Distribution and habitats of *Bulinus depressus* and possible role as intermediate host of economically important helminth parasites in South Africa

KN de Kock* and CT Wolmarans

School of Environmental Sciences and Development, Zoology, Potchefstroom Campus of the North-West University,
Private Bag X6001, Potchefstroom 2520, South Africa

Abstract

This article documents the large-scale spatial distribution and ecological descriptors of associated habitats of *Bulinus depressus* by analysis of samples taken from 552 collection sites on record in the database of the National Freshwater Snail Collection (NFSC) at the Potchefstroom Campus of the North-West University. This snail species is experimentally susceptible to *Schistosoma margrebowiei*, a helminth parasite of game animals and cattle and can possibly also exploit humans as definitive hosts. The 125 different loci (½ degree squares) on record reflect a geographical distribution that is largely limited to the central and western part of the Limpopo Province and westwards down the basins of the Vaal and Orange Rivers. Details of each habitat as described by collectors during surveys, as well as altitude and mean annual air temperature and rainfall for each locality, were processed and chi-square and effect size values calculated. A decision tree constructed from all the available data indicated that temperature and altitude, followed by the type of water-body, seemed to be the more important factors that significantly influenced the distribution of this species in South Africa. The possible role of this species as intermediate host of economically important helminth species is briefly looked at and the urgent need to update the geographical distribution of host snails is emphasised. It is recommended that efforts be made to determine the exact role of *B. depressus* in the epidemiology of economically important helminth parasites.

Keywords: Bulinus depressus, geographical distribution, habitat preferences, Schistosoma margrebowiei

Introduction

Bulinus depressus belongs to the family Planorbidae that has a wide geological distribution and is the most heterogeneous group of snails in the Basommatophora (Demian, 1960). Originally described by Haas (1936) from Lake Bangweulu, Zambia, this author drew attention only to those conchological characteristics that to him seemed distinctive of the new species. These features unfortunately subsequently proved to be insufficient to characterise it and resulted in a disagreement between malacologists as to what B. depressus actually was (Hamilton-Attwell and Van Eeden, 1969). Mandahl-Barth (1968) regarded B. depressus as a subspecies of Bulinus tropicus after examining similar snails from Zambia with non-angular mesocones on the radula. However, in the course of large-scale freshwater snail surveys conducted in South Africa since 1956 many samples identified as B. depressus (Van Eeden et al., 1965), were found to have strongly angular mesocones and to be often aphallic (Schutte, 1966; Hamilton-Attwell and Van Eeden, 1969). At that stage B. depressus was considered to be part of the B. truncatus group. Electrophoresis of proteins from the eggs of B. depressus (Hamilton-Attwell, 1976), however, yielded a single-banded main fraction consistent with the diploid chromosome number (Brown, 1994) in contrast to the tetraploid chromosome number characteristic of B. truncatus. According to Brown (1994) the Bulinus species may be divided into groups that have their origins in the four species groups of Mandahl-Barth (1957), namely B. africanus, B. truncatus, B. tropicus and B. forskalii.

2 +2718 299 2380; fax: +2718 299 2370;

e-mail: drkkndk@puknet.puk.ac.za

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However, the groups discussed by Brown (1994) differ in the separation of a *B. reticulatus* group (Wright, 1971) and the fusion of Mandahl-Barth's groups for *B. truncatus* and *B. tropicus* into a single *B. truncatus/tropicus* complex (Brown, 1980; 1981). *Bulinus depressus* is regarded by Brown (1994) as part of this complex.

This report focuses on the geographical distribution and habitats of B. depressus as reflected by the 552 samples in the database of the National Freshwater Snail Collection of South Africa (NFSC). Details of each habitat, as well as mean altitude and mean annual air temperature and rainfall for each locality, were processed to determine chi-square and effect size values. An integrated decision tree that is a statistical model enabling the selection and ranking of those variables that can maximally discriminate between the frequency of occurrence of a given species under specific conditions as compared to all other species in the database was also constructed. The results indicated that altitude, temperature and type of water-body seemed to be some of the major factors determining the distribution of this species in South Africa. On account of the great variation among species of the genus Bulinus in respect of compatibility with schistosomes (Brown, 1994), the role of B. depressus as intermediate host of economically important helminth parasites in South Africa is briefly discussed.

