 | 1 | 1 | 1 | 2 | 0 | 3 | 2 | 5 | 1 | 1 | 2 | 4 | 3 | 5 | 1 | 2 | 2 | 6 | 2 | 4 | 2 | 3 | 0 | 1 | 5 |
| PFEI4 | 1 | 1 | 5 | 2 | 2 | 3 | 2 | 4 | 1 | 1 | 2 | 4 | 3 | 5 | 1 | 2 | 2 | 6 | 2 | 4 | 2 | 3 | 0 | 1 | 0 |
| STRA1 | 2 | 1 | 2 | 0 | 0 | 5 | 3 | 0 | 2 | 4 | 2 | 1 | 4 | 4 | 1 | 2 | 1 | 1 | 0 | 5 | 2 | 4 | 3 | A | 1 |
| STRA2 | 2 | 1 | 2 | 0 | 0 | 5 | 3 | 0 | 2 | 4 | 2 | 3 | 5 | 4 | 1 | 3 | 1 | 1 | 0 | 5 | 2 | 3 | 2 | A | 4 |
| STRA3 | 2 | 1 | 4 | 0 | 0 | 6 | 2 | 0 | 3 | 4 | 2 | 3 | 4 | 4 | 1 | 2 | 1 | 1 | 0 | 5 | 2 | 3 | 2 | A | 0 |
| SUDA1 | 1 | 2 | 1 | 2 | 0 | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 3 | 3 | 1 | 2 | 2 | 5 | 4 | 4 | 2 | 1 | 0 | 3 | 0 |
| SUDA2 | 1 | 2 | 1 | 3 | 0 | 1 | 2 | 3 | 1 | 1 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 5 | 1 | 3 | 2 | 2 | 0 | 3 | 0 |
| SUDA3 | 1 | 2 | 1 | 3 | 1 | 2 | 2 | 3 | 1 | 3 | 2 | 2 | 3 | 3 | 1 | 2 | 2 | 5 | 1 | 3 | 2 | 2 | 0 | 4 | 0 |
| SUDA4 | 1 | 2 | 1 | 2 | 0 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 3 | 3 | 1 | 2 | 2 | 4 | 1 | 4 | 2 | 2 | 0 | 4 | 3 |
| SUDA5 | 1 | 2 | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 3 | 2 | 1 | 2 | 2 | 1 | 1 | 3 | 2 | 1 | 0 | 1 | 0 |
| TENA1 | 1 | 1 | 2 | 1 | 3 | 7 | 2 | 0 | 3 | 1 | 1 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 2 | 4 | 0 | 0 | 0 | 2 |
| TENA2 | 1 | 1 | •1 | 0 | 3 | 7 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 3 | 0 | 2 | 1 | 0 | 0 | 0 | 1 |
| TENA3 | 1 | 1 | 2 | 1 | 3 | 7 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
| HEL1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 |

Figure 1. A Nelson consensus tree summarizing the sixty phylogenetic trees obtained. Population names follow Table 1.

Figure 2. The six major topologies obtained when relationships among B. alexandrina, B. choanomphala and B. sudanica are held constant. Population names follow Table 1, and supporting character states for each branch are listed in the Appendix.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CHOA2
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SUDA2
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SUDA4
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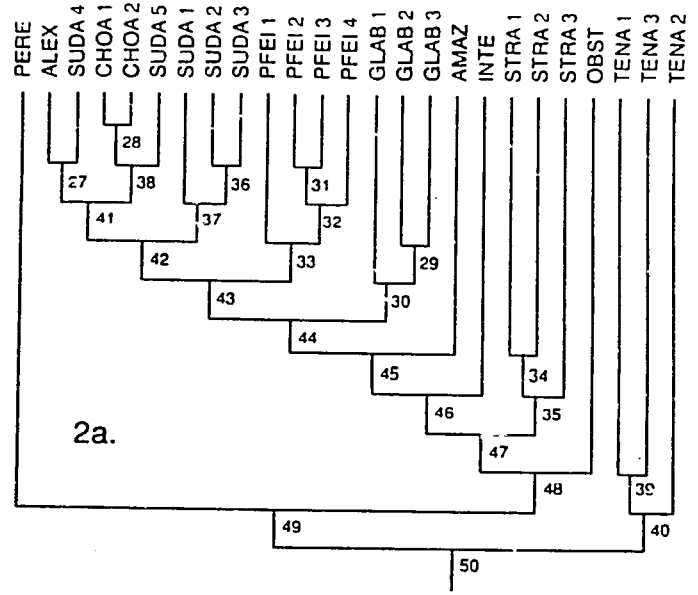
PFEI2
PFEI3
PFEI4
GLAB1
GLAB2
GLAB3
AMAZ
INTE
STRA1
STRA2
STRA3
OBST
PERE
TENA1
TENA3
TENA2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CHOA 2
SUDA 5
SUDA 1
SUDA 2
SUDA 3
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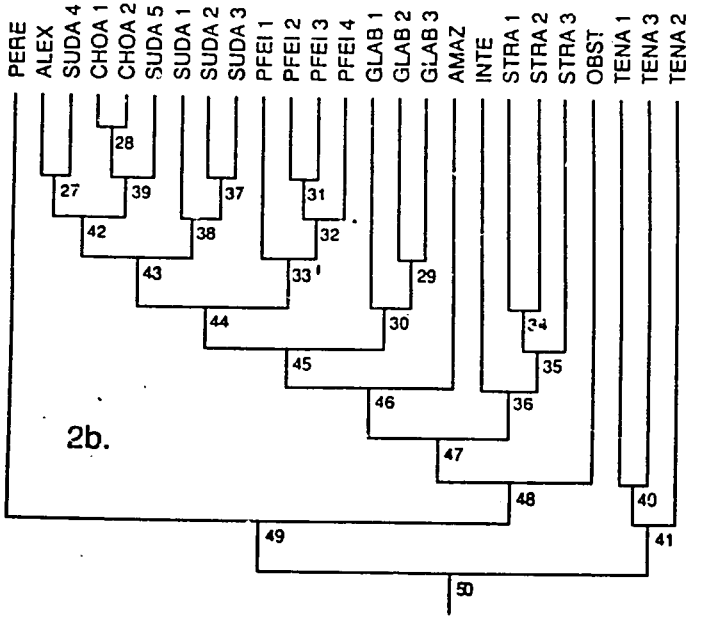
PFEI 2
PFEI 3
PFEI 4
GLAB 1
GLAB 2
GLAB 3
AMAZ
INTE
STRA 1
STRA 2
STRA 3
OBST
TENA 1
TENA 3
TENA 2
2a.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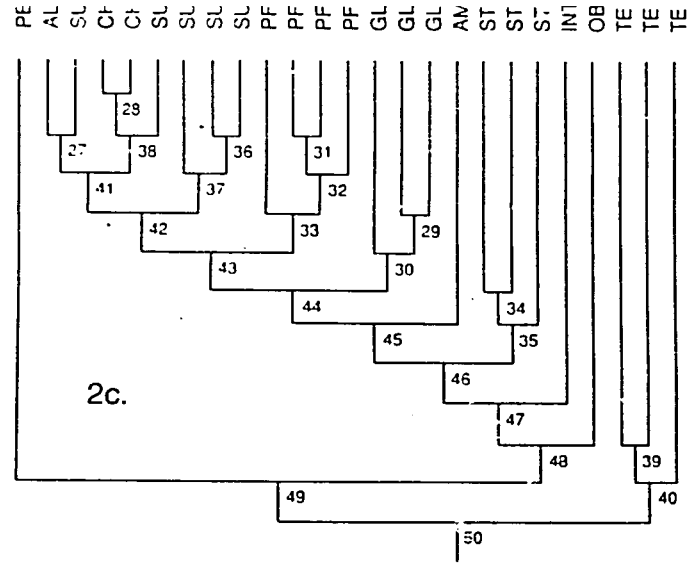
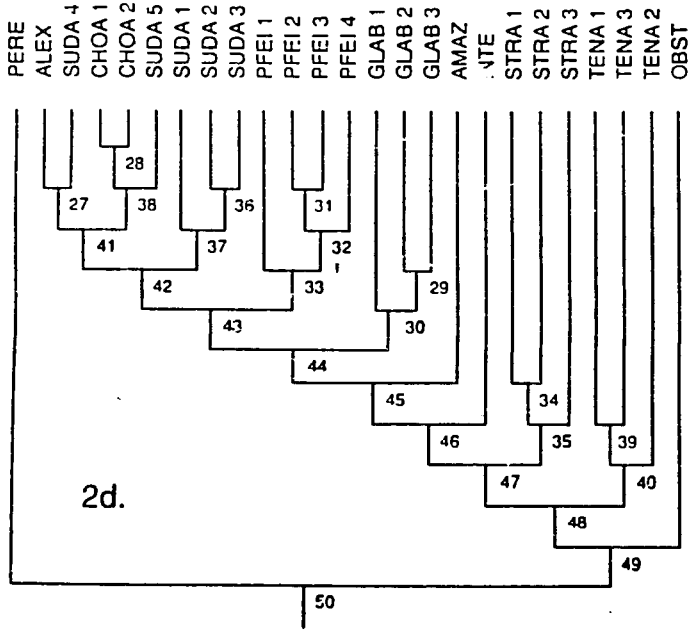
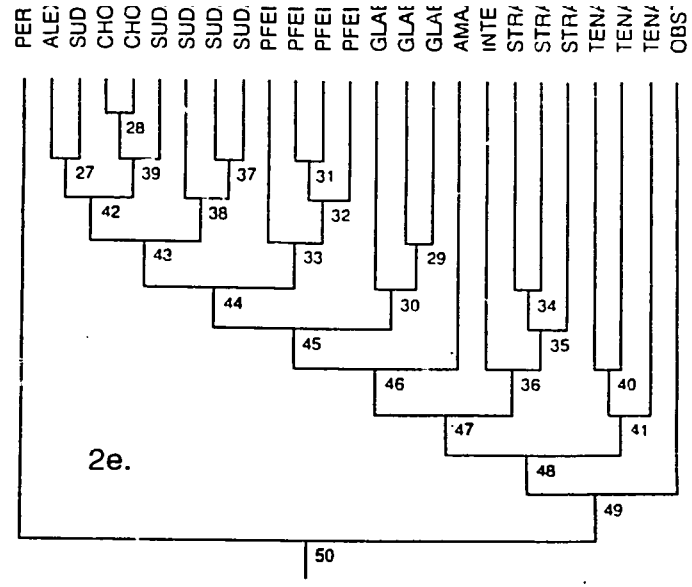
CHOA 2
SUDA 5
SUDA 1
SUDA 2
SUDA 3
PFEI 1
PFEI 2
PFEI 3
PFEI 4
GLAB 1
GLAB 2
GLAB 3
AMAZ
INTE
STRA 1
STRA 2
STRA 3
OBST
TENA 1
TENA 3
TENA 2
2b.


2c.


PERE
ALEX
SUDA 4
CHOA 1
CHOA 2
SUDA 5
SUDA 1
SUDA 2
SUDA 3
PFEI 1
PFEI 2
PFEI 3
PFEI 4
GLAB 1
GLAB 2
GLAB 3
AMAZ
STRA 1
STRA 2
STRA 3
TENA 1
TENA 3
TENA 2
OBST
2d.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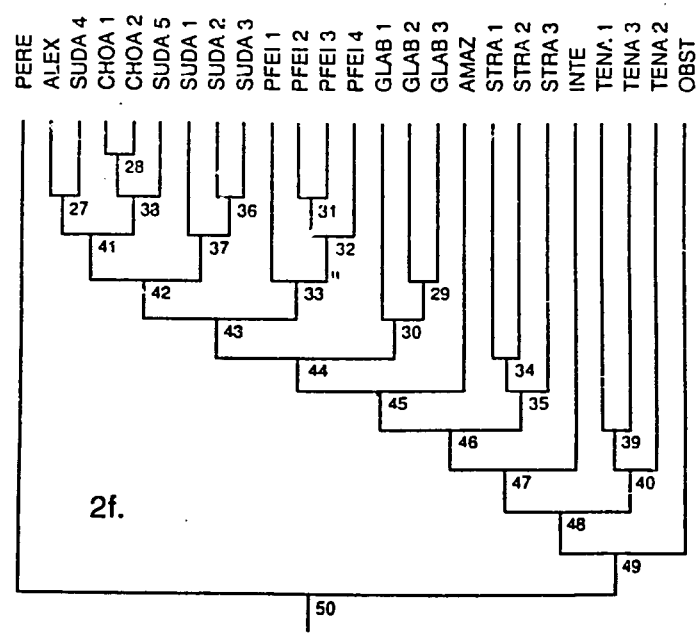
PERE
ALEX
SUDA 4
CHOA 1
CHOA 2
SUDA 5
SUDA 1
SUDA 2
SUDA 3
PFEI 1
PFEI 2
PFEI 3
PFEI 4
GLAB 1
GLAB 2
GLAB 3
AMAZ
STRA 1
STRA 2
STRA 3
INTE
TENA 1
TENA 3
TENA 2
OBST
2f.

Appendix Apomorphy lists for trees illustrated in Figure 2.

Tree 2a

| Node | Ancestor | Character |
|---|---|---|
| PERE | 49 | CK GR MD-1 MDH-2 PEPGL PGD PGM |
| ALEX | 27 | ACON-2 CAT CK NSP PEPLGG |
| AMAZ | 45 | AAT-2 HBDH PGM |
| CHOA1 | 28 | ACON-2 GR LDH MDH-1 NSP |
| CHOA2 | 28 | |
| GLAB1 | 30 | NSP PEPPP PGD PGI |
| GLAB2 | 29 | CAT CK HBDH IDH |
| GLAB3 | 29 | ACON-1 ACON-2 PGI PGM |
| INTE | 46 | IDH PEPLGG PGD XDH |
| OEST | 48 | EST AAT-1 GR PEPGL PGM |
| PFEI1 | 33 | CAT LDH PGM |
| PFEI2 | 31 | ACON-2 |
| PFEI3 | 31 | |
| PFEI4 | 32 | ACON-1 CK |
| STRA1 | 34 | GR PGD PGI XDH |
| STRA2 | 34 | HBDH MDH-1 |
| STRA3 | 35 | ACON-1 EST |
| SUDA1 | 37 | PEPGL |
| SUDA2 | 36 | LDH |
| SUDA3 | 36 | AKP CA GAPDH PGM |
| SUDA4 | 27 | PGD XDH |
| SUDA5 | 38 | |
| TENA1 | 39 | CAT EST HBDH PEPPP |
| TENA2 | 40 | NSP |
| TENA3 | 39 | PEPLGG |
| 27 | 41 | NSP PGM XDH |
| 28 | 38 | ACON-1 PGM |
| 29 | 30 | ACON-1 CA EST GAPDH |
| 30 | 44 | AAT-1 HBDH XDH |
| 31 | 32 | AKP XDH |
| 32 | 33 | GR IDH PEPGL |
| 33 | 43 | AKP CA NSP |
| 34 | 35 | CA CAT XDH |
| 35 | 47 | CA GAPDH GR HBDH IDH |

Tree 2a Continued

| Node | Ancestor | Character |
|---|---|---|
| 36 | 37 | ACON-2 GR PEPLGG PGD |
| 37 | 42 | NSP PGM |
| 38 | 41 | AKP IDH PEPLGG |
| 39 | 40 | ACON-1 ACON-2 CK XDH |
| 40 | 50 | AKP CA CAT GAPDH AAT-1 GPD GR HBDH IDH LDH PEPLGG PEPPP PGM |
| 41 | 42 | CK |
| 42 | 43 | CK AAT-2 PEPGL PGD |
| 43 | 44 | ACON-1 CK GR PEPGL PEPLGG PGI PGM |
| 44 | 45 | ACON-2 CA GAPDH MDH-2 |
| 45 | 46 | ACON-1 CA EST |
| 46 | 47 | AAT-1 PGM |
| 47 | 48 | ACON-1 CA CK LDH MDH'1 PGD XDH |
| 48 | 49 | GAPDH GPD HBDH IDH LDH PEPLGG PEPPP |
| 49 | 50 | PGD PGI |
| 50 | OG | ACON-1 CAT EST GAPDH AAT-1 AAT2 HBDH IDH MDH'1 MDH'2 NSP PEPLGG PEPPP XDH |

Tree 2b

| Node | Ancestor | Character |
|---|---|---|
| PERE | 49 | CK AAT-1 GR MDH-1 MDH-2 PEPGL PGD |
| ALEX | 27 | ACON-2 CAT CK NSP PEPLGG |
| AMAZ | 46 | AAT-2 HBDH PGM |
| CHOA1 | 28 | ACON-2 GR LDH MDH-1 NSP |
| CHOA2 | 28 | |
| GLAB1 | 30 | NSP PEPPP PGD PGI |
| GLAB2 | 29 | CAT CK HBDH IDH |
| GLAB3 | 29 | ACON-1 ACON-2 PGI PGM |
| INTE | 36 | PEPLGG PGD XDH |
| OBST | 48 | EST AAT-1 GR PEPGL |
| PFEI1 | 33 | CAT LDH PGM |
| PFEI2 | 31 | ACON-2 |
| PFEI3 | 31 | |
| PFEI4 | 32 | ACON-1 CK |
| STRA1 | 34 | GR PGD PGI XDH |
| STRA2 | 34 | HBDH MDH-1 |
| STRA3 | 35 | ACON-1 EST |
| SUDA1 | 3 | PEPGL |
| SUDA2 | 37 | LDH |
| SUDA3 | 37 | AKP CA GAPDH PGM |
| SUDA4 | 27 | PGD XDH |
| SUDA5 | 39 | |
| TENA1 | 40 | CAT EST HBDH PEPPP |
| TENA2 | 41 | NSP |
| TENA3 | 40 | PEPLGG |
| 27 | 42 | NSP PGM XDH |
| 28 | 39 | ACON-1 PGM |
| 29 | 30 | ACON-1 CA EST GAPDH |
| 30 | 45 | AAT-1 HBDH XDH |
| 31 | 32 | AKP XDH |
| 32 | 33 | GR IDH PEPGL |
| 33 | 44 | AKP CA NSP |
| 34 | 35 | CA CAT XDH |
| 35 | 36 | CA GAPDH AAT-1 GR HBDH IDH PGM |
| 36 | 47 | ACON-1 IDH |
| 37 | 38 | ACON-2 GR PEPLGG PGD |

Tree 2b Continued

| Node | Ancestor | Character |
|---|---|---|
| 38 | 43 | NSP PGM |
| 39 | 42 | AKP IDH PEPLGG |
| 40 | 41 | ACON-1 ACON-2 CK XDH |
| 41 | 50 | AKP CA CAT GAPDH GPD GR HBDH IDH LDH PEPLGG PEPPP PGM |
| 42 | 43 | CK |
| 43 | 44 | CK AAT-2 PEPGL PGD |
| 44 | 45 | ACON-1 CK GR PEPGL PEPLGG PGI PGM |
| 45 | 46 | ACON-2 CA GAPDH MDH-2 |
| 46 | 47 | CA EST |
| 47 | 48 | ACON-1 CA CK LDH MDH-1 PGD PGM XDH |
| 48 | 49 | GAPDH GPD HBDH IDH LDH PEPLGG PEPPP PGM |
| 49 | 50 | PGD PGI |
| 50 | OG | ACON-1 CAT EST GAPDH AAT-1 AAT-2 HBDH IDH MDH-1 MDH-2 NSP PEPLGG PEPPP XDH |

Tree 2c
Apomorphy lists

| Node | Ancestor | Character |
|---|---|---|
| PERE | 49 | CK AAT-1 GR MDH-1 MDH-2 PEPGL PGD |
| ALEX | 27 | ACON-2 CAT CK NSP PEPLGG |
| AMAZ | 45 | AAT-2 HBDH PGM |
| CHOA1 | 28 | ACON-2 GR LDH MDH- |
| CHOA2 | 28 | |
| GLAB1 | 30 | NSP PEPPP PGD PGI |
| GLAB2 | 29 | CAT CK HBDH IDH |
| GLAB3 | 29 | ACON-1 ACON-2 PGI PGM |
| INTE | 47 | IDH PEPLGG |
| OBST | 48 | EST AAT-1 GR PEPGL PGD |
| PFEI1 | 33 | CAT LDH PGM |
| PFEI2 | 31 | ACON-2 |
| PFEI3 | 31 | |
| PFEI4 | 32 | ACON-1 CK |
| STRA1 | 34 | GR PGD PGI XDH |
| STRA2 | 34 | HBDH MDH-1 |
| STRA3 | 35 | ACON-1 EST |
| SUDA1 | 37 | PEPGL |
| SUDA2 | 36 | LDH |
| SUDA3 | 36 | AKP CA GAPDH PGM |
| SUDA4 | 27 | PGD XDH |
| SUDA5 | 38 | |
| TENA1 | 39 | CAT EST HBDH PEPPP |
| TENA2 | 40 | NSP |
| TENA3 | 39 | PEPLGG |
| 27 | 41 | NSP PGM XDH |
| 28 | 38 | ACON-1 PGM |
| 29 | 30 | ACON-1 CA EST GAPDH |
| 30 | 44 | AAT-1 |
| 31 | 32 | AKP XDH |
| 32 | 33 | IDH PEPGL |
| 33 | 43 | AKP CA NSP |
| 34 | 35 | CA CAT XDH |
| 35 | 46 | GAPDH AAT-1 GR HBDH IDH PGM |
| 36 | 37 | ACON-2 GR PEPLGG PGD |

Tree 2c Continued
Apomorphy lists

| Node | Ancestor | Character |
|---|---|---|
| 37 | 42 | NSP PGM |
| 38 | 41 | AKP IDH PEPLGG |
| 39 | 40 | ACON-1 ACON-2 CK XDH |
| 40 | 50 | AKP CA CAT GAPDH GPD GR HBDH IDH LDH PEPLGG PEPPP PGM |
| 41 | 42 | CK |
| 42 | 43 | CK AAT-2 PEPGL PGD |
| 43 | 44 | ACON-1 CK GR PEPGL PEPLGG PGI PGM |
| 44 | 45 | ACON-2 CA GAPDH MDH-2 |
| 45 | 46 | ACON-1 CA EST |
| 46 | 47 | CA PGD XDH |
| 47 | 48 | ACON-1 CA CK LDH MDH-1 PGM XDH |
| 48 | 49 | GAPDH GPD HBDH IDH LDH PEPLGG PEPPP PGM |
| 49 | 50 | PGD PGI |
| 50 | OG | ACON-1 CAT EST GAPDH AAT-1 AAT-2 HBDH IDH MDH-1 MDH-2 NSP PEPLGG PEPPP XDH |

Tree 2d

| Node | Ancestor | Character |
|---|---|---|
| PERE | 50 | AAT-1 GR PEPGL PGD PGM |
| ALEX | 27 | ACON-2 CAT CK NSP PEPLGG |
| AMAZ | 45 | AAT-2 HBDH PGM |
| CHOA1 | 28 | ACON-2 GR LDH MDH-1 NSP |
| CHOA2 | 28 | |
| GLAB1 | 30 | NSP PEPPP PGD PGI |
| GLAB2 | 29 | CAT CK HBDH IDH |
| GLAB3 | 29 | ACON-1 ACON-2 PGI PGM |
| INTE | 46 | IDH PEPLGG PGD XDH |
| OBST | 49 | EST GR PEPGL PGD PGM |
| PFEI1 | 33 | CAT LDH PGM |
| PFEI2 | 31 | ACON-2 |
| PFEI3 | 31 | |
| PFEI4 | 32 | ACON-1 CK |
| STRA1 | 34 | GR PGD PGI XDH |
| STRA2 | 34 | HBDH MDH-1 |
| STRA3 | 35 | ACON-1 EST |
| SUDA1 | 37 | PEPGL |
| SUDA2 | 36 | LDH |
| SUDA3 | 36 | AKP CA GAPDH PGM |
| SUDA4 | 27 | PGD XDH |
| SUDA5 | 38 | |
| TENA1 | 39 | CAT EST HBDH PEPPP |
| TENA2 | 40 | ACON-1 CK NSP |
| TENA3 | 39 | PEPLGG |
| 27 | 41 | NSP PGM XDH |
| 28 | 38 | ACON-1 PGM |
| 29 | 30 | ACON-1 CA EST GAPDH |
| 30 | 44 | AAT-1 HBDH XDH |
| 31 | 32 | AKP XDH |
| 32 | 33 | GR IDH PEPGL |
| 33 | 43 | AKP CA NSP |
| 34 | 35 | CA CAT XDH |
| 35 | 47 | CA GAPDH AAT-1 GR HBDH IDH |
| 36 | 37 | ACON-2 GR PEPLGG PGD |
| 37 | 42 | NSP PGM |

Tree 2d Continued

| Node | Ancestor | Character |
|---|---|---|
| 38 | 41 | AKP IDH PEPLGG |
| 39 | 40 | ACON-2 XDH |
| 40 | 48 | AKP CAT GAPDH GPD GR HBDH IDH LDH PEPLGG PEPPP PGI PGM |
| 41 | 42 | CK |
| 42 | 43 | CK AAT-2 PEPGL PGD |
| 43 | 44 | ACON-1 CK GR PEPGL PEPLGG PGI PGM |
| 44 | 45 | ACON-2 CA GAPDH MDH-2 |
| 45 | 46 | ACON-1 CA EST |
| 46 | 47 | PGM |
| 47 | 48 | MDH-1 PGD XDH |
| 48 | 49 | ACON-1 CA CK AAT-1 LDH |
| 49 | 50 | CK GAPDH GPD HBDH IDH LDH MDH-1 MDH-2 PEPLGG PEPPP |
| 50 | OG | ACON-1 CAT EST GAPDH AAT-1 AAT-2 HBDH IDH MDH-1 MDH-2 NSP PEPLGG PEPPP PGI XDH |

Tree no 34

Tree 2e

| Node | Ancestor | Character |
|---|---|---|
| PERE | 50 | AAT 1 GR PEPGL PGD |
| ALEX | 27 | ACON-2 CAT CK NSP PEPLGG |
| AMAZ | 46 | AAT-2 HBDH PGM |
| CHOA1 | 28 | ACON-2 GR LDH MDH-1 NSP |
| CHOA2 | 28 | |
| GLAB1 | 30 | NSP PEPPP PGD PGI |
| GLAB2 | 29 | CAT CK HBDH IDH |
| GLAB3 | 29 | ACON-1 ACON-2 PGI PGM |
| INTE | 36 | PEPLGG PGD XDH |
| OBST | 49 | EST GR PEPGL PGD |
| PFEI1 | 33 | CAT LDH PGM |
| PFEI2 | 30 | ACON-2 |
| PFEI3 | 31 | |
| PFEI4 | 32 | ACON-1 CK |
| STRA1 | 34 | GR PGD PGI XDH |
| STRA2 | 34 | HBDH MDH-1 |
| STRA3 | 35 | ACO-1 EST |
| SUDA1 | 38 | PEPGL |
| SUDA2 | 37 | LDH |
| SUDA3 | 37 | AKP CA GAPDH PGM |
| SUDA4 | 27 | PGD XDH |
| SUDA5 | 39 | |
| TENA1 | 40 | CAT EST HBDH PEPPP |
| TENA2 | 41 | ACON-1 CK NSP |
| TENA3 | 40 | PEPLGG |
| 27 | 42 | NSP PGM XDH |
| 28 | 39 | ACON-1 PGM |
| 29 | 30 | ACON-1 CA EST GAPDH |
| 30 | 45 | AAT-1 HBDH XDH |
| 31 | 32 | AKP XDH |
| 32 | 33 | GR IDH PEPGL |
| 33 | 44 | AKP CA NSP |
| 34 | 35 | CA CAT XDH |
| 35 | 36 | CA GAPDH AAT-1 GR HBDH IDH PGM |

