Aquatic Parasite Information – a Database on Parasites of Freshwater and Brackish Fish in the United Kingdom

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by

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Declaration

This thesis is being submitted in partial fulfilment of the requirements of Kingston University, London for the award of Doctor of Philosophy.

I confirm that all work included in this thesis was undertaken by myself and is the result of my own research and all sources of information have been acknowledged.

Bernice Brewster

2nd June 2016

Abstract

A checklist of parasites of freshwater fish in the UK is an important source of information concerning hosts and their distribution for all aspects of scientific research. An interactive, electronic, web-based database, *Aquatic Parasite Information* has been designed, incorporating all freshwater and brackish species of fish, parasites, taxonomy, synonyms, authors and associated hosts, together with records for their distribution. One of the key features of *Aquatic Parasite Information* is this checklist can be updated.

Interrogation of *Aquatic Parasite Information* has revealed that some parasites of freshwater and brackish species of fish, such as the unicellular groups or those metazoans that are difficult to identify using morphological characters, are under reported. *Aquatic Parasite Information* identified the monogenean family Dactylogyridae and the cestodes infecting UK freshwater fish as under-represented groups, owing to the difficulties identifying them morphologically. Both the Dactylogyridae and cestodes have implications for pathology, outbreaks of disease and morbidity in freshwater fish in the UK, therefore accurate identification is critical. Studies were undertaken using both standard morphological techniques of histology and molecular techniques to identify dactylogyrid species and tapeworms commonly found parasitizing fish in the UK. Morphological studies demonstrated that histological processes could lead to distortion of the specimens and permanent mounting may affect the orientation which may obscure vital characters. Molecular techniques were successfully employed using ITS1 for the Dactylogyridae and cox1 and r28s for the cestodes, to demonstrate genetic variability for the interspecific identification of species. Histology, scanning electron microscopy and molecular techniques have also

identified an *Atractolytocestus* sp. tapeworm, parasitizing carp in the UK, as a potentially new species.

Analysis of parasite records extracted from *Aquatic Parasite Information* has implicated freshwater fishery management policies as impacting on the dissemination and distribution of parasites, resulting in the spread of some species and decline of others.

Acknowledgments

I gratefully acknowledge the support, guidance and dedication of my Supervisors Dr Ruth Kirk, Dr Scott Lawton, Dr James Denholm-Price, the late Professor Angela Russell-Davies and Dr Chris Williams. I am indebted to Mr Sivasankara Desikan for designing the front-ends for *Aquatic Parasite Information*, enabling the content of the database to be accessible on the internet and for his patience throughout the process. I am also indebted to Amy Reading and Nigel Hewlett, Environment Agency, National Fisheries Laboratory for providing me with cestode specimens for molecular work and data relating to fish movements.

This project could never have taken place without all the fish traders and fish farmers who have unknowingly contributed by delivering samples of fish for examination for movement consent.

Finally, a special thanks to my family and friends for putting up with the fish stuff, my constant mess and chaos and also to Finzi who has had to wait ‘forever’, every day for a walk.

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General Introduction

1.1 Current legislation and regulations

Since Kennedy's 1974 checklist of parasites associated with freshwater fish in the UK was published, there have been many changes in public attitude and legislation which have impacted on fish movements within and outside the UK. Recreational fishing for freshwater fish has a long history in the UK. Clubs and angling societies were formed in the 1800's primarily to protect rivers and canals from poaching but also to allow recruitment of fish for further stocking of these waters (Bradfield, 1883). For many years recreational fishing was confined to rivers and canals but in 1953 the publicity associated with Richard Walker's capture of a carp (*Cyprinus carpio)* from Redmire Pool, Herefordshire with a weight of 20kg, initiated a greater interest in coarse fishing but carp in particular (Taverner, 1957). As a direct result of the capture of this record breaking carp, interest in recreational fishing transferred from rivers and canals to lakes and still waters and the hobby of coarse fishing, especially for carp, has grown significantly from the late 1980's onwards, with over 30,000 still water fisheries now in operation (Williams, 2007, Brewster, 2009, 2014). Coupled with the increased popularity of recreational fishing, has been the movement and translocation of fish from within the UK and mainland Europe both legitimately and illegally to satisfy the demand for coarse fish, notably carp weighing in excess of 9kg.

Until recently all movements of freshwater fish inland were regulated under The Salmon and Freshwater Fisheries Act (SAFFA, 1975) Section 30, (www.legislation.gov.uk) which required Environment Agency consent to remove or

stock fish into rivers, lakes and canals. A further requirement of Section 30 regulations, included health examination of a sample of fish from the population destined for stocking into rivers, canals or lakes which were either connected to the catchment, or within a floodplain. Whilst adequate at the time it was introduced, with the increasing numbers of fish movements associated with the popularity of pleasure angling, it became evermore apparent that SAFFA 1975 needed updating. This has recently been achieved through use of the Marine and Coastal Access Act 2009. Introduced in January 2015 as 'The Keeping and Introduction of Fish (England and River Esk Catchment Area) Regulations 2015 No. 10' (www.legislation.gov.uk) replaces SAFFA 1975. Under these regulations, every angling club, society or commercial fishery, or persons keeping fish in inland waters has to hold a permit issued by the Environment Agency, which states the fish species on the site, the maximum number of fish which may be introduced and which species may be introduced. All consents for fish movements under this new legislation are also issued by the Environment Agency. This new legislation allows the Environment Agency some control over coarse fish welfare, which was not covered under the SAFFA regulations and was of increasing concern (Brewster, 2000, 2009, 2014). The Keeping and Introduction of Fish (England and River Esk Catchment Area) Regulations also compliments other legislation previously introduced, which includes Animal Welfare Act (2006) (http://www.legislation.gov.uk/ukpga/2006/45), Welfare of Animals in Transport (2006) (www.gov.uk/government/publications/welfare-of-animals-during-transport) and Aquatic Animal Health Regulations 2009, 2011 (http://www.legislation.gov.uk/uksi/2009/463/pdfs/uksi_20090463_en.pdf).

For the first time since legislation concerning animal welfare was introduced, the Animal Welfare Act (2006) includes fish, although Item 59 specifically excludes the 'normal course of fishing', that is catching fish on rod and line. The Act incorporates the 'five freedoms' of animal welfare:

1) Hunger and thirst
2) Discomfort
3) Pain, injury and disease
4) Ability to behave normally
5) Fear and distress

Many of the issues relating to the stocking densities of fish contravene these five freedoms but Item 3, Freedom from pain injury and disease is also related to the pathology caused by certain parasites. The Environment Agency classifies particular freshwater parasites as significant pathogens which, together with novel parasites, are regarded as 'Category 2 and Novel Parasites'. Any fish infected with these parasites are subject to movement restriction (s. 30 SAFFA; s.2 Diseases of Fish Act, 1983 www.uk-legislation.hmso.gov.uk/RevisedStatutes/Acts/ukpga/1983/cukpga Fish Health Regulations SI1992/3300 www.legislation.gov.uk/uksi/1992/3300/made; Diseases of Fish (Control) Regulations SI 1994/1447 http://www.legislation.gov.uk/uksi/1994/1448/made; s. 14 Wildlife and Countryside Act 1981, www.legislation.gov.uk/ukpga/1981/69 Salmon and Freshwater Fisheries Review 2000; Carty & Payne 1998). Historically, predecessors of the Environment Agency, that is the Water Authorities, then the National Rivers Authority (NRA), carried out the majority of fish health examinations and collated data regarding the distribution of the English and Welsh freshwater fish parasite fauna. In 2013, the National

Assembly for Wales, united the Environment Agency, Countryside Council for Wales and Forestry Commission Wales into a single organization, entitled 'Natural Resources Wales', which is completely independent of the Environment Agency in England. In addition to loss of responsibility for freshwaters in Wales, the Environment Agency now carries out limited numbers of fish health examinations for movement consent, the bulk of the work being conducted by private individuals, commercial enterprises and institutions. With the private sector now involved with most fish health examinations, there is a danger that important information on the changing distribution of novel or pathogenic parasites, or significant changes in the occurrence of native parasites, may be overlooked.

1.2 Importation of freshwater fish

In the last thirty years there have been substantial changes in the variety of species and numbers of freshwater fish imported into the UK from Europe and third countries (Salmon and Freshwater Fisheries Review, 2000; Brewster, 2000; Baldock *et al.* 2008; Davenport, 2008; Walster, 2008). Import of live fish represents a potential risk to endemic fish species through the introduction of novel parasites or diseases. The reasons for introduction of live fish are threefold: for aquaculture, the ornamental industry or recreational angling.

1.2.1. Aquaculture

Species imported for aquaculture include Atlantic salmon (*Salmo salar*) rainbow trout (*Onchorhynchus mykiss*) brook trout (*Salvelinus fontinalis*), Nile tilapia (*Oreochromis niloticus*) and common carp (*C. carpio*) (http://www.defra.gov.uk/foodfarm/fisheries/farm-health/aquaculture.htm; Jeffrey,

2008; Jeffrey, 2009) and more recently barramundi (*Lates calcarifer*) (Ellis, 2006) although the culture of barramundi in the UK proved to be an unsuccessful venture. Atlantic salmon (*S. salar*) tend to be imported as eggs, or eyed ova, from the Centre for Environment, Fisheries and Aquaculture (Cefas) approved sources and therefore are regarded as low risk to native fish species. Imported tilapia destined for aquaculture and the food industry are generally held in purpose-built fish farms and unlikely to enter any natural water body but commercial ventures supplying the public with hydroponic vegetable growing systems stocked with tilapia are now being marketed. Importation of fish for aquaculture pose a higher risk, as most suppliers to the food industry are based outside of the UK.

Scholz *et al*., (2015[1]) consider the rising trend for aquaculture has been accompanied by an increase in the diversity of parasites, including newly introduced parasites, infecting farmed fish. The ntroductions of these non-native parasites into new regions and countries have an unpredictable effect on both known and novel hosts.

1.2.2. Ornamental market

Import of fish for the ornamental industry represents a high risk to native fish through possible introduction of exotic parasites, since there are occasions when pet fish have been released into rivers or lakes, either deliberately or accidentally such as through flooding. The main exporters of both coldwater and tropical freshwater fish to the UK are North America, Singapore, Israel, Japan, Indonesia and Thailand (http://www.ornamentalfish.org/wp-content/uploads/2015/02/UK-Trade-Statistics-2014.pdf) although over recent years there has been a decline in imports (Figure 1.1). Nonetheless, according to the Ornamental Aquatic Trade Association (OATA) trade statistics, freshwater imports dominate the trade comprising 80% of the total import

value. Survival of many tropical freshwater fish species is limited in the UK, as they are unable to withstand the variable temperatures associated with this temperate region. Notwithstanding the effects of climate change, which in future may allow some of the more temperature tolerant tropical species and their parasites to survive in the UK.

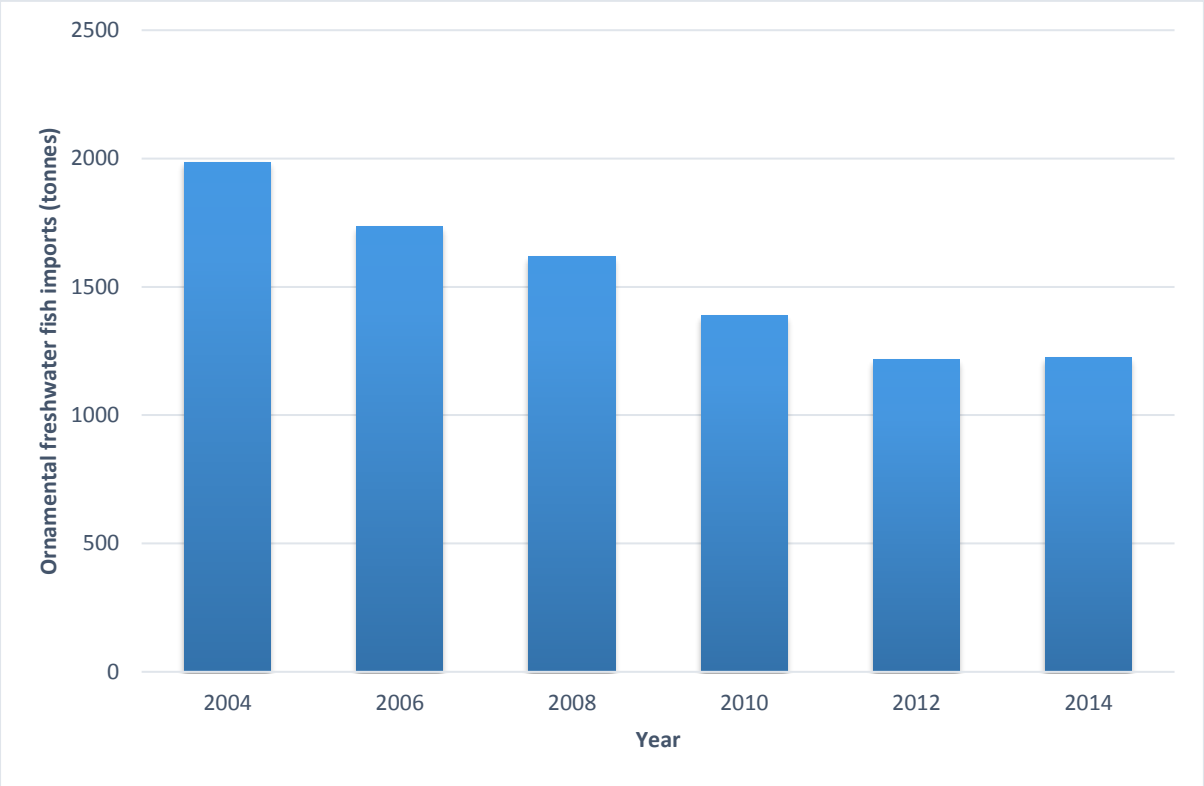

Figure 1.1. Coldwater and tropical freshwater fish tonnage imported into the UK 2004 – 2014 (Source: Ornamental Aquatic Trade Association)

However, coldwater ornamental species such as goldfish (*Carassius auratus*) and its many fancy varieties, coloured varieties of carp, popularly known as 'koi' (*C. carpio*), sterlet (*Acipenser ruthenus*) and various hybrid sturgeon species (Acipenseridae) do survive in fresh water in the UK (Giles, 1994; Farr-Cox, Leonard & Wheeler, 1996; Bolton, Wheeler & Wellby, 1998; Wheeler, 1998[a] Wheeler 1998[b]; Copp, Stakénas & Davison, 2006).

Whilst koi and goldfish are legitimately imported for retail by aquatic stores and garden centres for stocking domestic ponds they are frequently encountered in both fisheries and rivers. In ignorance, some members of the public have illegally released these ornamental fish into the wild but regrettably angling clubs and commercial fisheries have knowingly legally and illegally introduced koi into fishing lakes (*pers. obs.* http://www.celticlakesresort.com/celticlakes_fishing_lake1.html). In recent years, heavy rainfall has caused rivers to overflow banks and flood into adjacent properties and gardens resulting in the accidental release of pond fish into the wild. The River Medway in Kent has significant numbers of koi which have been released from captivity in winter flooding in 2000, 2012, 2013 and 2014 (*pers.obs.*) http://www.maggotdrowni_g.com/forum/topic.asp?ARCHIVE=true&TOPIC_ID=87904 Accidentally or illegally released ornamental fish have not been screened for parasites or other infectious diseases.

