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Covering Period: August, 1986 - August, 1989

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

BIOLOGICAL CONTROL OF SNAIL-BORNE PARASITES IN KENYA

Principal Investigator: Eric S. Loker
Grantee Institution: University of New Mexico

Collaborator: Davy K. Koech
Institution: Kenya Medical Research Institute

Project Number: 5.186
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A.I.D. Grant Project Officer: Judith D. Johnson

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I. Executive Summary

Schistosomiasis is a widespread, debilitating parasitic disease of humans and domestic animals in sub-Saharan Africa. Children are particularly likely to suffer from the effects of schistosome infection. Schistosomiasis is difficult to control, and new strategies are required for eliminating the freshwater pulmonate snails that serve as obligatory hosts for the larval stages of these parasites. The purpose of this project is to explore the potential of using competitor/predator gastropods in a program of biological control of schistosomiasis and other significant snail-transmitted parasites in Kenya. Biological control offers several potential advantages to developing countries including relatively low cost, avoidance of toxic chemicals, and sustainability. The project involves a combination of field and laboratory studies undertaken jointly by scientists at the Kenya Medical Research Institute in Nairobi, and the Department of Biology, University of New Mexico.

One of the important goals was to determine if exotic species of gastropods already introduced by others into East Africa have potential in the biological control of disease-transmitting pulmonates. Two such species were considered, the ampullariid Marisa cornuarietis and the pulmonate Helisoma duryi. An extensive study of freshwater snail distributions in Kenya revealed that neither exotic species occupies natural habitats in Kenya. We conclude that M. cornuarietis is not deserving of additional consideration because its use in biological control programs has produced mixed results in other parts of Africa; also, our laboratory studies indicate that indigenous ampullariids such as Pila ovata are just as efficacious as M. cornuarietis in achieving control of pulmonates. Regarding H. duryi, although this species was found in several locations in Kenya in cement-lined artificial habitats, it seems to be incapable of colonizing natural habitats and therefore has little potential to achieve meaningful snail control in the field.

We also considered the biological control potential of gastropods indigenous to Kenya, including Melanoides tuberculata, P. ovata, and Lanistes carinatus. Other workers have reported that M. tuberculata excludes disease-transmitting pulmonate snails from freshwater habitats in the Caribbean islands. Our studies of snail distribution in Kenya have revealed that M. tuberculata frequently coexists with pulmonates and that such coexistence is relatively stable. However, M. tuberculata typically remains numerically dominant over pulmonates, suggesting that this species deserves additional study. Also deserving of additional study is P. ovata which was shown to be an effective predator of pulmonate egg masses; this species also effectively limits pulmonate populations in laboratory studies and was observed to be negatively associated with some medically important pulmonates in natural habitats. The related ampullariid L. carinatus is not considered to be a promising control agent because it was less efficacious in our laboratory studies in controlling pulmonates than P. ovata. Additional field studies to assess the biological control potential of indigenous African gastropods are still underway. Further details regarding our activities are provided in the attached appendices.

Finally, we noticed that the presence of decapod crustaceans, particularly the introduced crayfish Procambarus clarkii, seemed to exclude pulmonate snails from natural habitats. Our current efforts are now focused primarily on P. clarkii.

In the course of our studies, we have had the opportunity to work side-by-side with several Kenyan scientists at both KEMRI and the Division of Vector

Borne Diseases, Ministry of Health. Some of these individuals are now initiating their own investigations of the potential of various biological control agents in Kenya.

II. Research Objectives

A. The Problem Posed by Snail-Transmitted Parasites in Kenya

Snail-transmitted parasites of medical and veterinary significance are abundant in Kenya (Table 1) and inflict an unacceptable burden of suffering and diminished productivity on both humans and domestic animals.

Table 1: Trematodes of medical and veterinary significance in Kenya and their snail intermediate hosts.

Schistosoma mansoni	
	Biomphalaria pfeifferi, *B. sudanica*
	B. choanomphala
Schistosoma haematobium	
	Bulinus (*Physopsis*) species group
Schistosoma bovis and *S. mattheei*	
	several *Bulinus* species
Fasciola gigantica	
	Lymnaea natalensis
Paramphistomatids - several species	
	Bulinus, *Biomphalaria*, *Lymnaea*

It has been estimated that at least 15,000 km^2 of Kenya are suitable for transmission of the human blood fluke *Schistosoma mansoni*, and 13,000 km^2 for *S. haematobium* (Brown et al., 1981). About 42% of Kenya's rapidly-growing population of over 22 million live in areas where transmission of *S. mansoni* or *S. haematobium* is possible (Diesfeld and Hecklau, 1978; Iarotski and Davis, 1981). As documented in older reviews by Webbe and Jordan (1966) and in detailed cross-sectional studies of particular communities (e.g. Arap Siongok et al., 1976), East African strains of these parasites are capable of inflicting serious pathological damage, particularly in children which typically bear much of the brunt of infection (Wilkins, 1987).

In addition to the human-infecting schistosomes, at least two ruminant schistosomes are found in Kenya, *S. bovis* and *S. mattheei*. Both parasites are capable of causing significant loss of condition and death in cattle, sheep, and goats (Dinnik and Dinnik, 1965). Another significant parasite of domestic animals in Kenya is the liver fluke *Fasciola gigantica*. *Lymnaea natalensis*, the snail host of this parasite, frequently inhabits the same waterbodies as *Biomphalaria pfeifferi*, the intermediate host of *S. mansoni* (van Someren, 1946). Still other significant parasites of domestic ruminants in Kenya are *F. hepatica* (e.g. Brown, 1980), and several species of paramphistome flukes such as *Calicophoron microbothrium* (Dinnik, 1964; Malek, 1980; Southgate et al., 1989).

B. Control of Snail-Transmitted Parasites

Regarding the control of snail-transmitted parasites such as the schistosomes, there is no panacea. The use of relatively new chemotherapeutic agents such as metrifonate, oxamniquine and praziquantel offers considerable hope in combatting schistosomiasis in Kenya and will serve as the cornerstone for future nation-wide control programs, but excessive reliance on the use of drugs to control this problem is short-sighted. The available drugs are expensive, they are not 100% effective (Marshall, 1987), and it is not realistic to expect that all infected individuals or reservoir hosts will be treated. Therefore, it is unlikely that chemotherapy alone will break the cycle of transmission; prevalence rates are likely to revert to pre-control levels following cessation of chemotherapeutic control (e.g. Sturrock et al., 1987). Furthermore, if drugs are given continually to control transmission, the chances of selecting for drug resistant parasites is increased enormously. Coles et al. (1987) have already provided disturbing evidence for oxamniquine tolerance in Kenyan S. mansoni. A far better strategy is to use other methods of control to interrupt transmission, and to reserve the use of currently effective drugs for treating individuals who are clinically ill with schistosomiasis.

The long-standing goal of much of the research on schistosomiasis has been to develop an effective vaccine (reviewed by Capron et al., 1987; Hagan, 1987; Sher et al., 1989). A vaccine would in theory alleviate many of the problems associated with reliance on chemotherapy, and should remain a priority for work in this area. However, it is our opinion that a field-worthy schistosome vaccine is not likely to be deployed on a large scale in Kenya for many years. Indeed, none of the current vaccine immunogens induce levels of resistance sufficient to significantly reduce human infection or subsequent development of disease (Sher et al., 1989). Also, a vaccine will pose distributional problems and its expense may prove to be prohibitive; it also seems likely that booster injections will be necessary to sustain protective levels of immunity to such a complex helminth parasite. These sobering considerations emphasize the continual need to develop alternative and complementary approaches to schistosomiasis control.

One such approach is to reduce or eliminate populations of snail intermediate hosts. The role of snails in transmission is of paramount importance because the parasite has enormous multiplicative potential within this host, and because snails produce the infective stage in the life cycle for mammals, namely the cercaria (Jourdane and Theron, 1987). Traditional methods of snail control based on the use of naturally-occurring or synthetic chemical molluscicides have many potential drawbacks (see reviews by Andrews et al., 1983; Combes and Cheng, 1986; Klumpp and Chu, 1987). Molluscicides are broad-spectrum toxins that can kill valuable non-target species such as fish which may naturally prey on snails. Molluscicides provide another example of expensive, imported technology that developing countries can not afford over the extended periods of time needed to achieve control. Even focal use of molluscicides often requires repeated applications and heroic efforts on the part of local control personnel. Indeed, it can be argued that freshwater snails are highly adapted to repopulate habitats following disturbance which is a pervasive feature of freshwater habitats. Molluscicides represent just another example of temporary habitat disturbance. Also, use of molluscidices, just as with chemotherapeutic agents, may encourage the development of resistance in the target organisms (discussed by Duncan and Brown, 1988).

C. The Option of Biological Control of Snails

Regarding snail control, an alternative method that is philosophically and financially compatible with conditions in developing countries is biological control. This approach, which has enjoyed considerable success in controlling insect and weed pests (e.g. Waage and Greathead, 1988) offers several advantages including relatively low cost, use of indigenous resources that are potentially self-renewing and long-lasting in their effect, simplicity of application, avoidance of dependence on imported technology, and avoidance of the use of toxic chemicals (Huffaker and Messenger, 1976). Also, some of the organisms considered as potential biological control agents, such as crustaceans or fish, can serve as human food sources, and propagation of such organisms could provide financial income and added incentive for maintenance of control efforts.

It is also imperative to realize that there are certain risks inherent in the application of biological control, particularly if exotics are used as the controlling species, and these must be weighed relative to the benefits. For example, introduction of exotic species could create new problems if they were capable of transmitting parasites of medical or veterinary significance, or if they consumed food plants. It is for this reason that the specific research we have undertaken has not emphasized exotic species. We also wish to stress that our proposal to investigate the biocontrol potential of indigenous species does not constitute an endorsement for their immediate use in large-scale control operations. Indeed, the focus of our work is to determine, under controlled conditions, if these putative biological control agents do, in fact, effectively control snails. If this is found to be the case, further study would then be warranted to determine if their use in control programs should be implemented.

Several different putative biological control agents have been suggested for use against freshwater snails of medical or veterinary significance (Michelson, 1957; Berg, 1973; Hairston et al., 1975; Jordan et al., 1980; McCullough, 1981; Combes and Cheng, 1986; Pointier and McCullough, 1989; Madsen, 1990). In our opinion, the most promising control agents for freshwater snails are multitrophic organisms that can 1) directly or indirectly compete for resources such as food or oviposition sites, and 2) function as facultative predators of snails. Such control agents, because of their generalist properties, are able to establish relatively stable populations, even if the target species undergoes temporary reductions in number. Potentially unstable cycles of abundance of target and control species are thereby avoided and the possibility of sustained, long-term control is created (Murdoch et al., 1985). It is important that the control agent can inhabit a variety of habitats including temporary waterbodies, and that they can rapidly re-colonize habitats following cessation of unfavorable conditions. Specificity of action of the control agent for the target species is also highly desirable.

D. Studies With Putative Biological Control Agents in Kenya

Described below are various invertebrates contemplated for use in biological control programs in Kenya, including several with at least some of the favorable characteristics described above. Unfortunately, no microbial pathogens known to be specific for freshwater snails are currently available for use in field trials. Also, certain groups of vertebrate control agents

such as molluscivorous fishes have been considered as possible biological control agents in Kenya (e.g. McMahon et al., 1977), but are not considered further at this time, primarily because so little is known about them.

I. Predator/Competitor Snails

Such snails can negatively affect target snails and associated trematodes by preying upon eggs, juveniles and adults of the target species, by competing for food or oviposition sites, by producing noxious secretions that interfere with growth and/or reproduction, and by serving as "decoys" or "sponges" of the miracidia of trematode parasites (Frandsen and Madsen, 1979). Predator/competitor snails that might be suitable for use in Kenya include Helisoma duryi, Marisa cornuarietis, Thiara granifera, and species indigenous to Kenya including Melanoides tuberculata and the ampullariid snails Pila ovata and Lanistes carinatus. Some of the salient features of each of these species are presented below:

Helisoma duryi - This species, which is native to southern North America, has been widely introduced into Africa, including Kenya (e.g. Brown, 1980). Its potential for controlling trematode-transmitting snails has been thoroughly discussed by Frandsen and Madsen (1979) and Jordan et al. (1980). We were particularly interested in determining if this snail has been successful in colonizing natural habitats in Kenya since the time of its first introduction there in approximately 1970.

Thiara granifera and Melanoides tuberculata - These two related snails are both cosmopolitan (Pace, 1973), and both have been considered as potential biological control agents (reviewed by Pointier and McCullough, 1989). Both species have become very common on some Caribbean islands and in other areas in the Neotropics, and have been implicated in the competitive displacement of Biomphalaria glabrata in several locations, including Puerto Rico, St. Lucia, and Guadeloupe (Butlers et al., 1980; Prentice, 1983; Pointier and McCullough, 1989). Because of lingering doubts regarding the role of thiarids in transmitting human lung- or liver-flukes, possibly in Africa (e.g. Bayssade-Dufour et al., 1982), and because T. granifera does not presently occur in East Africa, we can not condone the introduction of this species for use in biological control studies in Kenya.

In contrast, M. tuberculata is a natural component of the snail fauna of Kenya (Brown, 1980), and is the most commonly collected prosobranch snail in the country (see Appendix 1). Further studies regarding the possible role of this species in control in cental Kenya are warranted, particularly in view of recent reports from the Caribbean (Pointier et al., 1984; Pointier and McCullough, 1989) implicating this species as an effective control agent. Some of our observations regarding M. tuberculata are summarized in Appendix 2.

Marisa cornuarietis - This large ampullariid snail originates in northern South America, and has already been introduced into several countries because of its effectiveness as a biological control agent of pulmonate snails. Its successful use in control has been discussed by several authors (e.g. Ferguson 1977; WHO/VBC/82.837, 1982). It has been introduced in the Sudan to the north of Kenya (Haridi et al., 1985) and in Tanzania to the south where it successfully displaced several pulmonate species in a single pond (Nguma et al., 1982).

Native Kenyan Ampullariid Snails - In Kenya, the most common ampullariids are Pila ovata, Lanistes carinatus and various subspecies of L. ovum (Appendix 1). Because other ampullariids such as Marisa and Ampullaria have properties favorable for use in biological control (e.g. Pointier et al.,

1988), indigenous African ampullariids deserve consideration in this context.

II. Freshwater Decapods

Based on both field and laboratory studies of Kenyan material, we have become convinced that freshwater decapods deserve careful consideration as biological control agents. In particular, the Louisiana red swamp crayfish, Procambarus clarkii, which was introduced into Kenya several year ago, is deserving of additional study. However, for the funding period of the present grant, we have chosen to emphasize the study of competitor/predator gastropods.

E. Innovative Aspects

This proposal is innovative in emphasizing a relatively novel type of schistosomiasis control, one that deemphasizes reliance on imported technology and potentially toxic and expensive chemicals. Biological control also offers the hope of sustainability of action, a component that is frequently lacking in infectious disease control programs in developing countries.

Another innovative aspect of this proposal is that it deliberately focuses attention on indigenous organisms that may have unrealized potential as biological control agents. Also, we propose to investigate the biocontrol potential of certain exotic organisms that have already been introduced by others into African freshwater habitats.

III. Methods and Results

A. Introduction

Several appendices have been provided below that outline the methods used and results obtained for this proposal. The results are also extensively discussed in these appendices. Most of these appendices represent manuscripts that have been prepared for publication.

B. Conclusions and Recommendations

In the original proposal, we stated that we wished to formulate recommendations regarding several issues pertaining to biological control considered during the three-year tenure of the grant. These recommendations are as follows:

1. We remain convinced that biological control of schistosomes and other snail-borne parasites is a viable option, and definitely deserves additional consideration. Our work indicates that the indigenous ampullariid Pila ovata is the predator/competitor gastropod species most deserving of future consideration in the context of snail control in Kenya.

2. We conclude that if competitor/predator gastropod species are to be used as control agents in Kenya, that native species such as P. ovata or perhaps Melanoides tuberculata should be used rather than exotics such as Marisa cornuarietis or Helisoma duryi.

3. It is unlikely that P. ovata plays any significant role in transmission of parasites of medical or veterinary significance in Kenya. If used in a program of biological control, M. tuberculata should receive further study with respect to its role as a host of trematodes of public health significance. It is very unlikely, however, that this species could transmit Paragonimus westermani in Kenya.

4. Since our proposal was submitted, other studies have shown that

introduction of exotic ampullariids can create problems for rice cultivation (Ampullaria glauca in Japan, for example). As Marisa has been shown to eat rice seedlings, such a problem could conceivably occur in Kenya as well. Especially given that other efficacious ampullariids are already present in Kenya, Marisa should not be introduced into Kenya.

5. If P. ovata is to be used in biological control in Kenya, it is able to resist habitat drying and should be appropriate for use in ephemeral habitats. It is also able to survive in permanent habitats, including large lakes such as Lake Victoria. It should not be deliberately introduced near rice-growing schemes in Kenya such as Ahero or Mwea. However, our conversations with rice cultivators in Ahero, an area where P. ovata is naturally abundant, did not indicate that this species caused any problems for rice cultivation. If introductions of gastropods for biological control are ever contemplated for rice-growing areas, M. tuberculata would probably be the species of choice.

6. Future priorities for biological control work in Kenya, and sub-Saharan Africa in general, are as follows: a) further explore the potential of P. ovata and M. tuberculata to displace medically-important pulmonates in natural habitats. Habitats already containing these gastropods as well as habitats into which they are introduced should be studied; b) our survey work of gastropod habitats suggested that the presence of the introduced North American crayfish, Procambarus clarkii, in natural habitats was often correlated with a lack of pulmonate snails. Additional field and laboratory observations of the impact of this species on pulmonates are required.

IV. Impact, Relevance, and Technology Transfer

The findings of this study will be useful to Kenya in providing essential background information which may someday be used to facilitate planning of a national schistosomiasis control program. Results of the present proposal, in conjunction with results from ongoing studies, will help to provide alternative methods of snail control which are very much needed if broad-scale control programs are to be implemented. As the cost of mollusciciding for large scale programs of snail control is prohibitive, biological control offers virtually the only viable alternative method of snail control. We believe our ongoing work has pointed out at least two viable control agents that are already widespread in Kenya, and that can be used in a cost-effective manner in select habitats without causing any substantial environmental problems.

All of our work has been undertaken with the full cooperation and advice of both KEMRI and the Division of Vector-Borne Disease (DVBD) of the Ministry of Health. Dr. John Ouma, head of schistosomiasis operations at the DVBD, has been invaluable as a consultant for this project. One of Dr. Ouma's long-term goals is to develop a national schistosomiasis control program and he has indicated his strong interest in using biclogical control as part of this program. Unfortunately, such a program awaits further commitment of funds from both government and extramural sources.

In our opinion, larger scale trials further testing our ideas should not be undertaken until our small-scale trials and experiments (and possible additional work by Kenyan scientists) have been completed. As some of these experiments are undertaken under field conditions, it may take until the mid-1990s to complete them. At that time, and before biological control is applied on a large scale under more operational conditions, this whole topic should be the subject of an interdisciplinary meeting in which experts from disciplines

other than parasitology and vector biology are involved.

Formal training of Kenyan students was not included as part of the original proposal. However, several Kenyan parasitologists and malacologists have worked side-by-side with American participants in the field, and have gained first-hand knowledge of medical and veterinary malacology as a result. They have also been made aware of the potential of biological control to serve as an alternative to exisiting methods, and have learned about some of the more likely putative control agents. They have also been made aware of some of the limitations and potential problems that may exist. A partial list of some of the Kenyan scientists who have worked with us on this project are: Dr. John Ouma, Dr. Gerald Mkoji, Mr. Benson Mungai, Mr. Francis Mungai, Mr. Curtis Kariuki, and Mr. Jimmy Kihara. All of these individuals remain professionally active as parasitologists or infectious disease control specialists in Kenya.

V. Project Activities/Outputs

Owing to the long-term nature of some of the experiments, to unexpected findings that we uncovered, and to inherent logistical constraints of field and laboratory work involving personnel in both the U.S. and Kenya, we have not been as prompt as we would have hoped for in disseminating all the results of our work. However, we are highly motivated to do so, and as the appendices indicate, there has been a great deal written up and readied for publication. Our major task is now to submit the remaining manuscripts for publication.

Appendix 3, has been accepted for publication and is in press in Acta Tropica. Two others, 1 and 4, will soon be submitted for publication. Appendix 2 presents information relating to experiments that are ongoing and that have not undergone final analysis; the nature of these experiments requires long-term observations that extend beyond the granting period. Appendix 5 requires revision before submission. Appendix 6 is a manuscript that is in press in the Journal of Molluscan Studies and, although it does not bear directly on the goals stated above, this study could not have been undertaken without the support of this project.

Some of the results have also been presented at regional and national parasitology meetings, as follows:

Hofkin, B.V., Koech, D.K. and Loker, E.S. 1988. Distributional patterns of freshwater snails and associated trematodes in Kenya. Presented at the 21st Annual Meeting of the Southwestern Association of Parasitologists, Lake Texoma, Oklahoma, 14-16 April 1988.

Stryker, G.A., Dominguez, C.A., Koech, D.K., and Loker, E.S. 1988. Consumption of eggs and juveniles of Biomphalaria by African ampullariid snails and by Thiara granifera: a laboratory study. Presented at the 21st Annual Meeting of the Southwestern Association of Parasitologists, Lake Texoma, Oklahoma, 14-16 April 1988.

Bandoni, S.M., Mulvey, M., Koech, D.K. and Loker, E.S. 1988. Electrophoretic and morphological variation in Kenyan populations of Biomphalaria pfeifferi (Krause, 1848), Dunker, 1894 and Biomphalaria sudanica (Martens, 1870) Pfeiffer, 1870-76. Presented at the 21st Annual Meeting of the Southwestern Association of Parasitologists, Lake Texoma, Oklahoma, 14-16 April 1988.

Loker, E.S., Koech, D.K., and Hofkin, B.V. 1988. Distributional patterns of freshwater snails and associated trematodes in Kenya. Presented at the 63rd

Annual Meeting of the American Society of Parasitologists, Winston-Salem, North Carolina, 31 July - 4 August 1988.

VI. Project Productivity

When the U.S. PI originally wrote this proposal, he had not had the opportunity to visit Kenya to make certain first-hand observations relevant to the proposal. Consequently, the original proposal is naive in certain ways. For example, the proposal emphasizes study of the exotic snail Helisoma duryi which we subsequently discovered does not exist in natural habitats in Kenya. This discovery rendered some of our proposed experiments impossible and others irrelevant given the apparent inability of this species to exist in natural habitats in Kenya (see original objectives 1-3 of our revised proposal). One of our goals was also to investigate natural patterns of co-occurrence of pulmonate snails with the putative biolcgical control agent Melanoides tuberculata. As our field observations suggested that this species coexisted in stable patterns with pulmonates, we switched the emphasis of our studies from M. tuberculata to indigenous ampullariid species such as Lanistes carinatus and Pila ovata. This meant that specific objective #5 in our revised proposal was not specifically met. Thus, to a large extent, we shifted priorities as we accumulated information; to do otherwise would have been totally wasteful of time and money. Consequently, the final results depart from experiments described in the original proposal but in the long run, provide information on control agents more likely to be employed successfully.

VII. Future Work

This project has definitely lead to additional work, which is being undertaken with the support of a PSTC-funded proposal entitled "The Role of Biotic and Abiotic Factors in Controlling Disease-Transmitting Snails in Kenya: An Experimental Study", (9.413). The primary thrust of the work described is to further explore the potential of indigenous decapods and especially the introduced crayfish P. clarkii to achieve control of pulmonate snails in small, man-made impoundments in Kenya. We also hope to apply innovative methods such as Geographical Information Systems (GIS) to the study of the distribution and abundance of snails and snail-transmitted parasites in Kenya.

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IX. Appendices

APPENDIX 1

OBSERVATIONS ON THE DISTRIBUTION OF FRESHWATER SNAILS IN SOUTHERN KENYA WITH IMPLICATIONS FOR THE BIOLOGICAL CONTROL OF SCHISTOSOMIASIS AND OTHER SNAIL-TRANSMITTED PARASITES

BY

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ABSTRACT

From 1987 through 1989, freshwater snails were collected from 195 natural and eight artificial habitats in the southern half of Kenya. These collections covered a broad geographic scale to allow assessment of the major distributional patterns of gastropods in this region. From these habitats a conservative total of 34 gastropod taxa were recognized, including 19 pulmonate and 15 prosobranch taxa. Of all habitats, 68.0% contained snails capable of transmitting human schistosomiasis 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. Overall, it was noted that prosobranch taxa dominated habitats below 300m whereas habitats above this elevation contained more pulmonate taxa. With respect to freshwater gastropods, a total of five faunal regions were recognized, including 1) the Coastal Lowland Fauna dominated by prosobranch taxa; 2) the Upland Fauna characterized by abundant representation of pulmonates, including several of medical and veterinary significance; 3) the Highland Fauna, containing simplified assemblages of snails consisting of only pulmonates; 4) the Nyanza Low Plateau Fauna, which is somewhat intermediate in composition between the Upland Fauna and the Lake Victoria Fauna; and 5) the Lake Victoria Fauna characterized by a relatively diverse mixture of both pulmonate and prosobranch taxa, including some strictly lacustrine species. With a few exceptions, most taxa collected occupied a diversity of different habitat types, suggesting most of these snails 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. Within faunal regions, coefficients of association were calculated for pairs of 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 trematode parasites. Included among such putative control agents in Kenya are Helisoma sp., Melanoides tuberculata, Pila ovata, Lanistes carinatus, and L. ovum. Helisoma sp. was found only in artificial habitats, suggesting it does not establish well in natural habitats. M. tuberculata had no significant negative associations with parasite-transmitting pulmonates and was observed to co-exist in some habitats with such snails over the three year period of study, yet typically remained numerically dominant to pulmonates. With respect to indigenous ampullariid species, L. ovum had no significant negative associations with pulmonates whereas both L. carinatus and P. ovata did. Although caution is required in interpretation of this result, this and other considerations suggest the latter two ampullariids deserves additional study as possible natural biological control agents in Kenya. Also, decapod crustaceans, both indigenous freshwater crabs and the introduced North American crayfish Procambarus clarkii, deserve consideration as possible biological control agents in southern Kenya.

INTRODUCTION

In recent years, there have been several reports advocating further study of biological methods for controlling the freshwater snails involved in the transmission of human and ruminant schistosomiasis and other snail-borne parasites (e.g. McCullough, 1981; Thomas and Tait, 1984; Combes and Cheng, 1986; Pointier et al., 1988). Among the putative control agents frequently mentioned are species of freshwater snails that may prey upon, or compete with, the snails transmitting schistosomiasis (McCullough, 1981; Pointier, 1983; Pointier and McCullough, 1989).

Kenya, in common with many African nations, is plagued by several freshwater snail-transmitted trematodes that compromise human health (most notably Schistosoma mansoni and S. haematobium) or that infest domestic livestock (especially S. bovis, S. mattheei and Fasciola gigantica (Diesfeld and Hecklau, 1978; Brown, 1980). The geographic distributions and habitat preferences of the pulmonate snails that transmit these parasites in Kenya have been relatively well-studied (e.g. Van Someren, 1946; Teesdale, 1962; Brown, 1980; Brown et al., 1981).

Also presently found in Kenya are several species of gastropods considered to have some potential as biological control agents. Included among these are Helisoma duryi, a North American pulmonate snail now present in scattered locations in Africa (Appleton, 1977c; Madsen, 1979; Brown, 1980), and Melanoides tuberculata, a prosobranch, which comprises part of the normal freshwater snail fauna of Kenya (Brown, 1980). The latter snail, 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 area (Prentice, 1983; Pointier et al., 1989; Pointier and McCullough, 1989). Also found in Kenya are representatives of the prosobranch family Ampullariidae, including snails of the genera Pila and Lanistes. Such snails are of relevance to the topic of biological control because several reports have implicated the related ampullariids Marisa cornuarietis and Ampullaria glauca as effective biological control agents of pulmonate snails (e.g. Ferguson, 1977; Nguma et al., 1982; Jobin et al., 1984; Pointier et al. 1988). Efforts to develop feasible methods of biological control in Kenya are currently hampered by the lack of information pertaining to putative control species which are poorly known with respect to current geographic distributions, habitat preferences, and potential to coexist with vector species.

From 1987 through early 1989, as part of a program to explore the potential of developing biological methods of control of snail-transmitted parasites in Kenya, we surveyed freshwater gastropod habitats in southern Kenya. In this paper, we first report general observations pertaining to the distributional patterns of snails collected in over 200 habitats in this region. We then report our observations pertaining to snail species considered to have potential in biological control, including recent distributional information and patterns of co-occurrence with parasite-transmitting snails of medical and veterinary significance.

