Acc. No.	PM no.	Species	Stage Sex	Collectors no.	Location	State	Environment	Collector	Date	Primary diagnosis	Concurrent diseases, parasites and comments	Bd Histo	Bd scrape	Extra tests
19960495	9.2	*L. tasmaniensis*	Tad		Mt. Barker	SA		MJT	23/8/96	Undiagnosed				
19960570	1	*L. lesueuri*	Adult M	N73050	Mary River	QLD	Forest	HBH	31/5/96	Chytridiomycosis	*Myxidium* sp.	Y		VI, Blood smear
19960570	2	*L. lesueuri*	Adult F	N73048	Mary River	QLD	Forest	HBH	26/5/96	Chytridiomycosis	Septicaemia? *Myxidium* sp.	Y		
19960570	3	*L. lesueuri*	Adult M	N730490	Mary River	QLD	Forest	HBH	25/5/96	Chytridiomycosis	*Rhabdias* sp.	Y		
19960570	4	*L. lesueuri*	Adult M	N73047	Mary River	QLD	Forest	HBH	25/5/96	Chytridiomycosis		Y		
19960570	5	*L. lesueuri*	Adult	N73055	Mary River	QLD	Forest	HBH	5/6/96	Chytridiomycosis		Y		VI
19960570	7.1	*Mixophyes* sp.	Tad		Scrubby Creek, Conondales	QLD	Rainforest	HBH	4/6/96	*Fibricola* sp.	Coccidia			
19960570	7.2	*Mixophyes* sp.	Tad	00121342	Conondales	QLD	Rainforest	HBH	4/6/96	*Fibricola* sp.	*B. dendrobatidis*	Y		
19960570	7.3	*Mixophyes* sp.	Tad	00121343	Conondales	QLD	Rainforest	HBH	4/6/96	*Fibricola* sp.	*B. dendrobatidis*, coccidia	Y		
19960570	7.4	*Mixophyes* sp.	Tad		Conondales	QLD	Rainforest	HBH	4/6/96	*Fibricola* sp.				
19960570	7.5	*Mixophyes* sp.	Tad		Conondales	QLD	Rainforest	HBH	4/6/96	*Fibricola* sp.	*B. dendrobatidis*	Y		
19960570	7.6	*Mixophyes* sp.	Tad		Conondales	QLD	Rainforest	HBH	4/6/96	*Fibricola* sp.				
19960570	7.8	*Mixophyes* sp.	Tad		Conondales	QLD	Rainforest	HBH	4/6/96	*Fibricola* sp.	Coccidia			
19960692	1	*L. caerulea*	Adult		Fairy Hill, Casino	NSW	Garden	D. Charley	1/7/96	Undiagnosed	Biliary hyperplasia, *Myxidium* sp. CNS protist	N		VI
19960692	2	*L. caerulea*	Adult		Fairy Hill, Casino	NSW	Garden	D. Charley	1/7/96	Chytridiomycosis	Biliary hyperplasia	Y		VI Blood smear
19960692	4	*L. caerulea*	Adult M		Fairy Hill, Casino	NSW	Garden	D. Charley	1/7/96	Chytridiomycosis	Biliary hyperplasia	Y		
19960692	5	*L. caerulea*	Adult F		Fairy Hill, Casino	NSW	Garden	D. Charley	1/7/96	Chytridiomycosis		Y		
19960961	11	*L. caerulea*	Adult F	N73086	Yamanto	QLD		HBH	29/7/96	Protistan encephalitis	Spargana, *Myxidium* sp., periportal inflammation, leech on forelimb	N		
19960961	12	*L. caerulea*	Adult F	N73072	Laidley	QLD	Rural	HBH	3/7/96	Chytridiomycosis	*Myxidium* sp., *Rhabdias* sp.	Y		
19960961	13	*L. caerulea*	Adult F	N73095	Logan Village	QLD	Urban	HBH	4/9/96	Chytridiomycosis		Y		VI
19960961	15	*L. caerulea*	Adult F	N73113	Ripley	QLD		HBH	20/9/96	Chytridiomycosis	Biliary hyperplasia, *Myxidium* sp.	Y		
19960961	16	*L. caerulea*	Adult M	N73112		QLD		HBH	6-9/96	Chytridiomycosis?		Y		
19960961	17	*L. caerulea*	Adult M	N73111		QLD		HBH		Ulcerative dermatitis	Biliary hyperplasia, *Myxidium* sp.	N		
19960961	18	*L. caerulea*	Adult F	N73073	Laidley	QLD	Rural	HBH	3/7/96	Chytridiomycosis	*Rhabdias* sp.			
19960961	19	*L. caerulea*	Adult F	N73081	Brassall	QLD	Rural	HBH	7/7/96	Chytridiomycosis	Focal *Mucor amphibiorum*	Y		
19960961	21	*L. caerulea*	Adult M	N73220	Brown's Plane Brisbane	QLD	Urban	HBH	4/1/97	Protistan myelitis	*Rhabdias* sp.	N		SEM
19960961	22	*L. caerulea*	Adult F	N73071	Laidley	QLD	Rural	HBH	3/7/96	Chytridiomycosis		Y		
19960962	1	*M. fleayi*	Adult M		Cunningham's Gap	QLD	Rainforest	HBH	5/9/96	Chytridiomycosis		Y		VI, Blood smear
19960962	10	*M. fleayi*	Adult M	N73129	Lamington	QLD	Rainforest	HBH	10/96	Undiagnosed	Healthy? *Rhabdias* sp.	N		
19960962	11	*M. fasciolatus*	Adult M	N73134	Goomburra	QLD	Rainforest	HBH	10/96	Undiagnosed	Healthy?	N		
19960962	12	*M. fasciolatus*	Adult M	N73133	Goomburra	QLD	Rainforest	HBH	10/96	Undiagnosed	Healthy?	N		
19960962	14	*M. fleayi*	Adult M	N73090	Cunningham's Gap	QLD	Rainforest	HBH	30/8/96	Chytridiomycosis	Biliary hyperplasia, Cosmocerca?	Y		

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19960962	2	*M. fleayi*	Adult M	N73100	Cunningham's Gap	QLD	Rainforest	HBH	5/9/96	Undiagnosed		N		VI
19960962	3	*M. fleayi*	Adult M	N73099	Cunningham's Gap	QLD	Rainforest	HBH	5/9/96	Undiagnosed	Healthy?	N		VI
19960962	4	*M. fleayi*	Adult M	N73123	Cunningham's Gap	QLD	Rainforest	HBH	30/8/96	Chytridiomycosis		Y		VI
19960962	5	*M. fleayi*	Adult M	N73097	Cunningham's Gap	QLD	Rainforest	HBH	5/9/96	Chytridiomycosis		Y		
19960962	6	*M. fleayi*	Adult F	N73125	Cunningham's Gap	QLD	Rainforest	HBH	2/10/96	Chytridiomycosis?		?	N	VI
19960962	7	*M. fleayi*	Adult M	N73131	Cunningham's Gap	QLD	Rainforest	HBH	10/96	Panopthalmitis		N		
19960962	8	*M. fleayi*	Adult M	N73130	Cunningham's Gap	QLD	Rainforest	HBH	10/96	Trauma to hind limb?	Slight biliary fibrosis	N		ZN –ve
19960962	9	*M. fleayi*	Adult M	N73132	Lamington	QLD	Rainforest	HBH	10/96	Undiagnosed		N		
19961136	1	*L. lesueuri*	Adult F		Eungella NP	QLD	Rainforest	R. Retallick	23/6/96	Chytridiomycosis		Y		Irido PAP
19961136	3	*L. nannotis*	Juv M		Bells Peak	QLD		R. Retallick	3/8/96	Chytridiomycosis		Y		
19961136	4	*C. signifera*	Juv M		Bool Lagoon	SA	Rural	R. Short	12/11/96	Trauma?		N	N	
19961136	6	*L. tasmaniensis*	Adult	R42959	west of Armidale	NSW		R. Sadlier	23/9/64	Multifocal dermatitis		N		
19961136	8	*L. tasmaniensis*	Adult	R42958	west of Armidale	NSW		R. Sadlier	23/9/64	Multifocal dermatitis		N		
19961136	9	*L. infrafrenata*	Adult M		Adelaide Uni	SA	Captive	R. Short	21/4/96	Severe chronic nephrosis		N		Warthin-starry
19961427	10	*Mixophyes sp.*	Tad F		Cunningham's Gap	QLD	Rainforest	HBH	6/12/96	Ulcers	Biliary hyperplasia	N		
19961427	7	*M. fasciolatus*	Adult F		Cunningham's Gap	QLD	Rainforest	HBH	2/12/96	HBC			N	
19961428	12	*Mixophyes* sp.	Tad		Cunningham's Gap	QLD	Rainforest	HBH	6/12/96	Blister				VI, PCR
19961428	14	*M. fleayi*	Met		Goomburra	QLD	Rainforest	HBH	5/12/96	Dermatitis		N	N	VI, PCR
19961428	15	*M. fleayi*	Met		Goomburra	QLD	Rainforest	HBH	5/12/96	Trauma to head				VI, PCR
19961428	16	*Mixophyes sp.*	Tad		Goomburra	QLD	Rainforest	HBH	5/12/96	Ulcerative dermatitis	*B. dendrobatidis*	Y		
19961428	18	*Mixophyes sp.*	Tad		Goomburra	QLD	Rainforest	HBH	6/12/96	White foci on tail	*B. dendrobatidis*, coccidia, Biliary hyperplasia	Y		
19961428	20	*Mixophyes sp.*	Tad		Goomburra	QLD	Rainforest	HBH	6/12/96	White foci on tail	*B. dendrobatidis*, coccidian?	Y		
19961428	7	*M. fleayi*	Adult M		Goomburra	QLD	Rainforest	HBH	2/12/96	Dermatitis	*Rhabdias* sp., *Maxvachonia flindersi*? Trypanosomes	N	N	Blood smear, VI, PCR
19961428	8	*Mixophyes sp.*	Tad		Cunningham's Gap	QLD	Rainforest	HBH	6/12/96	Ulcer	*B. dendrobatidis*	Y		VI, PCR
19961429	1	*L. dumerilii*	Adult M		Goomburra	QLD	Rainforest	HBH	3/12/96	Chytridiomycosis	*Myxidium* sp.	Y	Y	
19961429	3	*B. marinus*	Juv M		Sunny Bank	QLD	Urban	HBH	30/11/96	Mucormycosis	*Myxidium* sp. *Rhabdias* sp.	N	N	
19961429	4	*L. latopalmata*	Adult F		Mt Edwards	QLD	Forest	HBH	4/12/96	HBC		N		

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19961431	X31	*M. fasciolatus*	Met		ARC	VIC	Captive	GM	12/96-2/97	Chytridiomycosis		Y	Y	VI
19961431	10	*L. chloris*	Juv M		ARC	VIC	Captive	GM	9/12/96	Unilateral micropthalmia				
19961431	11	*L. chloris*	Juv		ARC	VIC	Captive	GM	9/12/96	Bilateral micropthalmia				
19961431	40	*N. kunapalari*	Met		ARC	VIC	Captive	GM	1/97	Chytridiomycosis	Microsporidia	Y		
19961431	41	*N. kunapalari*	Met		ARC	VIC	Captive	GM	1/97	Chytridiomycosis	Microsporidia	Y		TEM
19961431	42	*N. kunapalari*	Met		ARC	VIC	Captive	GM	1/97	Chytridiomycosis		Y		
19961431	46	*N. kunapalari*	Met		ARC	VIC	Captive	GM	1/97	Chytridiomycosis		Y		
19961431	47	*N. kunapalari*	Met		ARC	VIC	Captive	GM	1/97	Chytridiomycosis		Y		
19961431	48	*N. kunapalari*	Met		ARC	VIC	Captive	GM	1/97	Chytridiomycosis		Y		
19961431	49	*N. kunapalari*	Met		ARC	VIC	Captive	GM	1/97	Chytridiomycosis		Y		
19961431	51	*M. fasciolatus*	Met		ARC	VIC	Captive	GM	13/2/97	Undiagnosed			N	
19961431	55	*M. fasciolatus*	Met		ARC	VIC	Captive	GM	27/2/97	Acute myopathy		N		
19961431	59	*M. fasciolatus*	Met		ARC	VIC	Captive	GM	2/97	Acute myopathy		N		
19961431	62	*Lim. peronii*	Tad		ARC	VIC	Captive	GM	10/95	Undiagnosed				
19961431	65	*Lim. peronii*	Tad		ARC	VIC	Captive	GM	10/95	Undiagnosed				
19961431	66	*Lim. peronii*	Tad		ARC	VIC	Captive	GM	10/95	Undiagnosed				
19961431	88	*M. fasciolatus*	Met		ARC	VIC	Captive	GM	16/1/97	Chytridiomycosis	Hyphal mycotic dermatitis	Y		
19970574	1	*L. lesueuri*	Adult M	N73277	Goomburra	QLD	Rainforest	HBH	10/4/97	Chytridiomycosis		Y	Y	SEM
19970845	1	*M. fasciolatus*	Adult		Lone Pine KS	QLD	Captive	Vere Nicholson	30/12/96	Intestinal impaction		N		
19970845	10	*L. caerulea*	Adult F	N73318	Thagoona	QLD	Rural	HBH	2/7/97	Inflammatory dermatitis	Chytridiomycosis? *Myxidium* sp.	N	Y	
19970845	11	*L. caerulea*	Adult F	N73320	Thagoona	QLD	Rural	HBH	2/7/97	Chytridiomycosis		Y	Y	
19970845	12	*L. caerulea*	Juv F	N73321	Thagoona	QLD	Rural	HBH	2/7/97	Chytridiomycosis	Brain protists	Y	Y	
19970845	13	*L. caerulea*	Juv M		Mackay	QLD	Rural	K. Wilson	7/97	Chytridiomycosis	Septicaemia	Y		
19970845	14	*L. caerulea*	Adult F		Mackay	QLD		K. Wilson	7/97	Chytridiomycosis	*Myxidium* sp.	Y		
19970845	3	*L. ornatus*	Adult F		Lone Pine KS	QLD	Captive	Vere Nicholson	30/5/97	Undiagnosed	Parasitic nephritis	N		
19970845	4	*L. caerulea*	Adult F	N73316	Thagoona	QLD	Rural	HBH	24/6/97	Chytridiomycosis	Oligochaetes	Y	Y	SEM
19970845	6	*L. caerulea*	Adult	N73323	Borallen point	QLD	Urban	HBH	3/7/97	Chytridiomycosis	Protist in CNS? Biliary hyperplasia	Y	Y	
19970845	7	*L. caerulea*	Juv M	N73319	Thagoona	QLD	Rural	HBH	2/7/97	Biliary hyperplasia	*Myxidium* sp. Few *B. dendrobatidis*	Y	N	
19970845	8	*L. caerulea*	Juv F	N73317	Thagoona	QLD	Rural	HBH	2/7/97	Chytridiomycosis, hyphae	Biliary hyperplasia	Y	Y	
19971510	14	*P. pengilleyi*	Adult F		ARC	VIC	Captive	GM	3/11/97	Chytridiomycosis, hyphae	Immunosuppression	Y		
19971510	15	*M. fasciolatus*	Met		ARC	VIC	Captive	GM	3/12/97	Chytridiomycosis			Y	
19971697	1-6	*L. tasmaniensis*	Tad		Sthn Cross Uni	NSW	Captive	M. Healey		Undiagnosed				TEM, IPX
19971698	1	*L. caerulea*	Adult		Gracemere	QLD	Urban	JC	7/8/97	Chytridiomycosis		Y		
19971698	4	*L. caerulea*	Adult F		ARC -banana frog	VIC	Captive	GM		Chytridiomycosis		Y	Y	

