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DIVERSITY AND COMPARATIVE STUDIES ON BULINUS SNAILS COLLECTED FROM TWO LOCALITIES IN EGYPT

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Abstract

A comparative study of the shell structure, seasonal temperature and Calcium content of *Bulinus* snails from two areas (Damietta and Giza) in Egypt was done and compared with laboratory snails from Schistosome Biological Supply Center (SBSC). The shells of collected snails identified as *Bulinus truncatus*, showed a wide variation in shape. The results showed a significant differences were detected between the populations from SBSC and Damietta ($p<0.05$) for mean of measured shell width, aperture length, length of spire and number of whorls. The populations from Giza and Damietta governorates showed significant differences ($P<0.05$) in mean of measured length of diagonal, length of body whorl above aperture, length of spire and number of whorls. There were no statically significant differences between the populations from SBSC and Giza. The seasonal temperature affected on susceptibility of snails to infection with *Schistosoma haematobium*. The mean prepatent period was short in summer and long in winter. The shells of *S. haematobium* – infected *B. truncatus* snails showed hypocalcification from all localities.

Key words: *Bulinus truncatus, Schistosoma haematobium*, shell morphology, seasonal temperature, susceptibility, hypocalcification

Introduction

Planorbid freshwater snails that belong to the genus *Bulinus* act as the intermediate hosts for *Schistosoma haematobium* and related species. There are currently 37 species of *Bulinus* recognized (Brown, 1994), but the specificity of the snail parasite interaction is such that only certain species are involved in transmission of the parasite. Zein-Eddine *et al*. (2014) recorded the distribution of *Bulinus truncatus* along River Nile and its branches.

Bulinus species possesses a sinistral shell and a pseudobranch (Yaseen, 1993). The *Bulinus* snail's shell shape varies widely. Variation occurs in the height of the spire, the form of the columellar margin and the size of the umbilicus. Mollusca shell is capable of modification in response to subtle differences in the environment (Bertness, 1980). On the basis of characteristics and morphometric analysis of shell (Brown *et al.,* 1971a,b; Mimpfoundi and Ndassa*,* 2006) identified the population of *B. tropicus or B. natalensis* collected from crater lakes in the western mountainous volcanic region. Morphometric analysis showed that the ratio Length/Aperture length, with a low spire ($L/AL<1.45$) and angular mesocones being associated with *B. natalensis* and populations with a high spire ($L/AL>1.50$) and non-angular mesocones to *B. tropicus* (Brown *et al,* 1971a, b).

The seasonal pattern is mainly governed by climatic factors, especially rain and temperature. The seasonal temperature variations are particularly important in bilharziasis-endemic areas with a subtropical climate, e.g. Egypt, Iran, South Africa (Pflüger *et al*, 1984). Water temperature is an important determinant of the limits of snail distribution and population size because egg production, hatching, maturation and death rates of juvenile and adult were all affected by the temperature (El-Hassan, 1974). Ittiprasert and Knight (2012) suggested that susceptibility of snails to infection is temperature-sensitive. The rate of cercarial maturation inside infected snails is also affected by temperature, increasing as temperature in-

creases (Pflüger, 1980). Yousif *et al*. (1993) showed that longevity of infected snails, length of prepatent period of the parasite and duration of cercarial shedding are inversely proportional to the prevailing seasonal water temperature.

Calcium is a metal that exerts an essential role on the biology of the snails, once that, this ion is the main component of the snails shell, constituting an important limiting factor that determine the distribution and survival of the adult snails, oviposition rate, survival and development of eggs and the embryos (Davies and Erasmus, 1984). Freshwater snails obtain calcium from both the food and water (Mazuran *et al*, 1999). Mishkin and Jokinen (1986) reported that environmental calcium has positively effects on the fecundity and cercarial production of *Biomophlaria glabrata* infected with *S. mansoni*. The disturbance occurred in the metallic ion concentrations in the snails infected with trematode larva was considered as one of the causes of alterations occurred in the biological activities and the increase in the mortality of infected snails (Mahmoud *et al*, 2002; Mostafa, 2008; Mostafa and Bin Dajem, 2010; Mostafa *et al*, 2013).

