FIELD PREVALENCE AND LABORATORY SUSCEPTIBILITY OF SOUTHERN AUSTRALIAN LAND SNAILS TO *BRACHYLAIMA CRIBBI* SPOROCYST INFECTION

BUTCHER A.R.* & GROVE D.I.*

Summary:

Brachylaima cribbi is a terrestrial trematode of birds and mammals with helicid and hygromiid land snails reported as first and second intermediate hosts. However, reports describing the first intermediate host range of *B. cribbi* have been limited to those snail species present in a small number of geographical locations in South Australia. The natural first intermediate host range, distribution and prevalence of *B. cribbi* in land snails in southern Australia were determined. A total of 6,432 introduced and native land snails were collected from eight geographical districts across 3,000 km of southern Australia and examined microscopically for *B. cribbi* sporocysts. Four introduced European snails, *Theba pisana*, *Cernuella virgata*, *Cochlicella acuta* and *Cochlicella barbara* were natural first intermediate hosts. Sporocyst-infected snails were detected in all districts from Victoria to the west coast of South Australia, a distance of over 1,300 km. Natural sporocyst infection was not observed in introduced European snails *Microxeromagna armillata* and *Helix aspersa* or in native Australian land snails *Succinea australis* and *Strangesta gawleri*. Egg feeding experiments in the laboratory with *B. cribbi* confirmed the susceptibility of those species of snails found to be natural first intermediate hosts. Of those species not found to be infected in nature, only *M. armillata* could be infected in the laboratory. Although this study has shown that five different species of European land snails are suitable first intermediate hosts for *B. cribbi* there are as yet no reports of *B. cribbi* from these snails in Europe or from other countries where they have been introduced. Further investigations are needed in Europe to clarify the origins of this parasite.

KEY WORDS : Digenea, Brachylaimidae, *Brachylaima cribbi*, first intermediate host infection, sporocyst, helicid snails, hygromiid snails.

Résumé : PRÉDOMINANCE SUR LE TERRAIN ET PRÉDISPOSITION EN LABORATOIRE DES ESCARGOTS TERRESTRES DE L'AUSTRALIE DU SUD À L'INFECTION DE SPOROCYST DE *BRACHYLAIMA CRIBBI*

Brachylaima cribbi *est un trématode terrestre des oiseaux et des mammifères. Les escargots terrestres helicid et hygromiid ont étés identifiés comme étant les premiers et seconds hôtes intermédiaires. Cependant, les rapports qui décrivent la gamme du premier hôte intermédiaire de* B. cribbi *ont été limités aux espèces d'escargot qui se trouvent dans un petits nombre de zones géographiques en Australie Méridionale. La gamme naturelle du premier hôte intermédiaire, la distribution et la prédominance de* B. cribbi *dans les escargots terrestres de l'Australie du sud ont été déterminées. 6 432 escargots terrestres introduits et indigènes ont étés ramassés de huit zones géographiques sur une largeur de 3 000 kilomètres dans la partie sud de l'Australie et ont été examinés au microscope pour determiner la présence des sporocysts de* B. cribbi. *Quatre escargots européens introduits,* Theba pisana, Cernuella virgata, Cochlicella acuta *et* Cochlicella barbara *étaient les premiers hôtes intermédiaires naturels. Des escargots infectés par les sporocysts ont été détectés dans toutes les zones depuis l'état de Victoria jusqu'à la côte ouest de l'Australie méridionale, soit une distance de plus de 1 300 kilomètres. On n'a pas observé d'infection naturelle de sporocyst dans les escargots européens introduits* Microxeromagna armillata *et* Helix aspersa, *ni dans les escargots australiens indigènes* Succinea australis *et* Strangesta gawleri. *Les experiences d'alimentation en laboratoire avec les œufs de* B. cribbi *ont confirmé la prédisposition de ces espèces d'escargots qui s'avèrent être les premiers hôtes intermédiaires naturels. Parmi ces espèces qui n'ont pas été trouvées infectées dans leur milieu naturel seulement* M. armillata *a pu être infecté dans le laboratoire. Bien que cette étude ait montrée que cinq espèces d'escargots européens terrestres sont des premiers hôtes intermédiaires convenables pour* B. cribbi, *jusqu'à présent aucune présence de* B. cribbi *n'a été signalée dans ces escargots en Europe, ni dans d'autres pays où ils ont été introduits. Des recherches supplémentaires sont nécessaires en Europe pour clarifier les origines de ce parasite.*

