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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.

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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

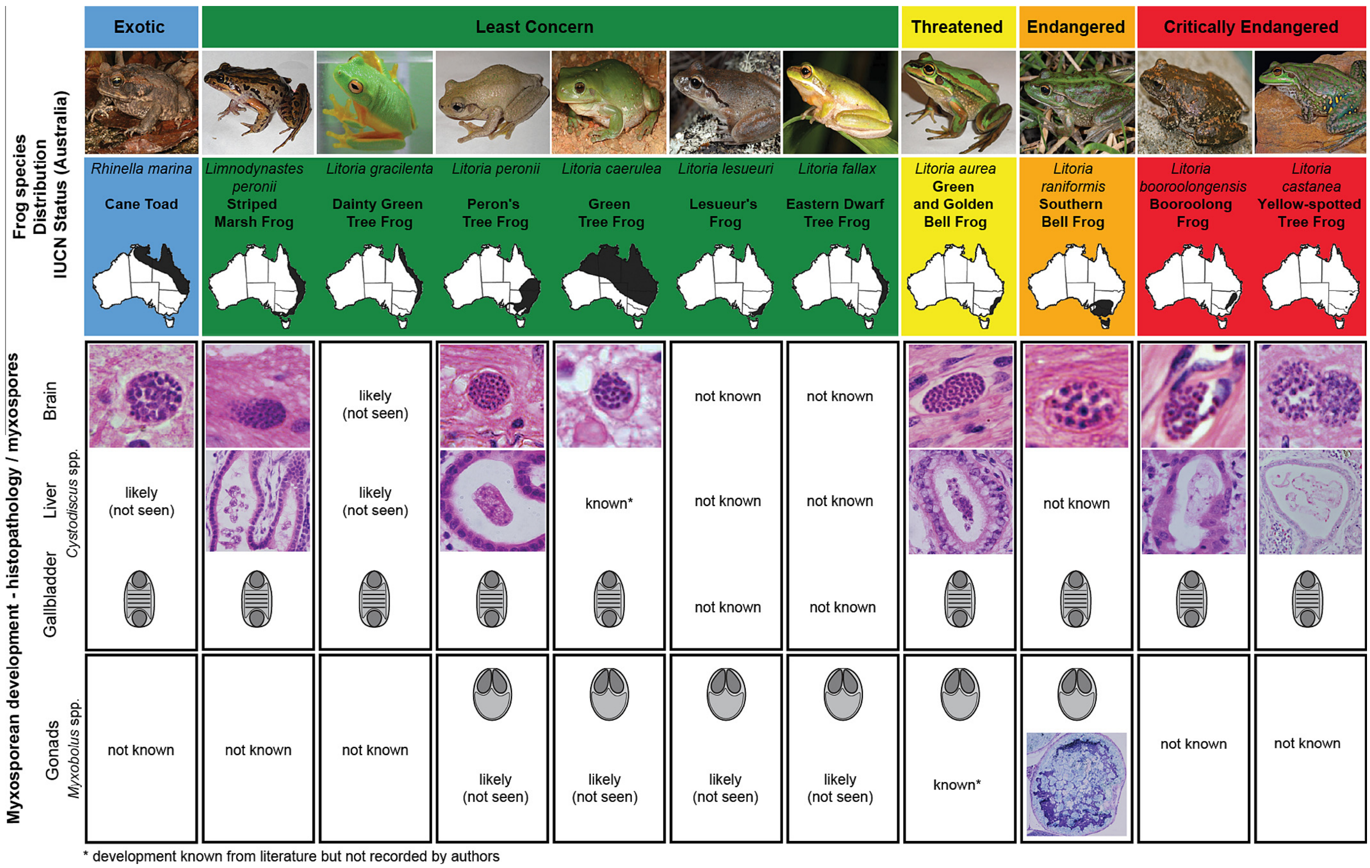

Fig. 1. Summary of myxosporean parasites recorded in Australian frogs. The frog species are sorted according to IUCN Red List conservation status [http://www.iucnredlist.org/], note, the Cane toad (*Rhinella marina*) is listed as exotic pest species introduced to Australia in 1935. Myxosporean frog host species belong to three out of five frog families present in Australia; genus *Litoria* (family Hylidae), *Limnodynastes* (family Myobatrachidae) and *Rhinella* (family Bufonidae). Distribution for each species in Australia (black) is shown according to Frogs Australia [www.frogs.org.au]. Myxosporean development and myxospores are shown as column under each frog species. For *Cystodiscus* species either brain (CNS) or liver development is shown in form of a histological section and line drawing of myxospore. Spore measurements according to Hartigan et al. 2012b for *C. australis* 15·0–18·0 × 8·0–10·0 μm, for *C. axonis* 13·0–15·0 × 8·0–10·0 μm. For *Myxobolus* species gonad development is shown in form of a histological section and line drawing of myxospore. Absence of a record of development or myxospores is shown as – not known. Presence of myxospores, but absence of a record of development is shown as – likely (not seen). Development of *M. hylae* has been published by (Johnston and Bancroft (1918) and (Berger, 2001. Diseases in Australian frogs. PhD thesis, James Cook University, Townsville), but not recorded by authors during 2007–2011. *M. fallax* spore measurements according to Browne et al. 2002 12.6–14.6 × 8.3–10.6 μm (no noticeable variation between fresh and formalin fixed material). *M. hylae* myxospores according to Johnston and Bancroft (1918) measured 8–10 × 7–8 μm. *L. lesueuri*, *L. raniformis* and *L. castanea* photos courtesy of David Hunter.