The aim of this study was to identify *Bulinus* snails collected from two localities from Egypt based on morphological characters, relation between seasonal temperature and susceptibility (infection rate) of collected snails; in addition to determine the calcium content in the uninfected and infected shells and soft parts with trematode parasite.

Materials and Methods

The snails were collected by the method recommended by Mandahl-Barth (1962). Populations of *Bulinus* snails were collected from two Egyptian governorates (Giza and Damietta) and the 3rd group was obtained from the Schistosome Biological Supply Center-Theodor Bilharz Research Institute (SBSC-TBRI) as a reference control.

Morphometric of *Bulinus* snail shell: Prior to measurements, the snail shells were cleaned in 10% oxalic acid using a tooth-brush to remove ferruginous deposits and foreign matter. All damaged shells, usually those with an incomplete apex, were removed from the samples. Measurements were made to the nearest 0.1 mm using vernier calipers according to the methods described by Kristensen *et al*. (1987). Variation was assessed for 9 characters: length (L); width (W); width of aperture (WA); aperture length (AL); width of shell at the level of the last suture (WS); length of diagonal (WD); length of body whorl above aperture (LH); length of spire (LS) (difference between length of shell and aperture) and number of whorls (NW). Spire length (L/AL), shell shape (L/W) and aperture shape (AL/AW) were calculated according to James (1968) in order to eliminate discrepancies resulting from size differences between individual samples. The recommended terms used by James (1968) and Gregoire (1972) to describe the latter three shell parameters are necessary to species.

Snail exposure and infection: The snails from each group were reared in the laboratory and their first generations (F1) were used throughout the infection experiment. Three replicates, each of 50 lab-bred sails (4-6mm in shell length), from each Governorate offspring were exposed individually to 10 newly hatched *S. haematobium* miracidia (SBSC-TBRI). After exposure, groups of the snails were maintained communally in plastic tray (20 x 30 cm) containing 1.5 liters of aerated tap water and supplied with lettuce leaves, blue green algae. The water temperature was recorded weekly.

Starting from the day 21 post miracidial exposure, the snails were examined individually and repeatedly for cercarial shedding in multi dishes containing 2 ml of dechlorinated tape water/ snail under artificial light for two hours (stimulated period). After initial shedding was observed, snails were screened individually once weekly till the death of snails. Infection rate, prepatent period, duration of cercarial shedding and cercarial production per snail per week were

observed. The classification of snail's susceptibility to infection was dependent on the infection rate of the snail according to the method of Saoud (1965) in which snails were considered refractory below 10%, low susceptible 10-25 %, moderate susceptible 25-50% and high susceptible at infection rate over 50%.

Calcium content of *Bulinus* snails: Snails shedding cercariae and clean, non-exposed snails used as control (nearly of the same age and size of the shedding ones) from SBSC, Damietta and Giza groups were dissected in deionized water to separate soft parts from shells. Shells and soft parts were rinsed, at least, three times with deionized water. Excess water was removed from the shells and soft parts by using filter papers. Shells and soft tissues were grounded with mortar and pestle, pooled to achieve a weight of 500 mg for each. Three pools of shells and soft parts from snails shedding cercariae and clean, non-exposed snails were prepared for analysis. Wet-weighted samples were digested in 10 ml of concentrated nitric acid by boiling to dryness. The residue from each digested sample was diluted to 25 ml with deionized water in a volumetric flask. Elemental analysis by flame atomic absorption spectrometry was performed to determine the calcium concentration. The flame wavelength and sample aspiration rate were optimized according to the manufacturer's recommendations, and four aqueous standards having analytic concentrations within the linear response range of the instrument and containing the same concentration of nitric acid as the samples were used for calibration. Each sample, standard and blank, was analyzed using three 10-s integrations. The reagent blank was prepared, and its value was subtracted to give the final concentration. The final calcium concentration (C) was calculated according to the following equation:

$$C = \frac{F \times V}{WT \times 1000}$$

Where F is the standard factor calculated from the standard curve, V is the volume of sample and WT is the wet weight of sample. Data are expressed in micrograms of calcium per gram of wet tissue or shell.