MOTS CLÉS : *Digenea, Brachylaimidae,* Brachylaima cribbi, *première infection d'hôte intermédiaire, sporocyst, escargots helicid, escargots hygromiid.*

INTRODUCTION

Trematodes of the subclass Digenea have a complex life-cycle involving both invertebrate and vertebrate hosts. Sexual reproduction occurs in definitive vertebrate hosts in which adult worms mature and produce eggs whereas one or more intermediate invertebrate hosts are required for larval proliferation through asexual reproduction. Molluscs, specifically gastropods and to a lesser extent bivalves and scaphopods, are first intermediate hosts of digenetic trematodes with many families showing strict miracidial specificity for a particular molluscan lineage (Gibson & Bray, 1994; Bargues & Mas-Coma, 1991; Adema & Loker, 1997). Host specificity is less stringent for cercarial infection of the second intermediate hosts. This

* Department of Clinical Microbiology and Infectious Diseases, Institute of Medical and Veterinary Science, The Queen Elizabeth Hospital, Woodville, South Australia 5011, Australia.
Correspondence: Andrew R. Butcher.
Tel.: 61 8 8222 6728 – Fax: 61 8 8222 6032.
E-mail: andrew.butcher@imvs.sa.gov.au

rigorous miracidial specificity for the first intermediate molluscan host is sometimes used as a taxonomic characteristic to separate morphologically similar species of digenetic trematodes. This has been the case for the terrestrial trematode family Brachylaimidae and in particular the large genus *Brachylaima*. Many species in this genus have morphologically similar adults. This makes species separation difficult and much reliance has been placed on characteristics of their life-cycle including molluscan and definitive host specificity (Mas-Coma & Montoliu, 1986; Bargues *et al.*, 1994; Gracenea & Gonzalez-Moreno, 2002).

We have recently established and described the laboratory life-cycle of *Brachylaima cribbi* using eggs recovered from the faeces of an infected human (Butcher & Grove 2001). The first intermediate molluscan hosts used in the laboratory life-cycle were *Theba pisana* Müller (Helicidae) and *Cernuella virgata* da Costa (Hygromiidae). These snails were selected on the basis that introduced European helicid and hygromiid land snails collected in South Australia were reported to be naturally infected with *Brachylaima* sporocysts (Cribb, 1990; Cribb & O' Callaghan, 1992). Also, these species, when collected from the local environment in the vicinity of human *Brachylaima* infections were found to be infected with larvae (Butcher *et al.*, 1996; Butcher *et al.*, 1998).

Although these two species of introduced snails were used successfully in the laboratory life-cycle as first intermediate hosts the exact extent of the natural host range of *B. cribbi* is unknown. It was postulated that *B. cribbi* was introduced to Australia through the arrival of alien snails or exotic animals at the time of European settlement of southern Australia or sometime thereafter (Cribb, 1990; Butcher & Grove, 2001). Clarification of the origins of *B. cribbi* requires a complete understanding of the range of definitive and intermediate hosts in southern Australia. We have therefore collected land snails from a wide range of locations across southern Australia and determined *B. cribbi* sporocyst prevalence rates in each species collected from each geographical location. Also, preliminary laboratory experiments were conducted to assess various methods for infecting first intermediate host snails with *B. cribbi* eggs in the laboratory. We then used the most reliable technique to examine the susceptibility of land snails from southern Australia to *B. cribbi* sporocyst infection in the laboratory.