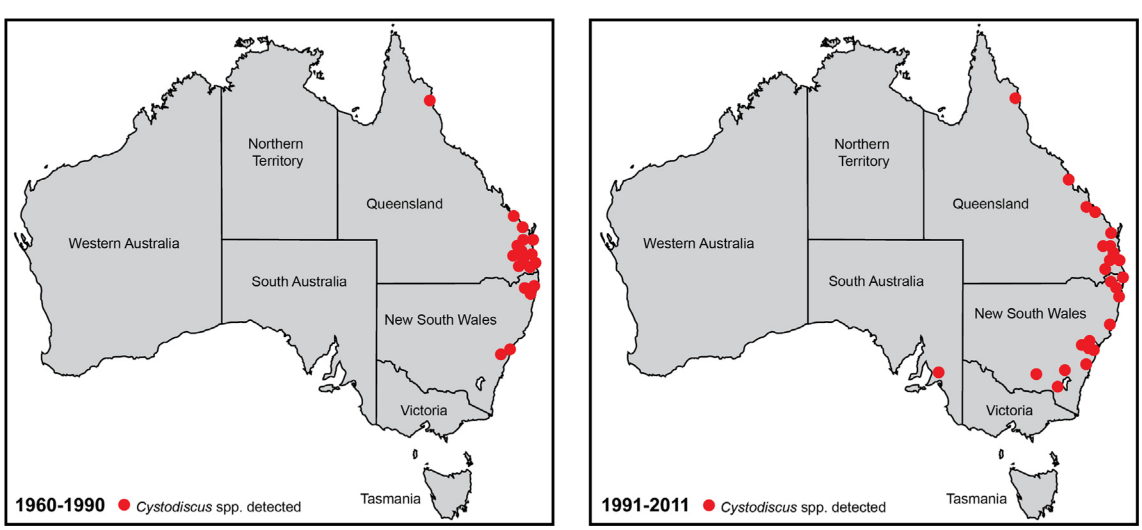

Fig. 2. Emergence of *Cystodiscus* parasites across eastern Australia from 1960–2011. All locations (red square) represent records for *Cystodiscus* spp. from one or more frogs. Both published and unpublished data were used to compile the distribution map (Delvinquier, 1986; Hartigan et al., 2010; , 2012a, 2012b, 2012c, 2012d; Supplementary Table 1). The data are split into 1960–1990 (A) and 1991–2011 (B) to show recent records in western New South Wales and South Australia.

is of concern as some of Australia's most endangered frog species are in southern states and species with no previous exposure to these parasites may be more susceptible to *Cystodiscus* infection and disease. Moreover *Litoria* species in particular *L. caerulea* are popular species within the international pet trade which can potentially spread *Cystodiscus* spp. around the world.

3. Are myxosporean parasites threats to amphibian health and conservation?

Emerging infectious diseases can have serious implications for the conservation of endangered species (Daszak et al., 2003). Pathogens have a greater impact when they are able to, (i) infect a large host range, (ii) can infect directly or through a single vector, and (iii) persist in the environment for long periods of time (Dobson and Foufopoulos, 2001). These characteristics are seen in the best studied and most devastating pathogens to frogs and salamanders around the world – the Chytrid fungus (*Batrochochytrium dendrobatidis*), ranaviruses (Ranavirus: Iridoviridae) and a trematode (*Ribeiroia ondatrae*) (Blaustein et al., 2012). These disease agents infect multiple amphibian species, remain in the environment for long periods of time either in the physical environment as seen in ranaviruses and Chytrid fungus (Mitchell et al., 2008; Nazir et al., 2012) or in reservoir hosts for the trematode (Collins et al., 2004; Briggs et al., 2010).

