Myxozoans Exploiting Homeotherms

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Abstract

Discoveries published in 2007 and 2008 expanded the known host range of myxozoans beyond poikilotherms to include mammals and birds. Here we review records of myxozoans from small terrestrial mammals, waterfowl and those associated with humans, and augment them with data from our ongoing studies. True myxosporean infections—those with active parasite development and sporogenesis—have been recorded for *Soricimyxum* spp. in central European shrews and *Myxidium* spp. in North American waterfowl. In all cases, bile ducts within the liver were the nidal tissue and complete life cycles are unknown. Incidental myxosporean infections—the presence of myxospores without parasite development—have been observed in humans, usually in association with the ingestion of infected fish. Clinical presentations of these cases range from no disease (e.g. *Henneguya* spp.), allergic responses (*Kudoa* sp.) or acute gastroenteritis (*Kudoa septempunctata*). Phylogenetically, myxosporean parasites of homeotherms cluster closely with *Myxidium* and *Cystodiscus* species known to infect other terrestrial vertebrates (reptiles and amphibians), which suggests a single evolutionary expansion from an aquatic *Myxidium*-clade ancestor to semi-aquatic and terrestrial hosts and environments. Given the diversity of potential mammalian and avian hosts, we expect additional myxosporean parasites

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to be discovered with further scrutiny of these homeotherms, especially in sparsely surveyed regions including Africa and South America.

Keywords
Homeotherms · Small mammals · Waterfowl · Humans · *Soricimyxum* · *Myxidium* · *Kudoa*

7.1 Introduction

For the first 150 years after their discovery, myxozoans were regarded exclusively as parasites of poikilothermic vertebrates and aquatic invertebrates. The overwhelming majority, some 2,000 species, have been described from fishes in marine, brackish and freshwater habitats from all continents except Antarctica. Myxosporeans in non-fish vertebrate hosts have been known for almost as long as those in fishes but account for <5 % of records, with some 50 species from chelonid reptiles and amphibians (Eiras 2005; Garner et al. 2005; Jirkù et al. 2006) (Fig. 7.1).

At the turn of the 21st century, the known host range of Myxozoa was broadened to include terrestrial homeotherms. These records from small mammals and birds are limited in both geographic and taxonomic diversity: two myxosporean species are known from three species of shrew in Europe (Prunescu et al. 2007; Dyková et al. 2007; Székely et al. 2011) (Table 7.1) and at least two species of myxosporeans are known from six species of waterfowl from North America (Bartholomew et al. 2008; Atkinson, unpublished data) (Table 7.2). The presence in small mammals and birds of a range of developmental stages, from presporogonic through to mature myxospores, is indicative of true host status rather than of a vector or incidental occurrence. The latter describes the nature of infections in humans, in which only myxospores have been observed in association with consumption of infected fish.

Herein, we review records of myxozoans from small terrestrial mammals, waterfowl and those associated with humans. We discuss the phylogenetic context of myxosporeans in homeotherms, possible sources of sample bias, factors that could influence host range expansion into homeotherms, possible modes of transmission and conclude with key questions for future studies.

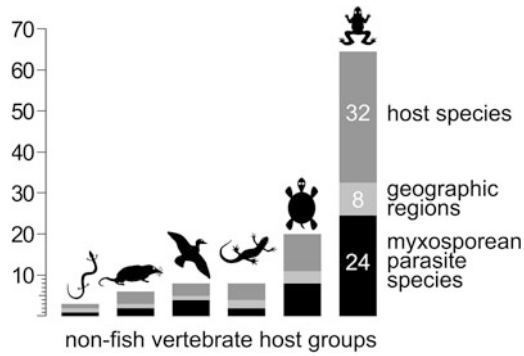

Fig. 7.1 Myxosporean parasitism in non-fish vertebrates, from, *right-to-left*: frogs/toads, turtles/tortoises, newts/salamanders, waterfowl, small mammals, primitive caudates (*Proteus*). The number of host species, the number of geographic regions from which they have been recorded (North America, South America, Africa, Europe, North Asia, South Asia (India), Southeast Asia, Australia) and the number of recorded myxosporean parasite species are indicated

7.2 Small Mammals

The first observation of a "myxozoan" developing in a host other than an aquatic poikilotherm was of myxosporean-like presporogonic stages (cells within cells) in the brains of wild Austrian moles (*Talpa europaea*; Friedrich et al. 2000). Xenomic (hypertrophic growth of an invaded host cell) pericytes of brain capillaries were found in 55 % of 55 moles, collected in 1982–1985 and 1994. Although this high prevalence is inconsistent with merely accidental infection, the lack of mature spores confounds definitive identification of the parasite and no DNA sequence data exist for comparison. Similar xenoparasitic complexes, again containing a

Table 7.1 Terrestrial mammalian hosts of myxozoans

Host common name	Host scientific name	Myxozoan	Reference
Common shrew	*Sorex araneus*	*Soricimyxum fegati*	Prunescu et al. (2007), Dyková et al. (2007, 2011), Székely et al. (2011)
Pygmy shrew	*Sorex minutus*	*Soricimyxum fegati*	Dyková et al. (2011)
		Soricimyxum minuti	Székely et al. (2011)
Lesser white-toothed shrew	*Crocidura suaveolens*	*Soricimyxum fegati*	Dyková et al. (2011)

