ARTICLE IN PRESS

International Journal for Parasitology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Phylogenetic and environmental DNA insights into emerging aquatic parasites: implications for risk management ☆

Salma Sana [a,*], Chris Williams [b], Emilie A. Hardouin [a], Adrian Blake [a], Phil Davison [a,c], Josie Pegg [a], Richard Paley [d], Tiantian Zhang [a], Demetra Andreou [a,*]

[a] Bournemouth University, Faculty of Science and Technology, Fern Barrow, Talbot Campus, Poole, Dorset BH12 5BB, UK
[b] Environment Agency, National Fisheries Laboratory, Bromholme Lane, Brampton, Cambridgeshire PE28 4NE, UK
[c] Centre for Environment, Fisheries and Aquaculture Science, Pakefield Road, Lowestoft, Suffolk NR33 0HT, UK
[d] Centre for Environment, Fisheries and Aquaculture Science, The Nothe, Barrack Road, Weymouth, Dorset DT4 8UB, UK

ARTICLE INFO

Article history:
Received 12 July 2017
Received in revised form 16 November 2017
Accepted 26 November 2017
Available online xxxx

Keywords:
Emergence
Invasion history
Environmental DNA
Rosette agent
Topmouth gudgeon
Epidemiology

ABSTRACT

Species translocation leads to disease emergence in native species of considerable economic importance. Generalist parasites are more likely to be transported, become established and infect new hosts, thus their risk needs to be evaluated. Freshwater systems are particularly at risk from parasite introductions due to the frequency of fish movements, lack of international legislative controls for non-listed pathogens and inherent difficulties with monitoring disease introductions in wild fish populations. Here we used one of the world's most invasive freshwater fish, the topmouth gudgeon, *Pseudorasbora parva*, to demonstrate the risk posed by an emergent generalist parasite, *Sphaerothecum destruens*. *Pseudorasbora parva* has spread to 32 countries from its native range in China through the aquaculture trade and has introduced *S. destruens* to at least five of these. We systematically investigated the spread of *S. destruens* through Great Britain and its establishment in native fish communities through a combination of phylogenetic studies of the host and parasite and a novel environmental DNA detection assay. Molecular approaches confirmed that *S. destruens* is present in 50% of the *P. parva* communities tested and was also detected in resident native fish communities but in the absence of notable histopathological changes. We identified specific *P. parva* haplotypes associated with *S. destruens* and evaluated the risk of disease emergence from this cryptic fish parasite. We provide a framework that can be applied to any aquatic pathogen to enhance detection and help mitigate future disease risks in wild fish populations.
© 2018 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Species translocation leads to disease emergence of considerable ecological and economic importance (Fisher et al., 2012). Generalist parasites are more likely to be transported, become established and infect new hosts, and pose a high risk to biodiversity across ecosystems. Freshwater systems are particularly at risk due to insufficient international legislation and system-inherent disease monitoring difficulties (Gozlan, 2012). This has resulted in the frequent introduction of non-native parasites to freshwater fisheries (Williams et al., 2013) with a risk of aquatic disease emergence and associated declines in wild fish populations (Peeler et al., 2011; Ercan et al., 2015). Non-native parasites with direct life-cycles, low host specificity, tolerant and long-lived environmental infectious propagules and a wide temperature tolerance are more likely to be translocated and become established in new environments (Andreou et al., 2009; Fisher et al., 2012).

The topmouth gudgeon, *Pseudorasbora parva*, is a small cyprinid fish that is naturally distributed in eastern Asia. It was introduced into Europe from China in the 1960s through a succession of accidental introductions into the area around the Black Sea through the trade of Chinese carp in aquaculture (Gozlan et al., 2010). It has now invaded at least 32 countries, including most of Europe, plus Turkey, Iran and Morocco, with their long-distance dispersal also occurring via aquaculture trade routes (Gozlan et al., 2010). In 2005, *P. parva* was identified as a healthy reservoir of the generalist parasite *Sphaerothecum destruens* which has been identified as a threat to freshwater fish biodiversity (Gozlan et al., 2005).