MATERIALS AND METHODS

Most of the snails collected in this survey were obtained in January of 1987, 1988, and 1989 or in June and July of 1987 and 1988. A deliberate effort was made to search all types of waterbodies encountered during our survey trips; the habitats from which snails were recovered therefore represent a relatively unbiased selection of snail habitats in southern Kenya. To outline the major distributional patterns of snails in this region, our collection efforts emphasized obtaining an overview of representative regions of southern Kenya, rather than detailed collections on smaller scales. Snails were collected using standard wire mesh dip nets (mesh size of $5mm^2$), or were picked from vegetation or rocks pulled from the habitat. A minimum of one man-hour of collection time was expended in each locality. All snails encountered, regardless of species, were saved and were either preserved in 70% ethanol, frozen in liquid nitrogen for other studies, or were maintained alive and brought to the laboratory for further study. All snails were identified using keys and descriptions provided by the Danish Bilharziasis Laboratory (1973) or Brown (1980). All preserved snails will be deposited in the National Museum of Kenya in Nairobi.

The latitude and longitude of each collection locality was determined and recorded on a route map of Kenya (the SK 81 map, scale 1:1,000,000). Using the topographic information on this map (originally recorded in feet), each collection locality was assigned to one of the ten following altitudinal categories (in meters): 1 (0-152), 2 (153-305), 3 (306-610), 4 (611-915), 5 (916-1220), 6 (1221-1524), 7 (1525-1829), 8 (1830-2134), 9 (2135-2439), and 10 (2440-2743). For each collection location, the climatic regime was approximated by data collected by the nearest meteorological station (located by reference to the "Climatological Statistics for Kenya", issued by the Kenya Meterological Department, 1984). Each habitat in which snails were found was assigned to one of 15 recognized habitat categories; further information regarding habitats is provided in the attached list.

It was of interest to determine if certain taxa of gastropods occur together within the same natural habitat more or less often than would be predicted by chance alone. Therefore, for different pairs of gastropod taxa, we calculated a coefficient of association based on equation 19.44 provided by Zar (1984). This coefficient ranges from a value of +1 for complete co-occurrence to -1 for a complete lack of co-occurrence. The significance of the association was also assessed using the Fisher exact test (Zar, 1984). When calculating such coefficients, for each pair of taxa considered, we enumerated only habitats within a particular faunal region where both taxa were known to be present (see Figure 7). Coefficients could not be computed for some prominent pairs of taxa because they occupied different faunal regions.

RESULTS AND DISCUSSION

Comments on Identifications of Snails: As uncertainties persist regarding the taxonomy and identification of some groups of Kenyan freshwater gastropods, the following rationale is presented for the taxon designations used in this study. We did not attempt to differentiate between species of bulinid snails of the *Bulinus africanus* group (snails in this group are also known as the subgenus or genus *Physopsis*). All such snails in our collections were designated as *Bulinus* (*Physopsis*) sp. Similarly, we did not attempt to differentiate among representatives of the *Bulinus truncatus/tropicus* complex; for convenience, they are referred to below as the *Bulinus tropicus* complex. Although the taxonomic scheme used may influence to a minor degree some of the statements made below, it is conservative and does not obscure the general patterns. Many of the analyses were made at the generic or species group level and are therefore less subject to taxonomic bias.

Observations on General Patterns of Freshwater Snail Distributions: The northern-most collections were made around Kitale and Maralal; logistical constraints prevented collection in the relatively arid and remote regions of northern Kenya, including regions north and east of the Tana River. A total of 195 natural and 8 artificial habitats containing snails were sampled; the locations of the sampling sites are indicated in Figure 1 and are detailed in the attached list. The distribution of the collection localities with respect to altitude is indicated in Figure 2. Notice the relatively small numbers of collection sites located in altitude categories 3 and 4. These altitude ranges cover an extensive portion of eastern Kenya receiving less than 500 mm of rain per year (Ojany and Ogendo, 1973) and, despite extensive searching in this region, snail habitats were not abundant. Also, we collected in relatively few habitats above 2135 m because such habitats contain simplified and predictable assemblages of snails of less relevance to public health. From the 195 natural habitats in which snails were found, a total of 33 gastropod taxa were recognized (Table 1), including 18 pulmonate and 15 prosobranch taxa. An additional pulmonate taxon, designated *Helisoma* sp. (probably *Helisoma duryi*) was recovered from the artificial habitats. The altitudinal range and the total number of habitats in which each taxon was found are also indicated in Table 1.

One of the prominent patterns observed was the different overall distributions of prosobranchs and pulmonates, with the former group being more abundantly represented at lower altitudes and the latter at high altitudes. This pattern held true for both total number of taxa recovered (Figure 3) and the number of taxa per habitat (Figure 4). This pattern can not be attributed to differential representation of preferred prosobranch or pulmonate habitats at different altitiudes. For example, if a specified type of habitat is considered, the same trends hold true. Thus small dams in altitude categories 1 and 2 have significantly fewer pulmonates ($t = 9.297$, $p < 0.001$) and significantly more prosobranchs ($t = 3.477$, $p = 0.001$) than small dams in altitude categories 5 and 6. We did not find prosobranch snails at altitudes over 1800 m in this survey. For several prominent taxa of Kenyan snails, the proportion of habitats occupied, as a function of altitude, is indicated in Figure 5.

Not surprisingly, there is a strong inverse relationship between altitude and air temperature (Figure 6), over the range of altitudes examined in our survey, implying that temperature (acting directly, or perhaps indirectly on parameters such as dissolved oxygen concentration) plays a large role in defining the major patterns of geographic distributions of Kenyan freshwater snails. Brown (1980, p. 393) has noted that it is likely that temperature is of paramount importance in shaping gastropod distributions in southern Africa, and that the effect of temperature becomes particularly obvious in areas where large climatic transitions occur (p. 361). Such is the case in a 200 km-wide zone in eastern Kenya where both dramatic climatic and faunal transitions co-occur.

The important biogeographic role of temperature is further supported by several studies of individual species of African freshwater snails, particularly those involved in transmission of schistosomiasis (reviewed by Appleton, 1978 and Brown, 1980). For example, Sturrock (1966) concluded that high temperatures are a major barrier to the colonization of the coastal plain of Tanzania by Biomphalaria pfeifferi. Appleton (1977a,b) observed that absence of B. pfeifferi in shallow waterbodies on the sub-tropical coastal plain of Natal and Mozambique was due to the adverse effects of high temperature on gametogenesis and development of the gonad (Appleton and Eriksson, 1984). Although comparably detailed temperature data derived from snail habitats in coastal Kenya are not presently available, it is probable that temperature regimes experienced in this region would normally prevent colonization by B. pfeifferi. This view is supported by the work in coastal Kenya of O'Keefe (1985a,b) who noted a strong inverse relationship between the the intrinsic rate of natural increase of Bulinus globosus and mean monthly water temperature. Several of the monthly means reported by O'Keefe are above the 29°C maximum optimum for B. pfeifferi discussed by Appleton and Eriksson (1984). Distributions can also be limited by low environmental temperatures, as exemplified by studies with B. globosus in southern Africa; this species does not breed at higher altitudes during the cool season (Shiff, 1964b), and laboratory studies have shown that maturity is delayed and fecundity sharply reduced at low temperatures (Shiff, 1964a). Although it is generally appreciated that cool temperatures are unfavorable to many genera of African prosobranchs (e.g. Brown, 1980, p. 391), there are no detailed studies to assess the effects of temperature on their distributions.

It is important to consider that the role of temperature in shaping geographic distributions may be less direct than envisioned above, and may actually affect other parameters such as dissolved oxygen concentration. For example, van Someren (1946) expressed the opinion that temperature per se had little direct effect on L. natalensis, but that high temperatures may have reduced the oxygen tension in the water to detrimental levels, particularly by increasing rates of plant growth and organic decay.

Temperature is certainly not the only factor involved in controlling broad distributional patterns of gastropods in East Africa. One example of the potential complexities involved is provided by Lanistes carinatus, which in our survey was recovered only from the coastal lowlands. Although this distribution would imply a strict dependence on warm tropical conditions,

Mandahl-Barth (1954) reports this species from Lake Kyoga and adjacent areas in Uganda, and it is abundant in the Nile drainage (Brown, 1980). We were able to establish self-perpetuating colonies of this species in concrete-lined outdoor aquaria on the grounds of the Division of Vector-Borne Diseases in Nairobi (unpublished observations). In this case, it would appear that the requirements for tropical conditions as experienced on the coast are not absolute. For large dioecious prosobranchs such as Lanistes and Pila, limitations in geographic range may well be imposed by relatively poor dispersal ability, by the activity of biological factors such as predation, or of some as yet unappreciated combination of these factors acting in concert with temperature constraints (see Davis, 1982 for a general discussion of factors shaping biogeography of freshwater snails).

Major Regional Faunas Within Southern Kenya: While collecting snails across the broad geographic scale presented by southern Kenya, it became apparent that different regions possessed characteristic molluscan faunas, and, at least in some cases, sharp discontinuities could be identified between regions. Based on our collections which, as noted above, were intended to reveal large-scale patterns, we recognized five distinct regional freshwater gastropod faunas within southern Kenya. Further in-depth collections in specific regions, such as those performed by Brown (1975) on the Kano Plains in western Kenya, may result in the recognition of additional characteristic assemblages, or may allow the boundaries of different faunal regions to be more precisely defined. Data pertaining to the relative abundance of different gastropod taxa in each fauna are presented in Figure 7, and were used to define the five groups described below:

1) Coastal Lowland Fauna: This fauna is dominated by the prosobranch genera Lanistes and Cleopatra, and to a lesser extent by Bellamya, Pila and Melanoides. The single most abundant taxon, however, is the Bulinus (Physopsis) species group which is also the only common pulmonate taxon in this region. This fauna is confined to altitudes below approximately 300m (it includes altitude categories 1 and 2). Biomphalaria is absent from the coastal fauna, and both Lymnaea and the Bulinus tropicus group are relatively uncommon.

2) Upland Fauna: This fauna is separated from the coastal fauna by a relatively sharp transitional zone between 300 - 600 m (altitude category 3) which is arid and contains relatively few snail habitats. Pila, Cleopatra and Bulinus (Physopsis) are found in the transitional zone; Biomphalaria may also extend down to altitudes of 300 m (Brown et al. 1981) although we did not observe this genus below 600 m in our survey.

The upland fauna defined here is found at altitudes between 600 and 1830 m (altitude categories 4-7) and is bounded approximately by Voi to the east, and the Nyanza Low Plateau to the west. Our collections do not permit us to define the northern limits of the upland fauna, although collections around Isiolo are typical of this faunal region. It extends to the Tanzanian border to the south. This fauna is characterized by relatively abundant representation of pulmonate genera including Biomphalaria (especially B. pfeifferi), Lymnaea, and Bulinus. Among the species groups of Bulinus, the tropicus group, forskali group, and the Physopsis group are all well-represented. The prosobranch genera Bellamya, and Melanoides are represented in this region, but are more rarely encountered than the most common

pulmonates. These two genera are absent in our upland collection sites lying west of Nairobi. The introduced species Physa acuta appears in several natural habitats in this region, again especially in the eastern portion of the upland region.

3) Highland Fauna: This faunal region is found at altitudes above 1830m, an altitude range that is not well-represented in our collections. It is not sharply demarcated from the upland fauna defined above but is nonetheless distinguishable for the following reasons: 1) prosobranchs are lacking; 2) Biomphalaria is rare and Bulinus (Physopsis) is apparently absent, meaning that human schistosomiasis transmission in this region is rare; 3) Lymnaea and the Bulinus tropicus group are the dominant gastropods; 4) small pulmonates including Ceratophallus and Ferrissia sp. are relatively more common than in the previously mentioned faunal regions, and 5) the average number of taxa per habitat is relatively low (Figures 3 and 4).

4) Nyanza Low Plateau Fauna: This region consists of areas ranging from the shore of Lake Victoria (altitude 1240 m) up to an altitude of approximately 1520 m, in western Kenya. Its boundary with the upland fauna is not sharp but it is considered distinct on the following grounds: 1) the ampullariid genus Pila is common in this region; 2) other prosobranchs including Gabbiella humerosa and Cleopatra guillemei are common here but lacking in the upland fauna. To a lesser extent, Melanoides and Bellamya are also represented (our original collections do not include these latter two genera from this region but recent observations made near Makalders Mines west of Migori indicate they are present); 3) Bulinus (Physopsis), Bulinus forskali, Ceratophallus, and Biomphalaria sudanica are relatively more abundant than in the upland fauna; and 4) the small pulmonate genus Segmentorbis is present in this region but rare or absent in the upland fauna. The fauna of the Nyanza low plateau is clearly influenced by Lake Victoria but differs from the lake in several ways, including: 1) the absense of endemic lacustrine species such as Biomphalaria choanomphala; 2) the relative scarcity of the prosobranchs (especially noticeable in this regard are Melanoides tuberculata and Bellamya sp., both of which are abundantly represented in the lake; and 3) lower overall numbers of taxa per collection site.

5) Lake Victoria Fauna: The freshwater gastropod fauna of Lake Victoria has been relatively well-characterized (e.g. Mandahl-Barth, 1954; Brown, 1980) and includes five prosobranch and nine pulmonate genera. Relative to collecting sites in the other identified faunal regions of Kenya, the diversity of species in our six collections from the Kenyan shore of the lake is high (Figure 4). Both pulmonates and prosobranchs contribute to this greater diversity. Similarly, as compared to other recognized habitat types, the shore of Lake Victoria supports a diverse fauna (Figure 8). Our collections under-represent the known diversity of the lake's gastropod fauna as they did not include limpets of the genus Burnupia, Lentorbis junodi, or Cleopatra cridlandi (Brown, 1980).

Observations on Habitat Types Occupied By Particular Taxa: The number of times each taxon was recovered from each of the 15 designated habitat types is indicated in Table 2. The average number of taxa recovered from the 15 different recognized habitat categories are indicated in Figure 8. As mentioned above, collection localities on the shores of Lake Victoria contained

the most diverse gastropod assemblages. Most other habitat types contained on average between 2 and 3 gastropod species. Ephemeral habitats (categories 8 and 9) had relatively low numbers of species. The three most frequently represented habitat types in our survey were small dams, small flowing streams, and small intermittent streams; the total number of different taxa collected in these three habitat types were 22, 20 and 18, respectively. The potential for involvement of these three habitat types in transmsision of human schistosomiasis or fascioliasis is emphasized in Figure 9. With a few obvious exceptions such as B. choanomphala which is endemic to Lake Victoria, most common taxa were collected in a variety of habitat types (Figure 10) and were commonly found in both impounded water and stream courses. For example, B. forskali was found in 14 of the 15 recognized habitat types, in sites as diverse as the Lake Victoria shoreline and small, ephemeral pools.

Within Habitat Associations Between Taxa: Coefficients of associations between the prominent taxa of gastropods relevant to our study are summarized in Table 3. First it should be noted that such coefficients have been calculated separately for each faunal region (except for Lake Victoria). Such coefficients can not be calculated for some pairs of taxa simply because they occupy different regions. For example, the geographic ranges of Biomphalaria and Lanistes do not overlap in Kenya, so they do not co-occur within the same habitat. Among the coefficients calculated, relatively few significant associations were noted, suggesting that even within a particular faunal region, the species composition within habitats is not highly predictable.

There are some exceptions to this generalization. In the coastal lowlands, L. carinatus has a significant negative association with both Bulinus (Physopsis) and L. ovum In the upland fauna, B. pfeifferi has a significant positive association with both L. natalensis and the Bulinus tropicus complex. In the Nyanza low plateau fauna, snails of the Bulinus tropicus complex show significant negative associations with both B. pfeifferi and Bulinus Physopsis), and B. pfeifferi is negatively associated with P. ovata. Our results regarding a significant positive association between L. natalensis and B. pfeifferi in the upland fauna are in essential agreement with those of van Someren (1946) who also noted strong positive associations in Kenya. This further implies that Kenyan habitats involved in transmission of Schistosoma mansoni are likely also to be involved in transmission of Fasciola gigantica as well (see van Someren, 1946 for additional discussion). It should be noted however that L. natalensis has a broader altitudinal range in Kenya than B. pfeifferi such that the presence of the former can not necessarily be used to predict the potential suitability of the habitat for the latter.

In South Africa, van Eeden and Combrinck (1966) noted strong positive associations between L. natalensis and Bulinus (Physopsis), and observed similar distributions of these two taxa with respect to altitude; for example, both taxa showed peak abundance at approximately 3000 ft. They concluded that in South Africa L. natalensis can be regarded as an indicator species of the suitability of a particular habitat for colonization by Bulinus (Physopsis). In our study, these two taxa did not show significant positive associations, and their altitudinal distributions were somewhat different with L. natalensis being proportionately more abundant at higher altitudes and Bulinus (Physopsis) more abundant at lower altitudes. However, our results are are in essential

agreement with their conclusion that with increasing altitude, Bulinus (Physopsis) first drops out followed by Lymnaea and finally Bulinus (van Eeden and Combrinck, 1966). Other significant associations observed are discussed below.

Observations on Specific Taxa of Potential Significance in Biological Control:

Helisoma sp.: Snails of this North American genus, perhaps more than one species, have been present in scattered localities in Africa for at least 22 years, and have been present in Kenya since at least 1974 (Brown, 1967; Haller, 1974; Appleton, 1977c; Brown, 1980). Potential use of this snail in biological control of schistosome-transmitting snails in Africa has been discussed by several authors (Appleton, 1977c; Frandsen and Madsen, 1979; Brown, 1980; Madsen, 1983). Appleton (1977c) and Brown (1980) noted that the ability of Helisoma to persist in other than artificial or semi-artificial habitats in Africa can be doubted as it has not spread into natural habitats from such restricted foci. Also, Karoum (1988) noted that Helisoma duryi populations did not establish well in field breeding sites in the Sudan.

Our recent collections support this conclusion. Helisoma was recovered only in habitats that were lined with concrete, typically in ornamental pools. Populations of Helisoma were found in Malindi, Mombasa, Nairobi, and near Lake Naivasha. Natural habitats in the vicinity of such ornamental pools were searched but were consistently negative for this species. We did not find Helisoma in the Mazeras Botanical Garden where it has been previously reported (Brown, 1980). We have not extensively surveyed artificial habitats such as ornamental pools in other locations in Kenya so we can not comment on its presence in locations to the west and north of Lake Naivasha. In Kenya, Helisoma therefore differs from other exotic species such as Physa acuta or Lymnaea truncatula in being rare or absent from natural habitats. Although hermaphroditic, African Helisoma are apparently obligatory out-crossers (Madsen et al., 1983). This property may limit its ability to indiscriminately spread into new habitats which can be interpreted as a favorable characteristic for a putative biological control candidate (e.g. Madsen, 1983). In our opinion, this or other as yet unresolved factors significantly impair colonization by Helisoma of natural habitats in Kenya, and consequently cast serious doubt on its ability to function effectively in a biological control context.

The potential difficulty for field workers in differentiating some specimens of Helisoma from Biomphalaria (e.g. Brown, 1980, p. 180) deserves reiteration. In Bamburi, we found specimens that could be confirmed as Helisoma only after dissection and examination of the genitalia. Conversely, in habitat #190 in western Kenya, specimens of B. pfeifferi with unusually high whorls required dissection to avoid misdiagnosis as Helisoma.

Melanoides tuberculata: Recent studies by Prentice (1983) and Pointier and co-workers in the Caribbean area indicate that this species, and the closely related Thiara granifera, are capable of rapidly colonizing habitats and reducing and even eliminating populations of Biomphalaria (Pointier, 1983; Pointier et al., 1989; Pointier and McCullough, 1989). For example, in water-

cress beds in Martinique, M. tuberculata established dense populations in less than one year, and apparently eliminated B. glabrata within two years (Pointier et al., 1989). The exact mechanism whereby thiarids snails displace Biomphalaria in the Neotropics is not known.

In Kenya, M. tuberculata was the most common prosobranch snail overall in our collections. Its altitudinal range is relatively broad and it was found at a higher altitude (1100 m, near Kitui) than any other Kenyan prosobranch except Bellamya unicolor. As indicated in Figure 1, this species is most commonly encountered in natural habitats in eastern Kenya. It is also frequently observed in ornamental pools. Its relative abundance in any altitudinal category never approached that of the common pulmonates or low-altitude prosobranchs (Figure 5). Although this species is common in Lake Victoria, it is relatively rare in small habitats adjacent to, but separate from the lake. Mandahl-Barth (1954) found M. tuberculata to be abundant in large lakes and rivers in Uganda, but rare in smaller habitats throughout the country. We noted no significant patterns of association, either positive or negative, for this snail with other gastropod taxa. In 24 of the 33 natural habitats where M. tuberculata was encountered, it coexisted with snails capable of transmitting human schistosomiasis or fascioliasis (Table 4). In the Kitui region, we have made periodic observations of three habitats where both M. tuberculata and B. pfeifferi are present. The former species has remained much more abundant than the latter over a three year period of observation.

The potential role of M. tuberculata in biological control in Africa in general, and Kenya in specific, remains enigmatic, and deserves additional study. Thomas and Tait (1984) indicated that this species may be useful in biological control in lotic environments based on their field and laboratory studies in Nigeria, although the observed interactions of M. tuberculata with target species were much less dramatic than observed in the Caribbean area. Karoum (1988) noted no negative associations between M. tuberculata and B. pfeifferi in the Gezira, and one year noted a positive association. Pulmonates of medical significance apparently coexist with this species in stable associations in Kenya, but this does not rule out the possibility that M. tuberculata exerts a negative effect under such circumstances. A more dramatic competitive effect, perhaps similar to results obtained in the Caribbean area, might be observed if Kenyan specimens of M. tuberculata were transplanted into habitats were they presently do not occur. Such introductions may favor a rapid and extensive proliferation capable of causing elimination of target species. However, it must also be considered that African pulmonates have probably co-existed with M. tuberculata over long time intervals, and may have adjusted to any adverse effects of this species. Also, snails recognized as M. tuberculata collectively have a huge geographic range (Pace, 1973; Brown, 1980), and genetic studies are needed to determine if African specimens are, in fact, closely related to specimens from the Caribbean islands.

East African specimens of M. tuberculata harbor trematode infections (e.g. Fain, 1953; Loker et al., 1981; Sommerville, 1982), a fact that must be borne in mind should these snails be considered for extensive use in a program of biological control. Although this snail has been implicated as a possible host of Paragonimus westermani and Clonorchis sinensis (Tucker-Abbott, 1952), it is unlikely to be involved in transmsission of either parasite in East

Africa (Malek, 1980; Brown, 1980). Also, it is known to transmit the heterophyid trematode Haplorchis pumilio in Kenya, which utilizes fish such as Tilapia as a second intermediate host (Sommerville, 1982). Finally, should M. tuberculata prove to be an effective biological control agent, one distinct advantage it would enjoy over other proposed control agents such as ampullariids and crustaceans is that it is very unlikely to damage rice or other aquatic crops (e.g. Pointier et al., 1989).

Cleopatra: Consideration of this thiarid genus in the context of biological control may be relevant considering that related snails seem to be successful control agents in the Neotropics. Furthermore, C. ferruginea and C. bulimoides are both abundant in the coastal lowlands and in many of the streams in this area are the dominant gastropod species. In some intermittent streams, such as the Rare River (#42), they virtually cover the substrate. At higher altitudes, this genus is relatively rare; representatives were recovered in Lake Baringo (designated as C. bulimoides congener) and in the Nyanza low plateau (C. guillemei).

Our distributional data indicate that thriving populations of Bulinus (Physopsis) often coexist with Cleopatra, even in locations where the latter genus is very abundant. In the coastal region, in 21 of 29 habitats where Cleopatra was found, Bulinus (Physopsis) was also present. These two taxa occupy different microhabitats, the former being heavy-shelled snails confined to substrates while the latter is typically noted attached to vegetation or other objects higher in the water column, or along the margin of the habitat. Such microhabitat differences may prevent any effective form of competition from occurring.

Pila ovata: Other ampullariid species including Marisa cornuarietis and Ampullaria glauca have been implicated as effective biological control agents of pulmonate snails. For example, Nguma et al. (1982) introduced Marisa into a pond in northern Tanzania and noted the complete disappearance of B. pfeifferi, Bulinus tropicus, and L. natalensis in approximately 24 months. A relatively recent study by Pointier et al. (1988) in a small lake in Guadeloupe demonstrated the near complete displacement of Biomphalaria glabrata by Ampullaria glauca, an effect that was attributed to the voracious comsumption by the ampullariid of plants favorable to B. glabrata. In addition to destroying aquatic vegetation that may serve as food sources or oviposition sites for pulmonates, ampullariids may also exert a control effect by acting as predators of egg masses, juveniles and even adult pulmonates (Chernin et al., 1956; Demian and Lutfy, 1965, 1966; Cedeno-Leon and Thomas, 1983). We have noted that native Kenyan ampullariids P. ovata and L. carinatus share with Marisa the ability to consume egg masses and juveniles of planorbid snails (unpublished data). Consequently it was of interest to examine the patterns of association of these ampullariids with pulmonates in Kenya.

The distribution of P. ovata in Kenya is discontinuous (Figure 1). This species is relatively common in collections from the coastal region of Kenya, and is also common both in Lake Victoria and in adjacent habitats in the Nyanza low plateau. It was recovered in other scattered localities in eastern Kenya, typically in altitude categories 4 or below. Although we did not recover specimens that could unambiguously be classified as Pila wernei, this species is known to exist in Kenya along the northern coastal region (Brown, 1980) and is found in swamps on the north end of Lake Turkana, actually in Ethiopia (Cohen, 1986).

In 18 of our 28 collection localities for P. ovata, pulmonate snails capable of transmitting either human schistosomiasis or fascioliasis were found (Table 4). It was commonly found with Bulinus (Physopsis) and L. natalensis. P. ovata was noted to have a significant negative coefficient of association with B. pfeifferi in the Nyanza low plateau, otherwise this species had no significant association with other gastropods in our collections. Although this result suggests that P. ovata may exert some control influence over B. pfeifferi, further collection data are required to substantiate this finding. Also, other interpretations are possible and should be considered in future studies. For example, the ability of P. ovata to exist in temporary habitats is well known (Brown, 1980), and the negative association could simply reflect differences in habitat preferences, as B. pfeifferi is more confined to permanent water bodies. However, in several of the P. ovata habitats where B. pfeifferi was absent, L. natalensis was present, suggesting that habitat conditions existed that were generally favorable to B. pfeifferi. Finally, the negative association of P. ovata and B. pfeifferi may simply reflect differences in climatic preferences; over much of Kenya, the ranges of these two species do not coincide.

Based on the present distributions of P. ovata and target pulmonate species in Kenya, we conclude that any effect this species has as a control agent is subtle at best. For the future, long-term laboratory studies are needed to establish if P. ovata can exert a significant effect on populations of target species. Such studies typically favor the control species and enhance the chance of observing a favorable effect. If such studies produce negative results, it seems very doubtful that P. ovata could be effective under field conditions. Also, experimental introductions of P. ovata into suitable sites containing target species should also be undertaken, to determine if this species can establish outside its present range, and if can exert a negative effect on populations of target species. Habitats chosen for such studies should be far removed from rice-growing areas.

Lanistes carinatus: This ampullariid, in common with other members of the genus, has a restricted distribution in Kenya, presently being found only in coastal Kenya at altitudes below approximately 200 m. We found it only in habitats to the north of Mombasa, particularly in the vicinity of Malindi. This species was typically found in lentic habitats, often in association with the floating leaves of water lilies (Nymphaea). We have noted that the stomach contents of L. carinatus consist largely of detritus, and thereby differ from the stomach contents of P. ovata which consist primarily of macrophytes (unpublished data). Karoum (1988) also noted that L. carinatus consumes significantly fewer aquatic macrophytes than Marisa in the Gezira. Often it

could be predicted if a particular habitat contained this species by noting the presence of flocks of open-bill storks (Anastomus lamelligerus) in the vicinity. Such storks are conspicuous predators of this snail species in Kenya.

This species has received some consideration as a potential control agent of pulmonates in the Gezira Agricultural Scheme in the Sudan where it occurs naturally (Karoum, 1988). It was noted that L. carinatus populations became established in all three experimental canals where it was introduced, but it was found at low densities, and its introduction did not result in any clear impact on the target species, Biomphalaria pfeifferi and Bulinus truncatus. Although a gradual reduction in the density of B. pfeifferi was noted in canals containing L. carinatus, weed-clearing operations were underway at the same time, rendering conclusions difficult (Karoum, 1988). Madsen et al. (in press) noted a positive correlation between L. carinatus and B. pfeifferi in the Gezira and Rahad irrigation schemes in the Sudan.

In our studies it was found to have a significant negative coefficient of association with both Lanistes ovum (all subspecies considered together) and Bulinus (Physopsis). Nonetheless, in 17 of the natural habitats where we found L. carinatus, it coexisted with Bulinus (Physopsis) in 6 (Table 4). Once again, even though this species can consume pulmonate egg masses and juveniles under laboratory conditions, and it may be a effective food competitor for schistosome-transmitting pulmonates, its ability to control natural populations of target species below a level that would prohibit schistosomiasis transmission can be severely doubted. Other factors, including predation by birds, and avoidance behavior by bulinid snails, may effectively nullify any beneficial effect of this species in controlling Bulinus (Physopsis) in Kenya. As previously noted, the distribution of this species in East Africa is peculiar and, at least in Kenya, it does not appear to overlap the distribution of Biomphalaria. Experimental introductions to determine if L. carinatus can displace existing populations of B. pfeifferi in Kenya should be attempted.