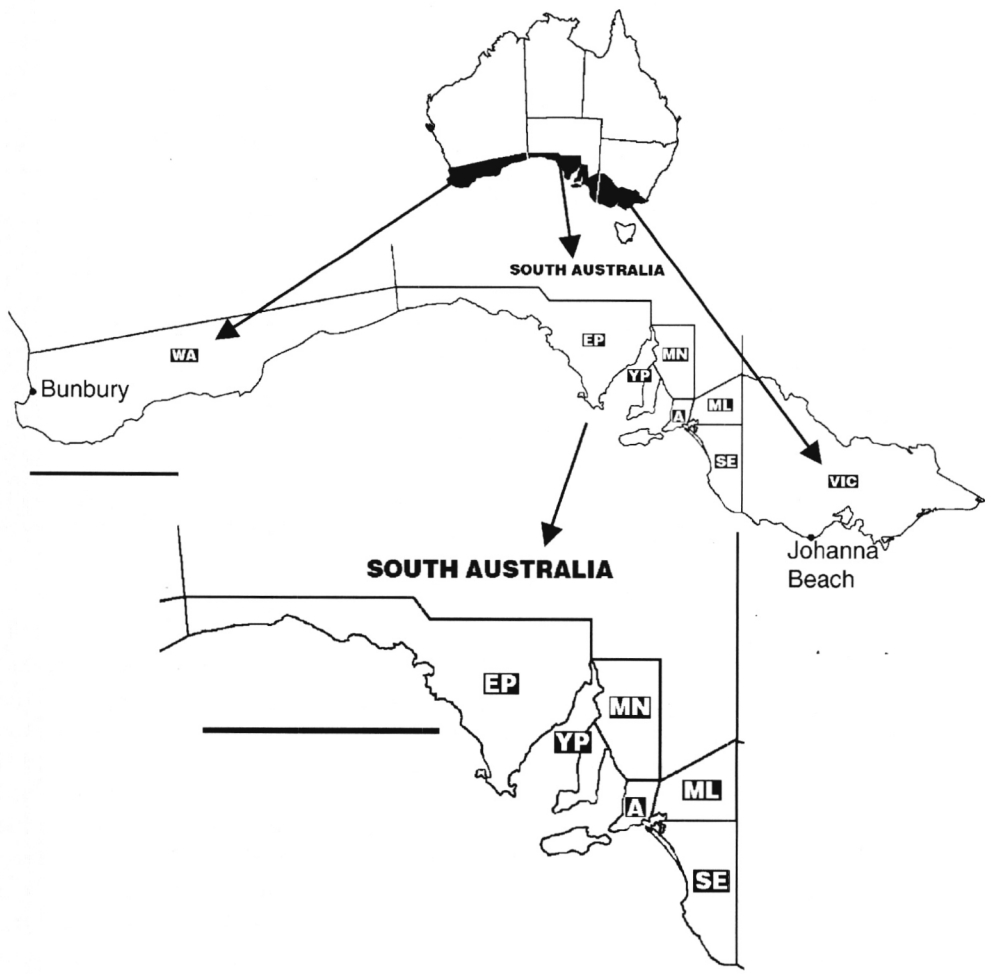

Fig. 1. – Line map of southern Australia showing eight geographical districts where land snails were collected and examined for *Brachylaima cribbi* sporocysts. The collection districts were southern Western Australia (WA) and western Victoria (VIC) with collection sites in South Australia grouped into six districts: Eyre Peninsula (EP); Yorke Peninsula (YP); Mid-North (MN); Adelaide/Adelaide Hilis/Barossa Valley/Fleurieu Penmsula (A); Murray Lands (ML); the South East (SE). Bars = 500 km.

MATERIALS AND METHODS

FIELD LAND SNAIL COLLECTION AND EXAMINATION

Land snails were collected from multiple sites in the proximity of 88 towns in eight geographical districts across 3,000 km of coastal and inland Western Australia (WA), South Australia (SA) and western Victoria (VIC). In South Australia the collection sites were grouped into six districts: Eyre Peninsula (EP); Yorke Peninsula (YP); Mid-North (MN); Adelaide/Adelaide Hills/Barossa Valley/Fleurieu Peninsula (A); Murray Lands (ML); and the South East (SE) (Fig. 1). Each collection site was identified by means of Global Positioning System (GPS) or topographic map longitude-latitude coordinates with the most easterly collection site being Johanna Beach, Victoria and Bunbury, Western Australia being the most westerly site. Representative specimens of *T. pisana*, *C. virgata*, *Helix (Cryptomphalus) aspersa* Müller, 1774 (Helicidae), *Cochlicella acuta* Müller, 1774 (Hygromiidae), *Cochlicella barbara* Linnaeus, 1758 (Hygromiidae), *Microxeromagna armillata* (synonym vestita) Lowe, 1852 (Hygromiidae), *Succinea australis* Ferussac, 1821 (Succineidae) and *Strangesta gawleri* Brazier, 1872 (Rhytididae) were collected when present at each of the collection sites. The large majority of snails (78 %) were collected in the warmer months of spring, summer and autumn from November to May over a period of five years. Snail identification and taxonomic classification were based on conchological and malacological morphologies as described by Manga-González (1978), Smith & Kershaw (1979), Robinson (1999) and using the Integrated Taxonomic Information System (ITIS) on-line database, http://www.itis.usda.gov (2001).