In 2010, *Garra rufa*, small cyprinid fish native to Turkey, were being commercially promoted as 'Doctor Fish' or 'Doctor Loach' in human health spas in many high streets and shopping centres in the UK and quickly became very popular for removing hard skin on the legs and feet, or for reducing symptoms of psoriasis and eczema. Turkish conservation authorities had concerns over exploitation of native *G. rufa* which led to a ban on the export of this species from Turkey, which allowed for a thriving trade in breeding this fish species in the far-east (Wildgoose, 2012). Specialist wholesalers were importing *G. rufa* which were then sold to health and beauty stores in filtered self-contained units. The customer would sit on the unit with feet immersed in the 'aquarium' allowing the fish to exfoliate the skin. Staff running these pedicure salons were beauticians, not aquarists, thus the health status of the imported *Garra rufa* was unknown but unwanted, sick or dying fish were illegally discarded into convenient

waters in ignorance of the Wildlife and Countryside Act (1981) and SAFFA (1975) (J. Skilleter pers. com). Subsequent public health concerns led to a decline in the number of the spas offering *G. rufa* as a means of exfoliating human skin, although there are still some businesses in operation (e.g. www.fishspasolutions.com). To date, no examples of *G. rufa* escapes have been recorded in the UK, although survey of inner city ponds and open waters for this recent introduction have not taken place.

A new UK business venture, rearing and wholesaling tilapia (*Oreochromis niloticus*), which are sold to the public in conjunction with hydroponic cultivation of fruit and vegetable systems (www.livetilapia.co.uk), has the potential for another non-native fish species to be released into the wild, either accidentally or deliberately. Although it is assumed tilapia would not thrive in the temperate conditions of the UK, one of the reasons their farming has been so successful worldwide, is their ability to adapt to a range of habitats.

Whilst the introduction of *G. rufa* and *O. niloticus* appear to be low risk, it demonstrates that despite all legislation, there are routes other than *via* the ornamental fish industry that non-native fish of unknown disease status, can be accidentally or deliberately introduced to inland waters.

1.2.3. Recreational angling

One of the highest risk factors for introduction of exotic parasites is associated with angling, one of the UK's most popular sports. In the last 50 years, angling has changed its focus from rivers and canals to lakes and reservoirs where there is a greater likelihood of catching specimen fish or maximum catches (Brewster, 2009; Brewster 2014; Environment Agency, 2009). Pressure for increasing numbers and

size of fish has created a market for both legitimate and illegal movements of fish within the UK. Ornamental species of coldwater fish originating from Japan, Israel, Indonesia, Malaysia, Sri Lanka, Singapore, North America and South Africa have been purposely, but illegally, stocked into fisheries with no knowledge, or concern, for the exotic parasite fauna being introduced. Rushton-Mellor (1992) recorded the Japanese fish louse, *Argulus japonicus* Thiele, 1900 (Crustacea, Maxillopoda) in wild stocks of fish in isolated localities in Dorset, Hampshire, Hereford and Kent and stated this exotic species was not found in conjunction with native *Argulus foliaceus* (L.). This work was undertaken almost 20 years ago and *A. japonicus* may now be more widely distributed owing to the stocking of koi into UK fisheries.

Although there are stringent regulations governing the import of fish, these can be flouted by anglers returning from continental Europe, smuggling coarse fish species (http://www.gofishing.co.uk/Angling-Times/Section/News--Catches/General-News/March-2010/Carp-haul-at-Dover-docks/). These include carp usually in excess of 9kg weight, tench (*Tinca tinca*) of any size and exotic species such as Wels catfish (*Siluris glanis*), grass carp (*Ctenopharyngodon idella*) and various species of sturgeon (Acipenseridae) which are then stocked illegally into UK fisheries. Recently, Cefas in conjunction with the Environment Agency using the Aquatic Animal Health Regulations 2009, 2011, have commenced the enforced removal of Wels catfish and various sturgeon species from fisheries which are not licensed to hold these exotic fish. The health status of fish and parasite fauna associated with these illegal imports is unknown, but occasionally unusual parasites such as *Aspidogaster limacoides* Diesing, 1835 (Platyhelminthes, Trematoda) from eastern Europe in a sample of roach (*Rutilus rutilus*) may indicate the non-native origins of fish stocked into a fishery (pers. obs.). Cropping and translocation of coarse fish are regular activities which can readily

disperse parasites to other parts of the country. For example, Table 1.1 shows the figures for freshwater fish movements England and Wales for the period between January 2008 and December 2009, however, Natural Resources Wales are now responsible for Welsh freshwater fish movement consent.

Table 1.1 Freshwater fish movements in England and Wales for the period January to December 2008 – 2009*

	No. consented movements	**Total fish number of fish moved**	**Approximate value (million £)**
2008	5552	7.1 million	13.5
2009	5390	8.1 million	14

*Figures courtesy Nigel Hewlett, Environment Agency

1.3 Introduction of novel parasites

Coldwater ornamental fish varieties readily adapt to the UK climate, and if released into the wild, may pose a risk by introduction of parasites to native fish which have little or no resistance. The goldfish (*Carassius auratus*) is the native host to a nematode parasite *Philometroides sanguinea* Rudolphi, 1819 (Nematoda: Secernentea) (Figure 1.2). The mature females are found between the bony rays of the caudal fin from September to March and, in heavy infections, in other fins (Chris Williams pers.com).

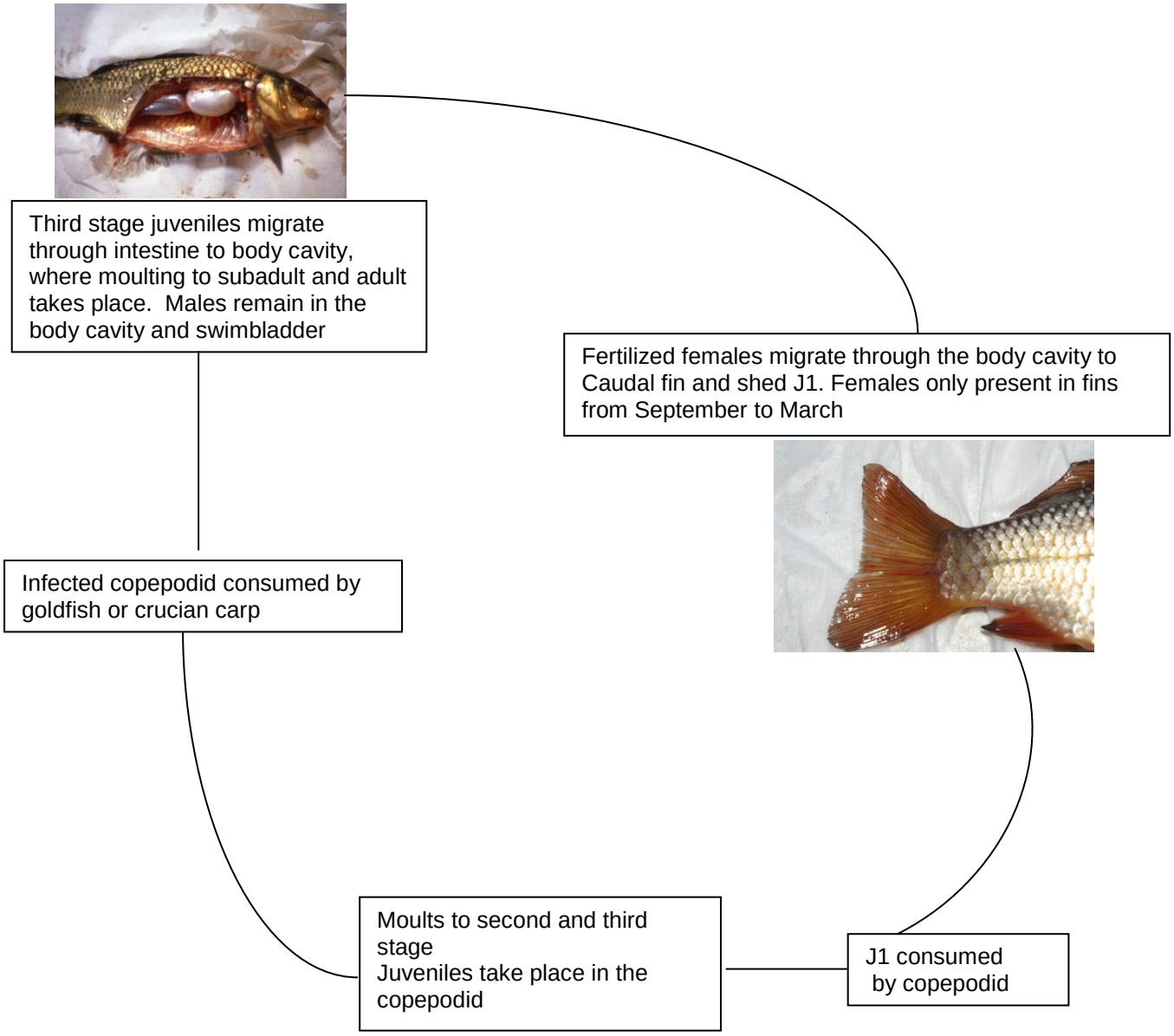

Figure 1.2 Life cycle of *Philometroides sanguinea* (Photographs B. Brewster)

Males are found in the abdominal cavity year round in infected fish, but these are easily overlooked. Following release of infected goldfish to the wild, there is evidence of transmission of *P. sanguinea* to the closely related crucian carp (*C. carassius)*, a species native to the UK (Nigel Hewlett, Environment Agency pers. com.; pers.obs.). *Philometroides sanguinea* is regarded by the Environment Agency as a 'novel parasite' (Hewlett pers. com.) so infected fish are subject to movement restriction. However, the distribution of the parasite in the UK is unknown so it is difficult to carry

out risk assessments. A further recent example is the introduction of the topmouth gudgeon or clicker barb (*Pseudorasbora parva*) to the wild which harbours *Sphaerothecum destruens* Arkush, 2003 (Mesomycetozoea, Dermocystida), an intracellular parasite which can infect a variety of native cyprinids, but appears to adversely affect reproduction in dace (*Leuciscus leuciscus*) and the introduced sunbleak (*Leucaspius delineata*) (Gozlan *et al.* 2009). In North America, *S. destruens* has been responsible for outbreaks of diseases and mortalities in salmonids (Gozlan *et al.* 2009). However, the distribution and epidemiology of *S. destruens* in the UK are unknown.

1.4 Aquaculture in the UK

Worldwide, there is an increasing trend for aquaculture production, according to the Food and Agriculture Organization of the United Nations (FAO) with carp and other cyprinid species, the highest freshwater production, which has been increasing annually (Figure 1.3).

In the UK, Atlantic salmon (*S. salar*) dominates production in Scotland, with a production of 158,018 tonnes and value of £584.7 million in 2011, in contrast, finfish production in England for the same year was just 8,000 tonnes comprising mostly rainbow trout (*Onchorhynchus mykiss*) (http://www.cefas.defra.gov.uk/industry-information/aquaculture.aspx). Farmed rainbow trout (*Onchorhynchus mykiss*) are either destined for the table of for 'put and take trout fisheries', which are stocked with 300 – 500g trout, the anglers may catch any number of fish but are restricted, usually to two, which they may take for their consumption. In Scotland, farmed rainbow trout are being grown in pens in lochs to produce marketable fish in excess of 1.5kg weight

but escapes happen, often associated with predation but also through accidental release (Figure 1.4).

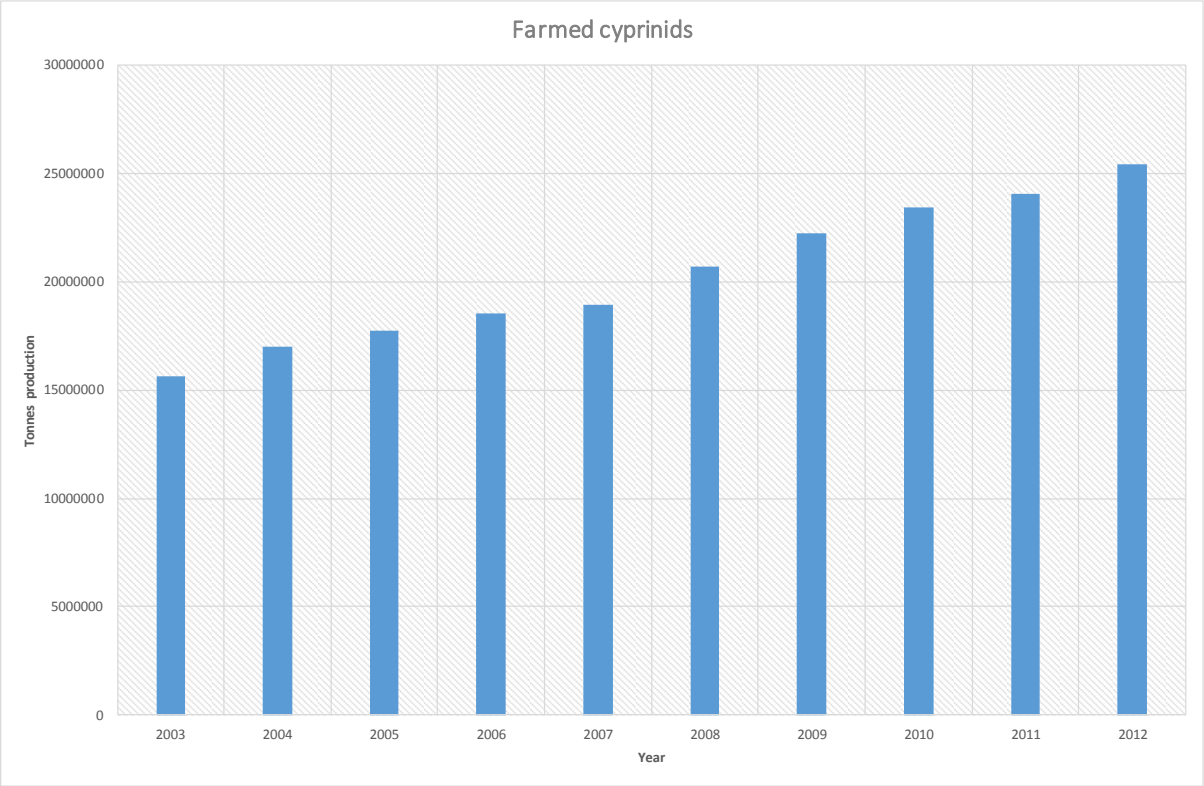

Figure 1.3. World carp and cyprinid production 2005 – 2012 (Source FAO)

