Apparent interspecific transmission of *Aphanomyces astaci* from invasive signal to virile crayfish in a sympatric wild population

James J[1*], Mrugała A[2], Oidtmann B[3], Petrusek A[2]#, Cable J[1]#

[1]School of Biosciences, Cardiff University, Cardiff, CF10 3AX, United Kingdom.

[2]Department of Ecology, Faculty of Science, Charles University, Prague, Viničná 7, CZ-12844 Prague 2, Czech Republic

[3]Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, Weymouth, Dorset, DT4 8UB, United Kingdom

*Corresponding author. E-mail: jamesj12@cardiff.ac.uk

Joint last authors

Running title: Interspecific transmission of *Aphanomyces astaci* between invasive crayfish

Abstract

The crayfish plague pathogen (*Aphanomyces astaci*) causes mass mortalities of European crayfish when transmitted from its original North American crayfish hosts. Little is known, however, about interspecific transmission of the pathogen between different American crayfish species, although evidence from trade of ornamental crayfish suggests this may happen in captivity. We screened signal and virile crayfish for *A. astaci* at allopatric and sympatric sites in a UK river. Whilst the pathogen was detected in signal crayfish from both sites, infected virile crayfish were only found in sympatry. Genotyping of *A. astaci* from virile crayfish suggested the presence of a strain related to one infecting British signal crayfish. We conclude that virile crayfish likely contracted *A. astaci* interspecifically from infected signal crayfish. Interspecific transmission of *A. astaci* strains differing in virulence

between American carrier species may influence the spread of this pathogen in open waters with potential exacerbated effects on native European crayfish.

Keywords: *Pacifastacus leniusculus*; *Orconectes* cf. *virilis*; Wildlife disease; Transmission pathways.

1. Introduction

The crayfish plague agent, *Aphanomyces astaci,* is arguably one of the most devastating invasive parasites in European freshwaters (Lowe, 2004; DAISIE, 2009). Since its first introduction in the mid-19th century (Alderman, 1996; Holdich, 2003), the pathogen has spread throughout Europe, facilitated in recent decades by movements of invasive North American (henceforth referred to as American) crayfish (Souty-Grosset *et al.,* 2006; Holdich *et al.,* 2014; James *et al.*, 2014). Whilst American crayfish are often asymptomatic carriers of *A. astaci* infection, the disease is usually lethal in European species (Unestam & Weiss, 1970; Diéguez-Uribeondo *et al.,* 1997; Bohman, *et al.*, 2006; Kozubíková *et al.,* 2008). Once introduced, *A. astaci* can spread rapidly, transmitted through zoospores that are released into water (Oidtmann *et al.,* 2002) and can survive for at least 14 days (CEFAS, 2000). Spores are mainly released during host moulting or death (Svoboda *et al.,* 2013), and within a cadaver *A. astaci* can remain viable for several days (Oidtmann *et al.,* 2002). Therefore, the movement of infected carcasses by predators could facilitate pathogen dispersal. If fish ingest infected tissue, the pathogen can even survive passage through the gastro-intestinal tract, providing an additional transmission pathway (Oidtmann *et al.,* 2002).

Whilst the transmission of *A. astaci* from non-native American to European crayfish has been widely documented (e.g. Alderman *et al.*, 1990; Diéguez-Uribeondo, Temiño & Múzquiz, 1997; Vennerström *et al.*, 1998; Bohman *et al.,* 2006), little is known about

50 interspecific pathogen transmission between these invasive carriers. Until now, four different
51 *A. astaci* genotype groups have been isolated in Europe; group A was obtained from infected
52 native European crayfish (*Astacus astacus* and *A. leptodactylus*) and groups B, D and E from
53 different American crayfish species (*Pacifastacus leniusculus*, *Procambarus clarkii* and
54 *Orconectes limosus*, respectively) (Svoboda *et al.,* 2017). The genotype groups infecting
55 additional *A. astaci* carriers known from European waters, calico (*Orconectes immunis*),
56 marbled (*Procambarus fallax* f. *virginalis*) and virile (*Orconectes* cf. *virilis*) crayfish
57 (Filipova *et al.,* 2013; Schrimpf *et al.,* 2013; Keller *et al.,* 2014; Tilmans *et al.,* 2014), are so
58 far unknown. Existing data suggest that *A. astaci* genotype groups are host-specific among
59 American crayfish (Grandjean *et al.,* 2014). There is no evidence of strains transmitting
60 between these crayfish in the wild, although it seems to occur in the aquarium trade (Mrugała
61 *et al.,* 2015).