Snails were dissected by removing their shell and then, with the aid of a dissecting microscope, the internal organs were inspected for the presence of Brachylaima sporocysts. Particular attention was placed on the digestive gland and hepato-pancreatic lobes, as these are the sites of sporocyst growth for *B. cribbi*. Species identification of *Brachylaima* sporocysts was confirmed for each snail species found infected from each district by examining cercarial chaetotaxy patterns of argentophilic papillae of emerging cercariae and by comparing these results with those for *B. cribbi* reported by Butcher & Grove (2001). In addition, cercariae from infected snails from each district were used to infect laboratory-reared *H. aspersa* to obtain mature metacercariae which were fed to C57BL/6J mice to isolate adult worms and confirm the species identification.

LABORATORY LAND SNAIL CULTURES

Snails of each species collected from the field were maintained in the laboratory at room temperature in opaque plastic terrariums 305 mm × 235 mm × 115 mm or 500 mm × 290 mm × 230 mm with a flooring of paper hand towels and a lid of brass flywire mesh. Snail food formulation was a modification of that described by Baker (1991) and consisted of equal parts of rolled oats, skim milk powder (both Black and Gold brand, Australian Asia-Pacific Wholesalers, Parramatta, Australia), calcium carbonate (BDH, Poole, England) and powered mouse-rat pellets (Ridley Agri Products, Murray Bridge, South Australia). Food mix was placed in the terrariums in plastic dishes to allow snails to feed on demand and water dispensed by spraying the paper twice weekly. The only exception to this culture procedure was for the carnivorous snail *S. gawleri*. These snails were maintained in similar terrariums but with a flooring of sterile soil and a food supply of live *T. pisana* or *C. virgata*. Before commencement of any intermediate host susceptibility experiments, all wild snails were maintained in laboratory culture for a minimum of 10 weeks and individual slime trails were examined for emergent cercariae during this time to eliminate any snails with pre-existing *Brachylaima* sporocyst infection.

LABORATORY SUSCEPTIBILITY OF LAND SNAILS TO *B. CRIBBI* INFECTION

Three sources of *B. cribbi* eggs were used to assess the most reliable method for infecting snails. These were: 1. Worm dissection (WD); eggs were dissected from gravid adult *B. cribbi* worms and suspended in distilled water. 2. Faecal suspension (FE); eggs purified from 1-2 g of faeces from C57BL/6J mice infected with *B. cribbi* using the method described by Butcher *et al.* (2002) except that the diethyl ether sedimentation was replaced by two distilled water washes and the resulting pellet re-suspended in 5 ml of distilled water. 3. RPMI worm cultures (RPMI); eggs excreted from *B. cribbi* worms in RPMI cell culture media were washed and suspended in distilled water. Live adult worms dissected from C57BL/6J mice 4 wpi were placed in RPMI-1640 cell culture media (Gibco, Paisley, UK) supplemented with 10 g/L glucose, 25 mmol/L HEPES (ICN, Aurora, Ohio, USA), 30 mg/L penicillin, 100 mg/L gentamicin and 25 mg/L amphotericin B and incubated at 35° C in 5 % CO_2 for 48 hours. Following incubation, worms were removed and the culture medium was centrifuged at 500 × g for one minute, supernatant fluid discarded, the egg pellet washed twice with distilled water then re-suspended in 5 ml of distilled water. An aliquot of each egg suspension was examined microscopically to ensure there were greater than 1,000 *B. cribbi* eggs per ml.