Two myxosporean species infect European shrews

Table 7.2 Avian hosts of myxozoans, all of which are waterfowl from North American localities

Host common name	Host scientific name	Myxozoan *Myxidium* spp.	USA locality	Source
Pekin duck*	*Anas platyrhynchos*	*M. anatidum*	Georgia	Wild
Mallard duck*	*Anas platyrhynchos*	*Myxidium* sp. 2	California	Wild
Baikal teal*	*Anas formosa*	*Myxidium* sp. 2	Texas	Captive
Wood duck*	*Aix sponsa*	*Myxidium* sp. 2	California	Wild
African yellow-billed duck	*Anas undulata undulata*	*Myxidium* sp.	California	Captive
African yellow-billed duck	*Anas undulata undulata*	*Myxidium* sp.	California	Captive
Mallard duck	*Anas platyrhynchos*	*Myxidium* sp.	California	Wild
Mallard duck	*Anas platyrhynchos*	*Myxidium* sp.	California	Wild
Smew	*Mergus albellus*	*Myxidium* sp.	California	Captive
Cape teal	*Anas capensis*	*Myxidium* sp.	Florida	Captive

Spore morphologies from histology or TEM images show infections are characteristic of genus *Myxidium* (Bartholomew et al. 2008)
SSU sequence data from four birds (*) indicate that at least two myxosporean species are present (Atkinson unpublished data)

range of parasite developmental stages excluding mature spores, were observed subsequently in European shrews with overt myxosporean infections (Dyková et al. 2011).

Common shrews (*Sorex araneus*) collected in Poland from 2001–2005 were infected with a novel species of an undoubtedly myxosporean parasite *Soricimyxum fegati*, which was observed in livers of 41 % of 46 shrews (Prunescu et al. 2007). Both developmental stages and mature spores were documented, which indicated that myxosporeans can infect homeothermic, wholly terrestrial hosts. *Soricimyxum fegati* was found subsequently in common shrews (42 % of 24; 52 % of 98) in both the Czech Republic (Dyková et al. 2007, 2011) and Hungary (36 % of 21; Székely et al. 2011) (Fig. 7.2) and in pygmy shrews *Sorex minutus* (20 % of 70) and the lesser white-toothed shrew *Crocidura suaveolens* (10 % of 10) in the Czech Republic (Dyková et al. 2011). A second species, *Soricimyxum minuti*, was discovered in the pygmy shrew (33 % of 3) in Hungary (Székely et al. 2011).

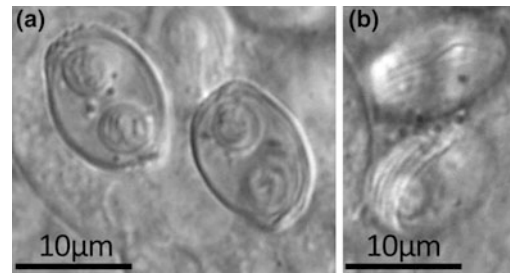

Fig. 7.2 *Soricimyxum fegati* myxospores from bile ducts of a common shrew (*Sorex araneus*). **a** Brightfield showing spore internal details, and **b** valve cell surface ridges and thickened suture

Myxospores of *S. fegati* develop asynchronously as disporic pansporocysts in polysporic plasmodia in the hepatic bile ducts and

parenchyma. Plasmodia in both locations elicit a vigorous inflammatory host response (Prunescu et al. 2007; Dyková et al. 2011). Proliferative stages cause hepatic lesions during their migration towards the lumina of bile ducts; lesions are similar in the different shrew species (Dyková et al. 2011). In 11 % of cases, covering all three species, xenoma-like formations (XLFs) containing presporogonic stages damaged the blood vessels in other organs, particularly the myocardium (Dyková et al. 2011). The role of XLFs in the myxozoan developmental cycle is unclear and atypical in fish infections (e.g. *Myxidium lieberkuehni* in renal corpuscles of pike, *Esox lucius*; Lom et al. 1989). Xenomas are associated most commonly with microsporidian infections, in which all developmental stages are present including mature spores (Lom and Dyková 2005). In the few shrew xenomal infections, no mature spores were visible (Dyková et al. 2011), which lends some weight to the original assignation of mole xenomas as myxozoan infections. In the absence of spores, the parasites within the shrew XLF's were identified by DNA sequencing (Dyková et al. 2011); technology not readily available at the time of the mole report.

Myxospores of the closely-related shrew parasite, *Soricimyxum minuti*, also develop within plasmodia in the bile ducts (Székely et al. 2011). This species is indistinguishable phenotypically from *S. fegati*, but differs by 4 % in its SSU gene sequence. Given the lack of clear phenotypic differences between these *Soricimyxum* species, taxonomists should remain vigilant for cryptic species when describing further occurrences of these homeotherm parasites.

Although myxosporean parasites occur at relatively high prevalence in European shrews, a limited survey in North America of East coast shrews ($N = 28$ *Sorex* sp.) and West coast shrews ($N = 3$ *Sorex* sp.) and voles ($N = 6$ *Microtus canacaudis*) did not reveal any visible myxosporean infections (Atkinson unpublished data). Molecular analyses and more extensive host sampling are needed to better assess myxosporean presence and diversity in small North American mammals.