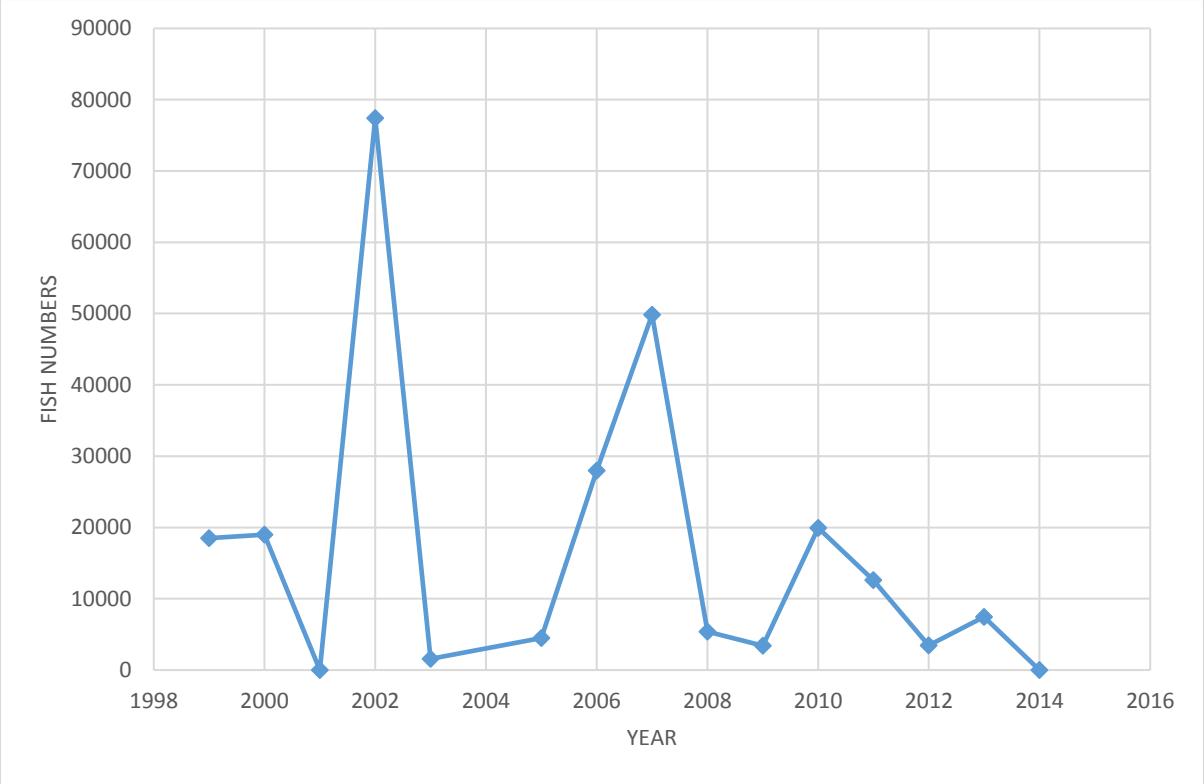

Figure 1.4 Scottish farmed rainbow trout escapes 1998 – 2014 (Source Scotlands Aquaculture http://aquaculture.scotland.gov.uk/data/fish_escapes.aspx

Whilst aquaculture production of rainbow trout (*Onchorhynchus mykiss*), brown trout (*Salmo trutta*) for 'put and take fisheries' or table, and cyprinids for re-stocking is generally regarded as low risk, some parasitic diseases, such as amoebic infections which cause Nodular Gill Disease (NGD), are probably largely either incorrectly identified or unrecorded (Nowak *et al.*, 2014).

In the UK, cyprinid aquaculture is dominated by rearing carp (*C. carpio*) and what are popularly known as 'F1's', which are hybrids of carp, crucian carp (*C. carassius*) and/or brown goldfish (*C. auratus*), with lower production of bream (*A. brama*), tench (*T. tinca*), crucian carp (*C. carassius*), chub (*S. cephalus*) and barbel (*B. barbus*) for stocking into recreational fisheries. The F1 hybrids are extremely popular with match anglers, competing for the largest total weight of fish caught over a 5 hour period.

These hybrids may be host to parasites more usually associated with one of the three parental species of fish (pers. obs). In recent years, many commercial fisheries and angling clubs have preferred to buy farmed carp which have been reared to minimum size of 40cm and in excess of 3kg weight, in order to reduce the impact of predation by cormorant (*Phalacrocorax* spp.). Biosecurity on coarse fish farms is variable, in some instances fish cropped from lakes, ponds and other freshwater sources are mixed with existing stocks, which may result in the introduction of parasites regarded by the Environment Agency as Category 2 parasites (pers. obs.).

Although recreational fisheries are not regarded as sites of aquaculture, there is an issue regarding fish biomass in many lakes and still waters. Marlow (1996) stated that some intensive fisheries were stocking at densities of 1500 – 2000kg per hectare but according to Brewster (2014) changes in attitude have driven many intensive fisheries to stock in excess of 3,000kg fish weight per hectare and one site with a biomass in excess of 5000kg per hectare (R. Oliver *pers. com*.), densities more commonly associated with aquaculture. Shinn *et al.* (2015) noted in marine aquaculture that stock densities and other production pressures cause farm reared fish to suffer a range of eukaryotic parasitic diseases. Certainly the stock densities and stress associated with angling pressure, predation and environmental degradation, particularly variable dissolved oxygen availability, eutrophication, habitat degradation and impoverished aquatic macroinvertebrate populations leads to many intensive fisheries also suffering a range of eukaryotic parasitic disease.

1.5 Changing populations of fish predators

Two subspecies of cormorant occur in the UK, *Phalacrocorax carbo carbo* and *Phalacrocorax carbo sinensis* which are either native or migrants from Europe. Cormorant were given protected status under the Wildlife and Countryside Act (1981) owing to the decline in numbers but by 1996, there were over 6000 overwintering in the UK and the effect of increasing numbers of these fish predators was beginning to impact on many fisheries (Britton *et al.* 2003). At about the same time, many commercial fisheries and angling clubs began increasing fish stock densities to satisfy the demands of recreational anglers (Brewster, 2000) with the Institute of Fisheries Management deeming it acceptable for lakes and still waters to have a fish biomass of 2000kg ha^{-1} (www.ifm.org.uk/sites/default/files/page/Still%20Waters%20Codes%20of%20Practice.pdf). The design of many recreational fisheries is to facilitate easy catch and release of coarse fish, with few islands, underwater obstacles, or macrophytes, which coupled with an increased fish biomass has resulted in intense predation by cormorant (Brewster, 2014). Denser fish biomass and especially bottom feeding species such as carp and bream create turbid water, which enables the cormorant to herd and forage on the fish (Grémilett *et al.,* 2012). Recreational fishing tends to be a fair weather sport and most fisheries are devoid of anglers in the winter months (*pers. obs.*), leaving the cormorant to feed at leisure on the biomass, sometimes completely stripping a lake of fish, leading to the popular angling press dubbing cormorant the 'Black Plague'.

Routine examination of coarse fish species for movement consent has identified the presence of metacestodes of *Paradilepis scolecina* (Rudolphi, 1819), *Valipora*

campylancristrota (Wedl, 1855) and *Neogryporhynchus cheilancristrotus* (Wedl, 1855) (Cestoda, Cyclophyllidea). *Paradilepis scolecina* and *V. campylancristrota* are found on the external surface of the gut, gall bladder and heart (Environment Agency, National Fisheries Laboratory; *pers. obs.*), whereas *N. cheilancristrotus* is found encysted within the intestinal tract. Fish are the intermediate hosts of gryporhynchids for whom the definitive hosts are fish eating birds, particularly cormorant and heron (*Ardea* species) (Scholtz *et al.*, 2004).

In the 1960's, the populations of European otter (*Lutra lutra*) in the UK went into serious decline, with only a few animals left in England by 1988 but in the latter part of the 20th century, otters began to increase in number (Kruuk, 2006) and according to the Environment Agency by 2011 were present in all English counties. At the same time that otters were increasing in number their preferred prey, European eels (*Anguilla anguilla*), were declining in number very rapidly (Beaton, 2013). In Scotland, Beaton found over a 30 year period from 1977/78 to 2012, otter predation on European eel showed the greatest decrease, followed by predation on minnows (*Phoxinus phoxinus*), accompanied by significant increase in predation on salmonids, perch (*Perca fluviatilis*), stickleback (*Gasterosteus aculeatus*), amphibians and birds, demonstrating that otters were adaptable in selecting available prey. In England, trout farms and recreational fisheries have become the source of prey for the increasing otter population. The fish biomass found on most recreational fisheries have made it easy for otters to hunt and capture fish, with specimen carp the preferred target but often consuming just some of the prey (Figure 1.5) with other smaller coarse fish such as roach (*Rutilus rutilus*) forming part of their diet.

Figure 1.5 Otter predation of carp (photograph: B. Brewster)

Whilst the recovery of otter populations is to be welcomed, Sherrard-Smith et al., (2009) have reported incidences of otters, but also a few American mink (*Mustela vison*), infected with *Pseudamphistomum truncatum* (Rudolphi, 1819) and *Metorchis albidus* (Braun, 1893) (Opistorchioidea; Opistorchidae) in a number of sites in England and Wales. The intermediate hosts of these digeneans are *Bithynia* species, freshwater snails and cyprinid fish. Hawkins et al. (2010) state that Simpson *et al*. in 2005 have proposed these digeneans were introduced with non-native sunbleak (*Leucaspius delineatus*) and topmouth gudgeon (*Pseudorasbora parva*). *Pseudamphistomum truncatum* and *M. albidus* are potentially zoonotic, although transmission to humans requires eating raw or poorly cooked infected fish however, all recreational coarse fishing is catch and release but in the last few years coarse fish have become an illegal source of food for European migrant workers.

1.6 *Aquatic Parasite Information*

The freshwater fish parasite fauna in the UK has changed since Kennedy's (1974) checklist was published over 40 years ago, due to release, either accidentally or deliberately of non-native, or illegally imported freshwater fish and a revised update on the distribution of both native and introduced parasites is overdue. Information resource technology has advanced significantly since this checklist was published. Electronic database software now enables a vast amount of data to be stored, rapidly updated, readily accessed and intensively interrogated for specific data retrieval. Parasitological databases such as the Natural History Museum's *Host-Parasite Database* (www.nhm.ac.uk/research-curation/projects/host-parasites) and the *GyroDB* database on gyrodactylid fish parasites (www.gyrodb.net) have provided a range of resources enabling dissemination of knowledge and data on parasites and their hosts. However, the *Aquatic Parasite Information* database has been designed specifically for freshwater and brackish fish species in the UK (collectively referred to as 'freshwater fish' throughout this thesis). The database incorporates historic records of freshwater parasites recorded from a number of sources, including data from the Water Authorities who were responsible for all freshwaters in the UK until their privatisation in 1989, research information (with permission), published records and independent consultants. Post mortem examination of fish samples has been carried out throughout the project and entered into the database. The generation of an electronic information resource on parasites of British freshwater fish and development of diagnostic techniques facilitates the monitoring of novel and pathogenic parasites, as well as native species.

Whilst checklists are an important source of information regarding parasites, fish hosts, host-parasite associations and distribution, published checklists in journals quickly become obsolete. Because *Aquatic Parasite Information* is an electronic database taxonomic changes, new records of parasites species, hosts and distribution data can be easily entered, which makes this a contemporary source of information.

1.6.1 Database design

A relational database has been designed to include all relevant information regarding the source of fish, the nature of the waterbodies from which the samples have been taken, the parasite species and where they were located in the tissues and the date these were recorded. Fish parasitology has an extensive history, with new species being recorded from the time of Linnaeus (1758). Over the years, improvement in optical equipment, available technologies and dissemination of knowledge, has resulted in the realization that some parasites have been described more than once, with some parasites having multiple synonyms. In order to reduce confusion, the synonyms associated with the parasite species have been included in the database, facilitating a search for all the names associated with any particular species. References to the first description of a species can be particularly useful but some of the early publications can prove difficult to trace, thus the database includes all references to the first published description of the parasite species.

The Environment Agency regards parasites which have significant disease potential, or exotic parasites of unknown pathogenicity and distribution found in freshwater fish as Category 2 parasites. There is industrial sensitivity and stigma surrounding the

presence of Category 2 parasites on any commercial site or angling club or society waters, as a consequence, the distribution of parasites is given at the level of vice-county or county.

1.6 2 Interrogating the database

The most important function of any database is retrieval of information. *Aquatic Parasite Information* has a comprehensive search facility, enabling the database to be mined for information on parasite species, distribution, hosts, target organ(s), synonyms, first recorded occurrence in the UK of novel and exotic parasites and reference to the first description. Demonstrating the ability of *Aquatic Parasite Information* to be an effective, contemporary checklist, records of the parasite species, hosts and distribution entered into the database are presented in this study. The entries for the Category 2 parasites provided the data for analysis of annual records, parasite host associations and distribution.

1.7 Morphological and molecular study of species of *Dactylogyrus* (Monogenea; Dactylogyroidea) associated with coarse fish

Dactylogyrus species are common parasites mostly associated with coarse fish, but identification in the UK has been overlooked because these gill parasites have been presumed to be of low pathogenicity. Studies by Buchmann & Bresciani (2006) and Rastiannasab *et al.* (2015) have shown that *Dactylogyrus extensus* suppresses the immune system and affects liver and kidney function in carp. Whilst non-parasitic

diseases of fish receive investigation, it is possible that *Dactylogyrus* species play a greater role in outbreaks of disease and morbidity than previously perceived.

Identification of dactylogyrids is problematic based on the morphometrics of the sclerotized haptor and copulatory organ (Simkova *et al.*2001). Identification based on the sclerotized parts is challenging as histological processing can distort the tissues and the orientation of the specimen on a microslide may obscure the haptor or copulatory organ and the size of these organs is approaching the limits of resolution for the compound microscope. Whilst molecular studies have been undertaken for a number of European species, the DNA sequencing of British *Dactylogyrus* species has not been studied and genomics may prove a better method to identify these parasites. An integrative approach, therefore, using morphological and molecular methods was employed to identify UK dactylogyrids in this study.

1.8. Cestodes of freshwater fish in the UK

As a result of introductions of freshwater fish, chiefly cyprinids, from the Far East and Europe, the number of cestode species parasitizing fish in the UK has increased since Chubb, Pool and Veltkamp's 1987 identification keys to species. Most of the native tapeworms associated with freshwater fish are thought to be of low pathogenicity but but large numbers of *Caryophyllaeus laticeps* (Pallas, 1878) can cause pathology and mortalities in bream (Karanis & Taraschewski, 1993, Williams & Jones, 1994) and Schaperclaus (1992) reports heavy infections have caused carp mortalities by occluding the intestine. Some of the non-native introductions such as *Schizocotyle acheilognathi* (Yamaguti, 1935) are known pathogens (Scholz et al., 2012; Pegg, *et*

al., 2015). Other species such as *Khawia japonensis* (Yamaguti, 1934) are of unknown pathogenicity and have potentially been introduced to the UK.

Apart from an enlarged drawing of *Caryophyllaeus fimbriceps* taken from the original Annenkova-Khoplina (1919) description, Chubb, Pool and Veltkamp (1987) relied on electron microscopy to identify morphological characters, to distinguish the species. Morphological characters are the preferred method for identifying tapeworms in the field, however accuracy of identification may depend on experience, plus identification keys make no allowance for phenotypic variability both of which can result in misidentification.

The emergence of genomics has resulted in the ability to easily extract, amplify and sequence DNA from cestodes which have been collected during routine screening of fish for movement consent. The use of DNA sequences should result in a reliable method for the identification of cestodes associated with freshwater fish in the UK.

