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SECOND INTERMEDIATE HOST LAND SNAILS AND DEFINITIVE HOST ANIMALS OF *BRACHYLAIMA CRIBBI* IN SOUTHERN AUSTRALIA

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Summary:

This study of infection of southern Australian land snails with *Brachylaima cribbi* metacercariae has shown that all commonly encountered native and introduced snails are susceptible second intermediate hosts. The range of infected snails is extensive with metacercariae-infected snails being present in all districts across southern Australia. *C. virgata* has the highest average natural metacercarial infection intensity of 6.1 metacercariae per infected snail. The susceptibility of birds, mammals and reptiles to *B. cribbi* infection was studied in South Australia by capturing, dissecting and examining the intestinal tract contents of animals which commonly eat land snails as a food source. Indigenous Australian little ravens (*Corvus mellori*), which are a common scavenger bird, and two other passeriform birds, the black bird (*Turdus merula*) and the starling (*Sturnus vulgaris*), which are both introduced European birds, were found to have the highest infection rates of all animals examined. Other birds found infected with *B. cribbi* were an emu (*Dromaius novaehollandiae*), chickens (*Gallus gallus*) and a pigeon (*Columba livia*). Natural infections were also detected in field mice (*Mus domesticus*) and shingleback lizards (*Tiliqua rugosa*) although the intensity of infection was lower than that observed in birds. Susceptibility studies of laboratory mice, rats and ducks showed that mice developed patent infections which persisted for several weeks, rats developed a short-lived infection of three weeks' duration and ducks did not support infection. This study has shown for the first time that a brachylaimid can infect a wide host range of birds, mammals and reptiles in nature.

KEY WORDS : Digenea, Brachylaimidae, *Brachylaima cribbi*, second intermediate host, definitive host animals, metacercaria, helicid snails, hygromiid snails.

Résumé : ESCARGOTS TERRESTRES, SECONDS HÔTES INTERMÉDIAIRES, ET ANIMAUX HÔTES DÉFINITIFS DE *BRACHYLAIMA CRIBBI* EN AUSTRALIE DU SUD

Cette étude de l'infection des escargots terrestres de l'Australie du sud par des métacercaires de Brachylaima cribbi *a montré que tous les escargots communs introduits et indigènes peuvent être le second hôte intermédiaire. La gamme des escargots infectés est étendue, et il y a des escargots infectés par des métacercaires dans toutes les régions de l'Australie du sud.* C. virgata *a l'intensité moyenne d'infection naturelle par métacercaire la plus élevée : 6,1 metacercaires par escargot infecté. La réceptivité des oiseaux, des mammifères et des reptiles à l'infection a été étudiée en Australie méridionale. On a capturé, disséqué et examiné les contenus intestinaux des animaux qui se nourrissent fréquemment d'escargots terrestres. Le petit corbeau Australien indigène* (Corvus mellori)*, un oiseau charognard commun, et deux autres passériformes, le merle noir* (Turdus merula) *et l'étourneau* (Sturnus vulgaris) *qui sont tous les deux des oiseaux européens introduits, avaient les taux d'infection les plus élevés de tous les animaux examinés. Les autres oiseaux infectés par* B. cribbi *étaient un émeu* (Dromaius novaehollandiae)*, quelques poulets* (Gallus gallus) *et un pigeon* (Columba livia)*. On a aussi détecté des infections naturelles chez des souris* (Mus domesticus) *et des scinques à queue tronquée* (Tiliqua rugosa)*, mais l'intensité des infections était plus basse que celle observée chez les oiseaux. Les expériences d'infestation de souris, de rats et de canards en laboratoire ont montré que les souris ont développé une infection manifeste de quelques semaines, les rats une infection de courte durée (trois semaines), et que les canards n'ont pas développé d'infection. Cette étude a montré pour la première fois qu'un brachylaimid peut infecter une grande gamme d'oiseaux, de mammifères et de reptiles hôtes dans la nature.*

MOTS CLÉS : *Digenea, Brachylaimidae,* Brachylaima cribbi, *second hôte intermédiaire, hôte définitif, métacercaire, escargot hélicide, escargot hygromiide.*

INTRODUCTION

Second intermediate host snails are an essential stage in the life-cycle of *Brachylaima cribbi*. Cercariae, which emerge from sporocyst-infected snails, infect a susceptible second intermediate host snail by migrating to the snail's kidney where they develop and grow to form metacercariae. This new larval stage is essentially a juvenile adult worm which parasitises the snail until it is eaten by a definitive host animal. Once in the intestinal tract of the definitive host, the metacercaria must evade the host's innate protective mechanisms and immune responses to attach to the intestinal tract wall, feed, mate and mature into a fertile adult worm.