62 Here, we investigate interspecific transmission of *A. astaci* upon contact of two
63 potential carrier species. Signal crayfish are widespread across the UK (James *et al.,* 2014)
64 and were initially stocked into the River Lea during the mid-1970s (Almeida *et al.,* 2014).
65 Conversely, virile crayfish are only found in the Lea catchment in the UK (James *et al.,*
66 2014), possibly unintentionally introduced there around 2004 (Ahern *et al.,* 2008). The two
67 species have been co-existing since at least 2011 (James *et al.,* 2015). Virile crayfish in this
68 river, as well as in a population from the Netherlands, have been reported to carry *A. astaci*
69 (Tilmans *et al.,* 2014), and it was suggested that these crayfish were already infected prior to
70 introduction. Here, we tested this hypothesis, the alternative hypothesis that virile crayfish
71 contracted *A. astaci* from co-existing signal crayfish, by evaluating the distribution of the
72 pathogen at allopatric and sympatric sites within the River Lea and an adjacent lake in
73 London, and by genotyping the pathogen from infected host specimens.
74

2. Methods

Invasive signal crayfish and virile crayfish were collected from the River Lea and an adjacent lake in London, UK, during September 2014. Using baited traps employed over two consecutive nights and checked daily, animals were caught from allopatric (Lat/Long: 51°45′14″N/ 000°00′16″E, 51°42′29″N/ 000°01′16″W for signal and virile crayfish respectively, n = 30 for each species) and a sympatric site (Lat/Long: 51°42′24″N/ 000°01′04″W, n = 9 signal and 30 virile crayfish) (Fig. 1). Upon capture, animals were transported individually to Cardiff University (UK), humanely euthanized by freezing at -80^{o}C and stored in ca. 95% molecular grade ethanol before transport to Charles University in Prague for further processing. For *A. astaci* screening, we harvested from each animal a section of tail fan, soft abdominal cuticle, two limb joints, and any melanised cuticle (as in Svoboda *et al.,* 2014). Tissue samples from each individual (40-50 mg) were ground together in liquid nitrogen from which DNA was extracted using a DNeasy tissue kit (Qiagen) as per manufacturer's guidelines.

All samples were screened for *A. astaci* presence using TaqMan MGB quantitative PCR (qPCR) on the iQ5 BioRad thermal cycler according to Vrålstad *et al.,* (2009), slightly modified to increase assay specificity (Strand *et al.,* 2011; Svoboda *et al.,* 2014). To check for potential inhibition (as in Kozubíková *et al.,* 2011; Svoboda *et al.,* 2014) each DNA isolate was analysed by qPCR at two concentrations (undiluted and 1:10 dilution). Negative controls were included at each step of the protocol, and in all cases these remained negative. Based on the estimated amount of *A. astaci* DNA in the isolates (expressed in PCR-forming units, PFU), the extent of the infection was expressed in semi-quantitative agent levels (A0-A7; according to Vrålstad *et al.,* 2009; Kozubíková *et al.,* 2011). Samples with agent levels of A2 or higher were considered positive for *A. astaci*.

For *A. astaci* genotype group identification, we analysed *A. astaci*-positive samples using nine *A. astaci*-specific microsatellite markers (Grandjean *et al.,* 2014). As amplification success depends on the amount of pathogen DNA in the sample, genotyping was only attempted for those with agent level A3 and higher (as in Grandjean *et al.,* 2014) and was repeated three times for each sample. In case of an initial lack of amplification, DNA isolates were concentrated on the Concentrator Plus 5305 (Eppendorf). The results of successful genotyping were compared with the *A. astaci* reference strains described by Grandjean *et al.,* 2014 and an *A. astaci*-positive DNA isolate from signal crayfish in Lake Mochdre (Newtown) Wales, UK (James *et al.,* 2017).