Lanistes ovum: The three recognized subspecies of L. ovum are confined in Kenya to altitudes less than 305 m. The possible role of these snails as biological control agents has received very little attention, although Teesdale (1962) observed that Lanistes olivaceous, a synonym for L. ovum (Brown, 1980), is often found in coastal habitats in Kenya where Bulinus (Physopsis) is absent. He noted that it was more likely that this reflected different habitat preferences rather than competitive exclusion by the ampullariid.

In the majority of our collection localities for L. ovum, it was coexisting with Bulinus (Physopsis) (Table 4), including locations where both snail taxa were thriving. Many of the same considerations discussed above also apply to L. ovum and, in our present opinion, any control effect exerted by this species must be subtle and at a level that would be unlikely to preclude successful transmission of schistosomiasis.

Marisa cornuarietis: To our knowledge, this snail presently is not found in Kenya. It has been introduced in habitats near Moshi, Tanzania, at a distance of approximately 100 km from the Kenya border (Nguma et al., 1982). Its establishment in a single dam there coincided with the disappearance of at least three pulmonate species of medical significance. For a variety of

reasons, it is unlikely to spread extensively beyond its point of introduction in Tanzania (Nguma et al., 1982). M. cornuarietis has also been introduced in the Sudan, and specimens have been introduced into the Gezira Agricultural Scheme (Haridi et al., 1985; Karoum, 1988). Karoum (1988) experienced difficulty in establishing dense populations in irrigation canals, but noticed that in sites where M. cornuarietis became established that submerged macrophytes disappeared and that significant reductions in B. truncatus populations were noted. A less obvious effect was noted for B. pfeifferi. Because of the difficulty in establishing thriving populations, Karoum (1988) advised caution in interpretation of their results and suggested that further long-term study is needed.

In our opinion, indigenous ampullariids are present in Kenya, and their potential in biological control operations should be thoroughly explored before the option of introducing an exotic snail such as Marisa is exercised. Our distributional studies provide some justification for further study of native species, particularly for the combinations of P. ovata and B. pfeifferi, and L. carinatus and Bulinus (Physopsis). Also, other laboratory studies suggest that indigenous ampullariid species consume egg masses and juvenile snails at rates comparable to Marisa. Experimental introductions of native ampullariids should be attempted and evaluated prior to use of Marisa. As rice is an important food crop in Kenya, the decision to use any ampullariid in biological control should be considered carefully, and rice-growing areas should be avoided as introduction sites, at least until further information on the extent of the risk becomes available. Relevant points regarding the danger of Marisa and other ampullariids to rice plants are summarized by Nguma et al. (1982) and Karoum (1988). Our concern about further introductions of Marisa into tropical Africa are heightened by recent reports from Japan regarding introductions there of the related South American snail Ampullaria glauca, which has become a serious pest in rice fields (Nishimura et al., 1986). Finally, any program of biological control based on the use of Marisa or other indigenous ampullariids should carefully consider the possible implications with respect to transmission of Angiostrongylus cantonensis and related nematodes. A. cantonensis is not presently known from East Africa, but its geographic range is expanding (Cross, 1987), and Lanistes carinatus snails in Egypt have been found naturally infected (Yousif and Ibrahim, 1978). Although we have not found any such nematodes in over 400 ampullariids snails from Kenya, the possibility may exist in the future for transmission of this parasite in East Africa.

Other Biological Control Agents: In addition to competitor/predator snails, a variety of other biological control agents for pulmonate snails have been proposed over the years (see reviews by Michelson, 1957; McCullough, 1981; Thomas and Tait, 1984; Combes and Cheng, 1986; Pointier et al., 1988. In Kenya, McMahon et al., (1977) have explored the potential for using fish in snail control, and noted some potential for Astatoreochromis alluaudi to reduce populations of B. pfeifferi in earthen dams in western Kenya. To our knowledge, these initial studies have not been pursued by other workers.

Another group of organisms deserving of additional study in a biocontrol context in Kenya are both indigenous and introduced decapod crustaceans. While collecting snails from different parts of Kenya, it became apparent that freshwater crabs were abundant in certain types of habitats,

particularly in streams in areas within 50 km of the coast or in western Kenya. Such habitats often either did not contain snails or supported predominantly populations of prosobranchs including Cleopatra, Bellamya, Melanoides and Lanistes. These four genera of snails all possess a tough operculum and a thickened shell that is difficult to crack even with pliers. In contrast, in most habitats sampled in upland and highland Kenya, particularly in isolated impoundments, crabs and most prosobranch genera are lacking, and relatively thin-shelled pulmonates such as L. natalensis and B. pfeifferi predominate.

Although we still believe that altitude and its effect on temperature play a major role in influencing gastropod distributions in Kenya, a role for predation by decapods has not been explicitly ruled out, and is compatible with the overall trends we have observed. Furthermore, we have noted that as yet unidentified crabs from a stream near Mazeras in the coastal region readily eat pulmonate snails in the laboratory (see also Lagrange and Fain [1952] and Deschiens et al. [1955]). Caution is warranted however, as such crabs co-exist with Bulinus (Physopsis). Another possible example of the impact of crustaceans on snail populations in Kenya is provided by Lake Naivasha where snails such as B. sudanica that used to be present there (e.g. Brown, 1980) have not recently been found. Such changes may be directly attributable to the presence of the North American crayfish Procambarus clarkii which has thrived in the lake since its original introduction in about 1970 (Lowery and Mendes, 1977). We have also observed smaller impoundments in the uplands of Kenya where crayfish are abundant and snails are virtually lacking, and have observed that P. clarkii readily consumes snails in the laboratory. It is also known for its voracious consumption of macrophytes, including possibly rice plants (Sommer and Goldman, 1983; Feminella and Resh, 1989).

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Appendix 1: Listed for each habitat is a local name, habitat type (indicated in parentheses with a number ranging from 1 to 15), and latitude and longitude. The habitat types were defined as follows: 1) artificial habitat, mostly concrete-lined ornamental pools; 2) Lake Victoria, collections made from shore; 3) Other large lakes, including Lakes Naivasha, Baringo, and Jipe, collections made from shore; 4) large permanent dams or small lakes; 5) small dams that permanently hold water; 6) dams in small stream channels; 7) large natural swamps and marshes; 8) borrow pits or overflow areas, typically adjacent to roads; 9) shallow and ephemeral pans; 10) shallow seepage areas around natural springs; 11) margins and pools of small, flowing streams; 12) margins and pools of large, flowing rivers; 13) pools in small, intermittent streams; 14) irrigation canals and ditches with slowly flowing water; 15) small man-made trenches with non-flowing water.

1. Matingani Stream (10), 1° 7' S, 37° 53' E;
2. Matingani Dam (9), 1° 7,S, 37° 53 E;
3. Muthamo Dam (5), 1° 8.4 S, 38° 2.7 E;
4. Muthamo Spillway (11), 1° 8,4 S, 38° 2,7 E;
5. Muthamo Seepage (1), 1° 8.4,S, 38° 2.7,E;
6. Migwani Seepage (11),1°,4.8 S, 38°,2.1 E;
7. Mbondoni Dam (4), 0° 57 S, 38° 2.4 E;
8. Kwakasoa Dam (9), 1° 29.7 S, 38° 9,5 E;
9. Kangonde Dam (4), 1° 2.1 S, 37° 43 E;
10. Kangonde Seepage (8), 1° 2,1 S, 37° 43,E;
11. Mwea East Pond (5), 0° 4.0,S, 37° 30.0,E;
12. Mwea West Pond (5), 0° 4,0 S, 37° 30.0 E;
13. Nairobi Dam (4), 1° 17.2 S, 36° 52 E;
14. Grogan Canal (11), 3° 25.5 S, 37° 44.5 E;
15. Lake Jipe (3), 3° 36.5 S, 37° 46.6 E;
16. Kwahoma Stream (11), 3° 23,5 S, 37° 39,4 E;
17. Mtawa Stream (11), 4° 20.7 S, 39° 29.5 E;
18. Muhaka Dam (4), 4° 17.8 S, 39° 30.2 E;
19. Provincial Headquarters Pond (1), 4° 3.9 S, 39° 40.7 E;
20. Mazeras Botanical Garden Pond (5), 3° 57.7 S, 39° 33.0 E;
21. Mazeras Garden Pool (1), 3° 57.7 S, 39° 33.0 E;
22. Bamburi Pond #1 (1), 3° 58.8,S, 39° 43.1,E;
23. Bamburi Pond #2 (1), 3° 58.8,S, 39° 43.1,E;
24. Bamburi Pond #3 (1), 3° 58.8,S, 39° 43.1 E;
25. Chem-Chem Swamp (7), 3° 12.3 S, 40° 2,3 E;
26. Stardust Ornamental Pool (1), 3°,11.6 S, 40° 6.9 E;
27. Eden Roc Hotel Pool (1), 3° 11.6 S, 40° 6.9 E;
28. Asawo Stream (11), 0° 16.3 S, 34° 59.4 E;
29. Ndori Dam (5), 0° 15.4 S,,34° 59.5 E;,
30. Kisumu Ditch (14), 0° 5.9 S, 34° 46.3 E;
31. Martin's Drain (11), 0° 5.9 S, 34° 46.3 E;
32. Old Prison Farm (14), 0° 6.5 S, 34° 44.5 E;
33. Dunga-L. Victoria (2), 0° 5.0 S, 34° 44.2 E;
34. Hippo Point (2), 0° 7,4 S, 34° 44,2 E;
35. ADC Farm (15), 0° 4.7 S, 34° 44.5 E;
36. West Kano Irrigation System (14), 0°,5.0 S, 34° 49.3 E;
37. Lake Jilori (7), 3° 11.4 S, 39° 53.2 E;
38. Dzimbwini-West (5), 3° 9.1,S, 39° 50.9,E;
39. Dzimbwini-East (5), 3° 9.1 S, 39° 50.9 E;
40. Mbaratum Goshi (7), 3° 11.0 S, 39° 45.4 E;
41. L. Gulamu Ng'onda (4), 3° 6.3 S, 39°,45.5 E;
42. Rare River (13), 3° 31.1 S, 39° 45.9 E;

43. Kachororoni Dam (6), 3° 29.0' S, 39° 42.7' E;
44. Ganze Dam (5), 3° 34.1 S, 39° 41.1 E;
45. Ndizovuni Stream (13), 3° 36.4 S, 39° 29.2 E;
46. Mwachi River (13), 3° 57.9,S, 39° 31.4,E;
47. Mbandi Stream (13), 4° 7.8 S, 39° 20.2 E;
48. Kiziamondo Stream (13),,4° 7.9 S, 39° 19.8 E;
49. Kinango Dam (5), 4° 7.9,S, 39° 18.8,E;
50. Bububu Dam (5), 4° 14.4 S, 39° 34.3 E;
51. Shamu Dam (7), 4° 17.3 S, 39° 30.4 E;
52. Mkurumuji Stream (11), 4° 21.5 S, 39° 26.9 E;
53. Kambe Stream (13), 4° 26.4 S, 39° 18.9 E;
54. Kiwegu Village (15), 4° 37.6 S, 39° 12.5 E;
55. Mwangwei (5), 4° 30.6 S, 39° 16.8 E;
56. Mangewa River (13), 3° 47.3 S, 39° 22.8 E;
57 Chumvi River (13), 2° 15.1 S,,37° 48.7 E;,
58. Kisumu-Homa Pond (5), 0° 10.1 S, 34° 54.9 E;
59. Kembija Dam (5), 0° 2.4 S, 34° 30.9 E;
60. Nyachido Stream (11), 0° 2.7 S, 34° 24.9 E;
61. Nyamguu Stream (13), 0° 1.8 S, 34° 26.7 E;
62. Dago Stream (15), 0° 0.6 N, 34° 32.7 E;
63. Otodo Stream (13), 0° 3.0 N, 34° 34.1 E;
64. Kendu Bay (2), 0° 20.4 S, 34° 39.0 E;
65. Oriang Stream (13), 0° 23,8 S, 34° 35,6 E;
66. Got Okii Dam (8), 0° 26.1 S, 34° 33.9 E;
67. Maugo Stream (15), 0° 28.5 S, 34° 32.7 E;
68. Sota Stream (13), 0° 30.6,S, 34° 28.8,E;
69. Nyalkiny Dam (5), 0° 31.2 S, 34° 28.2 E;
70. Rusinga Island (2), 0° 24.9 S, 34° 12.3 E;
71. Kamayoga Stream (11),,0° 24.3 S, 34° 9.8 E;
72. Sero Dam (5), 0° 35.6 S, 34° 29.7 E;,
73. Onsando Dam (11), 0° 42.8 S, 35° 2.4 E;
74. Kapcholyo Quarry (10), 0° 39.2,S, 35° 8.0 E;
75. Taita Hills Lodge (5), 3° 30.6 S, 38° 13.4 E;
76. Sagalla Magange Stream,(11), 3° 29,1 S, 38° 24.3 E;
77. Lata Dam (11), 3° 28.2 S, 38° 25.5 E;
78. Lugards Falls-Galana (12), 3°,2.4 S, 38° 45.7 E;
79. Pools-Voi River (12),,3° 26.7 S, 38° 30.6 E;
80. Meti Dam (5), 3° 39.0 S, 39° 10.2 E;
81. Mariakani Dam (5), 3° 46.8 S, 39° 22.2 E;
82. Silaluni Dam (4), 3° 36.0 S, 39° 17.1 E;
83. Chigutu Dam (5), 3° 31.8 S, 39° 17.1 E;
84. Hola Canals (14), 1° 22.2 S, 39° 58.2 E;
85. Tewe Pond "C" (5), 2° 27.6,S, 40° 41.1,E;
86. Kilongwe Pond (5), 2° 21.6,S, 40° 41.1,E;
87. Maketumu Pond (5), 2° 27.0 S, 40° 41.1 E;
88. Mapenya Pond (5), 2° 24.8 S, 40° 41.9 E;
89. Hongwe Pond (5), 2° 24.8 S, 40° 43.8 E;
90. Kwa Chikophe Pond (5), 3° 54.3 S, 39° 13.2 E;
91. Kanderi Swamp (7), 3° 21,0 S, 38° 40,8 E;
92. Aruba Lodge (5), 3° 19.8 S, 38° 50.4 E;
93. Kajiri Dam (6), 3° 35.4 S, 39° 28.2 E;
94. Nyore Dam (5), 3° 38.9 S, 39° 26.8 E;
95. Mwaremi River (13), 3° 49,8 S, 39° 28,2;
96. Shimba River (12), 4° 9.7 S, 39° 25.1 E;
97. Kanana Pond (6), 4° 31.8 S, 39° 21.6 E;
98. Kiwambale Stream (11), 4° 34.2,S, 39° 22.8,E;
99. Shimoni Road Pond (5), 4° 31.4 S, 39° 20.7 E;
100. Mwangutwa Stream (11), 3° 57.0 S, 39° 33.0 E;

101. Kambeni Pool (13), 3° 51.9′ S, 39° 38.1′ E;
102. Lutsangani Dam (11), 3° 54.8′ S, 39° 34.2′ E;
103. Lutsangani Dam (5), 3° 54.8′ S, 39° 34.2′ E;
104. Miyazeni Pool (11), 3° 51.3′ S, 39° 38.0′ E;
105. Kasidi Dam (5), 3° 49.1′ S,, 39° 43.4′ E;,
106. Marere Stream (11), 4° 9.8′ S,, 39° 22.0′ E;,
107. Mwandambala Pond (5), 4° 11.7′ S, 39° 25.0′ E;
108. Athi River-Mwala (12), 1° 13.6′ S, 37° 26.5′ E;
109. Marema East (5), 0° 47.5′ S, 37° 16.8′ E;
110. Kakuzi Dam (5), 0° 50.7′, S, 37° 14.1′ E;
111. Ruanda Dam (4), 0° 50.5′ S, 37° 8.1′ E;
112. Ariek Dam (5), 0° 51.3′ S, 37° 9.8′ E;,
113. Gachago Dam (5), 0° 53.5′ S,, 37° 10.9′ E;,
114. Makuyu Stream (11), 0° 53,5′ S, 37° 13,0′ E;
115. Kiriwiri Dam (5), 0° 53.5′ S, 37° 17.4′ E;
116. Ndura Dam (5), 1° 1.6′ S, 37°, 27.8′ E;
117. Mathauta Stream (11), 1° 8.5′ S, 37° 34.2′ E;
118. Kwa Mtungi Pond (5), 1° 9.8′, S, 37° 34.8′, E;
119. Kaituwa Stream (11), 1° 8.7′ S, 37° 29.3′ E;
120. Gitaru Dam Spillway (13), 0° 50.2′ S, 37° 45.9′ E;
121. Masinga Dam Spillway (13), 0° 48.9′ S, 37° 41.1′ E;
122. Marimanti River (11), 0° 9.7′ S, 37°, 58.9′ E;
123. Tana River (12), 0° 7.2′ S, 38° 12.5′ E;
124. Isiolo Ditch (14), 0° 21.6′ S, 37°, 34.6′ E;
125. Kambi Garba Stream (14), 0° 23.7′ N, 37° 35.7′ E;
126. Ngenya Dam (9), 0° 5.4′ N, 37° 20.3′ E;
127. Mwireri Farm Dam (5), 0° 2.7′ N, 37° 7.3′ E;
128. South Nanyuki Pond (8), 0° 3.8′ S, 37° 4.3′ E;
129. Aguthi Pond (8), 0° 13.5′, S, 37° 1.6′, E;
130. Tana River (12), 0° 27.6′ S, 37° 3.5′ E;
131. Thaithi Swamp (7), 0° 28.6′ S, 37° 5.7′ E;
132. Lower Kipkaren River (11), 0° 36.8′ N, 34° 58.3′ E;
133. Lambista River (11), 0° 15.1′ N, 34° 44.3′ E;
134. Isiukhu River (11), 0° 15.1′ N, 34° 46.5′ E;
135. Yala River (11), 0° 5.4′ N,, 34° 50.8′ E;,
136. Matuwe Stream (11), 0° 5.4′ N, 34° 59.1′ E;
137. River Jordan (11), 0° 5.4′ N, 34° 54.1′ E;
138. Itsava (11), 0° 5.4′ N,, 34° 53.0′ E;,
139. Asembo Bay (2), 0° 5,5′ S, 34° 32,7′ E;
140. Kombewa (11), 0° 5.9′ S, 34°, 31.9′ E;
141. Asembo Stream (13), 0° 10.3′ S, 34° 24.5′ E;
142. Maugo Rice Field (15), 0° 36,5′, 34° 29.4′ E;
143. Mwongori Scheme (5), 0° 41.6′ S, 35° 4.3′ E;
144. Bura Pond (5), 1° 15.8′ S, 39° 58.9′ E;
145. Hola Canal (14), 1° 31.6′, S, 40° 3.8′ E;
146. Tana River (12), 0° 27,8′ S, 39° 39,8′ E;
147. Borr-Algy (5), 0° 28.9′ S, 39° 40.9′ E;
148. Nairobi National Park (13), 1° 20,5′ S, 36° 44.3′ E;
149. Muoni Dam (5), 1° 33.3′ S 37° 20.7′ E;
150. Voi Safari Lodge (1), 3° 22.7′ S 38° 34.0′, E;
151. Aruba Lodge Dam (5), 3° 20.7′ S, 38°, 50.2′ E;
152. Lake Madina (5), 3° 12.0′, S, 40° 5.5′ E;
153. Marafa Ponds (6), 3° 2,2′ S, 39° 47,5′ E;
154. Dida Pond (5), 3° 24.9′ S, 39° 48.1′ E;
155. Dida Pond Two (5), 3° 23.6′ S, 39° 48.0′ E;
156. Vitengini Pond (5), 3° 21.3′ S, 39° 40.9′ E;
157. Lake Naivasha (3), 0° 46.2′ S, 36° 24.4′ E;
158. Lake Naivasha-Crater Bay (3), 0° 46.9′ S, 36° 24.0′ E;

159. Lake Naivasha (3), 0° 48.0′ S, 36° 17,5′ E;
160. Lake Baringo (3), 0° 36.8 N, 36° 1.6 E;
161. Enaptindinye Stream (11), 0° 22.5 S, 35° 17.3′ E;
162. Komorok Pond (8), 1° 16.9 S, 37° 1.1, E;
163. Tala Pond I (8), 1° 16.9 S, 37° 12.0 E;
164. Tala Pond II (8), 1° 16,9 S, 37° 12,5 E;
165. Katulu Dam (4), 1° 17.5, S, 37° 14.7, E;
166. Maombi Pond (5), 0° 3.8 S, 36°, 11.2 E;
167. Nyahururu Seepage (10), 0° 1.1 N, 36° 22.4′ E;
168. Chopi Pond Dam (5), 0° 10.9 N, 36°, 26.7 E;
169. Sukuta Naibor Spring (10), 0° 42.0 N, 36° 36.5′ E;
170. Mododo Dam I (4), 1° 4.9 N, 36° 39.8 E;
171. Mododo Dam II (4), 1° 4.9 N, 36° 38.2 E;
172. Siguta Spring (10), 0° 49.6 N, 36° 42.0 E;
173. Chuno Pond (5), 0° 46.9 N, 36° 22.9 E;
174. Kokwa Island, Lake Baringo (3), 0° 37.1 N, 36° 1.9′ E;
175. Perkerra Primary School Stream (11), 0° 26.7 N, 36° 3.8′ E;
176. Kebute Pond (5), 0° 29.5 N, 35° 45.3 E;
177. Chemalal Pond (5), 0° 36.5, N, 35° 12.0, E;
178. Kamagut Marsh (7), 0° 37.1 N, 35° 11.5 E;
179. Chipkoilel Stream (11), 0° 49.6 N, 35° 8.2 E;
180. Moi's Bridge Stream (11), 0° 53.2 N, 35° 7.6 E;
181. Sibwani River Swamp (10), 1° 4.9 N, 35° 4.6 E;
182. Saiwa Swamp National Park (7), 1° 4.3 N, 35° 8.7′ E;
183. Kimjnini River (11), 0° 54.5 N, 34° 57.3 E;
184. Webuye Borrow Pit (8), 0° 37.6 N, 34° 42.5 E;
185. Kapkiam Dam (5), 0° 15.8 S, 35° 10.4 E;
186. Cheptindini Pond (8), 0° 19.6 S, 35° 13.1 E;
187. Cheptangulgei Stream (11), 0° 40.4 S, 35° 9.8 E;
188. Yakamek Stream (11), 0° 41.4 S, 35° 9.8 E;
189. Sigor Dam (4), 0° 52.4 S, 35° 19.6 E;
190. Chebunyo Dam (4), 0° 58.4 S, 35° 10.9 E;
191. Karungu Bay-Lake Victoria (2), 0° 50.2 S, 34° 10.4′ E;
192. Ondiek Dam (5), 0° 44.7 S, 34° 19.1 E;
193. Umoja Dam (7), 0° 44.2 S, 34°, 21.8 E;
194. Kamagombo Quarry (5), 0° 46.9, S, 34° 37.1, E;
195. Kwo Lingira Pond (5), 1° 47,4 S, 36° 49,6 E;
196. Namanga River (13), 2° 32.2 S, 36°, 48.0 E;
197. Amboseli Serena Lodge (7), 2° 42.0 S, 37° 16.4 E;
198. Njukini Irrigation Canal (14), 3° 13.6 S, 37° 56.7′ E;
199. Engepoi Swamps (7), 3° 14.2 S, 37° 42.5 E;
200. Kimana Springs (11), 2° 42.5 S, 37° 32.7 E;
201. Sinet Springs (11), 2° 43.6 S, 37° 31.1 E;
202. River Kiboko (11), 2° 13.1, S, 37° 42.5, E;
203. Thwake River (11), 1° 16.9 S. 37° 20.7 E;

TABLE 1: Listed are the gastropod taxa collected from the 203 habitats found to contain snails. For each taxon is listed the number of habitats it was recovered from and the altitudinal range. The numbers listed below the altitudinal range are explained in the materials and methods section.

TABLE 1

	Number of Times Found	Altitudinal Range
PROSOBRANCH SNAILS		
Family Neritidae		
Neritina natalensis	1	1
Family Viviparidae		
Bellamya unicolor	14	1,2,5,6,7
B. trochlearis	1	5
Family Ampullariidae		
Pila ovata	28	1,3,5,6
Lanistes ovum ovum	18	1,2
L. ovum procerus	3	1
L. ovum purpureus	4	1,2
L. carinatus	17	1
L. ciliatus	4	1
Family Bithyniidae		
Gabbiella humerosa	3	5,6
G. parvipila	1	1
Family Thiaridae		
Melanoides tuberculata	40	1,2,3,4,5,6
Cleopatra ferruginea	18	1,2
C. bulimoides	17	1,2,3,5
C. guillemei	3	5
PULMONATE SNAILS		
Family Ancylidae		
Ferrissia kavirondica	1	5
F. clessiniana	8	1,5,6,7,10
F. isseli	7	6,8,9
Burnupia kempi	1	8
B. crassistriata	1	8
Family Planorbidae		
Biomphalaria choanomphala	2	5
B. sudanica	8	4,5,6,7
B. pfeifferi	46	4,5,6,7,8
Bulinus (Physopsis) group	63	1,2,3,4,5,6,7
Bulinus tropicus group	51	2,4,5,6,7,8,9,10
Bulinus forskalii	40	1,2,3,4,5,6,8
Ceratophallus natalensis	27	1,3,4,5,6,7,8
Segmentorbis kanisaensis	3	5
S. augustus	1	5
Gyraulus costulatus	4	5,6,8
Helisoma sp.	5	1,7,8
Family Lymnaeidae		
Lymnaea natalensis	75	1,3,4,5,6,7,8,9
L. truncatula	2	7,8
Family Physidae		
Physa acuta	9	3,4,5,6,7,8

TABLE 2: Listed for each taxon collected is the number of times it was recovered from each of the 15 different recognized habitat types. These habitat types are described in Appendix 1:

TABLE 2

	HABITAT TYPES													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
PROSOBRANCH SNAILS														
Family Neritidae														
Neritina natalensis											1			
Family Viviparidae														
Bellamya unicolor		1		1	4		3				1	1	3	
B. trochlearis		1												
Family Ampullariidae														
Pila ovata		4		1	7	2	1						4	5
Lanistes ovum ovum					8	3					2	1	4	
L. ovum procerus				1		1					1			
L. ovum purpureus				1	1								1	
L. carinatus	1			2	10		3					1		1
L. ciliatus					1	1					1		1	
Family Bithyniidae														
Gabbiella humerosa		2			1									
G. parvipila					1									
Family Thiaridae														
Melanoides tuberculata	7	5		4	9	1	2			1	5	2	1	3
Cleopatra ferruginea					4	1	1				2		9	1
C. bulimoides			2	1	10	1	1				1	1		
C. guillemei													1	
PULMONATE SNAILS														
Family Ancylidae														
Ferrissia kavirondica		1												
F. clessiniana				1	1			1	1		1	1	2	
F. isseli				1	1			1			4			
Burnupia kempi											1			
B. crassistriata												1		
Family Planorbidae														
Biomphalaria choanomphala		2												
B. sudanica		2	1	1							2		1	
B. pfeifferi				6	13		1			1	19		3	3
Bulinus (Physopsis) group	1	2		3	23	2	2	2	1		10	2	11	3
Bulinus tropicus group		4	2	7	16		3	2	2	5	5		2	2
Bulinus forskalii		4	1	1	9	1	2	3	2	1	6	2	3	4
Ceratophallus natalensis		4	1		7	1	2	1		3	3		1	2
Segmentorbis kanisaensis		1			2									
S. augustus		1												
Gyraulus costulatus		1									2		1	
Helisoma sp.	5													
Family Lymnaeidae														
Lymnaea natalensis	1	4	4	5	17		6			3	26		5	3
L. truncatula					1							1		
Family Physidae														
Physa acuta			1	1	2						2	2	1	
										29				

TABLE 3: For each faunal region, the coefficients of association, which range from -1 to +1 in value, between several pairs of prominent Kenyan gastropods are indicated. Values that are significant at the $p < 0.05$ level are indicated with an asterisk.

