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Myxosporean parasites in Australian frogs: Importance, implications and future directions

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ABSTRACT

Myxosporean parasites have been identified in amphibians around the world yet very little is known about their diversity, biology and host impact. Several species of Australian frogs have recently been shown to be affected by myxosporidiosis caused by two new *Cystodiscus* species. In this manuscript, we review what is known about the myxosporean parasites *Cystodiscus australis* and *Cystodiscus axonis* that produce myxospores in gallbladders of Australian frogs and *Myxobolus fallax* and *Myxobolus hylae* that produce spores in gonads and the potential impact of these parasites on the conservation of Australian frogs. By doing so, we aim to highlight the importance of amphibian myxosporean parasites, suggest directions for future research and argue that the lessons learned about these parasites in Australia are directly transferable to amphibians around the world.

1. Background

Parasites infect virtually every species, yet most are poorly studied unless they have been shown to have a medical, economic or conservation importance (Nichols and Gómez, 2011). This is particularly true for parasites of wildlife where baseline data on parasite diversity and ecology is minimal or absent for many species (Marcogliese, 2004; Thompson et al., 2010). This can mean that parasites with significant host health impacts are only recognized once the population of the host species has declined precipitously or when disease screening is undertaken in association with the establishment of a captive breeding program.

One of the most threatened taxa is the amphibians with over 40% of the approximately ~5743 amphibian species worldwide in decline (Stuart et al., 2004; Vié et al., 2009). Infectious diseases including those caused by fungi, viruses and parasites are major contributors to the decline of many of these species (Murray and Skerratt, 2012). As a result, captive breeding and disease control have been highlighted as important conservation tools that will be needed to protect global amphibian populations in the future (Murray and Skerratt, 2012). Captive breeding and disease control are closely interlinked. Without full understanding of pathogens causing diseases in amphibians, steps to avoid pathogens in captive breeding programs cannot be successful.

One group of pathogens that are known but poorly studied are the myxosporean parasites of amphibians. Myxosporean parasites are mostly known for causing diseases in fish and the significant losses that they cause to commercial aquaculture (Feist and Longshaw, 2006; Lom and Dyková, 2006). These parasites are metazoans that infect predominantly freshwater and marine fish as well as reptiles, small mammals, waterfowl and amphibians but information about the disease they cause in these other groups is limited (Feist and Longshaw, 2006; Lom and Dyková, 2006). Recently myxosporean infections have been suggested to represent a key threatening process that may be contributing to amphibian decline (Sitjà-Bobadilla, 2009; Hartigan et al., 2012a).

Myxosporean parasites have been observed in Australian frogs for over 100 years yet surprisingly there is very little knowledge about their ecology or impact on frog populations (Johnston and Bancroft, 1918; Delvinquier, 1986). This paper reviews what we know about myxosporean parasites in Australian frogs. We use the information acquired about Australian myxosporean species affecting the liver, brain and urogenital systems of native frogs to provide insights into the potential impact that amphibian myxosporean parasites may have in other ecosystems around the world. Lastly, we identify the gaps in the knowledge about these parasites that are necessary to be filled if their global impact on amphibian populations is to be understood.

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2. What do we know about the myxosporean parasites in Australian frogs?

2.1. Parasites of the urogenital system: Myxobolus hylae and Myxobolus fallax

Two *Myxobolus* species have been described in the urogenital system of Australian frogs. *M. hylae* Johnston and Bancroft, 1918 was described from cysts on the testes and oviducts of the Green and Golden Bell Frog (*Litoria aurea*) which were collected near Sydney, New South Wales in 1918 (Johnston and Bancroft, 1918). Frogs infected with *M. hylae* were reported to be lethargic, thin and, in cases of high parasite burden, the testes were distended. The second species was recognized during an investigation to preserve frog sperm of *Litoria fallax* that contained myxospores of *M. fallax* (Browne et al., 2002). The two species were distinguished from each other based on myxospore morphology of museum material of *M. hylae* and specimens of *M. fallax* (Browne et al., 2002). As yet, no other *Myxobolus* species has been identified in Australian frogs.

Myxobolus spp. have been reported in common (*Litoria caerulea*, *L. fallax*, *Litoria lesueuri*, *Litoria peronii*) and endangered (*L. aurea* and *Litoria raniformis*) species though only as incidental findings (Berger, 2001; Mann et al., 2010). Given the distribution of these frog species it is likely that *Myxobolus* spp. can be found across all of the east coast of Australia (Fig. 1A).

Myxobolus species infections in the gonads of frogs could have important impacts on the reproductive success of amphibians and thus could create problems for conservation programs (Sitjà-Bobadilla, 2009). *M. fallax* was found to be released with spermiation, possibly to maximize its transmission into the environment at a time of new host availability during spawning (Browne et al., 2006). Impacts on fecundity are unknown, however, myxosporean parasites in gonadal tissue of fish hosts have been associated with castration (Baudoin, 1975) e.g. *Myxobolus testicularis* (Sitjà-Bobadilla and Alvarez-Pellitero, 1993; Sitjà-Bobadilla, 2009). While castration of the frog by *M. fallax* has not been reported, even a decreased sperm count could have serious consequences for amphibian conservation and health of an already declining frog population. Decreased sperm numbers were reported for *Myxobolus chimbuensis* Ewers 1973 infections in *Litoria darlingtoni* of Papua New Guinea (Ewers, 1973). Determining the impact of myxosporidiosis caused by *Myxobolus* species of frogs will benefit the conservation outcomes of captive breeding projects by improving our understanding of infectious disease in amphibians.