In a series of experiments, a total of 407 *T. pisana* and 330 *C. virgata* were fed WD, FE and RPMI egg suspensions by distributing the suspensions evenly on the

surface of Whatman number one filter papers secured to the lids and bases of plastic petri dishes. Groups of 15-20 snails were placed in the petri dishes and allowed to feed for two days then returned to standard laboratory snail terrarium conditions. From one to 12 wpi, snails were examined weekly for emerging cercariae by placing the snails in a petri dish containing a thin film of distilled water and observing their slime trails microscopically for cercariae. Any dead snails were dissected and examined microscopically for *B. cribbi* sporocysts. In a second series of experiments, groups of 15-20 introduced European helicid and hygromiid land snails *T. pisana*, *C. virgata*, *H. aspersa*, *C. acuta*, *C. barbara* and *M. armillata* collected in South Australia were fed FE and RPMI egg suspensions as described above. Due to the low numbers of native Australian land snails present in the field, only eight *S. australis* were fed FE egg suspension and five *S. gawleri* were fed RPMI egg suspension. Snails surviving greater than 6 wpi were examined weekly for emergent cercariae as described above. Any dead snails were dissected and examined microscopically for *B. cribbi* sporocysts. At 12 wpi all surviving snails were dissected and examined microscopically for the presence of *B. cribbi* sporocysts. The percentage of sporocyst-infected snails that survived greater than 6 wpi was determined.

RESULTS

A total of 6,432 snails were collected from eight districts across southern Australia and examined microscopically for *B. cribbi* sporocyst infection. *T. pisana*, *C. virgata*, *C. acuta* and *C. barbara* were natural first intermediate hosts for *B. cribbi* (Table I). The highest sporocyst prevalence rate of 8.4 % was seen in *C. barbara* collected from the Yorke Peninsula. This district was also the region with the highest prevalence of *B. cribbi* sporocysts and was the only region in which four different species of snails were infected. *C. acuta* was generally restricted to coastal regions and sporocysts-infected snails were collected from only the Yorke and Eyre Peninsula regions. The most common snail collected was *C. virgata* but sporocyst infections were restricted to snails collected from the western and central regions of South Australia. Sporocysts were not found in any snails collected from Western Australia and *T. pisana* was the only species gathered from Victoria in which sporocysts were found. Natural sporocyst infection was not observed in the introduced snails *H. aspersa* and *M. armillata* or in the native Australian land snails *S. australis* and *S. gawleri*.

Preliminary experiments investigating the influence of egg source on the infectivity for *T. pisana* and *C. virgata* showed that eggs recovered from RPMI worm cultures always produced infections (Fig. 2). This was closely followed by eggs from mouse faeces with eggs obtained from dissected worms being the least efficient in producing sporocyst-infections. The pre-patent period from egg feeding to cercarial shedding was 7 to 11 wpi. Emergent cercariae were detected in a small number of snails at 7 wpi and all sporocyst-infected snails shed cercariae by 11 wpi. At 6 wpi it was possible, with the aid of a dissecting microscope, to observe in dead snails at post-

	YP	MN	ML	EP	A	SE	VIC	WA	Total
C. barbara	8.4 % (155)	0 % (50)	6.8 % (146)	0 % (50)	2.9 % (238)	3.3 % (60)	0 % (8)	0 % (19)	4.4 % (726)
T. pisana	6.2 % (289)	2.0 % (101)	3.6 % (223)	2.5 % (118)	1.0 % (199)	1.9 % (53)	0.6 % (162)	0 % (88)	2.8 % (1,234)
C. acuta	2.7 % (412)	–	–	1.8 % (228)	0 % (103)	0 % (5)	–	0 % (21)	2.0 % (769)
C. virgata	5.0 % (402)	4.2 % (451)	0.7 % (139)	1.0 % (1,184)	0.7 % (857)	0 % (216)	0 % (39)	0 % (38)	1.7 % (3,326)
H. aspersa	0 % (129)	–	0 % (40)	–	0 % (120)	–	–	–	0 % (289)
M. armillata	–	–	0 % (34)	–	–	–	0 % (30)	–	0 % (64)
S. australis	–	–	0 % (16)	–	–	–	–	0 % (3)	0 % (19)
S. gawleri	–	–	–	–	0 % (5)	–	–	–	0 % (5)
Total	4.5 % (1,387)	3.5 % (603)	3.2 % (598)	1.2 % (1,580)	1.0 % (1,522)	0.9 % (334)	0.4 % (239)	0 % (169)	2.2 % (6,432)

Table I. – Percentages of infection with *B. cribbi* sporocysts in land snails across eight geographical districts of southern Australia. Collection sites were grouped into six districts in South Australia: Yorke Peninsula (YP), Mid-North (MN), Murray Lands (ML), Eyre Peninsula (EP), Adelaide/Adelaide Hills/Barossa Valley/Fleurieu Peninsular (A) and the South East (SE). Other collection sites were in the states of Western Australia (WA) and Victoria (VIC). ( ) = number of snails examined.