7.3 Waterfowl

Routine post-mortem histological examinations of waterfowl in zoological collections first revealed myxozoan parasitism of these hosts in 1994 (Bartholomew et al. 2008). Over the 12 years that followed, myxozoans were found in the lumina of bile ducts of ten North American ducks of six different species (Anseriformes: Anatidae), which included free-flying native and captive exotic individuals (Table 7.2; Bartholomew et al. 2008). The myxozoans were associated with mild to severe pericholangeal hepatitis but were not the primary cause of death, although severe infections likely contributed to overall poor health. Polysporic plasmodia and in most cases free, mature spores were observed in the lumen of afferent bile ducts. Granulomas were present at foci of destroyed tissue, and severe inflammatory lesions centered on ruptured ducts extruded myxospores into the hepatic parenchyma. DNA sequenced from frozen or formalin-fixed material has shown at least two species of *Myxidium* are present: *M. anatidum* (Fig. 7.3; Bartholomew et al. 2008) and "*Myxidium* sp. 2" (Table 7.2; Atkinson, unpublished data). The SSU sequences of the two species vary by 5 % over ~1,100 bp. Both were detected in mallard/Pekin ducks (*Anas platyrhynchos*) and *Myxidium* sp. 2 was found also in a Baikal teal and a wood duck.

Myxozoan-infected waterfowl are distributed widely in North America, but there are no records of myxozoans in birds from any other continent. Examination of gall bladders and ureters from 23 waterfowl (including nine ducks and four swans) and other birds from Hungary did not reveal any myxozoan infections (Székely, unpublished data). However, the prevalence and diversity of bird myxozoans is difficult to determine, as all records are from non-randomly sampled bird mortalities and not from systematic parasitological surveys of larger waterfowl populations. Given the broad distribution of cases spatially and temporally in North America, myxozoan infections in waterfowl may be more common than currently documented.

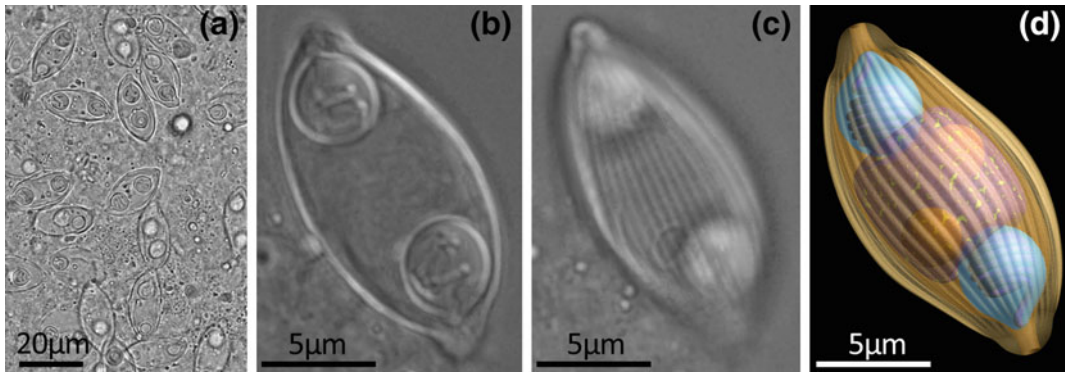

Fig. 7.3 Myxospores of *Myxidium anatidum* from the hepatic bile ducts of a Pekin duck (*Anas platyrhynchos*). **a** Spores viewed under bright field. (**b–c)** Nomarski interference contrast. **b** Polar capsules containing coiled polar filaments at either end of the elongate spore. **c** Spore surface ridges. **d** Composite graphic highlighting spore features including the binucleate sporoplasm

7.4 Humans

Humans are the only large mammal from which myxosporeans have been recorded, though none were viable infections (i.e. neither parasite proliferation nor spore development was observed). Myxospores have been detected in faeces of patients presenting abdominal pain and/or diarrhea, and from immunocompromised individuals. The first report of myxospores in humans was of the salmonid fish parasite *Henneguya salminicola* in stool samples of two Canadian patients with diarrhea; one sample was misidentified initially due to its resemblance to human spermatozoa (McClelland et al. 1997). The myxospores were likely ingested as part of infected fish flesh and passed through the gut undigested. These aberrant parasites were not considered responsible for illness in either patient. This study prompted a brief report of occasional cases of the closely related myxosporean *Henneguya zschokkei* in faeces of Swedish patients (Lebbad and Willcox 1998). There was no mention of symptoms or indication that the myxospores were a disease agent. Again, the myxospores were linked to consumption of fish, most often the salmonid whitefish (*Coregonus lavaretus*).

Three independent cases are reported from Australian patients whose faecal samples contained myxospores of *Myxobolus plectroplites*, known from the freshwater fish *Plectroplites ambiguus* (syn. *Macquaria ambigua*; Boreham et al. 1998). The patients had consumed cooked infected fish and *M. plectroplites* cysts were found in the remaining frozen fillets. The myxospores had passed unchanged through the alimentary tract and were considered unrelated to clinical symptoms of abdominal pain and/or diarrhea as other enteric pathogens were present in two of the patients. Myxospores of a different *Myxobolus* sp. were observed in stool samples, along with other pathogens, from an immunosuppressed patient (Moncada et al. 2001). Again, the myxospores were considered to be incidental and not associated directly with the abdominal symptoms.