Methods

Data pertaining to the habitats and geographical distribution of *B. depressus* were extracted from the database of the NFSC, which dates from 1956 up to the present. Only samples for which the collection sites could be pinpointed on the 1:250 000 topocadastral map series of South Africa, were included in the analy-

^{*} To whom all correspondence should be addressed.

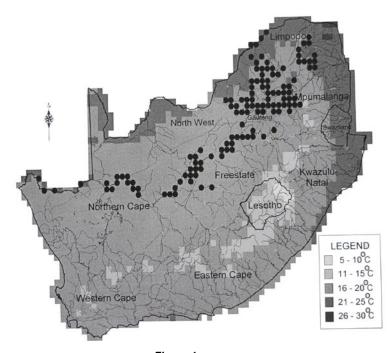


Figure 1 The geographical distribution of Bulinus depressus in 1/16 square degree loci and mean annual air temperature in South Africa

sis. The majority of these samples were collected during surveys conducted by staff of government and local health authorities and then dispatched to the former Snail Research Unit at the Potchefstroom University (now University of the North-West) for identification and to be added to the NFSC.

Details of the habitats were recorded by collectors during surveys by selecting the relevant options on forms compiled by the staff of the Snail Research Unit. The number of loci in which the collection sites were located was distributed in intervals of mean annual rainfall and air temperature, as well as intervals of mean altitude to illustrate the frequency of occurrence within specific intervals. Rainfall, temperature and altitude data were obtained in 2001 from the Computing Centre for Water Research (disbanded since), University of KwaZulu-Natal. A temperature index was calculated for all mollusc species in the database from their frequencies of occurrence within the selected temperature intervals and the results used to rank them in order of association with low to high climatic temperatures. This was done by allocating numeric values, ranging from one for the coolest to five for the warmest, to the five selected temperature intervals. The proportion of the total number of loci of each species falling within a particular temperature interval was then multiplied by the value allocated to that specific temperature interval. This was done for each temperature interval in which the species was recorded and the sum of these scores was then taken as the temperature index for that particular species and the results presented in Table 5 (Brown, 2002; De Kock and Wolmarans, 2005). Chi-square values were calculated to determine the significance in difference between the frequency of occurrence in, on, or at the different options for each variable, such as type of water-body, type of substratum and temperature interval. Furthermore, an effect size (Cohen, 1977) was calculated for all the different variables discussed in this paper. The effect size is an index which measures the degree of discrepancy between the frequency distribution of a given species in the set of alternatives of a given variable such as water-bodies, as compared to

TABLE 1							
Types of water-body from which Bulinus							
depressus was recorded by collectors							
during surveys							

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Water-bodies	Α	В	С	D
Concrete dam	5	0.9%	221	2.3%
Dam	168	30.4%	8 400	2.0%
Ditch	10	1.8%	636	1.6%
Pan	2	0.4%	306	0.7%
Pool	2	0.4%	225	0.9%
Quarry	1	0.2%	122	0.8%
River	163	29.5%	7 507	2.2%
Spring	1	0.2%	301	0.3%
Stream	55	10.0%	7 211	0.8%
Swamp/Marsh	7	1.3%	2 076	0.3%
aa	0.447		1 00	

- Effect size w = 0.44 (moderate to large effect)
- Number of times collected in a specific water-body
- % of the total number of collections (552) on record for this species
- Number of times any mollusc was collected in a specific water-body
- % occurrence of this species in the total number of collections in a specific water-body

the frequency distribution of all other mollusc species in the database in the set of alternatives of the same variable (Cohen, 1977). According to this author values for this index in the order of 0.1 and 0.3 indicate small and moderate effects respectively, while values of 0.5 and higher indicate practical significantly large effects. A value for this index in the order of 0.5, calculated for the frequency distribution of a given mollusc species in the different types of water-body, for instance, would indicate that this factor played an important role in determining the geographical distribution of this particular species as reflected by the data in the database.

The data were also processed to construct an integrated decision tree (Breiman et al., 1984). This is a statistical model that enables the selection and ranking of those variables that can maximally discriminate between frequencies of occurrence of a given species under specific conditions as compared to all other species in the database. This was accomplished by making use of the SAS Enterprise Miner for Windows NT Release 4.0, April 19, 2000 programme and Decision Tree Modelling Course Notes (Potts, 1999).