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19971698	5	*M. fasciolatus*	Adult F		ARC	VIC	Captive	GM	3/96	Chytridiomycosis		Y	Y	
19971698	6	*C. platycephala*	Adult F		ARC	VIC	Captive	GM	7/96	Chytridiomycosis		Y	Y	
19971747	1	*L. pearsoniana*	Adult M		Mebbin SF	NSW	Forest	MM	~8/12/97	Chytridiomycosis	Healthy?	Y	N	
19971754	1	*M. iteratus*	Adult F		Mororo River	NSW	Forest	MM	8/12/97	Dermatitis	Trypanosomes, Cosmocerca?	N	N	Blood smear
19971754	10	*L. chloris*	Adult M		Mobong Falls	NSW	Forest	MM	11/2/98	Ulcerative dermatitis	Septicaemia, chytridiomycosis?	N	Y	TEM,IPX, VI, PCR
19971754	11	*L. chloris*	Adult F		Mobong Falls	NSW	Forest	MM	11/2/98	Ulcerative dermatitis	Chytridiomycosis?	N	Y	TEM, VI, PCR
19971754	12	*L. chloris*	Adult F		Mobong Falls	NSW	Forest	MM	11/2/98	Healing dermatitis		?	N	VI, PCR
19971754	13	*L. chloris*	Adult M		Desert Creek, Washpool	NSW	Forest	MM	11/2/98	Dermatitis		N	N	Blood smear, VI, PCR
19971754	15	*L. chloris*	Adult M		Mobong Falls	NSW	Forest	MM	11/2/98	Dermatitis	Healthy? *Sphaerechinorhynchus rotundocapitatus*	N		
19971754	16	*L. chloris*	Adult M		Mobong Falls	NSW	Forest	MM	11/2/98	Dermatitis, thin	*Myxidium* sp.	N	N	
19971754	18	*L. chloris*	Adult M		Mobong Falls	NSW	Forest	MM	11/2/98	Dermatitis	Chytridiomycosis?	Y		
19971754	2	*L. chloris*	Adult M		Bo Bo River	NSW	Forest	MM	8/12/97	Dermatitis		N	N	
19971754	20	*L. chloris*	Adult M		Mobong Falls	NSW	Forest	MM	11/12/98	Enteritis?	Chytridiomycosis	Y		
19971754	3	*L. chloris*	Adult F		Bo Bo River	NSW	Forest	MM	8/12/97	Dermatitis	Kidney lymphoma?			Blood, VI, PCR
19971754	4	*L. chloris*	Adult M		Bo Bo River	NSW	Forest	MM	8/12/97	Chronic enteritis?		N		Blood, VI, PCR
19971754	7	*L. aurea*	Tad	9750087	Taronga Zoo	NSW	Captive	Bill Hartley		Protistan myelitis				TEM
19980222	09	*M. iteratus*	Adult F		Mebbin State Forest	NSW	Forest	DA Stewart	21/2/98	Trauma			N	
19980222	10	*L. pearsoniana*	Adult	49621	Castle Spur	NSW	Rainforest	MM	18/2/98	Dermatitis		N		
19980222	12	*L. pearsoniana*			Mebbin SF	NSW	Forest	DA Stewart	21/2/98	Chronic dermatitis		N		
19980222	14	*L. chloris*	Adult F	49466	Bo Bo River	NSW	Rainforest	MM		Vesicular dermatitis		N		TEM
19980222	15	*L. chloris*	Adult M	49465	Bo Bo River	NSW	Rainforest	MM		Dermatitis		N		
19980222	3	*L. pearsoniana*		49614	Big Scrub FR	NSW	Rainforest	MM	12/2/98	Dermatitis	Healthy			
19980222	4	*L. pearsoniana*	Adult M	49615	Big Scrub FR	NSW	Rainforest	MM	12/2/98	Undiagnosed dermatitis		N		
19980222	9	*L. pearsoniana*	Adult M	49620	Castle Spur	NSW	Rainforest	MM	12/2/98	Healthy?	Dermatitis	N		
19980320	1	*M. iteratus*	Adult M		Orara East SF	NSW	Forest	MM	12/2/98	Chlamydial pneumonia		N	N	TEM, PCR, VI, BC
19980320	10	*L. spenceri*	Adult M		Taponga River	VIC	Forest	GG	12/2/98	Chytridiomycosis		Y	Y	
19980320	11	*L. spenceri*	Adult M		Snake Ck	VIC	Forest	GG	18/3/98	Chytridiomycosis		Y		
19980320	12	*L. spenceri*	Adult		Snake Ck	VIC	Forest	GG	18/3/98	Chytridiomycosis		Y	Y	
19980320	13	*L. pearsoniana*	Adult M	1	Big Scrub FR	NSW	Forest	DA Stewart	20/3/98	Healthy?			N	Blood smear
19980320	14	*L. aurea*	Adult F		Homebush	NSW	Urban	M. Christy	30/3/98	Sparganosis	Microsporidiosis, septicaemia	N	N	BC
19980320	15	*L. lesueuri*	Adult F		Goomburra	QLD	Rainforest	HBH	2/4/98	Bee/wasp ingestion?	Autolysed		N	
19980320	16	*L. caerulea*	Adult M	CH/98/05	Emerald Beach	NSW		NS	2/4/98	Sparganosis	Brain protists, *Myxidium* sp.	N	N	Blood smear
19980320	2	*L. aurea*	Adult M		Kooragang Is.	NSW	Forest	MM	12/2/98	Ulcerative dermatitis	*Rhabdias* sp.	N	N	PAP, VI, PCR, Blood smear, TEM

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19980320	25	*L. lesueuri*	Adult	CH/98/07	Orara West S.F.	NSW	Forest	J. Turnbull	26/4/98	Panopthalmitis	*Myxidium* sp.	N	N	
19980320	26	*L. caerulea*	Adult F	CH/98/8	Lismore	NSW	H School	C. Taylor	14/5/98	Basal cell tumour	*Rhabdias* sp.	N	N	
19980320	27	*L. pearsoniana*	Adult F	N73458	Cunningham's Gap	QLD	Forest	HBH	20/5/98	Chytridiomycosis		Y		
19980320	28	*L. pearsoniana*	Adult M	N73946	Kroombit Tops	QLD	Forest	HBH	30/5/98	Chytridiomycosis	Encysted trematodes?	Y		
19980320	29	*L. pearsoniana*	Adult F	N73943	Kroombit Tops	QLD	Rainforest	HBH	20/5/98	Chytridiomycosis		Y		
19980320	5	*M. iteratus*	Met M		Mebbin SF	NSW	Forest	DA Stewart	24/3/98	Trematode metacercaria		N	N	Blood smear
19980320	6	*L. lesueuri*	Adult F		Buckwong Ck	VIC	Forest	GG	29/2/98	Chytridiomycosis		Y		
19980320	7	*L. lesueuri*	Adult F		Wheeler Ck	VIC	Forest	GG	2/26/98	Chytridiomycosis		Y	Y	
19980320	8	*L. lesueuri*	Adult F		Buckwong Ck	VIC	Forest	GG	28/2/98	Chytridiomycosis		Y		
19980320	9	*L. spenceri*	Adult M		Taponga River	VIC	Forest	GG	12/2/98	Chytridiomycosis		Y	Y	
19980871	23	*L. tasmaniensis*	Adult F		South Parklands, Adelaide	SA	Urban	MJT	19/8/98	Chytridiomycosis			Y	
19980871	25	*L. caerulea*	Adult M	G/98/5	Lanitza	NSW	Rural	D. Byrnes	10/8/98	Chytridiomycosis			Y	TEM
19980871	27	*L. caerulea*	Adult F	CH/98/18	Banville	NSW	Garden	D.Page/NS	12/8/98	Chytridiomycosis			Y	
19980871	28	*L. caerulea*	Adult M	CH/98/16	Coffs Harbour	NSW	Garden	D.Page/NS	14/8/98	Sparganosis	Helminth and bacterial cholangiohepatitis, Spiruroids	N		blood smear
19980871	29	*L. caerulea*	Adult F	CH/98/15	Korora, nr Coffs H.	NSW		D.Page/NS	14/8/98	Sparganosis	*Ophidascaris pyrrhus*, *Parathelandros* sp.	N	N	blood smear
19980871	30	*L. citropa*	Adult F		Walls cave, Black heath	NSW		D. Wother spoon	1990	Ulcerative dermatitis	Autolysed	?		
19980871	31	*H. australiacus*	Adult M		Springwood, Blue Mountains	NSW		D. Wother spoon	4/98	Chytridiomycosis		Y		
19980871	33	*L. tasmaniensis*	Adult F		South Parklands Adelaide	SA		MJT	19/8/98	Chytridiomycosis	Mixed mycotic and bacterial dermatitis, *Myxidium* sp.	Y	Y	
19980871	5	*L. caerulea*	Adult	CH/98/09	Bellingen	NSW	Garden	NS	1/7/98	Chytridiomycosis			Y	
19980871	6	*L. caerulea*	Adult F	CH/98/10	Bellingen	NSW	Garden	S. Williams	9/7/98	Chytridiomycosis	*Myxidium* sp., oligochaete – inflamed ureter	Y	Y	
19980927	41	*L. moorei*	Adult M		Uni of WA Perth	WA	Tax. Gardens	Michael Smith	2/9/98	Chytridiomycosis	Mixed mycotic dermatitis?	Y		
19981159	1	*M. fasciolatus*	Adult M	N73456	Mt Glorious	QLD	Rainforest	D. Driscold via HBH	9/8/98	Chytridiomycosis	Cosmocercidae	Y	Y	
19981159	10	*A. brevis*	Adult M	N73459	Ashgrove	QLD	Urban	J. Holdway & HBH	7/98	Chytridiomycosis		Y	Y	
19981159	11	*A. brevis*	Adult F	N73465	Ashgrove	QLD	Urban	J. Holdway	15/8/98	Chytridiomycosis		Y	Y	
19981159	12	*A. brevis*	Adult	N73461	Ashgrove	QLD	Urban	J. Holdway	7/8/98	Chytridiomycosis			Y	
19981159	2	*L. gracilenta*	Adult F	N73464	Kallangur	QLD	Urban	HBH	15/8/98	Spargana	Cataract, *Myxidium* sp, immunosuppression	N	N	
19981159	20	*L. ewingii*	Adult F		Snake valley	VIC	Forest	Karl U	7/6/98	Chytridiomycosis		Y	Y	
19981159	3	*A. brevis*	Adult M	N73457	East Brisbane	QLD	Urban	S. Durtschi	10/8/98	Chytridiomycosis			Y	
19981159	4	*A. brevis*	Juv	N73463	Ashgrove	QLD	Urban	J. Holdway	10/8/98	Chytridiomycosis		Y	Y	
19981159	5	*A. brevis*	Adult M	N73467	Ashgrove	QLD	Urban	J. Holdway	7/8/98	Chytridiomycosis			Y	