1. 9 Identification of *Atractolytocestus* (Cestoda: Caryophyllidea: Lytocestidae) species infecting common carp (*Cyprinus carpio*) in the UK

Atractolytocestus huronensis (Anthony, 1958) was first described from common carp in the Huron River, North America. Originally a monospecific genus, two additional species have been recognised, *A. sagittatus* (Kulakovskaya & Akhmerov, 1965) and *A. tenuicollis* (Li, 1964). *Atractolytocestus tenuicollis* was originally described as a species of *Khawia* but referred to the genus *Atractolytocestus* by Xi *et al*., (2009) The first recorded appearance of *A. huronensis* in the UK was in 1993 (Chubb, Kirk & Wellby, 1996) and was considered an exotic introduction, of unknown pathogenicity and included in the Environment Agency schedule of Category 2 parasites. Despite

the Environment Agency movement restrictions associated with Category 2 parasites, *A. huronensis* became widespread. In 2007 *A. huronensis* was removed from the Category 2 schedule by the Environment Agency Category 2 Review Group, based on evidence from Williams (2007) of low pathogenicity in infected carp.

During routine examination of a sample of carp, a tapeworm was found which appeared morphologically different from *A. huronensis* and was tentatively identified as *A. sagittatus*. A threefold approach has been taken to determine the validity of the species of this cestode by morphological comparison with cogeners using histological techniques, using scanning electron microscopy and finally genomics, sequencing the DNA and analysing the genetic variation in the *Atractolytocestus* species.

1. 10 Concluding Remarks

It is in excess of 40 years since Kennedy's checklist of freshwater fish parasites and their distribution was published, during this time there have been significant changes to the way fish have been imported and moved around the country. Changes in legislation have not kept pace with increasing numbers of fish translocations both from within the UK and importations predominantly from Europe, Israel and the Far East. As a consequence of non-indigenous fish translocations, there has been an increase in the non-native parasite fauna introduced to the UK. Freshwater fish parasites have also increased due to a recovery in numbers of fish predators. This study takes into account the changes in the freshwater fish parasite fauna since Kennedy's 1974 checklist was published through the development of an electronic information system, coupled with morphological and molecular work on some of the extant and exotic species which are found in the UK.

As a result of human persecution, cormorant (*Phalacrocorax carbo*) and otters (*Lutra lutra*) were in decline in the 1970's but populations of these fish predators have made significant recoveries and both are found countrywide. Freshwater fish are the intermediate hosts for a number of parasites, some with zoonotic potential, the distribution of which are largely undocumented.

Rapid advances in information technology has allowed for large volumes of data to be stored and retrieved electronically using readily available software. *Aquatic Parasite Information* has been designed to incorporate data on freshwater fish parasites, creating a web based checklist, which can be regularly updated and allow easy retrieval of information on parasite distribution together with access for data retrieval and mining.

Dactylogyrus species have proven difficult to identify using traditional methods because the sclerotized organs used for identification are not easy to visualize. Species of *Dactylogyrus* from UK hosts are the subject here of morphological and molecular study. The identification of a molecular marker by sequencing the DNA from the dactylogyids may provide a useful diagnostic method for identifying the species.

The number of species of cestode found in the UK has increased since Chubb *et al.* published their keys to this group on 1987, through the introduction of novel and exotic tapeworms associated with fish imports. Microscopy remains the basic tool for identification of the cestodes, particularly during routine examination of fish for movement consent thus there is a requirement to update the diagnostic key to common species. The molecular study of the cestodes is the first to be undertaken

for a number of species found in the UK and compared with European species, however genomics may also prove a valuable tool for diagnostic work.

Atractolytocestus huronensis (Anthony, 1958) is an exotic parasite first recorded in the UK in 1993 but is of particular interest following the more recent discovery of a similar species, tentatively identified as *A. sagittatus* (?). Comparison of the two species using microscopy and molecular work is undertaken to resolve the relationship between these species.

1. 11 Aims and Objectives

The aims of this study were to examine the changes in the freshwater fish parasite fauna of the UK, since Kennedy's 1974 checklist was published, through the development of an electronic database. An electronic information system can be continuously updated, allowing for interrogation of data and the compilation of a current checklist of the freshwater fish parasites in the UK. Information obtained from the database was used to monitor potentially serious fish pathogens regarded by the Environment Agency as Category 2 parasites. The extracted data indicates that commonly encountered fish parasites, notably *Dactylogyrus* species and the cestodes are poorly represented, owing to the difficulties in identification. Morphological and molecular techniques were therefore used to discriminate *Dactylogyrus* species and selected cestodes to develop improved methods of identification A combined morphological and molecular approach was also undertaken to identify a species of *Atractolytocestus* found parasitizing carp but which appeared on initial examination to be different from *A. huronensis* commonly found in the UK.

Chapter 2

Aquatic Parasite Information Database

2.1 Introduction

A checklist of parasites associated with freshwater fish provides a useful resource for researchers interested in the study of parasite taxa, parasites associated with a particular fish species, data for comparative host-parasite studies or for use as a parasite identification guide (Poulin *et al.*, 2016). Whilst important information is disseminated through checklists, this published information may not be regularly updated, if at all, consequently the frequency of reporting parasite distribution, or changes in taxonomy, may be poor, particularly as many peer reviewed journals no longer publish new distribution records (Poulin *et al.,* 2016). Control of the dissemination and spread of non-native or invasive parasites of freshwater fish is the responsibility of the Environment Agency in England, Natural Resources Wales and Marine Scotland but the information contained in published checklists is time sensitive. Organizing information concerning parasite and host distribution onto an interactive, electronic database enables all information relating to freshwater fish parasites in the UK to be readily and frequently updated, increasing the academic value of the content and enabling all of the regulatory bodies to access current data. The function of a database is to archive inter-related information, using software enabling a computer to link the component records, allowing data to be both stored and retrieved. The design chosen for the fish host and parasite information was the 'relational database', a multi-tabled database, commonly used because of its flexibility and ability to manage complex information by organizing data, based on the relationship between the component elements (Oppel, 2009). The organization of the relational database fields, contained within tables has the advantage of preventing duplication of data and a consistent lexicon of data entry.

Only the database design and contained data are the elements of this project, the implementation, maintenance and online system are not included.

A database enabling sample locality, fish host and parasite information to be stored electronically was designed at Kingston University initially using Microsoft Access® software as the platform for the fish host/parasite relational database. The design process is iterative, each stage providing the opportunity to test structure and component relationships, finally the functional database was uploaded to the internet. The component elements of the database for parasites of freshwater fish were both extensive and complex, to store and link the nomenclature, taxonomy, author of the parasite species, fish host, locality and information source obtained from published works, data contributed by scientists working in the field of fish parasitology, or records from routine fish health examinations. Published checklists refer to parasites identified from individual fish species together with a location (Chappell & Owen, 1969; Kennedy, 1974), whereas data from fish surveys or parasite survey work may comprise more than one fish species and the number of fish examined for parasites may vary, for example, 1 – 150 individuals. The relational database design is flexible and can link the classification and taxonomy of parasite species; parasites identified with individual fish; fish samples; locality data; water bodies and administrative details. Parasite taxonomy is constantly being revised and updated, particularly with the advent of molecular techniques, which are changing species concepts and relationships of taxa within higher classification, from genus to phylum or supergroup. The relational database allows for changes to be implemented at any taxonomic level and applied to all connected taxa.

The Environment Agency (1999 & 2007) consider some parasites to either cause significant pathologies in fish, or are exotic and of unknown pathogenicity to freshwater fish in the UK, these fish parasites are termed 'Category 2' parasites. All fish species on those sites recognised by the Environment Agency as having a Category 2 parasite present, are subject to movement restrictions. For many fisheries or fish farms, there is an economic penalty associated with the presence of a Category 2 parasite, leading to industrial sensitivity regarding such infected sites, the database search engines were therefore designed to restrict public access to this information.

As a web-based application, *Aquatic Parasite Information* can allow subscription controlled access for searching data creating a powerful tool for the regulatory bodies, academics, veterinarians and fish health professionals to keep pace with novel parasites introduced as a result of fish translocations, potentially pathogenic additions to the parasite fauna and the rapid changes taking place in the classification of parasites associated with freshwater fish in the UK.

2. 2 Database Software Design

Following identification of the entities (Appendix 1), a conceptual design for the database was created using Smartdraw® and DIA® software (Figure 2.1). The entities from the conceptual design were used to create tables in a relationship diagram using Microsoft® Access software. On completion of the relationship diagram, the tables were populated with fields, followed by the addition of records. Two hundred and ninety species of freshwater and brackish fish parasites were entered into the database and 36 species of fish, including hybrids. Details of the design method are given in Appendix 1.

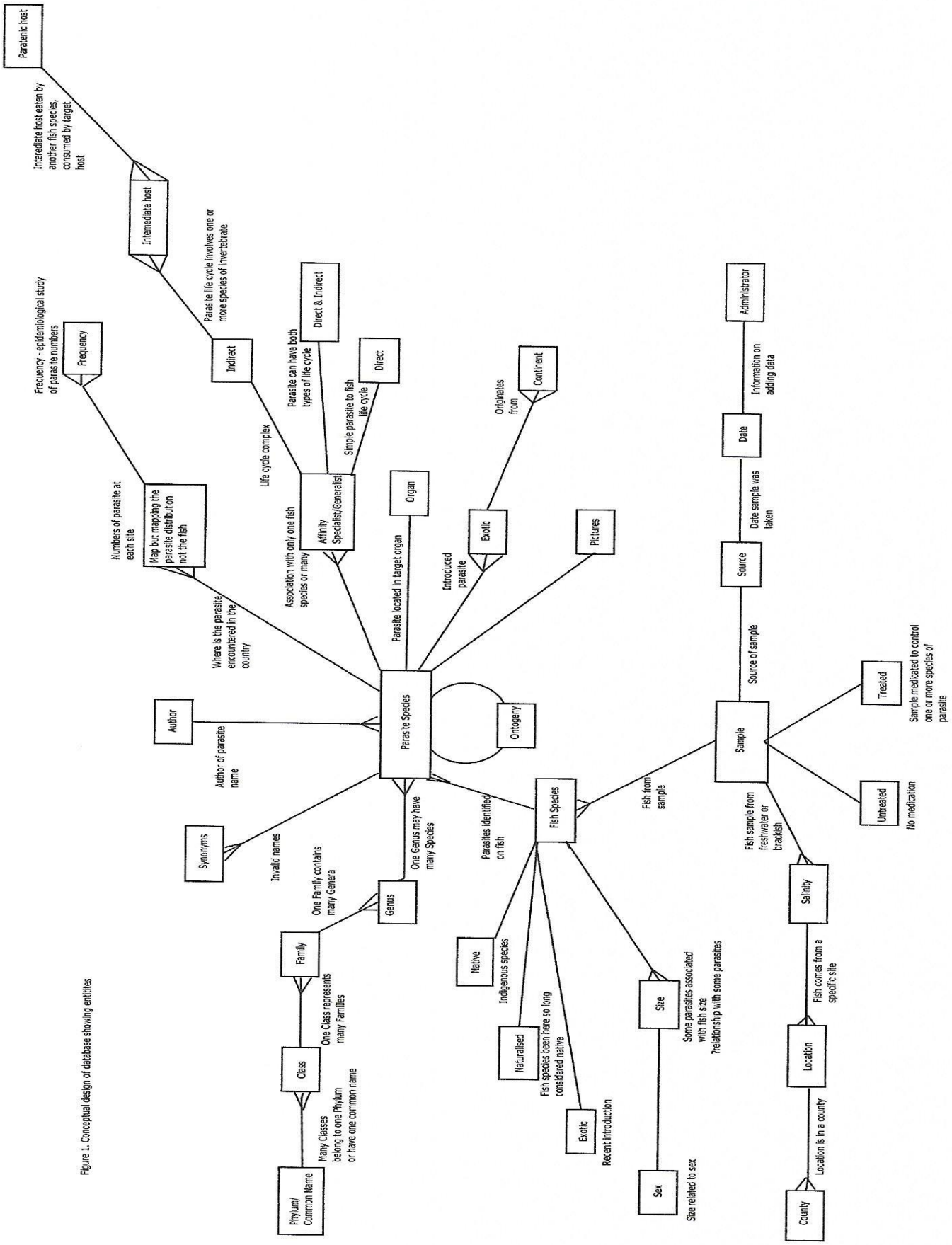

Figure 1: Conceptual design of database showing entities

Figure 2.1. Entities forming the conceptual design of the relational database for the parasites of freshwater fish

Public access of a database via the internet requires 'front-ends', which are the interface controlling how users interact with the data. Design of the front-ends was undertaken in collaboration with Mr Sivasankara Desikan, an MSc student also based at Kingston University, School of Computer Science and Mathematics, under the supervision of Dr J. Denholm-Price. The term 'front-ends' misrepresents the complexity of software design required to combine the Access® designed relational parasite database with an interactive website that enables users to register, log-in, access forgotten passwords and search the database. The search engines in *Aquatic Parasite Information* allow the user to conduct a general search for parasites, hosts, host common names, authors, synonyms, general distribution of parasites, whilst the advanced search allows information to be mined for particular parasite species, or parasites associated with fish species. Because of the sensitivity of sites affected by Category 2 parasites, regarded by the Environment Agency as serious pathogens, the distribution data provided by *Aquatic Parasite Information* is given as the vice county and not the locality. The published records of parasites associated with exotic fish such as Wels catfish (*S. glanis*), pumpkinseed (*L. gibbosus*) topmouth gudgeon (*P. parva*) and sunbleak (*L. delineatus*) have been included in the database because these non-native species are present in the UK (Reading *et al.*, 2011; Hockley, 2011; Gozlan *et al.*, 2009; Beyer *et al.*, 2005).

2.3 Data sources

Data on parasites from freshwater fish in various water bodies in the UK were entered onto the web based *Aquatic Parasite Information* database. The data was collated from published research papers including first records of introduced parasites (e.g. Fryer, 1967; Fryer 1968; Fryer & Andrews, 1983; Pool & Chubb, 1987; Kennedy &

Fitch, 1990) and published checklists (Chappell & Owen, 1969; Kennedy, 1974), unpublished routine fish health screening reports from independent fish health consultants and the Environment Agency. The aim was to create a useful tool in information technology to provide retrievable and updatable information relating to the distribution and status of parasites of freshwater fish in the UK.

2. 4. Data Overview

An internet based database can store a vast amount of information which can be readily accessed and interrogated *Aquatic Parasite Information* provides a collated data resource pertaining to the taxonomy of parasite species, fish hosts and host distribution in the UK. Records for 200 of the 290 species of freshwater and brackish fish parasites have been entered in the database (Table 2.1), these records are taken from 1285 fish samples, comprising 1 – 150 fish per sample and from 760 locations across the UK. Hosts and associated parasites and parasite distribution records are given in Appendices 2 & 3. Species of cyprinid comprise the hosts with the greatest number of recorded parasites. Whilst carp are preferred by the majority of anglers and are consequently regularly translocated between sites it would be assumed that they have the opportunity to have come into contact with the greatest recorded number of parasites but this is not the case. Data taken from *Aquatic Parasite Information* indicates carp are recorded as host to some 32 different species of parasite. Bream exceed this number with 36 recorded parasites but the species with the highest number is the roach which has 59 different parasite species.