Australian myxosporean parasites in amphibians fulfill all of the above characteristics. Firstly (i), *M. hylae*, *C. axonis* and *C. australis* have been described from multiple hosts. In fact, *Cystodiscus* species have been found in three out of the five frog families present in Australia (Fig. 1). Secondly (ii), although no myxosporean-amphibian life cycle has been determined, it is very likely that an invertebrate host is involved using the analogy from known lifecycles of myxosporea affecting fish (Eiras, 2005). Having multiple host species (vertebrate and invertebrate) in the environment increases the range and ease of spreading the infection. Thirdly (iii), myxosporeans are able to persist in the environment outside of their vertebrate host. They do this by surviving in their invertebrate host and through the ability of their myxospores to remain viable for months in sediment. In the case of *Myxobolus cerebralis*, development in the invertebrate host (oligochaetes) takes approximately 3 months before they start to release actinospores for the following 7 months (El-Matbouli et al., 1999; Stevens et al., 2001). In addition *Cystodiscus* spp. can persist as non-pathogenic infections in suitable hosts, some hosts do not present lesions and some can shed myxospores over time with no host health impacts observed (Hartigan et al., 2012a). This benefits transmission of the parasites into new populations and environments compared to other pathogens which kill or debilitate their hosts.

4. What can Australian frog myxosporean parasites teach us about other amphibian myxosporean parasites around the world?

There are a number of identified myxosporean parasite species in frogs around the world with more species to be described probably in Africa, Asia and Europe (Fig. 3). Several important aspects of myxosporean development in amphibians can be learned from the information known about *Cystodiscus* and *Myxobolus* species in Australian frogs.

Studies that resulted in the identification of the two Australian *Cystodiscus* species show that careful study of the developmental stages of myxosporean species are a necessary adjunct to characterization by spore morphology if cryptic species are to be identified. The key to the discovery that *C. axonis* from *C. australis* were separate species was demonstrating the intra-axonal plasmodial stages found in *C. axonis* infections but not in *C. australis* as well as using traditional scanning electron microscopy techniques to identify filiform polar appendages of *C. axonis* that were unique to this species (Hartigan et al., 2012b).

In addition to looking for developing stages in different organs, research into *Cystodiscus* species in Australia demonstrated the importance of looking for parasites in different host life cycle stages (Hartigan et al., 2012a, 2012b). Based on the findings in *Cystodiscus* spp., studying tadpoles can provide new insight into host-parasite development, transmission dynamics and host impact of myxosporean parasites. Despite this revelation, there is very limited information about what myxosporean species can infect tadpoles. There is a report of a myxosporean species in tadpole gallbladders of seven species in southwest US, unfortunately the identification of this parasite was not possible (Green and Dodd, 2007). *Sphaerospora ohlmacheri* is known to produce myxospores in the kidneys of tadpoles in North America and Canada whilst *Cystodiscus* spp. in Australia developed only presporogonic stages in tadpoles (Desser et al., 1986; Hartigan et al., 2012a). A European myxosporean *Sphaerospora ranae* infects about 75% of endemic frog species however no tadpoles from the same locality were infected, which could indicate that tadpoles are resistant to infection (Jirků et al., 2007). No *Myxobolus* species have been identified in tadpole surveys; this could indicate that tadpoles are not susceptible to infection due to undifferentiated gonadal tissue (McDiarmid and Altig, 1999). The effect of metamorphosis and tadpole biology on infection dynamics of myxosporean parasites is yet to be determined but may provide important insights into host parasite relationships.

Discoveries about *M. fallax* and its development in Australian frogs point to important relationships between myxosporean parasite development and host reproduction. This is the only species of myxosporean parasite that has been shown to have synchronization of spore shedding with spermiation, although it has been suggested that this occurs in some fish myxosporeans (Sitjà-Bobadilla and Alvarez-Pellitero, 1993). The regulatory factor and host interaction of this species is not well understood, *M. fallax* was not associated with any pathology disease or impact on fertility. Whether *M. fallax* or other amphibian *Myxobolus* species would cause disease in naïve hosts is not known (Eiras, 2005; Sitjà-Bobadilla, 2009).