Results

The 552 samples of B. depressus that could be pinpointed on our maps were collected from 125 different loci (Fig. 1). Although this species was recorded from 10 of the 14 water-body types represented in the database, dams (30.4%) and rivers (29.5%) accounted for nearly 60% of the total number of samples (552) for this species in the database (Table 1). However, the five times it was recovered from concrete dams represented 2.3% of the total number of collections of all species from concrete dams, which compares favourably with the percentages recorded for dams and rivers in this respect (Table 1). Consequently its frequency of occurrence in dams and rivers did not differ significantly from the figure for concrete dams (chi-square values: dams and concrete dams: $\chi^2 = 0.08$, df = 1; p > 0.05; rivers and

TABLE 2
Water conditions in the habitats of <i>Bulinus depressus</i> as described by collectors
during surveys

	Туре			Velocit	у	Co	lour	Salinity	
	Perennial	Seasonal	Fast	Slow	Standing	Clear	Muddy	Fresh	Brackish
A	354	46	25	153	207	284	94	252	5
В	64.1%	8.3%	4.5%	27.7%	37.5%	51.4% 17.0%		45.7%	0.9%
С	22 432	5 350	2 229	9 501	16 147	20 408	6 438	24 089	657
D	1.6%	0.9%	1.1%	1.6%	1.3%	1.4%	1.5%	1.0%	0.8%
Е	w =	0.30	w = 0.12		2	w = 0.02		w = 0.05	
	(modera	te effect)	(small effect		ect)	(small effect)		(small effect)	

- A Number of times collected in a specific water condition
- B % of the total number of collections (552) on record for this species
- C Number of times any mollusc was collected in a specific water condition
- D % occurrence of this species in the total number of collections in a specific water condition

concrete dams: $\chi^2 = 0.01$, df = 1; p > 0.05).

The majority of samples (64.1%) came from perennial habitats and from habitats with standing water (37.5%) (Table 2). Its frequency of occurrence in standing and slow-flowing water, however, did not differ significantly from each other ($\chi^2 = 4.7$, df = 1; p > 0.05). Although the majority of samples were collected in habitats with water recorded as clear (51.4%) and in water described as fresh (45.7%) (Table 2), chi-square values indicated no significant differences between these and the alternative water conditions.

Almost the same number of samples came from habitats of which the substratum types were described as either predominantly muddy (154) or stony (153) and together they represented more than half of the total number of collections of this species (Table 3). The 73 collections made in habitats with substrata consisting of predominantly decomposing plant material, however, represented a much higher percentage of the total number of collections in the database for all mollusc species recovered from habitats with a specific type of substratum than the other three substratum types (Table 3).

TABLE 3 Substratum types in the habitats of Bulinus depressus as described by collectors during surveys

	Substratum types									
	Muddy	Stony	Sandy	Decomposing material						
A	154	153	23	73						
В	27.9%	27.7%	4.2%	13.2%						
C	12 835	7 934	6 523	632						
D	1.2%	1.9%	0.4%	11.6%						

- Effect size w = 0.33 (moderate effect)
- A Number of times collected in a water-body with a specific substratum type
- B % of the total number of collections (552) on record for this species
- C Number of times any molluse was collected in a water-body with a specific substratum type
- D % occurrence of this group in the total number of collections in a water-body with a specific substratum type

TABLE 4 Frequency distribution of the 552 collection sites of *Bulinus depressus* in selected intervals of mean annual air temperature and rainfall and mean altitude in South Africa

	intervals of mean annual an temperature and rannan and mean antitude in oodin Africa													
	Temperature	intervals °C	Rainfall intervals (mm)				Altitude intervals (m)							
	16- 20	21- 25	0-300 301-600 601-900 901-1 2		901-1 200	0-500	501-1 000	1 001-1 500	1 501-2 000					
A	519	33	54	245	244	9	16	104	423	9				
В	94.0%	6.0%	9.8%	44.4%	44.2%	1.6%	2.9%	18.8%	76.6%	1.6%				
C	24 928	4 276	975	11 994	19 799	1 203	6 747	4 491	14 918	6 998				
D	2.1%	0.8%	5.5%	2.0%	1.2%	1.2%	0.2%	2.3%	2.8%	0.1%				
Е	w = 0.61 (large effect) $w = 0.48$ (moderate to large effect)					w = 0.77	(large effe	ct)						

- A Number of times collected in a locality falling within a specific interval
- B % of the total number of collections (552) on record for this species
- C Number of times any molluse was collected in a locality falling within a specific interval
- D % occurrence of this group in the total number of collections within a specific interval
- E Effect size values calculated for each factor

The presence of aquatic plants was reported from 406 (73.6%) of the collections sites at the time of survey.