COASTAL LOWLAND FAUNA

	Bulinus tropicus group	*Lymnaea natalensis*	*Pila ovata*	*Lanistes carinatus*	*Lanistes ovum*	*Melanoides tuberculata*	*Cleopatra* sp.
Bulinus (*Physopsis*)	0.102	0.151	0.009	-0.424*	0.118	-0.244	0.116
Bulinus tropicus group	-	-0.026	-0.048	-0.072	-0.115	-0.074	-0.155
Lymnaea natalensis		-	-0.068	-0.102	0.229	0.289	-0.221
Pila ovata			-		0.176	0.080	0.190
Lanistes carinatus				-	-0.359*	0.146	-0.157
Lanistes ovum					-	0.129	0.136
Melanoides tuberculata						-	0.140
Cleopatra sp.							-

UPLAND FAUNA

	Bulinus (Physopsis)	*Bulinus tropicus* group	*Lymnaea natalensis*	*Melanoides tuberculata*
Biomphalaria pfeifferi	-0.110	0.223*	0.239*	0.111
Bulinus (Physopsis)	-	-0.107	-0.137	-0.056
Bulinus tropicus group		-	-0.008	-0.032
Lymnaea natalensis			-	-0.211
Melanoides tuberculata				-

HIGHLANDS FAUNA

	Bulinus tropicus group	*Lymnaea natalensis*
Biomphalaria pfeifferi	-0.293	0.253
Bulinus tropicus group	-	-0.182
Lymnaea natalensis		-

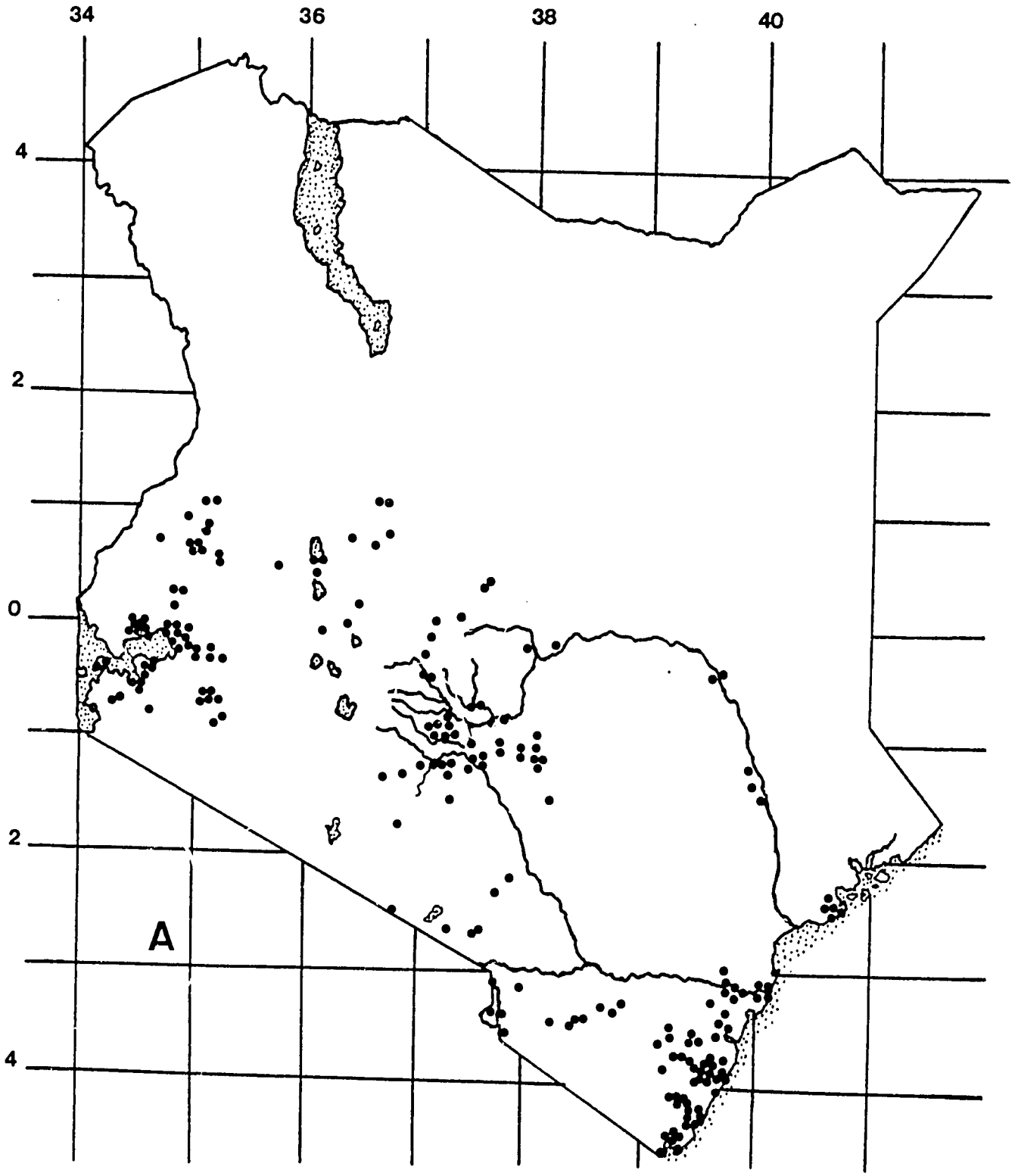
NYANZA LOW PLATEAU FAUNA

	Biomphalaria sundanica	*Bulinus (Physopsis)*	*Bulinus tropicus* group	*Lymnaea natalensis*	*Pila ovata*	*Cleopatra* sp.
Biomphalaria pfeifferi	-0.262	-0.030	-0.405*	-0.308	-0.589*	-0.175
Biomphalaria sudanica	-	0.120	0.120	0.030	0.228	-0.083
Bulinus (Physopsis)		-	-0.444*	-0.040	0.013	-0.175
Bulinus tropicus group			-	0.219	0.356	-0.175
Lymnaea natalensis				-	-0.243	-0.267
Pila ovata					-	0.228
Cleopatra sp.						-

TABLE 4: For each putative biocontrol species listed below, is listed the total number of natural habitats containing that species (N), and the number of habitats in which it co-exists with pulmonate taxa of medical or veterinary significance. The percentage of all habitats for the putative control agent in which coexistence occurs with the indicated pulmonate taxon is indicated in parentheses.

PUTATIVE CONTROL SPECIES	TARGET SPECIES				
	Biomphalaria pfeifferi	*Biomphalaria sudanica*	*Bulinus (Physopsis)*	*Bulinus tropicus*	*Lymnaea natalensis*
Melanoides tuberculata N = 33	9 (27.2)	2 (6.1)	12 (36.4)	8 (24.2)	11 (33.3)
Pila ovata N = 28	2 (7.1)	3 (10.7)	17 (42.9)	9 (32.1)	10 (35.7)
Lanistes carinatus N = 17	0 (0.0)	0 (0.0)	6 (35.3)	0 (0.0)	0 (0.0)
Lanistes ovum N = 23	0 (0.0)	0 (0.0)	17 (73.9)	0 (0.0)	2 (8.7)

FIGURE 1: A) All locations from which freshwater snails were collected are indicated. Additional collection locality data are provided in Appendix #1. B) Collection localities for ampullariid snails are indicated as follows: Pila ovata (solid circles), Lanistes carinatus (triangles), and L. ovum (open circles). C) Collection localities for representatives of the genus Bulinus are as follows: Bulinus (Physopsis) (solid circles), Bulinus tropicus complex (open circles), and Bulinus forskali (stars). D) Collection localities for Lymnaea natalensis (solid circles), L. truncatula (stars), and Physa acuta (open circles) are indicated. E) Collection localities for Biomphalaria pfeifferi (solid circles), B. sudanica (open circles), and B. choanomphala (stars) are indicated. F) Collection localities for Melanoides tuberculata (open circles) and Cleopatra sp. (solid circles) are indicated.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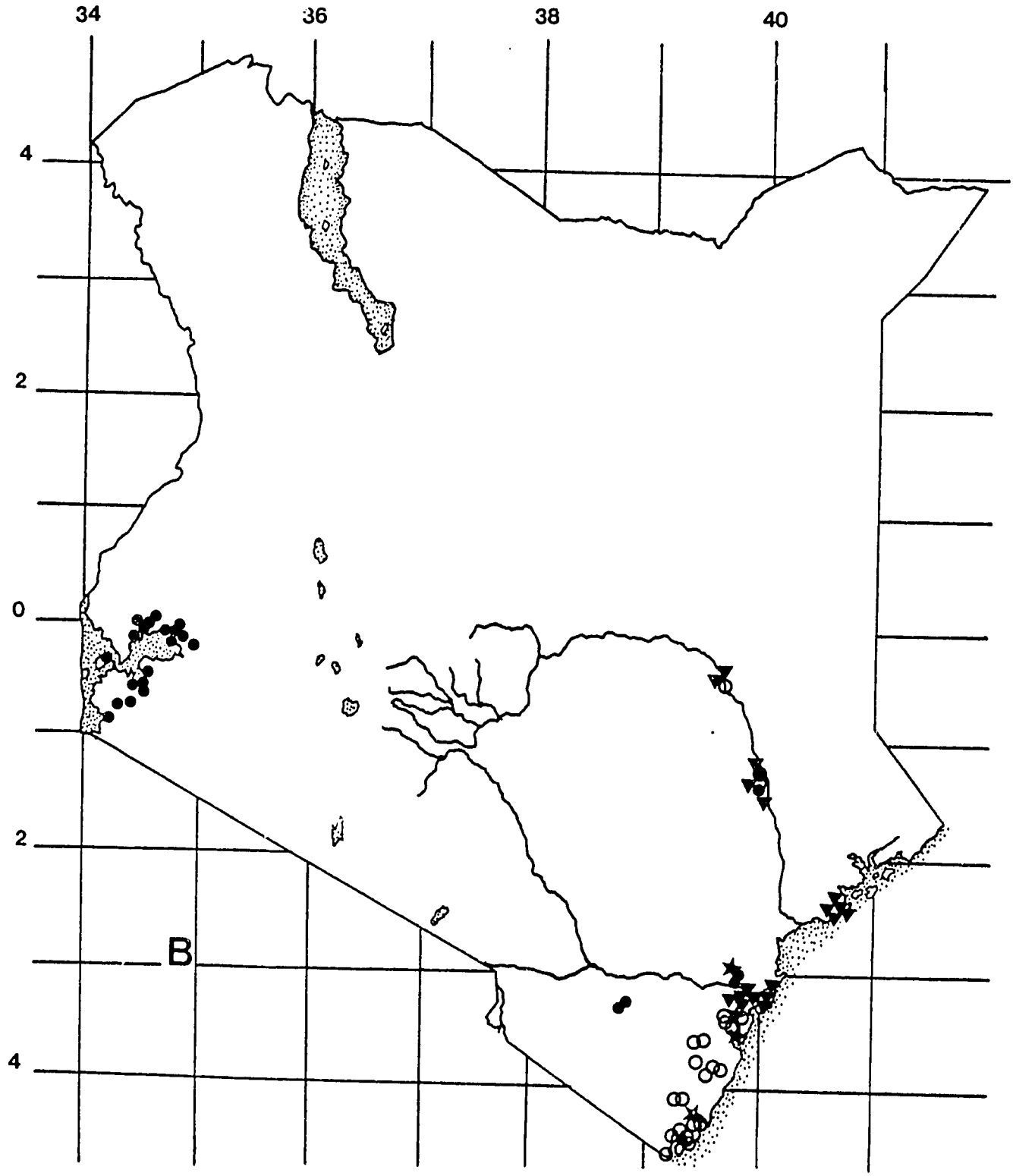
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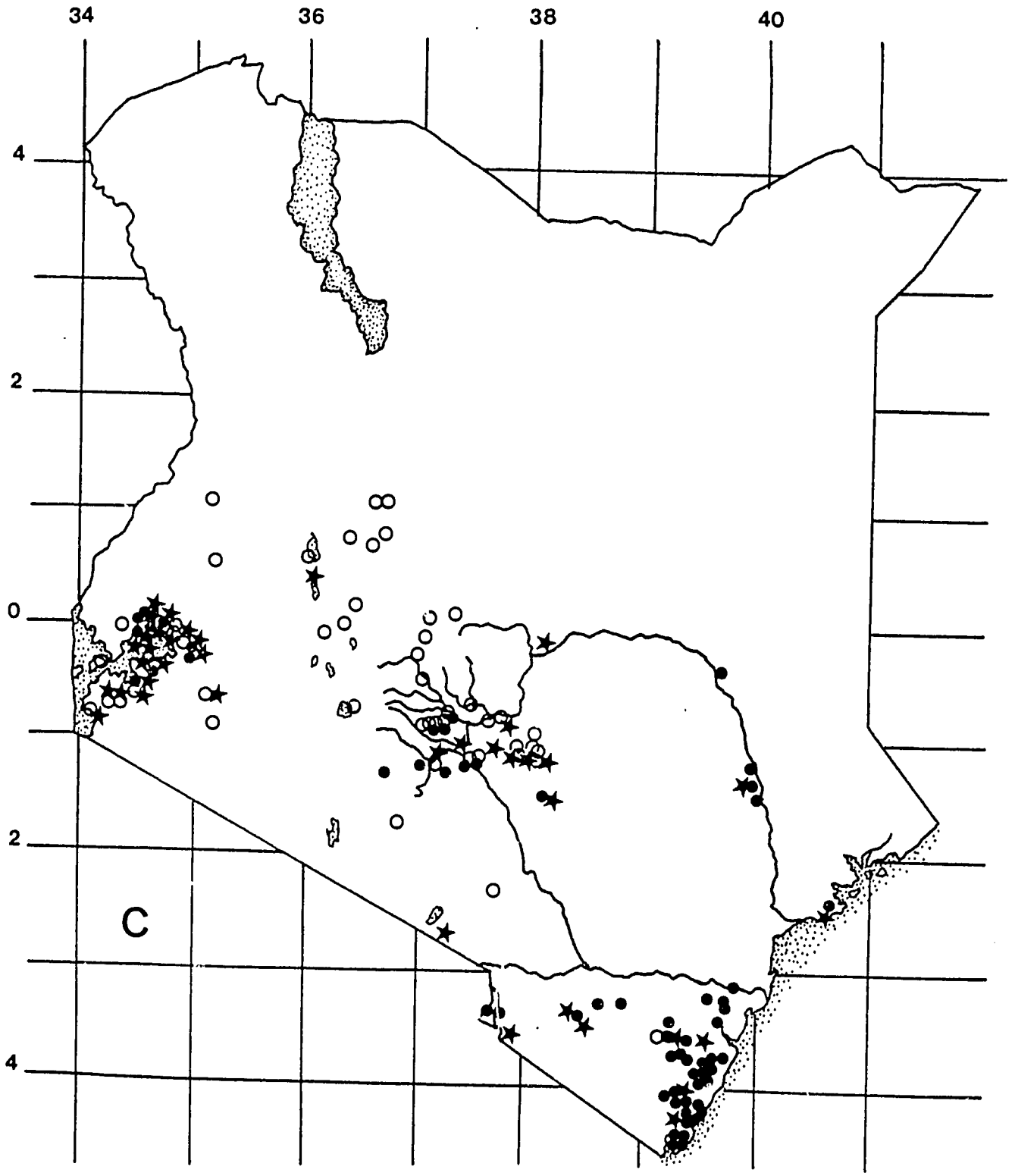
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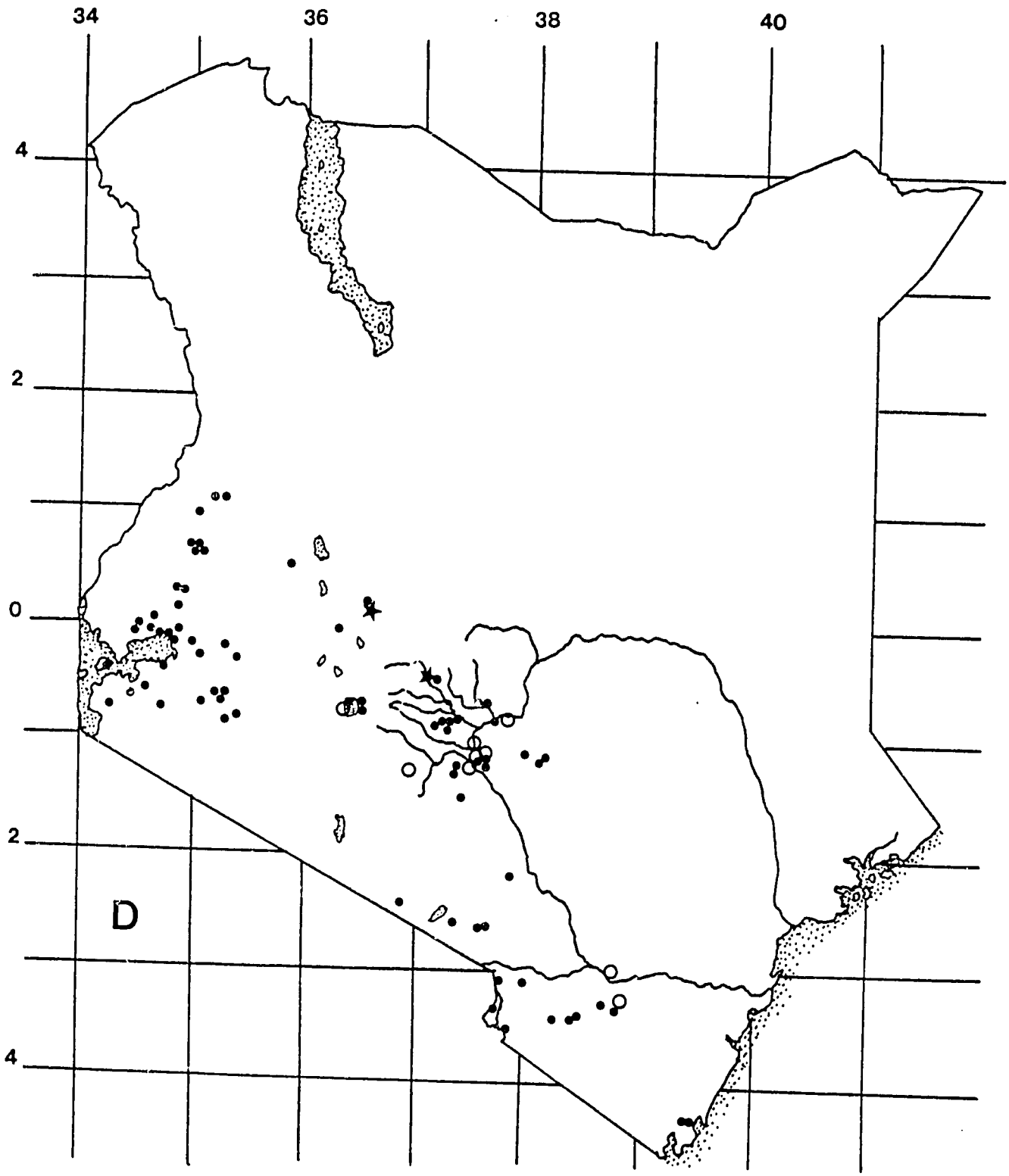
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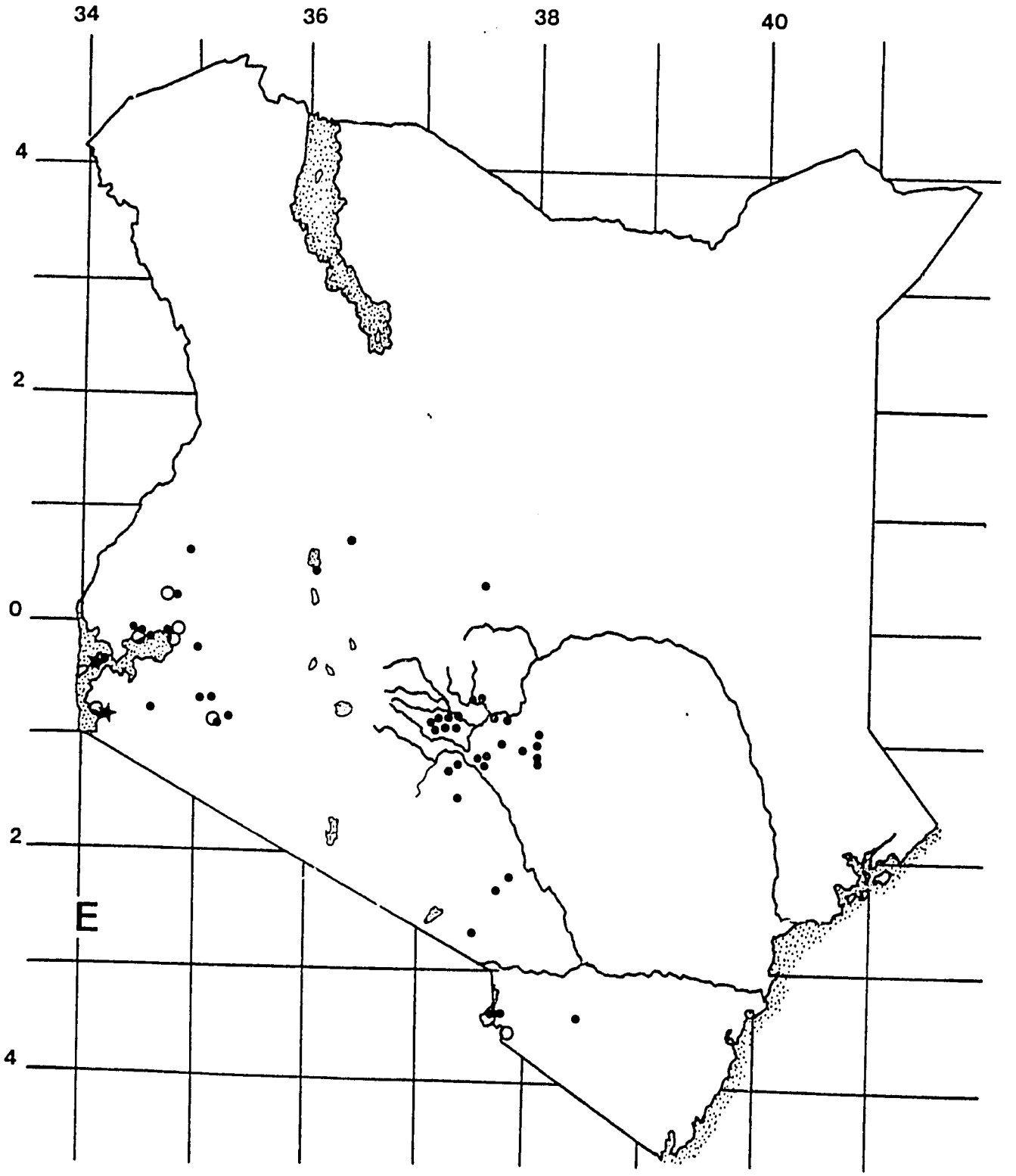
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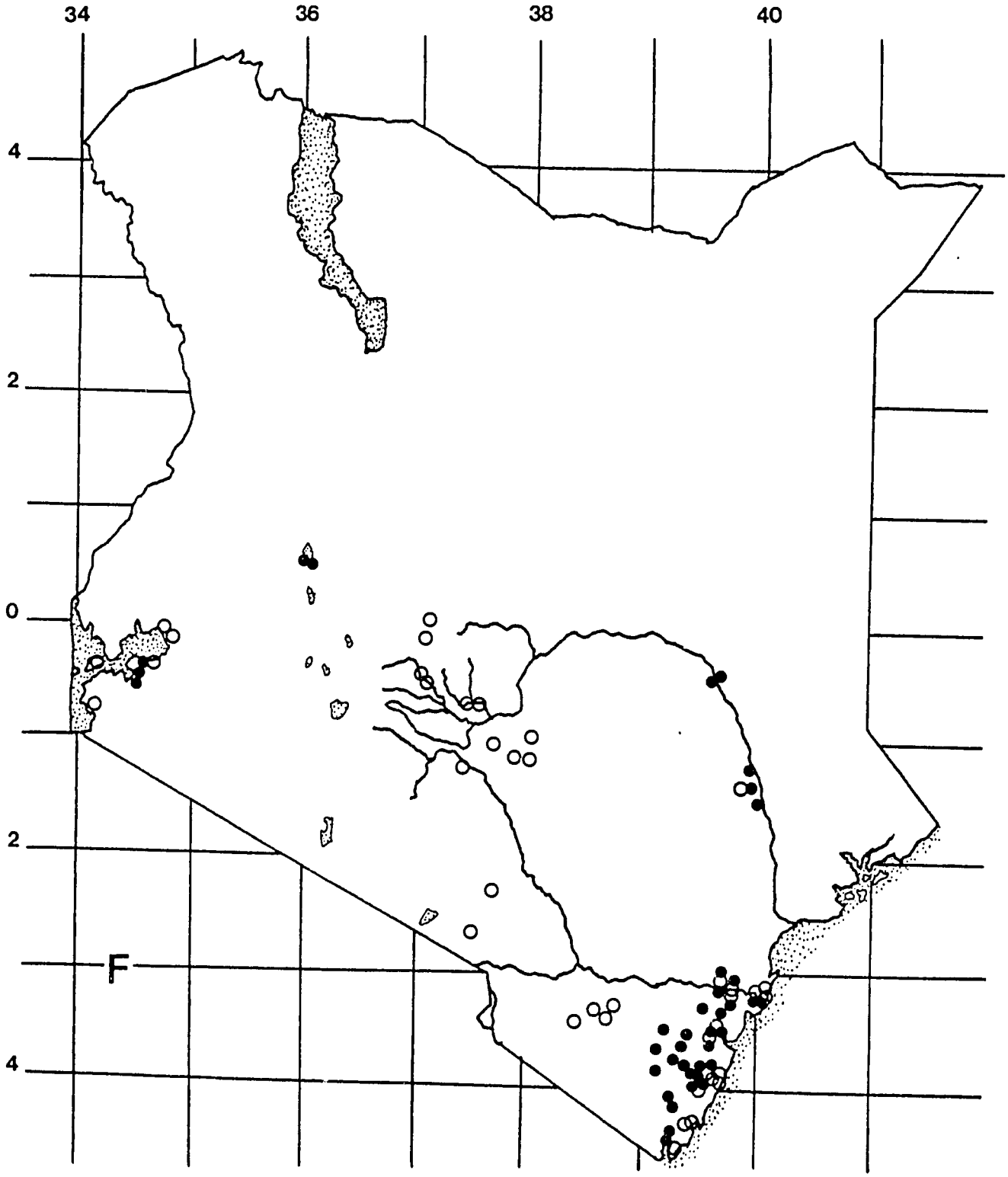
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FIGURE 2: Listed for each altitudinal category are the number of habitats from which snails were obtained. Altitudinal categories are defined in the materials and methods section.

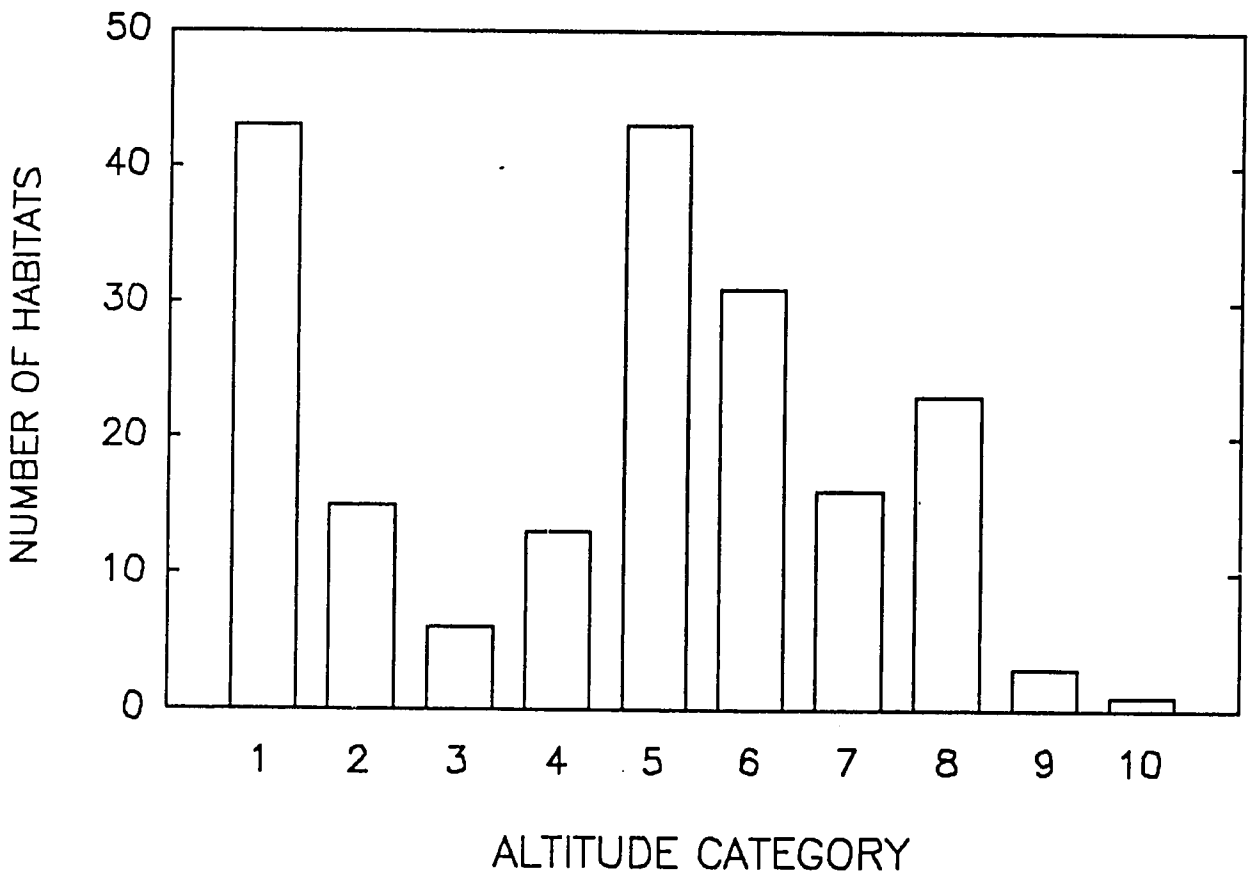


FIGURE 3: The total number of pulmonate or prosobranch gastropod taxa collected within each altitudinal category is listed.