Results

The examination of the shell revealed that all specimens collected from Damietta, Giza governorates, in addition to those collected from Schistosome Biological Supply Center (SBSC) as control one, identified as *Bulinus truncatus*. The shell is sinistral and narrow shaped, varies in thickness from fragile, thin to rather thick. It is highly variable in shape from elongated ovate to conical or subconical. The whorls are spirally coiled and somewhat flattened. The whorls are generally convex and rounded at the periphery and are separated by a deep suture. The body whorl has horizontal or slightly concave border and developed shoulder. The spire is clearly shorter than the aperture and the apex varies from obtuse to rather elevated. The sculpture is more conspicuous on the body whorl than on earlier whorls. It includes thick transverse irregular ribs, furrows and rather coarse growth lines. The umbilicus is visible and varies from small to rather big. The aperture varies from elongate ovate to ovoid and almost round. It is limited by an outer convex lip and inner sharp columellar margin which leaves a small umbilicus between it and the surface of shell. The shell is without an operculum (Fig.1).

Comparison between linear measurements (Tab. 1) showed that the parameters of shells (Fig.2) from the studied sits presented variations. The significant differences were detected between the populations from SBSC and Damietta ($p<0.05$) for mean of measured shell width (W), aperture length (AL), length of spire (LS) and number of whorls (NW). The populations from Giza and Damietta governorates showed significant differences ($P<0.05$) in mean of measured length of diagonal (WD), length of body whorl above aperture (LH), length of spire (LS) and number of whorls (NW). There were no

statically significant differences between the populations from SBSC and Giza in any measured character (Tab. 1).

Table 1: *B. truncatus* from Egypt, measurements of mean shell continuous characters.

Shell characters	SBSC	Damietta	Giza
L	7.2 ± 2.28[a]	8.09± 2.32 [a]	7.95± 2.04 [a]
W	4.66 ± 1.33 [a]	5.69± 1.44[b]	5.47 ± 1.29[a,b]
WA	3.25 ± 1.14[a]	3.38 ± 1.13[a]	3.75 ± 1.03 [a]
AL	4.24 ± 1.48 [a]	5.87 ± 2.11[b]	4.92± 1.46[a,b]
WS	1.65 ±0.56 [a]	1.42 ± 0.56[a]	1.7± 0.50 [a]
WD	3.27 ± 0.93 [a,b]	2.92± 0.48[b]	3.66 ± 0.88[a]
LH	1.97 ± 0.63 [a,b]	1.73 ± 0.37[b]	2.32 ± 0.67 [a]
LS	2.96 ± 0.91 [a]	2.22± 0.38 [b]	3.03 ± 0.87 [a]
NW	3.25 ±0.55 [a]	2.8 ± 0.29 [b]	3.15± 0.32 [a]

Data expressed as mean ± SD, number of snails for each tested group 21.
Means in same row, followed by different litters significantly different ($p<0.05$).

Table 2: Calcium content (M±SD) in micrograms per gram of wet shells and soft parts of clean, nonexposed, and cercariae-shedding *B. truncatus* snail.

Source	Snails	Shell of *B. truncatus*	Soft parts of *B. truncatus*
SBSC	Clean, nonexposed	1003.8±199.1	70.6± 5.9
	Shedding	489.72± 90.59*	110± 9.07**
Damietta	Clean, nonexposed	1050±132.28	72.33±3.77
	Shedding	695.08±98.57*	85.8±10.1
Giza	Clean, nonexposed	1066.7± 144.89	74.33±5.61
	Shedding	726.83±150.41*	80.33±9.71