☆ Note: Nucleotide sequence data reported in this paper are available in the GenBank™ database under the accession numbers MG283239-MG283252, MG386173-MG386179.

* Corresponding authors.
E-mail addresses: ssana@bournemouth.ac.uk (S. Sana), dandreou@bournemouth.ac.uk (D. Andreou).

https://doi.org/10.1016/j.ijpara.2017.11.002
0020-7519/© 2018 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite this article in press as: Sana, S., et al. Phylogenetic and environmental DNA insights into emerging aquatic parasites: implications for risk management. Int. J. Parasitol. (2018), https://doi.org/10.1016/j.ijpara.2017.11.002

The parasite has also been identified as non-native to Europe (Sana et al., 2017) having been introduced with the highly invasive fish *P. parva*. Great Britain (GB) is the first European country where *S. destruens* was identified in *P. parva* populations (Gozlan et al., 2005). *Pseudorasbora parva* was first recorded in the UK in an aquaculture facility in southern England in 1996 (Domaniewski and Wheeler, 1996) and has rapidly spread and colonized up to 23 UK water bodies (Britton et al., 2008). All *P. parva* populations in the UK have been associated with aquaculture or recreational fisheries with no recorded established populations in wild habitats such as streams, rivers or lakes. In response to the potential threats posed by *P. parva* (Britton et al., 2007), a national programme of eradication has been designed and administered (Britton et al., 2010). The programme aimed at complete eradication of *P. parva* from high risk sites (with high risk sites identified based on the conservation and fishery value of the adjacent water body) or containment in the case of medium risk sites (Britton et al., 2008). By 2014, 15 out of 23 confirmed *P. parva* sites had been eradicated, with a further six sites to be eradicated in England by 2017 (Britton et al., 2010; GBNNSS, 2015).

Despite *P. parva* having no wild populations in GB, several sites invaded by *P. parva* have water effluents which flow into wild freshwater habitats. This can have important implications for transmission of the parasite as epidemiological modelling has predicted that *S. destruens* can spread to and establish in connected downstream communities through environmental transmission of their infective spores and zoospores within 1 year post introduction of infected *P. parva* (Al-Shorbaji et al., 2016). The same work also predicted that *S. destruens* can establish in new hosts and maintain its transmission in the absence of the initial reservoir host - in this case *P. parva* (Al-Shorbaji et al., 2016). As *S. destruens* is a true generalist, it is highly probable that adjacent communities downstream from established *P. parva* populations, positive for *S. destruens*, have established *S. destruens* infections (Andreou and Gozlan, 2016). Despite an absence of disease detected in wild fish populations through existing monitoring activities, *S. destruens* has been proposed as a high risk parasite with the recommendation that its prevalence is closely monitored (Andreou and Gozlan, 2016). However, the cryptic nature of infections in fish can make the detection of *S. destruens* in asymptomatic fish problematic. Moreover, the sacrificial sampling of wild fish, in particular salmonids, is undesirable. As such, there is a need for a detailed epidemiological picture of *S. destruens* in GB, combining traditional methods of detection (e.g. DNA-based detection and microscopic examination of host tissue) with more novel approaches employing environmental DNA (eDNA) detection (due to its cost efficiency) (Andreou et al., 2011a). Here we used GB as a model country to determine how aquatic non-native parasites could spread through reservoir host translocation. Specifically, we (i) determined the distribution and presence of *S. destruens* in *P. parva* populations and deciphered the spread using the genetic diversity of the parasite and host and (ii) assessed the of risk of disease transfer to native fishes in water bodies with direct connection to *P. parva* holding waters, using a combination of in-tissue molecular detection, histopathology and a novel eDNA detection test for *S. destruens*.

2. Materials and methods

2.1. Detection of S. destruens in P. parva populations

Seven *P. parva* populations ($n = 210$ fish) were sampled from England and Wales prior to their eradication in 2013–2015 by the Environment Agency, National Fisheries Laboratory, Cambridgeshire, UK (Fig. 1; Table 1). Permission to sample these populations was granted by the Environment Agency, UK. Populations were sampled from six enclosed still water fisheries and two fisheries with outlets to streams. In two populations, roach (*Rutilus rutilus*) was also present and thus sampled ($n = 15$; Table 1). A number of native fish species were sampled from the stream adjacent to the proposed original site of *P. parva* introduction in 2015 and 2016 (Site 1; Table 1).