This species was reported from only two of the five selected temperature intervals (Table 5) with more than 90% recovered from sites that fell within the 16 to 20°C interval (Table 4).

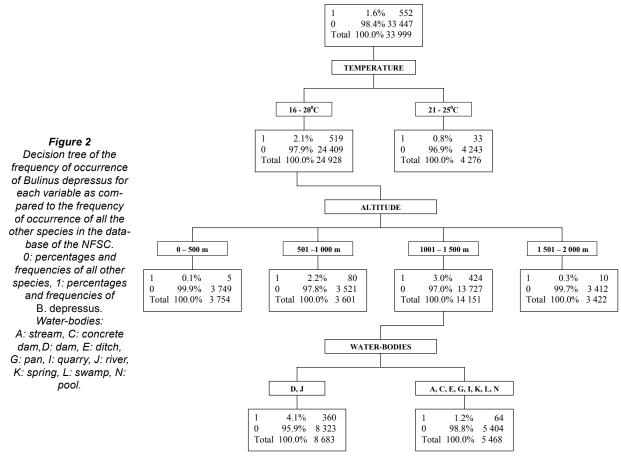
Although the nearly equal number of times this species was collected from sites which fell within the rainfall intervals ranging from 301 to 600 mm (245) and 601 to 900 mm (244) represented 88.6% of the total number of samples, the 54 times

collected within the 0 to 300 mm interval represented a higher percentage (5.5%) of the total number of collections within a specific interval (Table 4). The frequency of occurrence within the selected rainfall intervals differed significantly from each other except in the case of the 601 to 900 mm and the 901 to 1 200 intervals where the chi-square value indicated no significant difference ($\chi^2 = 2.2$, df = 1; p > 0.05).

The largest number of samples (423) were collected from sites which fell within the altitude interval ranging from 1 001

TABLE 5
Frequency distribution in temperature intervals and temperature index of *Bulinus depressus* as compared to all mollusc species in the database of the National Freshwater Snail Collection