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19981159	6	*A. brevis*	Adult F	N73460	Ashgrove	QLD	Urban	J. Holdway	7/98	Chytridiomycosis		Y	N	
19981159	8	*A. brevis*	Adult M	N73462	Ashgrove	QLD	Urban	J. Holdway	10/8/98	Chytridiomycosis	Nematode	Y	Y	
19981159	9	*A. brevis*	Adult	N73466	Ashgrove	QLD	Urban	J. Holdway	15/8/98	Undiagnosed			N	
19981231	1	*L. caerulea*	Juv M		ARC	VIC	Captive	GM	12/9/98	Chytridiomycosis			Y	
19981231	3	*L. caerulea*	Adult F	L/98/01	Treyone	NSW	Rural	L. Tarvey	6/98	Fungal ulcerative dermatitis		N	N	VI, PCR
19981231	4	*L. caerulea*	Adult		ARC	VIC	Captive	GM	22/9/98	Chytridiomycosis			Y	
19981231	6	*L. genimaculata*	Adult F		Topaz	QLD	Lawn	KM	27/7/98	Chytridiomycosis		Y	?	
19981231	7	*L. genimaculata*	Adult F		Topaz	QLD	Lawn	KM	27/7/98	Chytridiomycosis	Thorn in leg	Y	N	
19981331	1	*L. raniformis*	Adult F		Mt Compass	SA	Captive	MJT	30/9/98	Chytridiomycosis		Y	Y	
19981331	2	*L. dumerilii?*	Adult F		Adelaide	SA		C.Langman	21/9/98	Chytridiomycosis		Y	Y	
19981331	5	*L. ewingii*	Adult F		Woodville	SA	Urban	MJT	2/10/98	Chytridiomycosis	Nephrosis	Y	Y	
19981331	6	*L. raniformis*	Adult		Wayville	SA	Urban	MJT	2/10/98	Mycotic dermatitis	Chytridiomycosis	Y	N	
19981331	7	*L. dumerilii*	Adult M		Mt Compass	SA	Forest	MJT	15/10/98	Chytridiomycosis		Y	Y	
19981469	1	*L. peronii*	Adult F	CH/98/25	Bowraville	NSW	Urban	NS	8/98	Chytridiomycosis			Y	
19981469	10	*L. dumerilii*	Met		ARC	VIC	Captive	GM	18/11/98	Chytridiomycosis			Y	
19981469	11	*L. peronii*	Adult F	CH/98/27	Brambee	NSW	Garden	NS	20/11/98	Spargana/chytridiomycosis	Oxyurids	Y	Y	Blood smear-
19981469	12	*L. genimaculata*	Adult M		Tully Falls	QLD	Rainforest	KM & RS	14/11/98	Bacterial conjunctivitis	Vesicles in skin *Parapolystoma bulliense*	N	N	Blood smear, VI, PCR
19981469	13	*L. caerulea*	Adult F		Tully Falls	QLD	Rainforest	KM & RS	14/11/98	Ophthalmitis	*Myxobolus hylae*, Spargana	N	N	VI, PCR
19981469	2	*L. caerulea*	Adult	CH/98/24	Nth Casino	NSW	Urban	NS	3/8/98	Chytridiomycosis			Y	ELISA,
19981469	3	*L. terraereginae*	Adult F	L/98/03	Pottsville	NSW	Urban	L. Tarvey	5/98	Ingestion of spiders?		N	N	VI, PCR
19981469	4	*L. caerulea*	Adult		ARC	VIC	Captive	GM	7/11/98	Chytridiomycosis			Y	
19981469	5	*L. caerulea*	Adult		ARC	VIC	Captive	GM	9/11/98	Chytridiomycosis	Spargana		Y	
19981469	6	*L. caerulea*	Adult M			NSW	Urban	C. Taylor	28/10/98	Chronic dermatitis	Oligochaetes	N	N	IPX, Blood, VI, PCR
19981469	7.2	*C. georgiana*	Met		Uni of WA	WA	Captive	M. Smith	10/98	Undiagnosed		N		
19981469	7.3	*C. georgiana*	Met		Uni of WA	WA	Captive	M. Smith	10/98	Undiagnosed		N		
19981469	7.4	*C. georgiana*	Met		Uni of WA	WA	Captive	M. Smith	10/98	Undiagnosed		N		
19981810	1	*M. fasciolatus*	Met M		ARC	VIC	Captive	GM	17/12/98	Granulomatous dermatitis		N	N	
19981810	3	*N. dayi*	Adult M		Tully Ck	QLD	Rainforest	Alastair via KM	16/12/98	Chytridiomycosis	Monogenean trematodes	Y	Y	SEM
19990146	3	*L. caerulea*	Adult F		Bexhill	NSW	Rural	J. Morante	22/1/99	Protozoal dermatitis?		N	N	Blood, VI, PCR
19990232	1	*L. ewingii*	Adult M		?	SA	?	MJT & Jane Choat	6/1/99	Bacterial nephritis		N	N	
19990232	2	*L. tasmaniensis*	Adult M		Kadina	SA	Fish pond	MJT & Jane Choat	16/12/98	Undiagnosed	Parasites in kidney?	N		
19990232	20	*M. fasciolatus*	Met M		Lone Pine KS	QLD	Captive	PO'C	22/1/99	Obstruction	*Myxidium* sp.	N		

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19990232	21	*Lim. peronii*	Adult M		Chester hill	NSW	Suburb	L. Voigt	99	Chytridiomycosis		Y		
19990232	22.1	*M. fleayi*	Tad		Lone Pine KS	QLD	Captive	PO'C	14/3/99	Stress of handling?		N		
19990232	22.2	*M. fleayi*	Tad		Lone Pine KS	QLD	Captive	PO'C	14/3/99	Stress from handling?		N		
19990232	25	*L. tasmaniensis*	Juv M			SA	H school	MJT	16/12/98	Undiagnosed		N		
19990232	26	*L. infrafrenata*	Met			SA	Captive	MJT	20/10/98	Chytridiomycosis	Granulomatous pneumonia	Y		
19990232	28	*M. fleayi*	Tad		Lone Pine KS	QLD	Captive	PO'C	14/3/99	Unresorbed yolk sac	Bacterial yolk sacculitis	N		
19990232	29	*M. fleayi*	Tad		Lone Pine KS	QLD	Captive	PO'C	12/4/99	Stress from handling?		N		
19990232	29.1	*Lim. peronii*	Adult M			NSW		A Beezley & P Coupar	99	Fighting skin wound		N	N	
19990232	29.2	*Lim. peronii*	Adult			NSW		A Beezley & P Coupar	99	Fighting skin wound				
19990232	30	*L. dumerilii*	Adult M		Valley View	SA		MJT	12/99	Chytridiomycosis		Y	Y	
19990232	33	*L. citropa*	Juv			NSW	Captive	L. Voigt	16/5/99	Chytridiomycosis	Nematode larvae	Y	Y	
19990232	34	*L. citropa*	Juv M			NSW	Captive	L. Voigt	16/5/99	Chytridiomycosis	Nematode larvae	Y	Y	
19990232	35	*L. infrafrenata*	Met		Adelaide Uni	SA	Captive	MJT	20/10/98	Chytridiomycosis		Y		
19990232	36	*L. infrafrenata*	Met		Adelaide Uni	SA	Captive	MJT	1/22/98	Chytridiomycosis	Kidney parasite lesions	Y		
19990232	4	*L. caerulea*	Adult F			SA	Captive	MJT & Jane Choat		Chronic ulcerative dermatitis	FB cystitis, bacteria	N		
19990232	50	*L. peronii*	Adult F		ARC	VIC	Captive	GM	25/6/99	Osseous metaplasia		N		
19990951	1	*U. laevigata*	Adult M		Conondale Ranges	QLD	Captive	E. Vander duys	9/2/99	Chytridiomycosis	*Myxidium* sp.	Y		
19990951	10	*L. pearsoniana*	Adult F	N73268	U Tallebudgera Valley	QLD	Rainforest	HBH	2/4/99	Chytridiomycosis		Y		
19990951	11	*A. brevis*	Juv M	N73604	Mt Mee SF	QLD	Forest	HBH	7/1/99	Chytridiomycosis		Y		
19990951	12	*L. caerulea*	Adult	N73980	Bray Park	QLD	Suburban	HBH	19/4/99	Chytridiomycosis		Y		
19990951	13	*L. caerulea*	Adult M	N73473	The Caves	QLD	Semirural	JC & HBH	5/9/97	Chytridiomycosis		Y		
19990951	14	*L. caerulea*	Adult F	N73512	Old Toowoomba Rd, One Mile	QLD	Urban	HBH	21/4/99	Undiagnosed	Biliary hyperplasia	N		
19990951	2	*B. marinus*	Adult F	N73484	Cordalba SF	QLD	Forest	HBH	28/1//98	Mucormycosis		N		
19990951	3	*L. fletcheri*	Adult F	N73624	Cunningham's Gap	QLD	Rainforest	HBH	17/1/99	Chytridiomycosis		Y	N	
19990951	30	*L. caerulea*	Adult F		Alstonville	NSW	Rural	J. Miller	7/7/99	Chytridiomycosis			Y	
19990951	4	*L. genimaculata*	Adult M	N73411	Sth Johnston River	QLD	Rainforest	Ed Meyer, J-M Hero		Chytridiomycosis		Y		
19990951	5.4	*A. brevis*	Met	N73632	Caboolture	QLD	Semirural	HBH	12/98	Undiagnosed		N		
19990951	6	*L. terraereginae*	Adult	N73626	Little Liverpool Range, Rosewood	QLD	Rural-grazing	HBH	1/5/99	Chytridiomycosis	Septicaemia, immunosuppression	Y	Y	
19990951	7.1	*M. fasciolatus*	Tad	N73236	Goomburra	QLD	Forest	HBH	18/6/97	Biliary hyperplasia, coccidiosis	*B. dendrobatidis*	Y		

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19990951	7.2	*M. fasciolatus*	Tad	N73237	Goomburra	QLD	Forest	HBH	18/6/97	Coccidiosis	*B. dendrobatidis*	Y		
19990951	9	*A. brevis*	Adult M	N73235	Lamington NP	QLD	Rainforest	HBH	21/1/99	Chytridiomycosis	Protozoal gastritis	N		
19991385	1	*L. caerulea*	Adult F	CH/99/12	Bowraville	NSW	School	D Page/NS	2/8/99	Chytridiomycosis		Y	Y	
19991385	12	*L. caerulea*	Adult F	cap 15	North Rockhampton	QLD	Urban	JC & KT	24/8/99	Chytridiomycosis	*Rhabdias* sp.	Y	Y	
19991385	13	*L. chloris*	Adult F	N73640	Cunningham's Gap	QLD	Rainforest	HBH	24/8/99	Chytridiomycosis		Y	Y	
19991385	14	*L. gracilenta*	Adult F		ARC	VIC	Captive	GM	1/9/99	Pentastome skin nodule	Pentastomes			
19991385	2	*L. lesueuri*	Adult M		Canungra Ck	QLD		J-M Hero & Luke Shoo	4/5/99	Chytridiomycosis	Larval nematodes	Y	N	
19991385	3	*L. infrafrenata*	Adult F		Sydney Markets	NSW	Urban	Monica Wangmann	25/6/99	Septicaemia	*Batrachomyia* sp.	N	N	
19991385	35	*L. caerulea*	Adult M	cap 13	Kinka Beach	QLD	Forest	K. Taylor	28/7/99	Chytridiomycosis			Y	
19991385	9	*L. gracilenta*	Adult F		Sydney	NSW	Captive	Monica Wangmann	25/6/99	Nephroma?	Skin pox?	N	N	TEM
19991562	1	*A. brevis*	Adult M		Buderim	QLD	Garden	S. Wilson	23/8/99	Chytridiomycosis		?	Y	
19991562	21	*L. latopalmata*	Adult M		Mt Tamborine	QLD	Rainforest	P.Coupar		Spider ingestion?		N	N	
19991562	22	*L. caerulea*	Adult M	2	Dysart	QLD		P. Coupar	14/9/99	Sparganosis/ Chytridiomycosis	*Spirometra erinacei*	Y	Y	
19991562	23	*L. caerulea*	Adult F	1	Dysart	QLD		P. Coupar	23/8/99	Sparganosis	*Rhabdias* sp.	N	N	
19991562	24	*Lim. peronii*	Adult M	cap 05	Rockhampton	QLD	Urban	JC & KT	19/1/98	Mucormycosis		N	N	
19991562	5	*M. fasciolatus*	Juv M		Northcote	VIC	Captive	Jo Oakley	28/9/99	Chytridiomycosis			Y	
19991562	6	*L. pearsoniana*	Adult M	JC0026	Kroombit Tops	QLD	Rainforest	JC	23/9/98	Chytridiomycosis			Y	
19991562	7	*L. pearsoniana*	Adult	JC0125	Kroombit Tops	QLD	Rainforest	JC	23/9/99	Chytridiomycosis			Y	
19991562	8	*L. pearsoniana*	Adult M	JC0124	Kroombit Tops	QLD	Rainforest	JC	23/9/99	Chytridiomycosis			Y	
19991562	9	*L. caerulea*	Adult M		Maryborough	QLD	Forest	M. Wilson	7/9/99	Chytridiomycosis			Y	
19991743	1	*L. citropa*	Adult M		Watagan SF 6mo	NSW	Captive	R. Browne	20/9/98	Chytridiomycosis		Y		
19991743	10	*L. caerulea*	Adult F		Sydney	NSW	Captive	Monica Wangmann	13/11/99	Chytridiomycosis			Y	
19991743	2	*L. infrafrenata*	Adult F		Sydney Markets	NSW		Monica Wangmann	22/9/99	Septicaemia	Pentastomes			
19991743	3	*B. marinus*	Adult F		Crows nest	NSW	Rural	Dieter Hinz	20/10/99	Dermatitis, enteritis	Septicaemia, opthalmitis, *Rhabdias* sp.	N	N	IPX, Blood, TEM, PCR, VI
19991743	6	*L. caerulea*	Juv		Sydney	NSW	Captive	Monica Wangmann	30/10/99	Nephrosis	Heat stress?	N	N	
19991918	1	*L. aurea*	Adult F	6	Homebush Bay	NSW	Urban	Michelle Christy	22/7/97	Chytridiomycosis			Y	
19991918	13	*B. marinus*	Met M	2	Booie	QLD		A. Amey	winter	Chytridiomycosis	*Myxidium* sp.	Y	Y	
19991918	15	*B. marinus*	Met F	3	Booie	QLD		A. Amey	winter	Chytridiomycosis?	*Rhabdias* sp.	N	Y	