Most recently, a myxosporean has been linked directly to outbreaks of food poisoning in Japan. Infections were connected with consumption of raw olive flounder (*Paralichthys olivaceus*) imported from South Korea where it is grown in aquaculture (Kawai et al. 2012; Iwashita et al. 2013). Since 2003, an average of 100 incidents have occurred each year, reaching 158 in 2010 (Kawai et al. 2012) and food poisoning from olive flounder is now considered a major public health concern (Harada et al. 2012b). An extensive epidemiological analysis identified *Kudoa septempunctata* as the culprit; no alternative causative agents such as bacteria or viruses, or bacterial and other toxins were detected (Kawai et al. 2012). Before it was linked to human

illness, *Kudoa septempunctata* had been reported from the muscle of aquacultured olive flounder (Matsukane et al. 2010). The parasite spreads throughout the trunk muscle of the fish and has a range of stages present simultaneously within plasmodia in the myofibres (Iijima et al. 2012; Ohnishi et al. 2013b).

In 1–20 h after consumption of raw, infected olive flounder, humans develop self-limiting diarrhea and emesis (Kawai et al. 2012; Iwashita et al. 2013). The parasite induces a similar reaction in non-human mammals fed myxospores experimentally. Suckling mice had watery stools and an elevated fluid accumulation ratio after intragastric inoculation with a spore suspension, and house musk shrews exhibited vomiting after being fed fish slices spiked with myxospores (Kawai et al. 2012). In vitro inoculation of Caco-2 human intestinal cells with *K. septempunctata* myxospores revealed that the parasite's sporoplasm became active, emerged from the spore valves and invaded the intestinal cells, severely damaging them and compromising cell monolayer integrity (Ohnishi et al. 2013a). Similar assessments with *Myxobolus honghuensis*, common in an important Chinese food fish, allogynogenetic gibel carp (*Carassius auratus gibelio*), showed no adverse effects in suckling mice (Guo et al. 2014), indicating that toxicity to mammal cells is myxozoan species specific.

Identification of the causative agent of the food poisoning led to the development of several molecular assays to facilitate rapid detection: a PCR assay to differentiate *K. septempunctata* from other olive flounder *Kudoa* species (Grabner et al. 2012), two qPCR assays to detect the parasite in fish samples (Harada et al. 2012a; Iijima et al. 2012), and modification of the Harada qPCR to detect the parasite in patient clinical samples (faeces and vomitus) (Harada et al. 2012b). Ohnishi et al. (2013b) caution that since toxicity is provoked only by the myxospore stage, molecular assays that inherently quantify all genetically identical cells will overestimate the dose.

Although sushi and sashimi are popular in other countries, illness has been reported solely from Japan and in connection only with *K. septempunctata.* Flounder is the only known food fish infected with this parasite (Ohnishi et al. 2013a), but many *Kudoa* species occur in other marine food fishes (e.g. *Kudoa thyrsites* in Atlantic salmon; *Kudoa crumena* in yellowfin tuna; *Kudoa inornata* in spotted sea trout; *Kudoa islandica* in spotted wolffish), but these myxosporeans do not appear to illicit illness in humans nor do they adversely affect the health of the fish host. They can have economic impacts however, as some cause post-mortem myoliquefaction, which affects marketability of the fillets.

A third category of myxosporean associations with humans concerns apparent allergic reactions, again to consumption of fish infected with a *Kudoa* species. Infected Chilean hake (*Merluccius gayi gayi*) imported into Spain were linked with 4/15 patients with gastroallergic and/or allergic symptoms and who were positive to a *Kudoa*-pseudocyst skin prick test (Martínez de Velasco et al. 2008). The parasite material used in the skin tests was processed (frozen and homogenised) but uncooked. Whether the hake was consumed raw or cooked was not disclosed in the publication, but this species is usually eaten cooked (Gema Alama-Bermejo, pers. comm.). The elevated humoral response (IgG1 and IgE antibodies) of BALB/c mice immunised with pseudocyst extracts suggested that some components of the parasite could be allergenic and thus result in immunopathological effects in humans (Martínez de Velasco et al. 2002; Martínez de Velasco and Cuéllar 2003). This was followed up with a survey of the seroprevalence of anti-*Kudoa* sp. antibodies in human sera that supported an association between ingestion of *Kudoa* sp. and the allergic reaction (Martínez de Velasco et al. 2007).

In summary, no known myxosporean life cycle involves a human host. Developmental stages have never been observed, which indicates presence of myxospores is incidental from consumption of infected food, rather than the humans being natural hosts. The occurrence of *Myxobolus* and *Henneguya* myxospores in patients was coincidental and not related to symptoms, although reaction to these species by humans has not been investigated. Heat-treated (cooked) myxospores of both species passed intact through the human digestive tract.

Untreated myxospores of *Myxobolus cerebralis* retain both morphology and viability following passage through the gut of piscivorous fishes and birds (e.g. El-Matbouli and Hoffmann 1991). However, most myxospores were digested and none remained viable after passage of this species through mice (El-Matbouli et al. 2005). The spore activation exhibited by *Kudoa septempunctata* in the human gut is unique, and restricted to consumption of raw infected fish. Myxospores of *K. septempunctata* can be inactivated in 3–4 h at −15 to −20 °C, and after 5 min at 75 °C (Iwashita et al. 2013), and thus cooked infected fish should not pose a human health risk. The allergic reactions elicited by another species of *Kudoa* (see above) suggest that ingestion of these parasites can result in immunopathology.