3. Results

Within allopatric sites on the River Lea, *Aphanomyces astaci* was detected in 83% (25 out of 30) signal crayfish but was not detected in any virile crayfish (n = 30). From the sympatric site, 44% (4 out of 9) signal crayfish and 23% (7 out of 30) virile crayfish tested positive for *A. astaci* infection. All *A. astaci*-positive samples yielded low levels of infection (A2-A3; Vrålstad *et al.,* 2009). Of the *A. astaci* infected animals from the allopatric signal crayfish site A3 level infections were detected in four animals (estimated PFUs: 51, 71, 106 and 111). Within the sympatric site, A3 level infections were detected in three virile (estimated PFUs: 85, 167 and 1000) and two signal (estimated PFUs: 52 and 57) crayfish.

Due to low amount of *A. astaci* DNA, reliable amplification and scoring of the microsatellites were only possible for two specimen of virile crayfish. Of the nine microsatellite loci, amplification was achieved for seven loci for the first virile crayfish specimen, and for six loci for the second one. For the first virile crayfish specimen the multilocus genotype corresponded at five loci to the reference axenic culture of the genotype group B (Table 1). Differences were observed at the Aast9 and Aast12 loci, where

125 homozygotes rather than heterozygotes were scored. Such variation at the Aast9 locus has
126 been also observed in the *A. astaci*-positive DNA isolate from signal crayfish in Wales, UK
127 (Table 1; James *et al.* 2017). For the second virile crayfish specimen the multilocus genotype
128 corresponded at five loci to the reference axenic culture of genotype group B (Table 1). A
129 difference was observed at the Aast9 locus, where a homozygote rather than a heterozygote
130 was scored. All six of the amplified loci for this second specimen matched the *A. astaci*
131 positive DNA isolate from signal crayfish in Wales (Table 1).
132
133 **4. Discussion**
134 Here we present two lines of evidence suggesting the interspecific transmission of
135 *Aphanomyces astaci* between two invasive American crayfish species from the UK. Firstly,
136 virile crayfish were infected at a site in the River Lea where they coexisted with signal
137 crayfish, but were not found to be infected at an allopatric site. In contrast, signal crayfish
138 were infected with *A. astaci* both in regions of sympatry and allopatry. Secondly, the *A.*
139 *astaci* multilocus genotype identified in virile crayfish was similar, although not identical, to
140 the reference strain of the genotype group B, isolated in Europe from infected signal crayfish
141 (Huang *et al.,* 1994; Grandjean *et al.,* 2014), and closely matched the multilocus genotype
142 detected in a UK signal crayfish population (Table 1). Therefore, the current study may
143 represent the first report of *A. astaci* being interspecifically transmitted within a wild, mixed
144 species population of American crayfish in their invasive range.
145 Whilst the multilocus gentotypes obtained for the *A. astaci*-positive DNA isolates
146 from virile crayfish in the River Lea were not an identical match to the reference strain of the
147 group B from Europe, such within group variation has already been reported (Grandjean *et*
148 *al.,* 2014; Maguire *et al.* 2016; Mrugała *et al.,* 2016). This, combined with the fact that the *A.*
149 *astaci* genotype obtained from infected virile crayfish was not similar to any of the other four

150 genotype groups currently described (Grandjean *et al.,* 2014), make it a likely assumption
151 that the virile crayfish in this study harboured an *A. astaci* strain from the genotype group B
152 pathogen. Of all American crayfish species, this genotype group has only previously been
153 recorded from wild signal crayfish. In addition, the multilocus genotypes obtained from both
154 virile crayfish specimens closely matched that isolated from signal crayfish elsewhere in the
155 UK. Variation detected at the locus Aast 12 may have resulted inefficient amplification of
156 low concentration DNA template). Both virile crayfish specimens also shared a homozygote
157 pattern at locus Aast 9 in contrast to the heterozygote reported in the original reference
158 sequence (James *et al.,* 2017). Combined these data add to the evidence that virile crayfish
159 likely contracted *A. astaci* from infected signal crayfish within the UK.