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19991918	16	*L. dumerilii?*	Adult F	SM/99/02	Wedderburn	NSW	Rural	H. Jessup	19/11/99	Ophthalmitis	Acanthocephalan	N	N	
19992144	1	*L. tyleri*	Adult M		Lone Pine KS	QLD	Captive	PO'C	4/10/99	Chytridiomycosis	Bacterial cystitis	Y	Y	
19992144	10	*C. georgiana*	Adult M	R140210	West Cowaramup	WA		Peter Kirkpatrick	Winter99	Chytridiomycosis	Dermocystidiosis	Y		
19992144	11	*B. marinus*	Adult F	00/01	Mt Warning Rd, Uki	NSW		L. Tarvey	24/2/00	Chronic enteritis	*Rhabdias* sp.	N	N	VI, PCR
19992144	12	*L. caerulea*	Adult M	cap 12	Bouldercombe	QLD	Rainforest / rural	JC & KT	28/1/99	Chytridiomycosis			Y	
19992144	13	*L. caerulea*	Adult F	cap 12	Bouldercombe	QLD	Rainforest / rural	JC & KT	28/1/99	Chytridiomycosis			Y	
19992144	14	*L. pearsoniana*	Adult	N73471	Kroombit Tops, TP 40	QLD	Forest	JC & B. Tangey	31/8/98	Chytridiomycosis		Y	?	
19992144	15	*M. fleayi*	Adult M	N73470	Cunningham's Gap	QLD	Rainforest	HBH	10/9/98	Undiagnosed		N	Y	
19992144	16	*L. caerulea*	Adult F	N73472	Gladstone	QLD	Captive	JC & HBH	19/5/99	Intravascular lymphoma?		N	N	
19992144	17	*N. kunapalari*	Met		ARC	VIC	Captive	GM	16/1/97	Chytridiomycosis	Microsporidiosis	Y	Y	
19992144	18	*N. kunapalari*	Met		ARC	VIC	Captive	GM	16/1/97	Chytridiomycosis		Y	Y	
19992144	2	*M. fasciolatus*	Met	2	Lone Pine KS	QLD	Captive	PO'C	20/10/99	Severe hyphal mycotic dermatitis		N		
19992144	3	*M. fleayi*	Tad		Lone Pine KS	QLD	Captive	PO'C	8/11/99	Abdominal granulomas		N		
19992144	4	*M. fasciolatus*	Tad		Lone Pine KS	QLD	Captive	PO'C	20/11/99	Biliary hyperplasia	Coccidia	N		
19992144	5	*M. fasciolatus*	Met		Lone Pine KS	QLD	Captive	PO'C	27/11/99	Chytridiomycosis		Y	Y	
19992144	6	*M. fleayi*	Tad		Lone Pine KS	QLD	Captive	PO'C	4/12/99	Abdominal granulomas		N		
19992144	7.1	*L. gracilenta*	Adult F		Sydney Markets – Flemington	NSW	Urban	Monica Wangmann	1/9/99	Septicaemia	*Rhabdias* sp.	N	N	
19992144	8	*A. brevis*	Adult F	JC 126	Kroombit Tops SF	QLD	Rainforest	JC	30/11/99	Unidentified parasites	*Rhabdias* sp.	N		
20000545	11	*L. lesueuri*	Met		Genoa River-ARC	VIC	Captive	GM	22/3/00	Chytridiomycosis			Y	
20000545	13	*L. lesueuri*	Met		Genoa River-ARC	VIC	Captive	GM	24/3/00	Chytridiomycosis			Y	
20000545	6	*L. spenceri*	Met		Taponga R- ARC	VIC	Captive	GM	19/3/00	Undiagnosed		N	N	
20000671	1	*L. aurea*	Tad		Taronga Zoo	NSW	Captive	L. Voigt	1/4/00	Undiagnosed		N		
20000671	10	*L. aurea*	Juv		Sydney	NSW	Captive	L. Voigt	20/3/00	Mycobacteriosis	Ca deficiency	N	N	
20000671	11	*L. aurea*	Juv		Sydney	NSW	Captive	L. Voigt	20/3/00	Mycobacteriosis		N	N	
20000671	13	*L. caerulea*	Adult M	G/00/01	Grafton	NSW		Gina Hart	12/3/00	Ulcerated intestine	*Myxidium* sp.	N	N	
20000671	2	*L. aurea*	Tad		Taronga Zoo	NSW	Captive	L. Voigt	1/4/00	Undiagnosed		N		
20000671	3	*L. aurea*	Tad		Taronga Zoo	NSW	Captive	L. Voigt	1/4/00	Undiagnosed		N		
20000671	6	*L. verreauxii*	Met M	4	Sthern Tablelands	NSW		L. Voigt	7/99	Chytridiomycosis		Y	Y	
20000782	11	*L. aurea*	Adult F		Hoskinstown	NSW	Forest	R. Pietsch	2/5/00	Chytridiomycosis	Spargana	Y	Y	
20000782	12	*C. signifera*	Adult		Sale	VIC	Pine plantation	GM	14/9/99	Severe hyphal mycotic dermatitis		N	N	

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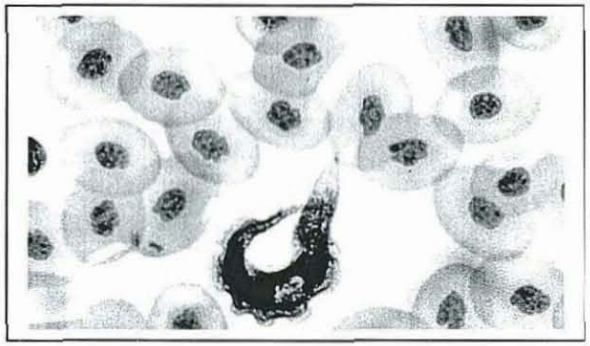
20000782	13	*C. signifera*	Adult M		Sale	VIC	Pine plantation	GM	19/9/99	Severe hyphal mycotic dermatitis		N	N	
20000782	14	*L. fallax*	Juv M		Penrith	NSW	Captive	N Mathews	1/5/00	Ca deficiency	Infected #	N	N	
20000782	15	*L. fallax*	Juv F		Penrith	NSW	Captive	N Mathews	1/5/00	Ca deficiency	Infected #	N	N	
20000782	17.5	*L. fallax*	Juv		Penrith	NSW	Captive	N Mathews	1/5/00	Ca deficiency		N	N	
20000782	21	*L. aurea*	Adult F		Hoskinstown	NSW		R. Pietsch	11/5/00	Chytridiomycosis		Y	Y	
20000782	30	*L. peronii*	Adult F		Dural plant nursery	NSW		Monica Wangmann	3/00	Vesicular dermatitis	*Myxobolus hylae*	N	N	
20001016	2	*L. aurea*	Adult F	3	Homebush Bay	NSW	Urban	M. Christy	21/7/97	Chytridiomycosis		Y	Y	
20001016	3	*L. aurea*	Adult M	10	Homebush Bay	NSW	Urban	M. Christy	28/7/97	Rhabdiasis?	*Rhabdias* sp.	N	N	
20001016	4	*L. aurea*	Adult F	11	Homebush Bay	NSW	Urban	M. Christy	28/7/97	Chytridiomycosis		Y	Y	
20001231	50	*L. caerulea*	Adult F		Qld Museum	QLD	Captive	C. Duck	1/96	Eleph beetle ingestion?	Nematode larvae			
20001266	10	*M. fleayi*	Tad	1	Lone Pine KS	QLD	Captive	PO'C	21/1/00	Parasitic granulomas	Fungal stomatitis	N		
20001266	13	*L. citropa*	Adult F		Werribee	VIC	Captive	Tim Portas	26/6/00	Chytridiomycosis		Y	N	
20001266	14	*B. marinus*	Adult F	N73673	Enoggera SF	QLD	Rainforest	HBH	4/9/99	Mucormycosis	*Mucor amphibiorum*, *Myxidium* sp, oxyurids?	N	N	
20001266	16	*L. lesueuri*	Adult F	N73676	Triunia Nat Park	QLD	Rainforest	R. Thomas & HBH	5/7/99	Chytridiomycosis		Y	Y	
20001266	17	*L. chloris*	Adult	N73514	Booloumba Ck	QLD	Forest	HBH	27/9/99	FB obstruction?		N	N	
20001266	4	*M. fleayi*	Tad	5	Lone Pine KS	QLD	Captive	PO'C	21/6/00	Granulomas- parasite				
20001266	5	*L. lesueuri*	Adult M	N73475	Six mile Ck, Yielo Bridge Rd	QLD		HBH	24/10/98	Chytridiomycosis	*Myxobolus hylae*, *Myxidium* sp.	Y	Y	
20001266	7	*L. caerulea*	Adult M	N73654	McDowell	QLD	Urban	HBH	8/12/99	Ascites	*Myxidium* sp.	N	N	
20001266	8	*M. fleayi*	Tad	4	Lone Pine KS	QLD	Captive	PO'C	3/3/00	Parasitic granulomas		N		
20001266	9	*M. fleayi*	Tad	6	Lone Pine KS	QLD	Captive	PO'C	21/1/00	Fungal stomatitis		N		
20001645	3	*L. caerulea*	Adult F	cap 19	Rockhampton	QLD	Bush property	JC & KT	21/9/00	Chytridiomycosis	Localised mucormycosis, *Myxobolus hylae* *Rhabdias* sp.	Y	Y	
20001645	4	*L. caerulea*	Adult	cap 10	Woodbury	QLD		JC & KT	20/7/99	Chytridiomycosis			Y	
20001645	5	*L. caerulea*	Adult	cap 13	Kinka Beach	QLD	Urban	JC & KT	?99	Chytridiomycosis			Y	
20001645	59	*P. corroboree*	Tad		ARC	VIC	Captive	GM		Two heads				
20001645	6	*L. caerulea*	Adult	cap 13	Kinka Beach	QLD	Urban	JC & KT	?99	Chytridiomycosis			Y	
20001645	7	*L. caerulea*	Adult	A/7/00	Grafton	NSW	Urban	D. Redman	13/1/00	Chytridiomycosis			Y	
20001645	8	*L. caerulea*	Adult		Alstonville	NSW		B. Woods		Chytridiomycosis			Y	
20001645	9	*L. caerulea*	Adult M	cap 18	Biloela	QLD	Rural	JC & KT	23/8/00	Chytridiomycosis				
20001879	1	*B. marinus*	Juv M	N73982	Pine Mountain	QLD	Semirural	HBH	19/8/00	Chronic enteritis	Acanthocephalan, *Rhabdias* sp.	N	N	TSE IPX
20001879	2	*L. peronii*	Adult F	AMH44359	Lockrose	QLD	Semirural	HBH	16/8/00	Chytridiomycosis	Nematode	Y	Y	

Acc. No.	PM no.	Species	Stage Sex	Collectors no.	Location	State	Environment	Collector	Date	Primary diagnosis	Concurrent diseases, parasites and comments	Bd Histo	Bd scrape	Extra tests
20001879	20	*M. fleayi*	Adult M	N73531	Lamington NP	QLD	Rainforest	HBH	19/7/00	Filarioidiasis	*B. dendrobatidis*? *Ochoterenella* or *Waltonella sp.*		Y	
20001879	21	*L. peronii*	Juv M	N73715	Lockrose	QLD	Semirural	HBH	7/2000	Chytridiomycosis		Y	Y	
20001879	3	*L. peronii*	Adult M	AMH44360	Lockrose	QLD	Semirural	HBH	19/8/00	Chytridiomycosis	Nephrosis	Y	Y	
20001879	30	*L. caerulea*	Adult M	N73717	Warana	QLD		HBH	18/7/00	Chytridiomycosis			Y	
20001879	31	*L. peronii*	Adult F	AMH44361	Lockrose	QLD	Rural residertial	HBH	5/8/00	Chytridiomycosis		Y	Y	
20001879	32	*L. peronii*	Juv	AMH44357	Lockrose	QLD	Semirural	HBH	7/00	Chytridiomycosis			Y	
20001879	33	*L. peronii*	Juv	AMH44358	Lockrose	QLD	Semirural	HBH	7/00	Chytridiomycosis			Y	
20001879	4	*L. peronii*	Adult M	AMH44365	Lockrose	QLD	Semirural	HBH	23/8/00	Chytridiomycosis		Y	Y	
20001879	5	*L. fallax*	Adult F	AMH44364		QLD		HBH	15/8/00	Undiagnosed		N	N	
20002051	1	*L. terraereginae*	Adult F	N73529	Camira	QLD	Urban	HBH	2/00	Ulcerative dermatits			N	
20002051	2	*T. eungellensis*	Adult		Eungella NP	QLD	Forest	GM	9/00	Chytridiomycosis			Y	
20010095	1	*L. lesueuri*	Adult		Kroombit Tops	QLD	Forest	JC	29/11/00	Chytridiomycosis		Y	Y	
20010095	10	*P. corroboree*	Adult F		ARC	VIC	Captive	GM	21/2/01	Bacterial pneumonia and peritonitis	Disrupted vitellinogenesis	N	N	
20010095	2	*L. chloris*	Adult M	TP58	Kroombit Tops	QLD	Forest	LB	29/11/00	Chytridiomycosis	*Myxidium* sp.	Y	Y	
20010095	3	*L. lesueuri*	Adult F		Eungella	QLD	Rainforest	HBH	1/12/00	Chytridiomycosis	Coccidia? *Rhabdias* sp.	Y	Y	
20010095	4	*M. fasciolatus*	Tad		Eungella	QLD	Forest	JC	4/12/00	*Fibricola* sp. Biliary hyperplasia	*B. dendrobatidis*, coccidia	Y		



Trypanosome from *Litoria nannotis* (95 656/2)

APPENDIX 2

List of helminths identified

During the disease survey, helminths were collected and sent to parasitologists for identification. This is an incomplete list as many are yet to be identified.

Key to parasitologists consulted for identification of helminths:
LS = Lee Skerratt, DB = Di Barton, RG = Robin Gasser, JS = John Sprent, AP = Adrian Pinder, IB = Ian Beveridge, XZ = Xingquan Zhu.

