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Research Article

Polyploidy in Bulinid Snails, with Emphasis on *Bulinus truncatus/tropicus* Complex (Planorbidae: Pulmonate Mollusks) from Various Localities in Ethiopia

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Freshwater snails of the genus *Bulinus*, mostly *Bulinus truncatus/tropicus* complex occur in Ethiopia in various ploidy levels (diploid, tetraploid, hexaploid and octoploid) which are otherwise rare in other animal phyla. This study provides data on the occurrence and distribution of various ploidy levels of *B. truncatus/tropicus* complex in Ethiopia based on meiotic bivalent chromosome counts. Emphasis was made on the role of shell morphology and chromosome number in species identification of *B.truncatus /tropicus* group. Specimens were collected from fourteen different localities in Ethiopia. Chromosome preparation was made from gonad tissue (ovotestis). The result showed that diploid species are mainly associated with low altitude, tetraploid with both low and medium altitudes whereas hexaploids and octoploids with high altitudes. Unexpected result was found in Lake Hora, where both diploid and tetraploid populations were occurring together in the same microhabitat which is in contrary to suggestions of some authors that two cytotypes of *Bulinus truncatus/ tropicus* complex do not occur in the same water body in Ethiopia. It was recommended that thorough investigation of each water body where snails are occurring, should be undertaken using cytological and molecular approaches and that the possibility of hexaploid *Bulinus*snails as potential host of human schistosome parasites in the highland of Ethiopia.

Key words: bivalent chromosome number, *Bulinus truncatus/tropicus* complex, gonad tissue (ovotestis), ploidy level.

INTRODUCTION

Many freshwater snails transmit schistosomiasis and other parasitic worm diseases of human and animals. The human schistosomes transmitted by snails include *Schistosoma mansoni* (intestinal schistosomiasis), *S. haematobium* (urinary schistosomiasis), *S. japonicum* (hepatosplenic schistosomiasis), *S. intercalatum* (rectal schistosomiasis), *S.mekongi* (hepatic schistosomiasis) (Baswaid, 2002). As far as human and other animal trematodiasis is concerned, bulinid, biomphlarid and lymnaeid snails are the most important intermediate hosts. In Ethiopia, all these groups have representative intermediate hosts and are widely distributed (Birrie *et al*., 1995). The genus *Bulinus*has a spired, sinistral shell like that in the Physidae and thus most bulinid species were originally referred to Physa (Wright, 1971). According to Brown (1980), the genus *Bulinus* is divided into four species groups/ complexes.

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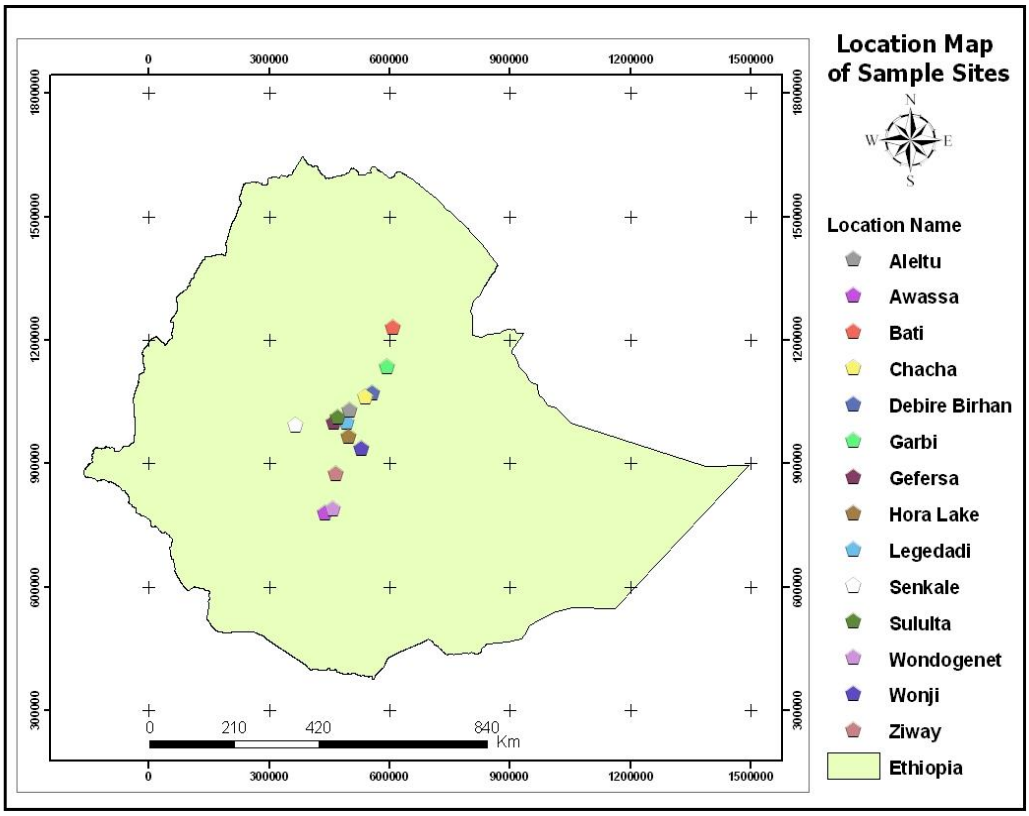

Fig 1. Map of Ethiopia showing geographical distribution of the studied samples of bulinid snails in fourteen water bodies.