The definitive host range for *Brachylaima* spp. is considered to be highly specific with the majority of species infecting only a narrow range of mammals or birds. Until recently, *B. pellucidum* and *B. erinacei*

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were the only species reported to infect both mammals and birds in nature (Yamaguti, 1971). More recently, the species that is now recognised as *B. cribbi* has been reported to infect feral house mice, the greater stick-nest rat and domestic chickens in nature (Angel & Mutze, 1987; Cribb, 1990; Cribb & O'Callaghan, 1992). In addition, *Mus musculus* and the Australian bush rat *Rattus fuscipes* have been infected in the laboratory (Cribb, 1990; Butcher & Grove, 2001; Butcher *et al.*, 2002b). These studies indicate that *B. cribbi* infects both mammals and birds but there have been no wide-ranging investigations of the involvement of these animals in the natural life-cycle. The aims of this study were to investigate both the natural host range of second intermediate host snails and definitive host animals commonly encountered in southern Australia and the distribution and intensity of metacercarial infection of second intermediate host. In addition, the ability of laboratory mice, rats and ducks to support the development of mature adult *B. cribbi* was assessed by examining faecal egg excretion and worm burden after oral inoculation with *B. cribbi* metacercariae. This information would increase our understanding of the potential sources of *B. cribbi* for infection of humans.

MATERIALS AND METHODS

Field land snail collection and examination

The collection sites and the snails species have been described previously in the report of first intermediate host snails infected with *B. cribbi* (Butcher & Grove, 2003). Briefly, 6,432 land snails were collected from southern Australia 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). Representative specimens of introduced European land snails *Theba pisana*, *Cernuella virgata*, *Helix aspersa*, *Cochlicella acuta*, *Cochlicella barbara* and *Microxeromagna armillata* and indigenous Australian land snails *Succinea australis* and *Strangesta gawleri* 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. Seasonal prevalence rates were not considered and are part of an on-going investigation.

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* metacercariae. If the snail was infected with metacercariae the number present was counted. Identification of *B. cribbi* metacercariae from each species of snail from each district was confirmed by infecting laboratory mice then recovering adult worms for the comparison of morphological features with those reported for *B. cribbi* (Butcher & Grove, 2001).

Field and laboratory susceptibility of potential definitive host animals

A range of common birds, mammals and reptiles including ravens, starlings, pigeons, rodents and shingleback lizards, which are likely to eat land snails as a food source, were collected from the Yorke Peninsula, South Australia. These animals were dissected and their internal organs examined for *B. cribbi* worms. Particular attention was placed on the alimentary tract with stomach contents being examined for snail remnants and the intestinal lining scraped, washed with saline and examined with the aid of a dissecting microscope. One shingleback lizard was collected and housed in the laboratory for two weeks prior to dissection to monitor faecal egg excretion. Other specimens of *B. cribbi* collected from various animals in South Australia and Victoria, Australia were kindly referred to us by Dr T.H. Cribb (Department of Microbiology, The University of Queensland) and Mr M. O'Callaghan (Parasitology Department, South Australian Research and Development Institute).

The susceptibility of laboratory mammals and birds was assessed by infecting rats and ducks with *B. cribbi* metacercariae. Nine out-bred Sprague Dawley rats (*Rattus norvegicus*) were infected with 15 to 20 *B. cribbi* metacercariae. At the same time eight Peking ducks (*Anas domesticus*) were inoculated via an intra-oesophageal tube with 40 to 60 metacercariae (six ducks were given 40-45 metacercariae, one duck was given 50 metacercariae and the last duck was given 60 metacercariae). Six C57BL/6J and ten non-obese diabetic severe combined immunodeficient NOD/Lt-Prkd-c^{scid} (NOD SCID) mice (*Mus musculus*) were also infected with 15 metacercariae each at the same time as control animals to ensure viability of the metacercariae. Faecal egg counts were determined weekly to assess the development of patent infections. Between 1-5 weeks post-infection (wpi), selected animals were killed and dissected and their intestinal tracts examined for the presence of adult *B. cribbi*. All remaining animals were dissected at 6 wpi.