7.5 Phylogenetic Context of Myxosporeans in Homeotherms

Figure 7.4 illustrates the positions of myxosporean parasites with non-fish vertebrate hosts in relation to a myxosporean phylogenetic tree (synthesised from Fiala 2006; Hartigan et al. 2012; Fig. 5.11 in Whipps 2013). DNA data are not yet available for many of the species isolated from homeotherms, so we placed them on the tree in clades with matching myxospore morphology. Figure 7.4 illustrates two primary patterns of species distribution: myxospores from frogs and toads belong to at least seven genera and are distributed widely across the tree, whereas myxospores from homeotherms and reptiles are from five genera and are restricted to only a few clades.

7.6 Diversity and Evolution of Homeotherm-Infecting Myxozoans

Myxosporean records from homeotherms cover limited host and geographic ranges: the two *Soricimyxum* species from mammals are recorded only from shrews in Europe and the two *Myxidium* species from birds are known only from waterfowl

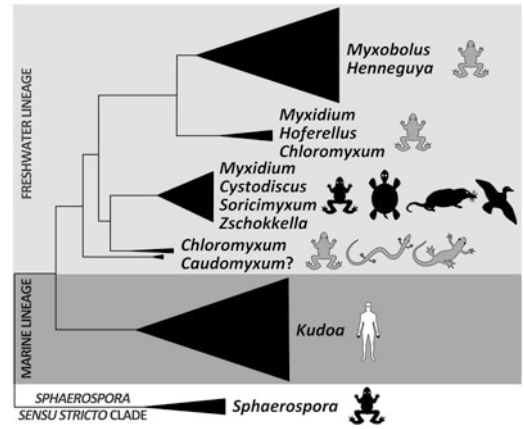

Fig. 7.4 Non-fish vertebrate hosts of myxosporeans shown relative to a generalized myxosporean phylogeny. *Dark* coloured icons represent hosts for which parasite DNA data are known. *Grey* icons indicate no parasite DNA data are available; hosts have been placed based on myxospore morphology. *White* icon (human) indicates incidental host. Host groups: frogs/toads, turtles/tortoises, small mammals, waterfowl, primitive caudate (*Proteus*), newts/salamanders, humans. [Tree topology synthesised from Fiala (2006), Hartigan et al. (2012) and Fig. 5.11 in Whipps (2013)]

in North America. All of these reports are from the last 15 years. This is in stark contrast with records of >2,200 myxosporean species in fishes and >50 in reptiles and amphibians, spanning more than 150 years and from most continents. But is the apparent paucity of taxa from homeothermic hosts an artifact of sample bias or does it reflect actual limited diversity due to barriers to myxosporean exploitation of warm-blooded hosts?

Fewer opportunities exist to reveal hosts in wild homeotherm populations, because surveys of wild populations of small mammals and birds are less frequent and involve fewer animals than fish surveys. In all hosts, disease caused by myxosporean infection is rare, so pathological examinations of mammals and birds typically reveal myxosporeans as incidental findings only, with other pathogens associated with disease or mortality, e.g. botulism in waterfowl. Another source of sample bias could be the failure to recognise myxosporean infections, because of either a lack of diagnostic stages, particularly mature spores, or unfamiliarity with the appearance of myxozoan infections by the examiner.

If the few records of myxosporeans in homeotherms are an accurate indication of diversity, then this suggests fundamental barriers exist to myxosporean exploitation of avian and mammalian hosts. These barriers include the relatively high body temperature of homeotherms, the natural host specificity of myxozoans, and physical restrictions which both limit opportunities for contact of aquatic myxosporeans with potential semi-aquatic or terrestrial hosts, and require unique solutions to problems of myxozoan transmission in semi-aquatic and terrestrial environments.

Previously, we considered body temperature of the host a barrier to host switching from fish to birds (Bartholomew et al. 2008), as most myxozoans have poikilothermic hosts with body temperatures within a few degrees of ambient, and typically much less than the body temperatures of warm blooded vertebrates (>35 °C). However, many myxozoan fish hosts exist in habitats where temperatures approach the body temperatures of homeotherms, especially species from tropical and subtropical regions. Common carp (*Cyprinus carpio*), which inhabit ponds in southern United States, can tolerate water temperatures up to 35.7 °C (McLarney 1998 in Ficke et al. 2007) and are host for a number of myxozoans including *Myxidium* species. Therefore, survival of some fish myxozoans at homeothermic body temperatures is fundamentally feasible and fish-to-homeotherm switching events are more likely to occur in warmer environments, where thermophilic myxosporeans are more prevalent. Indeed, the known cases of myxosporean infection in waterfowl are from warmer areas.