The shells showed a wide variation in shape which depend on certain characteristics namely: shell color, shape of the body whorl and certain conchological measurements. The morph from Giza, the shell varies in color from yellow to brownish. The body whorl is slightly rounded and moderate shoulder. The spire is medium, the mean L/AL ratio was 1.63 mm and the aperture is wide, the mean AL/AW ratio was 1.32 mm. Second form from Damietta, the shell varies in color from yellow to brownish. The body whorl is well rounded and shoulder is well developed. The spire is short and the calculated mean L/AL ratio was 1.42 mm. The aperture is very narrow and the calculated mean AL/WL ratio was 1.75 mm. SBSC population form has yellow shell and the body whorl is slightly rounded and slightly shoulder. The spire is tall, the calculated mean L/AL ratio was 1.72 mm and the aperture is wide, the calculated mean AL/AW ratio was 1.33 mm. This high correlation indicated that the calculated ration can be used with high reliability in predicting the length of different parts of the shell of *B. truncatus* from the shell length.

The offspring collected from SBSC, Damietta and Giza exposed to *S. haematobium* miracidia in summer and winter seasons and classification of snail's susceptibility according to infection rates. In summer (weekly mean temperature 25°C-29°C), SBSC snails showed moderate susceptibility with infection rate, survival rate & mean number of cercariae/snail/week (42.1%, 76%, 110± 33.16; respectively). Damietta snails exhibited moderate susceptibility characters by infection rate, survival rate and mean number of cercariae/snail/week (39.4%, 66%, 47.5±20.2; respectively). But, Giza snails showed low susceptibility with low infection rate, survival rate and mean number of cercariae/snail/week (17.85 %, 56%, 29.66± 13.65; respectively). The duration of cercarial shedding was nearly similar in SBSC and Damietta snails (3.3±1.0 & 3.11±1.05) respectively but decreased in G (2.57±0.53). In winter season (weekly mean temperature 16°C-21°C), the infection rate of SBSC

snails was lower than summer season, and these snails exhibited moderate susceptibility with infection rate, survival rate and mean number of cercariae/snail/week (31%, 58%, 88.75±30.1; respectively). The susceptibility of Damietta snails to infection with *S. haematobium* was decreased significantly ($p<0.05$) in winter in compared to summer. Snails were characterized by infection rate, survival rate and mean number of cercariae /snail/week (22.85%, 70% & 41.66±22.86; respectively). Giza governorate showed low susceptibility with low infection rate, survival rate and mean number of cercariae/snail /week (14.2%, 42 % & 20±7.54; respectively). The duration of SBSC cercarial shedding snails (2.63±0.8) followed by Damietta (2.44±0.72) but decreased in Giza (2.28± 0.48). Prepatent period was nearly similar for all groups. The infection rate and mean number of cercariae/snail/week were higher in summer in compared to winter (Figs.3 A & B). The mean prepatent period was short in summer season and long in winter season.

Calcium content in the shells of clean, non-exposed *B. truncatus* was significant higher if compared with the calcium content in the shells of cercariae-shedding snails. In contrast, the calcium content in the soft parts of cercariae shedding snails was higher than in the soft part of clean, non-exposed snails, the difference was statistically significant ($p<0.01$) in SBSC snails. Generally, calcium content was significantly higher in the shells than the soft parts of the snails, regardless were infected or non-infected (Tab. 2).

Discussion

Bulinus truncatus is one of the most common members of the freshwater snail fauna in Egypt, found most frequently along the margins and banks of small ponds with slow running or almost stagnate irrigation canals (Abd El-Wakeil *et al*, 2013). The present study showed that the populations of *B. truncatus* from Giza, Damietta and SBSC could be differentiated from morphometric characters. Conchological features proved to be useful in differentiating two populations of *B. truncatus* in Turkey (Sesen, 2004) and in Sudan (El Sheikh *et al*, 2010). Rollinson *et al*. (1998) managed to differentiate on morphologic basis *B. africanus* group from Lake Victoria. Presence of overlap in morphs among populations belonging to *B. truncatus*/*tropicus* complex from South Western Zimbabwe (Mukaratirwa *et al*, 1998) and among the *B. africanus* group from East Africa (Stothard *et al*, 1997).