Tree 2e Continued

| Node | Ancestor | Character |
|---|---|---|
| 36 | 47 | IDH |
| 37 | 38 | ACON-2 GR PEPLGG PGD |
| 38 | 43 | NSP PGM |
| 39 | 42 | AKP IDH PEPLGG |
| 40 | 41 | ACON-2 XDH |
| 41 | 48 | AKP CAT GAPDH GPD GR HBDH IDH LDH PEPLGG PEPPP PGI PGM |
| 42 | 43 | CK |
| 43 | 44 | CK AAT-2 PEPGL PGD |
| 44 | 45 | ACON-1 CK GR PEPGL PEPLGG PGI PGM |
| 45 | 46 | ACON-2 CA GAPDH MDH-2 |
| 46 | 47 | ACON-1 CA EST |
| 47 | 4 | MDH-1 PGD XDH |
| 48 | 49 | ACON-1 CA CK AAT-1 LDH PGM |
| 49 | 50 | CK GAPDH GPD HBDH IDH LDH MDH-1 MDH-2 PEPLGG PEPPP PGM |
| 50 | OG | ACON-1 CAT EST GAPDH AAT1 AAT2 HBDH IDH MDH-1 MDH-2 NSP PEPLGG PEPPP PGI XDH |

Tree 2f

| Node | Ancestor | Character |
|---|---|---|
| PERE | 50 | AAT-1 GR PEPGL PGD |
| ALEX | 27 | ACON-2 CAT CK NSP PEPLGG |
| AMAZ | 45 | AAT-2 HBDH PGM |
| CHOA1 | 28 | ACON-2 GR LDH MDH-1 NSP |
| CHOA2 | 28 | |
| GLAB1 | 30 | NSP PEPPP PGD PGI |
| GLAB2 | 29 | CAT CK HBDH IDH |
| GLAB3 | 29 | ACON-1 ACON-2 PGI PGM |
| INTE | 47 | IDH PEPLGG |
| OBST | 49 | EST GR PEPGL PGD |
| PFEI1 | 33 | CAT LDH PGM |
| PFEI2 | 31 | ACON-2 |
| PFEI3 | 31 | |
| PFEI4 | 32 | ACON-1 CK |
| STRA1 | 34 | GR PGD PGI XDH |
| STRA2 | 34 | HBDH MDH-1 |
| STRA3 | 35 | ACON-1 EST |
| SUDA1 | 37 | PEPGL |
| SUDA2 | 36 | LDH |
| SUDA3 | 36 | AKP CA GAPDH PGM |
| SUDA4 | 27 | PGD XDH |
| SUDA5 | 38 | |
| TENA1 | 39 | CAT EST HBDH PEPPP |
| TENA2 | 40 | ACON-1 CK NSP |
| TENA3 | 39 | PEPLGG |
| 27 | 41 | NSP PGM XDH |
| 28 | 38 | ACON-1 PGM |
| 29 | 30 | ACON-1 CA EST GAPDH |
| 30 | 44 | AAT-1 HBDH XDH |
| 31 | 32 | AKP XDH |
| 32 | 33 | GR IDH PEPGL |
| 33 | 43 | AKP CA NSP |
| 34 | 35 | CA CAT XDH |
| 35 | 46 | GAPDH AAT-1 GR HBDH IDH PGM |
| 36 | 37 | ACON-2 GR PEPLGG PGD |
| 37 | 42 | NSP PGM |

Tree 2f Continued

| Node | Ancestor | Character |
|---|---|---|
| 38 | 41 | AKP IDH PEPLGG |
| 39 | 40 | ACON-2 XDH |
| 40 | 48 | AKP CAT GAPDH GPD GR HBDH IDH LDH PEPLGG PEPPP PGI PGM |
| 41 | 42 | CK |
| 42 | 43 | CK AAT-2 PEPGL PGD |
| 43 | 44 | ACON-1 CK GR PEPGL PEPLGG PGI PGM |
| 44 | 45 | ACON-2 CA GAPDH MDH-2 |
| 45 | 46 | ACON-1 CA EST |
| 46 | 47 | CA PGD XDH |
| 47 | 48 | MDH-1 PGD XDH |
| 48 | 49 | ACON-1 CA CK AAT-1 LDH PGM |
| 49 | 50 | CK GAPDH GPD HBDH IDH LDH MDH-1 MDH-2 PEPLGG PEPPP PGM |
| 50 | OG | ACON-1 CAT EST GAPDH AAT-1 AAT-2 HBDH IDH MDH-1 MDH-2 NSP PEPLGG PEPPP PGI XDH |

# Appendix 12

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TITLE: Intraspecific and interspecific patterns of allozyme variation among species of Biomphalaria Preston, 1910 (Gastropoda: Planorbidae).

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Savannah River Ecology Laboratory

# Intraspecific and interspecific patterns of allozyme variation among species of Biomphalaria Preston, 1910 (Gastropoda: Planorbidae)

by

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KEY WORDS: Biomphalaria, Planorbidae, Gastropoda, ramshorn snails, Schistosoma mansoni, allozymes, population variation, systematics.

ABSTRACT

Freshwater snails in the genus Biomphalaria transmit Schistosoma mansoni, a causative agent of human schistosomiasis in Africa and the neotropical region. Twenty-five populations representing eleven species of Biomphalaria from both Africa and the neotropical region were examined using starch gel electrophoresis for twenty-two loci. The percentage of polymorphic loci observed per population varied from 0 to 59%, with a mean of 25%. The observed heterozygosity levels were similarly varied, ranging from 0.002 to 0.126, with an average of 0.052. Observed heterozygosity and polymorphism values fell within the range of values reported in previous studies of the genus, but observed heterozygosity in most cases was less than expected under Hardy-Weinberg equilibrium. The genetic variance was partitioned among species, populations and individuals using hierarchical G-statistics. For all of the populations considered together, the variance among species was 58.4%, among populations within species 24.9%, and among individuals within a population 16.7%. When the neotropical species alone were considered, the percentages of the total variance attributable to variation among species, among populations within species, and among individuals were 18.1%, 29.2% and 52.7% respectively. For the African species, 42.3% of the variance was among species, 27.8% among populations, and 29.9% among individuals. Intraspecific Rogers' genetic distances ranged from 0.009 to 0.377, and interspecific distances from 0.102 to 0.837.

Non-metric multidimensional scaling of Roger's distances revealed a clear separation of African species from neotropical species and of the neotropical species from each otehr The African species occupied overlapping spaces.



Freshwater snails belonging to the genus Biomphalaria transmit Schistosoma mansoni, the causative agent of human intestinal schistosomiasis in Africa and the neotropics [1]. Not all species of Biomphalaria are capable of transmitting schistosomiasis, and both susceptible and insusceptible species often occur in sympatry [2] Considerable attention has been given to systematic relationships among the species. The emphasis of such efforts has been on the recognition of species, determination of susceptibility, and mapping of the geographic distributions of susceptible species [2-6]. Taxonomists interested in species of Biomphalaria have worked primarily with the snails of a single region. For example, most of the neotropical species have been described or redescribed by Paraense [e.g., 5] or Malek [2], and most African species have been studied by Mandahl-Barth [3] or Brown [6]. In general, the African species are identified on the basis of conchological features, and the neotropical species on the basis of the soft anatomy and reproductive compatibility. However, the approach taken by each of these authors is unique. The result of regional specialization is that the classifications produced are not comparable.

Given the recent introduction of B. straminea into Hong Kong [7], there is clearly a need for a global perspective in the taxonomy of Biomphalaria species. The development of a unified taxonomy and the elucidation of evolutionary relationships within the genus will require the generation of a uniform database for species from various regions. As the first step toward achieving these goals, we have examined eleven species of Biomphalaria (seven neotropical and four African) using starch gel electrophoresis, in order to assess patterns of intra- and interspecific variation of allozymes. Of particular interest

are the comparisons of species from the two hemispheres, as few such comparisons have been performed previously [8].

## RESULTS

Twenty-five populations representing 11 species of Biomphalaria were examined. Both field and aquarium populations were included. The populations, collecting localities or sources of laboratory stocks, and abbreviations used are listed in Table 1.

Enzyme systems examined and buffer regimes are listed in Table 2. Because of the small size of many of the specimens available, a relatively small number of enzymes could be analyzed. Loci previously observed to be invariant (hemoglobin) or highly variable (some peptidases and esterases) among any of five species of Biomphalaria previously studied were not used [9-15]. Twenty-two loci could be scored reliably in all populations.

Our sample sizes per population were larger than the minimum considered acceptable by Gorman and Renzi [16] and in the range recommended by Archie et al., [17]. Sample sizes per locus vary from 2 to 57 (with an average of 17.7), due to factors such as the size of the animal, the quality of preservation of the tissue, and the difficulties of interpreting particular loci.

Genotypic counts for each population were analyzed using BIOSYS-1 [18]. Loci were considered polymorphic if the most frequently occurring allele occurred at a frequency less than or equal to 0.95. For the average sample size employed, this criterion was equivalent to a requirement that each allele be





observed at least twice per sample, either as one homozygote or two heterozygotes.

Frequencies of alleles for the 25 populations are reported in Table 3. The percentage of polymorphic loci per population, the average number of alleles per locus, and the observed heterozygosity are reported in Table 4. The percentage of polymorphic loci observed per population varied from 0 to 59 percent, with a mean of 25. The observed heterozygosity levels were similarly varied, ranging from 0.002 to 0.126, with an average of 0.052. The expected levels of heterozygosity under Hardy-Weinberg equilibrium were also calculated. Almost all values of observed heterozygosity are lower than the expected values. In a few cases the observed heterozygosity was the same as, or slightly higher than, the expected heterozygosity under Hardy-Weinberg equilibrium. The average number of alleles per locus ranged from 1.0 to 1.8, with an average of 1.3.

Among field samples, an average of 23.6% of the loci were polymorphic and the observed heterozygosity was 0.048. For laboratory populations the average percentage of polymorphic loci was 27.7%, and the average heterozygosity was 0.058. The heterozygosity values and the percentage of polymorphic loci of laboratory and field populations were compared using a Student's t-test after arcsine transformation. There was no statistically detectable difference in either heterozygosity or polymorphism between field and laboratory populations ($t = 0.86, 0.75$ respectively, $p > 0.05$).

Hierarchical analyses of genetic variance [19-22] were used to compare the intraspecific and interspecific contributions to the total genetic variance.

The hierarchy specified consisted of individuals within populations within species. The relationship among the variance components is:

$$H_L/H_T + D_{LS}/H_T + D_{ST}/H_T = 1$$

where $H_T$ is the total gene diversity, $H_L$ is the gene diversity within localities, $D_{LS}$ is the gene diversity among localities within species, and $D_{ST}$ is the gene diversity among species. Although the formulations of the hierarchical analysis of genetic variance of Nei [19,20] and Wright [21,22] are identical conceptually, the method of Nei allows the variance components to be expressed as percentages of the total variance.

Results of the first hierarchical analysis of genetic variance are presented in Table 5. The contribution of variation attributable to populations within species to the total variance was 24.9%, and the contribution of variation among individuals within a population to total variance was 16.7%. The variance among species therefore accounts for 58.4% of the total variance.

When the African species alone are considered, the percentages of the total variance attributable to variation among individuals, among populations within species, and among species were 29.9%, 27.8% and 42.3% respectively. For the neotropical species, 52.7% of the variance was among species, 29.2% among populations, and 18.1% among individuals. The observed heterozygosity and percentage of polymorphic loci of the African and neotropical species were compared using a Student's t-test, after arcsine transformation. There was no statistically detectable difference in either the observed heterozygosity or the percentage of polymorphic loci ($t = 0.056$, 1.000 respectively, $p > 0.05$).



Allele frequencies were also used to calculate Rogers' genetic distances (Table 6;23). Genetic distances within species ranged from 0.009 to 0.377. Interspecific genetic distances ranged from 0.102 to 0.837. Non-metric multidimensional scaling (an ordination technique similar to principal components analysis) was performed on the genetic distances using NT-SYS [24]. Populations are represented in a space of two or more dimensions in which the distances between pairs of points correspond to the original genetic distances as closely as possible. Thus, non-metric multidimensional scaling may represented complex patterns of resemblance better than a conventional phenogram.

An initial ordination in two dimensions was used to seed an analysis in three dimensions. Goodness of fit was measured using the stress coefficient stress 2, and iterations were repeated until the stress coefficient was less than 0.001 fewer than 40 iterations were required.

Distances derived from multidimensional scaling are plotted in three dimensions in Figure 1. African species form a tight cluster separated from the neotropical species, which form a more diffuse cluster. Individual neotropical species from discrete, non-overlapping clusters, although spaces occupied by some species pairs (e.g. B. straminea and B. intermedia) are extremely close. The African species occupy overlapping spaces, and thus are not so easily distinguished.

## DISCUSSION

The levels of heterozygosity and polymorphism observed here were similar to those observed in previous studies of Biomphalaria species [9-15, 25-

27] Values of observed heterozygosity previously reported for species of Biomphalaria range from 0.010 to 0.211 (see Table 7). Values of observed heterozygosity obtained for Biomphalaria are also similar to values obtained for other freshwater gastropods [28,29]. The choice of loci, number of loci and proportion of polymorphic loci all may influence the observed levels of heterozygosity, so some caution should be used in comparing across studies.

In most populations, the direct count heterozygosity was lower than the expected heterozygosity, a finding that is not surprising for self-compatible hermaphrodites, and is consistent with previous studies [28,29]. In a few populations, the direct count heterozygosity was identical to or slightly higher than the expected heterozygosity.

Three of four populations of B. pfeifferi exhibited heterozygosity values which were greater than or equal to the expected values under Hardy-Weinberg equilibrium, in contrast to previous reports of heterozygote deficiencies in this species [15, 26]. The failure to observe a marked deficiency of heterozygotes here most probably reflects sampling error, because of the very low levels of genetic variability observed in B. pfeifferi. Alternatively, greater heterogeneity in local population structure may be present in B. pfeifferi than was detected in previous studies.

No statistically detectable differences were found in the average percentage of polymorphic loci or the average observed heterozygosity for laboratory and field populations. The same pattern was reported previously for B. glabrata populations by Mulvey & Vrijenhoek [11,12]. These authors suggested that the effective population size may be similar in field and laboratory populations. The range of heterozygosity values observed was





slightly greater for field populations, perhaps because of a greater similarity in histories and environmental conditions among laboratory stocks.

There currently is considerable interest in the causes of variation in heterozygosity levels among taxa, and in attributing observed heterozygosity to stochastic factors or to selection [30]. For Biomphalaria species, too much remains unknown about the population biology of these organisms to be able to address the causes of variation in heterozygosity levels. Information that is especially needed for Biomphalaria species includes the age structure of populations, and the pattern of temporal variation in gene frequencies.

The largest component of the variance was attributable to differences among species (58.4%), as would be expected for well-delineated species. Nonetheless, the variance observed still represents only slightly more than half of the total genetic variance. Medical malacologists have been hopeful that electrophoresis would provide a reliable method for identification of Biomphalaria species [6,8]. Because the identification of species based on morphological characteristics requires considerable training and experience, electrophoretic markers for particular species have been sought as a supplement or even a replacement for morphological methods for an identification service [8]. Allozyme data are successfully employed for the identification of species in some taxa [31]. The present study suggests that the use of allozymes in identification will not be feasible for Biomphalaria species, at least for the loci sampled, because of the high level of intraspecific variation in comparison with the variation among species. Patterns of allozyme variation will nonetheless still be useful in taxonomic research on these snails.

The estimates of the contributions of intrapopulation (16.7%) and interpopulation (24.9%) variance obtained here are similar to values obtained with freshwater prosobranchs in the Goniobasis floridensis complex [32], and with land snails in the genus Solatopupa [33]. Both of these studies considered smaller numbers of species distributed over much smaller geographic scales, and, not unexpectedly, a slightly greater proportion of the variance was attributable to intraspecific variation in these studies.

The observation of substantial intraspecific genetic variance also has important implications for the transmission and control of schistosomiasis. Because susceptibility and resistance are under genetic control [34], these genes might be expected to exhibit similar levels of intra- and interpopulation variance, as the soluble enzyme products studied here. The possible effects of genetic drift on variation in susceptibility to infection with schistosomes were considered previously [12], but in that study, the populations of B. glabrata examined were relatively homogeneous. In a population of Helisoma anceps infected with the trematode Halipegus occidualis, Mulvey et al. [35] observed considerable differences in allozyme frequencies and heterozygosity between infected and uninfected snails. The observation of roughly comparable contributions of intra- and interpopulation variation to the total variance suggests that the relationship of intrapopulation variance in species of Biomphalaria to susceptibility to infection with S. mansoni should also be examined.

The contribution of variance attributable to variation among populations of a single species was highest for the African species alone. This observation may be attributable at least in part, to the larger number of populations per species, and hence to a greater probability of detecting variation among populations. The variance attributable to variation among species was lower than that observed for the neotropical species. Possible explanations for this include a lower degree of divergence among the four African species than among the neotropical species studied here, and problems with the current taxonomy of the African species.

The percentage of the total variance attributable to variation among individuals within a population was highest in the analysis of the African species alone. However, the results of the t-test indicate that the African species are more variable, as measured by the percentage of polymorphic loci and the average heterozygosity, than are the neotropical species. Thus, the individual populations of the African species are no more variable on average than are the populations of the neotropical species, and the difference in the sizes of the variance components instead may be attributable to the lower level of divergence among the African species.

Overlapping patterns of genetic variation were observed among many pairs of species. Often, the common allele of one species occurs as a low frequency allele in other taxa. For example, B. glabrata and B. intermedia are separated by some fixed differences, but a larger number of loci exhibit overlapping polymorphisms (Table 3). In B. sudanica and B. choanomphala, which occur in sympatry and are syntopic at one site, no fixed differences were observed. Similar patterns of genetic variation among two or more closely related species have been reported for several mollusks including unionid bivalves [36], freshwater prosobranchs of the genera Goniobasis and Neotricula [32,37], and pulmonate land snails of the genera Cerion, Partula, Samoana and

Solatopupa [33, 38-40]. Fixed differences may be less indicative of species boundaries in mollusks than in vertebrates. Alternatively, the current taxonomy may be in need of revision. Finally, Davis et al. [36] suggested that overlapping polymorphisms may also be attributable to selection or to a very recent origin. Further study will be needed in order to distinguish among these various possibilities.

The present study was not designed to test species boundaries, and therefore can provide only limited information with which to address this question. A few cases of apparent fixed differences among populations were observed in B. glabrata and B. pfeifferi over very large spatial scales. Similar results have been reported previously for Brazilian populations of B. glabrata [41] and for West African populations of B. pfeifferi [25]. As with previous studies, without populations from intervening localities, it is not possible to say whether intermediate stages also occur. Among the specimens included were members of three nominal species collected in sites where two of the species occurred syntopically. Taxonomic discrimination among these three species is treated elsewhere [42; Bandoni et al., in preparation).



Many of the observed differences among populations of a single nominal species appear to be the result of fixation of alternate alleles from an ancestral polymorphism (see Table 3), a pattern observed previously in the land snail Solatopupa [33]. This observation suggests that single populations alone may provide misleading estimates of taxonomic affinity, as alleles linking these to other species may be missing from any one sample studied.

Non-metric multidimensional scaling of genetic distances revealed a clear separation of African and neotropical species. If rates of evolution of isozymes



are constant, the species from each hemisphere would form monophyletic groups. However, if rates of evolution are variable, the phenetic relationships detected via non-metric multidimensional scaling would not be congruent with the phylogenetic relationships, and the similarities observed could be attributable to a common lack of evolutionary modification. Phylogenetic relationships among species of Biomphalaria are treated elsewhere [42].

The neotropical species occupy non-overlapping spaces in Figure 1, indicating that the pattern of isozyme variation is consistent with current taxonomy. Distances among populations within a species are generally less than distances among species, indicating concordance of the results of ordination and the hierarchical analysis of genetic variance. The African species occupy overlapping spaces in Figure 1, suggesting that the pattern of isozyme variation is not consistent with current taxonomy. Distances among populations within individual nominal species may be greater than interspecific distances, a pattern consistent with the hierarchical analysis of genetic variance, but suggestive of taxonomic problems. Neotropical species exhibit greater genetic diversity than do the African species studied.

Hillis [43] has argued that for the comparison of different species, small samples from many localities are preferable to large samples from only a few localities, particularly for species with high levels of genetic variation. The basis for his argument is that the variation lies among rather than within populations. The results of the present study may argue against the uniform application of this sampling design, and suggest instead that the sample sizes and sampling distribution needed may depend not only on the level of genetic variation present, as suggested by Archie et al. [17], but also on the distribution

of the variance components. In the present study, considerable overlap among species was detected that might not have been observed if smaller samples had been used, i.e., the predominant allele of one species often occurred at lower frequencies in other species. The larger sample sizes per population therefore appear to have contributed helpful information. Future efforts to address systematic relationships among species of Biomphalaria should be accompanied by further studies of the distribution of biochemical and molecular variation both within and among species.

## EXPERIMENTAL

Specimens were collected in the field or taken from aquarium stocks. Two methods of preparing specimens from the field were employed. Specimens were collected using a dip net, rinsed free of vegetation, and either wrapped in foil and frozen in liquid nitrogen for transport, or transported back to the laboratory in wide-mouthed jars and maintained in aquaria until they could be prepared for shipping. Snails were wrapped in damp surgical gauze or paper towels for shipping, and were shipped either live or frozen with dry ice. Voucher specimens have been prepared for all but one of the field populations included, and have been deposited in the collections of the Philadelphia Academy of Natural Sciences. The specimens of B. obstructa used were obtained from a site where no other species of Biomphalaria are known to occur. The identity of the specimens was determined by Dr. Emile Malek of Tulane University, who has written the most complete description of the species [43].



Electrophoretic protocols were similar to those described by Mulvey et al., [13] and Murphy et al., [45]. Whole snails were homogenized either with or without the shell. Extensive studies of allozyme variation in five species of Biomphalaria using the same or similar protocols enabled recognition of bands attributable to other organisms such as digenetic trematodes. Such additional bands did not interfere with scoring of the gels [9-15].

Individuals of many Biomphalaria species are quite small, and contain relatively low levels of enzyme activity, necessitating some modifications of the design of the study from that usually employed in electrophoretic comparisons of different species [31]. Some loci could not be reliably scored in samples refrozen and run a second day, so more comparative combinations of taxa were run per gel to maximize the number of interspecific comparisons. Larger sample sizes than are typical of systematic studies were used to enable these comparisons to be made.

Samples of the M-line stock of B. glabrata [46] and of the Salvadoran population of B. glabrata were run as standards. Three to five individuals of one or both stocks were run per gel to ensure that variation within the marker stocks did not interfere with the interpretation of the gel. Gels were photographed, and scores were confirmed from black and white prints. Gels stained for carbonate dehydratase, a fluorescent stain, were not photographed.

The following conventions were adopted for synonymizing alleles. If populations 1 and 2 shared their most common allele on one gel, and populations 1 and 3 shared their most common allele on another, then populations 2 and 3 were assumed to also share the same allele. Alphabetic

designations were assigned beginning with the most nodal allele. The mobility of each allele was measured relative to the marker stocks of B. glabrata. Relative mobility ranges of alleles overlapped for some loci, so the order was taken directly from their relative position on the gels wherever possible. Although the protocol used allowed most alleles to be observed together on one or more gels, some very rare alleles of similar relative mobility were not seen side by side or even with a common intermediate. These low frequency alleles were given unique designations to reflect their uncertain relationship to other alleles.