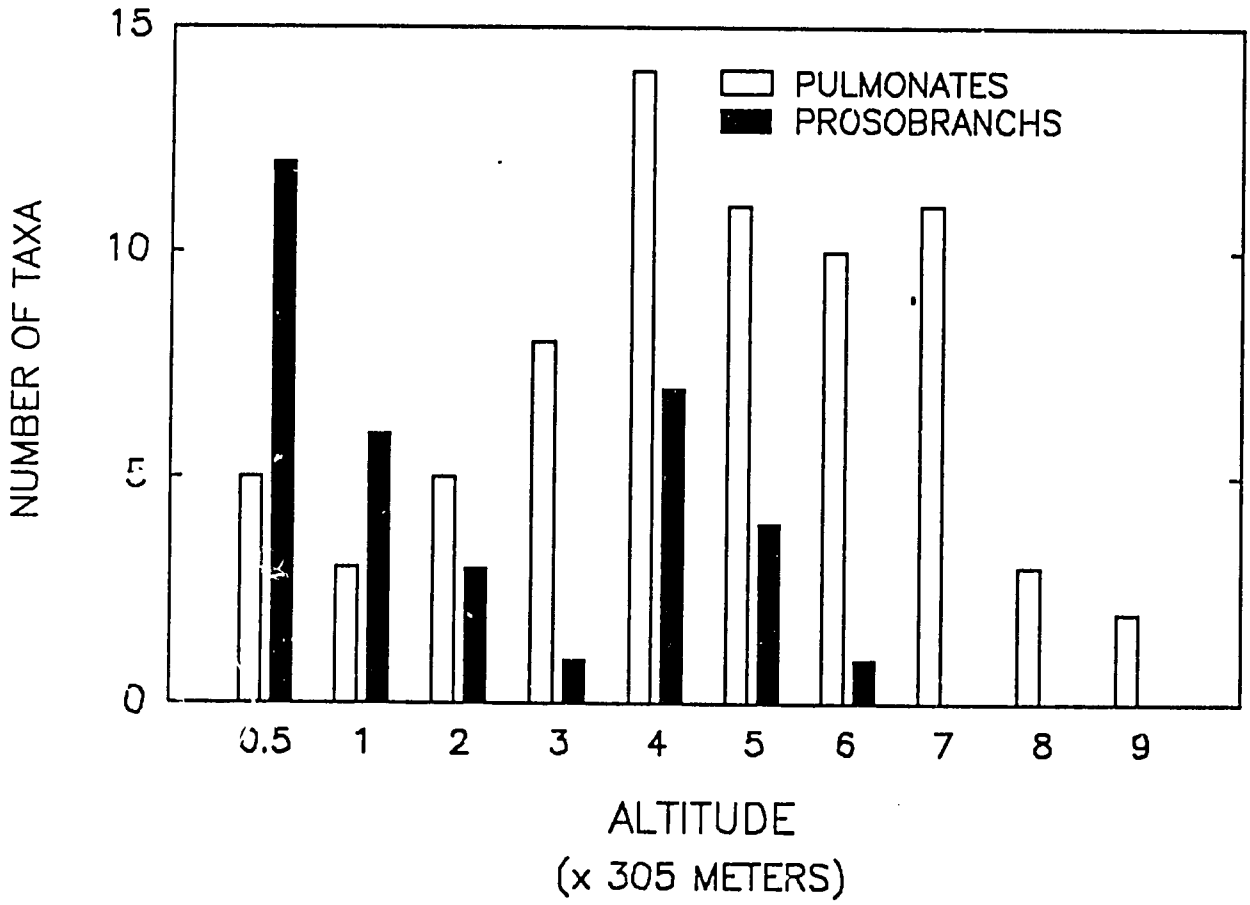
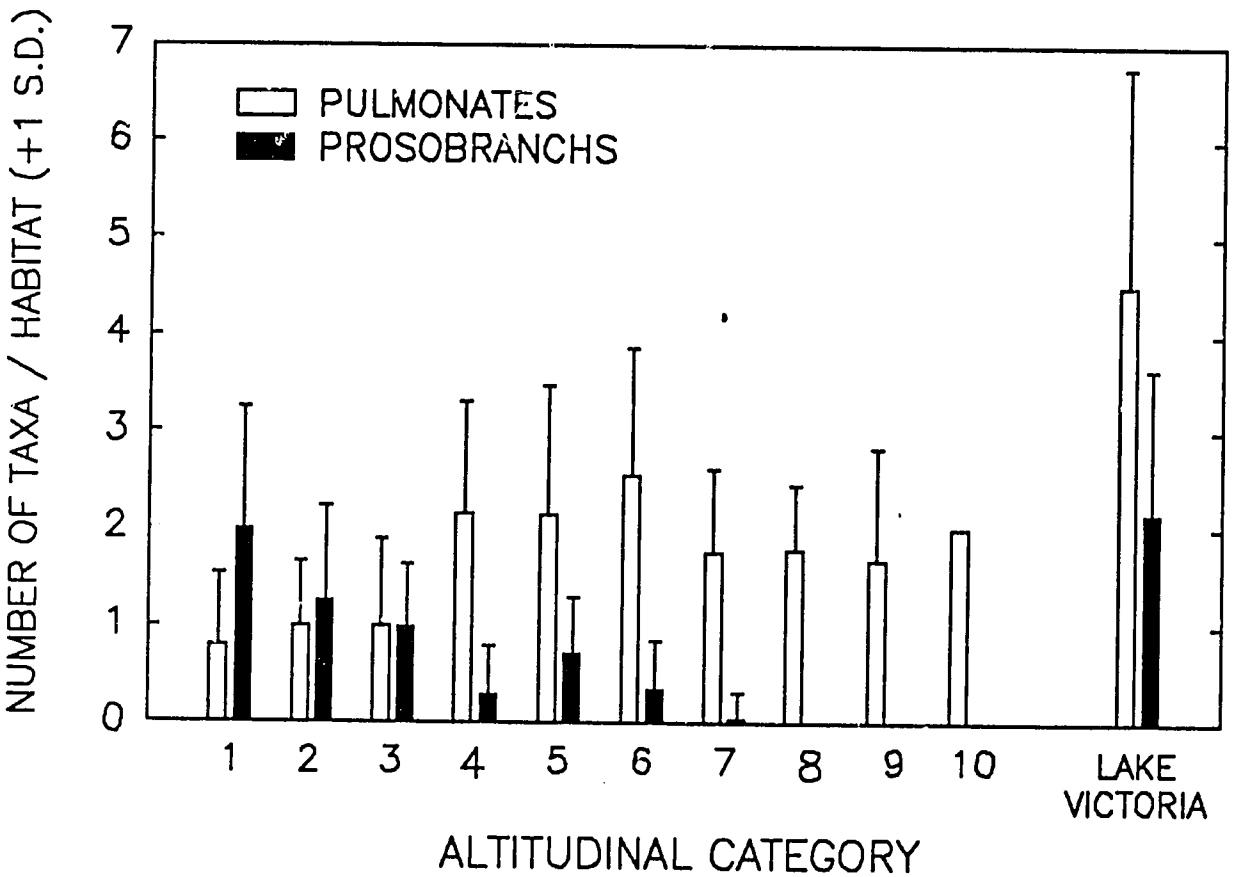


FIGURE 4: The mean number of pulmonate or prosobranch taxa collected per habitat is indicated for each altitudinal category. Lake Victoria collections are indicated separately.



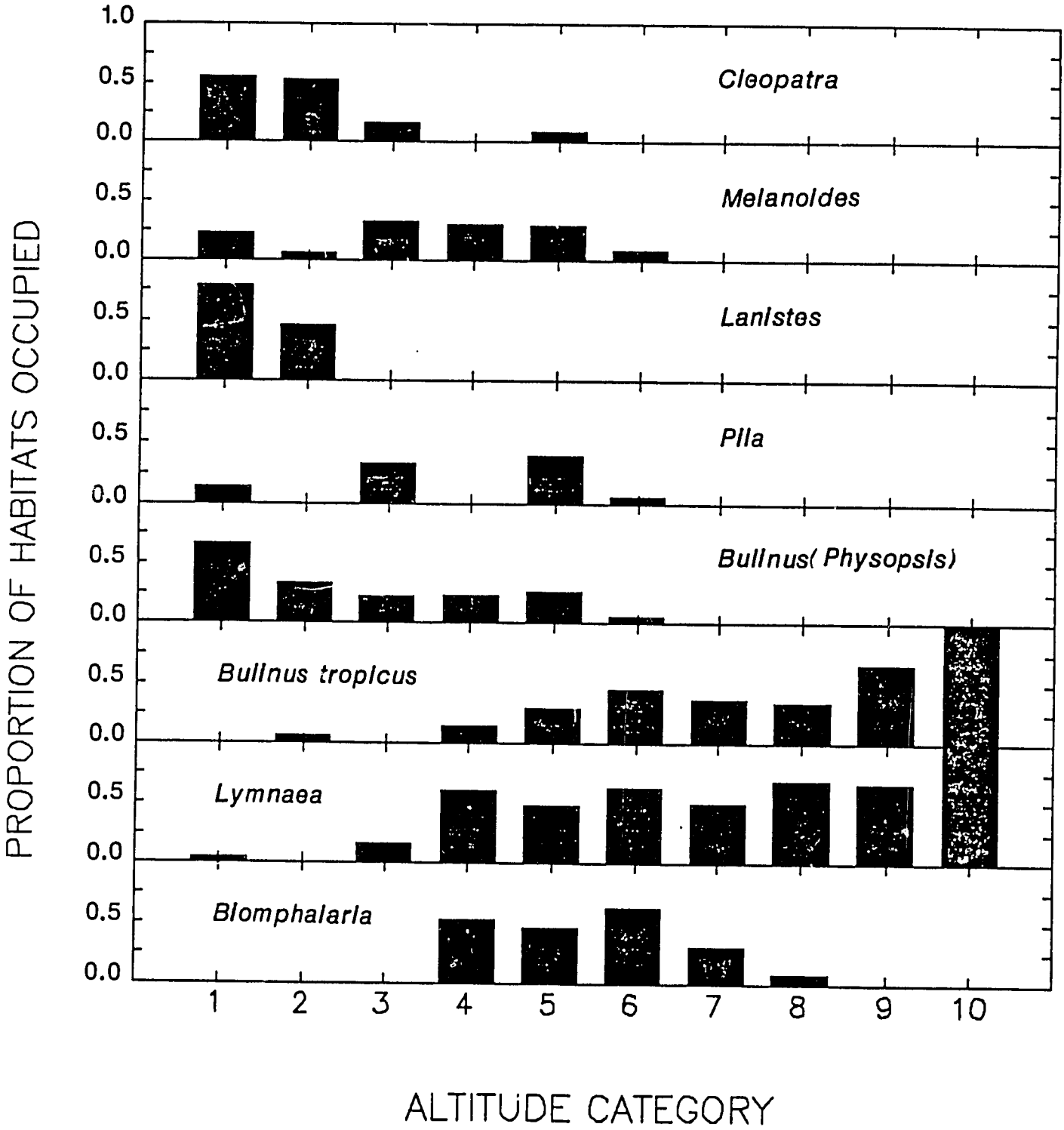
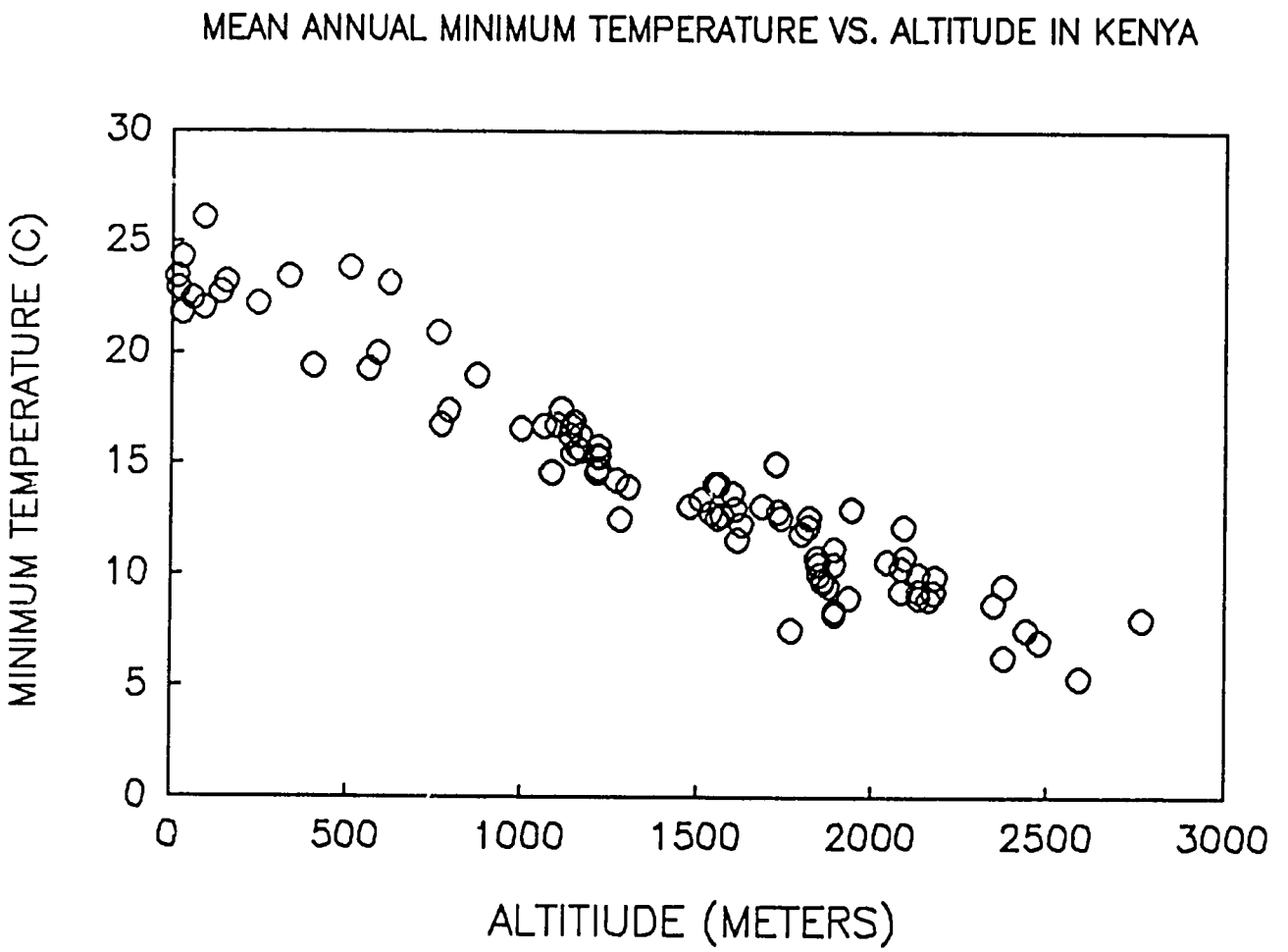


FIGURE 5: For several prominent gastropod taxa, the proportion of habitats within each altitudinal category containing that particular taxon is indicated.

FIGURE 6: For all weather stations covered in the "Climatological Statistics for Kenya", a plot related mean annual minimum temperature and altitude was constructed.



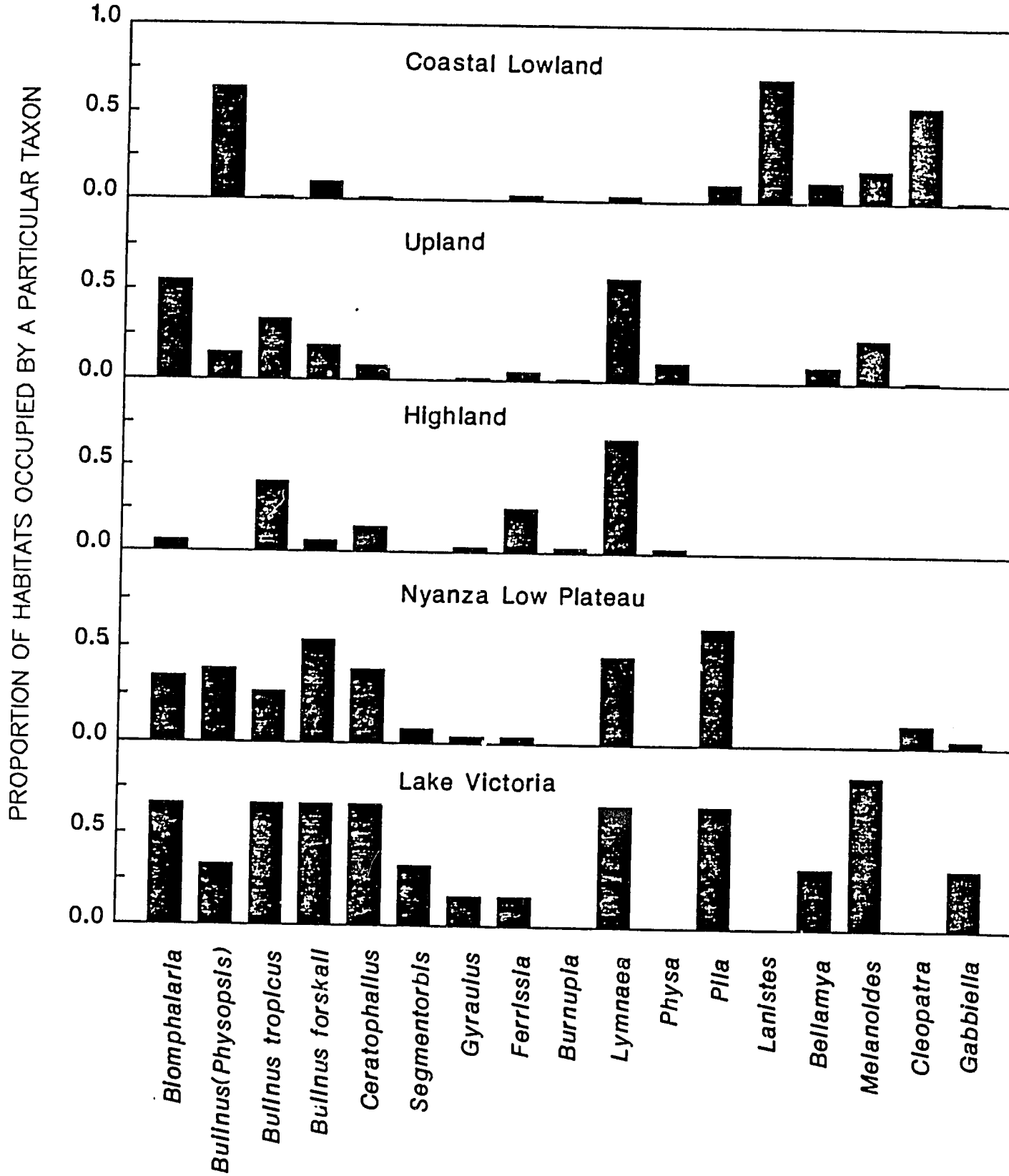


FIGURE 7: For each of the five faunal regions recognized, the proportion of all habitats within that region containing the indicated gastropod taxa, is indicated.

FIGURE 8: For each of the 15 recognized habitat types is indicated the mean number of taxa recovered per habitat. The habitat types are defined in Appendix #1.

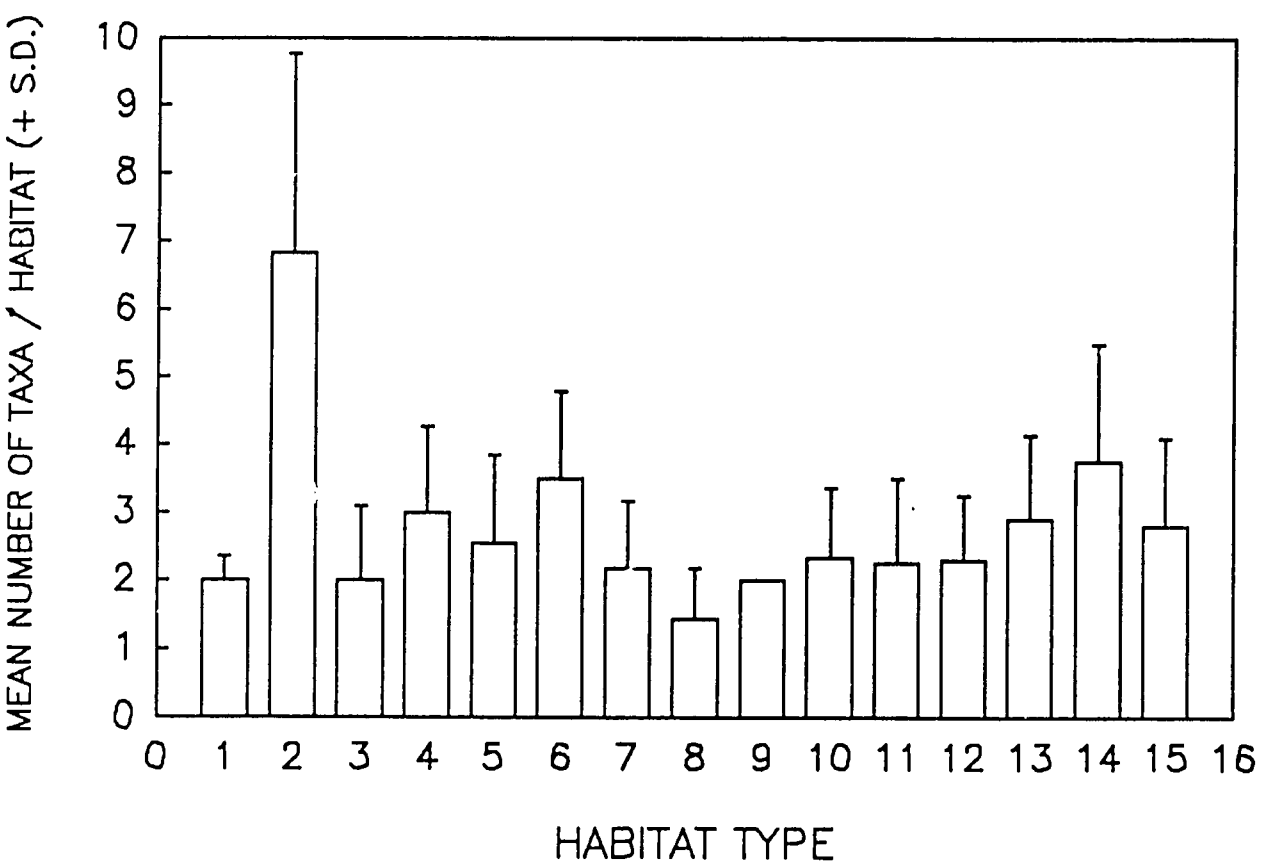


FIGURE 9: For each of the three indicated taxa of medical or veterinary significance, the percentage of all indicated habitats of a particular type (small dams, flowing streams, or intermittent streams) within the altitudinal range of the species that were found to contain that species is indicated. For example, 50% of all small dams in the altitudinal range of Biomphalaria pfeifferi contained that species.

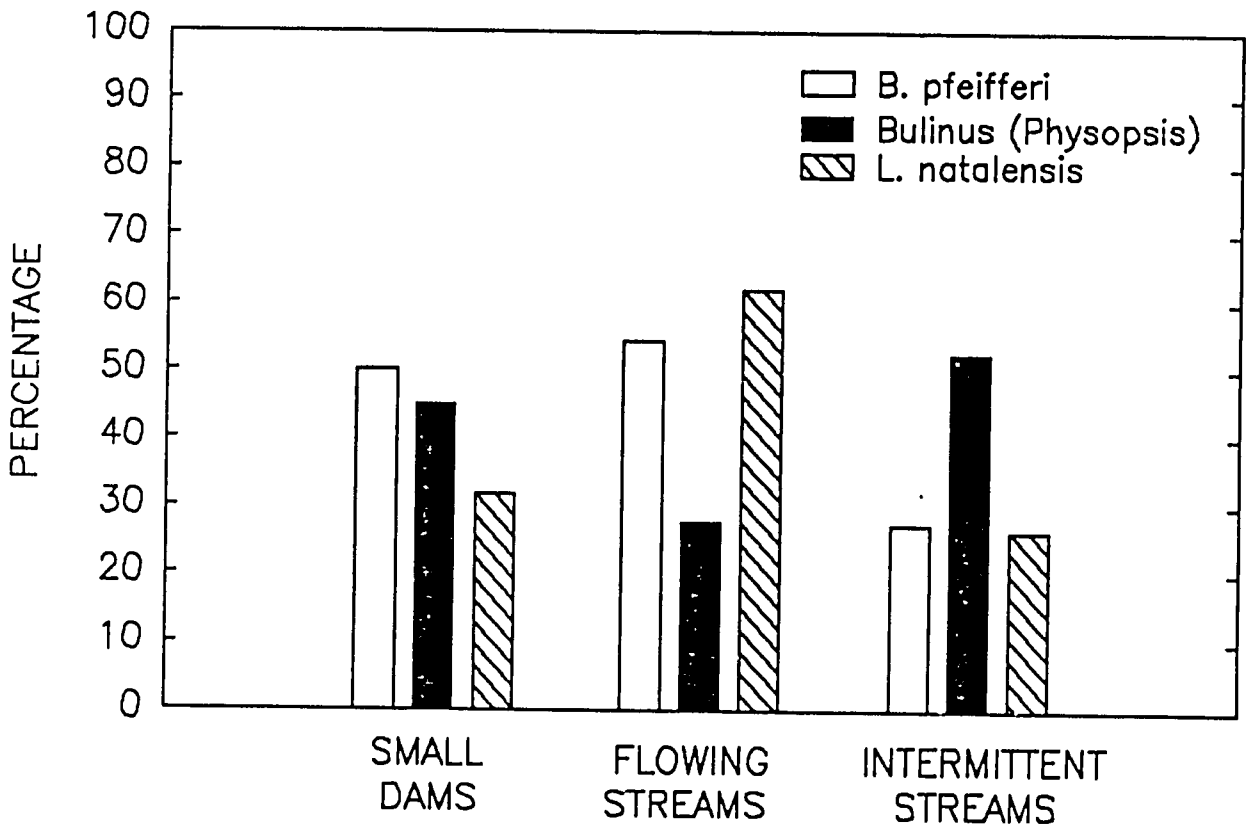
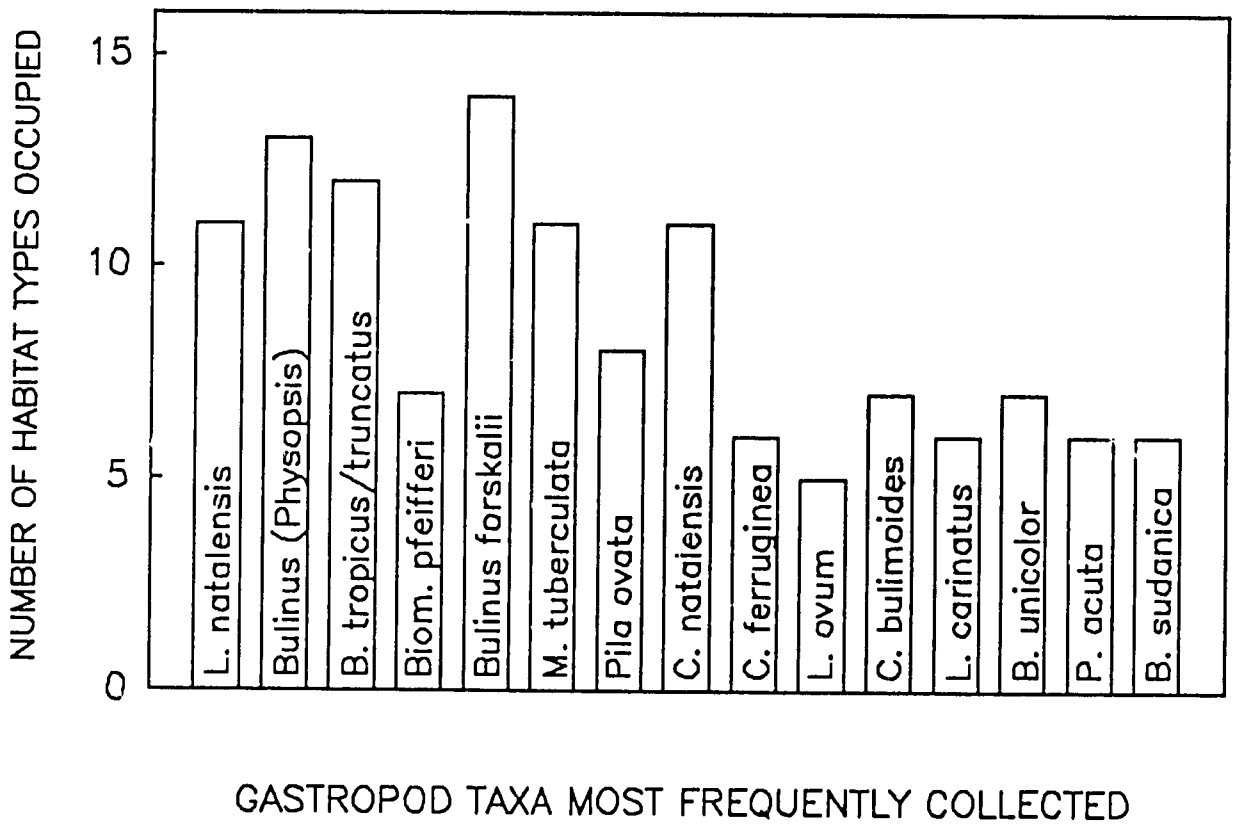


FIGURE 10: For each of several prominent gastropod taxa is listed the number of different habitat types from which that taxon was found.