These are *Bulinus africanus* group (2n=36), *Bulinus forskali* group (2n=36), *Bulinus reticulatus* group (2n=36), *Bulinus truncatus/tropicus* group (2n=36, 72, 108 and144). Intensive investigation of the *Bulinus truncatus/ tropicus* complex in East Africa was stimulated by the discovery of extensive polyploidy in Ethiopian highlands beside their importance as intermediate hosts for various trematode parasites (Brown, 1980).The *B.truncatus/tropicus* complex assumes special importance in Ethiopia because *B. africanus* group is poorly represented. In contrast, members of *B. truncatus/tropicus* complex are abundant on the highland plateau, where most of the human population lives (Brown and Wright, 1972).

The countrywide snail survey made in Ethiopia by Birrie *et al.* (1995) showed that *Bulinus truncatus/tropicus* complex and other snails of medical and veterinary importance have a wide geographical distribution. Besides supporting some *Schistosma* or *Bilharzia* (blood flukes) parasites in human, bulinid snails are burdened also by the immature stages of numerous other trematod parasites of man and other mammals such as *Fasciola* causing fascioliasis, lung flukes (*Paragonimus*) causing paragonimiasis, *Paramphistosomum* causing paramphistsomiasis, *Gastrodiscus* (intestinal flukes) causing gastrodisciasis, and also nematode parasite, *Angiostronglus* causing angiostrongloidiasis (Brown ,1980). Bulinid snails have experimentally been proved to be susceptible to these parasites and there is a need for correct identification of these snails and mapping of their distribution to enable prediction of the risk of trematodiasis introduction to areas free of the disease. For this purpose, Birrie *et al*. (1995) have recommended the use of chromosome study in the future. Therefore, the present cytological study has been conducted. Chromosome numbers have been reported since 1960 for species of genus *Bulinus* snails. Among bulinids, *B. truncatus/tropicus* group has been interesting because the complex comprises polyploidy series 2n=36, 72, 108 and 144. The two higher polyploids(hexaploid and octoploid) are known only from Ethiopia where their occurrence is associated with high altitude (Brown, 1980). Outside Ethiopia, octoploidy has reported also for a single population in southwest Arabia (Burch, 1964). This study was planned to determine ploidy levels existing among the *Bulinus* species in Ethiopia and their geographical distribution of different ploidy levels among *Bulinus* population, and assess the role of cytogenetics in taxonomy of these snails.

MATERIALS AND METHODS

Snails were collected from 14 localities (nine unstudied sites) in Ethiopia where bulinid snails were known to occur on the basis of previous studies and some additional sites. Snails were collected from upper Awash River in Wonji irrigation system, Lake Ziway, Lake Awasa and Wondogenet areas as representatives of low land populations. Other collection localities include Lake Hora (DebreZeyt), Senkale (Ambo region), stream flowing into Gefersa and Lagadadi reservoirs and Sululta streamnear Addis Ababa), Bati (Wollo), Gerbi, Debre Berhan, Chacha and Aleltu as highland representatives. Collections were made between February and June 2007. The collection sites are shown in Fig-1.