160 Possessing a wide host range is one of the key factors in determining the success of an
161 introduced parasite (Kennedy 1994). It has been speculated that the interspecific transmission
162 of *A. astaci* genotypes between American crayfish species may be limited by host-pathogen
163 incompatibilities (Tilmans *et al.,* 2014). Our findings cast doubt on this hypothesis, and
164 highlight the need for greater investigation into the host range of different *A. astaci* genotype
165 groups. Given that *A. astaci* genotypes different in their virulence (Makkonen *et al.,* 2012,
166 2014; Viljamaa-Dirks *et al.,* 2013) and climate requirements (Diéguez-Uribeondo *et al.,*
167 1995; Rezinciuc *et al.,* 2014) interspecific transmission may influence the spread of the
168 pathogen across Europe. This is likely to have implications for native crayfish conservation
169 given that *A. astaci* affords American crayfish species an even greater competitive advantage
170 over their susceptible European counterparts. Interspecific transmission may also result in
171 complex mixed genotype group infections with unknown consequences for both American
172 carrier crayfish and native European crayfish hosts. Ultimately, this may also influence
173 pathogen evolution.

174 This study provides the first indication of *A. astaci* being transmitted between
175 American carrier crayfish species in a wild population. We do, however, acknowledge the
176 limitations of our study in terms of incomplete genotyping and low sample size. Studies of *A.*
177 *astaci* interspecific transmission are particularly relevant given that many European countries
178 now harbour multiple non-native American crayfish species (Kouba *et al.,* 2014). With
179 microsatellite markers allowing the characterization of *A. astaci* genotypes from host tissue
180 samples, increased effort should be focused into genotyping the pathogen strains infecting
181 American carrier crayfish species. Characterising the strain(s) of *A. astaci* responsible for a
182 crayfish plague outbreak may also help identify the source of an epidemic and monitor the
183 spread of infection (see Kozubíková *et al.,* 2014, Vrålstad *et al.,* 2014).
184

185 **5. Acknowledgments**

186 We thank Nuria Viñuela-Rodriguez, Jiří Svoboda, Jasna Vukić, Radek Šanda, Robby
187 Mitchell, Graham Richardson and Nate Iliescu for laboratory and field assistance, Jana
188 Vokurková for technical support and two anonymous referees for useful comments on a
189 previous version of the manuscript. This study was funded by The Worshipful Livery
190 Company of Wales, Charles University in Prague and Cardiff University.
191

6. References

Ahern D., England J. & Ellis A. (2008) The virile crayfish, *Orconectes virilis* (Hagen, 1970) (Crustacea: Decapoda: Cambaridae), identified in the UK. *Aquatic Invasions,* **1**, 102-104.

Alderman D.J., Holdich D. & Reeve, I. (1990) Signal crayfish as vectors in crayfish plague in Britain. *Aquaculture*, **86**, 3-6.

Alderman D.J. (1996) Geographical spread of bacterial and fungal diseases of crustaceans. *Revue Scientifique et Technique de l'Office International des Epizooties,* **15**, 603-632.

Almeida D., Ellis A., England J. & Copp G.H. (2014). Time-series analysis of native and non-native crayfish dynamics in the Thames River Basin (south-eastern England). *Aquatic Conservation Marine and Freshwater Ecosystems*, **24**, 192-202.

Becking T., Mrugała A., Delaunay C., Svoboda J., Raimond M., Viljamaa-Dirks S., Petrusek A., Grandjean F. & Braquart-Varnier C. (2015) Effect of experimental exposure to differently virulent *Aphanomyces astaci* strains on the immune response of the noble crayfish *Astacus astacus. Journal of Invertebrate Pathology*, **132**, 115-124.

Bohman P., Nordwall F. & Edsman L. (2006) The effect of the large-scale introduction of signal crayfish on the spread of crayfish plague in Sweden. *Bulletin Français de la Péche et de la Pisciculture,* **380-381**, 1291-1302.

CEFAS (Centre for Environment, Fisheries and Aquaculture Science). (2000) *Summary final report: effects of exposure to high and low temperatures on the survival of the crayfish plague fungus A. astaci* in vitro *and* in vivo. Australian Quarantine and Inspection Service, Canberra.

DAISIE. (2009) *Handbook of Alien Species in Europe*. Springer, Dordrecht.