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Table 1. List of specimens, abbreviations used, sources, and original localities. Institutional affiliations of individuals providing snails are listed in the acknowledgements. Localities listed are collection sites for field populations, and sites of the original field source for laboratory stocks.

| | Species | Abbreviation | Source | Lab/Field | Locality |
|---|---|---|---|---|---|
| 1. | B. alexandrina (Ehrenberg, 1831) | ALEX | Bruce | L | Egypt, orginal locality unknown |
| 2. | B. amazonica Paraense, 1966 | AMAZ | Paraense | L | Barao de Melgaço, Brazil (16°13'S, 55°58'W) |
| 3. | B. choanomphala (Martens, 1879) | CHOA1 | Loker | F | Rusinga Island, Kenya (0°25'S, 34°12'E) |
| 4. | B. choanomphala (Martens, 1879) | CHOA2 | Loker | F | Karungu Bay, Kenya (0°50'S, 34°10'E) |
| 5. | B. glabrata (Say, 1818) | GLAB1 | Lewis | L | Belo Horizonte, Brazil (19°54'S, 34°54'W) |
| 6. | B. glabrata (Say, 1818) | GLAB2 | Dominguez | F | Jarabocoa, Dominican Republic (19°9'N, 70°, 39'W) |
| 7. | B. glabrata (Say, 1818) | GLAB3 | Paraense | L | Salvador, Brazil (12°58'S, 38°29'W) |
| 8. | B. intermedia (Paraense & Deslandes, 1962) | INTE | Paraense | L | Parapuã, Brazil (21°47'S, 50°50'W) |
| 9. | B. obstructa (Morelet, 1849) | OBST | Malek | F | New Orleans, USA (30°0'N, 90°3'W) |
| 10. | B. peregrina (Orbigny, 1835) | PERE | Paraense | L | Salto, Uruguay (31°23'S, 57°58'W) |
| 11. | B. pfeifferi (Krauss, 1848) | PFEI1 | Mimpfoundi | F | Yaounde, Cameroon (3°51'N, 11°31'E) |
| 12. | B. pfeifferi (Krauss, 1848) | PFEI2 | Loker | F | Mwea, Kenya (0°40'S, 37°10'E) |
| 13. | B. pfeifferi (Krauss, 1848) | PFEI3 | Loker | F | Churo Pond, Kenya (0°47'S, 37°10'E) |
| 14. | B. pfeifferi (Krauss, 1848) | PFEI4 | Loker | F | Chebwonyo Dam, Kenya (0°58'S, 35°11'E) |
| 15. | B. straminea (Dunker, 1848) | STRA1 | Paraense | L | Belem, Brazil (1°27'S, 38°54'W) |
| 16. | B. straminea (Dunker, 1848) | STRA2 | Paraense | L | Cajazeira, Brazil (6°54'S, 35°11'E) |
| 17. | B. straminea (Dunker, 1848) | STRA3 | Paraense | L | Paracatú, Brazil (17°13'S, 38°54'W) |
| 18. | B. sudanica (Martens, 1870) | SUDA1 | Loker | F | ADC Farm, Kenya (0°20'S, 34°39'E) |
| 19. | B. sudanica (Martens, 1870) | SUDA2 | Loker | F | Kendu Bay, Kenya (0°20'S, 35°11'E) |
| 20. | B. sudanica (Martens, 1870) | SUDA3 | Loker | F | Rusinga Island, Kenya (0°50'S, 34°12'E) |
| 21. | B. sudanica (Martens, 1870) | SUDA4 | Loker | F | Chebwonyo Dam, Kenya (0°58'S, 35°11'E) |
| 22. | B. sudanica (Martens, 1870) | SUDA5 | Loker | F | Karungu Bay, Kenya (0°50'S, 34°10'E) |
| 23. | B. tenagophila (Orbigny, 1834) | TENA1 | Paraense | L | Asunçion, Paraguay (26°26'S, 57°40'W) |
| 24. | B. tenagophila (Orbigny, 1834) | TENA2 | Bandoni | F | Niteroi, Brazil (22°54'S, 43°06'W) |
| 25. | B. tenagophila (Orbigny, 1834) | TENA3 | Bandoni | F | Rio de Janeiro, Brazil (25°55'S, 43°30'W) |

Table 2. Enzymes examined. List of enzymes examined, with Enzyme Commission numbers, number of loci scored, and gel conditions. In some cases, the number of loci listed is the number scored, not the number obtained. Buffers are as follows: (1) Amino-propylmorpholine citrate, pH 6.0 (Clayton & Tretiak, 1972), (2) Tris citrate EDTA, pH 7.0-7.1 (Ayala et al, 1972), (3) Tris-EDTA-borate, (Selander et al., 1971), (4) Lithium hydroxide, pH 8.2/8.4 (Selander et al, 1971), and (5) Poulik discontinuous tris-citrate/borate, pH 8.2/8.7 (Selander et al., 1971)

| Enzyme | Abbreviation | E. C. number | No. of loci | Gel type |
|---|---|---|---|---|
| Aconitate hydratase | ACON | 4.2.1.3 | 2 | 1 |
| Alkaline phosphatase | AKP | 3.1.3.1 | 1 | 2 |
| Aspartate amino transferase | AAT | 2.6.1.1. | 2 | 3 |
| Catalase | CAT | 1.11.1.6 | 1 | 2 |
| Creatine Kinase | CK | 2.7.3.2 | 1 | 1 |
| Carboxylesterase (α-Naphthyl acetate) | EST | 3.1.1.- | 1 | 3,4 |
| Glycerol-3-phosphate dehydrogenase | GPD | 1.1.1.8 | 1 | 1 |
| Glucose-6-phosphate isomerase | PGD | 1.1.1.44 | 1 | 1 |
| Glutathione reductase (NAD(P)H) | GR | 1.6.4.2 | 1 | 5 |
| 3-Hydroxybutyrate dehydrogenase | HBDH | 1.1.1.30 | 1 | 5 |
| Isocitrate dehydrogenase | IDH | 1.1.1.42 | 1 | 2 |
| Malate dehydrogenase | MDH | 1.1.1.37 | 2 | 1 |
| Purine nucleoside phosphorylase | NSP | 2.4.2.1 | 1 | 3 |
| Peptidase | PEP | 3.4.-.- | | |
| (glycylleucine) | PEP-GL | | 1 | 4 |
| (leucylglycylglycine) | PEP-LGG | | 1 | 4 |
| (phenylproline) | PEP-PP | | 1 | 4 |
| Phosphoglucoseisomerase | PGI | 5.3.1.9 | 1 | 5 |
| Phosphoglucomutase | PGM | 5.4.2.2. | 1 | 1 |
| Xanthine dehydrogenase | XDH | 1.1.1.204 | 1 | 3 |

Table 3. Allele frequencies for the twenty-two loci studied. Populations are placed in three groups, in an order identical to that presented in Table 1

| Locus | ALEX | AMAZ | CHOA1 | CHOA2 | GLAB1 | GLAB2 | GLAB3 | INTE | OBST | PERE | PFEI1 | PFEI2 | PFEI3 | PFEI4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ACN-1 | | | | | | | | | | | | | | |
| (N) | 24 | 11 | 46 | 6 | 13 | 3 | 37 | 20 | 16 | 19 | 25 | 14 | 13 | 14 |
| A | -- | 1.00 | -- | -- | 1.00 | -- | 0.31 | -- | -- | -- | -- | -- | -- | -- |
| B | 1.00 | -- | 0.72 | 0.83 | -- | -- | 0.38 | 0.68 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- |
| C | -- | -- | 0.28 | 0.17 | -- | 1.00 | 0.31 | 0.32 | -- | -- | -- | -- | -- | -- |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 |
| ACN-2 | | | | | | | | | | | | | | |
| (N) | 8 | 3 | 33 | 5 | 13 | 6 | 32 | 20 | 16 | 22 | 20 | 14 | 11 | 15 |
| A | 0.25 | 1.00 | -- | -- | -- | -- | -- | 1.00 | 1.00 | 1.00 | -- | -- | -- | -- |
| B | 0.75 | -- | 0.92 | 1.00 | 1.00 | -- | 0.95 | -- | -- | -- | 1.00 | 0.89 | 1.00 | 1.00 |
| C | -- | -- | 0.08 | -- | -- | -- | 0.05 | -- | -- | -- | -- | 0.11 | -- | -- |
| D | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- | -- | -- | -- | -- |
| AKP | | | | | | | | | | | | | | |
| (N) | 20 | 13 | 48 | 10 | 23 | 15 | 35 | 22 | 16 | 13 | 32 | 16 | 25 | 16 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | 1.00 | 1.00 | 0.94 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- | 1.00 | 1.00 | -- |
| C | -- | -- | 0.06 | 0.10 | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | 1.00 |
| CAT | | | | | | | | | | | | | | |
| (N) | 21 | 14 | 45 | 10 | 17 | 15 | 31 | 19 | 14 | 19 | 23 | 16 | 25 | 18 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | -- | 0.14 | 0.52 | 0.55 | 0.38 | -- | 0.79 | 0.37 | 0.11 | 0.92 | 0.02 | 0.88 | 0.86 | 0.92 |
| C | 1.00 | 0.86 | 0.48 | 0.45 | 0.62 | 1.00 | 0.21 | 0.63 | 0.89 | -- | -- | 0.12 | 0.14 | 0.08 |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.98 | -- | -- | |
| CK | | | | | | | | | | | | | | |
| (N) | 11 | 9 | 37 | 8 | 11 | 11 | 29 | 21 | 11 | 23 | 21 | 6 | 24 | 19 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | | -- | -- | 1.00 |
| B | -- | 0.22 | -- | -- | 0.14 | -- | 0.14 | 0.36 | -- | | | -- | -- | -- |
| C | -- | 0.78 | -- | -- | 0.86 | 1.00 | 0.86 | 0.64 | 1.00 | -- | -- | -- | -- | -- |
| D | -- | -- | 0.50 | 0.87 | -- | -- | -- | -- | -- | | 1.00 | 1.00 | 1.00 | -- |
| E | 1.00 | -- | 0.50 | 0.13 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| F | -- | -- | -- | -- | -- | -- | -- | -- | -- | | | | | |
| G | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | | -- | -- | |

Table 3 Continued

| Locus | ALEX | AMAZ | CHOA1 | CHOA2 | GLAB1 | GLAB2 | GLAB3 | INTE | OBST | PERE | PFEI1 | PFEI2 | PFEI3 | PFEI4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EST-2 | | | | | | | | | | | | | | |
| (N) | 16 | 15 | 40 | 4 | 14 | 15 | 35 | 18 | 16 | 15 | 27 | 13 | 19 | 16 |
| A | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- | -- | 0.03 | 0.81 | | 1.00 | 1.00 | 1.00 | 1.00 |
| B | -- | -- | -- | -- | -- | 1.00 | 1.00 | 0.97 | 0.19 | 1.00 | -- | -- | -- | -- |
| GOT-1 | | | | | | | | | | | | | | |
| (N) | 23 | 10 | 52 | 10 | 24 | 15 | 38 | 16 | 16 | 19 | 28 | 16 | 24 | 20 |
| A | 1.00 | 1.00 | 1.00 | 1.00 | -- | -- | -- | 1.00 | -- | 0.74 | -- | 1.00 | 1.00 | 1.00 |
| B | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- | -- |
| C | -- | -- | -- | -- | 1.00 | 1.00 | 1.00 | -- | -- | 0.26 | 1.00 | -- | -- | -- |
| GOT-2 | | | | | | | | | | | | | | |
| (N) | 9 | 3 | 43 | 8 | 13 | 10 | 20 | 12 | 10 | 13 | 18 | 6 | 20 | 16 |
| A | -- | 1.00 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | -- | -- | -- | -- | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| C | 1.00 | -- | 1.00 | 1.00 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| GPD | | | | | | | | | | | | | | |
| (N) | 24 | 10 | 47 | 10 | 17 | 7 | 39 | 16 | 13 | 13 | 30 | 16 | 21 | 9 |
| A | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- | 1.00 | 1.00 | 1.00 | 1.00 |
| B | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- |
| C | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| GR | | | | | | | | | | | | | | |
| (N) | 11 | 11 | 48 | 8 | 9 | 7 | 27 | 17 | 13 | 16 | 16 | 11 | 17 | 16 |
| A | -- | -- | 0.02 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | 1.00 | -- | 0.95 | 1.00 | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- |
| C | -- | 1.00 | -- | -- | 1.00 | 1.00 | 1.00 | 1.00 | -- | -- | -- | -- | -- | -- |
| D | -- | -- | 0.03 | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | 0.97 | 1.00 |
| E | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- |
| F | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | 0.03 | -- |
| G | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 3 Continued

| Locus | ALEX | AMAZ | CHOA1 | CHOA2 | GLAB1 | GLAB2 | GLAB3 | INTE | OBST | PERE | PFEI1 | PFEI2 | PFEI3 | PFEI4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HBDH | | | | | | | | | | | | | | |
| (N) | 24 | 2 | 48 | 8 | 19 | 15 | 38 | 17 | 15 | 20 | 28 | 16 | 25 | 20 |
| A | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- | -- | -- | -- | -- |
| B | -- | 1.00 | -- | -- | 0.90 | -- | 0.92 | -- | -- | -- | -- | -- | -- | -- |
| C | -- | -- | -- | -- | 0.10 | -- | 0.08 | -- | -- | -- | -- | -- | -- | -- |
| D | 1.00 | -- | 1.00 | 1.00 | -- | -- | -- | 1.00 | 1.00 | 0.77 | 1.00 | 1.00 | 1.00 | 1.00 |
| E | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.23 | -- | -- | -- | -- |
| IDH | | | | | | | | | | | | | | |
| (N) | 25 | 12 | 45 | 11 | 19 | 15 | 38 | 17 | 16 | 19 | 28 | 17 | 25 | 20 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | 1.00 | 1.00 |
| C | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- | -- | -- | -- | -- |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.82 | -- | -- | -- | -- |
| E | 1.00 | 0.96 | 0.78 | 0.68 | 1.00 | -- | 1.00 | -- | 1.00 | -- | 1.00 | -- | -- | -- |
| F | -- | 0.04 | -- | -- | -- | -- | -- | 1.00 | -- | 0.18 | -- | -- | -- | -- |
| G | -- | -- | 0.22 | 0.32 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| H | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| MDH-1 | | | | | | | | | | | | | | |
| (N) | 25 | 11 | 47 | 14 | 19 | 15 | 36 | 17 | 16 | 22 | 26 | 16 | 25 | 16 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- | -- |
| B | -- | -- | 0.18 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| C | 1.00 | 1.00 | 0.82 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- | -- | 1.00 | 1.00 | 1.00 | 1.00 |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- |
| MDH-2 | | | | | | | | | | | | | | |
| (N) | 24 | 10 | 47 | 14 | 18 | 15 | 37 | 17 | 16 | 22 | 27 | 16 | 25 | 16 |
| A | -- | 1.00 | -- | -- | -- | -- | -- | 1.00 | 1.00 | -- | -- | -- | -- | -- |
| B | 1.00 | -- | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 | -- | -- | -- | 1.00 | 1.00 | 1.00 | 1.00 |
| C | -- | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | -- | -- | -- | -- |
| D | -- | -- | 0.01 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 3 Continued

| Locus | ALEX | AMAZ | CHOA1 | CHOA2 | GLAB1 | GLAB2 | GLAB3 | INTE | OBST | PERE | PFEI1 | PFEI2 | PFEI3 | PFEI4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NSP | | | | | | | | | | | | | | |
| (N) | 25 | 12 | 48 | 9 | 15 | 14 | 37 | 18 | 16 | 19 | 28 | 16 | 25 | 20 |
| A | .. | . | .. | . | 0.93 | .. | .. | . | | | | | | |
| B | 0.96 | .. | 0.36 | .. | 0.07 | | .. | . | .. | . | 1.00 | 1.00 | 1.00 | 1.00 |
| C | 0.04 | 1.00 | 0.63 | 1.00 | . | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | .. | . | .. | .. |
| D | .. | .. | .. | .. | .. | . | .. | .. | . | . | .. | .. | .. | .. |
| E | .. | .. | .. | .. | . | .. | .. | .. | .. | . | | | | . |
| F | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | | | | .. |
| G | .. | .. | 0.010 | .. | .. | .. | .. | .. | .. | . | | . | . | . |
| PEPGL | | | | | | | | | | | | | | |
| (N) | 25 | 14 | 46 | 10 | 19 | 15 | 39 | 18 | 17 | 22 | 28 | 19 | 25 | 20 |
| A | 0.04 | 1.00 | 0.82 | 0.45 | 1.00 | 1.00 | 1.00 | 1.00 | 0.06 | .. | .. | .. | .. | .. |
| B | 0.96 | .. | 0.18 | 0.55 | .. | .. | .. | .. | 0.94 | 1.00 | . | 1.00 | 1.00 | 1.00 |
| C | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1.00 | .. | . | .. |
| D | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| LGGP | | | | | | | | | | | | | | |
| (N) | 23 | 11 | 42 | 8 | 12 | 15 | 27 | 12 | 16 | 13 | 14 | 16 | 17 | 19 |
| A | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | . |
| B | .. | .. | .. | .. | .. | .. | .. | .. | .. | 1.00 | .. | .. | .. | .. |
| C | 1.00 | 1.00 | 0.64 | 0.31 | 1.00 | 1.00 | 0.98 | 0.04 | 1.00 | .. | .. | .. | .. | 0.03 |
| D | .. | .. | 0.34 | 0.69 | .. | . | 0.02 | .. | .. | .. | 1.00 | 1.00 | 1.00 | 0.97 |
| E | .. | .. | .. | .. | .. | .. | .. | 0.96 | .. | .. | .. | .. | .. | .. |
| F | .. | .. | 0.01 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| PEPPP | | | | | | | | | | | | | | |
| (N) | 17 | 13 | 48 | 10 | 15 | 11 | 27 | 16 | 13 | 22 | 23 | 10 | 25 | 20 |
| A | .. | 0.04 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| B | 1.00 | 0.88 | 1.00 | 1.00 | 0.90 | 1.00 | 0.98 | 1.00 | 1.00 | .. | 1.00 | 1.00 | 1.00 | 1.00 |
| C | .. | 0.08 | .. | .. | 0.10 | . | 0.02 | .. | .. | .. | .. | .. | .. | .. |
| D | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.84 | .. | .. | .. | .. |
| E | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.16 | .. | .. | .. | .. |

Table 3 Continued

| Locus | ALEX | AMAZ | CHOA1 | CHOA2 | GLAB1 | GLAB2 | GLAB3 | INTE | OBST | PERE | PFEI1 | PFEI2 | PFEI3 | PFEI4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PGD | | | | | | | | | | | | | | |
| (N) | 18 | 10 | 47 | 10 | 6 | 11 | 30 | 15 | 16 | 20 | 24 | 13 | 16 | 20 |
| A | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.13 | .. | .. | .. | .. |
| B | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.50 | .. | .. | .. | .. |
| C | .. | .. | .. | .. | .. | .. | .. | .. | 0.84 | .. | .. | .. | .. | .. |
| D | 0.22 | 1.00 | 0.26 | 0.15 | 0.08 | 1.00 | 1.00 | 0.77 | 0.16 | 0.22 | 1.00 | 1.00 | 1.00 | 1.00 |
| E | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.15 | .. | .. | .. | .. |
| F | .. | .. | .. | .. | .. | .. | .. | 0.23 | .. | .. | .. | .. | .. | .. |
| G | 0.78 | .. | 0.74 | 0.85 | 0.92 | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| PGI | | | | | | | | | | | | | | |
| (N) | 25 | 14 | 48 | 10 | 16 | 15 | 38 | 18 | 16 | 23 | 26 | 16 | 25 | 20 |
| A | .. | .. | .. | .. | 0.81 | .. | 0.06 | .. | .. | .. | .. | .. | .. | . |
| B | . | 1.00 | .. | .. | 0.19 | 1.00 | 0.72 | 1.00 | 1.00 | 0.98 | .. | .. | .. | .. |
| C | 1.00 | .. | 1.00 | 1.00 | .. | .. | 0.22 | .. | .. | 0.02 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGM-1 | | | | | | | | | | | | | | |
| (N) | 15 | 9 | 39 | 5 | 13 | 3 | 36 | 21 | 13 | 16 | 22 | 7 | 17 | 8 |
| A | .. | .. | 0.01 | .. | .. | .. | .. | .. | .. | . | 1.00 | 0.71 | 0.76 | 0.31 |
| B | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.06 | .. | .. | .. | .. |
| C | .. | .. | .. | .. | .. | .. | .. | .. | 0.92 | .. | .. | .. | .. | .. |
| D | .. | .. | .. | .. | 1.00 | 1.00 | .. | 1.00 | .. | 0.19 | .. | .. | .. | .. |
| E | 1.00 | .. | 0.94 | 0.70 | .. | .. | .. | .. | .. | .. | .. | 0.29 | 0.24 | 0.69 |
| F | .. | .. | .. | .. | .. | .. | 1.00 | .. | 0.08 | 0.41 | .. | .. | .. | .. |
| G | .. | 1.00 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| H | .. | .. | 0.05 | 0.30 | .. | .. | .. | .. | .. | .. | . | .. | .. | .. |
| I | .. | .. | .. | .. | .. | .. | .. | .. | .. | 0.34 | .. | .. | .. | .. |
| XDH | | | | | | | | | | | | | | |
| (N) | 24 | 3 | 48 | 9 | 15 | 15 | 29 | 11 | 16 | 19 | 28 | 15 | 23 | 20 |
| A | .. | .. | .. | .. | 0.10 | 0.13 | 0.10 | .. | .. | 0.03 | . | . | .. | |
| B | .. | .. | .. | .. | .. | .. | .. | . | . | | . | .. | .. | .. |
| C | .. | .. | .. | . | 0.90 | 0.87 | 0.90 | .. | 1.00 | 0.95 | . | .. | .. | |
| D | .. | .. | .. | .. | . | . | . | 1.00 | .. | 0.02 | | | . | .. |
| E | 0.83 | 1.00 | 1.00 | 1.00 | .. | .. | | .. | .. | .. | 1.00 | .. | .. | 1.00 |
| F | . | .. | .. | .. | .. | . | . | .. | . | . | | 1.00 | 1.00 | |
| G | 0.17 | .. | .. | .. | .. | .. | .. | .. | .. | | | | | |

Table 3 Continued

| Locus | STRA1 | STRA2 | STRA3 | SUDA1 | SUDA2 | SUDA3 | SUDA4 | SUDA5 | TENA1 | TENA2 | TENA3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ACN-1 | | | | | | | | | | | |
| (N) | 12 | 15 | 16 | 10 | 28 | 22 | 14 | 16 | 11 | 18 | 11 |
| A | -- | . | -- | -- | . | . | . | | . | . | . |
| B | 0 75 | 0 13 | -- | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 0 91 | 1 00 | 0 91 |
| C | 0 25 | 0 87 | 1 00 | -- | -- | -- | -- | | 0 09 | -- | 0 09 |
| D | -- | . | -- | -- | -- | -- | . | . | | | |
| ACN-2 | | | | | | | | | | | |
| (N) | 8 | 11 | 7 | 10 | 25 | 14 | 15 | 7 | 9 | 15 | 16 |
| A | 1 00 | 1 00 | 1 00 | -- | -- | 0 04 | -- | . | 0 89 | 1 00 | 0 91 |
| B | -- | -- | -- | 1 00 | 0 94 | 0 93 | 1 00 | 1 00 | 0 11 | -- | 0 09 |
| C | -- | -- | -- | -- | 0 06 | 0 03 | . | -- | -- | -- | |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| AKP | | | | | | | | | | | |
| (N) | 14 | 15 | 18 | 10 | 33 | 22 | 11 | 16 | 9 | 18 | 17 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | 0 22 | 0 17 | 0 06 |
| B | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 0 86 | 1 00 | 0 94 | 0 78 | 0 83 | 0 94 |
| C | -- | -- | -- | -- | -- | 0 14 | -- | 0 06 | -- | -- | -- |
| CAT | | | | | | | | | | | |
| (N) | 16 | 16 | 17 | 10 | 25 | 22 | 11 | 14 | 14 | 18 | 17 |
| A | 0 09 | 0 13 | -- | -- | -- | -- | -- | -- | -- | . | -- |
| B | 0 57 | 0 65 | 0 41 | 0 200 14 | 0 36 | 0 18 | 0 04 | 0 11 | -- | -- | |
| C | 0 34 | 0 22 | 0 59 | 0 80 | 0 86 | 0 64 | 0 82 | 0 96 | 0 89 | 1 00 | 1 00 |
| Ck | | | | | | | | | | | |
| (N) | 11 | 11 | 16 | 10 | 21 | 22 | 15 | 15 | 9 | 5 | 14 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | . |
| B | 0 54 | 0 59 | 0 69 | -- | -- | -- | -- | -- | 0 56 | -- | 0 36 |
| C | 0 46 | 0 41 | 0 31 | -- | -- | -- | -- | -- | 0 44 | 1 00 | 0 64 |
| D | -- | -- | -- | 0 70 | 0 21 | 0 55 | 0 27 | 0 73 | -- | -- | -- |
| E | -- | -- | . | 0 25 | 0 71 | 0 41 | 0 73 | 0 27 | -- | -- | -- |
| F | -- | -- | . | 0 05 | 0 07 | 0 05 | -- | . | -- | -- | -- |
| G | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 3. Continued

| Locus | STRA1 | STRA2 | STRA3 | SUDA1 | SUDA2 | SUDA3 | SUDA4 | SUDA5 | TENA1 | TENA2 | TENA3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EST-2 | | | | | | | | | | | |
| (N) | 14 | 10 | 11 | 9 | 16 | 12 | 11 | 14 | 10 | 18 | 16 |
| A | -- | 1 00 | 0 59 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 0 75 | -- | -- |
| B | 1 00 | -- | 0 41 | -- | -- | -- | -- | -- | 0 25 | 1 00 | 1 00 |
| GOT-1 | | | | | | | | | | | |
| (N) | 13 | 16 | 16 | 10 | 28 | 22 | 23 | 16 | 14 | 18 | 16 |
| A | 0 08 | 0 94 | 0 72 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 |
| B | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| C | 0 92 | 0 06 | 0 28 | -- | -- | -- | -- | -- | -- | -- | -- |
| GOT-2 | | | | | | | | | | | |
| (N) | 10 | 11 | 10 | 10 | 17 | 18 | 15 | 16 | 13 | 17 | 17 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | 1 00 | 1 00 | 1 00 | -- | -- | -- | -- | -- | 1 00 | 1 00 | 1 00 |
| C | -- | -- | -- | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | -- | -- | -- |
| GPD | | | | | | | | | | | |
| (N) | 14 | 14 | 12 | 10 | 33 | 22 | 15 | 16 | 6 | 13 | 9 |
| A | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 | -- | -- | -- |
| B | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| C | -- | -- | -- | -- | -- | -- | -- | -- | 1 00 | 1 00 | 1 00 |
| GR | | | | | | | | | | | |
| (N) | 10 | 9 | 12 | 10 | 20 | 22 | 15 | 16 | 12 | 12 | 11 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | 0 95 | 0 78 | 0 13 | 1 00 | 0 95 | 0 89 | 1 00 | 1 00 | 1 00 | 1 00 | 1 00 |
| C | 0 05 | 0 22 | 0 87 | -- | -- | -- | -- | -- | -- | -- | -- |
| D | -- | -- | -- | -- | 0 05 | 0 09 | -- | -- | -- | . | -- |
| E | -- | -- | -- | -- | -- | -- | -- | -- | -- | . | -- |
| F | -- | -- | -- | -- | -- | -- | -- | -- | -- | . | -- |
| G | -- | -- | -- | -- | -- | 0 02 | -- | -- | -- | -- | -- |