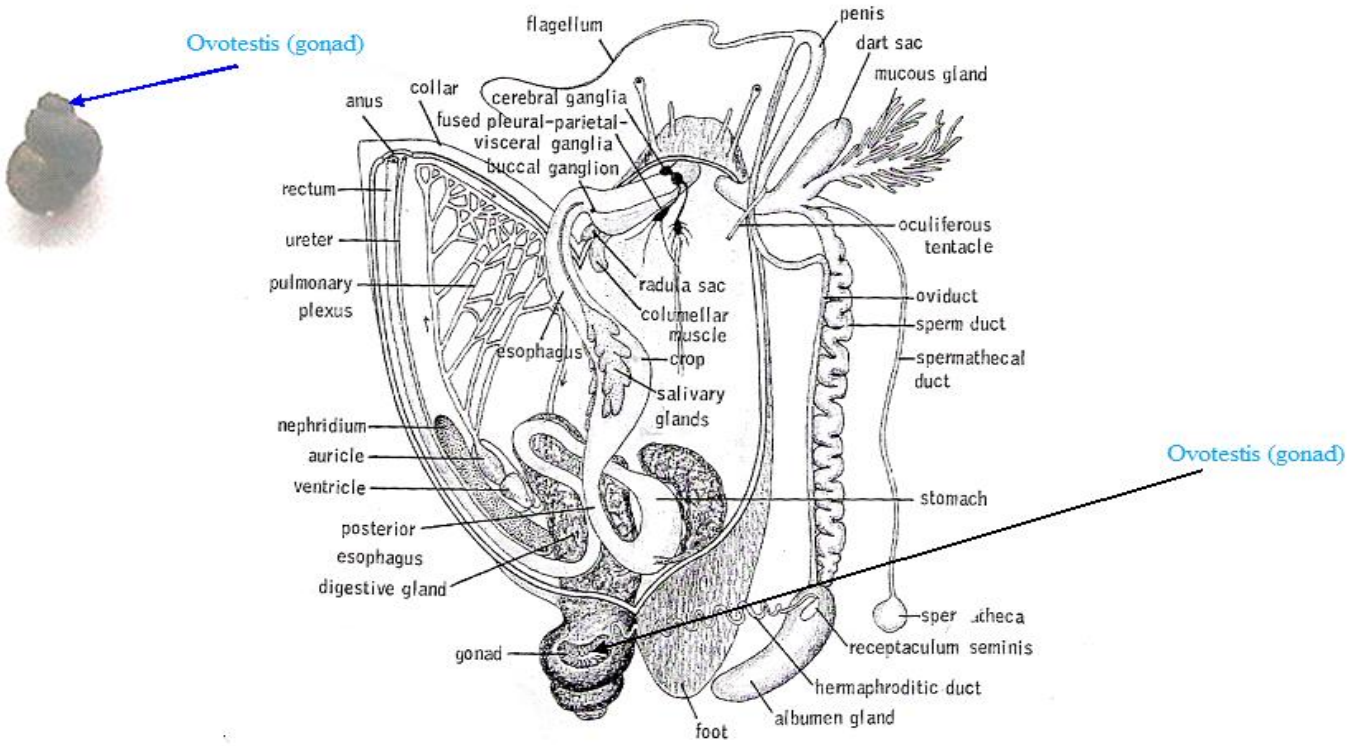

Fig 2. ***Location of ovotestis (gonad) tissue ofB. tropicuswithin shell anddissected pulmonate snails (adapted from Pechnick, 1996)***

Specimens collections were made by hand using gloves. Plastic dishes were used as aquaria for maintaining snails in lab in a well aerated place at room temperature. Dechlorinated water was used in the aquaria. Pipe water was dechlorinated by standing the water for at least two days. Snails were maintained on fresh lettuce leaves (Birrie *et al*., 1995). For each population a minimum of 20 specimens were investigated as a representative sample for each site and two slides were prepared for each of the specimen and slides with well spread bivalent chromosomes were made permanent. For future reference, soft parts were removed from shells, and then shells were preserved by drying.

Meiotic stages can be readily observed in slides prepared from reproductive gland (ovotestis) tissue which is located on the inner side of the first of several whorls of the body (Fig.2). The ovotestes can be distinguished by its white, gray or pale yellow color in contrast to the surrounding brown color of the digestive glands (Burch and Patterson, 1965).

Meiotic Chromosome Preparation from Gonad (ovotestis)

The following is a modification of various techniques reported by several workers (Burch, 1960; Choundhury and Mohapatrea, 1981; Goldman *et al*.,1983a; Yaseen *et al*., 1995).

1. Live animals were sacrificed, the shell was broken and ovotestis masses were taken out, homogenized in hypotonic solution (0.075M KCL) at room temperature for about 30 minutes.
2. The cell suspension was centrifuged at 1000 rpm for about 5 minutes and the supernatant was removed using Pasteur pipette.
3. To the centrifugant (pellet) about 1ml of freshly prepared fixative (1:3 glacial acetic acid: methanol, v/v) was added and after 10-15 minutes of fixation the pellet was resuspended in the fixative and recentrifuged.
4. To achieve better fixation, step three (3) above was repeated two or more times.
5. After the last centrifugation and removal of the supernatant, the pellet was resuspended in a very small volume of fresh fixative (producing the right concentration of cell suspension for slide preparation).
6. Slides were prepared, by splashing a few drops of the cell suspension on a glass slide from a height of about half a meter. Several slides were prepared in this way for each of the specimens studied from all localities.
7. Slides were allowed to air dry at room temperature and stored until needed for staining. The slides were stained with 3% Giemsa (diluted in phosphate buffer, PH 6.8) for about 30 minutes or more and rinsed thoroughly in distilled water , air dried and made permanent using appropriate mounting media such as DEPEX.
8. Photomicrographs of well spread meiotic bivalent chromosomes were taken and analyzed for chromosome number and ploidy levels.

Table 1. Collection sites of *Bulinus truncatus/tropicus* complex used for the present study