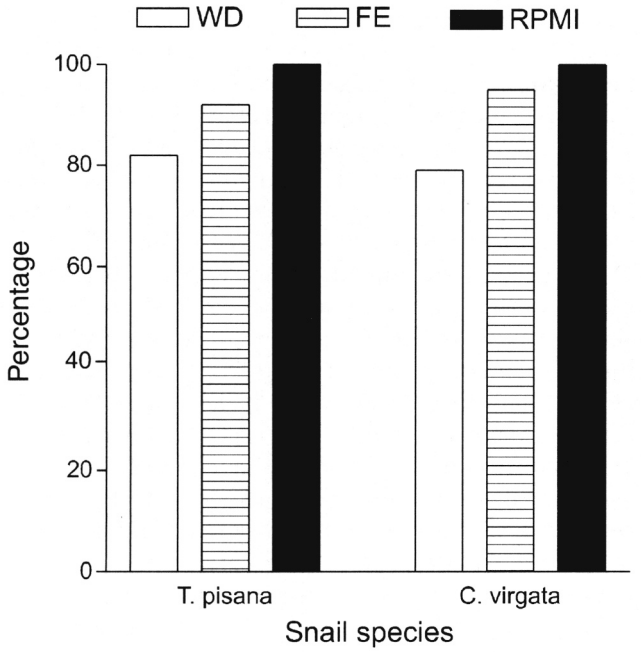

Fig. 2. – Percentages of *T. pisana* and *C. virgata* infected with *B. cribbi* sporocysts when examined six weeks after laboratory feeding experiments using eggs from three sources. 1. Eggs from dissected adult worms (WD, open bars, *T. pisana* n = 169, *C. virgata* n = 42). 2. Eggs recovered and purified from *B. cribbi* infected C57BL/6J mouse faeces (FE, striped bars, *T. pisana* n = 134, *C. virgata* n = 123). 3. Eggs recovered from RPMI *B. cribbi* worm cultures (RPMI, fihled bars, *T. pisana* n = 29, *C. virgata* n = 99).

mortem examination the presence or absence of a developing sporocyst and therefore distinguish infected from non-infected snails. Before this time the sporocyst is too small to allow reliable assessment of dead snails.

In subsequent experiments, the susceptibility in the laboratory of land snails from South Australia was assessed by feeding *B. cribbi* eggs recovered from RPMI worm cultures and mouse faeces except in the case of *S. gawleri* and *S. australis* when one or the other preparation was not available. Those snails surviving greater than 6 wpi were examined for sporocyst infection and cercarial shedding to determine the infection rate. The same four species of helicid and hygromiid European land snails which were natural first intermediate hosts for *B. cribbi* were also susceptible in laboratory feeding experiments. In addition, *M. armillata* was also susceptible although at a reduced intensity with only 42-50 % of snails becoming infected (Table II). *H. aspersa* and, in limited studies, the two native Australian land snails, *S. australis* and *S. gawleri* were not susceptible first intermediate hosts. Using both field and experimental data, the ability of *B. cribbi* eggs to develop into sporocysts in the eight species of introduced and native snails studied is summarised in Table III.

	Eggs from mouse faeces			Eggs from RPMI worm cultures		
		6-12 wpi			6-12 wpi	
	n1	n2	% SP	n1	n2	% SP
T. pisana	221	134	92 %	35	29	100 %
C. virgata	155	123	95 %	120	99	100 %
C. barbara	30	17	100 %	20	15	100 %
C. acuta	30	21	100 %	60	36	100 %
M. armillata	57	26	42 %	20	8	50 %
H. aspersa	133	133	0 %	50	50	0 %
S. australis	8	8	0 %	NT	NT	NT
S. gawleri	NT	NT	NT	5	5	0 %

Table II. – Experimental infection of land snails from southern Australia with *Brachylaima cribbi* eggs recovered from C57BL/6J mouse faeces and RPMI worm culture. n1 = number of snails infected; n2 = number of snails examined for *B. cribbi* sporocyst infection 6-12 weeks post infection; % SP = percentages of *B. cribbi* sporocysts-infected snails 6-12 wpi; NT = not tested.