Table 3 Continued

| Locus | STRA1 | STRA2 | STRA3 | SUDA1 | SUDA2 | SUDA3 | SUDA4 | SUDA5 | TENA1 | TENA2 | TENA3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HBDH | | | | | | | | | | | |
| (N) | 16 | 15 | 14 | 9 | 27 | 22 | 15 | 16 | 12 | 16 | 17 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | | |
| B | -- | -- | -- | -- | -- | -- | -- | -- | | | |
| C | -- | -- | -- | -- | -- | -- | -- | -- | 0.92 | 0.97 | 1.00 |
| E | -- | 0.60 | -- | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.08 | | |
| E | 1.00 | 0.40 | 1.00 | -- | -- | -- | -- | -- | -- | 0.03 | -- |
| IDH | | | | | | | | | | | |
| (N) | 16 | 15 | 16 | 10 | 29 | 21 | 14 | 16 | 14 | 18 | 16 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | 1.00 | 1.00 |
| B | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| C | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| E | -- | -- | -- | 1.00 | 1.00 | 0.98 | 0.96 | 0.72 | -- | -- | -- |
| F | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| G | -- | -- | -- | -- | -- | 0.02 | 0.04 | 0.28 | -- | -- | -- |
| H | 1.00 | 1.00 | 1.00 | -- | -- | -- | -- | -- | -- | -- | -- |
| MDH-1 | | | | | | | | | | | |
| (N) | 17 | 16 | 11 | 10 | 29 | 22 | 15 | 20 | 15 | 17 | 17 |
| A | -- | 0.06 | -- | -- | -- | -- | -- | -- | 1.00 | 1.00 | 1.00 |
| B | -- | 0.06 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| C | 1.00 | 0.88 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- | -- | -- |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| MDH-2 | | | | | | | | | | | |
| (N) | 16 | 16 | 16 | 10 | 29 | 22 | 15 | 20 | 15 | 18 | 17 |
| A | 1.00 | 1.00 | 1.00 | -- | -- | -- | -- | -- | 1.00 | 1.00 | 1.00 |
| B | -- | -- | -- | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- | -- | -- |
| C | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 3 Continued

| Locus | STRA1 | STRA2 | STRA3 | SUDA1 | SUDA2 | SUDA3 | SUDA4 | SUDA5 | TENA1 | TENA2 | TENA3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NSP | | | | | | | | | | | |
| (N) | 16 | 17 | 16 | 10 | 27 | 19 | 15 | 13 | 14 | 18 | 17 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| B | -- | -- | -- | -- | -- | 0.03 | -- | -- | -- | -- | -- |
| C | 1.00 | 1.00 | 1.00 | 0.65 | 0.93 | 0.89 | -- | 1.00 | 1.00 | 0.94 | 1.00 |
| D | -- | -- | -- | 0.35 | -- | 0.08 | 1.00 | -- | -- | -- | -- |
| E | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.06 | -- |
| F | -- | -- | -- | -- | 0.07 | -- | -- | -- | -- | -- | -- |
| G | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| PEPGL | | | | | | | | | | | |
| (N) | 15 | 17 | 16 | 10 | 15 | 22 | 15 | 16 | 17 | 18 | 17 |
| A | 1.00 | 1.00 | 1.00 | 0.60 | 0.53 | 0.52 | 0.97 | 0.44 | 1.00 | 1.00 | 1.00 |
| B | -- | -- | -- | 0.30 | 0.47 | 0.48 | 0.03 | 0.56 | -- | -- | -- |
| C | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| D | -- | -- | -- | 0.10 | -- | -- | -- | -- | -- | -- | -- |
| LGGP | | | | | | | | | | | |
| (N) | 12 | 10 | 18 | 10 | 23 | 21 | 12 | 15 | 13 | 13 | 15 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | 0.58 | 0.43 | |
| B | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| C | 1.00 | 1.00 | 1.00 | -- | 0.56 | 0.95 | -- | 0.70 | 0.38 | 0.65 | 0.50 |
| D | -- | -- | -- | 1.00 | 0.44 | 0.05 | 1.00 | 0.30 | -- | -- | -- |
| E | -- | -- | -- | -- | -- | -- | -- | -- | 0.04 | -- | 0.07 |
| F | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| PEPPP | | | | | | | | | | | |
| (N) | 16 | 15 | 13 | 10 | 20 | 22 | 15 | 16 | 16 | 18 | 17 |
| A | -- | -- | -- | -- | -- | -- | -- | -- | 1.00 | 0.94 | 0.65 |
| B | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | -- | 0.06 | 0.35 |
| C | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | |
| D | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | |
| E | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 3 Continued

| Locus | STRA1 | STRA2 | STRA3 | SUDA1 | SUDA2 | SUDA3 | SUDA4 | SUDA5 | STRA1 | STRA2 | STRA3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PGD | | | | | | | | | | | |
| (N) | 17 | 16 | 15 | 5 | 16 | 17 | 10 | 11 | 14 | 15 | 12 |
| A | : | : | : | : | : | : | : | : | : | : | : |
| B | : | : | : | : | : | : | : | : | 1.00 | 1.00 | 1.00 |
| C | : | : | : | : | : | : | : | : | : | : | : |
| D | 0.91 | 1.00 | 1.00 | 0.20 | : | 0.03 | : | 0.23 | : | : | : |
| E | 0.09 | : | : | : | : | : | : | : | : | : | : |
| F | : | : | : | : | : | : | : | : | : | : | : |
| G | : | : | : | 0.80 | 1.00 | 0.97 | 1.00 | 0.77 | : | : | : |
| PGI | | | | | | | | | | | |
| (N) | 19 | 18 | 19 | 10 | 16 | 16 | 11 | 16 | 18 | 18 | 17 |
| A | 0.21 | : | 0.03 | : | : | : | : | : | : | : | : |
| B | 0.11 | 1.00 | 0.97 | : | : | : | : | : | : | : | : |
| C | 0.68 | : | : | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGM-1 | | | | | | | | | | | |
| (N) | 16 | 14 | 16 | 10 | 20 | 18 | 14 | 16 | 9 | 15 | 10 |
| A | : | : | : | 0.50 | 0.50 | : | : | 0.09 | : | : | : |
| B | : | : | : | : | : | : | : | : | : | : | : |
| C | : | : | : | : | : | : | : | : | : | : | : |
| D | : | : | : | : | : | : | : | : | : | : | : |
| E | : | : | : | 0.40 | 0.40 | 0.97 | 1.00 | 0.91 | : | : | : |
| F | : | : | : | : | : | : | : | : | 1.00 | 1.00 | 1.00 |
| G | 1.00 | 1.00 | 1.00 | : | : | : | : | : | : | : | : |
| H | : | : | : | 0.10 | 0.10 | 0.03 | : | : | : | : | : |
| I | : | : | : | : | : | : | : | : | : | : | : |
| XDH | | | | | | | | | | | |
| (N) | 13 | 17 | 7 | 10 | 28 | 22 | 15 | 16 | 10 | 16 | 12 |
| A | : | : | : | : | : | : | : | : | 0.10 | 0.16 | 0.08 |
| B | : | : | : | 0.05 | : | : | 1.00 | : | : | : | : |
| C | 1.00 | 0.82 | : | : | : | : | : | : | 0.90 | 0.84 | 0.92 |
| D | : | : | : | : | : | : | : | : | : | : | : |
| E | : | 0.18 | 1.00 | 0.95 | 1.00 | 1.00 | : | 1.00 | : | : | : |
| F | : | : | : | : | : | : | : | : | : | : | : |

Table 4. Genetic variability measures. A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95. The estimate of expected hetozygosity under Hardy-Weinberg equilibrium follows Nei (1978). Standard errors are inparentheses.

| Population | Mean sample size per Locus | Mean no. of alleles per locus | Percentage of loci polymorphic | Mean heterozygosity observed | Mean heterozygosity expected |
|---|---|---|---|---|---|
| ALEX | 19.9 (1.2) | 1.2 (.1) | 13.6 | .016 (.008) | .054 (.026) |
| AMAZ | 9.5 (.8) | 1.2 (.1) | 13.6 | .034 (.021) | .042 (.022) |
| CHOA1 | 45.1 (.9) | 1.8 (.2) | 59.1 | .126 (.033) | .192 (.043) |
| CHOA2 | 9.0 (.5) | 1.4 (.1) | 40.9 | .088 (.033) | .155 (.044) |
| GLAB1 | 15.5 (.9) | 1.4 (.1) | 36.4 | .079 (.024) | .087 (.029) |
| GLAB2 | 12.0 (.9) | 1.0 (.0) | 4.5 | .006 (.006) | .011 (.011) |
| GLAB3 | 33.4 (1.1) | 1.5 (.1) | 27.3 | .088 (.031) | .099 (.038) |
| INTE | 17.2 (.6) | 1.3 (.1) | 18.2 | .051 (.021) | .087 (.037) |
| OBST | 14.9 (.4) | 1.2 (.1) | 22.7 | .035 (.016) | .048 (.021) |
| PERE | 18.6 (.7) | 1.6 (.2) | 36.4 | .110 (.043) | .137 (.047) |
| PFEI1 | 24.6 (1.0) | 1.0 (.0) | 0.0 | .002 (.002) | .002 (.002) |
| PFEI2 | 13.9 (.8) | 1.1 (.1) | 13.6 | .041 (.028) | .039 (.023) |
| PFEI3 | 21.5 (1.0) | 1.1 (.1) | 9.1 | .010 (.006) | .031 (.020) |
| PFEI4 | 17.2 (.7) | 1.1 (.1) | 9.1 | .038 (.029) | .030 (.022) |
| STRA1 | 14.1 (.6) | 1.4 (.1) | 31.8 | .043 (.017) | .109 (.041) |
| STRA2 | 14.3 (.6) | 1.5 (.1) | 36.4 | .083 (.030) | .127 (.041) |
| STRA3 | 14.2 (.7) | 1.3 (.1) | 22.7 | .019 (.008) | .098 (.040) |
| SUDA1 | 9.7 (.2) | 1.5 (.2) | 31.8 | .100 (.043) | .133 (.047) |
| SUDA2 | 23.9 (1.2) | 1.5 (.1) | 36.4 | .044 (.018) | .121 (.043) |
| SUDA3 | 20.1 (.6) | 1.7 (.2) | 31.8 | .088 (.032) | .117 (.038) |
| SUDA4 | 14.1 (.6) | 1.2 (.1) | 9.1 | .035 (.020) | .039 (.023) |
| SUDA5 | 15.3 (.6) | 1.4 (.1) | 31.8 | .057 (.024) | .114 (.039) |

Table 4. Continued

| Population | Mean sample size per Locus | Mean no. of alleles per locus | Percentage of loci polymorphic | Mean heterozygosity observed | Mean heterozygosity expected |
|---|---|---|---|---|---|
| TENA1 | 12.5 (.6) | 1.5 (.1) | 40.9 | .052 (.023) | .125 (.038) |
| TENA2 | 16.0 (.7) | 1.3 (.1) | 22.7 | .026 (.012) | .059 (.027) |
| TENA3 | 15.0 (.6) | 1.4 (.1) | 31.8 | .024 (.012) | .098 (.038) |

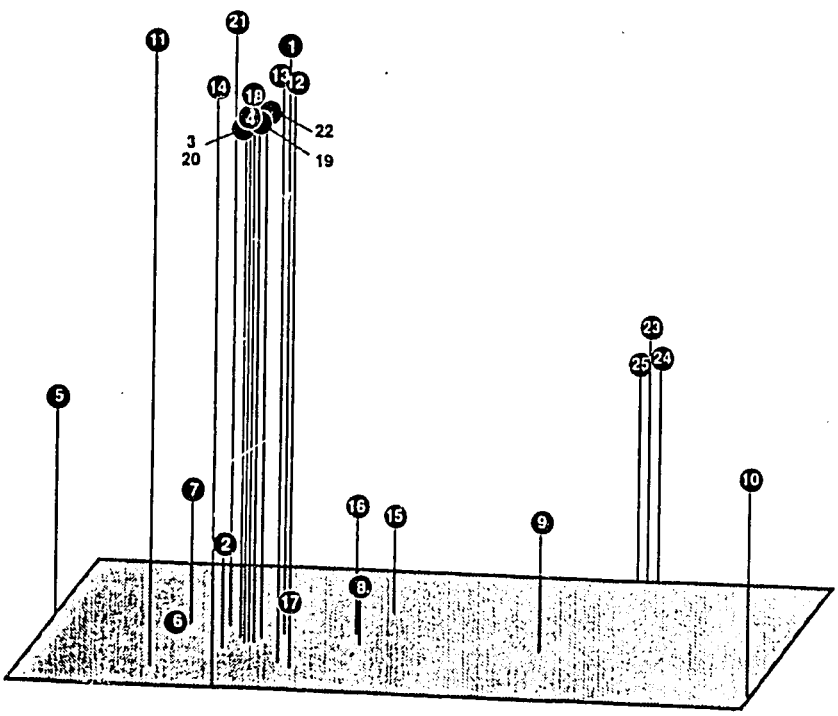
Table 5. Hierarchical G-statistics. The relative contributions of variation among individuals within populations, among populations within species, and among species is described below using the hierarchical G statistics of Nei (1973). The variance among species corresponds to $D_{ST}/H_T$, variance among populations to $D_{LS}/H_T$, and variance among individuals to $H_L/H_T$ (see text).

| | All species | African species | New World species |
|---|---|---|---|
| variance among species | 0.584 | 0.278 | .527 |
| variance among populations | 0.249 | 0.423 | .292 |
| variance among individuals | 0.167 | 0.299 | .181 |

Table 7 Levels of genetic variability in natural populations of Biomphalaria compared with B. glabrata M-line. $N_s$ = number of samples, $N_l$ = number of loci, P = Proportion of polymorphic, H = mean heterozygosity per sample.

| | $N_s$ | $N_l$ | P | H | Reference |
|---|---|---|---|---|---|
| B. glabrata | | | | | |
| Puerto Rico | 7 | 26 | 0.15 | 0-0.6 | [12] |
| Dom. Republic | 2 | 21 | 0.19-0.24 | 0.022-0.027 | [13] |
| St. Lucia | 2 | 21 | 0.09-0.24 | 0.003-0.037 | |
| Brazil | | | 0.18-0.48 | 0.076-0.211 | [11] |
| B. alexandrina | | | | | |
| Qalubia | 7 | 26 | 0.16-0.19 | 0.044-0.092 | [9] |
| B. straminea | | | | | |
| Hong Kong | 4 | 19 | 0.26 | 0.056-0.097 | [14] |
| B. pfeifferi | | | | | |
| Kenya | 12 | 10 | 0.00-0.40 | | [15] |
| B. glabrata | | | | | |
| M-line | 5 | 24 | 0.29-0.33 | 0.051-0.098 | [Mulvey & Bandoni, in preparation] |
| B. cameronensis | 32 | 15 | 0.00-.11 | 0.000-0.59 | [23] |
| B. pfeifferi | 19 | 18 | 0.00-.11 | 0.000-0.022 | [24] |

Figure 1 Three dimensional plot of distances among taxa after multidimensional scaling of Roger's genetic distances. Populations are identified by the same numbers used in Table 1.



## Appendix #13

Performance of the Ampullariid Snails, Pila ovata and Lanistes carinatus in Small Man-Made Impoundments Located in the Tala Area, Machakos District of Kenya.

The African ampullariid snails, Pila ovata and Lanistes carinatus are present in Kenya, but seem to occur only in the coastal lowlands and in or around Lake Victoria in western Kenya (Brown, 1980; Loker et al, in press), where warm tropical climatic conditions prevail. Whereas, P. ovata occurs in both coastal Kenya and within the Lake Victoria basin, L. carinatus is only restricted to the coastal area and at altitudes below 200m above sea level. These ampullariid species do not seem to occur at altitudes above 1500m were temperatures tend to be cool. Our interest in the two ampullariid species arises from observations that they may have a role in biological control of medically important pulmonate snails (Hofkin et al. 1991; Stryker et al, 1991; Loker et al, In Press; Mkoji, unpublished results). Loker et al (in press) observed that in coastal lowlands L. carinatus was negatively associated with the pulmonate snails, Bulinus africanus and Lymnaea natalensis. In the Lake Victoria area, P. ovata was negatively associated with the pulmonates, Biomphalaria pfeifferi and L. natalensis, B. pfeifferi, members of Bulinus africanus group and L. natalensis do also occur in localities outside the known geographical range of P. ovata or L. carinatus in Kenya. Some of these areas include the area occupied by the Machakos and Kitui Districts, where schistosomiasis is also endemic. This is a relatively dry area, and unlike the coast or Lake Victoria, the dry seasons here tend to be severe. If the African ampullariids prove to be promising in biological control of medically important snails in Kenya, will they be useful in localities outside their known distributional range here? Will they be able to survive and establish populations in the relatively dry areas of the country where schistosomiasis is a major public health problem? We introduced P. ovata and L. carinatus in small man-made impoundments located in the Tala area of Machakos District, Kenya, and we here provide a report on their performance at these sites.

P. ovata were collected from natural habitats in Kisumu on the shores of Lake Victoria, and L. carinatus was collected from Malindi on the Kenyan coast. They were transported within 48 hr of collection to the introduction sites in Tala, about 60 km east of Nairobi. The ampullariid introduction sites consisted of four small man-made impoundments, selected on the basis of presence of water, presence of pulmonate snail species, and easy accessibility. These sites were named Koma Rock, Tala Township, Maluva, and Kisukioni, and the following is a description of each of the sites: Koma Rock was a small shallow pond measuring about 0.1 ha when water level is highest. It is used mainly for watering domestic animals. It experiences large seasonal fluctuations in water levels, and may dry up completely for 2-3 months, in the dry seasons. Aquatic vegetation where present and included waterlily (Nymphaea). The snail species commonly found here, were Bulinus africanus, Bulinus forskalii and the prosobranch Bellamya unicolor. Tala Township site is a smaller dam about 0.8 ha in area, and fairly shallow along the shoreline. The site experiences minimal seasonal fluctuations in water levels, and retains water

throughout the year. At the time of ampullariid introductions, waterlily plants and Polygonum senegalensis were common. The site is used as a source of water for domestic use, building construction and watering animals. The species present at this site prior to ampullariid introductions, were members of Bulinus africanus group and L. natalensis. Maluva is a pond measuring about 0.2 ha in area. It is shallow along the shoreline and waterlily (Nymphaea) is common. It experiences marked seasonal fluctuations in water levels and during the dry seasons water level is usually very low. The site is used for watering domestic animals or as a recreational site for children. The snail species present here were B. africanus, L. natalensis and only occasionally, B. forskalii. Kisukioni is a dam measuring about 0.6 ha in area. About 40% of the shoreline is shallow and covered Nymphaea. The water level remains fairly stable for most of the year but towards the end of the long dry season (September-October) there is usually very little water here. The site is used as a source of water for domestic purposes, for watering animals, and as a recreational site for children. The snail species present here were B. africanus, L. natalensis and B. unicolor. Other aquatic species commonly present at the four sites included cichlid fish, frog tadpoles, several arthropod species and birds. Prior to ampullariid introductions, 5-6 sampling stations were selected along the shoreline of each site. Each station measured 10m long and was selected on the basis of its suitability for easy access for snail sampling. The same stations were sampled during the entire study duration. Ampullariids were introduced at the selected sites in the month of February 1989. Each station received 250 individuals of the ampullariid species. P. ovata was introduced in Koma Rock, Tala Township and Maluva, and L. carinatus in Kisukioni. Following ampullariid introductions the sites were visited for sampling regularly in the months of May, August, November, and February from 1989 to 1993. During sampling 5 min snail searches were conducted at each sampling station. The snails collected were identified, enumerated and subsequently returned to the habitat. For the purpose of presenting the results, the number of snails of each site was summed across the sampling stations, thus providing a measure of the overall relative abundance of different snail species in each of the study sites.

The patterns of snail abundance at each study site are shown in Figures 1-4. In general, the introduced ampullariid species survived in their new habitats for several months as evidenced by snail recoveries during sampling in subsequent months. Six to 8 months following the introductions, egg masses (for L. carinatus or juvenile snails (for P. ovata) were observed. Overall, there were variations in the performance of the ampullariids at the different sites. For instance, although P. ovata persisted in Koma Rock, it hardly increased in abundance (Figure 1). In Maluva Pond, abundance of P. ovata increased only slightly about 12 months after introduction. Ampullariid numbers then declined over the next 18 months, and remained low (Figure 2). Overall, ampullariid numbers recovered from Koma Rock or Maluva at any one time have, in general, been low. In contrast, performance of P. ovata in Tala Township Dam was impressive. Ampullariid numbers increased appreciably, reaching an impressive peak during the second year after introduction (Figure 3). Although abundance of P. ovata declined dramatically by the end of the second year, the ampullariid has remained relatively abundant here, four years after the first introduction. Based on results a the Koma Rock Pond, which dries up completely in the dry season, P. ovata is capable of surviving drying of a habitat. Although the snails disappear from the site

when it dries up, they return when the site receives water from the rains. Presence of P. ovata at Koma Rock or Maluva did not result in the decline in number or disappearance of pulmonate snail species previously present here. Although pulmonate populations fluctuated seasonally, they have remained common, and on many occasions, in greater abundance that the ampullariids. The prosobranch B. unicolor occurred sporadically in Koma Rock, and always in low numbers. The abundance of waterlily plants (Nymphaea) in Koma Rock or Maluva Ponds were also not affected noticeably by the presence of Pila here. On the other hand, the presence of P. ovata in Tala Township Dame resulted in a marked decline in the abundance of B. africanus and L. natalensis, which were initially common here during the first 18 months of ampullariid introduction. Pulmonate species have since become very rare here. The waterlily plants have also disappeared. Apparently, at Koma Rock and Maluva, P. ovata tended to be positively associated with the pulmonate species (see Table). At the Tala Township Dam, however, the ampullariid negatively correlated with the pulmonates species. At Kisukioni dam, L. carinatus increased appreciably, and was fairly abundant during the first 24 months (Figure 4). Since then, however, the ampullariid has declined in abundance and the numbers are fairly low here. As for the pulmonate species, L. natalensis was generally present in low numbers. It is therefore difficult to determine if L. carinatus abundance was accompanied with decrease in the relative abundance of B. africanus, and for several months the later species became rare at this site. However, with the decline in ampullariid abundance, B. africanus seems to have become common here again. Similarly, following L. carinatus introduction, B. unicolor, which was initially present here in great abundance, also decreased in number dramatically and became rare. A recent visit to this site, however, indicates that B. unicolor is coming back. L. carinatus was positively correlated with the pulmonate species and B. unicolor at this site (see Table). It is not clear why abundance of L. carinatus declined to such a low level in Kisukioni Dam. Interestingly, we noted that shells of many of the L. carinatus collected had holes a the shell umbilicus, suggesting weakness of shell structure. Also, on several occasions, we observed numerous broken shells of Lanistes at this site, indicating that predator may be present here. Overall, the ampullariid did not have any negative impact on waterlily plants here.

TABLE. Spearman's rank correlation coefficients between resident snail species and the introduced ampullariid species for the four study sites near Tala, in the Machakos District of Kenya.

| Resident Snail Species | Introduced Ampullariid Species | |
|---|---|---|
| | *P. ovata* | *L. carinatus* |
| KOMA ROCK | | |
| Bul. africanus | 0.321 | - |
| Bul. forskalii | 0.286 | - |
| Bellamyia unicolor | 0.236 | - |
| TALA TOWNSHIP | | |
| Bul. africanus | -0.418 | - |
| L. natalensis | -0.407 | - |
| MALUVA | | |
| Bul. africanus | 0.421 | - |
| L. natalensis | 0.154 | - |
| KISUKIONI | | |
| Bul. africanus | - | 0.082 |
| L. natalensis | - | 0.470 * |
| Bellamyia unicolor | - | 0.229 |

* indicates significant correlation

REFERENCES:Brown D.S. 1980. Freshwater snails of Africa and their medical importance. Taylor & Francis, Ltd. London.
Hofkin BV et al. 1991. Acta Trop. 49: 37-44.
Loker ES et al. J. Med. Appl. Malacology, In Press.
Stryker GA et al. 1991. Acta Trop. 49: 137-147.

FIGURE 1

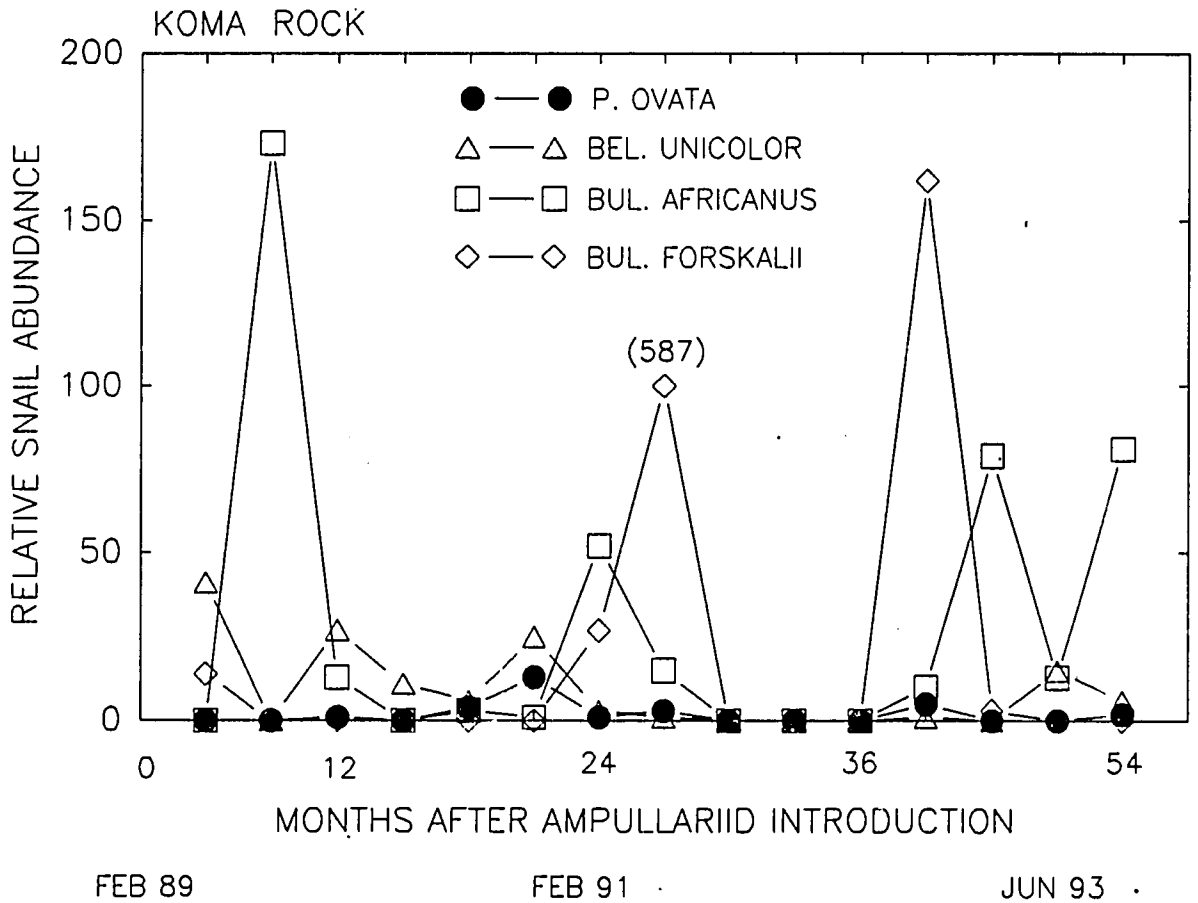

KOMA ROCK
200
150
100
50
0
RELATIVE SNAIL ABUNDANCE
P. OVATA
BEL. UNICOLOR
BUL. AFRICANUS
BUL. FORSKALII
(587)
0
12
24
36
54
MONTHS AFTER AMPULLARIID INTRODUCTION
FEB 89
FEB 91
JUN 93

FIGURE 2

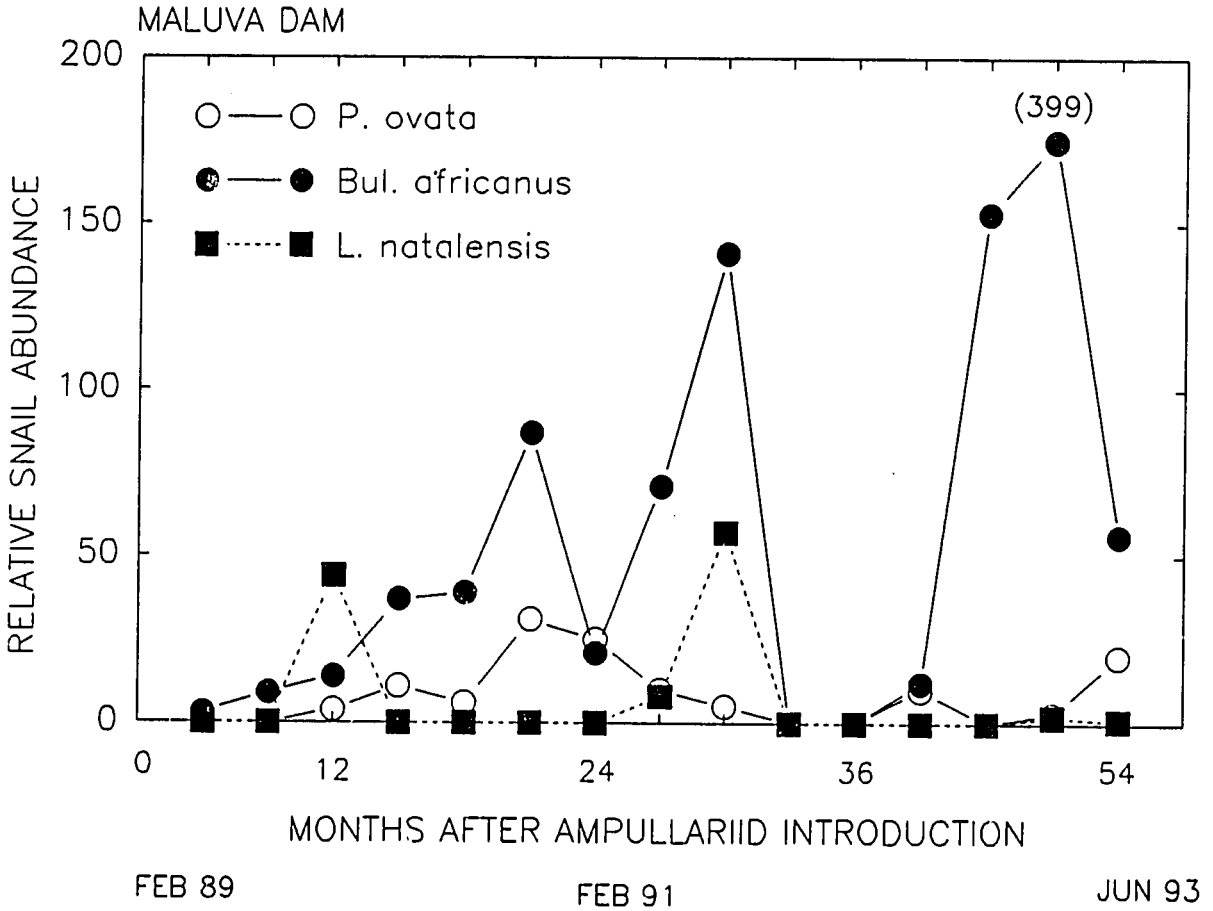

MALUVA DAM
200
150
100
50
0
RELATIVE SNAIL ABUNDANCE
P. ovata
Bul. africanus
L. natalensis
(399)
0
12
24
36
54
MONTHS AFTER AMPULLARIID INTRODUCTION
FEB 89
FEB 91
JUN 93