215 Diéguez-Uribeondo J., Temiño C. & Múzquiz J.L. (1997) The crayfish plague fungus
216 (*Aphanomyces astaci*) in Spain. *Bulletin Français de la Péche et de la Pisciculture,*
217 **347**, 753-763.

218 Diéguez-Uribeondo J., Huang T., Cerenius L. & Söderhäll K. (1995) Physiological
219 adaptation of an *Aphanomyces astaci* strain isolated from freshwater crayfish
220 *Procambarus clarkii. Mycological Research,* **99**, 574-578.

221 Filipová L., Holdich D. M., Lesobre J., Grandjean F. & Petrusek A. (2010). Cryptic diversity
222 within the invasive virile crayfish *Orconectes virilis* (Hagen, 1870) species complex:
223 new lineages recorded in both native and introduced ranges. *Biological Invasions,* **12**,
224 983–989.

225 Grandjean F., Vrålstad T., Diéguez-Uribeondo J., Jelić M., Mangombi J., Delaunay C.,
226 Filipová L., Rezinciuc S., Kozubíková-Balcarová E., Guyonnet D., Viljamaa-Dirks S.
227 & Petrusek A. (2014) Microsatellite markers for direct genotyping of the crayfish
228 plague pathogen *Aphanomyces astaci* (Oomycetes) from infected host tissues.
229 *Veterinary Microbiology,* **170**, 317-324.

230 Holdich D.M., James J., Jackson C. & Peay S. (2014) The North American signal crayfish,
231 with particular reference to its success as an invasive species in Great Britain.
232 *Ethology Ecology and Evolution,* **26**, 232-262.

233 Holdich D.M. (2003) Crayfish in Europe – an overview of the taxonomy, legislation,
234 distribution, and crayfish plague outbreaks. In: *Management and Conservation of*
235 *Crayfish* (Eds Holdich D.M., Sibley P.J.), conference proceedings, Nottingham, UK,
236 Nov 7th 2002.

237 Huang T., Cerenius L. & Söderhäll K. (1994) Analysis of genetic diversity in the crayfish
238 plague fungus, *Aphanomyces astaci*, by random amplification of polymorphic DNA.
239 *Aquaculture,* **126**, 1-9.

240 IUCN. (2015) The IUCN red list of threatened species [online]. Available at:
241 https::/www.iucnredlist.org. Accessed on: 31.08.15.

James J., Nutbeam-Tuffs S., Cable J., Mrugała A., Viñulea-Rodriguez N, Petrusek A. & Oidtmann B. (2017) The prevalence of *Aphanomyces astaci* in invasive signal crayfish from the UK, and implications for native crayfish conservation. *Parasitology*, **12**, in press.

242 James J., Thomas J.R., Ellis A., Young K.A., England J. & Cable J. (2015) Over-invasion in
243 a freshwater ecosystem: newly introduced virile crayfish (*Orconectes virilis*)
244 outcompete established invasive signal crayfish (*Pacifastacus leniusculus*). *Marine*
245 *and Freshwater Behaviour and Physiology,* **49**, 9-18.

246 James J., Slater F. & Cable J. (2014) A.L.I.E.N. databases: addressing the lack in
247 establishment of non-natives databases. *Crustaceana,* **87**, 1192-1199.

248 Keller N.S., Pfeiffer M., Roessink I., Schulz R. & Schrimpf A. (2014) First evidence of
249 crayfish plague agent in populations of the marbled crayfish (*Procambarus fallax*
250 forma *virginalis). Knowledge and Management of Aquatic Ecosystems,* **414**, 15.

251 Kennedy C.R. (1994) The ecology of introductions, in: Pike A.W., Lewis J.W. (Eds.),
252 Parasitic diseases of fish. Samara Publishing Limited, Great Britain, pp. 189-208.

253 Kozubíková E., Petrusek A., Ďuriš Z., Martín M.P., Diéguez-Uribeondo J. & Oidtmann B.
254 (2008) The old menace is back: Recent crayfish plague outbreaks in the Czech
255 Republic. *Aquaculture,* **274**, 208-217.