Species	Family	Sporocyst susceptibility
Theba pisana	Helicidae	Yes
Cernuella virgata	Hygromiidae	Yes
Cochlicella acuta	Hygromiidae	Yes
Cochlicella barbara	Hygromiidae	Yes
Microxeromagna armillata	Hygromiidae	Yes
Helix aspersa	Helicidae	No
Succinea australis	Succineidae	No
Strangesta gawleri	Rhytididae	No

Table III. – Summary of susceptibility of eight land snail species from southern Australia as first intermediate host to *B. cribbi* using field and experimental data.

DISCUSSION

This study has clarified the first intermediate host range of *B. cribbi*, defined the distribution of infected snails across southern Australia and determined the optimal methods for infecting snails with *B. cribbi* eggs in the laboratory. Helicid and hygromiid European land snails, which are introduced pests in southern Australia, have been reported as the first intermediate hosts for *B. cribbi* but such reports have been limited (Cribb, 1990; Cribb & O'Callaghan, 1992; Butcher *et al.*, 1996). They have not completely investigated all the species of common land snails present in southern Australia and have focused only on a limited number of geographical locations in South Australia. We have shown that the first intermediate host range for *B. cribbi* is extensive with five species of snails belonging to two families being suitable hosts and for the first time report *C. acuta* and *M. armillata* as first intermediate hosts. Natural sporocyst prevalence rates varied from 8.4 % for *C. barbara* on the Yorke Peninsula to 0.6 % for *T. pisana* in Victoria. The geographical range

of infected snails is extensive with sporocyst-infected snails found from Victoria to the west coast of South Australia, a distance of over 1,300 km. Although we have not found snails infected with sporocysts in Western Australia, we have observed *B. cribbi* metacercariae in these snails (unpublished observations). This discrepancy may be due to the low numbers of snails examined from Western Australia. All of these results must be interpreted with a degree of caution, as there may be seasonal variations in prevalences. Detailed analyses of seasonal variations in sporocyst infection rates are the subject of an ongoing study.

It is often considered that digenetic trematodes demonstrate strict miracidial specificity for their first intermediate host mollusc (Bargues & Mas-Coma 1991; Gibson & Bray, 1994; Adema & Loker, 1997). However, there are reports that miracidial specificity is not always limited to snail species belonging to one family. Gibson & Bray (1994) reviewed data related to digenean host specificity and reported that 22 of 119 families, of which Brachylaimidae was one, could exploit both prosobranch and pulmonate molluscan lineages. For example, the terrestrial trematode *Dicrocoelium lanceolatum* (*dendriticum*) displays a lack of first intermediate host specificity; Tarry (1969) studied the life-cycle of this fluke in Britain and produced a list of 38 species of snails from nine families which act as first intermediate hosts. *B. cribbi* is another terrestrial trematode which we have now shown to display an extensive first intermediate host range.