Table 2.1 Species of parasites of freshwater fish entered in *Aquatic Parasite Information*

Phylum/Supergroup	Class	Family	Genus	Species
Apicomplexa	Conoidasida	Eimeriidae	*Eimeria*	*anguillae*
Apicomplexa	Conoidasida	Eimeriidae	*Eimeria*	*rutili*
Apicomplexa	Conoidasida	Eimeriidae	*Epieimeria*	*anguillae*
Apicomplexa	Conoidasida	Eimeriidae	*Goussia*	*carpelli*
Apicomplexa	Conoidasida	Eimeriidae	*Goussia*	*metchnikovi*
Apicomplexa	Conoidasida	Eimeriidae	*Goussia*	*subepithelialis*
Ciliophora	Litostomatea	Amphileptidae	*Hemiophrys*	*branchiarum*
Ciliophora	Oligohymenophora	Epistylididae	*Apiosoma*	*piscicola*
Ciliophora	Oligohymenophora	Ichthyophthiridae	*Ichthyophthirius*	*multifilis*
Ciliophora	Oligohymenophora	Trichodinidae	*Paratrichodina*	*incisa*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*acuta*
Ciliophora	Oligohymenophora	Trichodinidae	*Tripartiella*	*copiesa*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*domerguei*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*epizootica*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*intermedia*
Ciliophora	Oligohymenophora	Trichodinidae	*Tripartiella*	*lata*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*megamicronucleata*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*modesta*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*mutabilis*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*nigra*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*pediculus*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*polycirra*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*reticulata*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*rostrata*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*tenuidens*
Ciliophora	Oligohymenophora	Trichodinidae	*Trichodina*	*urinaria*
Ciliophora	Phyllopharyngea	Chilodonellidae	*Chilodonella*	*cyprini*
Ciliophora	Phyllopharyngea	Chilodonellidae	*Chilodonella*	*hexasticha*
Ciliophora	Phyllopharyngea	Chilodonellidae	*Chilodonella*	*piscicola*
Euglenozoa	Kinetoplasta	Bodonidae	*Ichthyobodo*	*necator*
Euglenozoa	Kinetoplasta	Bodonidae	*Trypanosplasma*	*borelli*
Euglenozoa	Kinetoplasta	Bodonidae	*Trypanoplasma*	*keisselitzi*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*carassii*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*cobitis*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*elegans*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*granulosum*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*leucisci*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*percae*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*remaki*
Euglenozoa	Kinetoplasta	Trypanosomatidae	*Trypanosoma*	*tincae*
Retortamonada	Diplomonoidea	Hexamitidae	*Octomitus*	*truttae*
Retortamonada	Diplomonoidea	Hexamitidae	*Spironucleus*	*barkhanus*
Retortamonada	Diplomonoidea	Hexamitidae	*Spironucleus*	*salmonis*
Retortamonada	Diplomonoidea	Hexamitidae	*Spironucleus*	*vortens*
Microsporidia	Microsporea	Glugeidae	*Glugea*	*anomala*
Microsporidia	Microsporea	Glugeidae	*Glugea*	*gasterostei*
Microsporidia	Microsporea	Glugeidae	*Glugea*	*luciopercae*
Microsporidia	Microsporea	Pleistophoridae	*Pleistophora*	*longifilis*
Myxozoa	Malacosporea	Buddenbrockiidae	*Tetracapsuloides*	*bryosalmonae*

Table 2.1 Species of parasites of freshwater fish entered in *Aquatic Parasite Information* – continued

Phylum/Supergroup	Class	Family	Genus	Species
Myxozoa	Mesomycetozoa	Rhinosporideacae	*Dermocystidium*	*anguillae*
Myxozoa	Mesomycetozoa	Rhinosporideacae	*Dermocystidium*	*branchiale*
Myxozoa	Mesomycetozoa	Rhinosporideacae	*Dermocystidium*	*cyprini*
Myxozoa	Mesomycetozoa	Rhinosporideacae	*Dermocystidium*	*fennicum*
Myxozoa	Mesomycetozoa	Rhinosporideacae	*Dermocystidium*	*gasterostei*
Myxozoa	Mesomycetozoa	Rhinosporideacae	*Dermocystidium*	*percae*
Myxozoa	Mesomycetozoa	Rhinosporideacae	*Sphaerothecum*	*destruens*
Myxozoa	Myxosporea	Chloromyxidae	*Chloromyxum*	*esocinum*
Myxozoa	Myxosporea	Chloromyxidae	*Chloromyxum*	*phoxini*
Myxozoa	Myxosporea	Chloromyxidae	*Chloromyxum*	*truttae*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*giardi*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*lieberkühni*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*macrocapsulare*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*oviforme*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*pfeifferi*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*rhodei*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*scardini*
Myxozoa	Myxosporea	Myxidiidae	*Myxidium*	*truttae*
Myxozoa	Myxosporea	Myxidiidae	*Zschokkella*	*cyprini*
Myxozoa	Myxosporea	Myxidiidae	*Zschokkella*	*nova*
Myxozoa	Myxosporea	Myxobolidae	*Henneguya*	*creplini*
Myxozoa	Myxosporea	Myxobolidae	*Henneguya*	*oviperda*
Myxozoa	Myxosporea	Myxobolidae	*Henneguya*	*psorospermica*
Myxozoa	Myxosporea	Myxobolidae	*Henneguya*	*tegidiensis*
Myxozoa	Myxosporea	Myxobolidae	*Henneguya*	*zschokkei*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*actus*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*anurus*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*arcticus*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*artus*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*branchialis*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*cerebralis*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*cotti*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*cycloides*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*cyprini*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*dermatobius*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*dispar*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*ellipsoides*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*koi*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*kotlani*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*macrocapsularis*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*mülleri*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*neurobius*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*pseudodispar*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*subepithelialis*
Myxozoa	Myxosporea	Myxobolidae	*Myxobolus*	*volgensis*
Myxozoa	Myxosporea	Myxobolidae	*Thelohanellus*	*pyri*
Myxozoa	Myxosporea	Sphaerosporidae	*Hoferellus*	*carassi*
Myxozoa	Myxosporea	Sphaerosporidae	*Hoferellus*	*cyprini*
Myxozoa	Myxosporea	Sphaerosporidae	*Myxobilatus*	*gasterostei*

Table 2.1 Species of parasites of freshwater fish entered in *Aquatic Parasite Information* – continued

Phylum/Supergroup	Class	Family	Genus	Species
Myxozoa	Myxosporea	Sphaerosporidae	*Sphaerospora*	*dykovae*
Myxozoa	Myxosporea	Sphaerosporidae	*Sphaerospora*	*elegans*
Myxozoa	Myxosporea	Sphaerosporidae	*Sphaerospora*	*molnari*
Myxozoa	Myxosporea	Sphaerosporidae	*Sphaerospora*	*truttae*
Acanthocephala	Eoacanthocephala	Neoechinorhynchidae	*Neoechinorhynchus*	*rutili*
Acanthocephala	Palaeacanthocephala	Echinorhynchidae	*Acanthocephalus*	*anguillae*
Acanthocephala	Palaeacanthocephala	Echinorhynchidae	*Acanthocephalus*	*clavula*
Acanthocephala	Palaeacanthocephala	Echinorhynchidae	*Acanthocephalus*	*lucii*
Acanthocephala	Palaeacanthocephala	Echinorhynchidae	*Echinorhynchus*	*truttae*
Acanthocephala	Palaeacanthocephala	Polymorphidae	*Polymorphus*	*minutus*
Acanthocephala	Palaeacanthocephala	Pomphorhynchidae	*Pomphorhynchus*	*laevis*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*amphibothrium*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*anchoratus*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*auriculatus*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*cordus*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*crucifer*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*cryptomeres*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*difformis*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*extensus*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*gobii*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*nanus*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*phoxini*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*prostae*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*similis*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*sphyrna*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*suecicus*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*tincae*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*tuba*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*vastator*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*vistulae*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*wunderi*
Platyhelminthes	Monogenea	Dactylogyridae	*Dactylogyrus*	*zandti*
Platyhelminthes	Monogenea	Dactylogyridae	*Pellucidhaptor*	*pricei*
Platyhelminthes	Monogenea	Pseudodactylogyridae	*Pseudodactylogyrus*	*anguillae*
Platyhelminthes	Monogenea	Pseudodactylogyridae	*Pseudodactylogyrus*	*bini*
Platyhelminthes	Monogenea	Ancyrocephalidae	*Ancyrocephalus*	*paradoxus*
Platyhelminthes	Monogenea	Ancyrocephalidae	*Ancyrocephalus*	*percae*
Platyhelminthes	Monogenea	Ancyrocephalidae	*Onchocleidus*	*principalis*
Platyhelminthes	Monogenea	Ancyrocephalidae	*Thaparocleidus*	*vistulensis*
Platyhelminthes	Monogenea	Tetraonchidae	*Tetraonchus*	*borealis*
Platyhelminthes	Monogenea	Tetraonchidae	*Tetraonchus*	*monenteron*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*anguillae*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*aphyae*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*arcuatus*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*caledoniensis*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*cyprini*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*derjavini*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*elegans*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*gasterostei*

Table 2.1 Species of parasites of freshwater fish entered in *Aquatic Parasite Information* – continued

Phylum/Supergroup	Class	Family	Genus	Species
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*gurleyi*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*laevis*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*leucisci*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*limneus*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*longoacuminatus*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*lucii*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*macronychus*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*medius*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*minimus*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*pavlovskyi*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*phoxini*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*pungitii*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*rarus*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*rogatensis*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*salaris*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*sedelnikowi*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*sommervillae*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*thymalli*
Platyhelminthes	Monogenea	Gyrodactylidae	*Gyrodactylus*	*truttae*
Platyhelminthes	Monogenea	Diplozoidae	*Paradiplozoon*	*homoion*
Platyhelminthes	Monogenea	Diplozoidae	*Eudiplozoon*	*nipponicum*
Platyhelminthes	Monogenea	Diplozoidae	*Diplozoon*	*paradoxum*
Platyhelminthes	Monogenea	Discocotylidae	*Discocotyle*	*sagittata*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Glanitaenia*	*osculata*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*ambiguus*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*cernua*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*filicollis*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*longicollis*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*macrocephalus*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*neglectus*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*percae*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*pollanicola*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*sagittus*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*tetrastomus*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Proteocephalus*	*torulosus*
Platyhelminthes	Bothriocephalidea	Proteocephalidae	*Silurotaenia*	*siluri*
Platyhelminthes	Bothriocephalidea	Bothriocephalidae	*Schizocotyle*	*acheilognathi*
Platyhelminthes	Bothriocephalidea	Diphyllobothridae	*Diphyllobothrium*	*dendriticum*
Platyhelminthes	Bothriocephalidea	Diphyllobothridae	*Diphyllobothrium*	*ditremum*
Platyhelminthes	Bothriocephalidea	Diphyllobothridae	*Diphyllobothrium*	*latum*
Platyhelminthes	Bothriocephalidea	Diphyllobothridae	*Diphyllobothrium*	*norvegicum*
Platyhelminthes	Bothriocephalidea	Diphyllobothridae	*Ligula*	*intestinalis*
Platyhelminthes	Bothriocephalidea	Diphyllobothridae	*Schistocephalus*	*solidus*
Platyhelminthes	Bothriocephalidea	Hepatoxylidae	*Hepatoxylon*	*squali*
Platyhelminthes	Bothriocephalidea	Triaenophoridae	*Bathybothrium*	*rectangulum*
Platyhelminthes	Bothriocephalidea	Triaenophoridae	*Bothriocephalus*	*claviceps*
Platyhelminthes	Bothriocephalidea	Triaenophoridae	*Eubothrium*	*crassum*
Platyhelminthes	Bothriocephalidea	Triaenophoridae	*Eubothrium*	*salvelini*
Platyhelminthes	Bothriocephalidea	Triaenophoridae	*Triaenophorus*	*nodulosus*

Table 2.1 Species of parasites of freshwater fish entered in *Aquatic Parasite Information* – continued

Phylum/Supergroup	Class	Family	Genus	Species
Platyhelminthes	Spathibothridea	Acrobothridae	*Cyathocephalus*	*truncatus*
Platyhelminthes	Caryophyllidea	Caryophyllaeidae	*Archigetes*	*sieboldi*
Platyhelminthes	Caryophyllidea	Caryophyllaeidae	*Biacetabulum*	*appendiculatum*
Platyhelminthes	Caryophyllidea	Caryophyllaeidae	*Caryophyllaeus*	*fimbriceps*
Platyhelminthes	Caryophyllidea	Caryophyllaeidae	*Caryophyllaeus*	*laticeps*
Platyhelminthes	Caryophyllidea	Caryophyllaeidae	*Monobothrium*	*wageneri*
Platyhelminthes	Caryophyllidea	Lytocestidae	*Atractolytocestus*	*huronensis*
Platyhelminthes	Caryophyllidea	Lytocestidae	*Atractolytocestus*	*sagittatus*
Platyhelminthes	Caryophyllidea	Lytocestidae	*Caryophyllaeides*	*fennica*
Platyhelminthes	Caryophyllidea	Lytocestidae	*Khawia*	*japonensis*
Platyhelminthes	Caryophyllidea	Lytocestidae	*Khawia*	*sinensis*
Platyhelminthes	Cyclophyllidea	Dilepididae	*Neogryporhynchus*	*cheilancristrotus*
Platyhelminthes	Cyclophyllidea	Dilepididae	*Paradilepis*	*scolecina*
Platyhelminthes	Cyclophyllidea	Dilepididae	*Valipora*	*campylancristata*
Platyhelminthes	Trematoda	Allocreadiidae	*Allocreadium*	*isoporum*
Platyhelminthes	Trematoda	Allocreadiidae	*Allocreadium*	*transversale*
Platyhelminthes	Trematoda	Allocreadiidae	*Bunodera*	*lucioperca*
Platyhelminthes	Trematoda	Allocreadiidae	*Crepidostomum*	*farionis*
Platyhelminthes	Trematoda	Allocreadiidae	*Crepidostomum*	*metoecus*
Platyhelminthes	Trematoda	Allocreadiidae	*Macrolecithus*	*papilliger*
Platyhelminthes	Trematoda	Aporocotylidae	*Sanguinicola*	*armata*
Platyhelminthes	Trematoda	Aporocotylidae	*Sanguinicola*	*inermis*
Platyhelminthes	Trematoda	Aspidogastridae	*Aspidogaster*	*limacoides*
Platyhelminthes	Trematoda	Azygiidae	*Azygia*	*lucii*
Platyhelminthes	Trematoda	Bucephalidae	*Bucephalus*	*polymorphus*
Platyhelminthes	Trematoda	Bucephalidae	*Rhipidocotyle*	*campanula*
Platyhelminthes	Trematoda	Bucephalidae	*Rhipidocotyle*	*illense*
Platyhelminthes	Trematoda	Cyathocotylidae	*Holostephanus*	*lühei*
Platyhelminthes	Trematoda	Deropristidae	*Deropristis*	*inflata*
Platyhelminthes	Trematoda	Diplostomidae	*Diplostomum*	*gasterostei*
Platyhelminthes	Trematoda	Diplostomidae	*Diplostomum*	*mergi*
Platyhelminthes	Trematoda	Diplostomidae	*Diplostomum*	*paraspathaceum*
Platyhelminthes	Trematoda	Diplostomidae	*Diplostomum*	*petromyzi-fluviatilis*
Platyhelminthes	Trematoda	Diplostomidae	*Diplostomum*	*phoxini*
Platyhelminthes	Trematoda	Diplostomidae	*Diplostomum*	*pseudospathaceum*
Platyhelminthes	Trematoda	Diplostomidae	*Diplostomum*	*spathaceum*
Platyhelminthes	Trematoda	Diplostomidae	*Hysteromorpha*	*triloba*
Platyhelminthes	Trematoda	Diplostomidae	*Posthodiplostomum*	*cuticola*
Platyhelminthes	Trematoda	Diplostomidae	*Tylodelphys*	*clavata*
Platyhelminthes	Trematoda	Diplostomidae	*Tylodelphys*	*podicipina*
Platyhelminthes	Trematoda	Echinochasmatidae	*Echinochasmus*	*perfoliatus*
Platyhelminthes	Trematoda	Echinochasmatidae	*Petasiger*	*phalacrocoracis*
Platyhelminthes	Trematoda	Gorgoderidae	*Phyllodistomum*	*folium*
Platyhelminthes	Trematoda	Gorgoderidae	*Phyllodistomum*	*pseudofolium*
Platyhelminthes	Trematoda	Gorgoderidae	*Phyllodistomum*	*simile*
Platyhelminthes	Trematoda	Hemiuridae	*Lecithochirium*	*gravidum*
Platyhelminthes	Trematoda	Heterophyidae	*Cryptocotyle*	*concavum*
Platyhelminthes	Trematoda	Lissorchidae	*Asymphylodora*	*kubanicum*
Platyhelminthes	Trematoda	Lissorchidae	*Asymphylodora*	*tincae*