S.No	Site	Location	Elevation(m)	snails with slides preparedand observed	2n chromosomes observed	Ploidy level	Species
1	Lake Awasa	7^0 03'37.66"N 38^{0}28'38.04"E	1700	20$^+$/2/13*/9**	2n=36	diploid	*B. natalensis*
2	Lake Ziway	7^0 55'42.40"N 38^{0}42'35.52"E	1650	20$^+$/2/10*/6**	2n=36	diploid	*B. natalensis*
3	Wonji	8^0 27'01.86"N 39^{0}16'50.82"E	1580	20$^+$/2/12*/10**	2n=36	diploid	*B. tropicus*
4	Wondogenet♦	7^0 7'32.96"N 38^{0}38'04.08"E	2179	20$^+$/2/19*/19**	2n=36	diploid	*B. tropicus*
5	Lake Hora ♦ (Debre Zeit)	8^0 27'01.86"N 38^{0}59'23.06"E	1910	20$^+$/2/12*/6**	2n=36 2n=72	Diploid and tetraploid	*B. natalensis* *B.truncatus*
6	Gerbi river♦	10^0 6'34.64"N 39^{0}52'41.54"E	1500	20$^+$/2/15*/4**	2n=72	tetraploid	*B. truncatus*
7	Aleltu river ♦	9^{0}18˙58.14¨N 39^{0}01˙63.05¨E	2700	20$^+$/2/12*/3**	2n=108	hexaploid	*B.hexaploidus*
8	Chacha river♦	9^0 32^{1}08.39"N 39^{0}21'29.98"E	2818	20$^+$/2/25*/3**	2n=108	hexaploid	*B.hexaploidus*
9	Lega Dadi river♦	9^0 04'07.01"N 38^{0}57'50:43"E	2458	20$^+$/2/12*/3**	2n=108	hexaploid	*B.hexaploidus*
10	Senkale stream♦	8^0 59'26.29"N 37^{0}48'06.23"E	1938	20$^+$/2/25*/2**	2n=108	hexaploid	*B.hexaploidus*
11	Bati reservior♦	11^0 08'45.45"N 40^{0}01'15.5"E	1540	20$^+$/2/18*/5**	2n=144	octoploid	*B. octoploidus*
12	Debreberhan river	9^0 39'50.98"N 39^{0}31'55.21"E	2915	20$^+$/2/10*/2**	2n=144	octoploid	*B. octoploidus*
13	Gafarsa strem♦	9^0 03'55.75"N 38^{0}40'03.62"E	2580	20$^+$/2/15*/3**	2n=144	octoploid	*B. octoploidus*
14	Sululta stream	9^0 12'13.00"N 38^{0}46'11.56"E	2670	20$^+$/2/12*/11**	2n=144	octoploid	*B. octoploidus*

Key: $^+$indicates the number of samples investigated per site; 2-number of slides prepared per specimen; * number of slides observed; ** number of slides in which chromosome were obtained; n-haploid chromosome number; ♦ New report.

RESULT

Results of chromosome counts for samples of various bulinid snails collected from 14 localities are given in Table 1. All snails in fourteen water bodies belong to *B. truncatus /tropicus* complex. From a total of 14 localities from which *Bulinus truncatus/tropicus* complex have been investigated, different cytological forms were obtained having different ploidy levels as 2n=36 ; 2n=72; 2n=108 ; 2n=144. These are diploids, tetraploids, hexaploids and octoploids respectively. Diploids and tetraploids were mainly obtained from low and medium altitudes whereas hexaploids and octoploids were found at high altitudes, except for one population collected from Bati at an altitude of around 1500m, which belongs to lowland area elevation.

Shell Morphology

Shell morphology of snails studied shows two distinct shells of diploids, these are the low spired shells obtained from lakes Awasa, Ziway and Hora (Fig.3 A, B, and C respectively) whereas the high spired shells found in snails from Wondogenet and Wonji irrigation system (Fig 3. DandE respectively). The same shell type was obtained for tetraploids. Tetraploid shells, which appear to be moderately spired, were obtained from Lake Hora and Gerbi River (Fig 3.FandG respectively). One shell type was obtained for hexaploid populations. The hexaploid shells, which were found to be high spired, were obtained from Chacha, Aleltu, Legadadi and Senkele (Fig 3. H, I, J, and K respectively). There are two distinct shell morphologies of octoploid populations. These are the low spired shell (Fig. 3M) obtained for snail populations from Debreberhan whereas the relatively high spired shells were obtained for snail populations collected from Bati reservoir, Gefersa and Sululta streams (Fig. 3 L, N and O respectively).

Chromosome number

Diploid chromosome number of the genus *Bulinus* were found to be 18II (2n=36) (Fig 4A).Chromosome number was obtained from snail populations of Lake Hora, Lake Awasa, Wonji irrigation system, Lake Ziway and Wondogenet. Only diploid chromosome numbers were observed in snails from Awasa, Ziway, Wonji and Wondogenet. However, in addition to diploids, tetraploid chromosome numbers were obtained in snails from Lake Hora. Tetraploid chromosome number, 36II (2n=72) (Fig 4B), was observed for the specimens of snails collected from Gerbi river and Lake Hora . Only tetraploid chromosome numbers were observed in all the examined specimens of Gerbi. However, snails with tetraploid and diploid chromosome numbers were found in specimens from Lake Hora with the tetraploid forms being less frequent in number. Hexaploid chromosome number, 54II