The present results showed that the three populations are morphologically different, but *Bulinus* snails from Giza seems closely related to *Bulinus* snails from SBSC and both differ significantly from population sampled in Damietta. Yaseen (1993) found that a genetic difference in the chromosome numbers and karyotype of *B. truncatus* in Upper Egypt, this correlated to the morphological differentiation. According to shell attributes, the aothurs already came to the conclusion that *Bulinus* snails from Giza seemed to be closely related to *B. truncatus* in Upper Egypt (Yaseen, 1993) and Sudan (El Sheikh *et al*, 2010), but differences were found between populations in Sub-Saharan Africa (Nijokou *et al*, 2004; Zein-Eddine *et al*, 2014). The absence of migration from one site to another might favor the populations to evolve independently (Brown, 1994).

The dimensions recorded in the Damietta samples were close to those reported by Brown (1994) on the high-spired forms (9.5×6.5mm), but height of the spire is short (L/AL=1.42) in comparison with the variation shown by *B. truncatus*/*tropicus* complex in south-eastern Africa (L/AL>1.45) (Brown *et al,*1994). The mean value L/AL of shells from Giza and SBSC was similar to mean values calculated by Mukaratirawa *et al.* (1998) on the samples from South Western Zimbabwe.

Temperature considered an important role and a key factor in determining schistosome transmission potential (Martens *et al*, 1997; Mangal *et al*, 2008; Allana *et al*, 2013; McCreesh and Mark Booth, 2013). The susceptibility of snails and cerarial output are

important feature in the transmission patterns of schistosomiasis. These parameters are considerably affected by the prevailing temperature (Yousif *et al*, 1993). The temperature can act directly on disease by altering the susceptibility of hosts, the virulence of pathogens and the growth rates of both hosts and pathogens, which can in turn influence host pathology and disease emergence (Paull and Johnson, 2011).

The present results showed that the susceptibility (infection rate) and the mean number of cercariae/ snail/ week were higher in summer in compared to winter. In summer, SBSC and Damietta snails showed moderate susceptibility while Giza showed low susceptibility. In winter, SBSC snails showed moderate susceptibility while Damietta and Giza snails showed low susceptibility. This may be due to the snails exposed to high temperature had weaker immune defense, which potentially predisposes them to infections (Foster, 1964; Pflüger *et al,* 1984; Seppälä and Jokela, 2010; Aboelhadid *et al*, 2016) while at low temperature, cercarial development was slow or suspended, and the probability of cercariae maturing before the snails death (Pflüger, 1980; Kabatereine, *et al*, 2004).

The mean prepatent period was short in summer season and long in winter season. This result agreed with Yousif *et al*. (1993), who mentioned that the length of prepatent period of the parasite is inversely proportional to the prevailing seasonal water temperature. Mukaratirwa *et al*. (1996) explained the relation between prepatent period and parasite prevalence. Long prepatent periods are likely to generate low levels of prevalence, while short ones may lead to the majority of snails shed cercariae.

The calcium ion is one of the most important elements present in the shell of molluscs, mainly the gastropods. The calcium ion participates in many enzymatic reactions and is required to metabolic processes related to acid-basic equilibrium in the hemolymph (De With and Sminia, 1980). Moreover, large amount of calcium are used in reproduction of the snails (Mazuran *et al*, 1999). Also, reduction in calcium concentration in water can reduce occurrence of snails in aquatic system (Young and Harris, 1974). In the present observation, calcium content was significantly higher in the shell than in the soft part of the snails, regardless infected or non-infected. This observation was correlated with that of White *et al*. (2005), who mentioned that under conditions of variable concentrations in the water and trematode parasitism, pulmonate snails are able to maintain a high concentration of $CaCO_3$ in their shells.