Table 2.1 Species of parasites of freshwater fish entered in *Aquatic Parasite Information* – continued

Phylum/Supergroup	Class	Family	Genus	Species
Platyhelminthes	Trematoda	Opecoelidae	*Sphaerostoma*	*bramae*
Platyhelminthes	Trematoda	Opecoelidae	*Nicolla*	*gallica*
Platyhelminthes	Trematoda	Opistorchiidae	*Pseudamphistomum*	*truncatum*
Platyhelminthes	Trematoda	Strigeidae	*Apatemon*	*gracilis*
Platyhelminthes	Trematoda	Strigeidae	*Ichthyocotylurus*	*cucullus*
Platyhelminthes	Trematoda	Strigeidae	*Ichthyocotylurus*	*erraticus*
Platyhelminthes	Trematoda	Strigeidae	*Ichthyocotylurus*	*pileatus*
Platyhelminthes	Trematoda	Strigeidae	*Ichthyocotylurus*	*variegatus*
Nematoda	Camallanoidea	Camallanidae	*Camallanus*	*lacustris*
Nematoda	Camallanoidea	Camallanidae	*Echinorhynchus*	*salmonis*
Nematoda	Chromadorea	Anisakidae	*Contracaecum*	*aduncum*
Nematoda	Chromadorea	Anisakidae	*Contracaecum*	*microcephalum*
Nematoda	Chromadorea	Anisakidae	*Contracaecum*	*rudolphii*
Nematoda	Chromadorea	Daniconematidae	*Daniconema*	*anguillae*
Nematoda	Chromadorea	Quimperiidae	*Paraquimperia*	*tenerrima*
Nematoda	Chromadorea	Rhabdochonidae	*Rhabdochona*	*denudata*
Nematoda	Chromadorea	Rhabdochonidae	*Rhabdochona*	*oncorhynchi*
Nematoda	Chromadorea	Rhaphidascarididae	*Hysterothylacium*	*aduncum*
Nematoda	Chromadorea	Rhaphidascarididae	*Raphidascaris*	*acus*
Nematoda	Chromadorea	Rhaphidascarididae	*Raphidascaris*	*cristata*
Nematoda	Chromadorea	Thelaziidae	*Truttaedacnitis*	*truttae*
Nematoda	Chromadorea	Trichuridae	*Pseudocapillaria*	*brevispicula*
Nematoda	Chromadorea	Trichuridae	*Pseudocapillaria*	*salvelini*
Nematoda	Chromadorea	Trichuridae	*Pseudocapillaria*	*tomentosa*
Nematoda	Dracunculoidea	Anguillicolidae	*Anguillicoloides*	*crassus*
Nematoda	Dracunculoidea	Philometridae	*Philometra*	*ovata*
Nematoda	Dracunculoidea	Philometridae	*Philometra*	*rischta*
Nematoda	Dracunculoidea	Philometridae	*Philometroides*	*sanguinea*
Nematoda	Dracunculoidea	Skrjabillanidae	*Molnaria*	*intestinalis*
Nematoda	Dracunculoidea	Skrjabillanidae	*Skrjabillanus*	*scardinii*
Nematoda	Dracunculoidea	Skrjabillanidae	*Skrjabillanus*	*tincae*
Nematoda	Spiruroidea	Cystidicolidae	*Cystidicola*	*farionis*
Nematoda	Spiruroidea	Cystidicolidae	*Cystidicoloides*	*ephemeridarum*
Nematoda	Spiruroidea	Cystidicolidae	*Cystidicoloides*	*tenuissima*
Nematoda	Spiruroidea	Cystidicolidae	*Goezia*	*anguillae*
Nematoda	Spiruroidea	Cystidicolidae	*Spinitectus*	*inermis*
Annelida	Oligochaeta	Glossiphonidae	*Hemiclepsis*	*marginata*
Annelida	Oligochaeta	Piscicolidae	*Piscicola*	*geometra*
Mollusca	Unionidea	Unionidae	*Cygnaea*	*anodonta*
Arthropoda	Copepoda	Caligidae	*Lepeophthirius*	*salmonis*
Arthropoda	Copepoda	Ergasilidae	*Ergasilus*	*briani*
Arthropoda	Copepoda	Ergasilidae	*Ergasilus*	*gibbus*
Arthropoda	Copepoda	Ergasilidae	*Ergasilus*	*sieboldi*
Arthropoda	Copepoda	Ergasilidae	*Neoergasilus*	*japonicus*
Arthropoda	Copepoda	Ergasilidae	*Paraergasilus*	*longidigitus*
Arthropoda	Copepoda	Ergasilidae	*Thersitina*	*gasterostei*
Arthropoda	Maxillipoda	Argulidae	*Argulus*	*appendiculosus*
Arthropoda	Maxillipoda	Argulidae	*Argulus*	*coregoni*
Arthropoda	Maxillipoda	Argulidae	*Argulus*	*foliaceus*

Table 2.1 Species of parasites of freshwater fish entered in *Aquatic Parasite Information* – continued

Phylum/Supergroup	Class	Family	Genus	Species
Arthropoda	Maxillipoda	Argulidae	*Argulus*	*japonicus*
Arthropoda	Maxillipoda	Lernaeopodidae	*Lernaea*	*cyprinacea*
Arthropoda	Maxillipoda	Lernaeopodidae	*Salmincola*	*edwardsii*
Arthropoda	Maxillipoda	Lernaeopodidae	*Salmincola*	*gordoni*
Arthropoda	Maxillipoda	Lernaeopodidae	*Salmincola*	*percarum*
Arthropoda	Maxillipoda	Lernaeopodidae	*Salmincola*	*salmoneus*
Arthropoda	Maxillipoda	Lernaeopodidae	*Salmincola*	*thymalli*
Arthropoda	Maxillipoda	Lernaeopodidae	*Tracheliastes*	*maculatus*
Arthropoda	Maxillipoda	Lernaeopodidae	*Tracheliastes*	*polycolpus*

Roach breed prolifically in the confines of many angling waters and may be regarded as a nuisance species, especially on those sites which are fished for specimen carp. It is quite likely the numbers of roach on any water have a greater opportunity to come into contact with those parasites with a direct life cycle such as the *Ergasilus* species or those invertebrates on which roach feed, such as copepodids and which are intermediate hosts for parasites with an indirect life cycle.

In recent years the numbers and size of rudd appear to have been declining (Duncan Charman pers. com.; pers. obs.), this species will readily interbreed with roach which is a possible factor but it could also be due to interspecific competition for resources. The number of records for parasites on rudd, which shares a similar habitat preference to roach, is much lower with 22 recorded parasite species, which may also reflect declining populations with fewer numbers of fish coming into contact with parasites. The future addition of records of parasites infecting rudd to the *Aquatic Parasite Information* database may also prove significant in establishing whether rudd are a species of fish deserving conservation effort.

It was important the database was updatable so parasite species were uploaded to the database which have not yet been identified as present in freshwater fish in the UK. For example, the fluke *Pseudamphistomum truncatum* is a biliary parasite of otter (*L. lutra*), American mink (*Mustela vison*) and other mammals, found in otters in isolated areas of the UK and Ireland (Simpson *et al.*, 2009; Hawkins *et al.*, 2010). The intermediate host of *P. truncatum* has been identified as roach in Ireland (Hawkins *et al.,* 2010), but as yet no infected fish have been identified in this country. Given that *P. truncatum* has been identified in otters in the UK (Simpson *et al.,* 2009) it is anticipated that fish infected with this digenean are present, but the parasite has yet to be reported so there are currently no records or 'samples'. This parasite species has therefore been entered into *Aquatic Parasite Information*.

Other species, such as the cestode *Khawia japonensis,* have not been recorded from the UK, but may be present or introduced in the near future. *Khawia japonensis* originally from Japan, has already been recorded as present in Italy (Scholtz *et al*., 2011) and Slovakia (Oros *et al.,* 2015). This species is morphologically similar to another exotic tapeworm, *K. sinensis* (Hsu, 1935), with which it may be confused, allowing it to be overlooked, so is likely to be present in the UK, especially given the total import of ornamental fish in 2011 was 35 million fish from approximately 50 countries (source OATA, accessed 2016) including the Far East.

The ten parasite species with the highest number of entries in the *Aquatic Parasite Information* database are presented in Table 2.2. Records from published research projects for *E. sieboldi* (Nordmann, 1832)*, P. laevis* (Müller 1776) and *Sanguinicola inermis* Plehn1905 have been entered into the *Aquatic Parasite Information*, which

have inflated the number of entries in the database for these species. Five of the parasites included in Table 2.2 that is *I. necator* (Henneguy,1833), *Piscicola geometra* (L.), *Ichthyophthirius multifilis* (Fouquet, 1876) *A. foliaceus* (L) and *E. sieboldi* (Nordmann, 1832) have a direct life cycle, and the remaining five have indirect life cycles, with aquatic invertebrates as intermediate hosts. Eight of the parasites are euryxenous, whereas the preferred definitive hosts of *P. laevis* are barbel, chub and rainbow trout although it will infect other freshwater species of fish but not attain sexual maturity (Kennedy, 2006). Kirk (2012) stated that all varieties of carp were definitive host for *S. inermis,* but the parasite has also been reported from a number of other species of cyprinid.

Table 2.2 Parasites with the highest number of entries in *Aquatic Parasite Information*

Parasite species	No. API entries
Argulus foliaceus	211
Ergasilus sieboldi	141
Diplostomum spathaceum	130
Acanthocephalus lucii	94
Ichthyobodo necator	93
Piscicola geometra	86
Pomphorhynchus laevis	84
Ichthyophthirius multifilis	76
Posthodiplostomum cuticola	67
Sanguinicola inermis	65

Metazoa are readily visualized either by light microscope or eye and some species may be identified based on morphological features. For example, mining *Aquatic Parasite Information* on the fish louse, *A. foliaceus*, a commonly encountered parasite, reveals the highest number of entries in *Aquatic Parasite Information* (Table 2.2). Other common, readily identifiable parasites reported in the database include the fish leech, *P. geometra* with 86 entries, representing 8% of the total fish samples. *Posthodiplostomum cuticola* (Nordmann, 1832), a digenean parasite which commonly

causes blackspot on roach, rudd and bream has 67 fish sample entries, representing 5% of the total fish samples (Table 2.2).

The list of fish hosts shows a wide variety of species of parasite associated with them (Appendix 2). Some parasite species may have a large number of entries in the database, as illustrated by Table 2.2, whereas others are poorly represented. Reasons for the paucity of records for these include incomplete datasets for some species of fish such as the coregonids and those parasites which prove difficult to identify based on morphological features. Many unicellular parasites with complex inter- and intracellular life cycles appear to cause little obvious pathology at sub-clinical levels and are inadvertently overlooked during routine fish health screening. Most of these unicellular parasites require histological preparation of fish tissues for identification, procedures not used in routine fish health examination where a tissue squash is the most commonly used technique. Unicellular parasites often become the focus of research projects following either conspicuous outbreaks of disease or fish mortalities, which then generate distribution and other data, for example the non-native *S. destruens* (Gozlan *et al*., 2009).

Dactylogyrus species are common parasites of cyprinids and are readily visible under the light microscope. The low number of records extracted from *Aquatic Parasite Information* is indicative of the difficulty associated with identification using morphological characters. (Table 2.3) There are 18 species of *Dactylogyrus* reported in the UK, but only 4 of these species have been confirmed by molecular identification (Chapter 6). The recent publication of keys to the Monogenea by Galli *et al.* (2010)

may assist in preliminary identification of species of *Dactylogyrus* in the UK, but molecular genetics are required for definitive identification.

Dawes (1947) considered *Dactylogyrus* species were absent from freshwater fish in the UK, therefore no reports pre-date this publication, but after this date and probably associated with improved optical equipment, records of infection began to emerge. One of the issues raised by Poulin *et al.* (2016) is that after the first published record for a parasite and its associated host, the number of records are a frequency dependent function, cumulative over time and that common parasites should be over-represented and if absent, the records are based on weak or incomplete data. The data extrapolated from *Aquatic Parasite Information* (Table 2.3) illustrates that over the last 69 years, the reported presence of *Dactylogyrus* species in the UK are exceedingly poor, which corroborates the view of Poulin *et al.* (2016) concerning weak or poor data but these authors do not take into account that identification of many common parasite species based on morphological characters, can be very challenging and may also result in misidentifications.

Table 2.3. *Dactylogyrus* species in the UK from 1947 - 2016, data extracted from *Aquatic Parasite Information*

Dactylogyrus species	Host	UK Records
D. amphibothrium	*Gymnocephalus cernuus*	4
D. anchoratus	*Carassius carassius*	1
	Cyprinus carpio	1
D. auriculatus	*Abramis brama*	1
D. cordus	*Leuciscus leuciscus*	2
D. crucifer	*Rutilus rutilus*	7
	Abramis brama	1
D. extensus	*Cyprinus carpio*	2
D. gobii	*Gobio gobio*	1
D. nanus	*Rutilus rutilus*	1
D. phoxini	*Phoxinus phoxinus*	1
D. prostae	*Squalius cephalus*	1
D. similis	*Rutilus rutilus*	3
D. sphyrna	*Rutilus rutilus*	9
D. suecicus	*Rutilus rutilus*	1
D. tuba	*Squalius cephalus*	1
D. vastator	*Cyprinus carpio*	1
D. vistulae	*Squalius cephalus*	3
D. wunderi	*Abramis brama*	1
D. tincae	*Tinca tinca*	1

Poulin *et al.* (2016) hypothesised that over time the number of records of common parasites would increase exponentially but when they analysed published data they found instead that common parasites were reported just once in parasite diversity surveys. The number of entries for *Dactylogyrus* species extracted from *Aquatic Parasite Information* records would appear to conform with Poulin *et al*. (2016) data analysis that common parasites are reported infrequently. However, the multiple records of common parasites such as, *A. foliaceus, D. spathaceum, A. lucii* and *P. geometra* extracted from *Aquatic Parasite Information* (Table 2.2) would seem to contradict the Poulin *et al.* (2016) hypothesis that common parasite species tend to be recorded only once. Large metazoan parasites such as *Acanthocephalus lucii* (Müller, 1776), *A. foliaceus, E. sieboldi,* and *P. geometra* tend to be readily identified

and the unicellular species *I. necator* and *I. multifilis* although microscopic, have highly characteristic features rendering them recognizable. The dactylogyrids are difficult and time intensive to identify using morphological characters and therefore are frequently only identified to genus. The dichotomy in the reporting of common fish parasites can therefore be explained by the ease with which species can be identified.