APPENDIX 2

ADDITIONAL FIELD STUDIES

In addition to the research presented in the accompanying manuscripts, some additional field-oriented projects were undertaken, as follows:

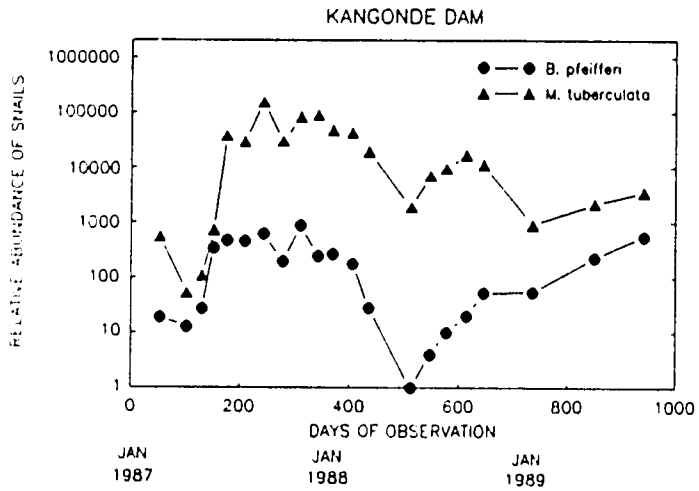
I. Field Observations of Melanoides tuberculata - Pulmonate Interactions

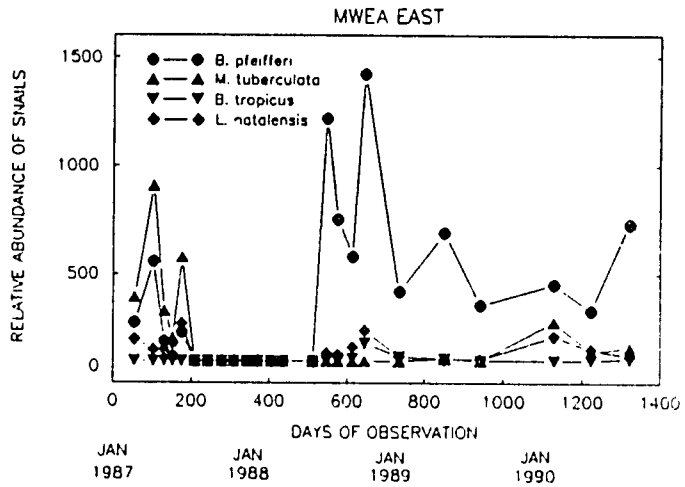
At the start of this project, we were interested to know if pulmonate snails could coexist in appreciable numbers over long intervals of time with the thiarid snail, Melanoides tuberculata, which seems to be so effective as a biological control agent of Biomphalaria glabrata in the Caribbean region. As shown in Figure 1F in Appendix 1, this species has a rather scattered distribution in Kenya with south-central Kenya, in the area around Kitui, being the location where this species is most predictably found. Consequently, we located three isolated impoundments (Kangonde Dam, Mbondoni Dam, Mwea East Pond) and one stream (Matingani Stream) in this region where M. tuberculata was present, and visited these locations repeatedly over a course of about 3 years. At each location, six regular sampling stations were established. At each visit, snails were for 10 min at each of the six sampling stations. All snails collected were identified, enumerated and returned to the habitat. The number of snails of each species was summed across the six sampling stations, providing a measure of the overall relative abundance of different snail species in each of the four habitats. The results of this study are presented in the four graphs below.

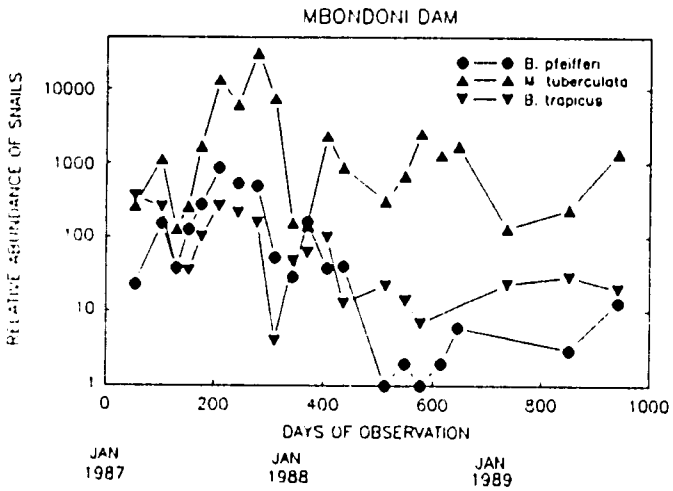
In Kangonde Dam, the only two snails present were M. tuberculata and B. pfeifferi. The former species was consistently more abundant than the latter (in some cases there was a 100- to 1000-fold difference in relative abundance), but B. pfeifferi persisted throughout the study. The one very low level of abundance noted for both species in mid-1988 was during the rainy season when snails were difficult to collect. Occasional visits to this habitat since late 1988 confirmed that the two species continued to coexist in Kangonde Dam.

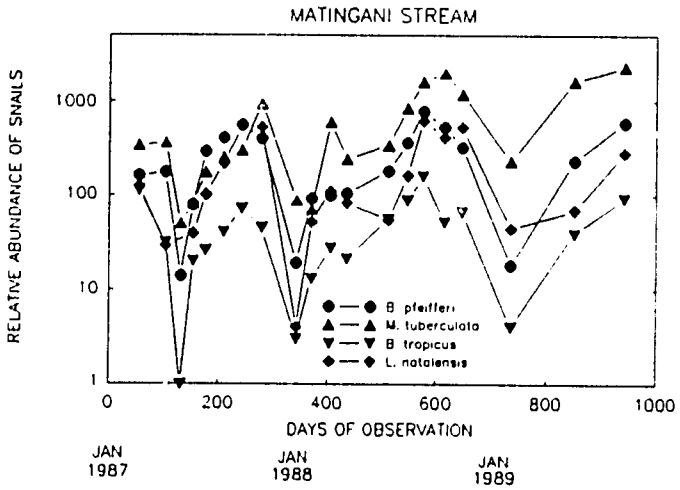
The patterns in Mbondoni Dam are more complex, in part because of the presence there of a third species, Bulinus tropicus. In this case, all three species persisted throughout the study and once again, M. tuberculata was numerically dominant, but not to the extent seen in Kangonde Dam. There was some suggestion that the two pulmonate species were showing 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.

The third impoundment, Mwea East, presents an interesting situation because after our study had begun, without our knowledge or participation, this habitat was treated with molluscicide (in mid-1987) by local health officials. Prior to treatment, M. tuberculata was the most abundant snail present but


KANGONDE DAM
B. pfeiffeni
M. tuberculata
RELATIVE ABUNDANCE OF SNAILS
DAYS OF OBSERVATION
JAN 1987
JAN 1988
JAN 1989


MWEA EAST
B. pfeiffeni
M. tuberculata
B. tropicus
L. natalensis
RELATIVE ABUNDANCE OF SNAILS
DAYS OF OBSERVATION
JAN 1987
JAN 1988
JAN 1989
JAN 1990


MBONDONI DAM
B. pfeiffeni
M. tuberculata
B. tropicus
RELATIVE ABUNDANCE OF SNAILS
DAYS OF OBSERVATION
JAN 1987
JAN 1988
JAN 1989


MATINGANI STREAM
B. pfeiffeni
M. tuberculata
B. tropicus
L. natalensis
RELATIVE ABUNDANCE OF SNAILS
DAYS OF OBSERVATION
JAN 1987
JAN 1988
JAN 1989

three pulmonates were also present, and B. pfeifferi was also relatively common. After application of molluscicide, all snails were scarce for almost one year. By mid-1988, B. pfeifferi populations had recovered and achieved levels of relative abundance considerably higher than before treatment. In contrast, M. tuberculata populations had still not recovered to before-treatment levels. This pattern raises several interesting points. First, it may suggest that M. tuberculata does exert a significant inhibitory effect on pulmonates, and when this species is absent, pulmonates are able to achieve large population sizes. Also, it is interesting to speculate that if M. tuberculata is exerting a controlling effect, molluscicidng may inadvertantly favor transmission by having a greater long-term impact on M. tuberculata than on pulmonates.

The situation in Mwea East is still being investigated and up to August, 1990, B. pfeifferi remains abundant and M. tuberculata has still not achieved its former abundance. It will be of interest to determine if a large increase in the population of M. tuberculata will be accompanied by a concomitant decline in the B. pfeifferi population.

Lastly, Matingani Stream exhibited a complex patterns of seasonal abundance of M. tuberculata and 3 different pulmonate species. Again it was noted that M. tuberculata was the most abundant snail present, but its dominance was less than reported in the three impoundments. Note also that all three pulmonate species persisted throughout the period of observation, and showed no sign of being eliminated by the presence of M. tuberculata.

To summarize these results, appreciable numbers of pulmonates were shown to coexist with M. tuberculata over long intervals of time; no obvious trend towards total displacement of pulmonates was noted. The results from Mwea East suggest that the presence of large populations of M. tuberculata may have the effect of keeping pulmonate populations low. However, even when M. tuberculata achieves extremely high abundance, as in Kangonde Dam, populations of schistosome-transmitting snails are able to persist. Thus it seems likely that Kenyan populations of M. tuberculata will not eliminate pulmonate snails but may, under circumstances where they attain large populations, limit pulmonate population sizes. This in turn might have the effect of reducing the rate of schistosomiasis transmission but certainly would not interrupt transmission.

II. Field Observations on Ampullariid Snails

Results of our survey work indicated that ampullariid snails are present in coastal Kenya and in west Kenya, but not in the higher, cooler, central region of the country. It was of interest to determine if either Lanistes carinatus or Pila ovata could establish self-perpetuating populations in central Kenya. In conjunction with the Ministry of Health, Division of Vector Borne Diseases, in January, 1988, we transplanted L. carinatus into Kangonde Dam, Mbondoni Dam, and Matingani Stream, all of which are far removed from rice growing schemes and isolated from other water bodies. A total of 100 snails were added to each of the six sampling stations in these three habitats. These introductions were relatively unsuccessful in that thriving populations of L. carinatus were not established in any of the three sites. In Matingani Stream, ampullariids were found sporadically in small numbers (less than 50 snails) through Aug. 1989, the latest sampling date available. In Mbondoni

Dam, they were reported in very low numbers through mid-1988, but have not been seen in the last four visits to that site. In Kangonde Dam, they apparently never became established as living L. carinatus were never recovered.

It should be noted that these habitats lacked lily pads (Nymphaea sp.) which seem to be associated with thriving populations of L. carinatus in coastal Kenya. We then tried an additional introduction of L. carinatus in a more appropriate habitat, Kusukioni Dam, north of Tala, Kenya, starting in January 1989. This habitat, which has a thriving Nymphaea population, is still being regularly observed and as of August 1990, contains an apparently self-perpetuating population of this ampullariid. We are monitoring the impact of L. carinatus on pulmonates in Kusukioni Dam as part of our ongoing AID-supported project.

At the same time L. carinatus was placed in Kusukioni Dam, we also placed P. ovata in three isolated dams in the Tala region, at Koma Rock Dam, Tala Township Dam, and Maluva Dam. As of August, 1990, P. ovata were persisting in each of these sites, but at relatively low numbers. The impact of P. ovata on pulmonate populations again is being monitored as part of our ongoing AID grant. This work is being done in conjunction with both KEMRI and the Division of Vector Borne Diseases.

APPENDIX 3

IN PRESS, ACTA TROPICA

CONSUMPTION OF BIOMPHALARIA GLABRATA EGG MASSES AND JUVENILES BY THE AMPULLARIID SNAILS PILA OVATA, LANISTES CARINATUS AND MARISA CORNUARIETIS

BY

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RUNNING HEAD: AMPULLARIID PREDATION OF PULMONATE EGG MASSES AND JUVENILES

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ABSTRACT

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 ± 0.5mm B. glabrata juvenile snails presented to them. P. ovata adults consumed significantly more 3.0 ± 0.5mm 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 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 hour light, 12 hour dark photoperiod was maintained for all experiments. Juvenile ampullariids used in these experiments ranged from 8 - 12mm in size (shell diameter for Lanistes and Marisa, shell height for Pila) and adults were 30-40mm 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 watt 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 (2x2cm), each containing a single B. glabrata egg mass, were floated at the surface. The squares were secured so 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 $\pm$ 0.5mm). 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 ± 0.5mm 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 juveniles 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, 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 ± 0.5mm 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 ± 0.5mm 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 1200m 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 that 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. Deposition of egg masses in spaces too small to be reached by ampullariids may also prevent attack.

All three ampullariid species consumed 1.5mm juveniles of B. glabrata, even when lettuce was continually available in the aquaria. Only P. ovata consumed appreciable numbers of larger juveniles in the 2.6-3.5mm 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 5mm.

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 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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Table 1: The percentage (± S.E.) of B. glabrata egg masses or juveniles consumed by adults of juveniles of Pila ovata, Lanistes carinatus, or Marisa cornuarietis. Each experiment was replicated six times. N.D. = not done. Experiment #3 is not represented as no B. glabrata egg masses or juveniles were consumed.

EXPERIMENT	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*	23.9 ± 11.9	4.0 ± 2.0
EXPERIMENT 2			
Adults			
low temperature	27.8 ± 8.5	8.3 ± 3.1	29.1 ± 8.2
high temperature	70.8 ± 10.2^#	23.6 ± 11.1	43.1 ± 10.0
Juveniles			
low temperature	8.4 ± 6.9	N.D.	9.7 ± 3.4
high temperature	69.7 ± 6.9*#	N.D.	29.1 ± 8.2#
EXPERIMENT 4			
1.5 ± 0.5mm juveniles	25.8 ± 11.6	26.7 ± 5.3	25.8 ± 10.5
3.0 ± 0.5mm juveniles	50.0 ± 14.4*	1.7 ± 1.7	1.7 ± 1.7

* significantly greater than other similar-sized ampullariids, $p < 0.05$
^ significantly greater than L. carinatus, $p < 0.05$
\# significantly greater than corresponding group at low temperature regime, $p < 0.05$

APPENDIX 4

GROWTH OF BIOMPHALARIA GLABRATA POPULATIONS IN THE PRESENCE OF THE AMPULLARIID SNAILS PILA OVATA, LANISTES CARINATUS AND MARISA CORNUARIETIS

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RUNNING HEAD: EFFECTS OF AMPULLARIIDS ON B. GLABRATA POPULATION GROWTH

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ABSTRACT

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 Domirican Republic. The presence of either 3 or 9 L. carinatus or 3 M. cornuarietis per 40 liter 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.

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., 1990). 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 30-40mm 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: Forty liter aquaria 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. A 12 hour light, 12 hour dark photoperiod was maintained for all experiments. All experiments were performed at 25 $\pm$ 2°C; 75 watt 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 <2mm, 2-9mm, and >9mm 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 one week. 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 Figure 1. For each experimental treatment, similar counts are provided in Figures 2-7. The mean weekly counts for control aquaria and for each experimental treatment are presented in Figure 8. Figure 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. Figure 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 Figure 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 Figure 12.

For the control aquaria, the number of B. glabrata generally rose throughout the 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; less than 10% 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 <2mm snails as compared to experimental aquaria. They also contained many 2-9mm snails.

Experimental aquaria containing 3 L. carinatus on average produced more B. glabrata than did aquaria of any other group, including controls. Total B. glabrata counts did not differ significantly from controls at any time, however. 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 large numbers of 2-9mm snails were found in such aquaria by the end of the experiment, indicating generally favorable 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 <2mm snails than controls although the presence of several 2-9mm 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 was 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 sifnificant; 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 <2mm and 2-9mm snails observed in such aquaria.

DISCUSSION

In all 30 aquaria, B. glabrata populations persisted throughout the eleven week period of study and in most, 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. We can not exclude the possibility that similar phenomena occurred in our experimental aquaria.

Whereas the effects of ampullariids on the total number of B. glabrata egg masses produced were generally not significant, the percentage of egg masses that subsequently disappeared was significantly higher than control values for each experimental group. The general inverse relationship between the total number of egg masses observed and the percentage of egg masses known to have disappeared also suggests that predation accounts for the reduced number of egg masses in aquaria with ampullariids. For example, nearly 90% of the observed egg masses present in aquaria with 9 P. ovata disappeared before they were due to hatch, suggesting that appreciable numbers of egg masses would have been produced and then consumed during the intervals between observation. Several other investigators have examined interactions between ampullariids and pulmonates under laboratory conditions and have noted similar

strong predatory effects (Chernin et al., 1956; Demian and Lutfy, 1965a and b, 1966; Paulinyi and Paulini, 1972; Cedeno-Leon and Thomas, 1983; Hofkin et al., 1990)

The fate of egg masses was in most cases not determined by direct observation so the conclusion that predation by ampullariids is responsible for the disappearance is indirect. However, a previous study has demonstrated that all three ampullariid species used in the present study consume B. glabrata egg masses; under comparable conditions, B. glabrata adults did not (e.g. Hofkin et al., 1990). Furthermore, some egg masses were observed to be consumed by ampullariids during the present study.

The results of the present study are in general agreement with Hofkin et al. (1990) who found that of the three ampullariids examined, P. ovata was generally a more efficient predator of B. glabrata egg masses and juveniles than M. cornuarietis or L. carinatus. Although caution is required in extrapolating the results of these two laboratory studies to field situations, together they suggest that with respect to the potential use of ampullariid snails for the biological control of schistosomiasis in Kenya, the indigenous ampullariid P. ovata is particularly deserving of additional study. The results also suggest that further introductions of M. cornuarietis into sub-Saharan Africa are particularly difficult to justify given the presence of an indigenous species with comparable, or superior, biocontrol properties.

Finally, it seems that L. carinatus has relatively little potential to function as an effective biological control agent in Kenya. Additional observations to support this claim are provided by Madsen et al. (1988) who noted a positive association between L. carinatus and B. pfeifferi in natural habitats in the Sudan. This ampullariid was also introduced into three canals in the Gezira Agricultural Scheme but had no obvious effects on pulmonate

populations (Karoum, 1988). L. carinatus had little effect on macrophyte densities in irrigation canals in the Gezira (Karoum, 1988), suggesting it is unlikely to function as an effective resource competitor with pulmonates. The ability of P. ovata to function as a voracious consumer of macrophytes and to displace or limit natural populations of pulmonates in sub-Saharan Africa is deserving of additional study.

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Figures 1-7

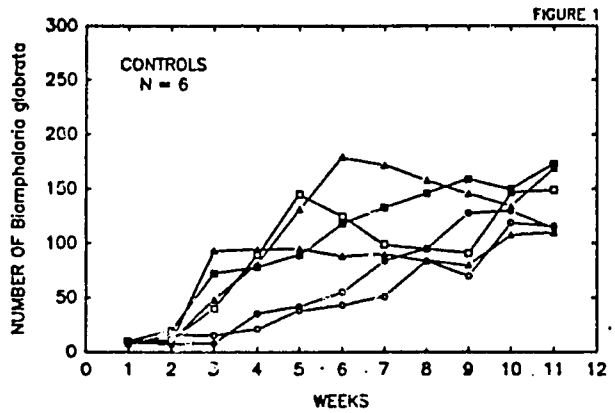


FIGURE 1
CONTROLS
N = 6
NUMBER OF Biomphalaria glabrata
WEEKS

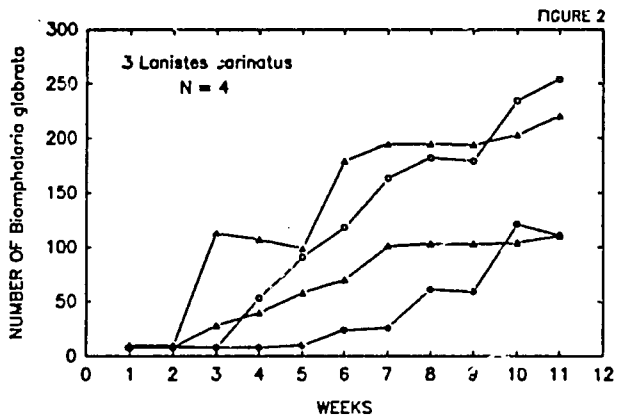


FIGURE 2
3 Lanistes carinatus
N = 4
NUMBER OF Biomphalaria glabrata
WEEKS

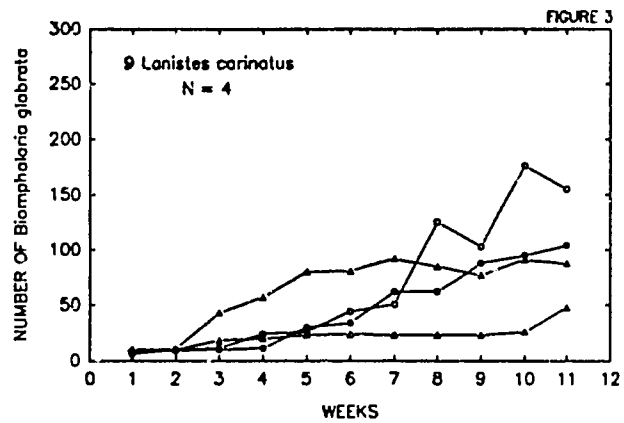


FIGURE 3
9 Lanistes carinatus
N = 4
NUMBER OF Biomphalaria glabrata
WEEKS

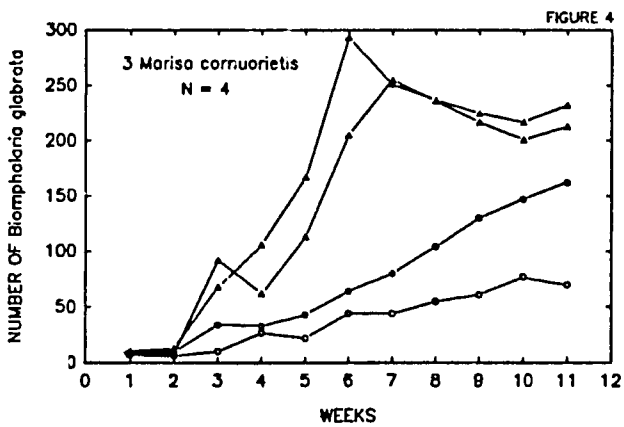


FIGURE 4
3 Marisa cornuarietis
N = 4
NUMBER OF Biomphalaria glabrata
WEEKS

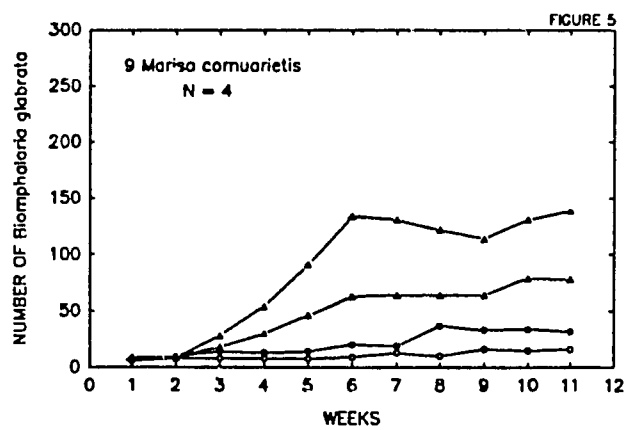


FIGURE 5
9 Marisa cornuarietis
N = 4
NUMBER OF Biomphalaria glabrata
WEEKS

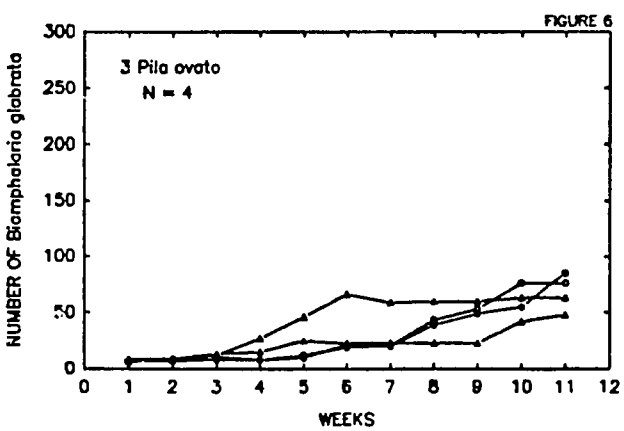


FIGURE 6
3 Pila ovata
N = 4
NUMBER OF Biomphalaria glabrata
WEEKS

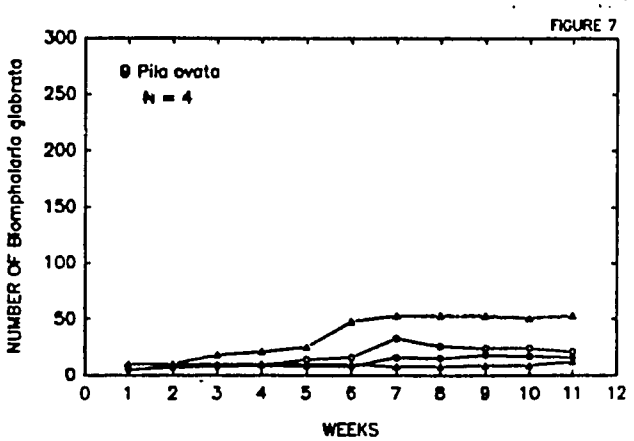


FIGURE 7
9 Pila ovata
N = 4
NUMBER OF Biomphalaria glabrata
WEEKS

Figure 8

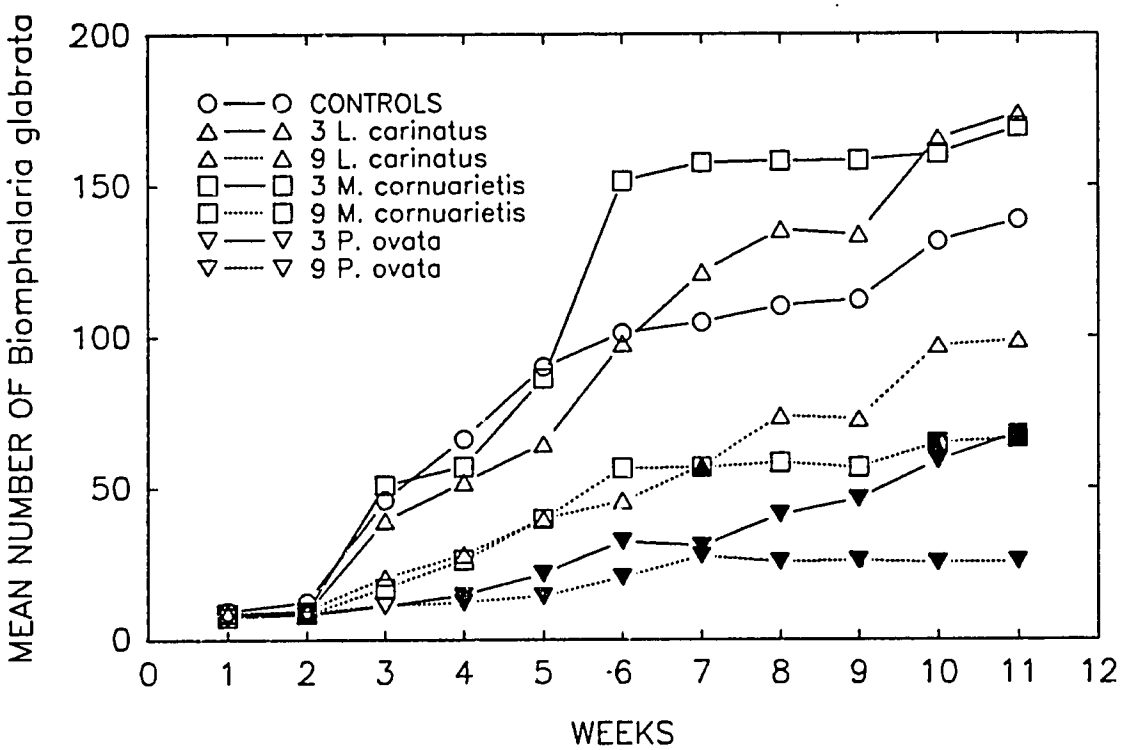

MEAN NUMBER OF Biomphalaria glabrata
200
150
100
50
0
0
1
2
3
4
5
6
7
8
9
10
11
12
WEEKS
CONTROLS
3 L. carinatus
9 L. carinatus
3 M. cornuarietis
9 M. cornuarietis
3 P. ovata
9 P. ovata

Figure 9

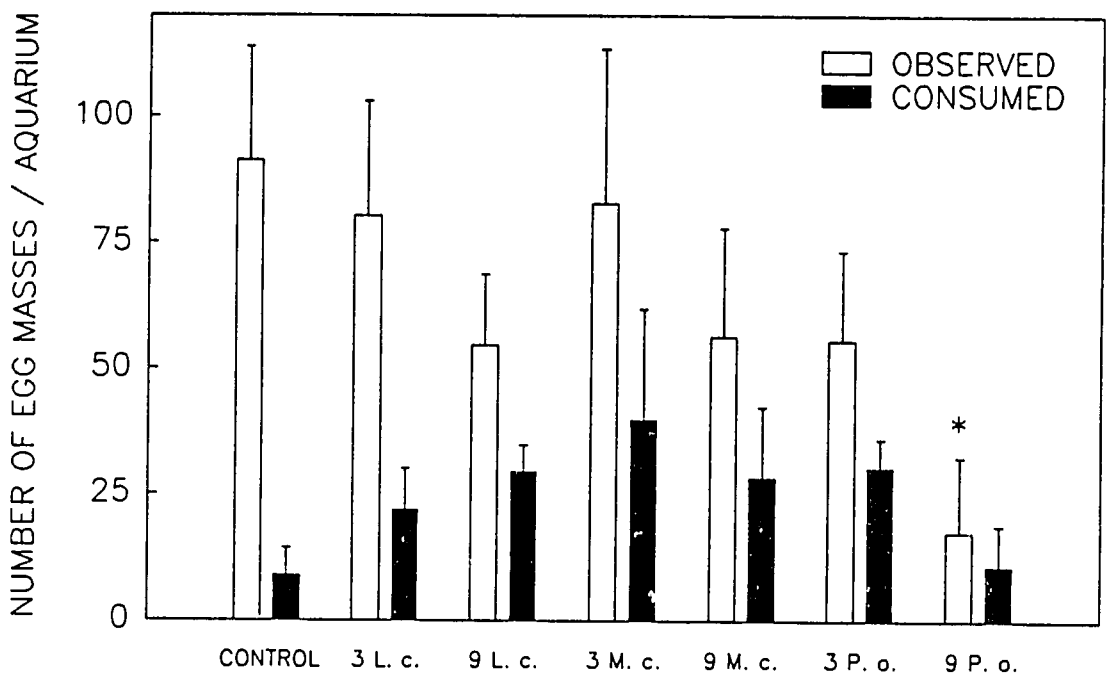

NUMBER OF EGG MASSES / AQUARIUM
100
75
50
25
0
OBSERVED
CONSUMED
*
CONTROL
3 L. c.
9 L. c.
3 M. c.
9 M. c.
3 P. o.
9 P. o.