The presence of myxosporean infections in shrews in the relatively cool environment of central Europe, may be the result of a homeotherm-homeotherm switching event—with a fish-to-migratory bird jump in tropical areas and subsequent bird-to-mammal transfer in temperate climates. This hypothesis is supported by the close phylogenetic relationship between *Soricimyxum* in shrews in Europe and *Myxidium* in migratory waterfowl in North America, which are sister taxa in the biliary *Myxidium* clade defined by Fiala (2006) (Hartigan et al. 2012; Fig. 7.3). This clade has a wide variety of non-fish vertebrate hosts, including reptiles and amphibians and the only myxozoans known from birds and mammals. This diversity of non-fish hosts suggests a single host switch from a common *Myxidium*-like ancestor in a fish to an amphibian, followed by radiation into semi-aquatic and terrestrial poikilothermic and homeothermic hosts.

At this early stage of homeotherm myxozoan research, it is unclear which fundamental characteristics of these *Myxidium*-clade myxosporeans enabled them to exploit this variety of non-fish hosts and what adaptations to their life cycles and transmission modes have allowed them to be successful in semi-aquatic and terrestrial environments.

Most myxozoan species infect a single family or species of fish (or annelid). This fundamental characteristic reduces the probability of successful cross-species infections. No myxozoan species from a non-fish vertebrate is known also from sympatric fish hosts, which suggests fish to non-fish host switches are infrequent, and genetic divergence after a host switch is rapid.

No life cycle is known from myxozoans that infect homeotherms, though they are most likely analogous to life cycles known throughout the Myxosporea (see Chap. 10), which involve an obligate, aquatic, annelid definitive host and two morphologically distinct waterborne spore stages. Transmission via waterborne spores may be the largest barrier to myxosporean host range expansion into semi-aquatic and terrestrial homeothermic hosts. Frogs and toads have aquatic larvae morphologically similar and sympatric with fish populations and are exposed naturally to fish-infecting waterborne actinospore stages, thus the diversity of myxosporeans in frogs and toads reflects these natural opportunities for cross-infection in the aquatic environment. Actinospores would infect the mammal or bird host through the thin epithelia of mouth, eyes or throat, like the epidermal surfaces of fish (El-Matbouli et al. 1999). Reinfection of the aquatic annelid host would occur via myxospores expelled from the vertebrate host by defecation or urination into water.

Adaptations would be required for transmission in a terrestrial setting. Alternation of invertebrate and vertebrate hosts and spore stages could still occur if the spore stages remained viable either within the host (i.e. not released) or within faeces in moist terrestrial habitats such as leaf litter or below ground. The parasite would utilize trophic transmission—spores would enter the host via direct consumption of infectious material, either in faeces or invertebrate prey items. The diet of *Sorex araneus* consists of a wide variety of invertebrates, dominated by earthworms, molluscs, beetles and spiders (Churchfield et al. 2012). Although the alternate stage of *Soricimyxum* is thought to involve a terrestrial annelid (Dyková et al. 2011), other invertebrates may be involved as *Sorex minutus* typically eat spiders not earthworms (Butterfield et al. 1981). This completely non-aquatic pathway of myxosporean transmission would require evolution of the parasite to exploit both non-aquatic vertebrate and invertebrate hosts. The inherent difficulty or low probability of these multiple adaptations may be the reason few myxozoans are known from terrestrial mammals.

An alternate parasite transmission strategy is direct vertebrate-to-vertebrate passage. Parasites could be transmitted through ingestion of myxospores shed from infected individuals, or from consumption of host tissues that contain spores or parasite proliferative stages. Direct fish-to-fish transmission has been shown to occur via developmental stages (but not spores) of several members of Family Myxidiidae: *Enteromyxum leei*, *Enteromyxum scophthalmi* and *Enteromyxum fugi* (Diamant 1997; Redondo et al. 2002; Yasuda et al. 2002). Direct transmission permits spread of infection in host populations in the absence of the alternate host. This does not eliminate the possibility that these myxosporeans could utilise an invertebrate host for amphimixis, which would maintain genetic diversity, as myxosporean sexual reproduction is known to occur only in the invertebrate host.

If the observed paucity of myxosporean parasites of homeotherms is due to a relative lack of sampling of non-fish versus fish hosts then many more taxa remain to be discovered in these hosts. The myxosporean pattern of infecting vertebrate hosts that are associated intimately with water (fish, frogs, turtles, ducks) suggests that myxozoan infections might occur in many animals that utilise aquatic habitats, including mammals such as cetaceans (dolphins and whales), carnivores (otters), monotremes (platypus) and rodents (beavers, nutria). The diversity of potential mammalian and avian hosts suggests many more myxosporean parasites remain to be discovered with further scrutiny of these homeotherms, especially in Africa and South America where relatively few myxosporeans have yet been described.

7.7 Key Questions for Future Studies

- Is direct transmission possible between small mammals, and does this explain the observed high infection rates in shrews?
- What is the 'terrestrial' life cycle? Are earthworms involved, or a different invertebrate?
- Could sampling shrew faeces be used to assess parasite geographic range?
- How do waterfowl become infected: trophic transmission via consumption of aquatic invertebrates or direct contact of actinospores with epithelial surfaces?
- What is unique about *Kudoa septempunctata* that it is the only known myxosporean whose myxospores cause gastroenteritis in humans?
- There are whole continents (Africa, South America) with rich non-fish vertebrate faunae —will the diversity of potential hosts be reflected in the discovery of novel myxozoans?