Information used to compile any checklist may owe its origin to a piece of research work based on a study of either a specific fish species or a parasite resulting in increased entries for host-parasite associations. For example, the European eel, *A. anguilla,* has 145 fish sample entries in *Aquatic Parasite Information*, however 82 of these entries are taken from two scientific studies, one based around Lough Erne, Northern Ireland and the second in East Kent. Whilst these records are an important inclusion, focussed research programmes may lead to incomplete distribution patterns for the fish and associated parasites. In addition, the conservation or economic status of a fish species will also influence the number of records of its parasite fauna. This is particularly significant for the European eel which is currently included in the International Union for the Conservation of Nature (IUCN) red list of threatened species as critically endangered (IUCN 2016). Under the Eel Management Plans (UK Government 2015) restrictions have been placed on the capture and movement of eels which may only be undertaken under licence, therefore information on the parasites of this species may become sparingly available.

The period for coarse fish translocation is relatively short, usually from October to March, when water temperatures are cooler, reducing the effect of stress on the fish but movements may be disrupted due to inclement weather, which leads to pressure

from industry for the rapid turn around of fish samples presented for examination by consultants. With speed of turn around being the essence, this results in many common parasites being identified to genus, or just family, with the main focus of routine fish health examination being absence or presence of Category 2 parasites. The presence of a Category 2 or an exotic parasite during routine fish health screening, results in termination of the process, as the population of fish from which the sample was taken then have a significantly deflated value. Where sampling is terminated the data on the incidence of these parasite infections is incomplete. Such is the sensitivity of the industry to Category 2 and exotic parasites that most fish suppliers are unwilling to provide locality data for infected sites, or will refuse permission for the data to be used. Government bodies such as Cefas and the Environment Agency are under no legal obligation to release data localities for sites affected by Category 2 parasites. The potential for such missing data concerning the distribution of parasites of freshwater fish in the UK will compromise the comprehensive basis of the database, but this issue is not unique to *Aquatic Parasite Information* as this is applicable to all checklists.

The study of fish parasitology has a long history and each technological advance has resulted in the realization that some species have been described on more than one occasion. Under the International Commission for Zoological Nomenclature (ICZN) rules, the species description which has priority is the one with the earliest publication date, usually post Linnaeus (1758), so all subsequent species names become synonyms. Poulin *et al.* (2016) note that nomenclatural changes impact on the reliability of a published checklist, but maintaining the valid name for a parasite species and the associated synonyms is readily tasked through *Aquatic Parasite Information*.

This data can be retrieved using the search tools, examples of which are shown in Chapter 3, Figures 3.1 & 3.2. The nomenclatural history of a parasite species is an important element of the taxonomy and forms an essential starting point for many research studies. The original description of a species forms the basis of many of these taxonomic revisions. *Aquatic Parasite Information* has facilitated access to an extremely useful resource for the authors and their references for all the UK parasite species. All taxa are subject to revision, especially with the advent of molecular studies which are rapidly changing established views of species and higher taxa. The versatility of *Aquatic Parasite Information* in enabling changes means all taxonomic data can be continuously updated.

Poulin *et al*. (2016) were concerned that many published checklists omit a time scale for the parasite records and view that it is significant to provide a date for the occurrence of a species as this indicates whether the checklist is both comprehensive and contemporary. A date and time line for the records of parasite species is useful evidential information in the distribution or even decline of a parasite species. For example, the digenean blood fluke, *S. inermis*, was first identified as an introduction into the UK in 1977 (Sweeting, 1979) after which it became quite widely dispersed in southern, eastern and central England (Kirk, 2012) throughout the 1980 to mid-1990's after which time numbers of carp infected with the parasite declined (Figure 2.2).

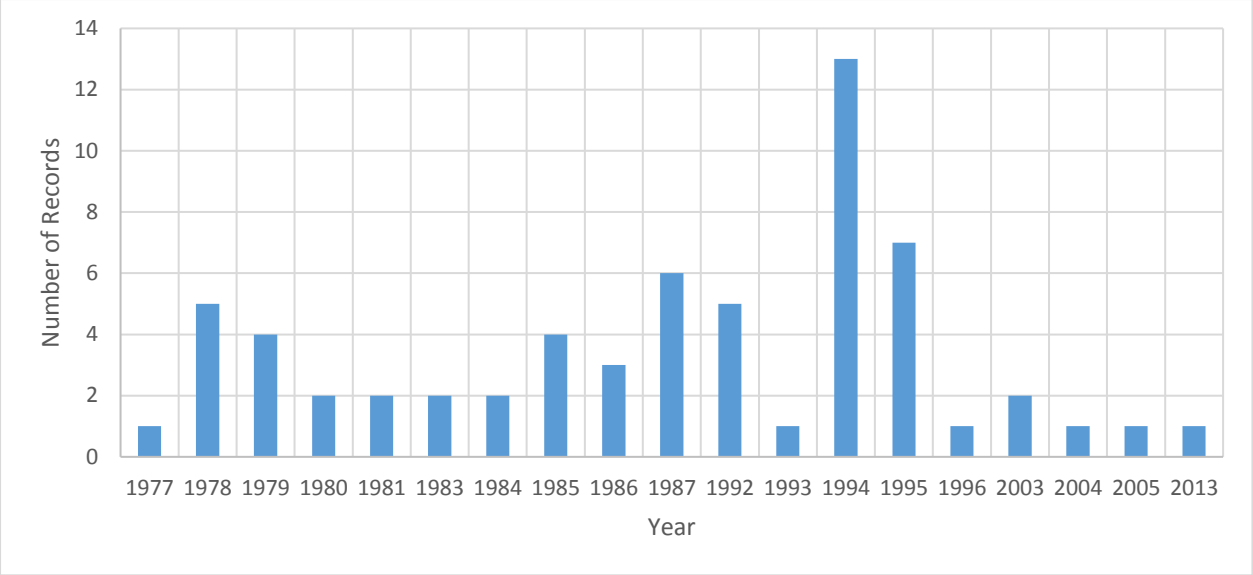

Figure 2.2. Annual records of carp infected with *Sanguinicola inermis*, extracted from *Aquatic Parasite Information*

Whilst the decline in records of *S. inermis* could be attributed to the completion of a research project, all varieties of common carp are the most sought after species for stocking angling clubs and commercial waters, which is reflected in the numbers of this fish submitted for routine health examination (pers. obs.). The decline in the number of annual records for *S. inermis* infecting carp is mirrored by the increasing trend of angling clubs, societies and commercial fisheries to heavily populate fishing venues with fish (Brewster, 2000; 2009; 2014). However, supplementary feeding is a poorly practiced aspect of fishery management resulting in malnourished and starving fish (Figure 2.3). The miracidia of the blood fluke infect lymnaeid snails, which emerge as cercaria to infect the fish. However, in these densely overpopulated fisheries, the starving fish consume all aquatic macroinvertebrates, including the snails, which in turn has probably reduced the incidence and distribution of the parasite.

Figure 2.3. Example of emaciated carp removed from an over-stocked fishery (Photograph: B. Brewster)

The data extrapolated from *Aquatic Parasite Information* indicates that the expansion and contraction of the blood fluke, *S. inermis,* have implications for current fishery management practices which have the potential to affect the freshwater fish parasite fauna. As an electronic database, therefore, potential changes occurring in the parasite fauna of freshwater fish can be monitored.

Concluding Remarks

Limitations in the data held in Aquatic Parasitic Information arise in the bias towards records for metazoan parasites, with unicellular species infrequently identified and with limited published records of their occurrence. Industrial sensitivity to the presence of Category 2 parasites means records for these species are incomplete. Nonetheless records included in the database enable the distribution of many parasite species to

be documented whilst the inclusion of a date associated with the parasite records enables the spread or decline of species to be tracked and documented. The spread and decline of parasite species appears to have a close relationship with both the translocation of fish and current fishery management practices. There is concern with regard to coarse fish welfare and whether these species are becoming a commodity in many angling clubs, societies and commercial fisheries (Brewster, 2014). Tracing the spread or decline of fish parasites through data entered in *Aquatic Parasite Information* has the potential to identify issues associated with fishery management policies of densely stocking with fish and the impact this may have on the parasitology, fish welfare and freshwater ecology of lakes. Consequently the *Aquatic Parasite Information* database has the capacity to become the most comprehensive source of parasite data associated with freshwater fish in the UK.

Chapter 3

Aquatic Parasite Information Database

Category 2 Parasite Distribution

3.1 Introduction

Monitoring the spread of any epizootic infecting fish is undertaken by the Organisation International Epizootique (OIE) an administrative body that recommends the regulation and control of those diseases which are regarded as emerging; of high pathogenicity to wild and cultured fish; or of economic significance to aquaculture and fisheries. Based on the advice of the OIE, the European Union regulates the control of fish disease under EU Council Directive 2006/88/EC (Europa Animal Health & Welfare, 2015) as List I, List II or List III diseases. Emerging diseases and diseases exotic to the EU are identified as List I and if identified as present, mandatory eradication measures are put into place. The diseases incorporated into List II are regarded as present in the EU but their distribution is limited and those affected areas are subject to control of the translocation of fish between infected and uninfected zones or countries. Lastly, those diseases included on List III are present in the EU but individual countries may apply for control programmes to eradicate them. List I – III diseases are 'notifiable', which means that if there is suspicion that one of these diseases is present, there is a legal requirement to notify the statutory body responsible, which in the UK is Cefas, who then undertake further investigative tests. During this investigation period an 'Initial Designation Notice' is placed on the site preventing any fish movements, if the disease is confirmed as present, a 'Confirmed Designation Notice' is imposed. Once the Confirmed Designation Order has been served either all stock is culled and the site is disinfected to the standard required by Cefas (Defra 2015) after which the Order is lifted, or if culling and disinfection is impractical, there is a mandatory, annual testing of the site until it has tested negative

for the disease for a minimum period of three years. These Lists include only one species of parasite, the skin fluke *Gyrodactylus salaris* Malmberg, 1957, which infects salmonids and is currently included on List III, however, it is considered to be absent from the UK (Paladini *et al.*, 2014).

Prior to 1989, any fish movements in the UK were undertaken by ten Regional Water Authorities, which had direct responsibility to Government but pre-dating the Salmon and Freshwater Fisheries Act 1975 (SAFFA), there were few restrictions on the movements of freshwater fish and health screening was minimal. Following the Water Act of 1989 (http://www.legislation.gov.uk/ukpga/1989/15/contents) the Water Authorities were privatized and responsibility for fresh and coastal waters was placed under the control of the newly established government body, The National Rivers Authority, which then implemented SAFFA, introduced consents for using an engine to catch fish, fish health examinations and movement consents. In 1996 the National Rivers Authority became a non-departmental public body, re-named the Environment Agency, with responsibility to the government through Defra. The Environment Agency has continued to implement all legislation concerning fish movements.

In England, the Environment Agency is the Government body responsible for coastal and fresh waters, in Wales this is the responsibility of Natural Resources Wales, with direct accountability to the National Assembly of Wales. Both agencies regulate fish movements under the previously discussed 'The Keeping and Introduction of Fish (England and River Esk Catchment Area) Regulations 2015 No. 10' (Environment Agency 2015), which requires health examination of fish being translocated to rivers, canals, lakes which are connected to the river catchment or are on a floodplain. In

Scotland the consenting for movement of fish is regulated by Marine Scotland, which is directly responsible to the Scottish Parliament, although the majority of freshwater fish stocked are salmonids with just four introductions of coarse fish in 2015 (Marine Scotland, 2015).

The Environment Agency groups all List I, II and III diseases as 'Category 1', the 'Category 2' diseases of freshwater fish are considered by the Agency to:

1) 'have a significant disease potential when introduced into waters where the disease or parasites do not already exist
2) be novel, non-indigenous diseases or parasites of unknown pathogenicity and distribution'

(Environment Agency 1999). Parasites currently regarded by the Environment Agency as Category 2 are given in Table 3.1. All freshwater fish translocations in England require authorisation by the Environment Agency and in Wales by Natural Resources Wales, under The Keeping and Introduction of Fish (England and River Esk Catchment Area) 2015. In accordance with this legislation, a sample of the fish scheduled for release into rivers, canals and lakes and which form part of a river catchment, or are situated on a flood plain, must be subject to routine health screening. If a Category 2 parasite is identified during the routine health screen, restrictions are placed on the movements of fish from the source site. Routine health screening is undertaken mostly by private individuals, plus some university parasitologists, but there is no legal requirement to notify the Environment Agency of the presence of any Category 2 parasite or the locality from which the fish sample originated.

Table 3.1. Category 2 parasites www.gov.uk/guidance/fish-health-checks

Significant disease potential	**Hosts**
Ergasilus sieboldi	Salmonids and coarse fish species
Ergasilus briani	Salmonids and coarse fish species
Ergasilus gibbus	European eel (*Anguilla anguilla*)
Pomphorhynchus laevis	Salmonids and riverine coarse fish species
Anguillicoloides crassus	European eel (*Anguilla anguilla*)
Monobothrium wageneri	Tench (*Tinca tinca*)
Schizocotyle acheilognathi	Mostly common carp (*Cyprinus carpio*) and carp variants
Novel parasites/disease	
Lernaea cyprinacea	Cyprinids
Pellucidhaptor pricei	Common bream (*Abramis brama*)
Philometroides sanguineus	Crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*)
Tracheliastes polycolpus & *T. maculatus*	Salmonids and coarse fish species
Lactococcus garvieae	Rainbow trout (*Oncorhynchus mykiss*)
Carp edema virus (CEV)	Common carp (*Cyprinus carpio*)
Herpesvirus anguillae (HVA)	European eel (*Anguilla anguilla*)

In the event of a novel or exotic parasite being recognised by the Environment Agency as present in the UK, it is automatically regarded as a Category 2 parasite to allow assessment of the pathogenicity.