Accession no.	PM no.	Helminth species	Infection site	Amphibian species	Location	State	Date collected	ID by
Order Rhabditida								
19950341	6	*Rhabdias* sp.	lung	*Crinia riparia*	Paralana Springs	SA	4/7/95	
19950341	7	*Rhabdias* sp.	lung	*Crinia riparia*	Paralana Springs	SA	4/7/95	
19950341	9	*Rhabdias* sp.	lung	*Crinia riparia*	Paralana Springs	SA	4/7/95	
19960144	4	*Rhabdias* sp.	lung	*Litoria infrafrenata*	Adelaide	SA	1/12/95	
19960214	1	*Rhabdias* sp.	lung	*Litoria nannotis*	Tully	QLD	1996	
19960282	1	*Rhabdias* sp.	lung	*Litoria caerulea*	Captive	SA	8/3/96	
19960284	2	*Rhabdias* sp.	lung	*Limnodynastes dumerilii*	Adelaide University	SA	1995	
19960284	4	*Rhabdias* sp.	lung	*Litoria caerulea*	Adelaide University	SA	20/2/96	
19960284	5	*Rhabdias* sp.	lung	*Litoria infrafrenata*	Adelaide University	SA	?	
19960495	8	*Rhabdias* sp.	lung	*Litoria caerulea*	Adelaide	SA	1996	
19960570	3	*Rhabdias* sp.	lung	*Litoria lesueuri*	Mary River	QLD	25/5/96	
19960961	12	*Rhabdias* sp.	lung	*Litoria caerulea*	Laidley	QLD	3/7/96	
19960961	18	*Rhabdias* sp.	lung	*Litoria caerulea*	Laidley	QLD	3/7/96	
19960961	21	*Rhabdias* sp.	lung	*Litoria caerulea*	Brown's Plane, Brisbane	QLD	4/1/97	
19960962	10	*Rhabdias* sp.	lung	*Mixophyes fleayi*	Lamington	QLD	10/96	
19961428	7	*Rhabdias* sp.	lung	*Mixophyes fleayi*	Goomburra	QLD	2/12/96	
19961429	3	*Rhabdias* sp.	lung	*Bufo marinus*	Sunny Bank	QLD	30/11/96	
19980320	2	*Rhabdias* sp.	lung	*Litoria aurea*	Kooragang Island	NSW	12/2/98	
19980320	26	*Rhabdias* sp.	lung	*Litoria caerulea*	Lismore	NSW	14/5/98	
19990951	12	*Rhabdias* sp.	lung	*Litoria caerulea*	Bray Park, Brisbane	QLD	19/4/99	
19991385	16	*Rhabdias* sp.	lung	*Limnodynastes peronii*	Chester Hill, Sydney	NSW	7/9/99	
19991562	23	*Rhabdias* sp.	lung	*Litoria caerulea*	Dysart	QLD	23/8/99	
19991743	3	*Rhabdias* sp.	lung	*Bufo marinus*	Crows Nest	NSW	20/10/99	
19991918	10	*Rhabdias* sp.	lung	*Bufo marinus*	North Stradbroke	QLD	18/8/99	
19991918	15	*Rhabdias* sp.	lung	*Bufo marinus*	Booie	QLD	Winter 99	
19992144	11	*Rhabdias* sp.	lung	*Bufo marinus*	Mt Warning Rd, via Uki	NSW	24/2/00	
19992144	7.1	*Rhabdias* sp.	lung	*Litoria gracilenta*	Sydney	NSW	1/9/99	
19992144	8	*Rhabdias* sp.	lung	*Adelotus brevis*	Kroombit Tops	QLD	30/11/99	
20001016	3	*Rhabdias* sp.	lung	*Litoria aurea*	Homebush Bay	NSW	28/7/97	
20001645	3	*Rhabdias* sp.	lung	*Litoria caerulea*	Rockhampton	QLD	21/9/00	
20001879	1	*Rhabdias* sp.	lung	*Bufo marinus*		QLD	19/8/00	
20010095	3	*Rhabdias* sp.	lung	*Litoria lesueuri*	Broken River, Eungella	QLD	1/12/00	
Order Oxyurida								
19980871	29	*Parathelandros* sp.	rectum	*Litoria caerulea*	Korora	NSW	14/8/98	LS
19960000	2	*Parathelandros mastigurus*	rectum	*Litoria caerulea*	Mt Edwards	QLD	7/12/96	LS
Order Ascaridida								
19961428	7	*Maxvachonia flindersi*	lung	*Mixophyes fleayi*	Goomburra	QLD	2/12/96	LS
19991385	16	*Falcaustra hylae*	rectum	*Limnodynastes peronii*	Chester Hill, Sydney	NSW	7/9/99	LS
19980871	29	*Ophidascaris pyrrhus*	SI serosa	*Litoria caerulea*	Korora	NSW	14/8/98	JS & LS
Order Echinorhynchida								
19971754	15	*Sphaerechinorhynchus rotundocapitatus*	peritoneum	*Litoria chloris*	Mobong Falls	NSW	11/2/98	LS & IB
Order Polyopisthocotylea								
19981469	12	*Parapolystoma bulliense*	bladder	*Litoria genimaculata*	Tully Falls	QLD	14/11/98	DB

Accession no.	PM no.	Helminth species	Infection site	Amphibian species	Location	State	Date collected	ID by
Order Digenea								
19960570	7.1	*Fibricola* sp.	subcutis, muscle	*Mixophyes sp.*	Scrubby Creek, Conondales	QLD	4/6/96	DB
19960000	2	*Pleurogenoides* sp.	rectum	*Litoria caerulea*	Mt Edwards	QLD	7/12/96	DB
Order Pseudophyllidea								
19960284	4	*Spirometra erinacei*	muscle*	*Litoria caerulea*	Adelaide University	SA	20/2/96	RG & XZ
19960961	11	*Spirometra erinacei*	muscle	*Litoria caerulea*	Yamanto	QLD	29/7/96	RG & XZ
19980320	14	*Spirometra erinacei*	muscle	*Litoria aurea*	Homebush	NSW	30/3/98	RG & XZ
19980320	16	*Spirometra erinacei*	muscle	*Litoria caerulea*	Emerald Beach	NSW	2/4/98	RG & XZ
19980871	28	*Spirometra erinacei*	muscle	*Litoria caerulea*	Coffs Harbour	NSW	14/8/98	RG & XZ
19980871	29	*Spirometra erinacei*	muscle	*Litoria caerulea*	Korora	NSW	14/8/98	RG & XZ
19981159	2	*Spirometra erinacei*	muscle	*Litoria gracilenta*	Kallangur	QLD	15/8/98	RG & XZ
19981469	11	*Spirometra erinacei*	muscle	*Litoria peronii*	Brambee	NSW	20/11/98	RG & XZ
19981469	13	*Spirometra erinacei*	muscle	*Litoria caerulea*	Tully Falls	QLD	14/11/98	RG & XZ
19981469	5	*Spirometra erinacei*	muscle	*Litoria caerulea*		QLD	7/11/98?	RG & XZ
19991562	22	*Spirometra erinacei*	muscle	*Litoria caerulea*	Dysart	QLD	14/9/99	RG & XZ
19991562	23	*Spirometra erinacei*	muscle	*Litoria caerulea*	Dysart	QLD	23/8/99	RG & XZ
20000782	11	*Spirometra erinacei*	muscle	*Litoria aurea*	Hoskinstown	NSW	2/5/00	RG & XZ
Class Oligochaeta								
19951165	1	*Dero (Allodero) litoria*	ureter	*Litoria genimaculata*	Big Tableland	QLD	?	AP
19951165	4	*Dero (Allodero) litoria*	ureter	*Litoria caerulea*	Townsville	QLD	11/1995	AP
19981469	6	*Dero (Allodero) litoria*	ureter	*Litoria caerulea*		NSW	28/10/98	
19970845	4	*Dero (Allodero) litoria*	ureter	*Litoria caerulea*	Thagoona	QLD	24/6/97	

* See Table 13.1 for a full description of infection sites in frogs with sparganosis.

APPENDIX 3

AMPHIBIAN AUTOPSY REPORT FORM

SPECIES ..... **STAGE**..... **AGE** ..... **PM NO**..... **SENDERS NO** .....
REASON FOR PM .....**RELATED PMs** .....
LOCATION & CONTACT .....
DATE & TIME COLLECTED .....
DATE & TIME OF PM .....
ENVIRONMENT .....
TRANSIT ..... **STORAGE** ..... **EFFECT OF HANDLING?** .....
HOW DIED? ..... **TIME DEATH TO AUTOPSY?** .....
LENGTH S-V ..... **S-Ur** ..... **WEIGHT** ..... **SEX** .....
CONDITION ..... **CLINICAL SIGNS:**

AUTOPSY FINDINGS:

FORMALIN FIXED MATERIAL: (tick if collected; comment on pathology in specific organs)

Heart **A-V fat?**
Liver **Size:** **Colour:**
Gall bladder **Size:**
Spleen
Kidney
Gonads **Stage of vitellinogenesis:**
Fat bodies **Size:**
Lung
Urinary bladder
Stomach **Stomach contents:**
Duodenum
Ileum
Colon **Consistency of faeces:**
Eyes
Brain
Skin - from belly: **thigh:** **feet:**
Leg
Feet
Brain
Spinal cord

GLUTARALDEHYDE FIXED

Liver kidney lung skin brain intestine other

UNFIXED MATERIAL:
FROZEN: liver kidney lung spleen heart GIT fat muscle 1/2 carcass
PARASITES:
Skin scrape:. ..... **% Ethanol** **Chytrids?**

BACTERIOLOGY.....
BLOOD smear.....**heparin** ..... **whole** .....
OTHER.....
.....
PHOTOGRAPHY:

SIGNIFICANT FINDING:

APPENDIX 4

List of publications and copies of papers

Journal publications

Speare, R., Berger, L. O'Shea, P., Ladds, P. W. Thomas, A. D. 1997. Pathology of mucormycosis of cane toads in Australia. Journal of Wildlife Diseases. 33: 105-111.

Berger, L., Speare, R., Humphrey, J. 1997. Mucormycosis in a free-ranging green tree frog from Australia. Journal of Wildlife Diseases. 33: 903-907.

Berger, L., Speare, R., Daszak, P., Green D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G., Parkes, H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences, USA. 95: 9031-9036. [1]

Creeper, J. H., Main, D. C., Berger, L., Huntress, S., Boardman, W. 1998. An outbreak of mucormycosis in slender tree frogs (*Litoria adelensis*) and white-lipped tree frogs (*Litoria infrafrenata*). Australian Veterinary Journal. 76: 761-762.

Berger, L., Speare, R., Hyatt, A. 1999. Chytrid fungi and amphibian declines: Overview, implications and future directions. Pp 23-33. In: Declines and Disappearances of Australian frogs. Ed A. Campbell, Environment Australia, Canberra.[1]

Berger, L., Speare, R., Kent, A. 2000. Diagnosis of chytridiomycosis in amphibians by histologic examination. Zoos' Print Journal. 15: 184-190.

Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E., Speare, R. 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases. 5: 735-748.[2]

Berger, L., Volp, K., Mathews, S., Speare, R., Timms, P. 1999. *Chlamydia pneumoniae* in a free-ranging giant barred frog (*Mixophyes iteratus*) from Australia. Journal of Clinical Microbiology. 37: 2378-2380.

Mutschmann, F., Berger, L., Zwart, P., Gaedicke, C. 2000. Chytridiomycosis on amphibians - first report from Europe. Berliner und Münchener Tierärztliche Wochenschrift. 113: 380-383. [1]

Berger, L., Speare, R., Thomas, A. Hyatt, A. 2001. Mucocutaneous fungal disease in tadpoles of *Bufo marinus* in Australia. Journal of Herpetology. 35: 330-335.

Berger, L., Hyatt, A. D., Olsen, V., Boyle, D., Hengstberger, S. G., Marantelli, G., Humphreys, K., Longcore, J. E. 2001. Production of polyclonal antibodies to *Batrachochytrium dendrobatidis* and their use in an immunoperoxidase test for chytridiomycosis in amphibians. Diseases of Aquatic Organisms (in press)

Zhu, X., Beveridge, I., Berger, L., Barton, D., Gasser, R. B. Single-strand conformation polymorphism-based analysis reveals genetic variation within *Spirometra erinacei* (Cestoda: Pseudophyllidea) from Australia. Molecular and Cellular Probes. (submitted)

Internet publications

Speare, R., Berger, L., Hines, H. How to reduce the risks of *you* transmitting an agent between frogs and between sites. 14 June 1998.
http://www.jcu.edu.au/dept/PHTM/frogs/prevent.htm

Berger, L., Speare, R. What to do with dead or ill frogs. 24 June 1998.
http://www.jcu.edu.au/dept/PHTM/frogs/pmfrog.htm

Berger, L., Speare, R. Bibliography of frog diseases and declines. 28 October 1998.
http://www.jcu.edu.au/dept/PHTM/frogs/bibliog.htm

1) These publications are reproduced in the following pages.
2) Daszak et al. (1999) is available on the web:
   http://www.cdc.gov/ncidod/eid/vol5no6/daszak.htm

Chytrid fungi and amphibian declines: Overview, implications and future directions

Lee Berger[1,2], Rick Speare[2] and Alex Hyatt[1]

ABSTRACT

A recently described chytrid fungus, genus *Batrachochytrium*, killed free-living and captive amphibians in Australia, Central America and the USA. There is epidemiological, pathological, and experimental evidence that some amphibian populations suddenly declined due to mass mortalities caused by chytridiomycosis.

These were notably high altitude, stream dwelling rainforest anurans in protected areas of Queensland and Panama. Chytrid fungi caused a widespread infection of the skin resulting in hyperkeratosis, sloughing and erosions of the epidermis, and occasional ulcerations. There was minimal inflammation in the skin. Infection occurs through waterborne zoospores that invade the superficial layers of epidermis, and experimentally infected frogs became terminally ill 10–47 days after exposure. Tadpoles appear to be unaffected by the fungus which infects their keratinised mouthparts. *Batrachochytrium* can probably also survive and grow in the environment. Based on the epidemiology of the amphibian declines, chytridiomycosis appears to be an emerging disease causing mortality in many species of anurans and has caused the disappearance and presumed extinction of some species. These species may have been more vulnerable to extinction due to a combination of characteristics of their distribution and biology which suited *Batrachochytrium*, as well as rendering them less able to recover from population declines. Here we present an overview of the published and unpublished data on the amphibian chytrid fungus, discuss the implications of these findings, and suggest future directions that should be taken to investigate and manage this problem.

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INTRODUCTION

Amphibian declines in some regions have been attributed to habitat disturbance including pollution, cattle damage, fish introduction and habitat destruction such as logging and wetland degradation (Hayes and Jennings 1986, Tyler 1997). However, habitat disturbance does not explain the rapid disappearance of high-altitude stream-dwelling rainforest amphibians from many protected areas in Australia (Richards *et al.* 1993, Mahony 1996) and Central America (Lips 1998). There was no correlation between frog population declines and changes in ground level solar UV-B radiation in Queensland (Moise, unpubl. data). Several factors in the declines indicated that a waterborne infectious disease, of high virulence to adults of some species, had entered a population previously unexposed to it. These factors were:

1) sudden, severe declines occurred over a few months;
2) declines were asynchronous and spread as a front;
3) adults died while tadpoles survived and metamorphs died when they subsequently emerged;
4) no environmental changes were detected;
5) only stream dwelling frogs disappeared and,
6) in two intensively monitored sites, mass mortalities were observed at the time of significant population declines (Laurance *et al.* 1996, Lips 1999, Trenerry *et al.* 1994).