In addition to the impact that Australian *Myxobolus* species may have on fertility in frogs, there is other important information about these parasites that is yet to be discovered. Given that in the original description of *M. hylae* spores considerable variation in spore morphology was observed (Browne et al., 2002), genetic studies need to be undertaken to prove that this variation can occur in one species and to rule out the possibility that the variation may actually be due to the presence of two or more species. Lastly, more field work needs to be done to determine the actual host ranges and distributions of these.

2.2. Parasites of the liver and brain: Cystodiscus australis and Cystodiscus axonis

C. australis Hartigan et al., 2012b and *C. axonis* Hartigan et al., 2012b are two species originally thought to be a single species *Cystodiscus immersus* Lutz, 1889 that was introduced to Australia courtesy of the exotic Cane Toad (*Rhinella marina*) in 1935 (Hartigan et al., 2010, 2011, 2012a, 2012b). This hypothesis of exotic introduction from South America was put forward after a survey of Australian frog gallbladders in 1986 demonstrated similar looking myxospores to *C. immersus* in native Australian frogs and *R. marina* (Delvinquier, 1986). Species identification was based on comparing the morphology of spores to line drawings for *C. immersus* from Brazilian specimens made in 1889 (Lutz, 1889) and 1940 (Kudo and Sprague, 1940), and scanning electron micrographs of Australian material (Delvinquier, 1986).

This hypothesis was not considered again until 2011 when genotyping of gallbladder myxospores and infected brain and liver tissue from several Australian frog species showed that the parasite thought to be *C. immersus* was in fact two novel parasites with similar spore morphology to each other and *C. immersus* (Hartigan et al., 2011, 2012c). Confirming the cryptic diversity of two Australian endemic species (*C. australis* and *C. axonis*) required several descriptive tools including comparison of multiple ribosomal DNA regions, transmission and scanning electron microscopy as well as histopathology. Included in these findings was the observation that not only could *C. australis* and *C. axonis* be distinguished genetically, but that *C. axonis* had not only liver developmental stages but also brain intra-axonal developmental stages (Hartigan et al., 2011, 2012b).

Both *Cystodiscus* species have been shown to infect and cause disease in tadpoles although not all host species are affected in the same way (Hartigan et al., 2012a). The lesions caused by either *Cystodiscus* species included inflammation and hyperplasia of the frog livers. The brain lesions attributed to *C. axonis* were more severe in some species (*Litoria booroolongensis*, *Litoria castanea* and *L. raniformis*) and included haemorrhage, gliosis and necrosis (Hartigan et al., 2012a). Frogs with severe disease exhibited neurological dysfunction, affected frogs lost the ability to right themselves and in some cases lost hindlimb movement. Moreover, it is speculated that the infection with *Cystodiscus* species may cause delayed metamorphosis leading to tadpole overwintering (Hartigan et al., 2012a). Prolonging the aquatic stages i.e. tadpole of frog development is at odds with the fitness of the species due to risk of predation and pond desiccation (Newman, 1992).

Both Australian *Cystodiscus* species appear to be emerging parasites. The similarity of the presporogonic stages on histological sections in the liver and myxospore morphology of *C. axonis* and *C. australis* species prevents species retrospective identification. However, we used existing historical data to plot the distribution of the genus *Cystodiscus* in Australia over time (Fig. 2; Supplementary Table 1; Berger, 2001. Diseases in Australian frogs. PhD thesis, James Cook University, Townsville; authors unpublished data). Examination of archived frogs in the Australian Museum revealed the absence of *Cystodiscus* spp. myxospores in specimens of the 19th and early 20th century with the first *Cystodiscus* positive frog detected in 1966 (Hartigan et al., 2010). Additionally, the examination of archival tissue combined with other reports describing *Cystodiscus* species parasite infections in native and exotic frogs from 1960 to 2011 suggests a southerly spread with invasion into South Australia being documented for the first time in 1998 (Fig. 2). No *Cystodiscus* positive records have been detected in the Northern Territory or Western Australia in the last 30 years (Delvinquier, 1986) and the first positive recorded for South Australia does not occur until 1998 (Fig. 2). Based on case reports and wildlife disease screening *Cystodiscus* species has not been identified in Western Australia or the Northern Territory (authors unpublished observation). The absence of *Cystodiscus* parasites in the Northern Territory, but the presence of known susceptible hosts in this area (*R. marina* and *L. caerulea*) may indicate the absence of an invertebrate host in this environment.

All the factors that have facilitated the emergence of these parasites are not known, however, movement of *Cystodiscus* species into new areas has been linked to frogs accidentally translocated with fresh produce (Hartigan et al., 2012c). The southward spread and increased prevalence in southern distributed frog populations