256 Kozubíková E., Viljamaa-Dirks S., Heinikainen S. & Petrusek A. (2011) Spiny-cheek
257 crayfish *Orconectes limosus* carry a novel genotype of the crayfish plague pathogen
258 *Aphanomyces astaci. Journal of Invertebrate Pathology,* **108**, 214-216.

259 Kozubíková-Balcarová E., Beran L., Ďuriš Z., Fischer D., Horká I., Svobodová J. & Petrusek,
260 A. (2014) Status and recovery of indigenous crayfish populations after recent crayfish
261 plague outbreaks in the Czech Republic. *Ethology Ecology and Evolution,* **26**, 299–
262 319.

263 Kouba A., Petrusek A. & Kozák P. (2014) Continental-wide distribution of crayfish species
264 in Europe: update and maps. *Knowledge and Management of Aquatic Ecosystems*,
265 **413**, 1-31.

266 Lowe S., Browne M., Boudjelas S. & De Poorter M. (2004). 100 of the world's worst
267 invasive alien species. A selection from the Global Invasive Species Database. The
268 Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival
269 Commission (SSC) of the IUCN, Gland.

270 Makkonen J., Jussila J., Kortet R., Vainikka A. & Kokko H. (2012) Differing virulence of
271 *Aphanomyces astaci* isolates and elevated resistance of noble crayfish *Astacus astacus*
272 against crayfish plague. *Diseases of Aquatic Organisms,* **102**, 129-136.

273 Makkonen J., Kokko H., Vainikka A., Kortet R. & Jussila J. (2014) Dose-dependent
274 mortality of the noble crayfish (*Astacus astacus*) to different strains of the crayfish
275 plague (*Aphanomyces astaci*). *Journal of Invertebrate Pathology,* **115**, 86-91.

276 Mrugała A., Kozubíková-Balcarová E., Chucholl C., Cabanillas Resino S., Viljamaa-Dirks
277 S., Vukić J. & Petrusek A. (2015) Trade of ornamental crayfish in Europe as a
278 possible introduction pathway for important crustacean diseases: crayfish plague and
279 white spot syndrome. *Biological Invasions,* **17**, 1313-1326.

280 Oidtmann B., Heitz E., Rogers D. & Hoffman R.W. (2002) Transmission of crayfish plague.
281 *Diseases of Aquatic Organisms,* **52**, 159-167.

282 Rezinciuc S., Galindo J., Montserrat J. & Diéguez-Uribeondo J. (2014) AFLP-PCR and
283 RAPD-PCR evidences of the transmission of the pathogen *Aphanomyces astaci*
284 (Oomycetes) to wild populations of European crayfish from the invasive crayfish
285 species, *Procambarus clarkii*. *Fungal Biology,* **118**, 612-620.

286 Schrimpf A., Maiwald T., Vrålstad T., Schulz H.K., Śmietana P. & Schulz R. (2013) Absence
287 of the crayfish plague pathogen (*Aphanomyces astaci*) facilitates coexistence of
288 European and American crayfish in central Europe. *Freshwater Biology,* **58**, 1116-
289 1125.

290 Souty-Grosset C., Holdich D.M., Noël P.Y., Reynolds J.D. & Haffner P. (2006) *Atlas of*
291 *crayfish in Europe.* Publications Scientifiques du Muséum National d'Histoire
292 Naturelle. Paris.

293 Strand D.A., Holst-Jensen A, Viljugrein H., Edvardsen B., Klaveness D., Jussila J. &
294 Vrålstad T. (2011) Detection and quantification of the crayfish plague agent in natural
295 waters: direct monitoring approach for aquatic environments. *Diseases of Aquatic*
296 *Organisms,* **95**, 9-17.

297 Svoboda J., Kozubíková-Balcarová E., Kouba A., Buřič M., Kozák P., Diéguez-Uribeondo J.
298 & Petrusek A. (2013) Temporal dynamics of spore release of the crayfish plague
299 pathogen from its natural host, American spiny-cheek crayfish (*Orconectes limosus*),
300 evaluated by transmission experiments. *Parasitology,* **140**, 792-801.

301 Svoboda J., Mrugała A., Kozubíková-Balcarová E., & Petrusek A. (2017) Hosts and
302 transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. *Journal*
303 *of Fish Diseases*, **40**, 127-140.