In these two montane rainforest locations — Big Tableland, Australia (1993) and Fortuna, Panama (1997) — sick and dying anurans (including *Taudactylus acutirostris, Litoria rheocola* and *L. nannotis*) were collected for pathological examination and found to be infected with chytrid fungi in the skin (Berger *et al.* 1998). This fungus has been placed in a new genus, *Batrachochytrium* (Longcore *et al.* 1999).

Along with the epidemiological evidence above, we present pathological and experimental data that demonstrate that chytrids are pathogenic to amphibians. The pattern of the population declines is consistent with being caused by *Batrachochytrium* as it is waterborne, is virulent to adults, does not kill tadpoles (Berger *et al.* 1999), prefers cooler temperatures (Longcore *et al.* 1999), and is not dependent upon the highly susceptible species for its continued existence. Similar waves of mass mortalities, described as the post-metamorphic death syndrome, have been reported in various amphibian populations in western North America (Scott 1993). Although the cause(s) was not determined, chytridiomycosis has recently been discovered in populations of endangered north American frogs, including leopard frogs (*Rana yavapiensis* and *R. chiricahuensis*) in Arizona (Nichols *et al.* 1998, Morell, 1999) and chytrids were also seen as incidental findings in six percent of a group of wild cricket frogs (*Acris crepitans*) in Illinois (Pessier *et al.* 1999).

Although *Batrachochytrium* has a broad amphibian host range and is currently widespread, not all susceptible species have declined. The selectivity of the declines may be due to a combination of environmental factors and host biology that provide the necessary conditions for expression of disease, as well as rendering species less able to recover after population crashes. Declining species from high altitude rainforests have restricted ranges and smaller clutch sizes (Williams and Hero 1998).

In this paper we collate the data on the amphibian chytrid and expand on previously presented hypotheses, with a focus on Australian circumstances.

BIOLOGY OF *BATRACHOCHYTRIUM* AND THE CHYTRIDIOMYCOTA

The amphibian chytrid has been placed in a new genus, *Batrachochytrium* (Phylum Chytridiomycota, Class Chytridiomycetes, Order Chytridiales) and an isolate from a captive blue poison dart frog (*Dendrobates azureus*) that died at the National Zoological Park in Washington has been described as *B. dendrobatidis* (Longcore *et al.* 1999). The ultrastructural morphology, amphibian host and 18S rDNA sequence of *Batrachochytrium* show that it is distinctly different from other chytrid fungi (Berger *et al.* 1998, Longcore *et al.* 1999).

Chytridiomycete fungi are a large and diverse group and have been found in almost every type of environment, including rainforests, deserts and arctic tundra (Powell 1993). They are frequently found in soil and water where they digest substrates such as chitin from insect cadavers, cellulose from vegetable matter, keratin from hair and skin, or pollen. These species function as important primary biodegraders and are possibly vital to the ecosystem. Others are parasites of insects, fungi, algae, plants and nematodes and a few of these cause significant disease (Barr 1990, Powell 1993).

Powell (1993) discusses the significance and inherent value of chytridiomycetes and reviews the ability of parasitic species to cause disease. The onset of chytridiomycete parasitism of phytoplankton is often correlated with a rapid decline in host population and so has a major impact on the ecology of the host. *Synchytrium endobioticum* causes black wart disease of potatoes in Europe and Canada, and was introduced to the USA in the early 1900's but has since been eradicated. *Coelomomyces* has been considered for use in biological control of mosquitoes. Apart from species found among the normal rumen flora of ruminants, chytridiomycetes have not been found in vertebrates other than amphibians (Barr 1990, Berger *et al* 1998).

Sparrow (1960) describes the evanescent nature of chytrid epidemics, with their sudden appearance, brief period of rapid multiplication and then decline and disappearance. This pattern is related to their virulence, ability for rapid reproduction, and the loss of optimal environmental conditions. Factors affecting the epidemiology of chytrid blooms include seasonal temperature changes, water pH, light, nutrition and dissolved oxygen (Sparrow 1968). These may be relevant considerations when attempting to isolate *Batrachochytrium* from the environment and when investigating the causes of outbreaks of chytridiomycosis. For example, epidemics in populations of *Litoria caerulea* in southern Queensland and northern NSW occurred in the winters of 1996, 1997 and 1998, demonstrating seasonal regularity (Table 1).

Findings from studies of other aquatic zoosporic fungi may be pertinent here. The abundance of Saprolegniaceae in California was correlated with altitude (Sparrow 1968). As aquatic phycomycetes are probably very sensitive to contaminants they are considered good biological indicators of pollution (Sparrow 1968).

Most chytrids (i.e. members of the order Chytridiales) occur in aquatic habitats. They have motile flagellated zoospores which develop within a stationary sporangium. Sporangia of some species form one or more discharge tubes through which the zoospores are released. Zoospores often display chemotaxis towards their particular substrate enabling them to reach hosts or nutrients in the vicinity which are not abundant, although water flow is probably the main method of dissemination (Sparrow 1968). Zoospores of *Batrachochytrium* are waterborne, can live for over 24 hours (Berger, unpubl.) and are infective to frogs and tadpoles. Zoospores of many fungi produce an adhesive as they encyst on their host (Bartnicki-Garcia and Sing 1986). Encysted zoospores of Batrachochytrium in culture take 4-5 days to grow into mature sporangia containing numerous zoospores (Longcore *et al.* 1999). Sporangia of *Batrachochytrium* grow in the keratinised epidermis of amphibians, but as they can be grown in culture and grew on boiled snake skin (keratin), they may also be able to exist and proliferate as saprobes in the environment (Longcore *et al.* 1999). Rhizoids supply the developing sporangia with nutrients, and are formed whether the sporangia are in the epidermis or in culture (Longcore *et al.* 1999). *Batrachochytrium* is inoperculate and develops either monocentrically or colonially (Longcore *et al.* 1999).

Some chytrids have a thick walled, resistant resting spore stage which can survive for decades in extreme conditions (Powell 1993) but such a stage has not been observed in *Batrachochytrium* (Longcore *et al.* 1999), which may be a relatively fragile species.

Culture media for the amphibian chytrid contained tryptone, gelatin hydrolysate and lactose (Longcore *et al.* 1999). In culture *B. dendrobatidis* developed most rapidly at 23C and grew at 28C, but did not grow significantly at 29C (Longcore *et al.* 1999). Cultures grew well at 15C and survived for more than three months at 4C (Longcore, unpubl. data). Chytridiomycetes do not generally survive freezing well, although some success with storage in liquid nitrogen has been achieved (Hohl and Iselin 1986). Species without resting spores are less able to be preserved in an inactive state (Hohl and Iselin 1986).

No significant ultrastructural morphological differences were observed between isolates from Australia, the USA and Central America (Longcore *et al.* 1999, Berger *et al.* 1998) and DNA comparisons are needed to determine how many amphibian chytrid species exist. It is likely all isolates belong to a single species. The 18S rDNA sequence of chytrids from a wild caught Australian *L. caerulea* and a captive American *D. azureus* had only five base pairs different out of about 1700 bp sequenced, and four of these differences were deletions which may be due to error (James, Porter and Longcore, unpubl.). Preliminary sequencing of a more variable region, the rDNA internal transcribed spacers (ITS), demonstrates that similar strains (<2% sequence divergence) can infect a range of Australian frog species (Morgan, unpubl.). More studies are needed to define significant variations.

DISTRIBUTION

In Australia, *Batrachochytrium* has been found in frogs since 1989 and has been observed in various regions — including rainforests of southern, central and northern Queensland and

Figure 1: Map of Australia with stars indicating where Batrachochytrium has been found on amphibians. This distribution correlates with searching intensity.

northern NSW, montane and foothill forests of Victoria, and suburban/semi-rural populated areas of Brisbane, Adelaide and Perth (Figure 1, Table 1). Captive axolotyls from Townsville (Speare, unpubl.) and Perth (Aplin and Kirkpatrick, unpubl.) were also found with chytridiomycosis.

Overseas, chytrids have been found in rainforest anurans in montane Panama in 1997 (Berger *et al.* 1998), in captive frogs in National Zoo, Washington DC and other American zoos since 1988 (Nichols *et al.* 1998, Pessier *et al.* 1999), and in wild frogs in Arizona (Nichols *et al.* 1998) and Illinois (Pessier *et al.* 1999). Recently, chytridiomycosis was diagnosed in anurana that died in high altitude regions of Equador, including adults of *Telmatobius niger* collected in 1989 from the Azuay province, adults of *Atelopus* sp. (bomolochos complex) collected in 1991 from the Riobamba province and captive metamorphs of *Gastrotheca pseustes* that died in 1999 (Merino-Viteri and Coloma, unpubl.). As investigations into frog disease become more common, the known distribution of the amphibian chytrid is likely to greatly expand, and by examining archived specimens the dates of first occurrence will be pushed back. Investigating the origin and historical range of the amphibian chytrid may determine whether it has been introduced or is endemic.

Apparently healthy frogs can exist as carriers; clinically normal frogs (including *L. genimaculata*) collected from the wet tropics in Queensland have been found with low levels of infection (Speare and Freeman, unpubl.). Although *Batrachochytrium* has not yet been found in non-amphibian hosts, it is possible that other cold-blooded animals with keratinised surfaces could harbour infections and play a role in the persistence and spread of the disease. As the chytrid is susceptible to desiccation (Berger, unpubl.), transportation of the disease long distances is most likely to have occurred via the movement of amphibians, either deliberately (for example in the pet trade), or accidentally (such as in fruit boxes). Both these scenarios have been observed and epidemics in captive collections have occurred after the arrival of a few infected frogs (Marantelli, pers. comm.). Cane toads are also potential

TABLE 1: Species, locations and dates of amphibians in Australia found infected with Batrachochytrium.

Hylidae				
Litoria adelaidensis	Perth, WA	Aug 1998	#	J
Litoria caerulea	Casino, NSW	July1996	*	A 96/692
	Brisbane, Qld	July–Sep1996	*	A 96/961
	Brisbane, Qld	June–July 1997	*	A 97/845
	Rockhampton, Qld	Aug 1997		A 97/845
	Mackay, Qld	Sep 1997		A 97/845
	Bellingen, NSW	July 1998	*	A 98/871
	Lanitza, NSW	Aug 1998		A 98/871
Litoria chloris	Dorrigo Plateau, NSW	Dec 1997–Feb 1998		A 97/1754
Litoria citropa	Captive, Sydney	May 1999		A 99/232
Litoria ewingi	Woodville, SA	Oct 1998		A 98/1331
Litoria genimaculata	Kirrama State Forest, Qld	Nov 1989		A 98/927
	Windsor Tableland, Qld	April 1993		J
	Topaz, Qld	July 1998		A 98/1231
	Wright's Creek, Qld	Oct 1998		J 98/398
	O'Keefe Creek, Qld	Dec 1998		J 99/28
Litoria infrafraenata	Captive, Adelaide, SA	Oct 1998		A 99/232
Litoria lesueuri	Mary River, Qld	May-June 1996	*	A 96/570
	Eungella NP, Qld	June 1996		A 96/1136
	Goomburra, Qld	April, 1997		A 97/574
	Buckwong Ck, Vic	Feb 1998	#	A 98/320
Litoria moorei	Perth, WA	Sept 1998	#	A 98/927
	Ferndale, Perth, WA	Nov 1998	#	J 99/12
	Manjimup, WA	Nov 1998	#	J 99/12
	Mt Barker, WA	Nov 1998	#	J 99/12
	Mt Helena, WA	Nov 1998	#	J 99/12
	Sawyers Valley, WA	Nov 1998	#	J 99/12
	Witchcliffe, WA	Nov 1998	#	J 99/12
Litoria nannotis	Big Tableland, Qld	Oct 1993	*	J 93/290
Litoria pearsoniana	Mebbin State Forest, NSW	Dec 1997		A 97/1747
	Kroombit Tops, Qld	May 1998		A 98/320
Litoria raniformis	Captive, Adelaide, SA	Sep 1998		A 98/1331
Litoria rheocola	Big Tableland, Qld	Oct 1993	*	J 93/290
(tadpoles & adults)	Westgid Ck, Qld	May 1999	®	J
(tadpoles & adults)	Frenchman Ck, Qld	July 1999	®	J
Litoria spenceri	Bogong Ck, Vic	March 1996		A 96/370
	Taponga River, Vic	Feb–March 1998	*	A 98/320
Nyctimystes dayi	Tully, Qld	Dec 1998		A 98/1810
Myobatrachidae				
Adelotus brevis	Brisbane, Qld	Nov 1995		A 99/927
	Brisbane, Qld	Aug 1998		A 99/927
	Lamington NP, Qld	Jan 1999		A 99/951
Crinia pseudinsignifera	Sawyers Valley, WA	Nov 1998	#	J
Heleioporus australiacus	Springwood, NSW	May 1998		A 98/871
Heleioporus eyrei	Captive, Perth, WA	Nov 1998	#	J
Limnodynastes dorsalis	Woodlands, Perth, WA	May 1998	#	J
Limnodynastes dumerilii	Goomburra, Qld,	Dec 1996	*	A 96/1429
	Mt Compass, SA	Oct 1998		A 98/1331
	Captive Melbourne, Vic	Nov 1998		A 98/1469
	Valley View, SA	Jan 1999		A 99/232
Limnodynastes tasmaniensis	Adelaide, SA	May–June 1996	*	A 96/495
	Captive, Adelaide	Aug 1998		A 98/871
Lechriodus fletcheri	Cunningham's Gap, Qld	Jan 1999		A 99/951
Neobatrachus kunapalari	Captive Melbourne, Vic	June–Aug 1996		A 96/1431
Mixophyes sp. (tadpoles)	Goomburra	Dec 1996		A 96/1428
Mixophyes fasciolatus	Captive Melbourne, Vic	Dec 1996–Feb 1997	*	A 96/1431
	Mt Glorious, Qld	Aug 1998		A 98/1159
Mixophyes fleayi	Cunningham's Gap, Qld	Aug–Oct 1996	*	A 96/ 962
Pseudophryne pengilleyi	Captive, Melbourne, Vic	Nov 1997		A 97/1510
Taudactylus acutirostris	Carbine Tableland, Qld	Nov 1990		A 98/927
	Big Tableland, Qld	Nov 1993–Jan 1994	*	J 93/290
	Captive Townsville, Qld	Dec 1993	*	J 93/290
Taudactylus eungellensis	Eungella National Park, Qld	Oct 1995	*	A 96/657
Bufonidae				
Bufo marinus	Captive Geelong, Vic	June–Aug 1996	*	A 96/673
Ambystomatidae				
Ambystoma mexicanum	Captive Townsville, Qld	Feb 1999		J

Accession numbers are provided: "A" refers to specimens deposited at AAHL, "j" refers to specimens deposited at JCU.