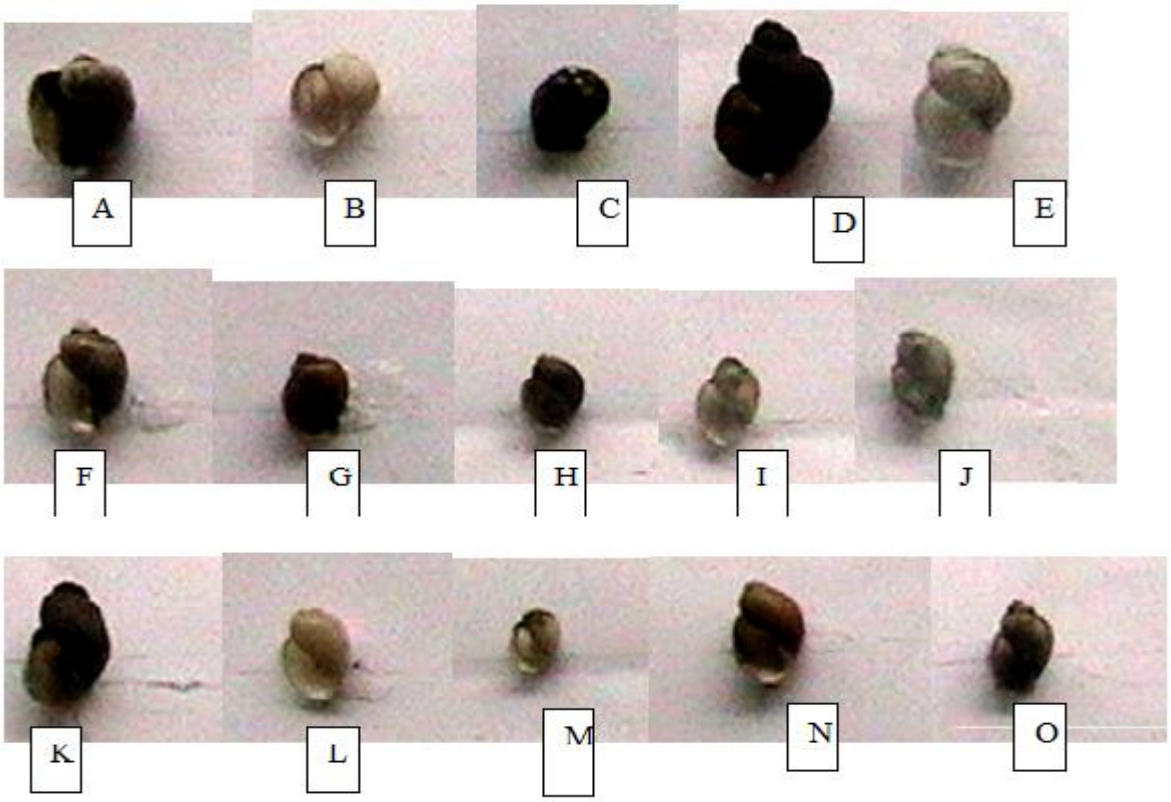

Fig 3. Representative shell types of snails collected from 14 different water bodies in Ethiopia: A.Awasa; B. Ziway; C. Hora(diploid);D. Wondogenet; E. Wonji; F. Gerbi; G. Hora (tetraploid); H. Chacha,; I. Aleltu; J. Lega Dadi; K. Senkale; L. Bati; M. Debreberhan; N. Gefersa; O. Sululta.

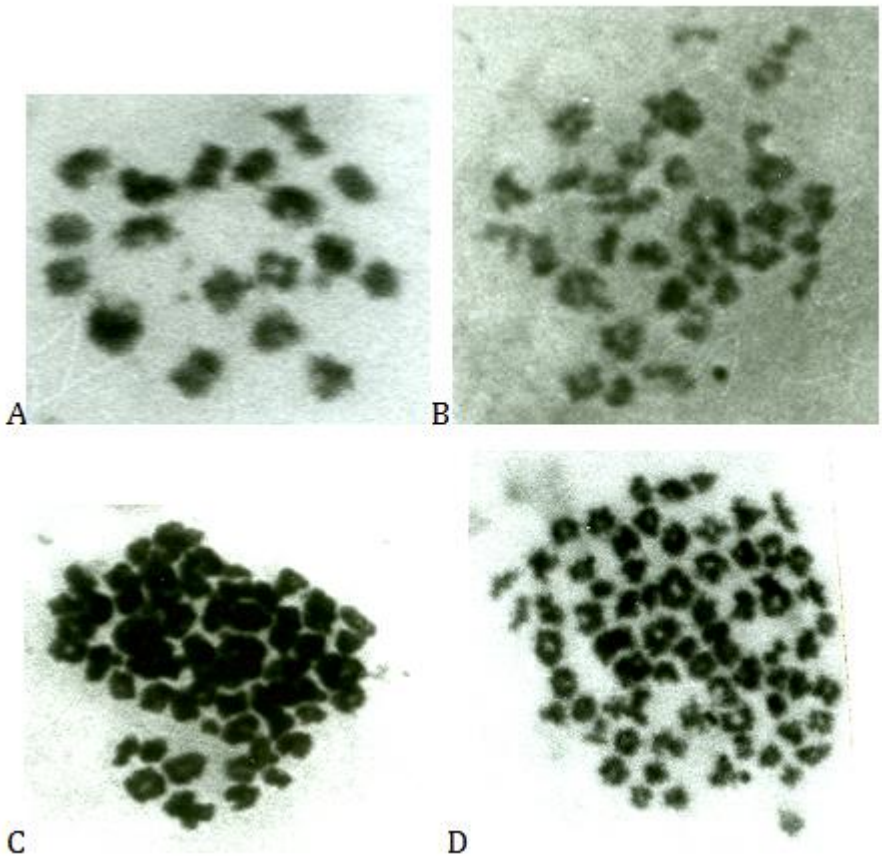

Fig 4. Bivalent chromosomes of snail studied; A. diploids, B.tetraploids, C. hexaploids, D. octoploids

or 2n=108 (Fig 4C), was observed in specimens of snail populations collected from Senkale, Aleltu, Lega Dadi and Chacha. No ploidy levels other than hexaploid chromosome numbers were observed in specimens from these sites. Octoploid chromosome number 72II or 2n=144 (Fig 4D) was observed for snail populations collected from Sululta River, Debreberhan, Gefersa and Bati. All of the snail specimens from these sites were found to be octoploid.