Figure 10

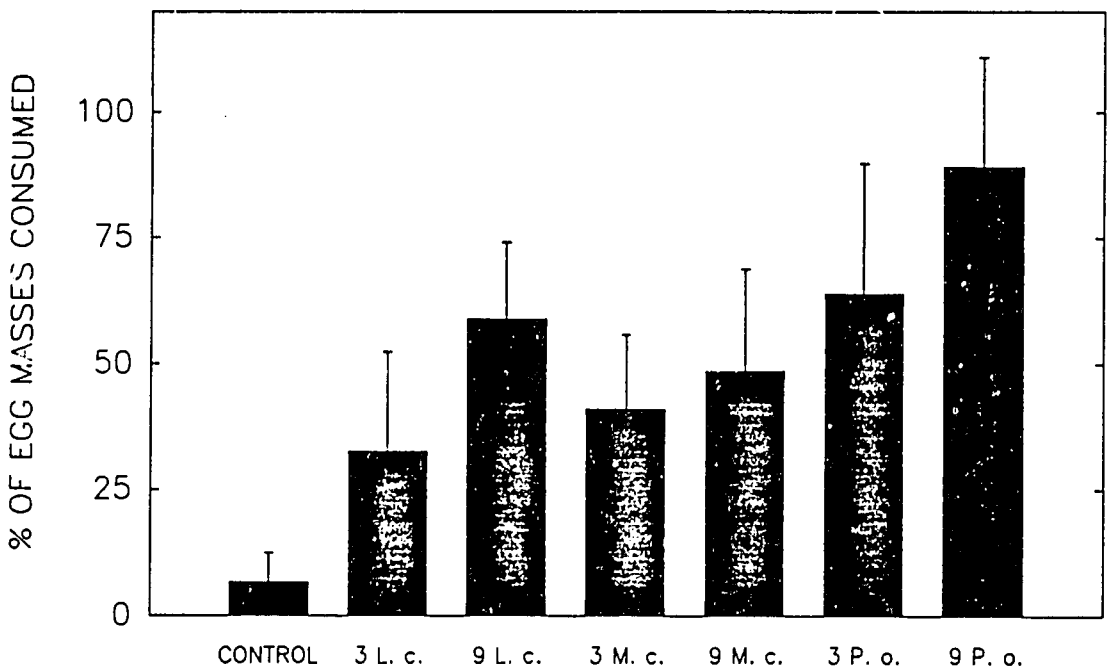

% OF EGG MASSES CONSUMED
100
75
50
25
0
CONTROL
3 L. c.
9 L. c.
3 M. c.
9 M. c.
3 P. o.
9 P. o.

Figure 11

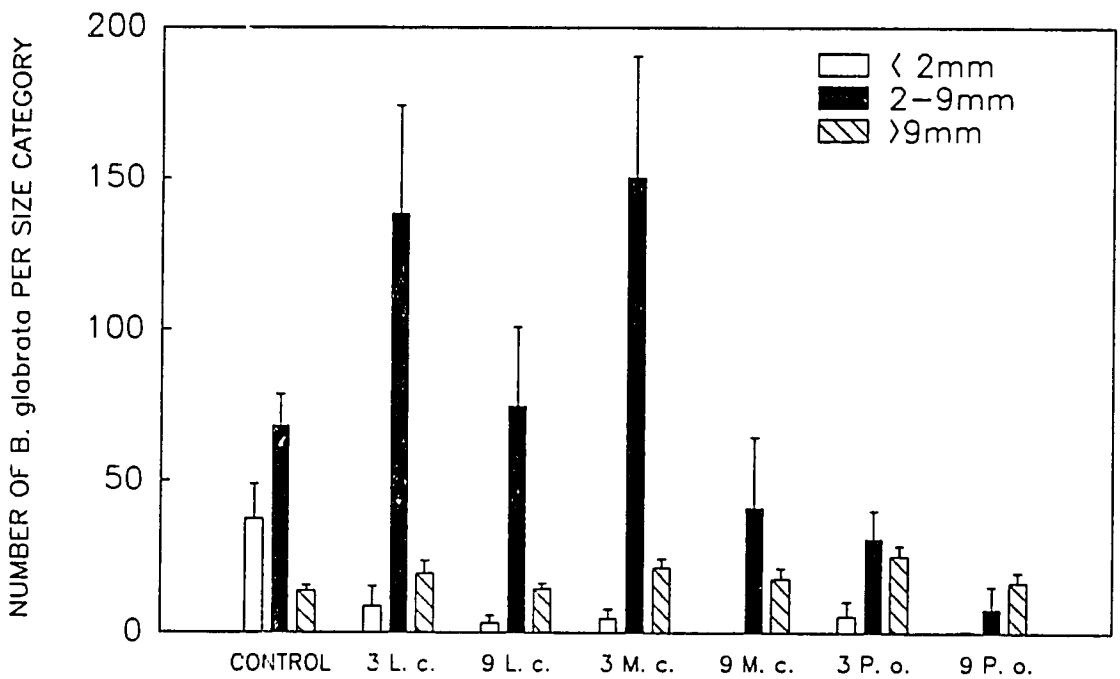
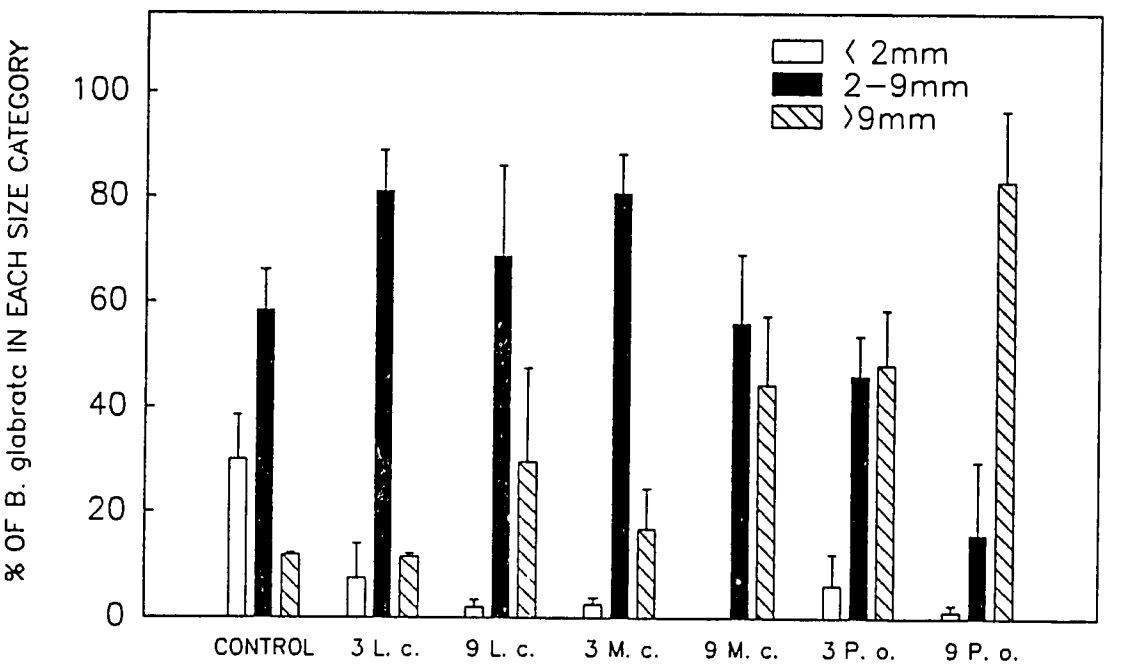

NUMBER OF B. glabrata PER SIZE CATEGORY
200
150
100
50
0
< 2mm
2–9mm
>9mm
CONTROL
3 L. c.
9 L. c.
3 M. c.
9 M. c.
3 P. o.
9 P. o.

Figure 12


% OF B. glabratc IN EACH SIZE CATEGORY
100
80
60
40
20
0
< 2mm
2–9mm
>9mm
CONTROL
3 L. c.
9 L. c.
3 M. c.
9 M. c.
3 P. o.
9 P. o.

APPENDIX 5

HELMINTH PARASITES AND DIETARY PREFERENCES OF KENYAN AMPULLARIID SNAILS, POSSIBLE AGENTS OF BIOLOGICAL CONTROL OF SCHISTOSOME-TRANSMITTING PULMONATES

BY

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ABSTRACT: Freshwater snails of the family Ampullariidae have often been considered as possible biological control agents of schistosome-transmitting pulmonate snails. Ampullariid species indigenous to Africa have been little studied in this context. A total of 424 snails representing four species of ampullariids (Pila ovata, Lanistes carinatus, L. ovum, and the relatively uncommon species L. ciliatus) were collected from 34 different habitats in Kenya. Two aspects of the biology of these snails which are pertinent to biological control studies were investigated, namely their role as intermediate hosts of helminths and their dietary preferences. Two different trematode cercariae were recovered from Kenyan ampullariids and are described. A third cercaria found previously in P. ovata from Tanzania is also described. None of these trematodes are believed to be of public health significance. No infections with Angiostrongylus or other nematodes were found. Relative proportions of various dietary items recovered from the stomachs of the three common ampullariid species were recorded. Lanistes carinatus consumed significantly more detritus than other species, and macrophytes comprised a significantly larger part of the diet of P. ovata. Lanistes ovum was intermediate with respect to the amount of both detritus and macrophytes consumed. Kenyan ampullariid species utilize food resources differently, and may therefore interact with pulmonate species in fundamentally different ways in natural habitats.

Many species of freshwater gastropods have been considered as possible biological control agents of the molluscan intermediate hosts of schistosomiasis (Michelson, 1957; Jordan et al., 1980; Combes and Cheng, 1986). The family of snails receiving the most attention in this context is the Ampullariidae, representatives of which are widely distributed in both the old- and new-world tropics (Michelson, 1961). Within this family, Marisa cornuarietis has been most thoroughly studied as a potential biological control agent. The ability of this snail to consume Biomphalaria and Bulinus egg masses and juveniles, and its voracious consumption of aquatic plants are well known (e.g. Ferguson, 1977). This gastropod has been implicated in reductions of Biomphalaria populations in streams and reservoirs in Puerto Rico (Oliver-Gonzalez et al., 1956; Jobin et al., 1977, 1984). Introductions of M. cornuarietis in Egypt (Demian and Kamel, 1973), Tanzania ((Nguma et al., 1982), and the Sudan (Haridi et al., 1985; Karoum, 1988) suggest that this snail has control potential outside of the neotropics.

However, the introduction of exotic gastropods into Africa or elsewhere carries with it potential risks that must be carefully weighed. Unfortunate instances of gastropod introductions, such as that of Achatina fulica (Mead, 1961) onto several Pacific islands, have been well documented. With respect to M. cornuarietis, this snail has been implicated as a potential pest in rice fields (Ortiz-Torres, 1962), although others have contested this finding (Demian and Ibrahim, 1969). Moreover, a control program based on the introduction of exotic gastropods must also consider the possibility that these snails may transmit helminth parasites of medical or veterinary importance.

For example, natural infections of Angiostrongylus cantonensis, causative agent of human eosinophilic meningoencephalitis, have been recovered from Ampullarius canaliculatus snails from Kyushu, Japan (Nishimura et al., 1986). This ampullariid was brought from Argentina to Japan in 1981 as a potential food source (Nishimura et al., 1986).

Another factor to be considered is that several ampullariid species are indigenous to Africa (Brown, 1980), and these snails have not been adequately studied in the context of biological control. If further study indicates that native ampullariids can serve as effective control agents, the continued introduction of exotic species such as Marisa into Africa would become particularly difficult to justify.

Several aspects of the biology of Pila and Lanistes, the two prominent genera of African ampullariids, are poorly known. Additional information pertaining to the efficacy or to the safety of these snails is required before their use in a program of biological control can be advocated. Consequently, we have collected four species of ampullariid snails from a variety of habitats in Kenya and have examined them for evidence of infection with helminths that might pose a public health risk. We have used the same snail specimens to ascertain the natural dietary preferences of Kenyan ampullariids. The feeding habits of such snails has a direct bearing on their ability to function as possible resource competitors for disease-transmitting pulmonate snails.

MATERIALS AND METHODS

Representatives of Pila ovata, Lanistes carinatus, L. ovum, and L. ciliatus were collected in Kenya in 1987 and 1988. Identification of snails to genus, species, and subspecies was based on keys and descriptions provided by Brown (1980). Snails were preserved in 70% ethanol immediately following collection. For each snail, both shell and shell aperature size (length and width) were measured to the nearest millimeter. Each snail was then removed from its shell; pieces of the shell were weighed to obtain total shell weight. After determining a snail's sex, the presence or absence of trematode parasites was assessed by dissection of the digestive gland and reproductive organs. Trematode larvae from positive specimens were examined further with the aid of a compound microscope. The same snails were also examined for infection with Angiostrongylus and other nematodes by slicing the snails into small (<0.5 cm) pieces which were digested in a solution of 1% pepsin (W:V) in 1% HCl (V:V) at 37°C for a minimum of two hours. The digest was then examined microscopically for Angiostrongylus larvae.

The descriptions of cercariae were based, in part, on observations of living material derived from P. ovata obtained from Mwanza, Tanzania (see Loker et al., 1981). Measurements were based on specimens fixed in hot 10% formalin.

Stomach contents of individuals of each species from each locality were also studied. A food sample from the proximal region of the stomach was suspended in water and examined under X100 magnification. The microscope used contained an ocular eyepiece fitted with a grid that measured 0.5mm by 0.5mm at this magnification. The food sample was scored for presence or absence of a

particular food type in each of twenty five squares on the grid (Kesler et al., 1986). Six dietary categories were recognized. These included inorganic material, macrophytes, detritus, single celled algae (including diatoms), filamentous algae, and animal matter. Seven grids per sample were randomly selected and examined. For a given food type, the total number of squares scored positive for that type was divided by the total number of squares scored positive for all food types. This value, compared to that for other food categories, was used to estimate the importance of a given type of food in the diet of a particular snail. Such data were transformed to arc-sine values and were analyzed by ANOVA, followed by Tukey's test when necessary to assess the significance of individual pairs of means.

RESULTS

Collection Localities: For each numbered collection locality below is listed the local name, collection date, its longitude and latitude, and a brief description of the habitat. For *P. ovata*, localities in western Kenya include: 1. Old Prison Farm (20 Jan 1987; 0° 6.5'S, 34° 44.5'E; series of shallow irrigation ditches) 2. ADC Farm (21 Jan 1987; 0° 4.7'S, 34° 44.5'E; series of shallow irrigation ditches) 3. B1-A1 Pond (12 July 1987; 0° 10.1'S, 34° 54.9'E; small borrow pit) 4. Kembija Dam (13 July 1987; 0° 2.4'S, 34° 30.9'E; man-made dam) 5. Nyamguu Stream (13 July 1987; 0° 1.8'S, 34° 26.7'E; small stream) 6. Dago Stream (13 July 1987; 0° 0.6'N, 34° 32.7'E; small stream) 7. Otodo Stream (13 July 1987; 0° 3.0'N, 34° 34.1'E; small stream) 8. Maugo Stream (14 July 1987; 0° 28.5'S, 34° 32.7'E; small stream) 9. Sota Stream (14 July 1987; 0° 30.6'S, 34° 28.8'E; small stream) 10. Nyalkiny Dam (14 July 1987; 0° 31.2'S, 34° 28.2'E; man-made dam) 11. Rusinga Island (14 July 1987; 0° 24.9'S, 34° 12.3'E; Lake Victoria) 12. Asembo Bay (20 June 1988; 0° 5.5'S, 34° 32.7'E; Lake Victoria. Collection localities for *P. ovata* in eastern Kenya include: 13. Lake Gulamu Ng'onda (2 July 1987; 3° 6.3'S, 39° 45.5'E; large, ox-bow lake) 14. Bura Pond (29 June 1988; 1° 15.8'S, 39° 58.9'E; man-made dam) 15. Hola Canal (30 June 1988; 1° 31.6'S, 40° 3.8'E; irrigation canal).

All collection localities for representatives of *Lanistes* were in eastern Kenya. For *Lanistes carinatus*, they were as follows: 13, 14 and 15. see above, 16. Chem-Chem Swamp (17 Jan 1987; 3° 12.3'S, 40° 23.0'E; large, natural swamp) 17. Lake Jilori (2 July 1987; 3° 11.4'S, 39° 53.2'E; large, natural swamp, nearly dry) 18. Dzimbwini (2 July 1987; 3° 9.1'S, 39° 50.9'E;

man-made dam) 19. Mbaratum (2 July 1987; 3° 11.0'S, 39° 45.4'E; large, natural swamp) 20. Tana River (1 July 1988; 0° 27.8'S, 39° 39.8'E; pools along river margin) 21. Borr-Algy (1 July 1988; 0° 28.9'S, 39° 40.9'E; pools along river) and 22. Lake Madina (22 July 1988; 3° 12.0'S, 40° 5.5'E; small permanent lake).

For L. ovum ovum, localities included: 14, 21 and 22. see above, 23. Mwachi River (4 July 1987; 3° 57.9'S, 39° 31.4'E; pools in intermittent stream) 24. Kinango Dam (4 July 1987; 4° 7.9'S, 39° 18.8'E; man-made dam) 25. Kambe Stream (5 July 1987; 4° 26.4'S, 39° 18.9'E; pools in intermittent stream) 26. Marafa Pond (23 July 1988; 3° 2.2'S, 39° 47.5'E; small ponds) 27. Dida Pond II (24 July 1988; 3° 25.6'S, 39° 48.0'E; man-made dam) and 28. Vitengini Pond (24 July 1988; 3° 21.3'S, 39° 40.9'E; large pond). For L. ovum procerus the localities were: 13. see above, 29. Mtawa Stream (15 Jan 1987; 4° 20.7'S, 39° 29.5'E; shallow, slow-flowing stream) and 30. Kachororoni Dam (3 July 1987; 3° 29.0'S, 39° 42.7'E; small dam on intermittent stream). Localities for L. ovum purpureus are: 31. Muhaka Dam (15 Jan 1987; 4° 17.8'S, 39° 30.2'E; man-made impoundment) 32. Kiziamondo Stream (4 July 1987; 4° 7.9'S, 39° 19.8'E; pools in intermittent stream) and 33. Mwangwei (5 July 1987; 4° 30.6'S, 39° 16.8'E; small, natural swamp).

For L. ciliatus, the two collection localities were: 26. see above, and 34. Rare River (3 July 1987; 3° 31.1'S, 39° 45.9'E; pools in intermittent stream).

Infection of Ampullariids with Helminth Parasites: From the 34 Kenyan ampullariid habitats, a total of 424 snails were collected and examined for helminth infection. Ampullariids are often considerably less abundant in natural habitats than smaller snail species; in many of the localities, fewer than ten specimens were found. Eighteen individuals (4.2%) were found to be

infected with trematode larvae (Table 1). Two different cercariae, designated Cercaria A and B, were found. Cercaria A is a furcocercous cercaria recovered only from two P. ovata from habitat #7 in western Kenya. Cercaria B was recovered from P. ovata in four habitats in western Kenya and from L. carinatus, L. ovum procerus and L. ovum purpureus in five habitats in the eastern portion of the country (Table 1).

For ten of the fifteen habitats containing P. ovata, females outnumbered males in our collections, and 61.8% of all mature snails collected were females. No trematode infections were found in males. Infected L. carinatus were recovered from two of the ten habitats where we found this species. Females outnumbered males in five of these habitats and comprised 57.9% of mature snails. Snails of both sexes were infected but more females were infected than males. Of the fifteen habitats where the three subspecies of L. ovum were found, three contained infected snails. Females comprised 60.3% of the mature snails in our collections. All mature, infected snails were females with one exception. Only sixteen individuals of the relatively uncommon species L. ciliatus were collected, all from localities #26 and #34. None were found to be infected.

Sexually immature snails accounted for 29.2% of all collected ampullariids. Furthermore, of the eighteen snails found to be positive for trematode infection, four (22.2%) were juveniles. Thus, although the sample sizes are small, these data suggest that juvenile ampullariids harbor larval trematodes at rates comparable to those seen in adults.

The tissues of all dissected snails were subsequently digested in pepsin-HCl to determine if Angiostrongylus or other nematode infections were present. No nematodes were found.

Description of Cercariae: Cercaria A is pharyngeate, has a ventral

sucker and lacks eyespots. It possesses conspicuous refractile granules within the excretory bladder and has thick-walled intestinal cecae which terminate at the level of the ventral sucker (Fig. 1). This cercaria is produced in daughter sporocysts. It closely resembles Cercaria megacoelia obtained from Melanoides tuberculata from Lake Albert (Fain, 1953). A cercaria very similar to C. megacoelia was reported by Fain (1953) from snails of the genus Pila, but was not given a formal name.

Cercaria B corresponds in morphology to a cercaria recovered from P. ovata in the Mwanza region of Tanzania (Cercaria #26 of Loker et al., 1981). Living representatives of this previously undescribed cercaria have been examined so a relatively complete description is possible (Fig. 2). It is an echinostome cercaria possessing 27 collar spines of approximately equal size. It has only a rudimentary tail which is not clearly demarcated from the body. The excretory bladder is spherical and contains a small, posterior bulb that extends into the "tail". The main excretory collecting ducts are sinuate and for each, the portion immediately anterior to the ventral sucker is expanded and filled with approximately 10 refractile granules. Approximately 26-27 pairs of flame cells were observed. Numerous cystogenous gland cells extend from the level of the pharynx to the posterior margin of the body; cystogenous cells are filled with needle-like structures arranged in bundles. Along the esophagus, 8-10 glandular structures were observed. This cercaria was recovered only from daughter rediae (Fig. 3) which were found in the digestive gland of infected snails. Metacercariae of this species (Fig. 4) have a regular oval outline (145-190 X 90-122 um) and are also found in the digestive gland of infected snails. This suggests that the essentially tailless cercariae of this species encyst to become metacercariae within the tissues of the host in which they are produced.

Additionally, we here describe a third cercaria (Cercaria C, Fig. 5) which, although not observed in ampullariid snails from Kenya, has been recovered from P. ovata collected in the Mwanza region of Tanzania (Cercaria #27 of Loker et al., 1981). This echinostome cercaria possesses a collar bearing 47 spines of similar length. A cluster of similarly-sized spines is located anterior to the pharynx. The esophagus bifurcates immediately anterior to the ventral sucker, and the ceca extend to the posterior margin of the body. Numerous cystogenous cells are found throughout the body and are filled with needle-like rods arranged in bundles. Refractile granules are also distributed throughout the body and are especially prominent between the pharynx and ventral sucker. A total of 13-15 unicellular penetration glands is observed on each side of the pharynx. The main excretory ducts are sinuate and inflated from the level of the ventral sucker to the pharynx; 10-20 refractile granules are located in each inflated excretory duct. Sixteen pairs of flame cells were observed in the body. The tail contains no prominent fin-folds. Metacercariae (Fig. 6) corresponding in morphology to this cercaria were found encysted in the salivary glands of P. ovata. Metacercarial cysts have a flattened oval shape and measure 198-228 long by 114-133 um wide (living specimens). Living daughter rediae have a relatively clear body with a dark gut which is approximately one-third the length of the body (Fig. 7).

Analysis of Stomach Contents: The stomach contents of P. ovata were relatively consistent between localities; in 12 of 14 habitats, the most abundant dietary component was macrophytes, and in no habitat did detritus contribute more than 30% of the total diet. Similarly, for L. carinatus, in six of the eight habitats where collected, detritus was the most abundant dietary component. In no case did macrophytes comprise more than 20% of the diet. For L. ovum, the three recognized subspecies did not differ

conspicuously from one another in dietary preferences, and are considered together below. Macrophytes were the most abundant dietary item in nine of 14 L. ovum habitats, with both detritus and one-celled algae contributing prominently in other locations.

For the interspecific comparisons described below and in Figure 8, diet data from different localities were pooled for each species. Significant interspecific differences in the relative abundance of dietary macrophytes were noted ($F = 27.286$, $p < 0.001$). All three species differed significantly from one another in this regard; P. ovata consumed the most macrophytic material and L. carinatus the least. Significant differences between species were also noted in the relative abundance of detritus in the diet ($F = 39.566$, $p < 0.001$). L. carinatus ate significantly more detritus than L. ovum which consumed significantly more than P. ovata.

Interspecific differences in the consumption of single celled algae (including diatoms), filamentous algae, and inorganic material were not statistically significant. Unicellular algae appeared to be a relatively important food source for all three ampullariid species, while filamentous algae typically made up only a small portion of the diet. All species consumed substantial amounts of inorganic material. The amount of recognizable animal material consumed was low for all three species and consisted mainly of ostracods, or the chitonous remains of arthropod exoskeletons. L. carinatus consumed significantly more animal material than the other two species, although the low values obtained for all three ampullariids indicate that this result should be interpreted cautiously.