The parasites identified in Australian frogs have provided important insights on the host-parasite relationship between myxosporean parasites and amphibians. Prior to the information about *C. australis* and *C. axonis*, liver and gallbladder infections with myxosporean parasites were not considered to have any host health outcome, we now know this is not true (Hartigan et al., 2012a). It has been shown that disease caused by *Cystodiscus* species is variable between host species, can extend to tadpoles, and potentially can cause tadpoles to overwinter which has serious ecological outcomes (Hartigan et al., 2012a).

Finally, *Cystodiscus* species have been shown to spread across large distances in live frogs hitch hiking in fresh produce (Hartigan et al., 2012c). It is worth noting that frogs are transported internationally for food and the pet trade (Schlaepfer et al., 2005; Warkentin et al., 2009). While it is increasingly common to test these frogs for chytridiomycosis, our findings suggest that testing for myxosporean parasites is of similar importance if additional spread is to be prevented.

In summary, there are a number of myxosporean parasites described from frog hosts around the world (Fig. 3), the majority of these have little information known other than host records and myxospore morphology. Increased efforts to understand the biology and host interaction of amphibian myxosporean parasites in frogs will assist conservation of frogs and salamanders around the world. Australian myxosporean species in amphibians exemplify what can be discovered about these parasites when scrutinised, to benefit frog conservation.

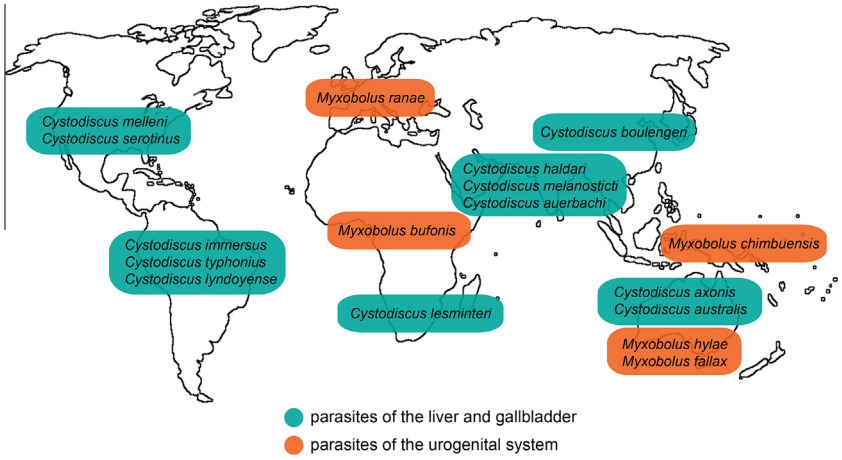

Fig. 3. *Cystodiscus* species and *Myxobolus* species of frogs. Worldwide records of described *Cystodiscus* species (blue) based on gallbladder myxospores and *Myxobolus* species (orange) based on myxospores in gonad of amphibians. Note: Only descriptions down to species level are included, i.e., *Myxobolus* sp. are not included.