Mollusc species	No. of	5-10°C	11-15°C	16-20°C	21-25°C	26-30°C	¹Index	² SD	³CV	Effect
Pisidium viridarium	samples 636	201	270	163	2		1.947	0.764	39.22	-4.698
Lymnaea truncatula	723	95	281	343	4		2.354	0.709	30.14	-2.979
Pisidium casertanum	5	93	201	343	4		2.600	0.709	21.07	-1.941
	627	18	173	430	6		2.676	0.544	20.33	-1.619
Pisidium langleyanum	425	10	138	282	4			0.344	18.34	
Pisidium costulosum							2.680			-1.603
Bulinus tropicus	8 448	32	2 326	5 860	230		2.744	0.502	18.31	-1.332
Gyraulus connollyi	969		185	777	7		2.816	0.406	14.40	-1.028
Ceratophallus natalensis	1 797		299	1 430	68		2.871	0.433	15.09	-0.796
Burnupia (all species)	2 778	7	287	2 384	100		2.928	0.380	12.97	-0.558
Ferrissia (all species)	540		72	420	47	1	2.957	0.476	16.09	-0.433
Bulinus reticulatus	296		6	287	3		2.990	0.174	5.83	-0.296
Assiminea umlaasiana	2			2			3.000	0.000	0.00	-0.253
Tomichia cawstoni	4			4			3.000	0.000	0.00	-0.253
Tomichia diferens	10			10			3.000	0.000	0.00	-0.253
Tomichia lirata	2			2			3.000	0.000	0.00	-0.253
Tomichia ventricosa	89			89			3.000	0.000	0.00	-0.253
Tomichia tristis	81			79	2		3.025	0.156	5.16	-0.149
Unio caffer	76		6	63	6	1	3.026	0.461	15.24	-0.142
Physa acuta	755			719	36		3.048	0.213	7.00	-0.052
Bulinus depressus	552			519	33		3.060	0.237	7.76	0.000
Arcuatula capensis	15			14	1		3.067	0.258	8.42	0.028
Lymnaea columella	2 302		81	1 977	243	1	3.071	0.371	12.07	0.047
Lymnaea natalensis	4 721		205	3 802	713	1	3.108	0.429	13.79	0.023
Assiminea bifasciata	17			15	2		3.118	0.332	10.65	0.243
Gyraulus costulatus	736		20	580	135	1	3.159	0.437	13.84	0.418
Bulinus forskalii	1 209		17	985	204	3	3.160	0.409	12.95	0.420
Pisidium ovampicum	6			5	1		3.167	0.408	12.89	0.450
Sphaerium capense	25		1	17	7		3.240	0.523	16.14	0.759
Bulinus africanus group	2 930		9	2 155	760	6	3.260	0.450	13.82	0.846
Corbicula fluminalis	389		1	291	94	4	3.267	0.437	13.38	0.875
Tomichia natalensis	23			16	7		3.304	0.470	14.24	1.031
Thiara amarula	10			6	4		3.400	0.516	15.19	1.435
Assiminea ovata	5			3	2		3.400	0.548	16.11	1.435
Melanoides victoriae	49			29	19	1	3.429	0.540	15.75	1.555
Biomphalaria pfeifferi	1 639		5	880	751	3	3.459	0.508	14.69	1.683
Septaria tesselaria	2			1	1		3.500	0.707	20.20	1.857
Coelatura framesi	6			3	3		3.500	0.548	15.65	1.857
Neritina natalensis	16			8	8		3.500	0.516	14.75	1.857
Bulinus natalensis	244		2	97	145		3.588	0.510	14.20	2.227
Segmentorbis planodiscus	27			9	18		3.667	0.480	13.10	2.560
Segmentorbis angustus	32			7	25		3.781	0.420	11.11	3.043
Melanoides tuberculata	305			64	237	4	3.803	0.430	11.30	3.136
Pisidium pirothi	23			4	19		3.826	0.388	10.13	3.232
Spathopsis wahlbergi	36			7	28	1	3.932	0.398	10.11	3.679
Aplexa marmorata	9			,	9	1	4.000	0.000	0.00	3.966
Bellamya capillata	31				31		4.000	0.000	0.00	3.966
Eupera ferruginea	169			6	157	6	4.000	0.267	6.68	3.966
Lentorbis carringtoni	8				8	0	4.000	0.207	0.00	3.966
Lentorbis junodi	12				12		4.000	0.000	0.00	3.966
Segmentorbis kanisaensis	9				9		4.000	0.000	0.00	3.966
	39			1	36	2		0.000	6.80	3.966
Spathopsis petersii	1			1	71	2	4.000	0.272		
Cleopatra ferruginea	73 41				38	3	4.027		4.08	4.082
Lanistes ovum		dord 1 - 1	otion: 301	V. Carff			4.073	0.264	6.47	4.275
¹ Index: Temperature index;	-SD: Stan	uaru devi	ation; 'C'	v. Coeinc	ent of var	тапсе				



to 1 500 m (Table 4). The frequency of occurrence within this interval differed significantly from the other intervals (chi-square values ranging from $\chi^2 = 158.0$, df = 1; p < 0.05 to $\chi^2 = 180.6$, df = 1; p < 0.05) except from the 501 to 1 000 m interval where the chi-square-value indicated no significant difference at all ($\chi^2 = 3.5$, df = 1; p > 0.05).

The effect size values calculated for all the factors investigated are given in Tables 1 to 4 and the temperature indexes of all mollusc species in the database, as well as the effect sizes to evaluate their significance in difference as compared to *B. depressus*, are listed in Table 5 and the decision tree analysis depicted in Fig. 2.

Discussion

Bulinus depressus was reported from all the provinces of South Africa except the Western and Eastern Cape and KwaZulu-Natal Provinces and is well represented in the basins of the Vaal and Orange Rivers and further westwards towards the mouth of the Orange River (Fig. 1). This is in general agreement with the geographical distribution reported by Brown (1994) for this species in South Africa. Initially described by Haas (1936) from specimens collected in a canal flowing into Lake Bangweulu, this species was subsequently reported from the same region by Mandahl-Barth (1968). Elsewhere in Africa it was reported from Zaire (Mandahl-Barth et al., 1972) and from the Okavango River and East Caprivi (Brown et al., 1992).