* denotes records from Berger *et al.* 1998.
\# denotes records from Aplin *et al.* 1999
" denotes records from Retallick 1999

carriers as they continue to expand their distribution in Australia, as evidence suggests that free-ranging toads are infected with chytrids[1] (Parkes, pers. comm.).

We hypothesise that *Batrachochytrium* was introduced to Australia in the 1970's around Brisbane (where the first precipitous declines occurred), although as yet there are no hard data to support this assumption. *Batrachochytrium* has since become established in many areas on the east coast, around Adelaide and in south west Western Australia.

CHYTRIDIOMYCOSIS: THE DISEASE

Typical clinical signs in Australian frogs with chytridiomycosis were lethargy, inappetence, skin discolouration, presence of excessive sloughed skin, and sitting unprotected during the day with hind legs held loosely to the body (Berger, Speare and Marantelli, unpubl.). Frogs became moribund in terminal stages with loss of righting reflex, and death usually occurred a few days after the onset of clinical signs. Most diseased frogs were in reasonable body condition and 45 out of 69 were considered to have moderate or large fat bodies. Gross pathology of internal organs was generally unremarkable. Some notable species variation in the clinical signs was observed. The rainforest frogs from Big Tableland, northern Queensland (Speare 1994) predominantly showed neurological signs; most commonly, abnormal sitting posture with hind legs adducted, lethargy, and slow response to tactile stimuli. When handled, these frogs (*Litoria rheocola*, *L. nannotis*, *Taudactylus acutirostris*) became rigid and trembled with extension of the hindlimbs and flexion of the forelimbs. Less common signs included minute skin ulcers and haemorrhage in skin, muscle or eye. Many frogs appeared anaemic, with pale muscles and internal organs. In contrast, individuals of *Litoria caerulea* often became intensely red on the belly, legs and feet. These frogs had marked congestion and reddening of organs internally, and skin ulcers were occasionally seen (Berger and Hines, unpubl.) Captive metamorphs of *Mixophyes fasciolatus* became slow and sloughing skin accumulated over the body (Figure 2). The presence of shedding skin on the body is a nonspecific sign which is seen in other diseases where frogs become lethargic and fail to wipe themselves, but with chytridiomycosis, excessive shedding may occur in response to infection of the skin.

In frogs with chytridiomycosis, light microscopy of unstained pieces of sloughing skin revealed masses of round to oval refractile fungi (Figure 3), enabling rapid diagnosis. On histologic examination, chytrid fungi were observed inhabiting the outer keratinised layers of the epidermis (Figure 4) and were associated with thickening or erosions and occasional ulcerations of the skin (Berger *et al.* 1998, Pessier *et al.* 1999). Different frogs varied greatly in their intensity of infection, but skin of the ventral body, limbs and feet were most consistently infected. Bacteria often colonised the layers of sloughing keratin. There was negligible inflammatory response in the dermis. Scanning electron microscopy of diseased skin revealed fungal discharge tubes emerging through the surface of the skin and confirmed

1 *Fourteen out of 63 wild-caught juvenile toads from Lennox heads, NSW which were brought into AAHL in june 1996 died between 1 and 44 days after arrival and chytridiomycosis was diagnosed in 5 which were examined histologically (Parkes, unpubl. data).*

FIGURE 2: Captive-bred metamorph of Mixophyes fasciolatus (AAHL accession no. 96/1431) from the Amphibian Research Centre with naturally acquired chytridiomycosis. This frog is in the terminal stages of the disease - note depressed attitude, half closed eyes and accumulations of sloughed skin over the body (arrow head). Bar = 0.5 cm.

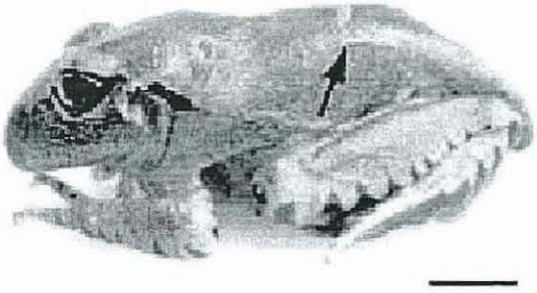


FIGURE 3: Unstained skin slough from a dead adult of Litoria caerulea from Thagoona, Qld (AAHL accession no. 97/845) examined by light microscopy. Note refractile round and oval chytrid fungi. Most are empty, but one contains developing zoospores (arrow). Occasionally empty sporangia are seen divided by thin septae. E = epidermal cell. Bar = 20 μm.

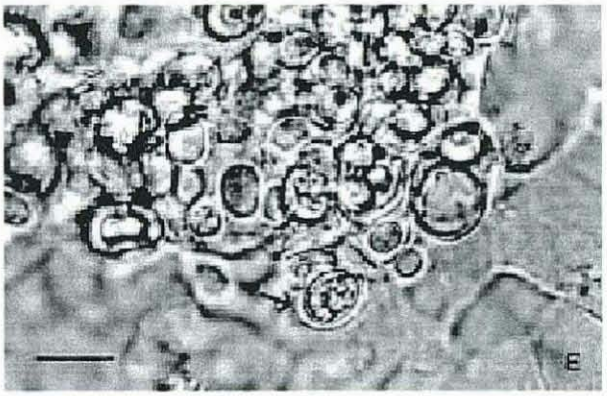


FIGURE 4: Histological section of heavily infected skin from the toe of a wild-caught adult of Litoria caerulea (AAHL accession no. 97/845). Batrachochytrium does not invade through the epidermis but occurs just under and within the superficial keratinised layer which becomes thickened. Solid, immature sporangia are present (arrow), as well as mature sporangia containing numerous dark zoospores (Z). Many sporangia are empty after the flagellated zoospores have swum out through the discharge tube (below the D). Immature stages are present in the superficial layer of viable epidermal cells and older, empty stages are commonly seen in the outer dead layers of keratin which are sloughed. Batrachochytrium does not form hyphae. (Haematoxylin & eosin). E = epidermis, K= keratin. Bar = 50 μm.

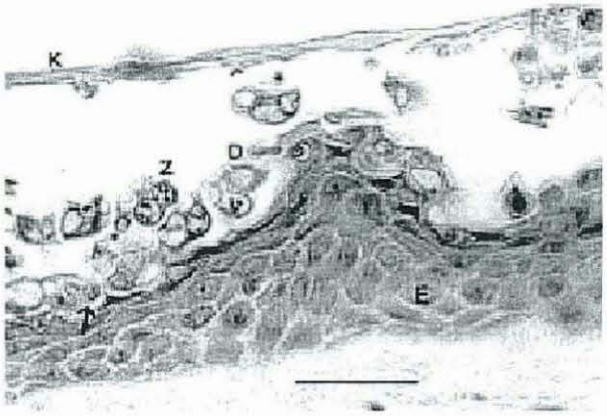
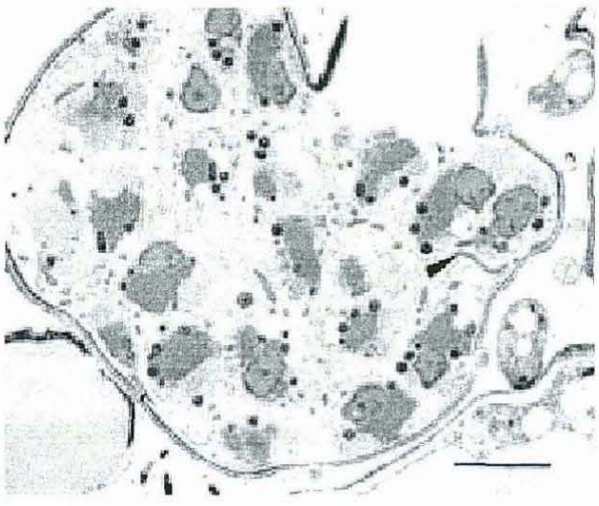


FIGURE 5: Scanning electron micrograph of skin from an infected adult of Litoria lesueuri from Goomburra, Qld (AAHL accession no. 97/5741), showing fungal discharge tubes protruding through the surface of epidermal cells. Bar = 10μm.

FIGURE 6: Scanning electron micrograph of skin from the toe of an infected adult of Litoria lesueuri (AAHL accession no. 97/5741) with extensive peeling and degeneration of the keratinised skin surface. The surface of healthy skin from control frogs appeared smooth. Bar = 100μm.

FIGURE 7: Transmission electron micrograph of a mature sporangium cultured from the skin of a sick Nyctimystes dayi from Tully, Qld (AAHL accession no. 98/18103). It is filled with developing zoospores, each with a single flagellum (arrow head). The discharge tube is closed with a gelatinous plug which deliquesces when the zoospores are ready to be released. Bar=3μm.



hyperkeratosis and extensive sloughing of the epidermis (Figures 5 and 6). The internal structure of *Batrachochytrium* was revealed by transmission electron microscopy (Figure 7).

The lack of inflammation in the skin could be due to a lack of stimulation of the host immune system – perhaps due to the superficial site of infection, insufficient epidermal damage, or the chytrid may have low inherent antigenicity.

There were few specific internal lesions in sick frogs, suggesting that the ultimate cause of death was metabolic or toxic. Focal necroses, vacuolation, or cloudy swelling were sometimes apparent in a range of internal organs (Speare and Berger, unpubl.).

Histologic examination of organs involved in immunity — i.e. spleen and bone marrow, revealed no evidence of immunosuppression, apart from in one frog. Tests (including viral and bacterial culture, electron microscopy and haematology) for infectious organisms did not detect any other significant pathogens. Of 147 wild and captive frogs with chytridiomycosis, concurrent disease was diagnosed in 16 (11%). However, apart from one frog with immunosuppression, these diseases were not considered to be the primary cause of death. The concurrent diseases included septicaemia (four frogs), microsporidial hepatitis (one frog) and hyphal mycotic dermatitis (two frogs), all of which may have occurred secondary to chytridiomycosis. The other diseases - biliary hyperplasia and/or fibrosis (six frogs), foreign body myositis (one frog) and mild, localised mucormycosis (one frog) — all appeared chronic and inactive and may not have contributed to the deaths. A variety of helminth and protozoan parasites were identified as incidental findings (Berger and Speare unpubl.).

During our survey of amphibian diseases in Australia, 272 wild and captive sick frogs were examined between 1989 and 1999, but the only disease that was found consistently was chytridiomycosis which occurred in 54% and accounted for almost all cases of unusual mortality in the wild. A range of diseases was diagnosed in individual sick frogs that did not have chytridiomycosis, including viral, bacterial, protozoal, fungal, tapeworm, neoplastic, traumatic and congenital diseases, and inappropriate ingestion (Berger and Speare, unpubl.).

Batrachochytrium is not a ubiquitous parasite. In a histological survey of toeclips from 348 apparently healthy frogs from Queensland and Victoria collected between 1989 and 1999 only 7 (2.0%) were infected (Speare, Berger and Kent, unpubl.).

In experimental infections in Australia using *Mixophyes fasciolatus* a terminal illness was reached in 6/6 frogs 10–18 days after exposure to infected skin scrapings at 24C (Berger *et al.* 1998). In the USA, 2/2 *Dendrobates tinctorius* died 23 and 31 days after being exposed to broth culture of *B. dendrobatidis* (Longcore *et al.* 1999). Small doses of the pathogen have now been shown to cause fatal chytridiomycosis in metamorphs of *Mixophyes fasciolatus* — 3/3 frogs each exposed to an estimated 1000 zoospores died or became terminally ill between 23 and 38 days post exposure, 3/3 frogs exposed to approximately 100 zoospores died between 35 and 47 days post exposure, however 3 frogs exposed to approximately 10 zoospores did not succumb to chytridiomycosis and 2 have remained healthy for over 3 months (the other frog died by misadventure) (Marantelli and Berger unpubl.). The metamorphs were

housed individually and were infected by bath exposure to zoospores (cultured from a captive *Limnodynastes dumerilli*) in 10ml water for 24 hours.

In epidemics in captive collections of *M. fasciolatus* and *Bufo marinus*, almost all tadpoles survived to metamorphose, but suffered high mortality rates 2-3 weeks after metamorphosis (Berger *et al.* 1998). Tadpoles were found infected with *Batrachochytrium*, but were never found dead or dying as a consequence of infection (Berger *et al.* 1998). Tadpoles were infected in the mouthparts (see Berger *et al.* 1999), the only keratinised area of tadpole skin. After metamorphosis, the skin over the body becomes keratinised allowing the fungal infection to spread. The resistance of tadpoles to chytrid fungi is consistent with the epidemiology of the amphibian declines where tadpoles have been seen after adults have disappeared (McDonald and Alford 1999, Lips 1999). During the declines in Panama in 1997, many abnormal tadpoles were seen with partial or complete loss of keratinised mouthparts, although tests for fungal infection in these animals were not reported (Lips 1999). Tadpoles with abnormal mouthparts have not been observed in Australia.