Systematic Description

The taxonomy of the mollusks intermediate hosts of schistosomes based on classical morphological features (conchological and anatomical), serological or biochemical comparison of proteins by means of electrophoretic and immunological techniques, cytological characteristics and genetic methods (Baswaid, 2002).The morphology of mollusks include the shell, tegument, operculum, radula, the genital system, paleal or mantle cavity, respiratory, digestive, circulatory, nervous and reproductive systems (Brown, 1980; Baswaid, 2002).The variation in shape of the shell (number of spirals, whorls, dimensions of the aperture, height and last whorls height) helps to identify different mollusk species (Baswaid, 2002). Our species identification in the present study, was based on shell morphology and determination of chromosome numbers. The classification and nomenclature was based on Brown (1980). Accordingly, diploid snails with highly spired shell classified as *B. tropicus* following Brown *et al*. (1967) and Brown (1980) while diploid snails with depressed or low spired shell were identified as *B. natalensis* following Brown and Wright (1972). In agreement with Brown and Burch (1967) and Brown and Wright (1972) Ethiopian teraploid populations with moderately spired shell and shouldered whorls were identified as *B. truncatus*. Hexaploid populations with highly spired shell were identified as *B. hexaploidus* whereas octoploid populations with both relatively high spired and low sppired shells were classified as *B. octoploidus*.

DISCUSSION

The purpose of this study is to provide recent chromosome data and distribution of Ethiopian *Bulinus truncatus/tropicus* from sites reported previously by different workers (Brown and Wright, 1972; Brown, 1964; Brown and Burch, 1967; Itagaki *et al*., 1975; Yasuraoka, 1973; Kloos and Lemma, 1974; Brown, 1980; Birrie *et al*., 1995) and some other new localities like Wondogenet, Lake Hora, Aleltu, Chacha, Lega Dadi, Senkele, Bati and Gefersa. Cytotaxonomic studies of Planorbid snails were strongly stimulated by the discovery of polyploid series (n = 18, 36,54 and 72) in bulinids from the Ethiopian highlands region (Brown and Burch, 1967).

Classification based on shell morphology may not be appropriate for identification of *Bulinus truncatus/tropicus* complex. Cytogenetic study has been used to resolve classification problems of such animals (Brown, 1980). In the present study also snails collected from different altitudes and having different ploidy levels appear to be similar in their shell morphology (Fig-3). As suggested byBrown, Shaw and Rollinson (1991), *B. tropicus* is highly variable in shell form, euphallic, with the radular mesocone or generally non-angular shape. In this study, also it was found that *B. tropicus* has varying high spired shell. *B. natalensis* has depressed shell spire, aphallic individuals are present in some populations and has predominantly angular mesocone (Mukaratirwa*et al*., 1998) In relation to this finding, in the present study, diploid *B. natalensis* were found to have low spired shell. In the present study four ploidy levels but only two types shell morphologies were observed showing that variation in ploidy levels have no relationship with variation in shell morphology. Since both types of shell morphologies were found in diploids and octoploids but only one type of shell morphology was found in tetraploids such variation may give some clue concerning their evolutionary relationship as previous biochemical studies (Jelnes, 1979b; Brown, 1980) who showed *B. hexaploidus* closely related to *B. tropicus* whereas *B. octoploidus* closely related to both diploid and tetraploid snails.

In the present study shell morphology of tetraploid snails is relatively moderately spired with shouldered whorls which is in line with the previous studies (Brown, 1980; Brown, Shawand Rollinson,1991). According to our observation, it appears that shouldered whorl may also be found in *B. tropicus*and *B. hexaploidus* which seem relatively highly spired. Shell morphology of hexaploid, which is highly spired, appears similar to that of *B. tropicus* and the highly spired octoploid populations. The low spired shell morphology of octoploid snails appears similar to low spired shell of *B. natalensis* whereas the high spired shell morphology of octoploid populations appears identical to that of *B. tropicus.*This finding might be related to the variation in shell morphology as suggested by Brown (1980). Brown and Wright (1972) suggested that variation in shell sculpture and size of the first lateral radular tooth showed no relation with chromosome number but some correlation is seen in other morphological characters such as male copulatory organ. Morphological and other information of Ethiopian polyploid snails are still lacking; we hope future studies should generate more information on these snails as they are known (especially hexaploids and octoploids) only from Ethiopia.