FIGURE 3

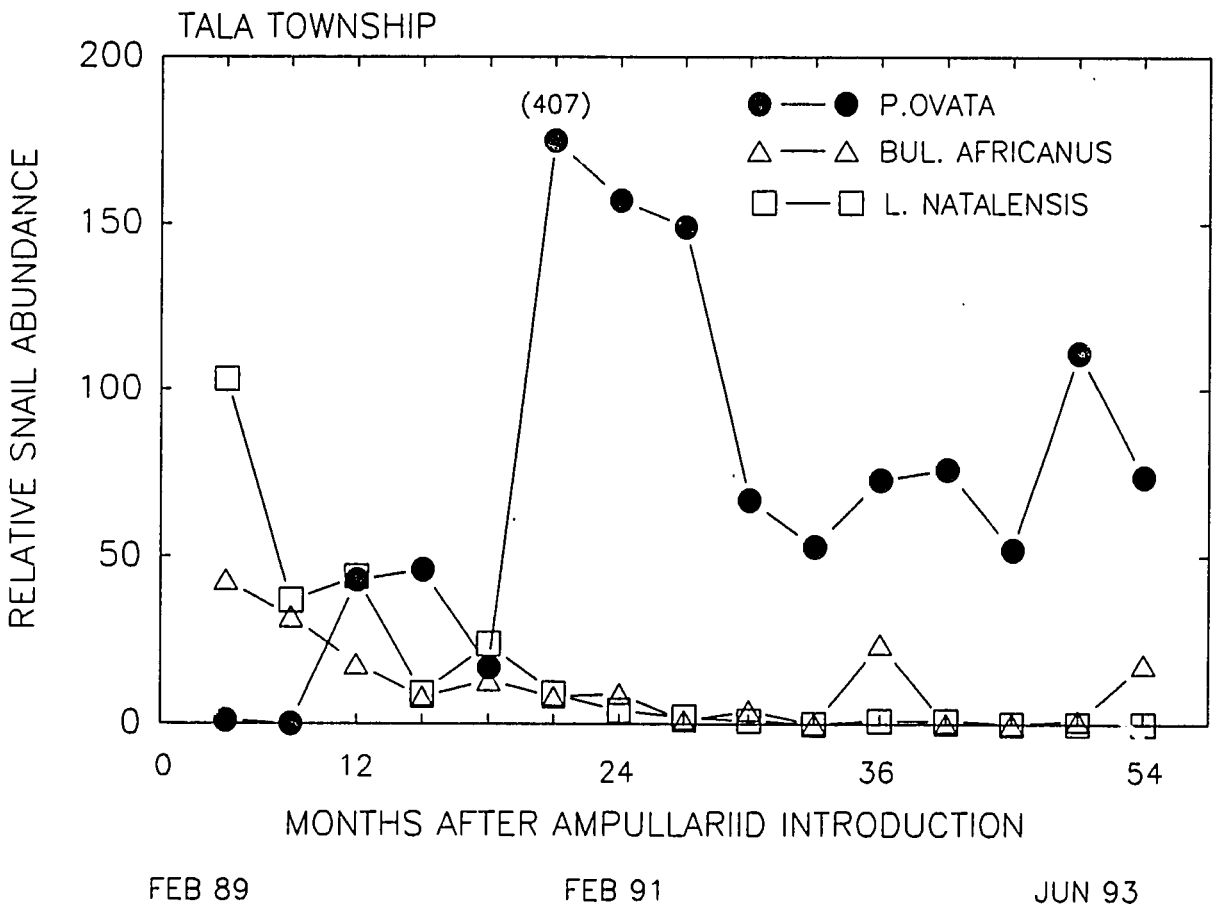
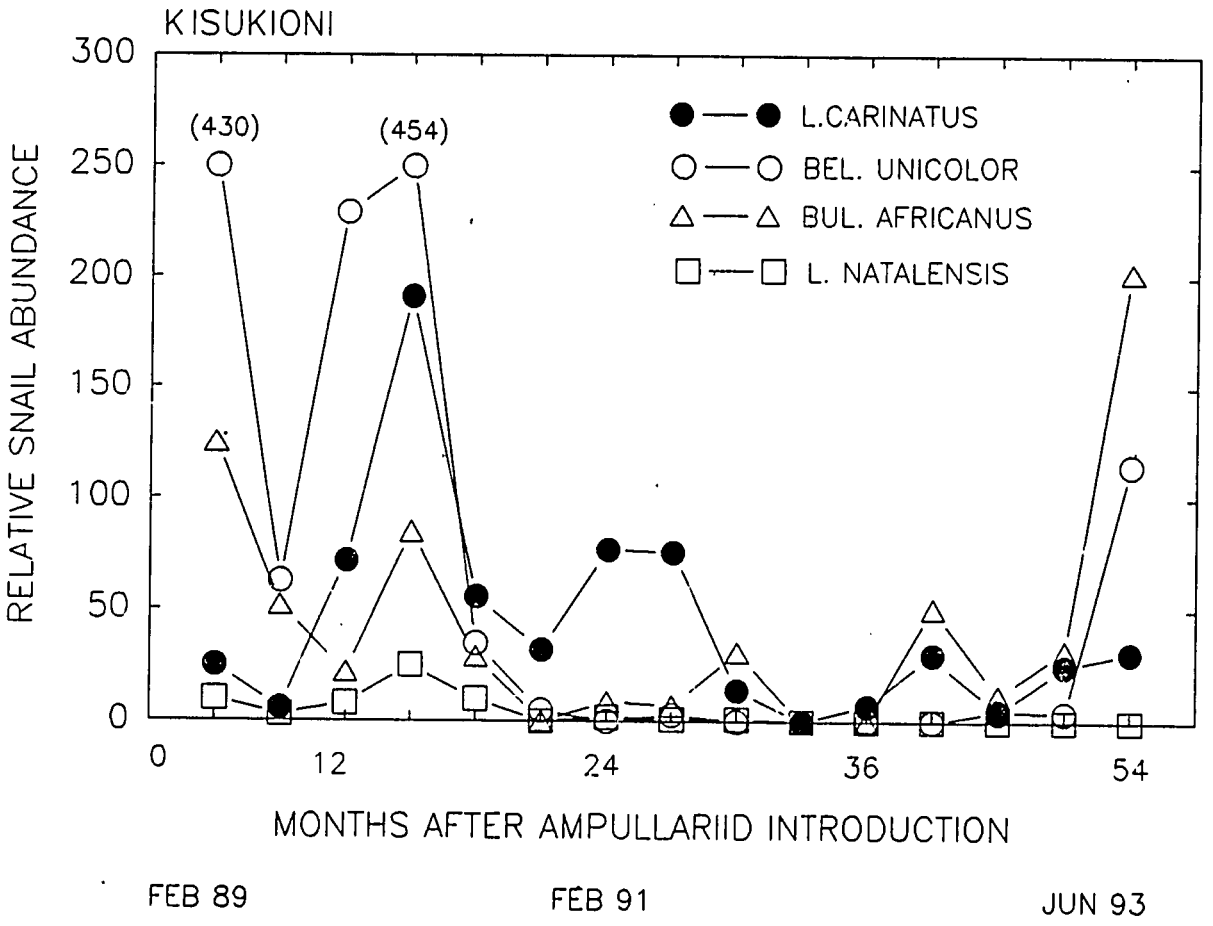

TALA TOWNSHIP
200
150
100
50
0
RELATIVE SNAIL ABUNDANCE
(407)
P.OVATA
BUL. AFRICANUS
L. NATALENSIS
0
12
24
36
54
MONTHS AFTER AMPULLARIID INTRODUCTION
FEB 89
FEB 91
JUN 93

FIGURE 4


KISUKIONI
RELATIVE SNAIL ABUNDANCE
300
250
200
150
100
50
0
(430)
(454)
L.CARINATUS
BEL. UNICOLOR
BUL. AFRICANUS
L. NATALENSIS
0
12
24
36
54
MONTHS AFTER AMPULLARIID INTRODUCTION
FEB 89
FEB 91
JUN 93

## Appendix #14

Predation of aquatic stages of the mosquito Anopheles gambiae by the crayfish Procambarus clarkii under laboratory conditions

The Louisiana red swamp crayfish, Procambarus clarkii, a decapod crustacean, is widespread in Kenya (Lowery & Mendes, 1977; Oluoch, 1990; Loker et al, 1992). Recent studies have indicated that, P. clarkii can control populations of freshwater pulmonate snails that transmit schistosomiasis or other medically important parasitic infections, through predation and/or consumption of macrophytes used by the snails for shelter, oviposition, or as food source (Hofkin et al, 1991a,b; 1992; Mkoji et al, 1992). Elsewhere, it has also been shown that crayfish can control aquatic stages of certain mosquito species, through elimination of macrophytes used by mosquito stages for shelter (see Feminella & Resh, 1986). In Kenya, and other tropical regions anopheline and culicine mosquitoes are considered to be of great medical significance. They serve as vectors for a number of medically important infections including malaria filariasis and certain viral infections. The use of biological agents to control mosquitoes is well documented, and among the organisms with potential in this regard include microbial pathogen, hydrozoans, mermithid nematodes and larvivorous fish (Champan, 1985). We report here that, P. clarkii is capable of consuming, under laboratory conditions, larvae or pupae of the anopheline mosquito, Anopheles gambiae, an important transmitter of malaria and lymphatic filariasis in eastern Africa. It, therefore has potential in biological control of medically important mosquito species.

The experiments were conducted using third instar larvae or newly transformed pupae of a strain of A. gambiae maintained in a colony at the Kenya Medical Research Institute. Crayfish were collected from reservoirs located within Nairobi using meat-baited crayfish traps. Juvenile crayfish (carapace length 10-20mm) or adult crayfish (carapace size, 30-40mm) were used, and the experiments were conducted in 10-liter glass aquaria containing water at a depth of 10 cm, at room temperature. A single male of female crayfish was introduced into the aquarium containing 10 larvae of pupae, plus or minus fresh lettuce leaves as alternative food for the crayfish. Crayfish were excluded in the control aquaria. Each experiment was replicated at least 12 times. The results are expressed as mean % (± SD) of larvae or pupae surviving. Where appropriate statistical comparisons were made using the Mann-Whitney test. Probability value $<0.05$ was considered significant.

Over 80% of mosquito larvae survived for at least 3 days in the absence of crayfish. Less than 50% of the pupae, however, survived beyond 2 days. Our results with pupae are, therefore based on 24 hr observations. As no significant sex differences were observed in ability of crayfish to affect survival of mosquito larvae or pupae, the results of male and female crayfish are pooled. In general, significantly fewer mosquito larvae survived in presence of either juvenile of adult crayfish, compared with controls, whether alternative food for crayfish was present or not, $P<0.001$ (Table 1). When alternative food was present, significantly more larvae survived in presence of adult crayfish (26 ± 8) than in presence of juvenile crayfish (6 ± 7), $P<0.001$. This suggests that the adult crayfish will not necessarily

choose mosquito larvae if alternative food is present. Thus, significantly more larvae survived when alternative food was present, than when it was absent (26 ± 8% vs 14 ± 13, $P<0.05$). On the other hand, juvenile crayfish preferred mosquito larvae even when they were offered lettuce leaves as alternative food.

Similarly, significantly fewer pupae survived in the presence of either juvenile or adult crayfish (Table 2). However, significantly more pupae survived in the presence of adult crayfish (40 ± 28%) than in the presence of juvenile crayfish (17 ± 20) when alternative food was excluded, $P<0.05$.

The present study indicates that P. clarkii have a negative impact on aquatic stages of A. gambiae. In Kenya, crayfish commonly occur in small man-made impoundments. Such habitats can potentially also serve as suitable breeding sites for Anopheles mosquitoes (see Hunter et al, 1993). Crayfish, therefore, have potential in biological control of mosquitoes of public health significance in such habitats in Kenya.

More larvae or pupae generally survived in the presence of adult crayfish than in the presence of juvenile crayfish, whether lettuce leaves where present or not. This implies that when alternative food is present , adult crayfish then consume fewer larvae, compared with juvenile crayfish. In contrast, juvenile crayfish preferred mosquito larvae even when lettuce leaves were present. This is not surprising because adult crayfish are know to feed predominantly on detritus and vegetation, whereas juvenile crayfish are, therefore, likely to be more efficient predators of aquatic stages of mosquitoes than adult crayfish. It is still premature to speculate on the efficacy of crayfish, in limiting the populations of aquatic stages of medically important mosquitoes under natural conditions. In a preliminary experiment (results not presented here), conducted in large out door plastic tanks, and in which the culicine mosquito, Culex quinquefasciatus was breeding naturally, we observed that crayfish could limit abundance of aquatic stages of this mosquito species.

Besides directly affecting aquatic forms of mosquitoes, crayfish can also have indirect negative impact on certain mosquito species through, for instance, eliminating aquatic macrophytes used by these species for shelter (see Feminella & Resh, 1986). In Africa, one mosquito species that can potentially be affected by crayfish in this way is Anopheles funestus, a transmitter of malaria and which prefers to breed in shaded habitats. Our results indicate the potential of crayfish in biological control of medically important mosquito species.

We thank mess Sofie Njuguna for her help in this study.

TABLE 1. Effect of the crayfish P. clarkii on survival of larval A. gambiae in laboratory aquaria..

| Experiment | Mean % Larvae ± SD Surviving at 3 Days | |
|---|---|---|
| | Experimental | Control |
| 1.Larvae + Juvenile Crayfish - Lettuce | 15 ± 12 | 83 ± 17 |
| 2.Larvae + Juvenile Crayfish + Lettuce | 6 ± 7 | 91 ± 11 |
| 3.Larvae + Adult Crayfish - Lettuce | 14 ± 13 | 85 ± 13 |
| 4.Larvae + Adult Crayfish + Lettuce | 26 ± 8 | 80 ± 16 |

TABLE 2. Effect of P. clarkii on survival of pupal A. gambiae in laboratory aquaria.

| Experiment | Mean % Pupal ± SD Surviving at 24 hr | |
|---|---|---|
| | Experimental | Control |
| 1.Pupae + Juvenile Crayfish - Lettuce | 17 ± 20 | 90 ± 10 |
| 2.Pupae + Juvenile Crayfish + Lettuce | ND | ND |
| 3.Pupae + Adult Crayfish - Lettuce | 40 ± 28 | 89 ± 18 |
| 4.Pupae + Adult Crayfish + Lettuce | 51 ± 27 | 83 ± 16 |

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Hofkin BV, et al. 1991a. Amer. J. Trop. Med. Hyg. 45: 339-344.
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Mkoji GM, et al. 1992. In: Aquaculture and Schistosomiasis. National Academy Press, pp 264-271. Washington, DC.

## Appendix #15

The crayfish Procambarus clarkii in Kenya: where it presently occurs

| | Site Name | Habitat Type | Geographical Location |
|---|---|---|---|
| A. | NZOIA BASIN, WESTERN KENYA: | | |
| 1. | Kibomet Farm | dam | 6km S.E. of Kitale Town |
| 2. | Maribadi Farm | swamp | 12km N.E. of Kitale Town |
| B. | EWASO NYIRO BASIN, CENTRAL KENYA | | |
| 3. | Kwa Kamawe | pond | Subukia, north of Nakuru Town |
| 4. | Kihoto Farm | stream | 3km north of Sbukia town, on Nyahururu Rd |
| 5. | Igwamiti | pond | 10km west of Nyahururu town, Nyandarua |
| 6. | Ewaso, Nyiro | river | 2km south of Nyahururu town, at the T.Falls, on Nyeri Rd |
| 7. | Kwa Thimiti | dam | 8km S.W. of Nyahururu town |
| 8. | Suguroi Farm | dam | 42km S.E. of Nyahururu town just off Nyeri Rd |
| 9. | Nanyuki Ranch | dam | near Nanyuki town, on Rumuruti Rd |
| 10. | Nanyjki | pond | 12 km from Nanyuki town on Rumuruti Rd |
| 11. | Rumuruti | stream | 8km from Rumuruti Td on Nanyuki Rd |
| 12. | Enjore | dam I | 24km N.W. of Rumuruti town |
| 13. | Enjore | dam II | 6km S.E. of Enjore Dam I |
| C. | ATHI BASIN | | |
| 14. | Hill Crest | dam | in the Langata area, Nairobi |
| 15. | Mathenge's | dam | in the Langata area, Nairobi |
| 16. | Jamhuri Park | stream | Langata area, Nairobi |
| 17. | Muchure Estate | dam I | Juja area, Kiambu District, off Thika Rd |
| 18. | Muchue Estate | dam II | Juja area, Kiambu District, off Thika Rd |

| | | | |
|---|---|---|---|
| 19. | Tigoni | dam | In Tigoni, near Limuru, Kiambu District |
| 20. | Athi | river | about 15km east of Kabaa Market, Machakos District on the road to Matuu town |
| 21. | Bushwacker's Camp | river | river Athi, 30km N.E. of Kibwezi town |
| D. | TANA BASIN | | |
| 22. | Kituto Mission | dam | Makuyu area |
| 23. | Mwitu Mberia | dam | Makuyu area |
| 24 | Matuu | Irrig. canal | Near Matuu town |

## Appendix #16

### Survival of the crayfish Procambarus clarkii in coastal Kenya under experimental conditions

In Kenya, the Louisiana red swamp crayfish, Procambarus clarkii, occupies all the major drainage systems of this country: the River Nzoia Basin in the west, the Ewaso Nyiro in the central region, and the Tana and Athi basins to the east (Lowery & Mendes, 1977; Loker et al, 1992). The crustacean presently only occurs in the cooler highland areas of the country, and appears to be absent in the relatively warm coastal Kenya or the Lake Victoria basin where tropical conditions occur. Our efforts to locate crayfish in these areas have so far been unsuccessful. Reports from officials of the Kenya Fisheries Department also indicate that crayfish are absent from these areas. We are, however, informed that crayfish were at one time kept in aquacultural ponds on the Baobab Farm in Bamburi, Mombasa (R.D. Haller, personal communication). It is not clear why they no longer exist here.

Our interest in crayfish arises from our observations that they have potential in biological control of freshwater snails that transmit schistosomiasis or other medically important parasitic infections in Kenya (Hofkin et al, 1991a, b; 1992; Mkoji et al, 1992). Schistosomiasis is a major public health problem in this country. It is endemic in the Lake Victoria Basin in the west, in the area occupied by Machakos and Kitui Districts in the south-central region, and in the coastal lowlands. A question often asked is whether crayfish will be able to survive in those areas with warm tropical climatic conditions such as the coastal Kenya or the Lake Victoria Basin, and where schistosomiasis is also endemic. Preliminary studies indicate that P. clarkii will survive, reproduce and control schistosome-transmitting snails in temporary man-made quarry pits located in an endemic locality in the Machakos District, an area which experiences relatively long dry seasons (Mkoji et al, 1992). Apparently, crayfish here are able to survive the dry seasons when the introduction sites dry completely. What is not clear is why crayfish do not appear to be present in the Lake Basin or in coastal Kenya. Is it because they cannot survive under the climatic conditions that occur here or is it simply because they have not dispersed into these areas? We undertook an experiment to determine if P. clarkii will survive, in controlled conditions, under the warm tropical conditions that prevail in coastal Kenya.

The experiment was set up in the town of Kilifi on the Kenyan coast, about 60 km north-east of Mombasa. A control experiment was also set up in Nairobi. Crayfish were kept in large plastic tanks measuring 100 X 60 X 60cm, each provided with a sand and soil substratum about 10 cm thick, and water added to a depth of about 30 cm. Several pieces of rock were also placed on the substratum to provide shelter or a crawling surface for the crayfish. The tanks were kept out-door in partially shaded locations. A tightly fitting cover, consisting of a wooden frame and a chicken wire mesh was placed on top of each tank to prevent the animals from escaping, and also to keep away possible predators. Aerated tap water was used in the tanks. The crayfish were given fresh lettuce leaves as food on a daily basis.

Juvenile crayfish (carapace length 7-12mm) were used in this experiment, and were collected from a pond in the Makuyu area, some 60 km north of Nairobi. One hundred and

ten animals were included in this experiment. They were randomly selected into 2 groups, on group consisting of 56 individuals was set up in Nairobi, and the other group of 54 individuals was transported to Kilifi. Forty eight out of the 54 (89%) juvenile crayfish survived the trip to the coast. The 2 groups were further divided into 2 sub-groups of roughly equal proportion and each of these was introduced into one experimental tank. Once every week the water was drained and replaced with fresh aerated tap water. Also, mid-day temperatures of the water in the tanks were taken twice every week. Observations were made for seven months from June to December, 1992.

The average water temperatures for the seven months (June-December, 1992) were 17.2C in Nairobi and 30.3C in Kilifi. Twenty five out of the 56 (about 45%) of the initial number of the crayfish kept in Nairobi, and 29 out of the 48 (about 60%) of those kept in Kilifi survived through the duration of the experiment. The mean (± SD) initial carapace length of the crayfish kept in Nairobi was 8.9 ± 1.1mm, and the carapace length of those kept at Kilifi was 8.4 ± 1.1mm. By the end of the experiment, seven months later, mean carapace length of those in Nairobi had grown to 25 ± 4.5mm, and those at Kilifi to 31.1 ± 2.5mm. The difference in carapace size between the two groups was significantly different ($P<0.001$, by Mann-Whitney test). An earlier experiment using adult crayfish (carapace length 30-40mm) indicated that survival of adult crayfish at Kilifi was comparable with that of adult crayfish kept in Nairobi (results not presented). At both places the crayfish reproduced.

Survival of juvenile P. clarkii in Kilifi, was comparable with that of the juvenile crayfish kept in Nairobi. This suggests that crayfish can survive in those parts of the country where warm tropical conditions occur. It is possible that crayfish are absent from coastal Kenya or the Lake Basin because they have not been given opportunity to disperse into these areas.

We thank Mr. Joseph Nzovu for his help in this study.

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## Appendix #17

Procambarus clarkii in temporary man-made quarry pits in Kangundo, Machakos District, Kenya: An Update

The crayfish, Procambarus clarkii was previously introduced into abandoned rain-filled quarry pits located in the Kangundo area of Machakos District (Mkoji et al, 1992). Prior to crayfish introduction, these sites supported thriving populations of pulmonate snails, predominantly Biomphalaria pfeifferi (Mkoji et al, 1992). The snail populations rapidly declined following crayfish introductions in the sites that received crayfish, but snails continued to be present in adjacent pits that did not receive crayfish. Although these sites experienced marked seasonal fluctuations in water levels, crayfish established populations at the introduction sites, and increased in abundance. As this are experiences long dry seasons, many of the habitats may dry up for 2-3 months every year. We have observed that when these habitats dry up, crayfish disappear but they return and increase in number when the sites receive water during rainy seasons. They probably survive the dry conditions by burrowing into the substratum. Crayfish have persisted at the introduction sites for the last 3 years or so, and continue to be present here. During this period, snails have remained absent from crayfish introduction sites, but snails continue to occur in adjacent habitats that never received crayfish. These results support our earlier observations (see Hofkin et al, 1991a,b; Hofkin et al, 1992) that crayfish have potential in biological control of medically important pulmonate snails. They further suggest that crayfish are likely to be useful in snail control even in the relatively dry localities of Kenya such as the Kangundo area.

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Mkoji GM et al. 1992. In: Aquaculture and Schistosomiasis, pp 264-271. National Academy Press, Washington, DC.

## Appendix #18

# The use of a geographic information system (GIS) to analyze the distribution of human schistosome-transmitting snails in Kenya

Boyce, T.G., Rizor, D.D., Mkoji, G.M. and Loker, E.S.

Based on a talk presented at the joint meetings of the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, 31 October - 4 November, 1993.

Schistosomiasis is a chronic and debilitating parasitic disease affecting approximately 8 million people in the East African country of Kenya. The causative agents of this disease in Kenya are the trematode flatworms Schistosoma mansoni and Schistosoma haematobium. In Kenya, Schistosoma mansoni is transmitted by the freshwater snails Biomphalaria pfeifferi, B. sudanica, and, to a lesser degree, B. choanomphala. The first two species are found primarily in the central and southwest regions of the country in a variety of habitats that do no regularly dry out, including streams, dams, irrigation ditches, swamps, marshes and man-made trenches. B. choanomphala is a lake-dwelling species that transmits S. mansoni in Lake Victoria.

The intermediate hosts of Schistosoma haematobium in Kenya are freshwater snails of the Bulinus (=Physopsis) africanus group. This group includes Bulinus africanus, B. globosus, and B. nasutus. Bulinus species are often associated with small waterbodies and rainpools that may contain water for only half a year and are found primarily in the coastal region and in regions adjacent to Lake Victoria.

There are several factors known to affect snail distribution and may be broken down into biological, chemical and physical factors. Although biological factors are indisputably important, they are not factors that necessarily control broad-scale geographic distribution but affect presence or absence within particular habitats. The same may be said of chemical factors. The most influential physical factors affecting snail distribution are temperature and rainfall. In contrast to biological and chemical factors, these do affect broad-scale geographic distribution. Also, temperature is influenced by elevation so gradients in abundance that exist in response to temperature are often manifested geographically by elevational gradients.

In Kenya, the distribution of the schistosome-transmitting snails is fairly well documented as well as key factors determining their distribution. However, there are gaps in our knowledge that make it difficult to pinpoint or predict snail presence or absence within a given geographic area. This is due primarily to the complex interactions of temperature, rainfall and all other factors affecting distribution. A geographic information system, or GIS, is a system that will allow the integration of the climatic and geographically-referenced data necessary to produce a visual display of snail distributions thus making it much easier to locate snail-inhabited areas.