The shells of *S. haematobium*-infected *B. truncatus* snails showed hypocalcification in the present study was agreed with Mostafa (2008) observed the hypocalcification in the *Lymnea. natalensis* infected with *Fasciola gigantica*. In the present study hypocalcification observed in the shells of *S. haematobium* infected *B. truncatus* snails may be due to that the snails used in this experiment were actively shedding cercariae snails. These cercariae utilized large amount of calcium, which may be compensated by calcium from the shell as suggested by Mostafa (2007). Davies (1983) revealed that the cercariae of *S. mansoni* sequester large amount of calcium in their pre-acetabular glands and such sequestration probably occurs at expense of calcium in the shell and hemolymph of the snail. In contrast the hypercalcification noted in the shells of *E. liei*-infected *B. alexandrina* snails may be due to the *E. liei* cercariae within the snails' utilized small amount of calcium (Mostafa *et al*, 2013). Davies and Erasmus (1984) reported that *B. glabrata* containing larval stages of *S. mansoni* at 40 days post-infection showed disintegration of the calcium corpuscles in Type-A calcium cells and erosion of the inner surface of the shell.

In the present study, the soft parts of uninfected snails had decidedly less calcium than did those infected. This may be due to the presence of sporocysts containing cercariae

and free cercariae that contained a large amount of calcium (Davies and Erasmus, 1984) in the tissues of infected snails. This result agreed with the observation of Layman *et al*. (1996). They reported that Ca, Cu, Fe, Na & Zn were present in the digestive gland–gonad complex at higher concentration in infected as compared with uninfected snails. Furthermore, Ong *et al*. (2004) found significantly higher amount of Ca, Cd, Mn & Na in soft parts of *B. glabrata* infected with *S. mansoni*. These results were in contrast with Evans *et al*. (2001) and Bergey *et al.* (2002) in which infection decreased the amounts of certain elements in infected hosts.

Conclusion

The morphometric analysis proved to be a useful tool to distinguish between populations. The seasonal temperature and calcium content in the water effect on schistosomiasis transmission. The current work has concentrated on specific regions of Egypt and could add a significantly to the knowledge of *B. truncatus* which serves as the main intermediate host of *S. haematobium* in Egypt.

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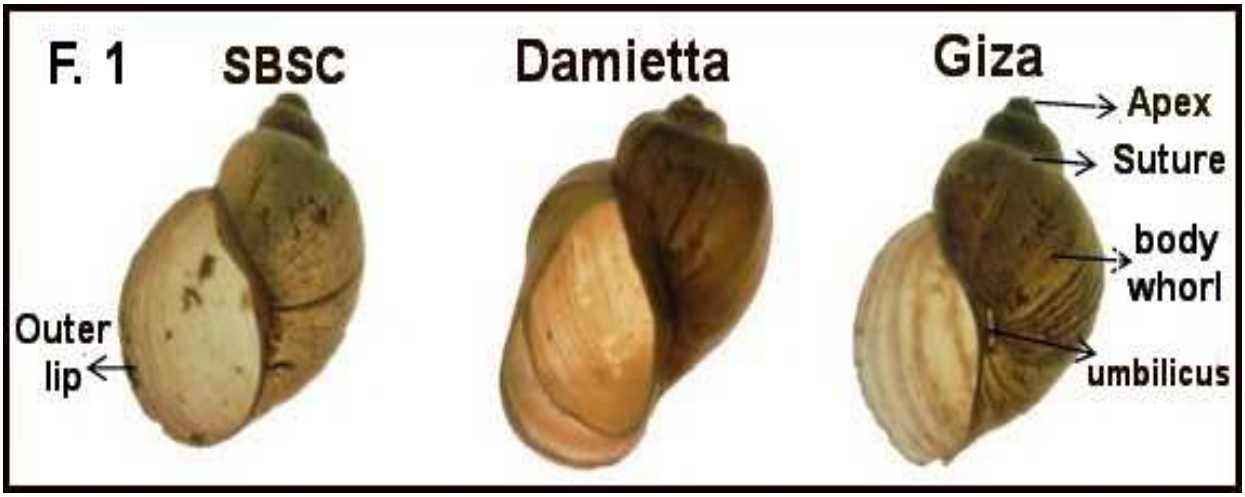
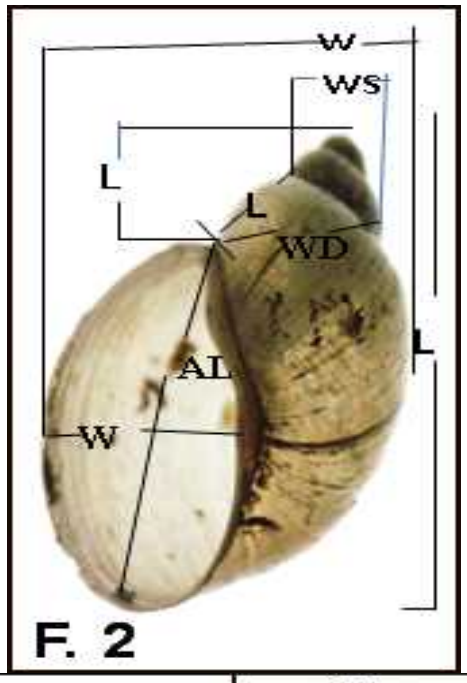
Explanation of figures