RESULTS

SECOND INTERMEDIATE HOST SNAILS

In all geographical districts each species of snail collected was infected with *B. cribbi* metacercariae with the exception of *M. armillata* collected from the Murray River region of Victoria and the small numbers of *C. barbara* and *S. australis* collected from Western Australia (Tables I & II). In South Australia the overall prevalence rates ranged from a low of 36 % for snails collected in the Adelaide district to a high of 60 % in the Murray Lands district. The overall prevalence rate, which does not take into account seasonal variation, was 47 % for all snail species collected from all districts across southern Australia. Non-encysted metacercariae of *B. cribbi* were observed exclusively contained within the kidney of infected snails. Metacercariae were not observed in the pericardial sac, which is in close proximity to the kidney nor were any observed in other organ systems. No other trematode metacercariae were observed in any snails.

Species	Family	n	% MC	MC/snail
C. acuta	Hygromiidae	769	38 %	2.7
C. barbara	Hygromiidae	726	53 %	4.0
C. virgata	Hygromiidae	3,326	55 %	6.1
H. aspersa	Helicidae	289	18 %	2.1
M. armillata	Hygromiidae	64	30 %	2.7
S. gawleri	Rhytididae	5	60 %	2.7
S. australis	Succineidae	19	63 %	2.5
T. pisana	Helicidae	1,234	36 %	3.4

Table II. – Summary of snail families and species examined for *B. cribbi* metacercariae from southern Australia. n = number of snails examined. % MC = percentages of snails infected with metacercariae. MC/snail = mean numbers of metacercariae per infected snail.

	YP	MN	ML	EP	A	SE	VIC	WA	Total
C. acuta	49 % 2.5 (412)	–	–	16 % 2.3 (228)	43 % 3.8 (103)	60 % 3.0 (5)	–	14 % 2.7 (21)	38 % 2.7 (769)
C. barbara	70 % 4.2 (155)	28 % 1.6 (50)	72 % 3.8 (146)	14 % 1.3 (50)	52 % 4.8 (238)	40 % 2.3 (60)	50 % 2.8 (8)	0 % 0 (19)	53 % 4.0 (726)
C. virgata	74 % 5.7 (402)	59 % 8.0 (451)	67 % 10.7 (139)	65 % 4.8 (1,184)	36 % 5.2 (857)	45 % 11.5 (216)	8 % 2.8 (39)	13 % 4.8 (38)	55 % 6.1 (3,326)
H. aspersa	15 % 1.8 (129)	–	48 % 2.2 (40)	–	13 % 2.5 (120)	–	–	–	18 % 2.1 (289)
M. armillata	–	–	56 % 2.7 (34)	–	–	–	0 % 0 (30)	–	30 % 2.7 (64)
S. australis	–	–	75 % 2.5 (16)	–	–	–	–	0 % 0 (3)	63 % 2.5 (19)
S. gawleri	–	–	–	–	60 % 2.7 (5)	–	–	–	60 % 2.7 (5)
T. pisana	58 % 3.2 (289)	31 % 2.6 (101)	48 % 2.9 (223)	30 % 4.6 (118)	28 % 2.7 (199)	13 % 1.6 (53)	24 % 6.6 (162)	1 % 1.0 (88)	36 % 3.4 (1,234)
Total	57 % 4.0 (1,387)	52 % 7.2 (603)	60 % 5.1 (598)	54 % 4.6 (1,580)	36 % 4.7 (1,522)	40 % 9.1 (334)	19 % 6.0 (239)	5 % 4.0 (169)	47 % 5.0 (6,432)

Table I. – Percentages of snails infected and mean numbers per infected snail of *B. cribbi* metacercariae 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.

Three species of introduced helicid and hygromiid snails, *C. barbara*, *C. virgata* and *T. pisana* were collected from all eight districts. With the exception of the 19 *C. barbara* collected from Western Australia, metacercariae-infected snails of these species were found in all districts. The highest prevalence rates were observed in *C. barbara* and *C. virgata* with metacercarial infection rates of 70 % and 74 %, respectively, in snails collected from the Yorke Peninsula.

The average intensity of metacercarial infection ranged from 2.5 metacercariae per infected snail for *S. australis* to 6.1 for *C. virgata*. Hyper-abundant *C. virgata* were found to have the highest mean numbers of metacercariae per infected snail with a range of 2.8 metacercariae per snail in Victoria to a high of 11.5 metacercariae per snail in the South-East of South Australia. These data however, must be interpreted with caution as seasonal variation in rates and intensity of metacercarial infection has not been assessed in this study. The majority of snails from the South-East were collected in early to mid-summer which could influence the prevalence rates. Nevertheless, this study has shown a consistent high rate of metacercarial infection in introduced helicid and hygromiid snails collected throughout southern Australia regardless of the season.