In line with previous studies (Burch, 1964, 1967; Brown and Burch, 1967) the present study also reported diploid population in lakes Awasa and Ziway. Brown and Wright (1972) reported tetraploid population in Wonji irrigation

system; however, in the present study snail population collected from this area was found to be diploid. The difference between this and previous studies may be due to difference in site of sample collection. Though we don't know the specific site of their collection as our sample was collected from a locality called "Sebategna Camp". The difference may also be accounted for by disappearance of teraploids from Wonji area or displacement of tetraploids by diploids. There was no previous cytological report for bulinid snails found in Wondogenet area; it is the first report for this area.

According to Lo and Lemma (1975) both diploid and tetraploid snails occur in Lakes Ziway and Awasa but the two forms appear to have distinct habitats. The tetraploid form in those lakes was occurring in stagnant marshes and differed in appearance from diploid forms which lived on open shores of the lakes. Tetraploid forms reported in the northeastern shore of Lake Awasa at the entrance of Tikurwuha River and in the northwestern shore of Lake Ziway. In the present study samples were taken from open shores of Lakes Awasa and Ziway, and dipliod chromosome numbers were observed which corresponds to habitats reported for diploids by Lo and Lemma (1975). This result agrees with the previous studies (Brownand Burch, 1967; Lo andLemma, 1975).

Both diploid (Brown and Burch, 1967) and tetraploid (Brown, 1964) populations were reported in Lake Bishoftu (Lake Beite Mengist) near Debre Zeit. Brown and Burch (1967) suggested that no more than one cytological form was observed in any samples of snails from a particular locality in Ethiopia; however, it was reported that cytologically different forms occur together in small water bodies in Tanzania. In contrary to the suggestion of Brown and Burch (1967) we have found both diploid and tetraploid populations occurring in the same microhabitat in Lake Hora.

According to some authors(Brown,1964; Brown and Burch,1969), hexaploid populations are rare and most likely restricted and unique to highland of Ethiopia. However, in this study we have obtained hexaploid populations as frequently as octoploid populations. Previous studies (Brown, 1964; Brown and Wright, 1972) reported both diploid and tetraploid population in streams (unspecified) near Addis Ababa; however, in the present study diploids were not found in streams studied from around Addis Ababa, but only it is found that hexaploid and octoploid populations were found near Addis Ababa. Hexaploid populations were found in Lega Dadi and Aleltu whereas octoploid populations in Sululta and Gefersa streams. Also, we didn't find tetraploids in those localities. Snail population at Senkale stream (near Ambo town) found to be hexaploid, supporting the report of Brown (1980) that hexaploid populations were present in Guder River, near Ambo.

Diploid, tetraploid and octoploid populations have been reported at more or less similar altitudes near Dessie town (Brown, 1980). In agreement with this finding, in the present study, snail population collected from Bati was found to be octoploid. Specimens of snails collected from Gerbi stream, the climate of which is more or less similar to the neighborhood of Dessie, were found to be tetraploid. On the other hands, diploids were not observed in the localities from where we have taken samples in the neighborhood of Dessie. There may be possibility of diploids occurring in this region but it may need more sites to be sampled. Previous studies (Brown and Burch, 1967; Brown and Wright, 1972; Wu, 1972) reported *B. hexaploidus* in Sululta River. In contrary to this, we obtained only *B. octoploidus* from Sululta. Brown and Burch (1967) reported both hexaploid and octoploid populations between Shano and Debreberhan but specific localities were not indicated. In agreement with this finding, the present study also got hexaploid population in Chacha, a locality between Shano and Debreberhan, but specimens collected from Debreberhan River were found to be octoploid.

According to the result, diploid and polyploidy forms of the snails have different altitudinal distribution patterns (Table 1). Hexaploids and octoploids are mainly found at high altitudes whereas diploids and tetraploids were obtained from sites located at low altitudes mainly in the rift valley. There is also a possibility of occurrence for other ploidy levels (diploids and tetraploid) at high altitudes as Brown (1964) suggested that altitudinal zonation does not completely segregate octoploid and hexaploid populations from those with lower chromosome numbers. For instance diploid, hexaploid and octoploid populations have been found at similar altitudes near Shano (Brown and Burch, 1967). However, in the present study, snails collected from high altitude regions were found to be hexaploids and octoploids except for one population of octoploid in Bati reservior. Likewise, diploids were largely obtained for lowland populations except that in Lake Hora, situated at a medium altitude contains both diploid and tetraploid forms. Tetraploids were obtained from low altitude, Gerbi River, and medium altitude in Lake Hora.

Burch and Huber (1966) studied meiotic metaphase cells of several polyploid species of *Bulinus* and in no case reported the occurrence of multivalents. They interpreted their results as suggestive of an allopolyploid origin for the higher polyploids of *Bulinus*. The lack of multivalent formation, however, cannot be considered incontrovertible evidence against autopolyploidy, since multivalent formation can be genetically suppressed (White, 1978 cited in Goldman *et al*., 1983a) and is rare in small

chromosomes which have only one chiasma (Goldman *et al.,* 1983a). In the present study, it was also observable that chromosomes were associated as to be bivalent which may indicate the occurrence of allopolyploidy or hybrid origin than autopolyploidy or chromosome doubling of same genome.