The effect size values calculated for the various factors investigated (Tables 1 to 4) indicated that altitude, temperature, rainfall and water-bodies, in that order, had a large to moderate effect (w > 0.05) on the geographical distribution of *B. depressus* in South Africa and these observations were corroborated by the

results of the decision tree analysis depicted in Fig. 2.

Dams and rivers seemed to be the type of water-body of choice as indicated by its frequency of occurrence in these types of water-body (Table 1). The results of both the decision tree and chi-square analyses indicated a significant difference between these two types of water-body and the rest of the types of water-body investigated. These observations and the fact that it was also recovered from cement-lined reservoirs and marshes (Table 1), is in accordance with reports in literature by several authors (Schutte, 1966; Mandahl-Barth, 1968; Hamilton-Attwell and Van Eeden, 1969; Mandahl-Barth et al., 1972).

Although *B. depressus* is not included in a list in which the common African freshwater snails were arranged in groups according to their ability to aestivate (Brown, 1994), 46 of the samples in the database of the NFSC were recovered from habitats described as seasonal (Table 2) and this species was also reported from a temporary marsh by Mandahl-Barth et al. (1972). From this it can be deduced that it might have some ability to aestivate, like the majority of the other *Bulinus* species.

The first records of both *B. depressus* and the closely related *B. tropicus* in the database of the NFSC date back to the early 1950s but the 552 samples on record for the former, compare poorly to the 8 448 samples on record for the latter (Table 5). Likewise the 125 different loci recorded for *B. depressus* (Fig. 1) compare poorly to the 939 different loci reported for *B. tropicus* by De Kock et al. (2002), reflecting a much narrower geographical distribution. A decision tree analysis (Fig. 2) selected temperature as one of the more important factors determining the geographical distribution of both these species in South Africa (Fig. 2; De Kock et al., 2002). Scrutiny of Table 5, however, reveals that the temperature index calculated for *B. tropicus*(2.744) differssignificantly(effectsize>0.5) fromthatof

B. depressus (3.060) and also indicates a closer association with cooler temperatures. This implies that the former species should be better adapted to colonise cooler parts of the country. Furthermore, B. depressus was recovered from sites falling into only two of the five selected temperature intervals as compared to the four recorded for B. tropicus (Table 5).

Field-collected and laboratory-reared specimens of B. depressus from the Limpopo Province, South Africa, were not susceptible to infection with either Schistosoma haematobium or S. mattheei (Schutte, 1966). However, laboratory infections with S. margrebowiei have been achieved by Pitchford and Du Toit (1976) and according to Pitchford (1976) the definite host range of this schistosome species includes six game genera, cattle and possibly man. Although B. depressus is not included in the list of snails found to harbour natural infections of amphistome parasites of grazing animals, specimens of two of the closely related diploid species, B. tropicus and B. natalensis were both reported to harbour natural infections of the conical fluke, Calicophoron microbothrium (Brown, 1994). In the process of the preparation of microscope slides of the radula and penis complex of the 552 samples of B. depressus in the NFSC, immature parasite stages were observed in many of these specimens and field specimens kept alive in the laboratory for experimental purposes also shed amphistome cercariae on several occasions (personal observations). Unfortunately the identity of these immature parasite stages was never established.

In an earlier publication (De Kock and Wolmarans, 2005) the authors emphasised the need for coordinated surveys to update the knowledge of the current distribution of the freshwater snails in South Africa. The most recent record of *B. depressus* in the NFSC dates back to 1989 and in view of the fact that this species is susceptible to schistosome parasites of certain game animals and possibly of man and could perhaps also play a role in the transmission of the conical fluke of cattle, *C. microbothrium*, it is recommended that efforts be made to update the geographical distribution of *B. depressus* and to clear up its role in the transmission of economically important helminth parasites in South Africa.

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References

BREIMAN L, FRIEDMAN JH, OLSHEN RA and STONE CJ (1984) Classification and Regression Trees. Chapman and Hall.

BROWN DS (1980) Freshwater Snails of Africa and their Medical Importance. Taylor & Francis, London.