Natural definitive host animals

Twelve species of birds were examined with six species being found infected with *B. cribbi* (Table III). The Australian native little raven (C*orvus mellori* Mathews) and the introduced black bird (*Turdus merula* Linnaeus) were the only birds examined in which all animals collected were infected with *B. cribbi*. Little ravens had the highest worm counts, with one bird infected with 250 adult worms. The number of worms recovered from black birds ranged from 2-49 with a mean of 16 worms per bird. Starlings (*Sturnus vulgaris* Linnaeus) are a common flocking bird of southern Australia with large numbers present in most agricultural districts of South Australia. *B. cribbi* infection was detected in 60 % of starlings examined with the numbers of worms per infected bird ranging from two to 30 with a mean number of 11 worms. All of these three bird species were found to have whole snails or snail shell remnants in their crop indicating they were feeding on snails. Specimens of *B. cribbi* were also identified from an emu (*Dromaius novaehollandiae* Latham), chickens (*Gallus gallus* Linnaeus) and a pigeon (*Columba livia* Gmelin). Dr T.H. Cribb and Mr. M. O'Callaghan supplied these worms but did not provide details of the number of animals examined when collecting the specimens. All

Animals	Collection location	n	Number infected	Total number of worms
Birds				
Little raven, *Corvus mellori*	Yorke Peninsula, SA	10	10	358
Black bird, *Turdus merula*	Yorke Peninsula, SA	12	12	195
Starling, *Sturnus vulgaris*	Yorke Peninsula, SA	11	7	78
Emu, *Dromaius novaehollandiae*	South East, SA	*	*	10
Chicken, *Gallus gallus*	Mid North, SA	* #	* #	6
Pigeon, *Columba livia*	Wirrabee, Victoria	#	#	10
Pigeon, *Columba livia*	Yorke Peninsula, SA	3	0	0
Crested pigeon, *Ocyphaps lophotes*	Yorke Peninsula, SA	3	0	0
Magpie, *Gymnorhina tibicen*	Yorke Peninsula, SA	2	0	0
Seagull, *Larus novaehollandiae*	Yorke Peninsula, SA	2	0	0
Sparrow, *Passer montanus*	Yorke Peninsula, SA	3	0	0
Stubble Quail, *Coturnix novaezealandiae*	Yorke Peninsula, SA	7	0	0
Willy Wag Tail, *Rhipidura leucophrys*	Yorke Peninsula, SA	1	0	0
Mammals				
Mouse, *Mus domesticus*	Yorke Peninsula, SA	35	5	8
Sheep, *Ovis* sp.	South East, SA	*	*	3
Cat (feral), *Felis catus*	Barossa Valley, SA	*	*	1
Reptiles				
Shingleback lizard, *Tiliqua rugosa*	Yorke Peninsula, SA	6	5	113

Table III. – Animals examined for *B. cribbi* infections and the total numbers of worms recovered from birds, mammals and reptiles collected from South Australia (SA) and Victoria. n = number of animals examined. * = Specimens supplied by M. O'Callaghan and # = Specimens supplied by T. H. Cribb; number of animals examined uncertain.

worms examined were gravid adults and satisfied the morphological criteria for the species as described previously (Butcher & Grove, 2001).

Mice were the predominant species of mammal collected. Only eight gravid worms were recovered from five (14 %) of the 35 mice examined with a mean number of 1.6 worms and a range of 1-2 worms per infected mouse. Mr M. O'Callaghan supplied gravid adult *B. cribbi* specimens from a sheep and a feral cat. No details about the number of animals examined to recover these worms are available.

Shingleback lizards (*Tiliqua rugosa* Gray) were collected from the Yorke Peninsula. This animal is a protected Australian native lizard and therefore only a small number of animals were taken for examination. Most lizards were examined immediately and all were infected with gravid *B. cribbi* worms. The mean number of worms per infected lizard was 23 with a range of two to 50 worms. Whole snails and shell remnants were present in the stomach and intestinal tract contents of all infected lizards. One lizard was housed in the laboratory for two weeks prior to dissection. *B. cribbi* eggs were present in faecal samples of this lizard at the time of capture but after two weeks in the laboratory eggs were no longer detected and at dissection no worms were recovered.