DISCUSSION

Not surprisingly, it was found that ampullariids in East Africa harbor larval trematodes. The most common ampullariid trematode species that we found, Cercaria B, produces a 27-spined echinostome cercaria that apparently encysts within the same snail in which it is produced. Although the life cycle of this fluke is not known, the openbill stork (Anastomus lamelligerus) and/or the hamerkop (Scopus umbretta) should be investigated as possible definitive hosts; both avian species are conspicuous consumers of ampullariid snails in Kenya (personal observations). As has been reported for other echinostome species in Asia (see Malek, 1980 for review), ingestion of raw or poorly-cooked ampullariids might result in human infections with this echinostome, though we know of no documented cases of echinostome infections in humans in Kenya. Also, although people in Africa do under some circumstances consume ampullariid snails (Pilsbry and Bequaert, 1927), this practice is not common in Kenya. Similar considerations apply for Cercaria C, the 47-spined echinostome cercaria recovered from P. ovata in Tanzania. This species encysts as metacercariae in P. ovata and could also possibly infect humans if such snails were ingested. Cercaria A, the furcocercous cercaria recovered from Pila ovata in western Kenya is not a schistosome cercaria as it possesses a pharynx. It is unlikely that this particular trematode infects humans or domestic animals with any regularity although additional experiments to elucidate its life cycle are needed.

Indeed, all trematodes of known medical or veterinary significance in Kenya are transmitted by pulmonate snails (Brown, 1980). Because of the distant relationship between pulmonate and prosobranch snails, it is very

unlikely that native or introduced ampullariid snails can serve as intermediate hosts of these pulmonate-transmitted trematodes. Marisa cornuarietis has been shown to be refractory to Schistosoma mansoni, S. haematobium and Fasciola hepatica (reviewed by Ferguson, 1977) but comparable experiments have not been undertaken with the African ampullariids.

Perhaps a more probable way in which native Kenyan ampullariids might become involved in the transmission of helminths of medical significance is by hosting nematodes of the genus Angiostrongylus. Although there are no records from East Africa of human infections with this nematode genus (Brown, 1980), the range of Angiostrongylus cantonensis is expanding (Cross, 1987). For example, A. cantonensis has been reported to infect Lanistes carinatus in Egypt, a country in which previous surveys had failed to report this parasite (Yousif and Ibrahim, 1978). Also, a worm described as Angiostrongylus costaricensis has recently been recovered from a man in Zaire (Baird et al., 1987).

None of the Kenyan ampullariids we examined were infected with nematodes, as assessed by examination of pepsin-HCl digestion of ethanol-preserved snails. Angiostrongylus larvae preserved in ethanol and subjected to the same treatment were not digested, so we are confident that nematode larvae would have been recovered if present. Further survey work of rodents, particularly domestic rats, and of Achatina fulica and other terrestrial mollusks in and around prominent port cities such as Mombasa is needed to determine if Angiostrongylus has been introduced in Kenya. If such an introduction were to occur, it is not inconceivable that ampullariid snails could play a role in transmission of infection to rats, and possibly to other mammals including man (see Yousif and Ibrahim, 1978 and Nishimura et al., 1986).

Biological control of pulmonate snails by ampullariids could theoretically be achieved in several ways. For example, ampullariids can consume the eggs, neonates, and even the adults of medically important pulmonate snails (Demian and Lutfy, 1966; Cedeno-Leon and Thomas, 1983). Alternatively, control might be achieved as a result of competitive interactions, either as resource or interference competition (Schoener, 1983; Madsen, 1986). Use of M. cornuarietis in biological control has been encouraged because it is able to exert both predatory and competitive effects on target species (Ferguson, 1977).

To date, little has been done to identify snails with such characteristics among the native ampullariid fauna of Africa. Preliminary studies from our laboratory indicate that P. ovata, L. carinatus, and L. ovum are capable of preying on egg masses and juveniles of pulmonate snails at rates comparable to those seen with M. cornuarietis. Regarding the potential competitive ability of African ampullariids, the results obtained from stomach content analysis indicate that significant dietary differences exist among species. For example, P. ovata consumes proportionately more macrophytes and L. carinatus more detritus. It should be noted that in the chosen method of dietary analysis, no attempt was made to quantify the percentage of a square on the grid that was occupied by a particular food type. A single-cell alga, for example, might fill only a fraction of a square and be scored in the same way as a piece of macrophyte that completely covers a square. Thus, the importance of some smaller food types may be over-estimated. Also, this method may underestimate the amount of animal material in the diet, particularly so for soft tissues which may not be recognizable among the stomach contents. Nonetheless, this method was applied consistently between species and provides an accurate estimate of the relative importance of the various classes of

dietary items.

The available data in the literature, although limited, generally support our observations. For example, Pila globosa is known to voraciously consume macrophytes in India (Thomas, 1975). In contrast, L. carinatus showed only a slight tendency to consume macrophytes taken from irrigation canals in the Sudan (Karoum, 1988). L. ovum, which we found consumed both macrophytes and detritus, has been shown to damage rice plants in Swaziland (Crossland, 1975).

The implications of the different dietary preferences of African ampullariids with respect to their possible use in biological control of pulmonates have yet to be clearly elucidated. Published reports (Dudgeon and Yipp, 1983; Thomas et al., 1985) as well as unpublished data from our laboratory indicate that both Biomphalaria and Bulinus consume relatively large quantities of detritus. L. carinatus might therefore be expected to function effectively in biological control as both an egg predator and a competitor for food in the form of detritus. However, studies of L. carinatus in Sudanese irrigation systems suggest its distribution in canals is positively associated with that of Biomphalaria pfeifferi (Madsen et al., 1988). Although such a distributional association does not necessarily imply that competitive interactions do not occur between these two species, it does suggest that L. carinatus is incapable of completely excluding B. pfeifferi from natural habitats. Karoum (1988) introduced L. carinatus into three canals in the Gezira Agricultural Scheme and, although populations of this snail did become established in each canal, their presence did not result in a clear reduction of schistosome-transmitting pulmonates. It should be noted, however, that L. carinatus did not attain high densities in these canals (Karoum, 1988), a problem that may plague all efforts to achieve control with ampullariids.

Alternatively, P. ovata may prove useful in control programs if it preys on pulmonate egg masses and unfavorably alters pulmonate habitats by lowering the biomass of macrophytes. The reported successes with M. cornuarietis, a known consumer of macrophytes, support this view. Similarly, the displacement of Biomphalaria glabrata by Ampullaria glauca in Grand Etang Lake in Guadeloupe was attributed to consumption by the ampullariid of floating macrophytes used by B. glabrata for food and shelter (Pointier et al., 1988). Perhaps two or more ampullariid species with different dietary preferences, that impinge in different ways on the target species, could ultimately be used in concert to achieve better control.

In conclusion, the Kenyan ampullariid specimens we examined did not harbor helminths of known medical or veterinary significance. The involvement of native ampullariids in Angiostrongylus transmission in Egypt, however, suggests that caution and vigilance are warranted should Pila or Lanistes be used in biological control programs in Africa. Our results also indicate that ampullariid food preferences vary between species, and we argue that such preferences will be important in assessing the biocontrol potential of different ampullariid species. Further trial studies of the efficacy of African ampullariids as control agents of pulmonate target species, and of their effects on aquatic food plants are warranted before their widespread use in biological control in Kenya can be condoned.

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Table 1. Listed for each ampullariid species are the collection localities, the number of juvenile snails, and adult male and female snails collected at each locality, and the number of snails in each category infected with larval trematodes. All infections indicated in this table refer to Cercaria B, with the exception of habitat #7, indicated by the asterisk, which refers to Cercaria A.

Locality Number	Number of Snails Collected	Number Infected / Number Collected			
		Juveniles	Males	Females	
		Pila ovata			
1	5	0/5	-	-	
2	11	-	0/7	0/4	
3	33	3/21	0/4	0/8	
4	3	-	0/2	0/1	
5	5	0/1	0/1	1/3	
6	25	0/1	0/9	1/15	
7	13	0/1	0/6	2/6*	
8	8	0/4	0/1	0/3	
9	2	-	0/1	0/1	
10	11	0/1	0/3	0/7	
11	11	-	0/3	0/6	
12	15	0/1	0/4	1/10	
13	6	0/3	0/1	0/2	
14	1	-	-	0/1	
15	1	-	-	0/1	
TOTALS	150	3/38	0/42	5/68	8/148 = 5.4%
		Lanistes carinatus			
13	15	-	0/6	0/9	
14	1	0/1	-	-	
15	1	-	0/1	-	
16	35	-	1/14	1/21	
17	10	-	0/4	0/6	
18	25	0/3	0/12	0/10	
19	33	-	1/12	3/21	
20	8	0/4	0/1	0/3	
21	6	0/2	0/2	0/2	
22	2	-	0/1	0/1	
TOTALS	136	0/10	2/53	4/73	6/136 = 4.4%
		Lanistes ovum ovum			
14	1	0/1	-	-	
21	2	0/2	-	-	
22	8	0/8	-	-	
23	1	0/1	-	-	
24	2	0/2	-	-	
25	15	0/13	0/1	0/1	
26	6	0/5	-	0/1	
27	12	0/7	0/1	0/4	
28	15	0/10	1/1	0/4	
TOTALS	62	0/49	1/3	0/10	1/62 = 1.6%

Table 1, continued

Locality Number	Number of Snails Collected	Number Infected / Number Collected			
		Juveniles	Males	Females	
		Lanistes ovum procerus			
13	24	0/1	0/10	0/13	
29	2	0/2	-	-	
30	8	1/1	0/2	1/5	
TOTALS	34	1/4	0/12	1/18	2/34 = 5.9%
		Lanistes ovum purpureus			
31	9	0/4	0/3	0/2	
32	8	0/5	0/1	1/2	
33	11	0/4	0/4	0/3	
TOTALS	28	0/13	0/8	1/7	1/28 = 3.6%
		Lanistes ciliatus			
26	1	-	-	0/1	
34	15	0/10	0/5	-	
TOTALS	16	0/10	0/5	0/1	0/16 = 0.0%

FIGURE LEGENDS

Figure 1. Cercaria A, a furcocercous, pharyngeate cercaria recovered from Pila ovata in habitat #7 in western Kenya. Scale bar = 200um

Figure 2. Cercaria B, an echinostome cercaria bearing 27 collar spines, recovered from ampullariids in both Kenya and Tanzania (see text). Scale bar = 100um

Figure 3. Daughter redia in which Cercaria B is produced. Scale bar = 75um

Figure 4. Metacercaria produced during encystment of Cercaria B. Scale bar = 80um

Figure 5. Cercaria C, an echinostome cercaria bearing 47 collar spines, recovered from Pila ovata from the Mwanza region of Tanzania. Scale bar = 70um

Figure 6. Metacercaria produced during encystment of Cercaria C. Scale bar = 125um

Figure 7. Daughter redia in which Cercaria C is produced. Scale bar = 675um

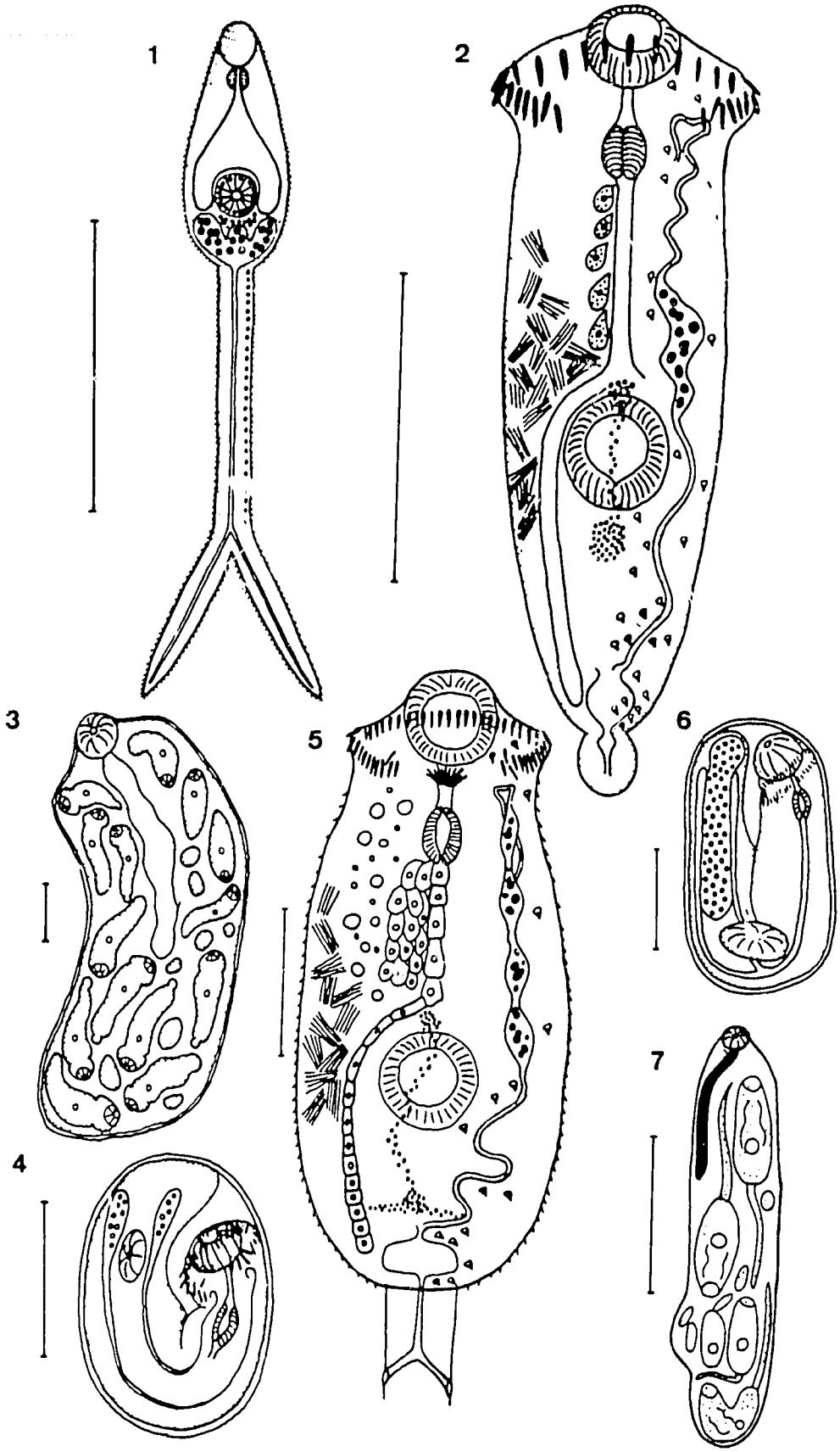
1
2
3
4
5
6
7

Figure 8. Indicated on this bar graph are the relative percentages (mean + 1 s.d.) of six categories of dietary items recovered from the stomachs of *Pila ovata*, *Lanistes carinatus*, and *L. ovum*. The sample size (pooled between collection localities) for each species is indicated on the graph. The dietary categories are inorganic material (inorg), macrophytes (macro), detritus (det), one-celled algae (one), filamentous algae (fil), and animal material (an). Significant differences between species were noted for both the macrophyte and detritus categories (see text for additional explanation).

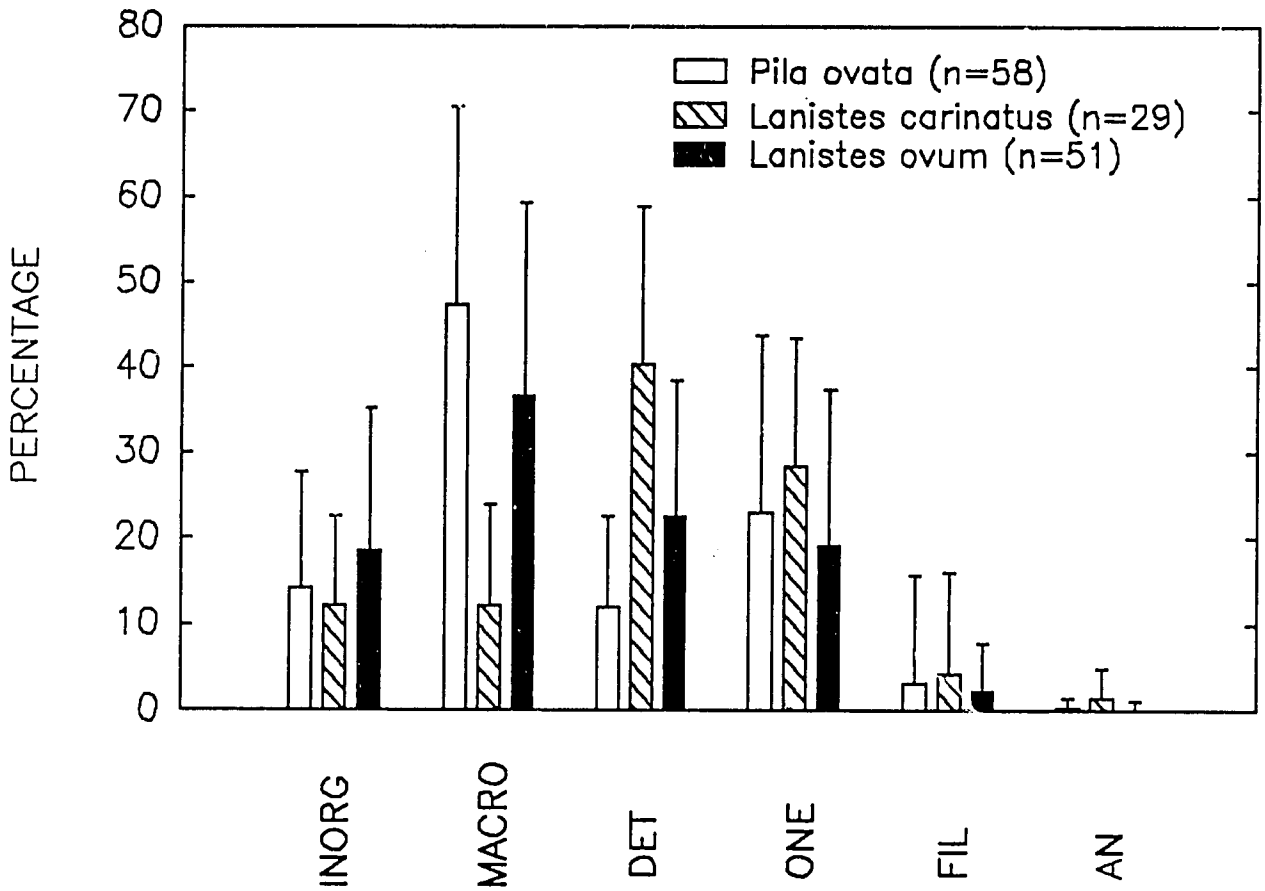
Pila ovata (n=58)
Lanistes carinatus (n=29)
Lanistes ovum (n=51)
PERCENTAGE
80
70
60
50
40
30
20
10
0
INORG
MACRO
DET
ONE
FIL
AN

Table 1: The ampullariid snails examined for this study originated from the collection localities listed below (see Table 2 for additional information). For each locality is listed the local name of the habitat, collection date, its longitude and latitude, and a brief description of the habitat. The freshwater gastropods found at each location are also indicated using the following abbreviations: BB = Bulinus (Bulinus) sp., Bc = Biomphalaria choanomphala, Bf = Bulinus forskalii, Bip = Biomphalaria pfeifferi, BP = Bulinus (Physopsis) sp., Bt = Bellamya trochlearis, Bu = Bellamya unicolor, Cg = Cleopatra guillemei, Cf = Cleopatra ferruginea, Cn = Ceratophallus natalensis, Fc = Ferrissia clessiniana, Gh = Gabbiella humerosa, Lca = Lanistes carinatus, Lci = Lanistes ciliatus, Ln = Lymnaea natalensis, Loo = Lanistes ovum ovum, Lopr = Lanistes ovum procerus, Lopu = Lanistes ovum purpureus, Mt = Melanoides tuberculata, Po = Pila ovata, Sk = Segmentorbis kanisaensis

#17: Mtawa Stream; 15 Jan 1987; 4° 20.7'S, 39° 29.5'E; shallow, slow-flowing stream; Bf, BP, Ln, Lopr, Mt.

#18: Muhaka Dam; 15 Jan 1987; 4° 17.8'S, 39° 30.2'E; man-made impoundment; BP, Ln, Lopu, Mt.

#25: Chem-Chem Swamp; 17 Jan 1987; 3° 12.3'S, 40° 23.0'E; large, natural swamp; Cf, Lca, Mt.

#32: Old Prison Farm; 20 Jan 1987; 0° 6.5'S, 34° 44.5'E; series of shallow irrigation ditches; Bip, Bf, BP, Cn, Ln, Po.

#37: Lake Jilori; 2 July 1987; 3° 11.4'S, 39° 53.2'E; large, natural swamp, nearly dry; Lca.

#38: Dzimbwini; 2 July 1987; 3° 9.1'S, 39° 50.9'E; man-made dam; BP, Cf, Gh, Lca, Mt.

#40: Mbaratum; 2 July 1987; 3° 11.0'S, 39° 45.4'E; large, natural swamp; Lca.

#41: Lake Gulamu Ng'onda; 2 July 1987; 3° 6.3'S, 39° 45.5'E; large, ox-bow lake; Cf, Lca, Lopr, Mt, Po.

#42: Rare River; 3 July 1987; 3° 31.1'S, 39° 45.9'E; pools in intermittent stream; Lci.

#43: Kachororoni Dam; 3 July 1987; 3° 29.0'S, 39° 42.7'E; small dam on intermittent stream; BP, Cf, Cn, Lopr.

#46: Mwachi River; 4 July 1987; 3° 57.9'S, 39° 31.4'E; pools in intermittent stream; BP, Bu, Cf, Fc, Loo.

#48: Kiziamondo Stream; 4 July 1987; 4° 7.9'S, 39° 19.8'E; pools in intermittent stream; BP, Cf, Lopu.

#49: Kinango Dam; 4 July 1987; 4° 7.9'S, 39° 18.8'E; man-made dam; BP, Bu, Loo.

#53: Kambe Stream; 5 July 1987; 4° 26.4'S, 39° 18.9'E; pools in intermittent stream; BP, Cf, Fc, Loo.

#55: Mwangwei; 5 July 1987; 4° 30.6'S, 39° 16.8'E; small, natural swamp; BP, Bu, Cf, Lopu.

#58: B1-A1 Pond; 12 July 1987; 0° 10.1'S, 34° 54.9'E; small, borrow pit pond; BB, Bf, Cn, Ln, Po, Sk.

#59: Kembija Dam; 13 July 1987; 0° 2.4'S, 34° 30.9'E; man-made dam; BB, Bf, Cn, Po, Sk.

#61: Nyamguu Stream; 13 July 1987; 0° 1.8'S, 34° 26.7'E; small stream; In, Po.

#62: Dago Stream; 13 July 1987; 0° 0.6'N, 34° 32.7'E; small stream; BP, Cn, Po.

#63: Otodo Stream; 13 July 1987; 0° 3.0'N, 34° 34.1'E; small stream; BP, Bf, In, Po.

#67: Maugo Stream; 14 July 1987; 0° 28.5'S, 34° 32.7'E; small stream; Bf, Cg, Cn, Po.

#68: Sota Stream; 14 July 1987; 0° 30.6'S, 34° 28.8'E; small stream; Po.

#69: Nyalkiny Dam; 14 July 1987; 0° 31.2'S, 34° 28.2'E; man-made dam; BP, Cn, Fc, Po.

#70: Rusinga Island; 14 July 1987; 0° 24.9'S, 34° 12.3'E; Lake Victoria; BB, Bc, Bt, Bu, Gh, In, Mt, Po.

#139: Asembo Bay; 20 June 1988; 0° 5.5'S, 34° 32.7'E; Lake Victoria; Bf, Bip, BP, In, Po.

#141: Asembo Stream; 20 June 1988; 0° 10.3'S, 34° 24.5'E; semi-permanent stream; Bf, BP, Bs, Cn, Po.

#142: Maugo Rice Field; 22 June 1988; 0° 36.5'S, 34° 29.4'E; rice field; Cg, Po.

#144: Bura Pond; 29 June 1988; 1° 15.8'S, 39° 58.9'E; man-made dam; BP, Cb, Lca, Loo, Po.

#145: Hola Canal; 30 June 1988; 1° 31.6'S, 40° 3.8'E; irrigation canal; BP, Cb, Lca, Po.

#146: Tana River; 1 July 1988; 0° 27.8'S, 39° 39.8'E; pools along river margin; Cb, Lca, Po.

#147: Borr-Algy; 1 July 1988; 0° 28.9'S, 39° 40.9'E; pools along river; BP, Cb, Lca, Loo.

#152: Lake Madina; 22 July 1988; 3° 12.0'S, 40° 5.5'E; small permanent lake; Cb, Lca, Loo.

#153: Marafa Pond; 23 July 1988; 3° 2.2'S, 39° 47.5'E; small ponds; Cb, Lci, Loo.

#155: Dida Pond II; 24 July 1988; 3° 25.6'S, 39° 48.0'E; man-made dam; Cb, BP, Lci, Loo.

#156: Vitengini Pond; 24 July 1988; 3° 21.3'S, 39° 40.9'E; large pond; BP, Cb, Loo.

Appendix 6

Loker

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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 ($D < 0.018$), but divergent from western populations ($D < 0.097$). Two of three western populations were quite similar ($D = 0.001$), the third being more divergent ($D < 0.258$). 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*.

D < 0.001 see p. 3

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 Stream (0°24.3'S, 34°9.8'E, intermittent stream); (12)

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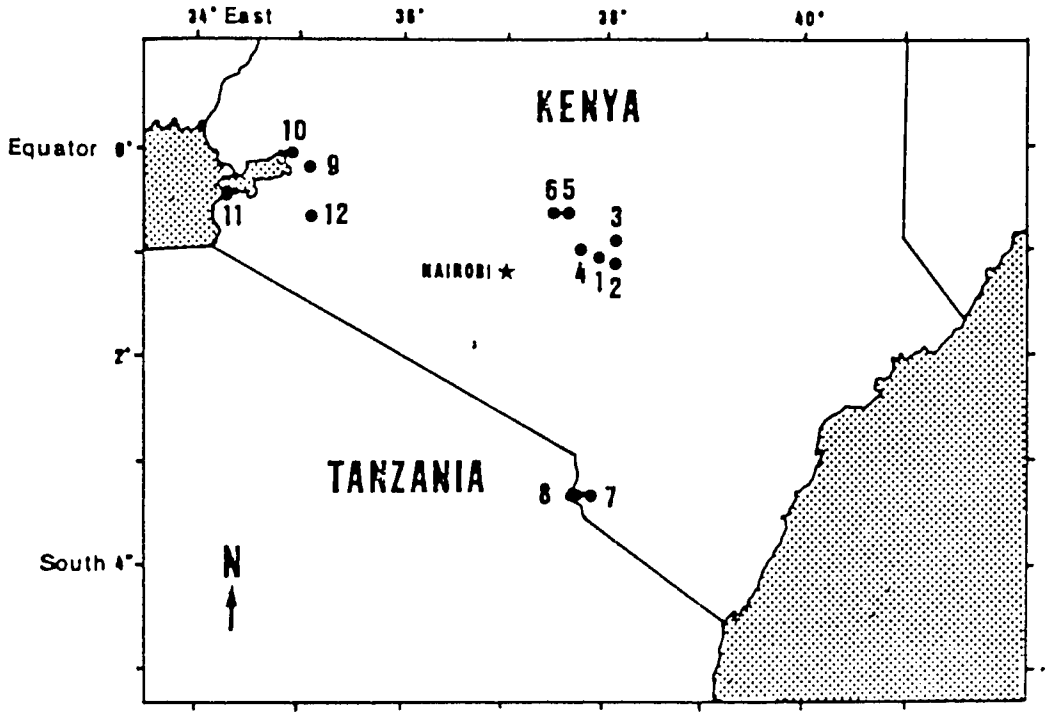

Fig. 1. Map of Kenya, showing collecting localities: (1) Matingani Seepage; (2) Muthamo Seepage; (3) Mbondoni Dam; (4) Kangonde Dam; (5) Mwea East; (6) Mwea West; (7) Grogan Canal; (8) Kwahoma Stream; (9) Asawo Stream; (10) Martin's Drain; (11) Kamayoga Stream; (12) Onsando Dam.

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, 3.4.11.11, 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 sensitivity of allozyme 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. 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 breeding coefficient will approach one.

inbreeding

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 breeding 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, and can be either positive, indicating a deficiency of heterozygotes, or negative, 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:

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

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 ($D < 0.10$).

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,

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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) Kangondo 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
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			
98/98	(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
42/432										0.87	0.85		0.08
	(N)	29	21	20	8	51	16	50	34	31	27	7	13
Est-2													
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.

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	AA	0.91	1.00	1.00		1.00	1.00	1.00	0.56	1.00	0.40	1.00	1.00
175/100	AB								0.33				
100/100	BB	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 (10.48)		
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)		

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Table 3. Fixation Indices.

Population	Mean Heterozygosity Observed (Expected)	Fixation
Matingani Seepage	0.01 (0.02)	0.26
Muthamo Seepage	0.01 (0.07)	0.85
Mbandoni Dam	0.00 (0.01)	1.00
Kangonda 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

Kangonde

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	X^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 Kangonda	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	0.015	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	0.081	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

Kangonde

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 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, *Nm*, 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, *Nm* equals 0.176, which is equivalent to one migrant every 5 to 6 generations. This estimation 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 the 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 specie 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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