The origins of *B. cribbi* in southern Australia have not been clarified but it has been postulated to be a European species of brachylaimid introduced to Australia (Cribb, 1990; Butcher & Grove, 2001). If this is the case, it is necessary to explain why this parasite, which is so prolific in southern Australia and infects such a wide range of common European snails, has gone unnoticed in Europe since the time the genus, *Brachylaima* Dujardin, 1843, was first described. Also, these same species of European snails have been introduced to other continents around the world including southern Africa and North and South America (Baker, 1986; Robinson, 1999). As with the snails from Europe, there have been no reports of brachylaimids resembling *B. cribbi* from these helicid and hygromiid snails. Environmental factors, host-parasite interactions and/or limited studies of these snails in Europe and around the world could explain why *B. cribbi* has not been observed elsewhere. From environmental and host-parasite interaction perspectives, *B. cribbi* may have expanded in helicid and hygromiid snails introduced to Australia due to the favourable conditions for proliferation of these snails in the Mediterranean climate of southern Australia. The population explosion of these introduced snails has also be compounded by the fact that there are no natural predators for these alien snails in Australia (Baker, 1986). As the introduced snail population expanded, animals feeding on land snails would have been exposed to a greater proportion of introduced snails compared to their normal diet of native snails. This would give a competitive advantage for any parasite utilising introduced snails as an intermediate host. Both mammals and birds are suitable definitive hosts for *B. cribbi* (Angel & Mutze, 1987; Cribb, 1990; Cribb & O' Callaghan, 1992; Butcher & Grove, 2001) feeding on the abundant supply of introduced land snails and shedding eggs back into the environment. It is likely that this has resulted in the spiralling life-cycle of *B. cribbi* across southern Australia where helicid and hygromiid snails have colonised. In order to complete our understanding of the dynamics of the life-cycle of this parasite in southern Australia further studies are required to determine the complete range of natural animal hosts. It is also possible that *B. cribbi* was an indigenous Australian brachylaimid which adapted to helicid and hygromiid snails as its natural native snail host(s) declined and the local habitat changed during European settlement of southern Australia. This would explain why no brachylaimids resembling *B. cribbi* have been reported from helicid and hygromiid snails in Europe or from other countries where they have also been introduced. However, this seems unlikely as no native Australian snails were found to be natural or experimental hosts for *B. cribbi*. It should be noted, however, that only relatively low numbers of native snails have been collected and examined. Native snails are hard to find in contrast to the hyper-abundant introduced snails which have adapted to Australian conditions and displaced indigenous snail species. The original host(s) of *B. cribbi* may no longer be present in the natural environment in sufficient numbers to permit detection. According to Cribb (1990) there are three species of trematodes introduced to Australia since European settlement which have adapted to native snails. To our knowledge there are no examples of an indigenous trematode adapting to an introduced snail but this still remains a possibility. A search for helicid and hygromiid snails infected with *B. cribbi* in their natural habitat in Europe and in other countries where they have been introduced may shed more light the origins of *B. cribbi*.

Until now, first intermediate host experiments for brachylaimids in the laboratory have essentially relied on the feeding of eggs from dissected gravid adults worms to test snails (Mas-Coma & Montoliu, 1986; Mas-Coma & Montoliu, 1987; Bargues & Mas Coma, 1991; Bargues *et al.*, 1994) although we used *B. cribbi* eggs recovered from human faeces (Butcher & Grove, 2001). Snails ingest passively embryonated eggs which then hatch in the digestive tract allowing the miracidium to escape and infect the hepatopancreatic acinic cells. Bargues & Mas Coma (1991) described the intracellular development of *B. ruminae* and the subsequent invasion of connective

tissue spaces in the digestive gland to give rise to the sporocyst. To evaluate the efficacy of this feeding process, we exposed snails to eggs from dissected worms, in faecal suspension and eggs excreted by gravid worms in RPMI cell culture medium. The greater infectivity of the last two methods may be attributable to the fact that the majority of eggs from mouse faeces and RPMI worm cultures were fertile eggs containing a developed miracidium whereas eggs dissected from gravid worms had of a greater proportion of immature or infertile eggs. Snail survival after egg feeding varied considerably. Snails fed eggs from RPMI worm cultures generally showed the highest survival rates at 6 wpi. Faecal egg suspension may expose the snails to other pathogens which could reduce survival. *M. armillata* had the highest mortality in laboratory cultures; this may reflect sub-optimal conditions for this snail or the effects of miracidial infection. Survival rates in non-susceptible snails (*H. aspersa*, *S. australis* and *S. gauleri*) were 100 % which indicates that miracidial infection causes some morbidity and mortality in susceptible snails. If the course of infection in *B. cribbi* follows the same process as that described by Bargues & Mas-Coma (1991) for *B. ruminae*, it is likely that the rate of snail death is influenced by the intensity of infection and the intracellular damage which occurs in the snail. It is not clear if multiple miracidial infections occur in an individual snail although Bargues & Mas-Coma (1991) did demonstrate multiple intracellular parasite cell clusters in the early stages of infection with *B. ruminae* eggs. Eggs recovered from RPMI worm cultures should be used in future first intermediate host experiments as this will allow greater control over the numbers of embryonated eggs presented to each snail.

In summary, this study has shown that *B. cribbi* can utilise *T. pisana*, *C. virgata*, *C. acuta*, *C. barbara* and *M. armillata* as first intermediate hosts in southern Australia. These European snails have been introduced to many countries around the world but to date *B. cribbi* has only been observed in introduced helicid and hygromiid snails in Australia. The origin of *B. cribbi* is still unclear, but may be clarified by further studies in Europe and other locations around the world where these snails have colonised.

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