SUSCEPTIBILITY OF LABORATORY ANIMALS

Previous studies of inbred, out-bred and immunodeficient mice (Butcher *et al.*, 2002b; Butcher *et al.*, 2002a; Butcher *et al.*, 2003) showed that C57BL/6J mice were the most susceptible immunocompetent mouse to *B. cribbi* infection with the infection persisting for 9-16 wpi whereas in NOD SCID immunodeficient mice infections persisted for the life of the animal. In this experiment C57BL/6J and NOD SCID mice showed a similar response to *B. cribbi* infection with all animals developing patent infections and gravid worms recovered at necropsy. These animals were used as controls to assess the viability of metacercariae given to the rats and ducks. Out-bred Sprague Dawley rats showed limited susceptibility with faecal egg counts negative in all animals except two in which eggs were seen transiently in the first three weeks post infection. When these animals were dissected at 5 wpi, no eggs or worms were recovered. Peking ducks did not develop patent infections with no birds excreting *B. cribbi* eggs at any stage during the observation period and no worms were recovered at dissection six weeks after infection.

DISCUSSION

All species of commonly encountered introduced and native land snails from southern Australia are susceptible second intermediate hosts for *B. cribbi*. In all districts across 3,000 km of southern Australia, snails infected with metacercariae were observed. The spread of *B. cribbi* is extensive and is most likely related to the diverse definitive host range. Both rodents and chickens have been reported as definitive hosts for *B. cribbi* (Angel & Mutze, 1987; Cribb, 1990; Cribb & O'Callaghan, 1992) and this study has shown that a range of birds (ravens, black birds and starlings) commonly found throughout southern Australia as well as various mammals and lizards are susceptible definitive hosts. The high prevalence of snails infected with metacercariae in nature would expose many animals which feed on snails to potential *B. cribbi* infection. Birds would be a likely vehicle for wide dissemination of the parasite while ground-feeding mammals and lizards spread the parasite in the local environs.

The success of the final stage in the life-cycle of *B. cribbi* from metacercaria to adult worm relies on there being a sufficient number of snails capable of being second intermediate hosts present in the environment as well as susceptible definitive host animals which feed on these snails. Second intermediate host specificity of digeneans is generally low with many species being capable of infecting a range of different molluscan lineages (Gibson & Bray, 1994; Adema & Loker, 1997). Factors influencing susceptibility of second intermediate hosts and, to a greater extent, definitive hosts are mainly driven by host ecology (Adamson & Caira, 1994; Jousson *et al.*, 2000; Jousson & Bartoli 2001). The hosts' microhabitat, range, feeding preferences, mating habits, seasonal changes in population and local climate are just a few ecological factors which can influence the susceptibility of potential second intermediate and definitive hosts. Also, extent of exposure of susceptible second intermediate host snails to cercariae from first intermediate hosts is an important consideration. The first intermediate host range and distribution of *B. cribbi* is extensive with sporocyst-infected snails present in most districts across southern Australia (Butcher & Grove, 2003). Snail species which are first intermediate hosts have been shown in this study to also be susceptible second intermediate hosts. This provides the parasite with a microhabitat for the amplification of the life-cycle where a small number of cercariae shedding snails can infect large numbers of second intermediate host species. However, the relationship between co-infection within the one snail of sporocyst and metacercariae is complex and is the subject of further studies. Helicid and hygromiid snails are found in large groups during the summer months when they aestivate on fence-posts, trees and vegetation. With the start of autumn rains, snails move *en masse* from their resting points to feed, mate and lay eggs (Smith & Kershaw, 1979; Baker,

1986; Baker, 1991). This continues over the winter and early spring and provides ideal conditions for dissemination of cercariae. The life-cycle of the snail population, and the population densities allow sufficient contact between the different species to permit cercarial infection. Seasonal conditions could also play a significant role and is currently the topic of ongoing studies.

The range of second intermediate host snails, which includes both introduced and indigenous snails does not offer any information about the origins of *B. cribbi*. It has been shown that the host specificity of digenean families can change with adaptive pressures and parasites which have been introduced to a new environment can adapt to new host systems (Boray, 1969; Lively, 1989; Adema & Loker, 1997; Cribb *et al.*, 2001). Consequently, no conclusions can be reached as to whether the original second intermediate hosts of *B. cribbi* were introduced or indigenous snails.