GIS may be formally defined as 'an integrated system to capture, store, manage,

analyze and display information relative to concerns of a geographic nature'. Essentially what a GIS provides is the ability to associate information with a feature on a map and to create new relationships that can, for example, determine the suitability of various sites for development, evaluate environmental impacts, calculate harvest volumes, or identity and predict the distribution of a parasite or its vectors.

This computer-based technology has been used to analyze the distribution of other parasitic diseases and vectors, but at the onset of this project, had not been utilized to analyze the distribution of the human schistosome-transmitting snails in sub-Saharan Africa. This kind of a study is particularly useful because if the distribution of the snails are better known in Kenya, the information produced by using a GIS can be used to predict areas of snail-inhabitation and, thus, areas where snail control efforts are most needed. Also, areas can be pinpointed where transmission may be more likely and where public education or human chemotherapy may need to be implemented or increased. Therefore, in order to 1) graphically display the distribution of the snails, 2) define the limits of the snails within Kenya and 3) specify the areas in Kenya and the parameter or parameters which most clearly delineate the distribution of the snails, we have constructed a GIS in ARC/INFO, a GIS software, incorporating a recent snail survey. The emphasis of this study is to try to define factors dictating distributions over a broad-scale rather than focusing on a more detailed view of a small geographic area.

The snail data obtained includes the latitude and longitude of 218 freshwater sites sampled for snails in southern Kenya and the species found at each site. The sample sites ranged from irrigation canals, ditches and small dams to large lakes and rivers. For the purpose of this study, we have focused on Biomphalaria pfeifferi rather than Biomphalaria sudanica which was found in only six of the 218 sites and Biomphalaria choanomphala which is restricted to Lake Victoria. We will also treat the Bulinus (=Physopsis) africanus group as a single taxon just as they were in the original survey due to problems in identification.

The United Nations Environmental Programme in Nairobi provided mean annual rainfall data at scale 1:2 million as well as the general geographic data at scale 1:1 million, the boundary of Kenya and the location of lakes, roads and towns. Mean annual temperature at an unknown scale and elevational data at scale 1:1 million were digitized from maps. All data were projected into Universal Transverse Mercator.

The 218 sampling sites are located primarily in the southern half of the country (Fig. 1). These represent habitats that were searched and found to harbor at least one living species of freshwater snail. The northern half of the country was not sampled due, in part, to the unavailability of water but primarily due to political instability in the area. Biomphalaria was found in 56 sites while Bulinus was found in 67 of the 218 sites.

The map in figure 2 is of the rainfall zones of Kenya. Note the large area where rainfall is quite low. Figure 3 is a map of the temperature zones of Kenya, and figure 4 maps the elevational contours of the country.

The first step in this process was to overlay the sites where B. pfeifferi and Bulinus (Physopsis) were found each with the rainfall, temperature and elevational zones, generate reports and evaluate the base-line ranges encompassing 100% of each snail species. In doing so, we found the ranges within each parameter indicated in table 1.

Gaps in ranges may be explained in two ways: 1) sampling bias in which areas coinciding with 7000 to 8000 ft. were not sampled due to the unavailability of water, or 2) the result of a slight misplacement in the latitude or longitude of a point. Because the elevation and rainfall data are at such a large scale, a slight miscalculation may offset the point by 1 to 2 miles, thus putting it in an incorrect zone.

The next step in the process was to combine two or more coverages - rainfall & temperature, temperature & elevation, etc. - to create additional coverages which allowed us to even more tightly define the areas inhabited by these two snails. However, in order to more easily focus on the snail distributions, a polygon of approximately 251,000 square kilometers was constructed which enclosed all 218 sites plus a 10k buffer and is referred to as the "study area" (figure 5). The polygon was clipped to the Kenyan border in areas where it crossed the border. The study area included all B. pfeifferi points.

In order to evaluate whether one or a combination of two parameters best defined the distribution of the snails, it was determined that the polygons encompassing 100% of snail points with the least amount of area was the best "definer" of a snail's geographic range. We then proceeded to overlay the snail coverages with the different single and combination coverages. In doing so, we were able to generate reports that gave us the number of snail sites found within each range and also the specific range combinations for the combination coverages.

Before presenting the numeric findings from these overlays, we first will show a sampling of the graphic display of these overlays. These plots are generated in a module of ARC/INFO which allows the selection of specific polygons within each coverage. So, for example, we can select all polygons with a temperature range from 18-28 C in the temperature coverage or polygons with a temperature range from 18-28 and an elevation from 2000-6000 ft. in the combination temperature/elevation coverage. In this way one can graphically display the ranges generated in the reports. Figures 5 - 10 were designed to illustrate the power of graphic display.

The results of the summation of areas of the specific polygons generated in the reports created when snail distributions were overlayed with the various single and combination coverages are shown in table 2. Both B. pfeifferi and Bulinus distributions are best defined with the least area when rainfall and elevation are combined.

When viewing this information on its own, it seemed that we had reached the conclusions we had been seeking. However, at this point it was crucial to examine where and how this kind of information would be used. In other words, who cared? At present, the Division of Vector Borne Diseases in Nairobi in conjunction with the Kenya Medical Research Institute is attempting to focus snail control resources: time, manpower, transportation, and supplies. Although we can now give them specific ranges within elevation and rainfall to help direct their efforts, the reality is that current information about rainfall may be difficult to come by. The rainfall data used in my project, which was the only complete data base available, dates back to 1966. Also, rainfall is of course notoriously variable from year to year. Is it reasonable to expect that any agency in Kenya would be able to obtain accurate and complete information there? The answer is "probably" but it may be very difficult.

Based on this reality, we would offer that elevation is the most appropriate predictor

of snail distributions. This information is stable and readily available. Virtually every bookstore in Nairobi carries road maps with elevation contours included. Therefore, although a combination of rainfall and elevation best define snail distributions, elevation alone is the most suitable predictor of snail distributions in Kenya and provides a straightforward and generally reliable guide as to where schistosomiasis will occur in Kenya.

Table 1. Summary of ranges for both *Biomphalaria pfeifferi* and *Bulinus* (*Physopsis*) as determined through frequency reports run in ARC/INFO on initial overlays of point data with rainfall, temperature and elevation coverages.

Table 1

SUMMARY OF RANGES

| | *Biomphalaria pfeifferi* | *Bulinus* (*Physopsis*) |
|---|---|---|
| Rainfall | 200-2400 mm | 200-2000 mm |
| Temperature | 18-34° C | <18 and 22-34+° C |
| Elevation | 2000-7000 and 8000-9000 ft. | Sea level - 5000 and 8000-9000 ft. |

Table 2. Summary of least areas for *Biomphalaria pfeifferi* and *Bulinus* (*Physopsis*) as determined through reports run in ARC/INFO when areas of inhabitation for each species were summed for single and combination coverages. For simplicity, decimals were dropped from actual area values.

Table 2

SUMMARY OF LEAST AREAS
(TOTAL STUDY AREA = 250,919 SQ. KILOMETERS)

| | *Biomphalaria pfeifferi* | *Bulinus* (*Physopsis*) |
|---|---|---|
| Rainfall | 250,875 sq. km | 249,115 sq. km |
| Temperature | 243,560 sq. km | 237,633 sq. km |
| Elevation | 149,697 sq. km | 178,612 sq. km |
| Rain/Temp | 168,646 sq. km | 164,985 sq. km |
| Rain/Elev | 106,831 sq. km | 124,936 sq. km |
| Temp/Elev | 138,418 sq. km | 166,789 sq. km |

Figure 1. The location of the 218 sampling sites, indicated with black stars, within the boundary of Kenya.

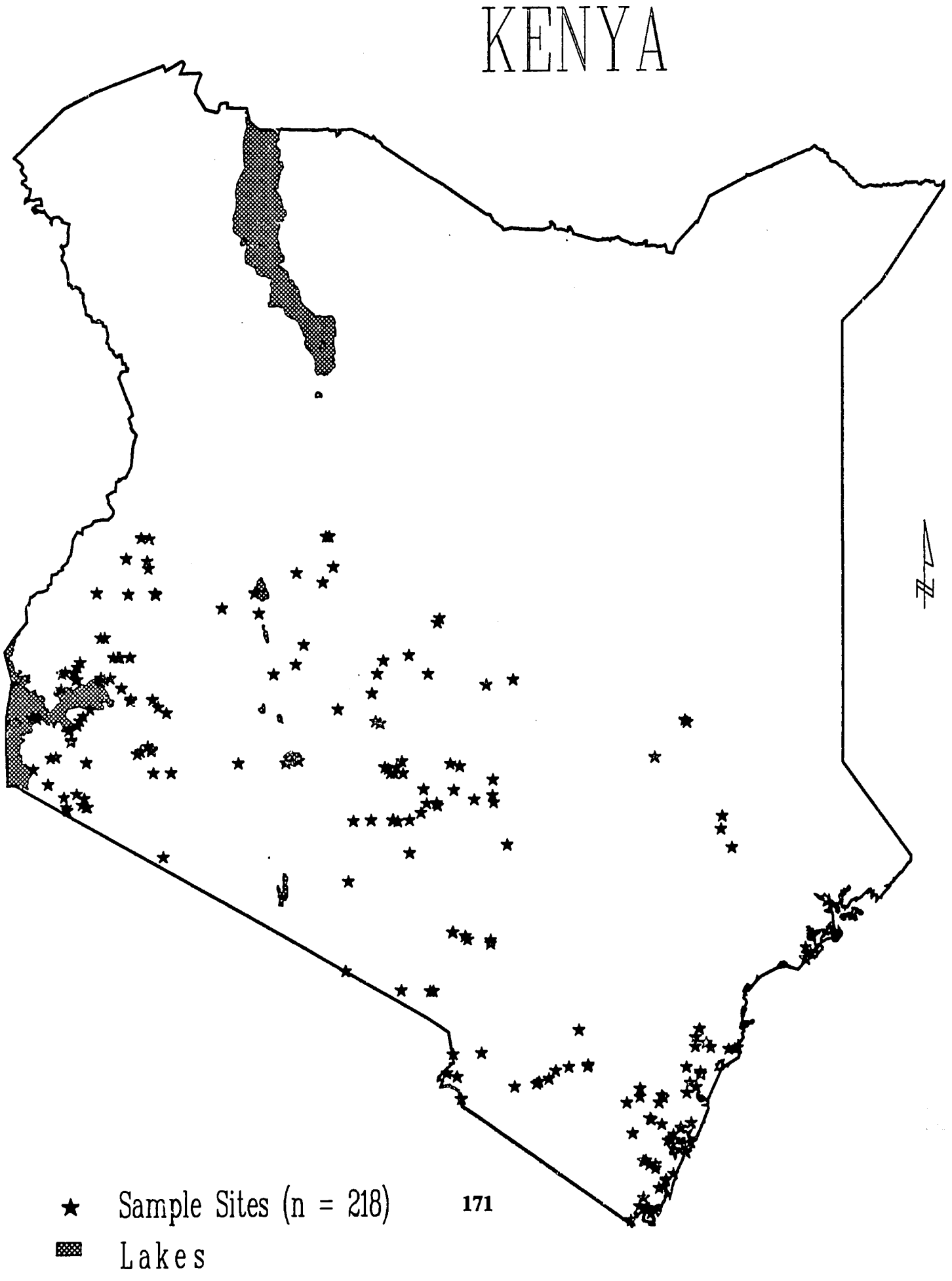

KENYA
N
Sample Sites (n = 218)
Lakes

Figure 2. The mean annual rainfall zones of Kenya based on data collected in 1966.

# The Mean Rainfall Zones of Kenya

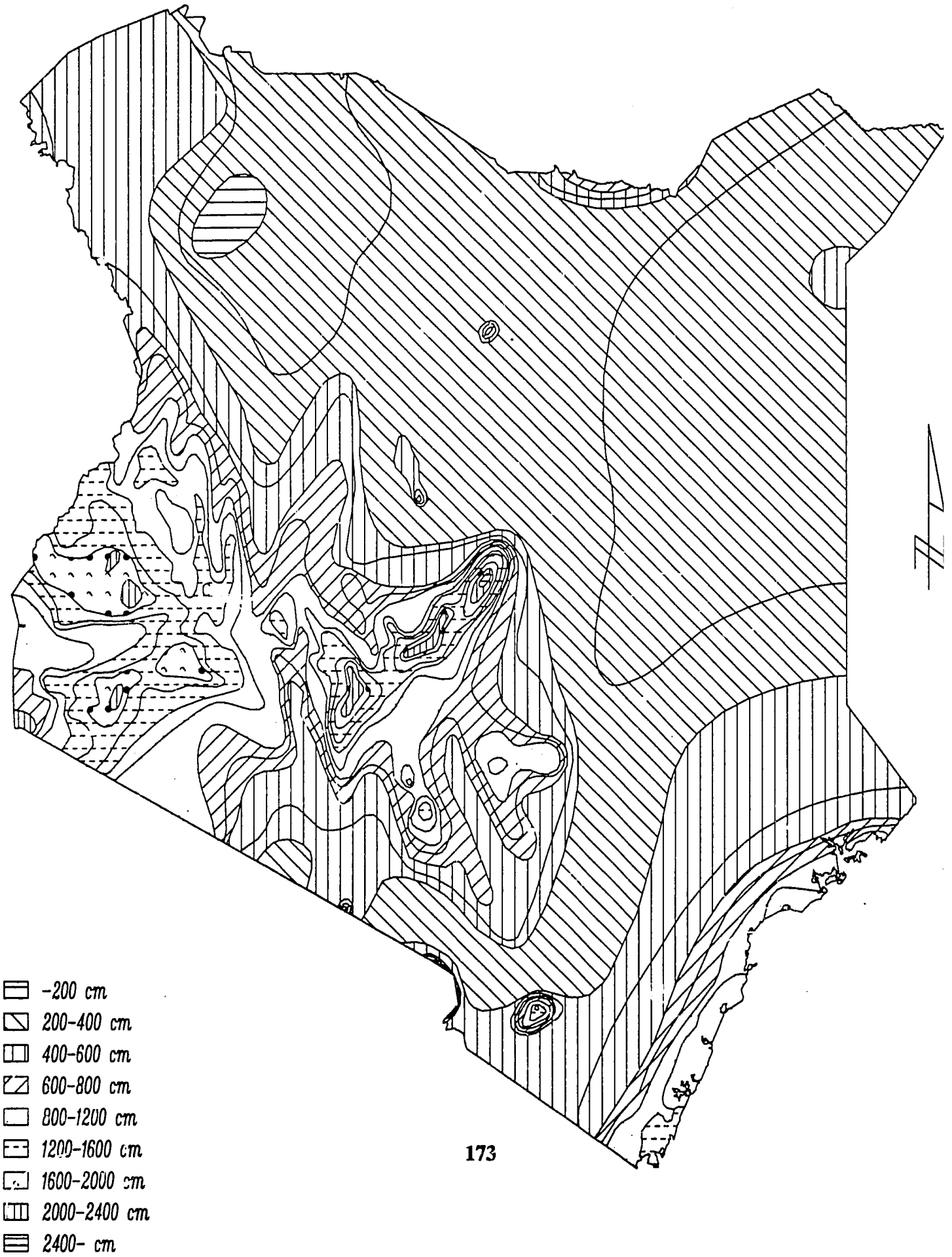


Figure 3. The mean annual temperature zones of Kenya (unknown data source).

# The Mean Temperature Zones of Kenya

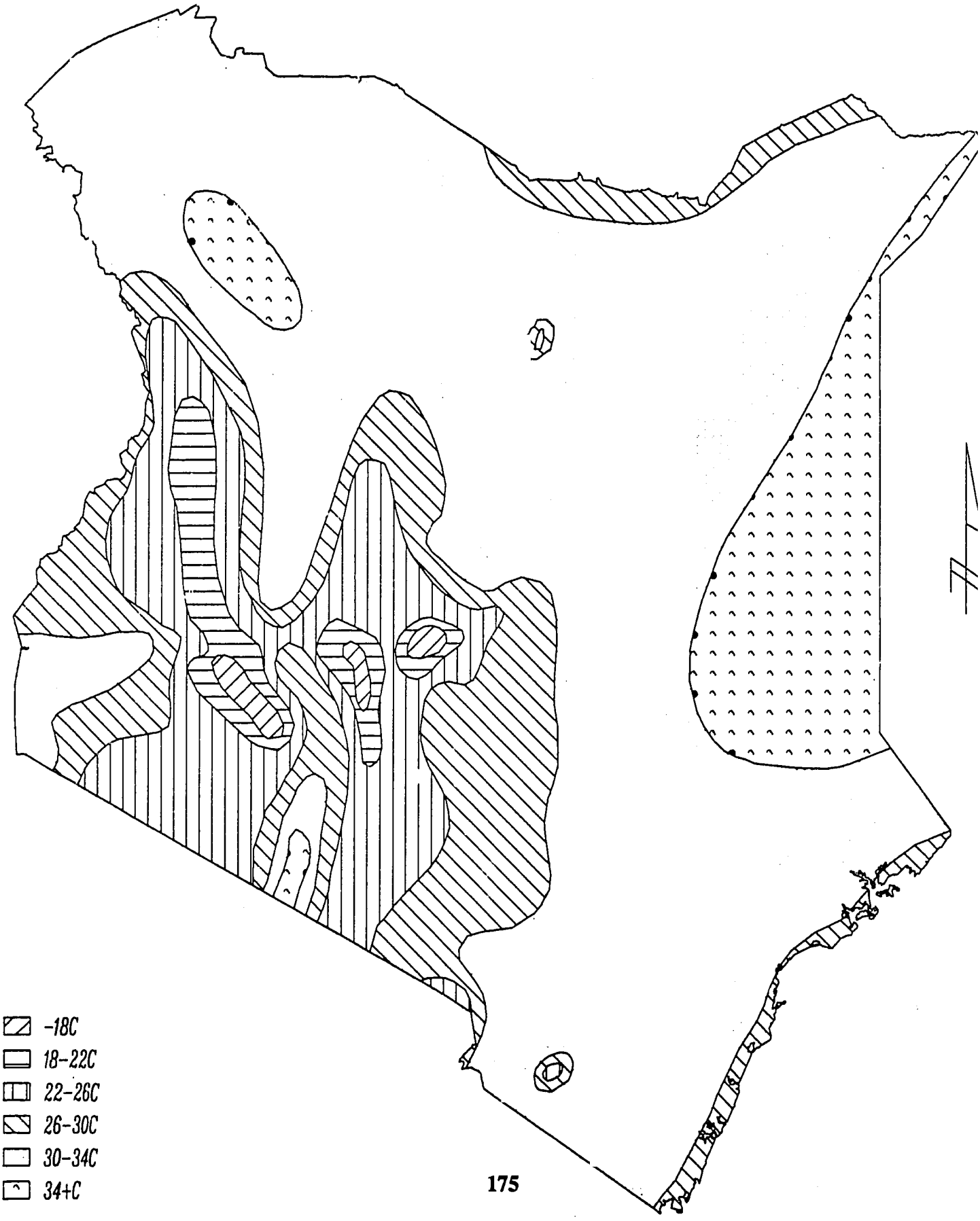


Figure 4. The elevational ranges (ft.) of Kenya digitized from a map at scale 1:2 million.

# The Elevation Ranges of Kenya

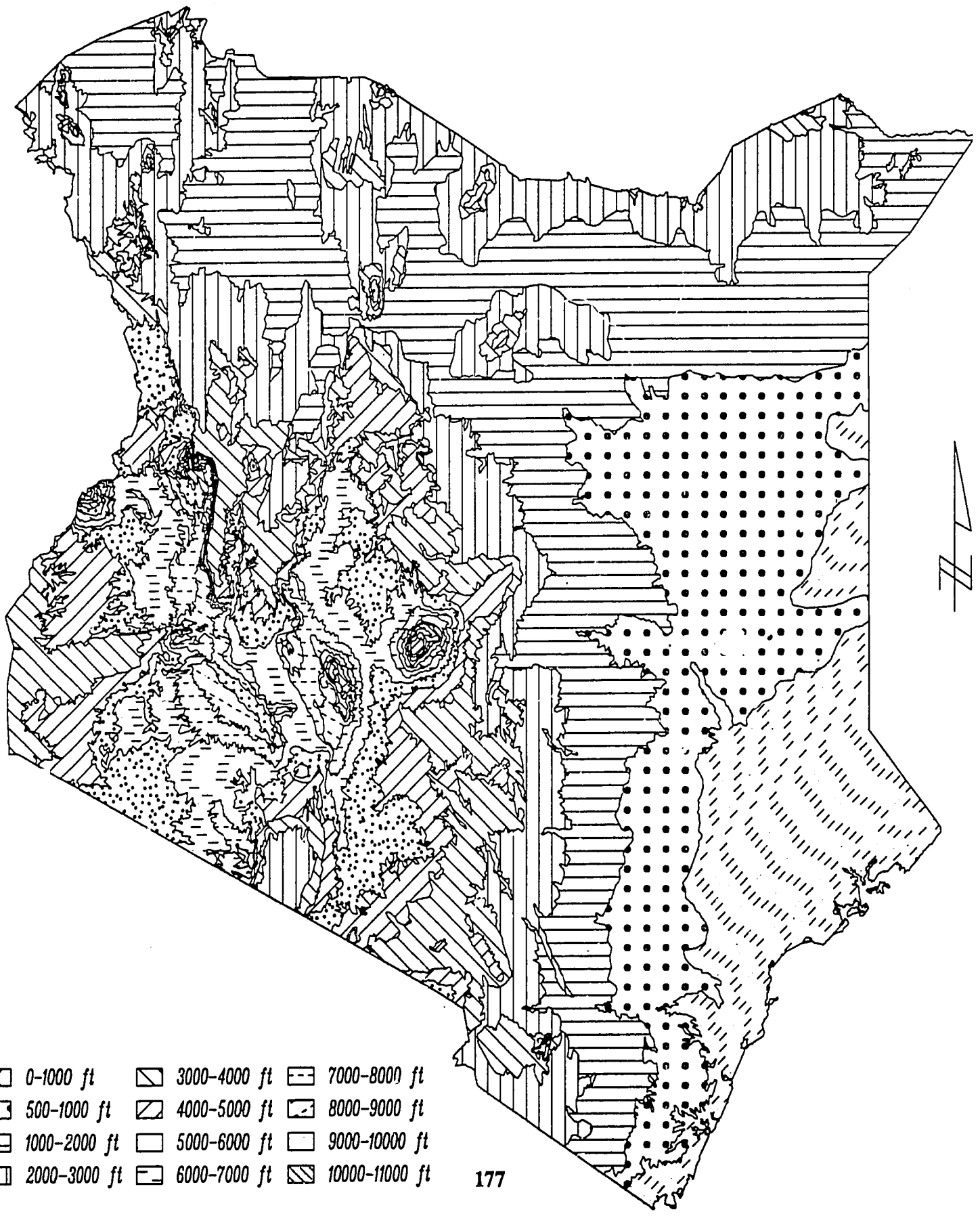


Figure 5. Biomphalari pfeifferi sites, indicated with black stars, within the Kenyan study area.

Biomphalaria pfeifferi Points Within The Kenyan Study Ar

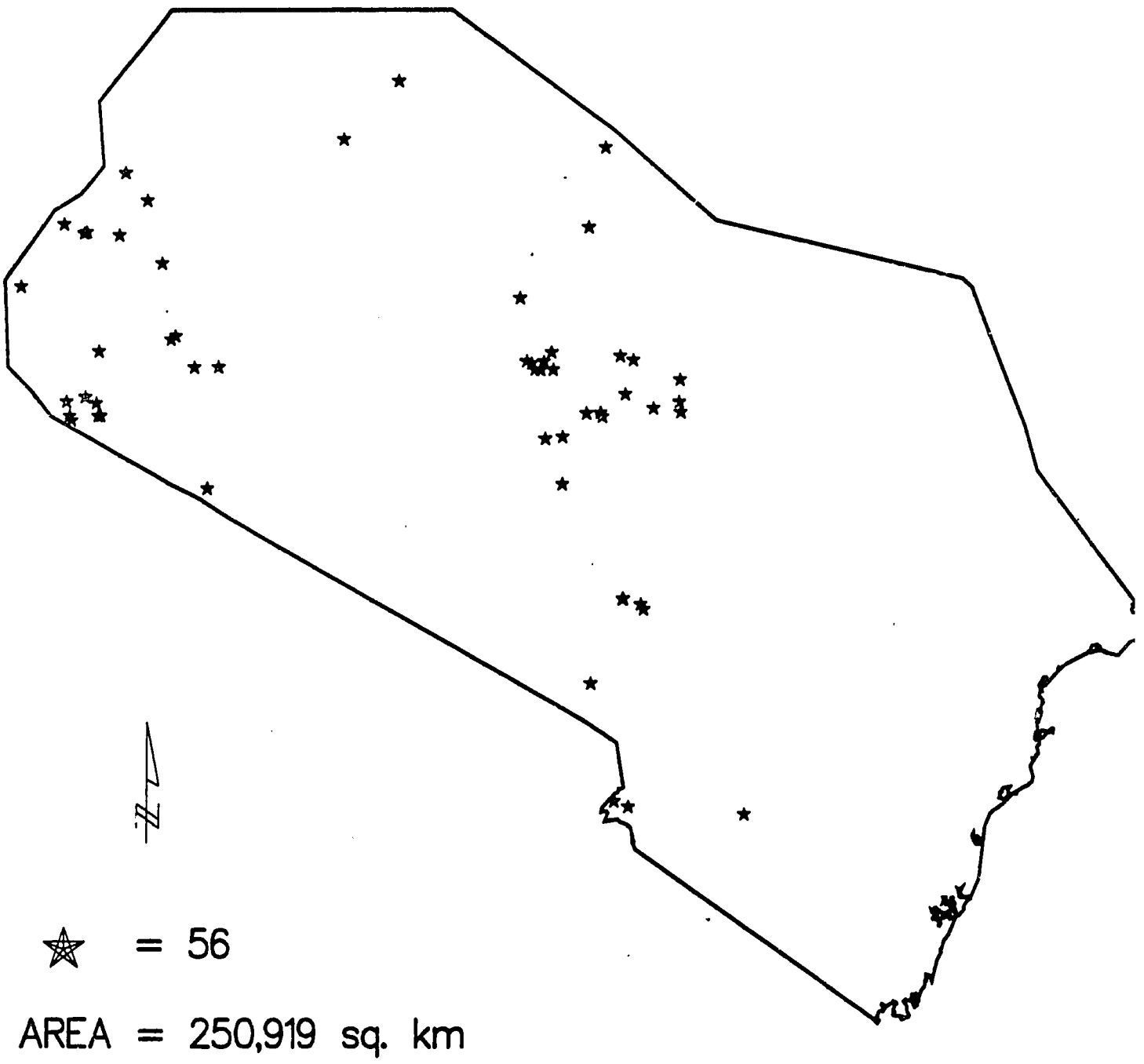


Figure 6. Bulinus (Physopsis) sites, indicated with black stars, within the Kenyan study area.