In previous studies (Burch, 1960; Burch, 1964; Brown and Burch, 1967) chromosome number determination from meiotic bivalents of the ovotestis employed squashing technique using acetoorcein staining and splashing technique after colchicine treatment followed by hypotonic solution. The drawbacks associated with squashing technique used in the previous studies (Burch, 1960; Brown and Burch, 1967) are the difficulty in making the slides permanent and difficulty in getting well spread meiotic prophase chromosomes. In the present study, we used hypotonic treatment of cells before fixation in order to spread the bivalents within the cells and thus minimize chromosome overlapping. The technique provides an easy way to determine chromosome number. Well spread meiotic prophase bivalent chromosomes were obtained by this method particularly the diploid forms. Owing to the large chromosome number, the method has only minimized the chromosome overlapping, but could not be completely avoided.

It seems that chromosomes study can best be done during the active reproductive period of snails, that is, when laboratory culture of snails laying many eggs on the floater that is placed on the surface of the water for snails to lay eggs on. In this study we have got better results when snails were in the stage of egg laying since meiotic cells are active during this time. No chromosomes were observed on slides prepared from young snails. During collection of samples, it was observed that in areas where only bulinid populations were found there were very large numbers of individuals whereas in areas where different populations of snails (*Bulinus, Lymna, Biomphlaria*) were occurring together there were smaller number of bulinid snails. This may be related to factors such as competition brought about by difference in reproductive rate, resistance to environmental conditions and parasitic burden. Nevertheless, future studies are required to confirm this observation.

In agreement with the previous studies (Brown and Burch, 1967; Brown, 1980; Brown *et al*., 1991) in the present study, it was also observed that bulinid snails are found on the surface of aquatic plants mostly on leaves, under or on the surface of rocks submerged in water, or on the surface of mud at the bottom of the water. They are common in disturbed water where there are contact activities by man and cattle, in small stream that has been diverted from main rivers for irrigation or for some other purpose. In lakes, they are easily found at the shore where there are aquatic plants. They are less common in running waters and large streams and rivers, and also less common in rivers that are flooded during rain seasons and in lakes where there are no aquatic plants. It was observed that *B. natalensis* collected from lakes Awasa and Ziway were less adapted to laboratory to room temperature in Addis Ababa, which may be related to cold temperature as they are adapted to warm climate. Such observation is in line with Brown and Wright (1972) who suggested that *B. natalensis* appears to be less tolerant of cool climate than *B. tropicus*.

In most previous studies (Burch and Brown, 1967; Brown and Wright, 1972; Berrie *et al.,* 1995) the name of local water bodies and localities were not specified. The present study overcomes such problem. We have tried to use specified names. Sampling technique, collecting season, cytological techniques, and developmental stage of snails that may affect the result of the study. One has to take into account such constraints. Collecting season should be varied as breeding of snails is greatly affected by seasonal changes. Since the distribution of snails is largely affected by water current, running water and flooding as a result snail can be found in new areas. Frequent and updated cytological data are important to control snails that are intermediate hosts of schistosome parasites.

CONCLUSION AND RECOMMENDATIONS

The genus *Bulinus* has basic chromosomal complement of 18 (n=x=18) (Brown and Burch, 1967). In the present study snails collected from fourteen water bodies in Ethiopia were found to have the basic chromosome number of 18, but exist in four ploidy levels: diploid, tetraploid, hexaploid and octoploid. All snails collected in the present study were belonging to *B.truncatus/tropicus* complex. Based on shell morphology and chromosome number determination, bulinid snails in the *B.truncatus/tropicus* complex were classified into different species. These are *B. tropicus, B. natalensis, B. truncatus, B.hexaploidus, B. octoploidus*. Meiotic bivalent chromosomes were studied from the ovotestis using splashing technique after swelling the cell with a hypotonic solution, which is better than squashing method, as it gives well spread chromosomes. The present study developed reviewed and easier technique for chromosome number determination. Despite variation in ploidy levels, bulinid snails studied show similarity in their shell morphology. Generally, two shell morphologies (low spired and highly spired were found in this study irrespective of ploidy levels. Snails collected from localities belonging to different altitudes show that diploids and tetraploids were obtained from low and medium altitudes whereas hexaploids and octoploids were obtained from high altitude areas. The present study provides chromosome data for workers interested in studying

epidemiology, parasitology of schistosome parasites, evolution and ecology of parasites and host relationship in Ethiopian water bodies, taxonomy and evolution of animals. The present study has given insight into problems associated with cytotaxonomy and distribution of Ethiopian *B. truncatus/tropicus* complex.

The present study has not done exhaustive investigation on a particular water body. Samples were taken from one or a few sites due to resource constraints. Future studies should be conducted tolerable investigation of each water body. The present study also covers only a small number of water bodies in Ethiopia. Future studies should include other Ethiopian water bodies and generate cytological, biochemical and molecular data.

B. hexaploidus and B. octoploidus are known only from Ethiopian highlands except for one *B.octoploidus* population in south Arabia (Brown, 1980). Little is known also about susceptibility of *B. hexaploidus* to schistosome parasites. Future studies are indicated to investigate the susceptibility of this species complex, especially *B. hexaploidus* to schistosome parasites. The occurrence and evolution of higher polyploidy forms of the genus *Bulinus* only in Ethiopian highlands should better be studied by molecular techniques.

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