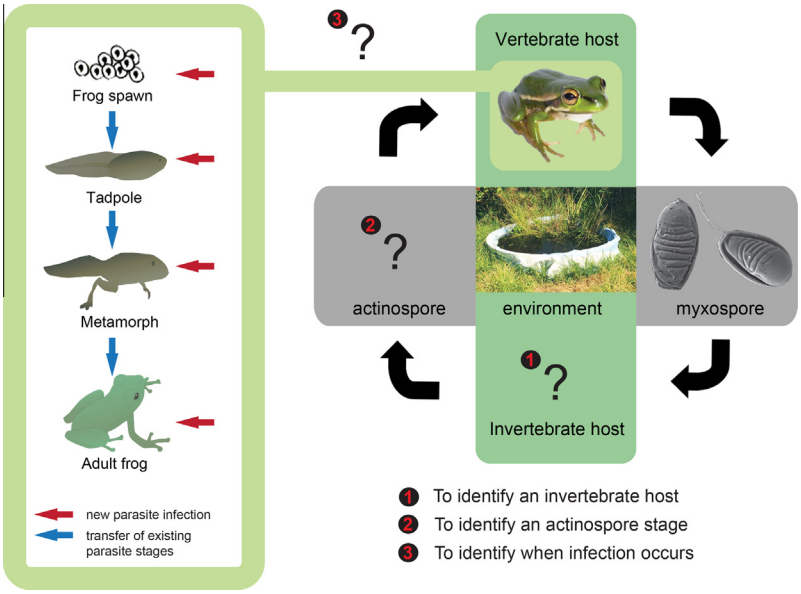

Fig. 4. Hypothetical life cycle and transmission routes of *Cystodiscus* species. Understanding of the life cycle and timing of infection will provide invaluable information to wildlife disease managers to mitigate the impact of myxosporean parasite. The majority of myxosporean parasite life cycles alternate between a vertebrate host and an invertebrate host, in the case of *Cystodiscus* it is frogs and an unknown invertebrate (green box) (1). The vertebrate host sheds myxospores (grey square, right) that infect an invertebrate host that produces actinospores (grey square, left) (2). Actinospores are the infective stages for vertebrate hosts (frogs). It is unknown at what stage of development amphibians are susceptible to infection (3), *Cystodiscus* spp. have been found in both tadpoles and adults. It is possible that spawn, tadpole, metamorph or an adult frog can be infected by actinospores (red arrows, green outlined square). It is unknown if infection can be shed during development or if it is passed on during metamorphosis or spawning (blue arrows, green outlined square).

5. Future directions

Often parasites are neglected until they cause large scale decline, however, effective monitoring can reduce the blind spots in our understanding of parasites (Nichols and Gómez, 2011). Making sure that amphibians are routinely screened for myxosporean parasites by pathologists and disease investigators could avoid overlooking a serious threat for amphibian conservation. This may be particularly important for areas of high amphibian diversity, such as Central America and Asia, where there is a paucity of data about the prevalence and diversity of myxosporean parasites in these populations. Surveys of these areas and thorough description of their myxosporean parasites should be prioritized.

In addition to understanding the diversity of pathogens in amphibians, future research should prioritise improving the quality of species descriptions and detection methods. The majority of new species descriptions recognize the need for molecular identification together with spore morphology. There are likely to be cryptic species complexes masked by shared myxospore morphology in the currently described species for both *Myxobolus* and *Cystodiscus* species. Genetic studies using multiple markers demonstrated that the internal transcribed spacers (ITS) of the rDNA, in addition to the commonly used small subunit rDNA (18S) can be powerful tools for identifying new species within myxosporean cryptic species complexes (Henderson and Okamura, 2004; Whipps et al., 2004; Whipps and Kent, 2006; Hartigan et al., 2012b). To

facilitate studies on myxosporean parasite infections in endangered species where examining tissues of collected specimens is not an option, non-invasive methods of detecting myxosporeans should be further developed such as screening faecal smears of captive frogs for *Cystodiscus* myxospores (Hartigan et al., 2012c). Furthermore, the authors advocate the evaluation of tissue distribution, development stages and associated disease within the host tissue in new species descriptions.

The work with *C. australis* and *C. axonis* highlights the importance of considering the impact of season on prevalence of infection and disease (Hartigan et al., 2012a). There is also a need to identify the timing and earliest stage of frog development susceptible to myxosporean infection (Fig. 4). An example of the importance of timing to infection can be seen in tadpoles infected with the trematode parasite, *R. ondatrae* (Johnson et al., 2011). Tadpoles can be infected with *R. ondatrae* at different times however, if infected at a prelimb stage of development the host has a higher mortality risk than a tadpole infected at a later development stage (Schotthoefer et al., 2003). Moreover, it is unknown whether spawn or newly hatched tadpoles can be infected with myxosporean parasites, if metamorphs can be infected and what impact myxosporean parasites have on metamorphosis (Fig. 4). Finally, the impact myxosporean parasites have on host reproduction requires investigation to elucidate what level of threat is posed to captive breeding programs. Answers to these questions cannot be addressed without controlled experimental infections requiring the yet unknown mode of transmission.

New research needs to develop and evaluate practical methods for mitigating the impact of myxosporean parasites in captive and wild populations. Practical steps toward eliminating myxosporean parasites will only be achievable once the life cycle is understood (Fig. 4). Identification of the invertebrate host is an essential aspect of understanding the development of the parasite (Fig. 4). Given that *Cystodiscus* species infect tadpoles their invertebrate host would appear to be a freshwater aquatic invertebrate. Given that *Myxobolus* spp. of amphibians have only been reported in adult frogs, their intermediate host might either be an aquatic or terrestrial species.

In conclusions, baseline data are needed to make useful decisions about the translocation, captive breeding and conservation of wildlife populations of amphibians (Thompson et al., 2010). If the data gained from the Australian myxosporean parasites are any kind of example, it is likely that many of the other known amphibian myxosporean parasites in other areas around the world are more diverse than currently known. It is possible that other myxosporean parasites can infect tadpoles and frogs with unknown ecological consequences. Understanding myxosporean parasites infecting amphibians and their importance to wild and captive populations is essential for benefit disease surveillance and conservation programs such as captive breeding in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijppaw.2012.12.002.

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