- BROWN DS (1981) Generic nomenclature of freshwater snails commonly classified in the genus *Bulinus* (Mollusca: Basommatophora). *J. Nat. Hist.* **15** 909-915.
- BROWN DS (1994) Freshwater Snails of Africa and Their Medical Importance (Revised 2nd edn.) Taylor & Francis, London.
- BROWN DS (2002) Personal communication. Dept. of Zoology, The Natural History Museum, London (Dr. David Seymour Brown passed away on 25 March 2004).
- BROWN DS, CURTIS BA, BETHUNE S and APPLETON CC (1992) Freshwater snails of East Caprivi and the lower Okavango River Basin in Namibia and Botswana. *Hydrobiol.* **246** 9-40.
- COHEN J (1977) Power Analysis for the Behaviour Sciences (revised edn.) Academic Press, Orlando.
- DE KOCK KN and WOLMARANS CT (2005) Distribution and habitats of the *Bulinus africanus* species group, snail intermediate hosts of *Schistosoma haematobium* and *S. mattheei* in South Africa. *Water SA* 31 (1) 117-126.
- DE KOCK KN, WOLMARANS CT, BORNMAN, M and MAREE DC (2002) Verspreiding en habitats van *Bulinus tropicus*, tussengasheerslak van die peervormige bot *Calicophoron microbothrium*, in Suid-Afrika. *SA Tydskr. Natuurwet. Teg.* **21** (4) 114-120.
- DEMIAN ES (1960) Morphological studies on the Planorbidae of Egypt. 1. On the microscopic anatomy of *Bulinus (Bulinus) truncatus* (Audouin). *Ain Shams Sci. Bull.* **5** 1-84.
- HAAS F (1936) Binnen-Mollusken aus Inner-Afrika. Abh. Senckenber. Naturforsch. Gesell. 431 1-156.
- HAMILTON-ATTWEL VL (1976) Electrophoresis of the perivitelline fluid of molluscan eggs: 4. Protein characteristics determining the taxonomic position of *Bulinus (Bulinus) depressus* Haas and *B. (B.) natalensis* (Küster). Wetenskaplike Bydraes van die PU vir CHO, Reeks B: Natuurwetenskappe 76 1-37.
- HAMILTON-ATTWEL VL and VAN EEDEN JA (1969) The shell, radula pallial organs and reproductive system of *Bulinus (Bulinus) depressus* Haas (Mollusca: Basommatophora). *Wetenskaplike Bydraes van die PU vir CHO, Reeks B: Natuurwetenskappe* **9** 1-54.
- MANDAHL-BARTH G (1957) Intermediate hosts of *Schistosoma*. African *Biomphalaria* and *Bulinus*. II. *Bull*. *World Health Org.* 17 1-65.
- MANDAHL-BARTH G (1968). Freshwater molluscs. *Explor. Hydrobiol. Bangweulu-Luapala* 12 1-68.
- MANDAHL-BARTH G, MALAISSE F and RIPERT C (1972) Etudes malacologiques dans la région du lac de Retenue de la Lufira (Katanga), etc. *Bull. Soc. Pathol. Exot.* **65** 146-156.
- PITCHFORD RJ (1976) Preliminary observations on the distribution, definitive hosts and possible relation with other schistosomes, of *Schistosoma margrebowiei*, Le Roux, 1933 and *Schistosoma leiperi*, Le Roux, 1955. *J. Helminthol.* **50** 111-123.
- PITCHFORD RJ and DU TOIT JF (1976) The shedding pattern of three little known African schistosomes under outdoor conditions. *Ann. Trop. Med. Parasitol.* **70** (2) 181-187.
- POTTS WJE (1999) Decision Tree Modeling Course Notes. SAS Institute Inc., Cary, USA.
- SCHUTTE CHJ (1966) Observations on two South African bulinid species of the *truncatus* group (Gastropoda, Planorbidae). *Ann. Trop. Med. Parasitol.* **60** (1) 106-113.
- VAN EEDEN JA, BROWN DS and OBERHOLZER G (1965) The distribution of freshwater molluses of medical and veterinary importance in south-eastern Africa. *Ann. Trop. Med. Parasitol.* **59** 413-424.
- WRIGHT CA (1971) Bulinus on Aldabra and the subfamily Bulininae in the Indian Ocean area. Philosoph. Trans. Roy. Soc. B 260 299-313.