Two theories have been proposed to explain how a fungus restricted to the superficial epidermis could kill frogs (Berger *et al.* 1998, Pessier *et al.* 1999). The first hypothesis is that the chytrid releases proteolytic enzymes or other active compounds that are absorbed through the permeable skin of the frog. The second hypothesis is that damage to skin function results in disturbance of water or electrolyte balance resulting in death. We tend to favour the first explanation. Anurans have evolved to manage water stress by shifting water and electrolytes from the brain and cerebrospinal fluid and can withstand levels of dehydration that would be fatal to other classes of vertebrates (Hillman 1988). The epidermal damage caused by chytridiomycosis does not appear severe enough to result in the major changes in water and electrolyte balance required to cause death.

DISCUSSION AND IMPLICATIONS

The observations of high mortality rates resulting in the disappearance of populations suggests there has been some recent change in the balance between host and pathogen. Three explanations are suggested:

1. *Batrachochytrium* has been introduced recently to these areas,
2. it has suddenly become more pathogenic to the host, or
3. the frogs have a lowered resistance due to environmental changes allowing the endemic parasite to cause mass mortalities.

The first explanation seems the most plausible, due to the epidemiological factors already discussed, and acute or chronic environmental problems in these protected rainforest areas were not detected (Richards *et al.* 1993). The amphibian chytrid fungus may have originated by the escape of a pathogen from a local environment, transfer to new hosts, followed by rapid progression through these non-essential hosts. Two recent examples of emerging infectious diseases following this pattern are Ebola virus in Africa and *Vibrio cholerae* 0139. The first has had several episodes of escape, from an as yet undetected reservoir host into the human population of Zaire (Dobson and Carper 1996), while the second, an environmental bacterium, arose from the Bay of Bengal in 1992 and has spread rapidly through the human population of Asia (Lee *et al.* 1996).

Once a new pathogen has emerged, the rapid global transport by air of people, goods and animals will assist in its dissemination both within and between continents.

If long-term degradation of the environment was the key problem in declines of amphibian populations, we would expect reproductive and nutritional status to be affected before fatal immunosuppression occurs. However, moribund frogs were found which were gravid (Mahony 1996), and we have seen many with adequate fat reserves. Also, with severe immunosuppression a range of opportunistic infections is likely to be involved, rather than a solitary chytrid fungus. The experiments in captivity demonstrate that the chytrid fungus can cause 100% mortality in conditions where uninfected animals remained healthy. There is no evidence that predisposing immunosuppression is necessary for epidemics of chytridiomycosis to occur.

Although *Batrachochytrium* has a wide distribution and broad host range, we suspect only some species had the necessary combination of characteristics to render them vulnerable to decimation by disease. The declining species from high altitude rainforests in eastern Australia have significantly smaller clutch sizes, occupy restricted geographic ranges, have aquatic larvae associated with streams, and many spend a large proportion of their time in or adjacent to streams (Williams and Hero 1998, McDonald and Alford 1999). Populations of these species are therefore less able to recover from declines due to any cause, and also inhabit environments that would support *Batrachochytrium* i.e. cooler, riparian habitats.

Other aspects of host biology, genetic factors (see Leberg and Vrijenhoek 1994), humidity and complex ecological factors affecting the lifecycle of the chytrid, could be important in determining the selectivity of the declines.

As *Batrachochytrium* can probably exist as a free-living organism in the environment (Longcore *et al.* 1999), can be carried by healthy tadpoles and has a broad host range (Berger *et al.* 1998), this may explain an ability to persist and cause disease even when the density of adults from particular species has been greatly reduced (Daszak *et al.* 1999).

Although most of the frog species that have disappeared from Queensland rainforest since 1979 (e.g. *Rheobatrachus vitellinus, R. silus, Taudactylus diurnus, T. rheophilus, Litoria lorica* and *L. nyakalensis* (Tyler 1997)) were not found dying and tested for disease, the epidemiological evidence suggests that chytridiomycosis caused mass mortalities of these species, as was observed in declining frogs (including *T. acutirostris*) at the Big Tableland site in north Queensland in 1993 (Berger *et al.* 1998). The possible role of chytridiomycosis in frog declines in southern Australia is less clear.

The episodes of population declines described above now appear to have passed in many areas. Numbers of some species, decreased significantly when other species disappeared, but are now increasing. These species include *T. eungellensis* (McDonald and Alford 1999), *L. genimaculata* on Big Tableland (McDonald unpubl.) and *L. pearsoniana* from southeast Queensland (Hero *et al.* 1998). As residual populations are increasing after significant declines although chytrids are still present (Berger *et al.* 1998), this suggests resistance is present in some members of the population. A balance between *Batrachochytrium* and the frogs may be

developing in areas where we suspect it has been present for at least five years, and although disease still occurs, it does not devastate the populations. *Batrachochytrium* may now behave as an endemic pathogen with outbreaks of disease occurring when conditions are optimal. The long term prognosis may be good for species which have survived and are recovering, as long as remaining habitats are protected to allow damaged populations to reestablish. However, since we currently know little about the interaction between the amphibian chytrid and hosts, and many frog species are currently considered threatened or their status is insufficiently known (Tyler 1997), we should not be complacent. Also, if uninfected areas still exist in Australia then locally endemic species in those areas may be at high risk.

Infectious disease is important in the population biology of wild animals, as it is in humans and domestic animals (May 1988). A review of infectious disease and animal populations concluded that disease is an important factor affecting survival, reproduction, dispersal, community structure and genetic diversity, and should therefore be considered by ecologists examining host population-dynamics (Scott 1988). Disease becomes a threatening force when environmental degradation puts pressure on populations, and international trade and smuggling continually threaten to introduce new pathogens to which the native fauna has no resistance (Scott 1988, Daszak *et al.* 1999). Exotic diseases can have effects similar to those of feral predators, with susceptible native species facing extinction while the ecosystem readjusts, and are another example of how increased global homogeneity leads to reduced biodiversity. Although the initial >99% mortality rate of myxomatosis was not sufficient to exterminate rabbits from Australia (Fenner and Ratcliffe 1965), it is plausible that a similar mortality rate could wipe out frog species with limited distributions and relatively infrequent breeding. The introduction of avian malaria is suspected to have caused the extinction of birds in Hawaii (Warner 1968). A protozoan parasite of cats (*Toxoplasma gondii*) was probably introduced to Australia during the European invasion, and marsupials are among the most susceptible animals (Reddacliff *et al.* 1993). *Phytophthora cinnamomi* is an example of a pathogenic, introduced zoosporic fungus which threatens many native Australian plant species, and quarantine measures are recommended to prevent the invasion of the pathogen into new areas. Some plant species are highly susceptible, whereas others only become diseased after periods of stress such as a drought (Dawson and Weste 1985; Wills 1993). Infrastructure exists to prevent and manage exotic disease outbreaks in domestic animals, but little concern is shown for wildlife where many diseases are yet to be discovered and understood, and monitoring the disease status of populations currently appears to be the responsibility of no one.

FUTURE DIRECTIONS

Work on amphibian chytridiomycosis must continue — to confirm or reject the hypothesis that it is the primary cause of the declines, to determine how the epidemic began, to find ways to manage the problem in areas where the fungus is established, and to prevent it occurring in new regions. Knowledge gained from these investigations will be useful in preventing similar population crashes occurring in frogs again, and in developing management strategies for other wildlife species.

We will continue to map the temporal and geographic distribution of chytridiomycosis by examination of frogs preserved in museum collections to determine whether chytrids were introduced to Australia or to these habitats and to determine from where and how chytrids have spread. Information on the current distribution may also help in formulating management plans to prevent new outbreaks — for example, in deciding where exposed or uninfected frogs should be released after captive breeding. This survey relies on a collaborative approach (see Appendix 1). As many healthy tadpoles may carry chytrids for extended periods, sampling of tadpoles may provide a more sensitive means of assessing a location. Examination of sick frogs is the most sensitive way to detect chytrids, but these are not always available.

Mycological studies on the chytrid are needed to learn more about its lifecycle, requirements and survival in the wild. Knowledge about the ecology and hosts of the fungus is essential to understanding the spread, and therefore to the management of the disease. Information about the particular conditions that encourage growth may help in understanding the factors which precipitate disease epidemics.

Further transmission experiments are required to confirm the pathogenicity in a range of species, and also to determine what environmental conditions e.g. temperature, are required for expression of the disease in frogs. Treatments for adults and tadpoles are also being tested which will aid captive breeding of endangered species. By producing large numbers of frogs in captivity, it may be possible to help species to survive and evolve immunity.

Studies of the pathogenesis of chytridiomycosis are important to understanding this disease, and immunologists in the USA will investigate the innate and acquired immune response of frogs to *Batrachochytrium*.

Further work will be done using DNA analysis to compare chytrids from various species from localities across Australia, Central America and the USA. The number of species of *Batrachochytrium* can be determined by using this information combined with morphological taxonomy. Molecular biology as a tool for molecular epidemiology can also be used to provide clues about the origins and spread of the fungus.

Data are being collected to evaluate diagnostic tests. Histology and examination of skin scrapings are highly specific tests, but may not be very sensitive when used to detect chytrids on healthy specimens. Production of antibodies has commenced to enable more sensitive testing, and perhaps for use in detecting chytrids in the environment.

Regulations regarding quarantine, testing, treatment and movement of amphibians need to be introduced to prevent further spread of *Batrachochytrium* within Australia and internationally. Although our understanding of the role of chytridiomycosis in amphibian declines is far from complete, we believe it is crucial to take immediate preventative measures rather than risk waiting for more scientific data to be accrued.

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APPENDIX 1

What herpetologists can do to assist

SURVEY OF SICK AND HEALTHY FROGS

We wish to examine any diseased frogs or cane toads that are found in order to determine the cause of death and to screen for the presence of chytrids. Frog tissues deteriorate very rapidly after death, so if a sick frog is found that is likely to survive another 24 hours, it should be sent by courier to the Australian Animal Health Laboratory or James Cook University after contacting us. Frogs or tadpoles found dead should be fixed or frozen immediately to preserve the tissues. They should be fixed in 10% buffered neutral formalin, but 70% ethanol can also be used. It is important to slit open the belly, and to ensure the frog is well covered in fixative so that tissues are preserved rapidly. Details of what to do with sick or dead frogs have been posted on the World Wide Web at http://www.jcu.edu.au/dept/school/phtm/PHTM/frogs/pmfrog.htm.

Please send collection data with any frog submitted, and we will keep you informed about the results of the post mortem. Pathology is required for diagnosis, as the clinical signs of chytridiomycosis are not highly specific.

We have prepared a frog mortality questionnaire (http://www.jcu.edu.au/school/phtm/PHTM/frogs/pmques.htm) which details the type of data that are important to observe and record if you encounter a mass mortality event.

For our examination of archived frogs for chytrids, we need skin samples from amphibians from a wide range of localities and dates. We want skin from the pelvic areas and toes from any formalin-fixed or ethanol-fixed frog that has collection data. We especially require frogs from inland Australia, Northern Territory and northern WA, as none have been examined from these regions. A protocol is available at http://www.jcu.edu.au/school/phtm/frogs/pmskin.htm.

People doing skeletochronology on histological sections of toes could simultaneously check the skin for chytrids (see Figure 4). In healthy frogs, the level of infection may be very low, with only occasional sporangia present along the skin surface. For detailed diagnostic histological features, see Berger *et al.* (1999).

We are attempting to maintain a comprehensive list of confirmed cases of chytridiomycosis (http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyspec.htm) and hope that data will be submitted for inclusion. This list will enable management decisions to be made based on current knowledge.

MANAGEMENT OF CHYTRIDIOMYCOSIS IN CAPTIVITY

If any epidemics of chytridiomycosis occur in captive collections, various antifungal drugs could be administered, and the results communicated.

Benzalkonium chloride is a disinfectant that has been used at 2 mg/l to successfully treat a similar superficial mycotic dermatitis in dwarf African clawed frogs (*Hymenochirus curtipes*) reported to be caused by *Basidiobolus ranarum* (Groff *et al.* 1991). The regime used experimentally was 30 minutes of bath treatment, on three alternate days. This was repeated in 8 days (i.e. 6 treatments in total). Oral itraconazole has also been used to treat *B. ranarum* infections (Taylor *et al.* 1999). One micro bead from 100mg itraconazole capsules was administered daily for 9 days to Wyoming toads (*Bufo baxteri*) at the first signs of disease. Benzalkonium chloride (1mg/L), amphotericin and fluconazole are effective against *Batrachochytrium in vitro* (Berger, unpubl.).

In captivity, routine quarantine procedures (Marantelli 1999) have been adequate in restricting outbreaks to certain tanks, and no airborne transmission has been observed (Marantelli, unpubl.). Each group of frogs should be kept completely separate to ensure no water borne transmission of disease can occur. By changing and discarding gloves between every tank, avoiding splashing water between tanks, and disinfection of tanks and implements before reuse using 2% hypochlorite, many frogs have been housed in close proximity without transmission of disease.

PREVENTING SPREAD OF DISEASE IN THE WILD

To prevent the spread of chytrids or other diseases when performing field work, disinfection of equipment should be performed. We need more information on the resistance of this fungus to heat, desiccation and disinfectants; so at present are recommending measures for disinfection that have been proven against highly resistant organisms. (see protocol — http://www.jcu.edu.au/school/phtm/PHTM/frogs/prevent.htm).

Disease status should always be considered when translocating animals (Marantelli 1999, Viggers *et al.* 1993) and attempts should be made to reduce the chance of introducing disease to a naïve population. We recommend the screening of healthy frogs by histologic examination of toe clips, or by sacrificing a few in the group for more extensive skin examination (Berger *et al.* 1999). Testing for chytrids on sick frogs is more sensitive, so any deaths in a valuable group of animals should be submitted to a pathology laboratory for testing. To screen a group of healthy tadpoles, some need to be sacrificed so that their mouths can be examined histologically (see Berger *et al.* 1999). We have little information on the sensitivity of these tests, so it is impossible to recommend a statistically significant number of animals. Also, until we have more data on the distribution of chytrids in amphibian populations around Australia, it will be difficult to make decisions about the release of infected animals.

New regulations are being proposed to control the movement and trade of adult amphibians and tadpoles, but before these are introduced, quarantine measures should become routine.