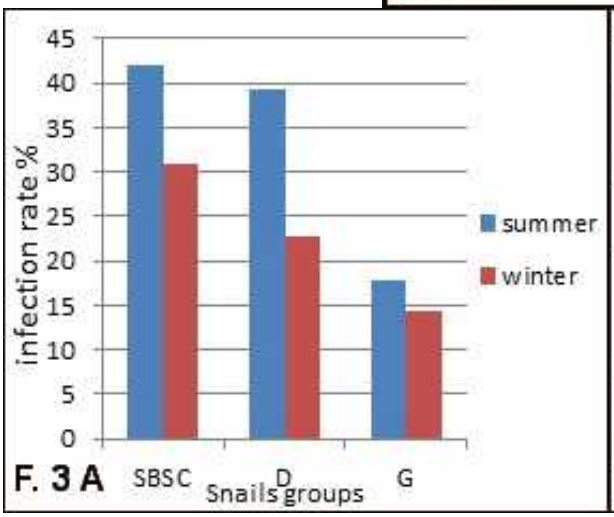
Fig. 1: Shells of *B. truncatus* from three different populations, each randomly chosen to show variation from population to population.

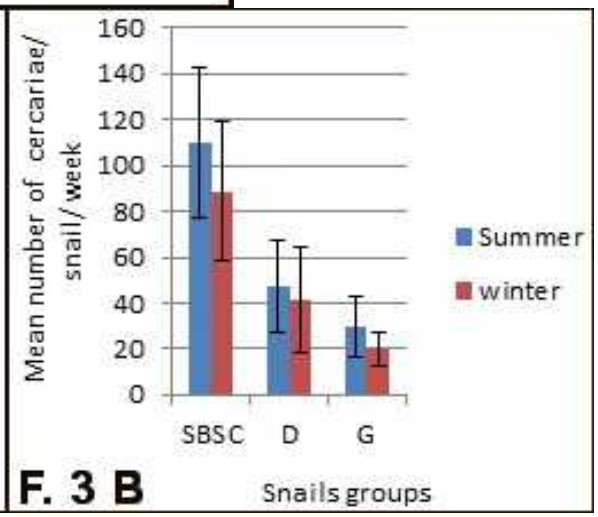
Fig. 2: Linear measurements of shell of *B. truncatus* used in discriminant analysis. Shell length (L); shell width (W); width of aperture (WA); aperture length (AL); width of shell at level of last suture (WS); length of diagonal (WD); length of body whorl above aperture (LH); length of spire (LS) (difference between length of shell and aperture) and number of whorls (NW).

Fig. 3: Seasonal variation of infection rate percent (A) and mean number of cercaria/snail/week (B) of snail *B. truncatus* collected from investigated sites of *B. truncatus* from Egypt.


F. 1
SBSC
Damietta
Giza
Apex
Suture
body whorl
umbilicus
Outer lip


W
WS
L
L
WD
L
AL
W
F. 2


45
40
35
30
25
20
15
10
5
0
infection rate %
summer
winter
SBSC
D
G
Snails groups
F. 3 A


160
140
120
100
80
60
40
20
0
Mean number of cercariae/ snail/ week
Summer
winter
SBSC
D
G
Snails groups
F. 3 B