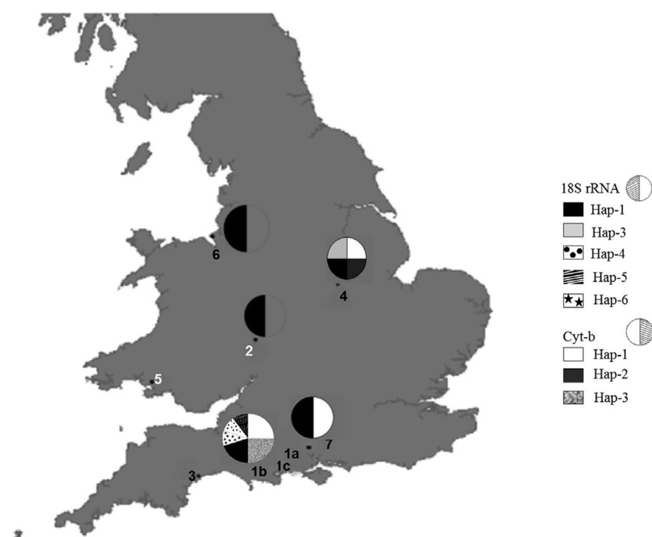

Fig. 1. Distribution of sampled *Pseudorasbora parva* populations across Great Britain (GB). Population 1a is the hypothesised first *P. parva* population in GB to have been introduced in the mid-1980s (Domaniewski and Wheeler, 1996). Details of each sampled population can be found in Table 1. The black and white numbering for each population represents the two genetically different metapopulations of the host *P. parva* in GB (Blake et al., unpublished data). The 18S rRNA haplotypes for *Sphaerothecum destruens* are represented in the left half of each circle, the cytochrome b (Cyt-b) gene haplotypes are in the right half of each circle. The different patterns and shadings represent different haplotypes.

All fish were euthanised through a lethal dose of benzocaine. From all *P. parva*, samples of liver and kidney tissues were divided with one half fixed in 100% ethanol for molecular detection and the remaining half fixed in 10% Neutral Buffered Formalin (NBF) for histopathology. From all native fish species, detailed post mortem examinations were performed to detect gross pathological changes and the presence of parasites. Liver and kidney samples were taken in 100% ethanol for molecular detection, with additional samples of liver, kidney, spleen, gut, heart and gill for histopathological assessment. Molecular detection using the 18S rRNA gene was performed in pooled kidney and liver samples as described in Andreou et al. (2012). All *S. destruens*-positive samples had their cytochrome b gene (Cyt-b) amplified as per Sana et al., (2017) and their tissues were histopathologically checked for the presence of *S. destruens* (Andreou et al., 2011a). For clarity, all *S. destruens* Cyt-b haplotypes will be named as *S. destruens*_Cytb. Samples for histopathology were trimmed, dehydrated in alcohol, embedded in paraffin wax, sectioned at 3 μm, stained using H&E and examined microscopically for pathological changes and the presence of *S. destruens.*

2.2. Deciphering invasion history through host and parasite phylogenetic relationships

In order to investigate any potential links between specific *P. parva* populations or genetic lineages and the spread of *S. destruens*, all *P. parva* had their Cyt-b gene amplified and sequenced as per Simon et al. (2011). For clarity, all *P. parva* Cyt-b haplotypes will be named as *P. parva*_Cytb. The obtained Cyt-b sequences for *P. parva* were then aligned with all the available *P. parva* Cyt-b

Please cite this article in press as: Sana, S., et al. Phylogenetic and environmental DNA insights into emerging aquatic parasites: implications for risk management. Int. J. Parasitol. (2018), https://doi.org/10.1016/j.ijpara.2017.11.002

Download English Version:

https://daneshyari.com/en/article/8500116

Download Persian Version:

https://daneshyari.com/article/8500116

Daneshyari.com