This study has shown that birds are a significant definitive host for *B. cribbi*. Australian little ravens (*C. mellori*), which are a common scavenger bird in many parts of South Australia, were heavily infected with *B. cribbi*. These birds have been observed sitting on snail-encrusted fence posts systematically eating the post clean of snails (unpublished observations). During the hot summer months, when helicid and hygromiid snails aestivate on fence posts, vegetation, shrubs and trees, they provides an easy food source for ravens and other scavenger birds during this dry season. Two other passeriform birds, the black bird (*T. merula*) and starling (*S. vulgaris*), which are both introduced animals to Australia from Europe, were also commonly infected with *B. cribbi*. As the origins of *B. cribbi* are still unclear (Cribb, 1990; Butcher & Grove, 2003) it can not be determined if this parasite has adapted to native little Australian ravens or to the introduced black birds and starlings. Regardless of the origins of the species, birds have provided a vehicle for the spread of parasite eggs back to the environment. Also, birds provide a mechanism for the transport of the parasite over vast distances and to islands where introduced helicid and hygromiid snails have colonised. This would account for the considerable geographical distribution of *B. cribbi* across southern Australia as all of these birds are present in the same habitats where *B. cribbi*-infected snails have been detected with the exception of starlings which are not present in Western Australia. The extent of the involvement of emus, chickens and pigeons as definitive hosts is still unclear, as the number of animals examined was very low. However, these data do show that *B. cribbi* has the ability to infect a wide host range of birds.

The low *B. cribbi* infection rate in field mice (*M. domesticus*) observed in this study was similar to that observed by Angel and Mutze (Angel & Mutze, 1987) who reported that nine of 95 mice examined were infected with what is now considered to be *B. cribbi*. Those authors indicated that the number of worms recovered ranged from one to 34 with the greatest number of infected mice being found in September/October. Seasonal prevalence of *B. cribbi* in mice was not assessed in this study, as animals were collected whenever they could be captured throughout the year. It is likely that there is variation in infection rates over the seasons which is influenced by the seasonal variation in food supply. Furthermore, laboratory susceptibility studies have shown that age, sex and prior exposure to *B. cribbi* infection can influence infection rates (Butcher *et al.*, 2002a; Butcher *et al.*, 2003). Other than the reported human infections (Butcher *et al.*, 1996; Butcher *et al.*, 1998), a cat and a sheep are the only two large mammals found to be infected with *B. cribbi*. The susceptibility of mammals other than rodents remains an area that requires further investigation.

Lewin in 1992 (Lewin, 1992) discussed the parasite fauna of the ground lizard *Lacerta agilis* in Poland and recorded that one lizard of the 150 examined was infected with a *Brachylaima* sp. There were no morphological details given to assess the identification of the *Brachylaima* recovered from the lizard and it was merely reported as being probably *B. helicis*. Our study confirms that brachylaimids can develop to a mature gravid worm in lizards as *B. cribbi* was found to infect shingleback lizards in their natural environment. It is likely, however, that it is a short-lived parasite in this host; when an infected shingleback lizard was removed from its natural environment and housed in the laboratory, the parasite was eliminated within two weeks. The mechanisms underlying this expulsion of worms are unclear. Land snails are a common food source for the large shingleback lizards in southern Australia with the hyper-abundant introduced helicid and hygromiid snails providing a constant supply of food. Whole snails and shells remnants were found in the intestinal tract contents of the lizards confirming that snails are a food source. The consumption of snails and the high prevalence of metacercariae-infected snails in nature would expose these lizards to a constant inoculum of *B. cribbi*. Furthermore, there are many other species of reptiles throughout southern Australia which need to be investigated. *Brachylaima pellucidum* (Werby, 1928) Joyeux *et al.*, 1934 and *B. erinacei* Blanchard, 1847 are the only species of *Brachylaima* previously reported to infect both mammals and birds in nature (Werby, 1928; Yamaguti, 1971). *B. cribbi* can now be further differentiated from all other brachylaimids including *B. pellucidum* and *B. erinacei* by the finding that birds, mammals and reptiles are natural definitive hosts.

In summary, this study has shown that six species of introduced European helicid and hygromiid snails and two species of Australian native land snails are suitable second intermediate hosts for *B. cribbi*. A range of mammals, birds and lizards that feed on land snails are suitable definitive host. This is the first time a *Brachylaima* species has been shown to infect mammals, birds and reptiles.

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