Bulinus (Physopsis) Points Within The Kenyan Study Area

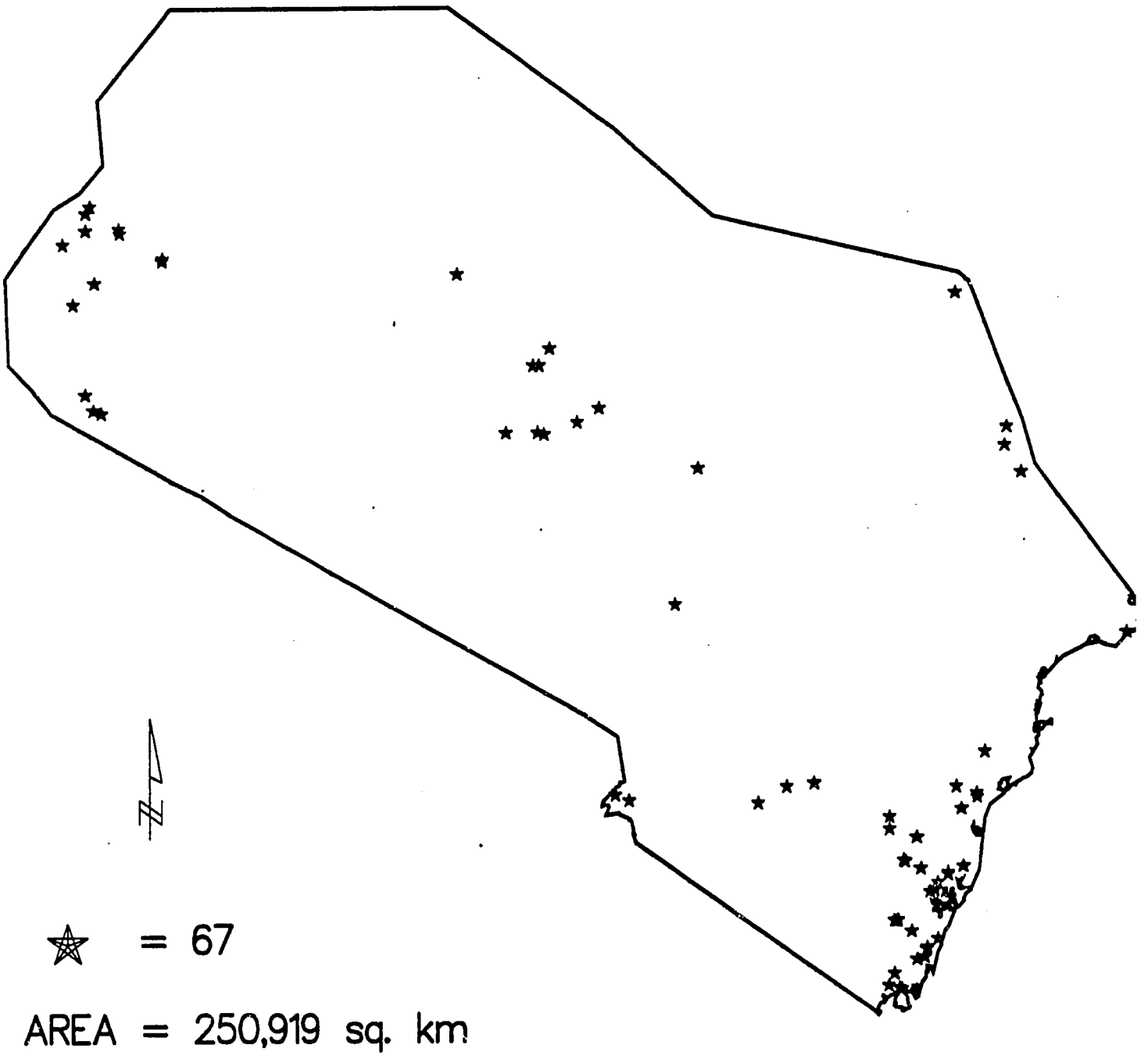


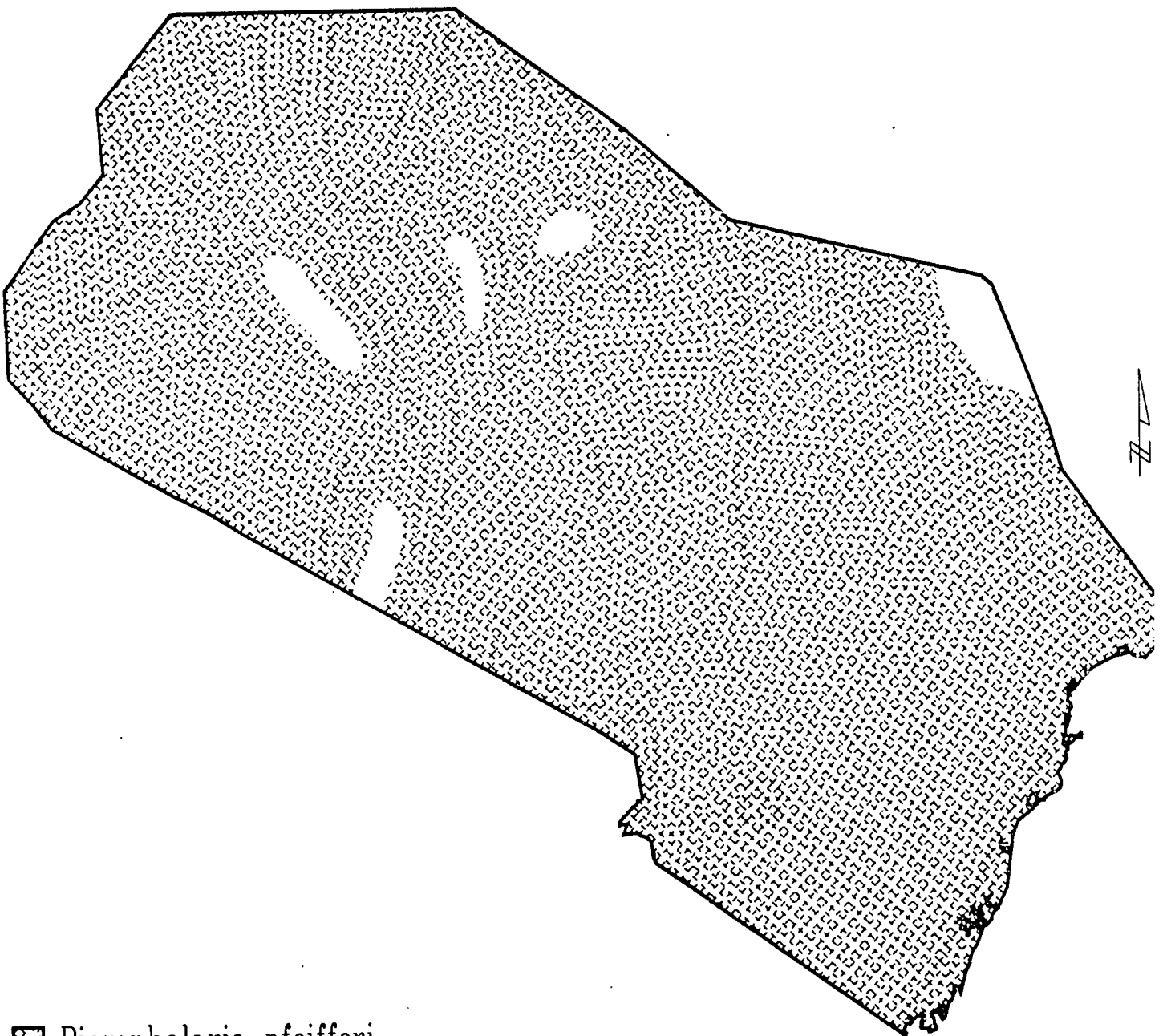
Figure 7. The rainfall zones encompassing 100% of *Biomphalaria pfeifferi* points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 250,875.034 square kilometers.

The Rainfall Zones Encompassing 100% of
Biomphalaria pfeifferi Points Within The Kenyan Study Area

Biomphalaria pfeifferi
AREA = 250,875.034 sq. km

Figure 8. The temperature zones encompassing 100% of *Biomphalaria pfeifferi* points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 243,560.08 square kilometers.

The Temperature Zones Encompassing 100% of
Biomphalaria pfeifferi Points Within The Kenyan Study Area



Biomphalaria pfeifferi
AREA = 243,560.08 sq. km

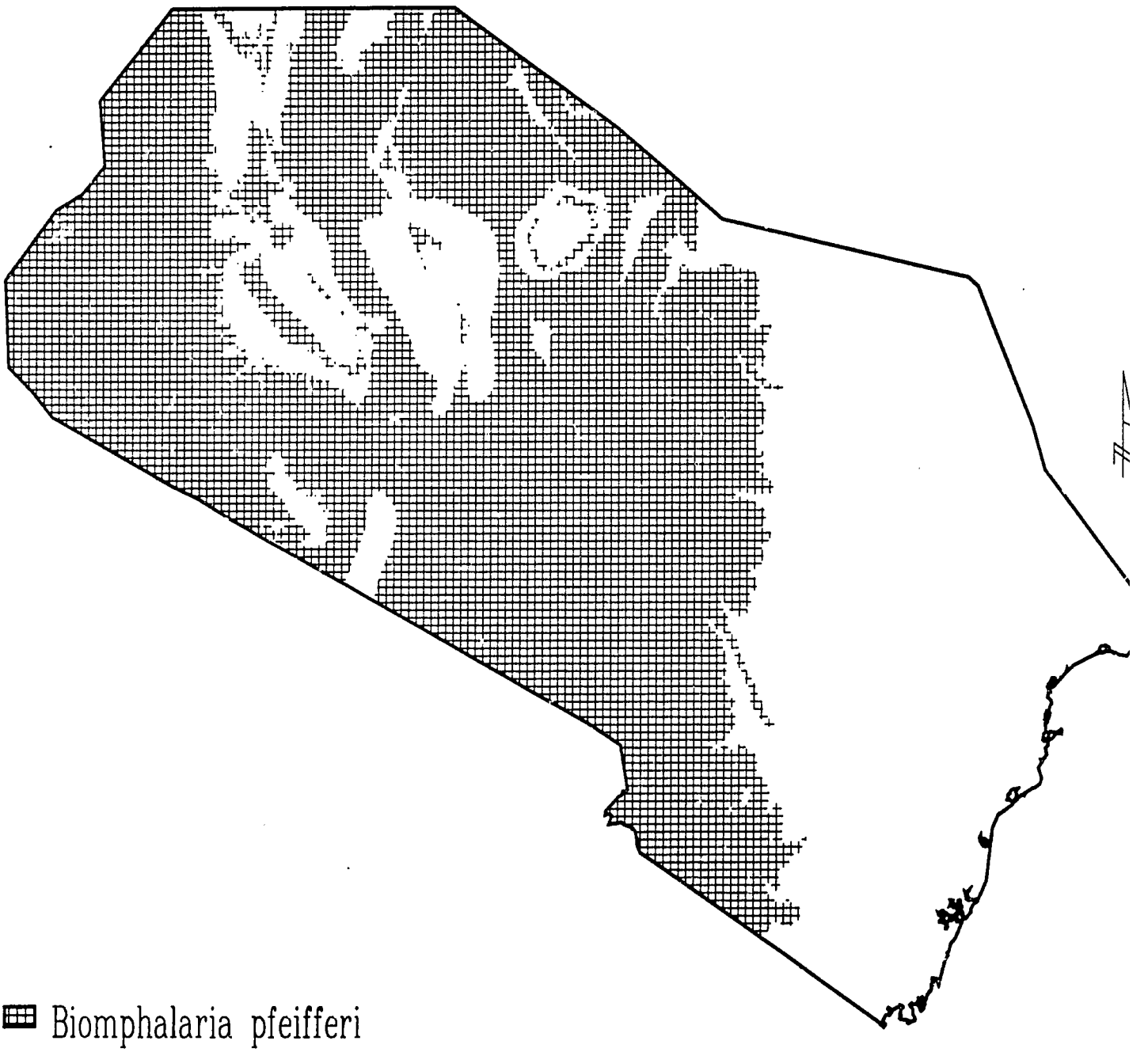
Figure 9. The elevation zones encompassing 100% of Biomphalaria pfeifferi points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 149,697.692 square kilometers.

The Elevation Zones Encompassing 100% of
Biomphalaria pfeifferi Points Within The Kenyan Study Area

Biomphalaria pfeifferi
AREA = 149,697.692 sq. km

Figure 10. The temperature and elevation zones encompassing 100% of *Biomphalaria pfeifferi* points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 138,418.999 square kilometers.

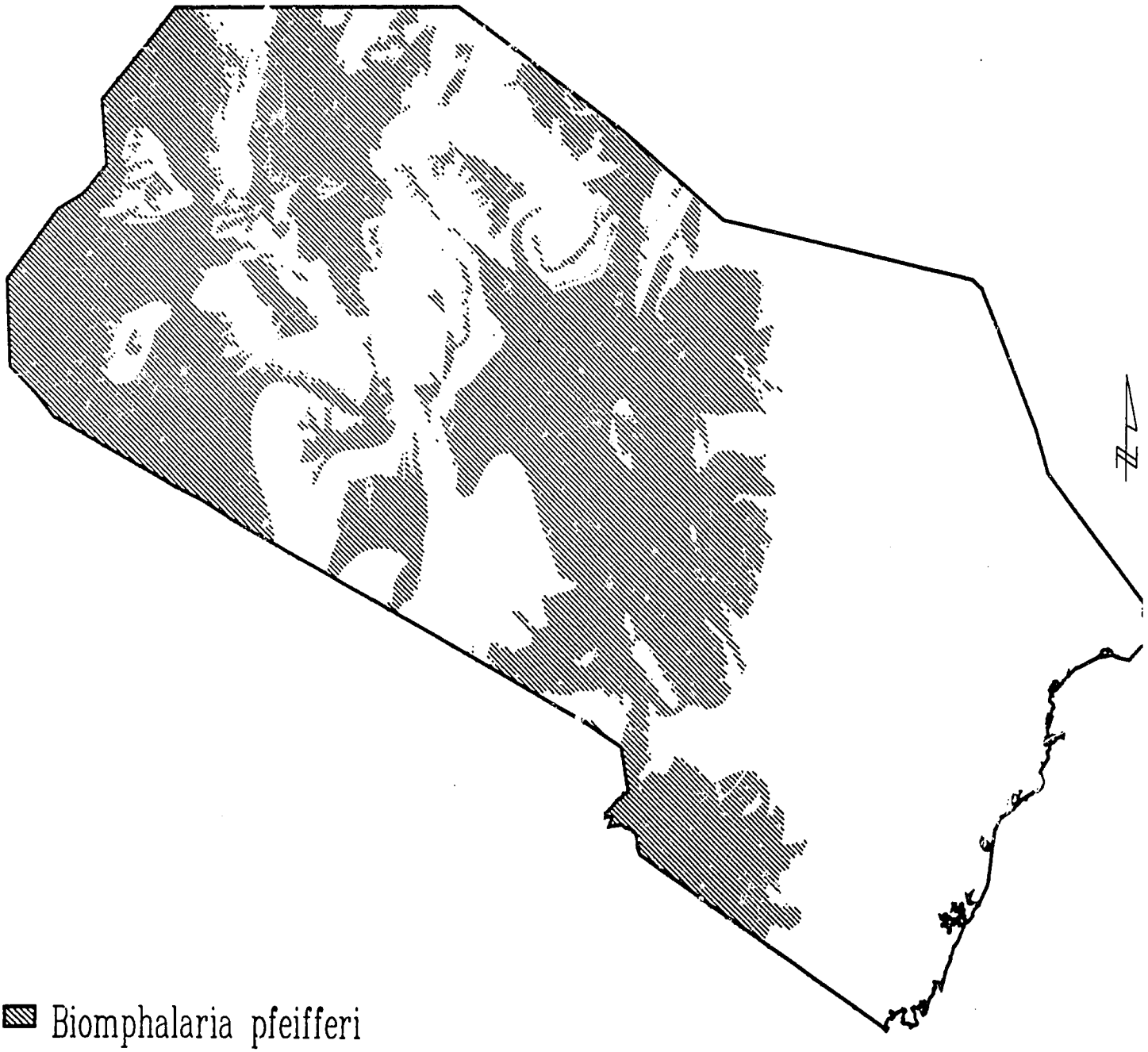
The Temperature and Elevation Zones Encompassing
100% of Biomphalaria pfeifferi Points Within The Kenyan Study Area



Biomphalaria pfeifferi
AREA = 138,418.999 sq. km

Figure 11. The rainfall and elevation zones encompassing 100% of *Biomphalaria pfeifferi* points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 106,831.919 square kilometers.

The Rainfall and Elevation Zones Encompassing
100% of Biomphalaria pfeifferi Points Within The Kenyan Study Area




Biomphalaria pfeifferi
AREA = 106,831.919 sq. km

Figure 12. The rainfall and temperature zones encompassing 100% of Biomphalaria pfeifferi points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 168,646.204 square kilometers.

The Rainfall and Temperature Zones Encompassing
100% of Biomphalaria pfeifferi Points Within The Kenyan Study Area

Biomphalaria pfeifferi
AREA = 168,646.204 sq. km

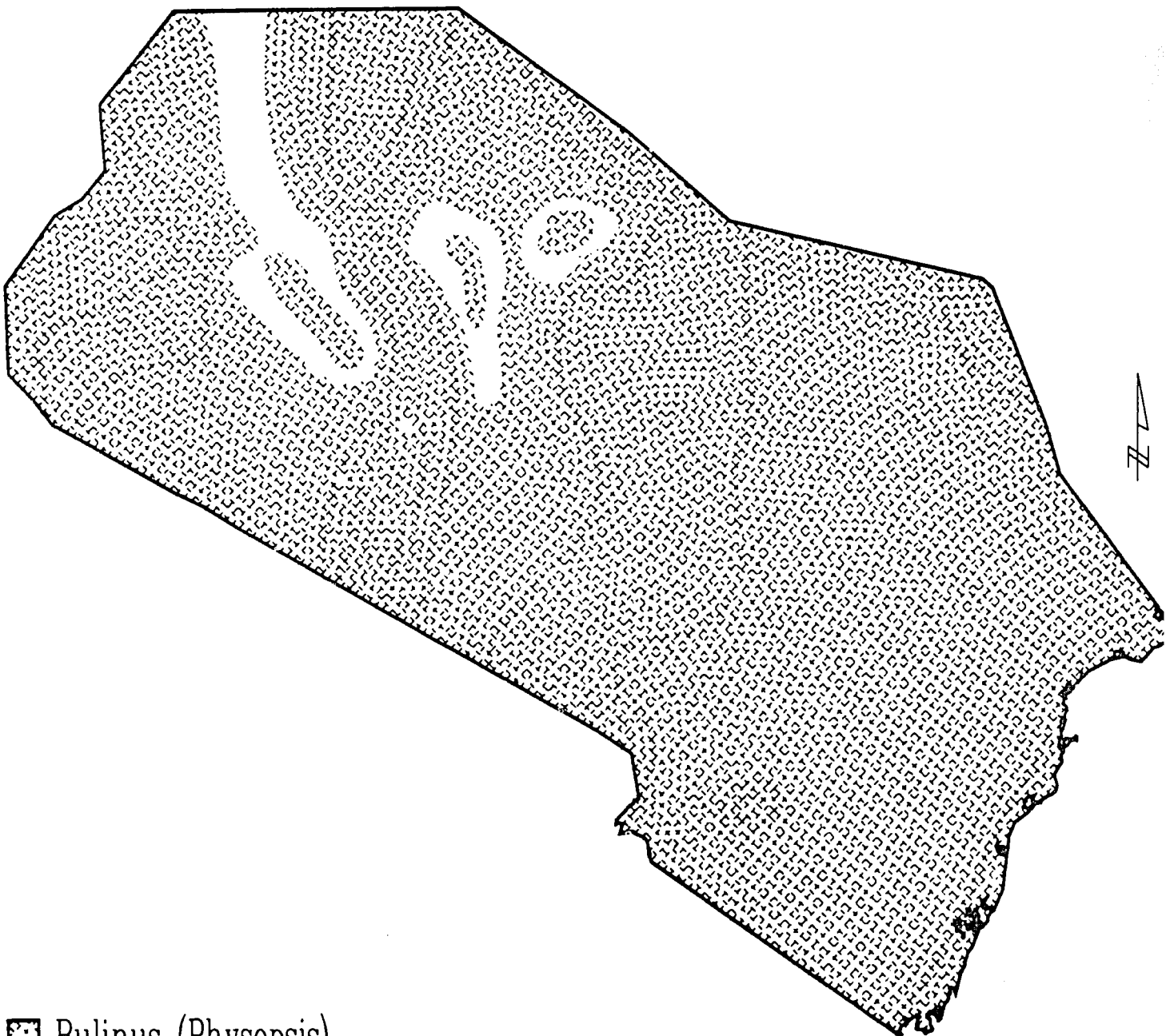
Figure 13. The rainfall zones encompassing 100% of Bulinus (Physopsis) points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 249,115.264 square kilometers.

The Rainfall Zones Encompassing 100% of
Bulinus (Physopsis) Points Within The Kenyan Study Area

Bulinus (Physopsis)
AREA = 249,115 264 sq. km

Figure 14. The temperature zones encompassing 100% of Bulinus (Physopsis) points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 237,633.326 square kilometers.

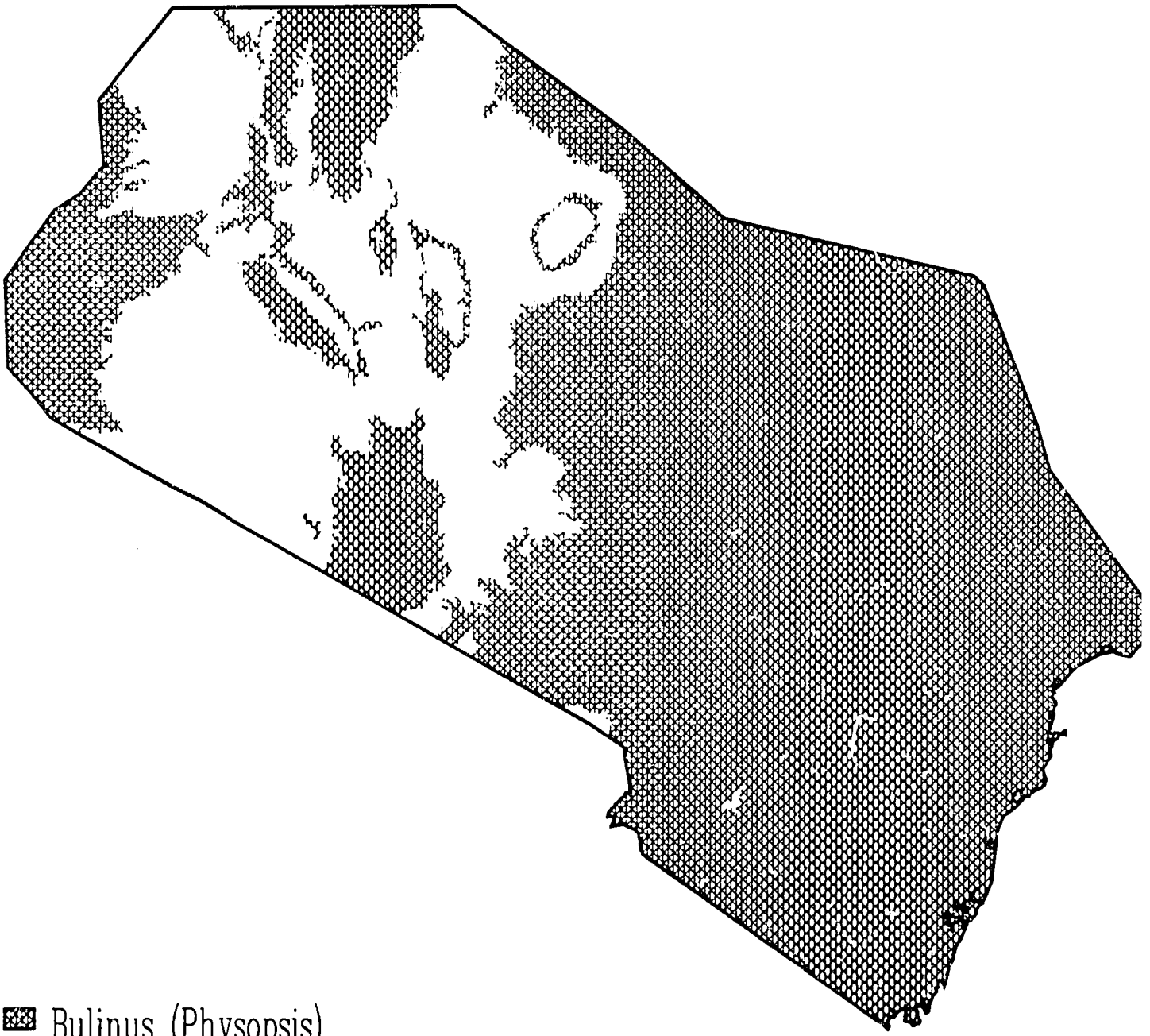
The Temperature Zones Encompassing 100% of
Bulinus (Physopsis) Points Within The Kenyan Study Area



Bulinus (Physopsis)
AREA = 237,633.326 sq. km

Figure 15. The elevation zones encompassing 100% of Bulinus (Physopsis) points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 178,612.777 square kilometers.