304 Svoboda J., Strand D.A., Vrålstad T., Grandjean F., Edsman L., Kozák P., Kouba A., Fristad
305 R.F., Koca S.B. & Petrusek A. (2014) The crayfish plague pathogen can infect
306 freshwater-inhabiting crabs. *Freshwater Biology,* **59**, 918-929.

307 Tilmans M., Mrugała A., Svoboda J., Engelsma M.Y., Petie M., Soes D.M., Nutbeam-Tuffs
308 S., Oidtmann B., Rossink I. & Petrusek A. (2014) Survey of the crayfish plague
309 pathogen presence in the Netherlands reveals a new *Aphanomyces astaci* carrier.
310 *Journal of Invertebrate Pathology,* **120**, 74-79.

311 Unestam T. & Weiss D.W. (1970) The host-parasite relationship between freshwater crayfish
312 and the crayfish disease fungus *Aphanomyces astaci*: responses to infection by a
313 susceptible and a resistant species. *Journal of General Microbiology,* **60**, 77-90.

314 Vennerström P., Söderhäll K. & Cerenius L. (1998) The origin of two crayfish plague
315 (*Aphanomyces astaci*) epizootics in Finland on noble crayfish, *Astacus astacus*.
316 *Annales Zoologici Fennici,* **35**, 43-46.

317 Viljamaa-Dirks S., Heinikainen S., Torssonen H., Pursiainen M., Mattila J. & Pelkonen S.
318 (2013) Distribution and epidemiology of the crayfish plague agent *Aphanomyces*
319 *astaci* genotypes from noble crayfish *Astacus astacus* in Finland. *Diseases of Aquatic*
320 *Organisms,* **103**,199-208.

321 Vrålstad T., Knutsen A. K., Tengs T. & Holst-Jensen A. (2009) A quantitative TaqMan®
322 MGB real-time polymerase chain reaction based assay for detection of the causative
323 agent of crayfish plague *Aphanomyces astaci*. *Veterinary Microbiology,* **137**, 146-
324 155.

325 Vrålstad T., Strand D.A., Grandjean F., Kvellestad A., Håstein T., Knutsen A.K., Taugbøl T.
326 & Skaar I. (2014) Molecular detection and genotyping of *Aphanomyces astaci* directly

from preserved crayfish samples uncovers the Norwegian crayfish plague disease history. *Veterinary Microbiology,* **173**, 66–75.

Figure 1. Sample sites along the River Lea (UK) and an adjacent lake for invasive signal (*Pacifastacus leniusculus*) and virile (*Orconectes* cf. *virilis*) crayfish. River flow direction is indicated by the black arrow. Allopatric sites shown for signal (●) and virile crayfish (▲) respectively, and the sympatric site (■). Note: the location of the allopatric virile crayfish site is a lake adjacent to the river. More details about virile and signal crayfish distribution in this region can be found in James *et al.,* (2016). Image courtesy of Maps data 2016 @Google.

Table 1. Comparison of allele sizes of nine microsatellite loci from the original European reference strain of *Aphanomyces astaci* genotype group B (Grandjean *et al.,* 2014), a reference strain from *Pacifastacus leniusculus* from Wales, UK (James *et al.,* 2017) and *A. astaci*-positive samples of *Orconectes* cf. *virilis* examined in the current study.

Locus	**Original European reference strain VI03555 (*P. leniusculus*)**	**UK population (*P. leniusculus*)**	**UK population (*O.* cf. *virilis*) specimen 1**	**UK population (*O.* cf. *virilis*) specimen 2**
Aast 2	142	142	142	142
Aast 4	87	87	87	n/a
Aast 6	148	148	n/a	148
Aast 7	215	215	215	n/a
Aast 9	164/182	164	164	164
Aast 10	132	132	n/a	132
Aast 12	226/240	226/240	240	226/240*
Aast 13	202	202	202	202
Aast 14	248	248	248	n/a

*both alleles were scored but respective peaks were observed in separate PCR runs. n/a – loci with no amplification, likely due to low concentration of *A. astaci* DNA in the isolate.