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References

Bartholomew JL, Atkinson SD, Hallett SL, Lowenstine LJ, Garner MM, Gardiner CH, Rideout BA, Keel MK, Brown JD (2008) Myxozoan parasitism in waterfowl. Int J Parasitol 38:1199–1207

Boreham RE, Hendrick S, O'Donoghue PJ, Stenzel DJ (1998) Incidental finding of *Myxobolus* spores (Protozoa: Myxozoa) in stool samples from patients with gastrointestinal symptoms. J Clin Microbiol 36:3728–3730

Butterfield J, Coulson JC, Wanless S (1981) Studies on the distribution, food, breeding biology and relative abundance of the pygmy and common shrews (*Sorex minutus* and *S. araneus*) in upland areas of northern England. J Zool Lond 195:169–180

Churchfield S, Rychlik L, Taylor JRE (2012) Food resources and foraging habits of the common shrew, *Sorex araneus*: does winter food shortage explain Dehnel's phenomenon? Oikos 121:1593–1602. doi:10.1111/j.1600-0706.2011.20462.x

Diamant A (1997) Fish-to-fish transmission of a marine myxosporean. Dis Aquat Org 30:99–105

Dyková I, Tyml T, Fiala I, Lom J (2007) New data on *Soricimyxum fegati* (Myxozoa) including analysis of its phylogenetic position inferred from the SSU rRNA gene sequence. Folia Parasitol 54:272–276

Dyková I, Tyml T, Kostka M (2011) Xenoma-like formations induced by *Soricimyxum fegati* (Myxosporea) in three species of shrews (Soricomorpha: Soricidae), including records of new hosts. Folia Parasitol 58:249–256

Eiras JC (2005) An overview on the myxosporean parasites in amphibians and reptiles. Acta Parasitol 50:267–275

El-Matbouli M, Hoffmann RW (1991) Effects of freezing, aging, and passage through the alimentary canal of predatory animals on the viability of *Myxobolus cerebralis* spores. J Aquat Anim Health 4:44–47

El-Matbouli M, Hoffmann RW (1999) Whirling disease: host specificity and interaction between the actinospore stage of *Myxobolus cerebralis* and rainbow trout *Oncorhynchus mykiss*. Dis Aquat Org 35:1–12

El-Matbouli M, Sobottka I, Schumacher U, Schottelius J (2005) Effect of passage through the gastrointestinal tract of mice on the viability of *Myxobolus cerebralis* (Myxozoa) spores. Bull Eur Assoc Fish Pathol 25:276–279

Fiala I (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. Int J Parasitol 36:1521–1534

Ficke AD, Myrick CA, Hansen LJ (2007) Potential impacts of global climate change on freshwater fisheries. Rev Fish Biol Fisher 17:581–613

Friedrich C, Ingolic E, Freitag B, Kastberger G, Hohmann V, Skofitsch G, Neumeister U, Kepka O (2000) A myxozoan-like parasite causing xenomas in the brain of the mole, *Talpa europaea* L., 1758 (Vertebrata, Mammalia). Parasitol 121:483–492

Garner MM, Bartholomew JL, Whipps CM, Nordhausen RW, Raiti P (2005) Renal myxozoanosis in crowned river turtles *Hardella thurjii*: description of the putative agent *Myxidium hardella* n. sp. by histopathology, electron microscopy, and DNA sequencing. Vet Pathol 42:589–595

Grabner DS, Yokoyama H, Shirakashi S, Kinami R (2012) Diagnostic PCR assays to detect and differentiate *Kudoa septempunctata*, *K. thyrsites* and *K. lateolabracis* (Myxozoa, Multivalvulida) in muscle tissue of olive flounder (*Paralichthys olivaceus*). Aquaculture 338–341:36–40

Guo QX, Gu ZM, Jia L, Qing JH, Li H (2014) Pathogenicity studies of *Myxobolus honghuensis* (Myxosporea: Bivalvulida) using suckling mice model. Paper presented at the 7th international symposium on aquatic animal health, Portland Oregon, USA, 31 Aug—4 Sept 2014

Harada T, Kawai T, Sato H, Yokoyama H, Kumeda Y (2012a) Development of a quantitative polymerase chain reaction assay for detection of *Kudoa septempunctata* in olive flounder (*Paralichthys olivaceus*). Int J Food Microbiol 156:161–167

Harada T, Kawai T, Jinnai M, Ohnishi T, Sugita-Konishi Y, Kumeda Y (2012b) Detection of *Kudoa septempunctata* 18S Ribosomal DNA in patient fecal samples from novel food-borne outbreaks caused by consumption of raw olive flounder (*Paralichthys olivaceus*). J Clin Microbiol 50:2964–2968

Hartigan A, Fiala I, Dyková I, Rose K, Phalen DN, Šlapeta J (2012) New species of Myxosporea from frogs and resurrection of the genus *Cystodiscus* Lutz, 1889 for species with myxospores in gallbladders of amphibians. Parasitology 139:478–496

Iijima Y, Nakanishi N, Furusawa H, Ohnishi T, Sugita-Konishi Y (2012) Inter-laboratory validation and applications of quantitative real-time PCR for the detection of *Kudoa septempunctata* in olive flounder (*Paralichthys olivaceus*). Jpn J Infect Dis 65:436–438