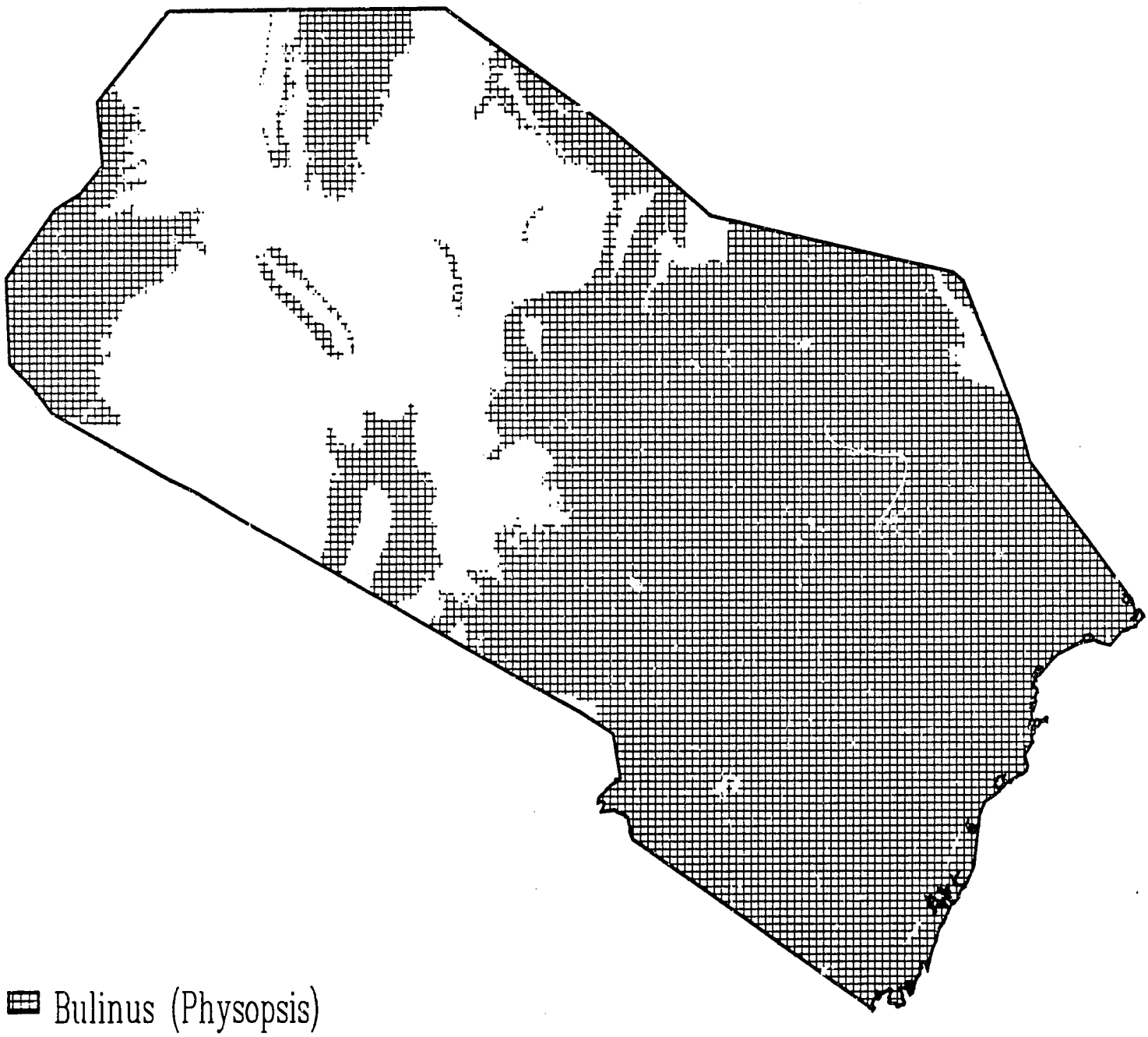
The Elevation Zones Encompassing 100% of
Bulinus (Physopsis) Points Within The Kenyan Study Area



Bulinus (Physopsis)
AREA = 178,612.777 sq. km

Figure 16. The temperature and elevation zones encompassing 100% of Bulinus (Physopsis) points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 166,789.271 square kilometers.

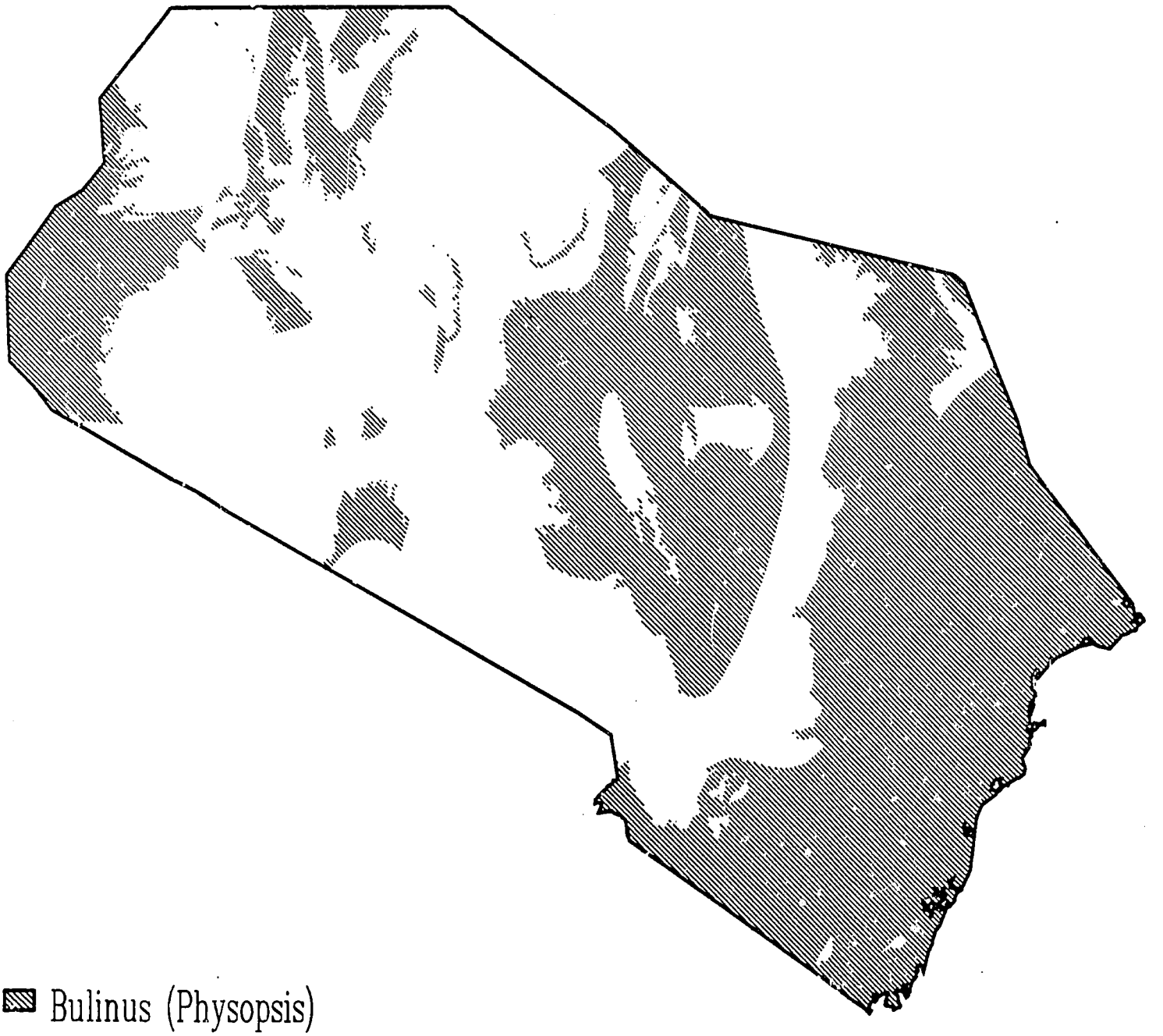
The Temperature and Elevation Zones Encompassing
100% of Bulinus (Physopsis) Points Within The Kenyan Study Area



Bulinus (Physopsis)
AREA = 166,789.271 sq. km

Figure 17. The rainfall and elevation zones encompassing 100% of Bulinus (Physopsis) points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 124,936.495 square kilometers.

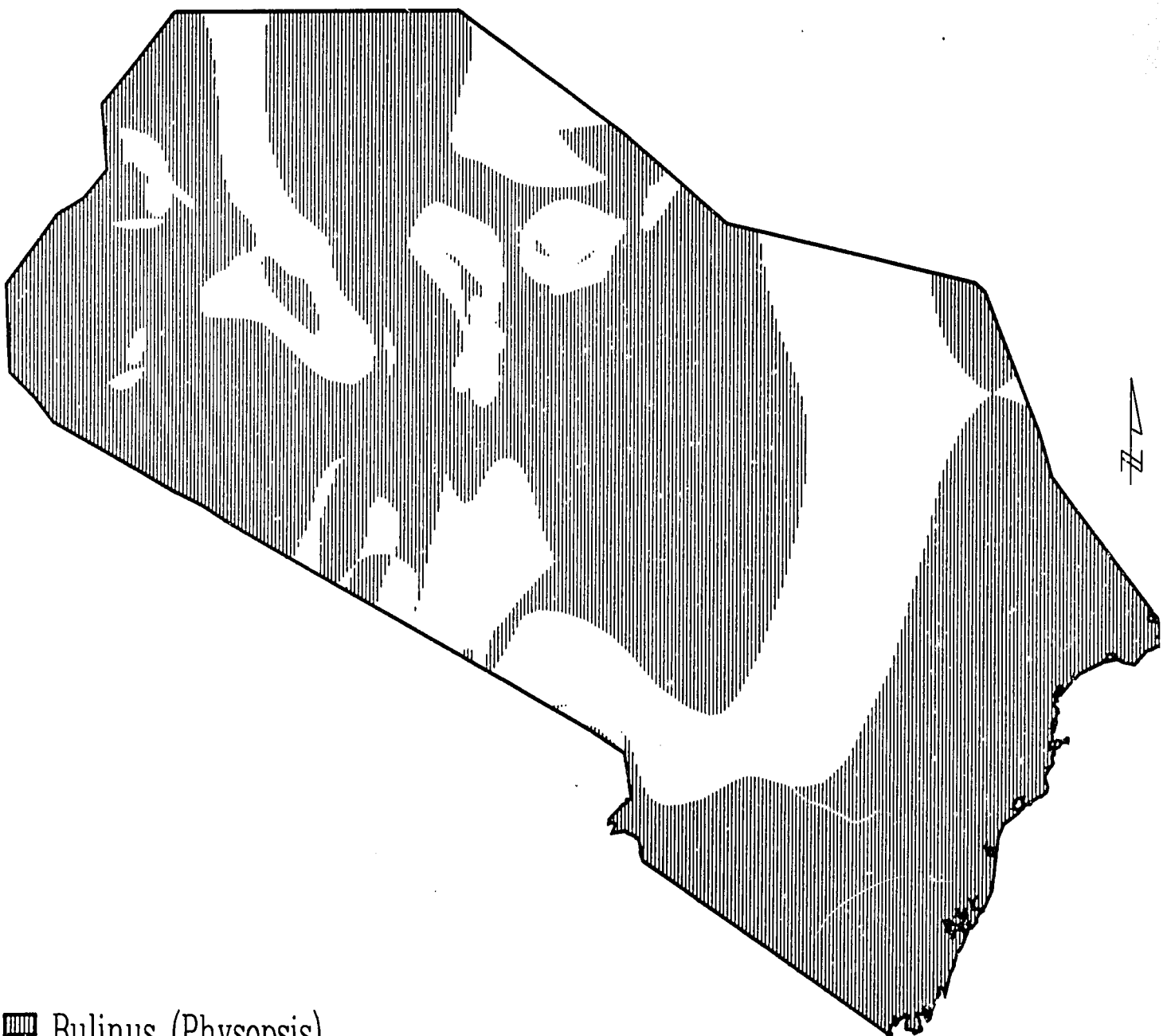
The Rainfall and Elevation Zones Encompassing
100% of Bulinus (Physopsis) Points Within The Kenyan Study Area



Bulinus (Physopsis)
AREA = 124,936.495 sq. km

Figure 18. The rainfall and temperature zones encompassing 100% of Bulinus (Physopsis) points within the Kenyan study area as determined through frequency reports run in ARC/INFO. Total area encompassed by these zones equal 164,985.683 square kilometers.

The Rainfall and Temperature Zones Encompassing
100% of Bulinus (Physopsis) Points Within The Kenyan Study Area



Bulinus (Physopsis)
AREA = 164,985.683 sq. km

## Appendix #19

Report on a visit to Marisa introduction site in northern Tanzania

On 24 June 1993, we visited Kisangara village, in the Kilimanjaro Region, northern Tanzania, to see the site where the south American ampullariid snail, Marisa cornuarietis was introduced in June 1977 (see Nguma et al, 1982). The site is a small pond less than 0.2 ha in area and located on a sisal estate, about 65 km south-east of the town of Moshi. According to Nguma and colleagues, introduction of Marisa to this site resulted in the elimination of the pulmonate snails, Biomphalaria pfeifferi, Lymnaea natalensis and Bulinus tropicus, which were present here. Although Marisa was first brought into Tanzania in 1970 (Msangi & Kihaule, 1972), this appears to have been the first detailed field study on this ampullariid in the country. Since then, no further reports have appeared in the literature about this species in Tanzania. the purpose of our visit was to determine whether Marisa is still present in Tanzania, in particular Kisangara, and whether there have been further introductions to other sites within the country. Our visit was facilitated by the Tropical Pesticide Research Institute (TPRI) in Arusha.

With the help of two members of staff of the TPRI, we located the site. At the time of our visit, it had very little water which appeared muddy and dark in color. The site was also, almost completely covered bulrush reeds, and the shoreline was fairly steep. We conducted a search for snails along the entire shoreline but we did not collect any snails or shells. We learned from TPRI staff that Marisa had not been recovered from this site in recent years. We also learned that a few years ago, prior to the disappearance of the ampullariid from this site, TPRI staff involved in schistosomiasis research collected several individuals of the snail and transferred them to an irrigation canal in a sugar plantation, located about 2km from the town of Moshi. When we visited the sugar plantation we found that Marisa was present in great abundance in one of the canals that supply water for irrigating the plantation. We were informed that there were plans by TPRI to establish a field colony of Marisa in small shallow pits at the TPRI field station located near Moshi. The colony will provide snails for further field studies on biological control of schistosome-transmitting snails in Tanzania.

We acknowledge the assistance of Dr. F. Mosha, Director, TPRI, Mrs Ritha Njau, and Messrs. P.A.S. Mnkai and M.A. Kipande

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## Appendix #20

# Recommendations for the selection of control agents for the biological control of aquatic vector organisms

Bruce V. Hofkin

Based on a talk presented at the joint meetings of the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, 31 October - 4 November, 1993.

In what is commonly known as "classical biological control", an exotic agent is introduced into an area where a particular pest is creating a problem. The goal is to establish long-term pest regulation. This strategy has enjoyed a great deal of success in controlling many agricultural and forest pests. According to theory, successful biological control is associated with two main factors. The first of these factors is depression of pest numbers below some acceptable level. The second is stability. The pest population levels should be held at this new stable level. It is important that the pest not be driven to extinction, as the control agent itself would subsequently die out.

How is such stability achieved? One important factor is the type of control agent utilized. To get the desired density-dependent stable equilibrium between the control agent and the target, certain characteristics in the control agent are sought. These include a high degree of prey specificity and searching, both of which lead to a stabilizing (type III) functional response, and a generation time shorter than that of the prey, which leads to a stabilizing numerical response.

In the mid-1980's, a serious challenge to the concept of classic biological control was made by Dr. William Murdoch and his colleagues from the University of California. They said that in many cases, such as in the biological control of mites or scale insects, there is no density-dependent stability, but pest extinction. Some pests may survive, but they do so by colonizing new patches, not due to any inherent stability in the system. If these patches are discovered by predators, the prey in such patches will likewise be destroyed. Any pest survival, therefore becomes merely a stochastic process, and is not the result of a stable equilibrium between predator and prey. Murdoch concluded by saying that the most efficient agents are not those that leave some prey safe, but those that find and kill prey at any density. This would suggest a very different sort of control agent than that indicated by the classical model.

This debate has led to some rethinking about what constitutes the best biological control agent. Murdoch's challenge may not mean that no predator/prey systems result in stable equilibrium, but he has caused us to consider the possibility that in different sorts of situations, biological control may operate differently. If he is correct, it means that the most favorable attributes for natural enemies would likewise vary between systems, and this would have obvious implications for the selection of control agents.

It is important to note here that all theory discussed so far stems from a study of terrestrial pests, using parasitoids or predacious arthropods as the controlling agent. No theory has been developed specifically for the control of aquatic disease vectors.

Consequently, in the search for biological control agents for schistosome-transmitting snails, the World Health Organization and other interested parties generally look to terrestrial-based theory for guidance. The criteria that are used for agent selection are usually those used in "classic" control. In other words, the best predators are again thought to be short-lived snail specialists that would regulate snail numbers in a density-dependent manner, through both functional and numerical responses.

However, few snail specialists are known, and those that have been studied, lack other important qualities of a "classical" biological control agent. For example, there are many species of molluscivorous fish, but their relatively long generation time rules out a numerical response. Sciomyzid fly larvae are snail specialists, but the species that have thus far been studied feed only in the top few centimeters of the water column, meaning many snails would escape predation.

Consequently, since there are no appropriate predators that conform to classic criteria, snail competitors are considered more promising. Also, in theory, competition is non-oscillatory and capable of complete prey displacement, whereas density-dependent predation is not.

But it is unclear how common or important interspecific competition between snails is. Although it's been demonstrated in the laboratory, it's been difficult to document in the field.

A model developed by Dr. David Lodge and colleagues in the late 1980's explains snail distribution based on biotic and abiotic factors. According to the model, competition will only be important when either disturbance or predators don't already reduce snail populations below levels at which resources become limiting. Competition, therefore, will only play an important role in snail distribution in relatively undisturbed or permanent habitats lacking predators.

Recent use of snail predators to control schistosome-transmitting snails lends support to the Lodge model. For example, Pointier and colleagues have successfully controlled Biomphalaria glabrata on Caribbean islands using competitor snails. But successful control has only been assured in small, stable waterbodies like watercress beds. Control has been much less successful in more complex habitats.

It therefore appears clear that competitors can play a role in the control of schistosome-transmitting snails, but what about the large number of habitats where competition will not work and schistosomiasis is still a problem?

The notion that biological control interactions operate differently in different environments may be especially true in freshwater environments, which may be different from terrestrial systems in a variety of ways. Constraints, therefore, as to predator type, may not apply.

For example, predators may be different in aquatic systems than they are in terrestrial systems. It has already been mentioned that there are few known snail specialists. In fact, the vast majority of snail predators are generalists. Such predators are usually discounted as promising snail control agents, because it's believed that they would switch

foods as snail numbers decline. Also, most snail predators, such as fish and macroinvertebrates are long-lived relative to snails, precluding a numerical response. But Murdoch and colleagues argue that such long-lived generalist predators are typical aquatic predators, and that such generalists often drive their prey to extinction. Because predator numbers would not track prey numbers, there would be no density-dependent stability. In fact, there are numerous examples of such predators driving their prey to extinction. For example, Orchonectes rusticus, the rusty red crayfish, has driven many native species extinct in midwestern lakes. Other well-known examples involve lampreys and lake trout, and large mouth bass and native fish species.

Another way that aquatic and terrestrial systems may be different is in the nature of the habitats themselves. At least some aquatic habitats probably have less spatial heterogeneity than terrestrial habitats, as well as lower immigration rates. Such factors would lead to increased biotic interactions, which may translate into higher extinction rates. If such differences between terrestrial and aquatic systems are real, then using "classical" criteria to find biological control agents for aquatic vectors may be inappropriate.

One very widely used biological control agent in freshwater supports this idea. The mosquitofish Gambusia, has been used successfully to control mosquito larvae for decades. This is in spite of the fact that Gambusia is a long-lived generalist. Populations of the fish do not track that of the prey, and there is none of the predator/prey stability associated with "classical" biological control. In fact, successful control is often equated with target extinction.

Our work in Kenya with Procambarus clarkii, the Louisiana red swamp crayfish, is also inconsistent with "classical" control. P. clarkii is also a long-lived generalist, and we have found that this crayfish can rapidly control or eliminate schistosome-transmitting snails in the laboratory, in field enclosures, and in small field habitats, even when alternative foods are abundant.

Obviously, because P. clarkii is a generalist, it may pose a risk to non-target species. Consequently its use is not appropriate in all habitats and careful examination of safety considerations are necessary, before introduction of this crayfish can be advocated. Such concerns dictate that it's use be restricted to isolated waterbodies, where the crayfish is unlikely to spread to areas where it's use is not indicated. Furthermore, any habitats where the introduction of crayfish is being considered should not contain other species of ecological or economic importance. In other words, crayfish should be used only in those situations where their introduction will not cause problems worse than those their introduction was meant to solve.

In East Africa, the ideal site in this regard may be small man-made impoundments, used by local inhabitants for domestic and livestock needs. Such impoundments are very numerous and serve as important schistosomiasis transmission foci. They are relatively isolated from other waterbodies, and they are extremely disturbed, containing little, if anything of ecological or economic importance. P. clarkii is likewise adapted to such lentic habitats.

Furthermore, there is reason to believe that control will be most assured in such habitats. They are generally small and stable with very low levels of spatial heterogeneity. Because of their isolation, there is reduced colonization. Such factors as small size, low

spatial heterogeneity, habitat permanence, and isolation will lead to increased predator effects, and should therefore increase the probability of prey extinction.

In conclusion, it appears that when it comes to biological control, what works best on land may not be appropriate in water, and no one set of criteria will be valid in all situations. It is important to consider both the appropriate agent, which may be very different in water than it is on land, as well as the habitat type, not all of which are equally amenable to biological control.

## Appendix #21

Comments Pertaining to the Theft of the Last KEMRI Subcontract Payment

In January, 1993, a check for the amount of $3,579.52 was sent to the Kenya Medical Research Institute (KEMRI) as the last payment on the KEMRI subcontract set up for this project. This payment was never received by KEMRI and was apparently stolen and cashed illegally. Details regarding this theft, including the results of an investigation carried out in Kenya, are reported in the attached documents. Inquiries made in the United States regarding the stolen check led to the conclusion that it would be difficult if not impossible to recover the funds and Dr. Loker was advised by appropriate financial officials at the University of New Mexico and by international banking experts at the university's bank (the Sunwest Bank of New Mexico) that further pursuit of the stolen funds was likely to be a waste of additional money and time. This unfortunate incident had the effect of denying KEMRI funds for legitimate services rendered during year four of the project. It is also fair to say that the loss of these funds curtailed KEMRI's project-related activites some during year four. It was for this reason in a letter to Mike Atsalinos of USAID that Dr. Loker wondered if USAID or KEMRI should be reimbursed for the stolen money in some way by UNM.

In trying to resolve this issue, it seemed clear that in spite of not receiving these much-needed funds, KEMRI had completed the scope of work outlined in the original proposal, as subsequently modified as a result of discoveries and developments occurring during the tenure of the project (see explanations above and previous annual reports). In short, KEMRI did everything requested of it by the U.S. counterparts. Activites curtailed in year four such as acquisition of additional crayfish survey data or further information on the ability of crayfish to survive in tropical Kenya, although not trivial, were in many ways tangential to the central thrust of the project. Also, it should be realized that during year four, KEMRI did continue to support project-related activites in many ways. For example, KEMRI vehicles were made available to Dr. Mkoji and his colleagues as they continued to visit project-related study sites. These vehicles and petrol were also made available to Dr. Loker during his visit to Kenya in June of 1993. KEMRI has also continuously made their personnel available to our project without demanding that the project compensate their salaries. Dr. Mkoji and Mr. Ben Mungai both have essentially donated long hours of their time to the project. For example, Dr. Mkoji contributed substantially to writing portions of the final report. It should also be mentioned that KEMRI demanded no institutional overhead for services rendered for this project. They have continued to generously donate laboratory space, aquarium facilities, and scientific equipment to the project, including year four. In our view, the stolen payment did not deny USAID a return on their investment. Rather, it was KEMRI that was denied the money they so much deserve for continuing to support our project.

Also, if there is a remaining concern at USAID that the agency was not adequately compensated for their investment, we argue that the project has been extremely productive. Eleven papers have already been published or are in press, one more has been submitted,

and at least two more are in preparation. Promising results pertaining to biological control of schistosome-transmitting snails in East Africa were obtained. We argue that the overall productivity of the project has given USAID an amazing return for their investment. In many respects, this lamentable theft can be viewed as one of the unfortunate costs of undertaking research in a developing country like Kenya.

UNM officials are neither willing nor legally bound to compensate KEMRI for their legitimate loss. In support of its position, UNM has in its possession a canceled check to verify that payment was sent to KEMRI. The case has been made above that because KEMRI continued to be actively involved in the project and that the project has been productive, USAID has been fairly compensated for their investment. It is suggested that KEMRI must bear the brunt of this unfortunate loss. To at least partially compensate them for their loss, we note that Dr. Loker has initiated a new collaborative project with KEMRI with $61,535 earmarked for a new KEMRI subcontract. KEMRI will also receive equipment to be purchased on the new grant. As an aside, with respect to our new project, we have instituted a policy that all payments due to KEMRI will either be sent by telegraphic transfer directly to KEMRI's bank in Nairobi, or will be hand-carried by Dr. Loker to Kenya.

# KENYA MEDICAL RESEARCH INSTITUTI

Mbagathi Road,
Biomedical Sciences Research Centr
P.O. Box 54840,
Nairobi, Kenya.

Date 20th May, 19 93

Address all correspondence
to Director
Telephone: 722541/2/3/4
Telegrams: KEMRI
Our fax: 254-2-720030 or 715105

Our Ref: BSRC/RES/4

Your Ref:

Dr. Eric S. Loker
Department of Biology
University of New Mexico
167 Castetter Hall
Albuquerque, NM 87131

FAX NO: (505) 277-0304

Dear Dr. Loker,

This refers to UNM cheque No. 693895 for US$ 3579.52 which was to come to Biomedical Sciences Research Centre, (BSRC) early this year in payment of KEMRI's subcontract for the project on biological control of snails. This is to inform you that the cheque was intercepted and we never received it. From the xerox copy of the cheque you sent to Dr. Mkoji it looks like the cheque has already been cashed in favour of a company in Japan. We suspect fraud in this case.

The administrative authorities at KEMRI have, therefore, taken up the matter, and are investigating the case. To assit in this regard, we suggest that UNM Accounts office and possibly the UNM Bank also initiate investigations from that end. It might be easier for your bank to request the bank in Japan in whose favour the cheque was made.

Yours sincerely,

DR. JOHN I. GITHURE
DIRECTOR, BSRC



cc: Director, KEMRI

# KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840, Tel: (02)722541, Fax: (02)720030, Tlx. 25696 KEMRI, NAIROBI, Kenya.


18th October, 1993

Dr. Eric S. Loker
Associate Professor
Department of Biology
University of New Mexico
167 Castetter Hall
ALBUQUERQUE, NM 87131-1091
U.S.A.

Fax No. (15)05277-0304

Dear Dr. Locker,

YOUR CHEQUE NO.693895 OF 13TH JANUARY, 1993

Dr. Gerald Mkoji of our Biomedical Sciences Research Centre has requested us to write to you and up-date you on the position with the above said cheque.

As you may have learnt, we never received the cheque. The cheque was fraudulently intercepted and diverted to a Company called Iwata Kiito Co. Ltd., in Japan. We understand that this company deals mainly with the export of second-hand (reconditioned) motor vehicles primarily to Kenya. The cheque then appears to have been endorsed by the said Iwata Kiito Co. Ltd. for payment to the Bankers Trust Company, New York for the Ogaki Kyoritsu Bank Ltd., Gifu Branch, Japan.

From investigations carried out by the Embassy of Kenya in Japan, the said Iwata Kiito Co. Ltd. in Japan have indicated that they received the cheque from a certain Mr. Dick Bisaso of P.O. Box 11393, Kampala, Uganda whom they claim to be a new customer with them. They indicate that they do not know how the said Dick Bisaso got the cheque. They claim that the cheque was, however, one of three cheques received from Dick Bisaso by the said Iwata Kiito Co. Ltd., all amounting to US$ 20,286.30 for export of 3 units of reconditioned used cars to Uganda via the port of Mombasa. The Kenya Police are carrying out further investigations on the matter and they have indicated that they will link up with Interpol in Uganda to try and get the culpits behind this fraudulent deal.

The loss of the cheque has been a very irritating and exasperating affair for us and we are keen to get to the bottom of the investigations relating to its loss so that

the culprits can be unearthed and punished. We are equally disappointed that the work for which the cheque was intended could not be carried out.

In future kindly send any payments to us by telegraphic transfer to our K.C.B. Kipande House External Account Number 241-970-312 and advise us accordingly to avoid similar incidents. We would also be grateful if you would kindly consider replacing the lost cheque.

We shall let you know of any further developments on the matter.

Yours sincerely,

D. M. NGUMO
for: DIRECTOR, KEMRI