Iwashita Y, Kamijo Y, Nakahashi S, Shindo A, Yokoyama K, Yamamoto A, Omori Y, Ishikura K, Fujioka M, Hatada T, Takeda T, Maruyama K, Imai H (2013) Food poisoning associated with *Kudoa septempunctata*. J Emerg Med 44:943–945. doi:10.1016/j.jemermed.2012.11.026

Jirkù M, Bolek MG, Whipps CM, Janovy J Jr, Kent ML, Modrÿ D (2006) A new species of *Myxidium* (Myxosporea: Myxidiidae), from the western chorus frog, *Pseudacris triseriata triseriata*, and Blanchard's cricket frog, *Acris crepitans blanchardi* (Hylidae), from eastern Nebraska: morphology, phylogeny, and critical comment. J Parasitol 92:611–619

Kawai T, Sekizuka T, Yahata Y, Kuroda M, Kumeda Y, Iijima Y, Kamata Y, Sugita-Konishi Y, Ohnishi T (2012) Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in raw fish. Clin Infect Dis 54:1046–1052

Lebbad M, Willcox M (1998) Spores of *Henneguya salminicola* in human stool specimens. J Clin Microbiol 36:182

Lom J, Dyková I (2005) Microsporidian xenomas in fish seen in wider perspective. Folia Parasitol 52:69–81

Lom J, Dyková I, Feist S (1989) Myxosporea-induced xenoma formation in pike, Esox lucius L., renal corpuscles associated with *Myxidium lieberkuehni* infection. Eur J Protistol 24:271–280

Martínez de Velasco G, Cuéllar C (2003) Humoral immune responses induced by *Kudoa* sp. (Myxosporea: Multivalvulida) in BALB/c mice: oral administration, immunization and cross-reactions with *Myxobolus aeglefini* (Myxosporea: Bivalvulida). Parasite Immunol 25:449–456

Martínez de Velasco G, Rodero M, Zapatero L, Cuéllar C (2002) Humoral immune responses induced by *Kudoa* sp. (Myxosporea: Multivalvulida) antigens in BALB/c mice. Memórias do Instituto Oswaldo Cruz 97:1091–1095

Martínez de Velasco G, Rodero M, Chivato C, Cuéllar C (2007) Seroprevalence of anti-*Kudoa* sp. (Myxosporea: Multivalvulida) antibodies in a Spanish population. Parasitol Res 100:1205–1211

Martínez de Velasco G, Rodero M, Cuéllar C, Chivato T, Mateos JM, Laguna R (2008) Skin prick test of *Kudoa* sp. antigens in patients with gastrointestinal and/or allergic symptoms related to fish ingestion. Parasitol Res 103:713–715

Matsukane Y, Sato H, Tanaka S, Kamata Y, Sugita-Konishi Y (2010) *Kudoa septempunctata* n. sp. (Myxosporea: Multivalvulida) from an aquacultured olive flounder (*Paralichthys olivaceus*) imported from Korea. Parasitol Res 107:865–872

McClelland RS, Murphy DM, Cone DK (1997) Report of spores of *Henneguya salminicola* (Myxozoa) in human stool specimens: possible source of confusion with human spermatozoa. J Clin Microbiol 35:2815–2818

Moncada LI, Lo´pez MC, Murcia MI, Nicholls S, León F, Guío OL, Corredor A (2001) *Myxobolus* sp., another opportunistic parasite in immunosuppressed patients. J Clin Microbiol 39:1938–1940

Ohnishi T, Kikuchi Y, Furusawa H, Kamata Y, Sugita-Konishi Y (2013a) *Kudoa septempunctata* invasion increases the permeability of human intestinal epithelial monolayer. Foodborne Pathog Dis 10:137–142. doi:10.1089/fpd.2012.1294

Ohnishi T, Furusawa H, Yoshinari T, Yamazaki A, Horikawa K, Kamata Y, Sugita-Konishi Y (2013b) Electron microscopic study of *Kudoa septempunctata* infecting *Paralichthys olivaceus* (olive flounder). Jpn J Infect Dis 66:348–350

Prunescu C-C, Prunescu P, Pucek Z, Lom J (2007) The first finding of myxosporean development from plasmodia to spores in terrestrial mammals: *Soricimyxum fegati* gen. et sp. n. (Myxozoa) from *Sorex araneus* (Soricomorpha). Folia Parasitol 54:159–164

Redondo MJ, Palenzuela O, Riaza A, Macías Á, Álvarez-Pellitero P (2002) Experimental transmission of *Enteromyxum scophthalmi* (Myxozoa), an enteric parasite of turbot *Scophthalmus maximus*. J Parasitol 88:482–488

Székely C, Cech G, Ostoros G, Gubányi A (2011) First occurrence of myxozoan parasites in small mammals (*Sorex araneus* and *Sorex minutus*) in Hungary. In: Abstract book of the 15th international conference on diseases of fish and shellfish, Split, Croatia, p 436, 12–16 Sept

Whipps CM (2013) Myxozoa: the spore-forming cnidarians. In: Goater TM, Goater CP, Esch GW (eds) Parasitism: the diversity and ecology of animal parasites, 2nd edn. Cambridge University Press, New York, p 109

Yasuda H, Ooyama T, Iwata K, Tun T, Yokoyama H, Ogawa K (2002) Fish-to-fish transmission of *Myxidium* spp. (Myzozoa) in cultured tiger puffer suffering from emaciation disease. Fish Pathol 37:29–33