The parasites or diseases included in the Environment Agency Category 2 are subject to periodic review. In 1995 the blood fluke, *S. inermis* was removed from the list by an internal review group, followed in 1997 by removal of the tapeworm *K. sinensis* from this list. In 2006 the Environment Agency convened a Category 2 Review Group

inviting academics and independent consultants to participate in revising the list, based on Williams (2007) impact assessment of non-native freshwater fish parasites. Williams (2007) introduced a risk assessment and matrix analysis for determining the status of non-native parasites introduced into the UK, based on the following criteria:

A) **Scoping process** - to evaluate whether the distribution of the parasite can be managed, using a 'Decision Tree' to assess the feasibility of management

B) **Hazard** – whether there is evidence of pathogenicity, or rapid dispersal in other countries, involving a 10 step questionnaire, focussing on three areas 1) the ecological and economic value of natural resources; 2) distribution potential of the parasite; 3) potential disease risk. Each question is given an individual score, which is then used to produce a total hazard score for any non-native parasite presents to fish populations in the UK

C) **Impact assessment** – what effect does the parasite have on both individual fish and populations of fish, using defined criteria and creating an impact matrix for each non-native parasite based on these standards, finally creating a risk assessment based on the impact matrix

D) **Risk management** – can the parasite dispersal be managed or controlled, based on the risk assessment devised from the impact matrix and creating six options: 1) Reliance on national control measures, that is notifiable disease status; 2) eradication for example, on importation at Border Inspection Point (BIP), or if the parasite is infecting fish within a restricted site where draining, culling and disinfection is feasible; 3) control measures are not implemented unless clinical disease is observed; 4) implementation of temporary movement control until the impact studies and risk assessment can be carried out; 5)

permanent movement restriction of fish imposed on susceptible or high risk fisheries; 6) permanent movement restrictions to all fisheries

On the basis of Williams (2007) risk assessment and matrix analysis work, the Category 2 Review Group approved the Environment Agency's removal of *Paraergasilus longidigitus* in 2007 followed by *Neoergasilus japonicus* and *Atractolytocestus huronensis*, in 2008. The Category 2 Review Group has not reconvened since 2008.

The first occurrence of non-indigenous fish parasites and their disease potential are usually the subject of publication but subsequent monitoring of their dispersal, spread, and establishment are lacking. The importance of Category 2 parasites and their potential impact on native fishes cannot be underestimated. Whilst the Environment Agency reduce the spread of non-indigenous parasites through movement control of infected fish, in recent years excessively heavy rainfall has resulted in many fisheries and ornamental ponds becoming flooded by adjacent rivers and streams, mixing captive and wild fish populations. *Aquatic Parasite Information* provides a readily accessible source of evidence for changes in the distribution and spread of Category 2 parasites and accession to data for detailed analysis. The data pertaining to those parasite species currently included on the Environment Agency, Category 2 list are the subject of interrogation of the records held on *Aquatic Parasite Information* and discussion of the information retrieved to evaluate the current status of Category 2 parasites in UK fish.

3.2 Methods

Data for each Category 2 parasite held in *Aquatic Parasite Information* was extracted using the parasite search engine (Figure 3.1) for records detailing the author of the species, reference to the original description, synonyms, hosts and UK distribution based on the British vice county recording schemes (www.brc.ac.uk).

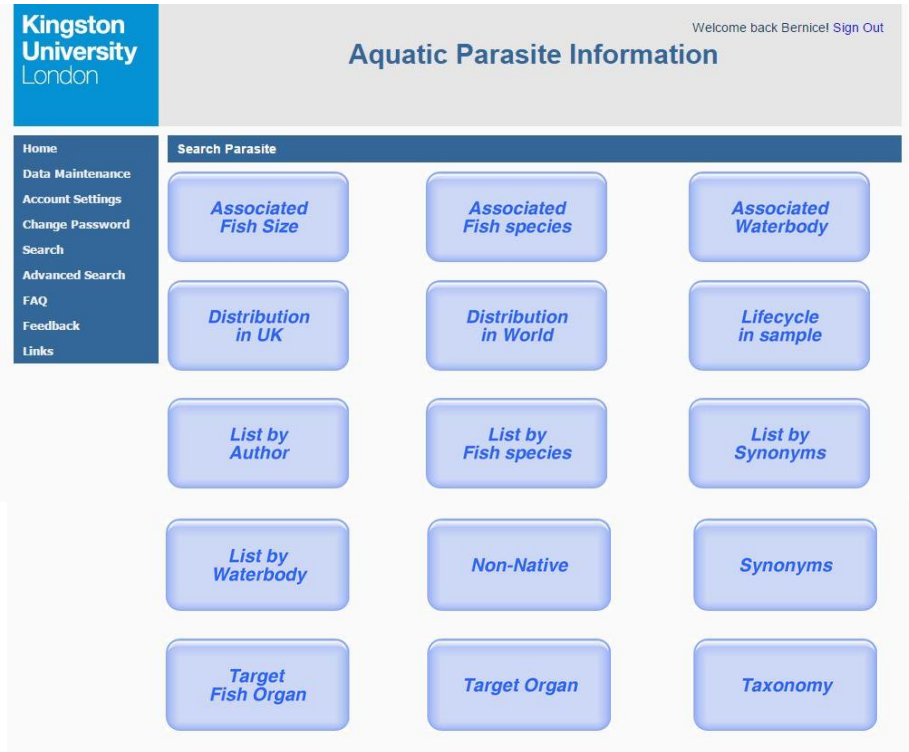

Figure 3.1 *Aquatic Parasite Information* parasite search engine offering multiple choices for mining information

The distribution of Category 2 parasites listed records the vice county in which the parasite has been identified but there may be multiple database entries for various localities within each vice county or, the record is for the same locality within a vice county but at different periods of time. Because of the industrial sensitivity and potential impact on revenue concerning sites infected with Category 2 parasites, *Aquatic Parasite Information* search engines present restricted distribution data, to protect the identity of affected fish farms, commercial fisheries and angling clubs.

Annual records and where relevant, numbers of fish infected with Category 2 parasites were accessed using the *Aquatic Parasite Information* advanced search engine (Figure 3.2). The number of records for *Ergasilus sieboldi* Nordmann 1832 and *E.briani* Markevich 1933 enabled a detailed analysis of fish hosts, prevalence and intensity and preference for host size, based on the work of Alston and Lewis (2003). Records for other Category 2 parasites were not as extensive as those for *E. sieboldi* and *E.briani.*

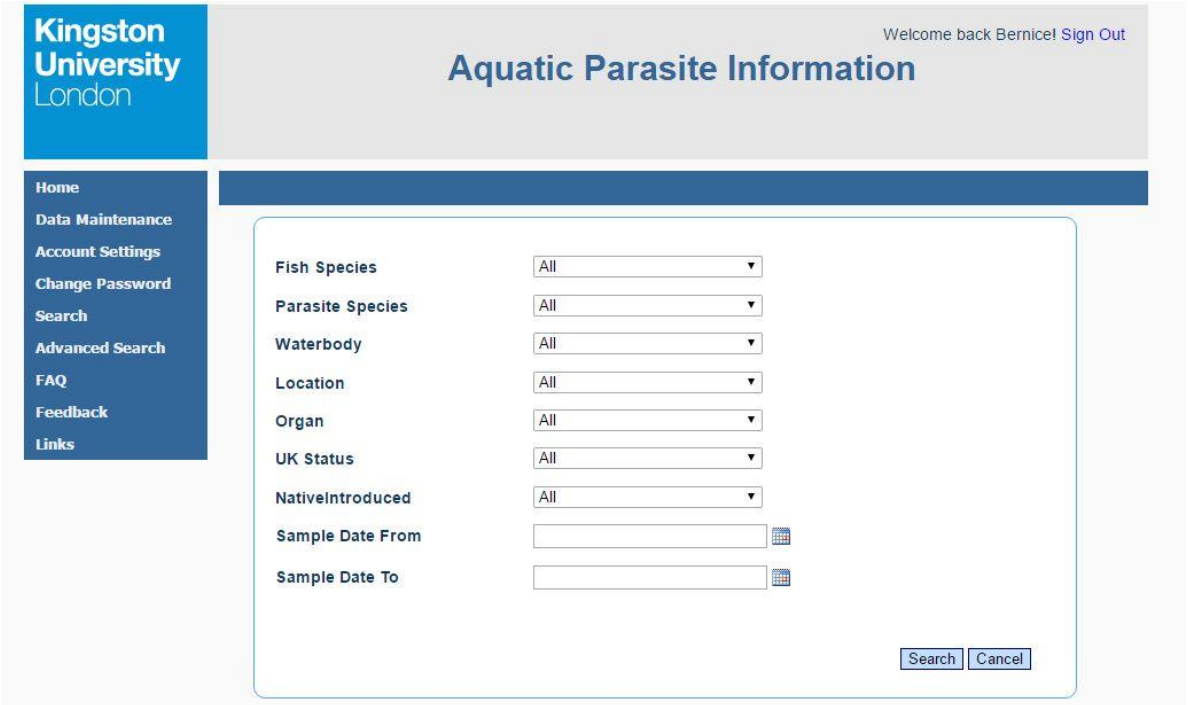

Figure 3.2 *Aquatic Parasite Information* advanced search engine, facilitating search for records in specific fields within the database

Data on the origin of Category 2 parasites was based on a literature search.

3.2 Results and discussion

The data for each of the extant Category 2 parasite species was obtained from *Aquatic Parasite Information* using the Search and Advanced Search engines, then downloaded for analysis.

Ergasilidae

The life cycle of the Ergasilidae is direct and generally the introduction of the parasites is through translocation of infected fish. However, a number of fisheries, which were previously uninfected, have tested positive for ergasilids after periods of flooding, when local rivers have inundated the lakes, allowing lotic and captive lentic fish species to mix and implying these crustacean parasites are present in some river catchments (pers. obs.).

***Ergasilus sieboldi* Nordmann 1832 (Copepoda: Ergasilidae)**

Reference: Nordmann, A. von, (1832) *Mikrographische Beiträge zur Naturgeschichte der wirbellosen Thiere. First Part*. (Berlin) 118pp

Synonyms: *Ergasilus baicalensis*; *E. esocis?; E. hoferi; E. surbecki; E. trisetacus*

Hosts: *Anguilla anguilla; Salmo trutta; Onchorhynchus mykiss; Abramis brama; Rutilus rutilus; A. brama x R. rutilus hybrids; Erythrophthalmus scardinius; Leuciscus; Tinca tinca; Cyprinus carpio; Carassius carassius; Gobio gobio; Squalius cephalus; Esox lucius; Perca fluviatilis; Barbatula barbatula* (*Aquatic Parasite Information*)

Distribution: Berkshire, Buckinghamshire, Cambridgeshire, Derbyshire, East Gloucestershire, East Suffolk, East Sussex, Flintshire, Hertfordshire, Huntingdonshire, London, Middlesex, Mid-west Yorkshire, North Essex, North Hampshire, North Lincolnshire, North-east Yorkshire, Nottinghamshire, South Essex, South-east Yorkshire, South-west Yorkshire, Staffordshire, Surrey, Warwickshire, West Kent, West Lancashire, West Sussex (*Aquatic Parasite Information*)

Origin: Non-native; native range, continental Europe (Kabata, 1979)

***Ergasilus briani* Markewitsch 1933 (Copepoda: Ergasilidae)**

Reference: *Bulletin de l'Institute Ocèanographique de Monaco* no. 638: 1 – 27

Synonyms: *E. minor*

Hosts: *Rutilus rutilus; Abramis brama; Scardinius erythrophthalmus; Tinca tinca; Gobio gobio; Leuciscus leuciscus; Carassius carassius; Cyprinus carpio; C. carassius x Cyprinus carpio; Perca fluviatilis;* (*Aquatic Parasite Information*)

Distribution: Berkshire, Buckinghamshire, North Hampshire, North Lincolnshire, Northamptonshire, Nottinghamshire, Oxfordshire, South Essex, South Hampshire, South Lancashire, South Lincolnshire, South Somerset, South-west Yorkshire, Surrey, West Kent, West Suffolk (*Aquatic Parasite Information*)

Origin: Non-native; native range Eurasia (Kabata, 2003)

Ergasilus sieboldi was first identified in the UK in 1967 (Fryer 1969), a time when there were few restrictions on the movement of freshwater fish, with coarse fish angling predominantly based on the rivers and canals, the reservoirs being preferred for 'put and take' trout fishing, where game fish anglers take two fish for consumption and the remainder are returned to the water. Kabata (1979) notes the initial record for *E. sieboldi* was on the gills of a dead brown trout (*S. trutta*) but the fish was free of infection when introduced to Howbrook Reservoir and this author makes the deduction the gill parasite was already present in some numbers at this location and in the River Don catchment. *Ergasilus briani* was first recorded by Fryer (1982) and Fryer & Andrews (1983) in the UK, infecting bream. This species is smaller than *E. sieboldi* and morphologically very similar to *Neoergasilus japonicus*, the two being differentiated by a spine on the basal segment of the antenna and specialized structure of the first leg in the latter (Fryer, 1982, Kabata 2003).

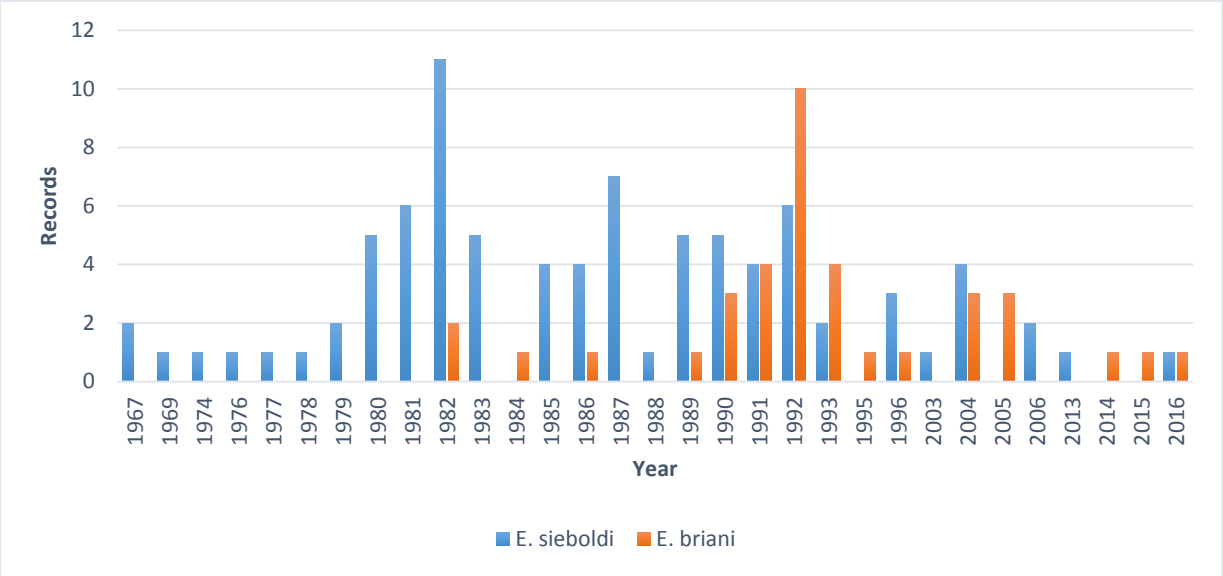

Figure 3.3. *Aquatic Parasite Information* annual records 1967 – 2016, for *Ergasilus sieboldi* and *E. briani*

Annual records for *E. sieboldi* and *E. briani* extracted from *Aquatic Parasite Information* are illustrated in Figure 3.3, the annual entries for both species for 1989 – 1993 originate from Alston's 1994 study of these ergasilids, all other records are from Regional Water Authority and independent fish health examination records. Williams (2007) and Alston & Lewis (1994) stated that both species of *Ergasilus* are euryxenous as shown by the host records extracted from *Aquatic Parasite Information* (Figure 3.4).

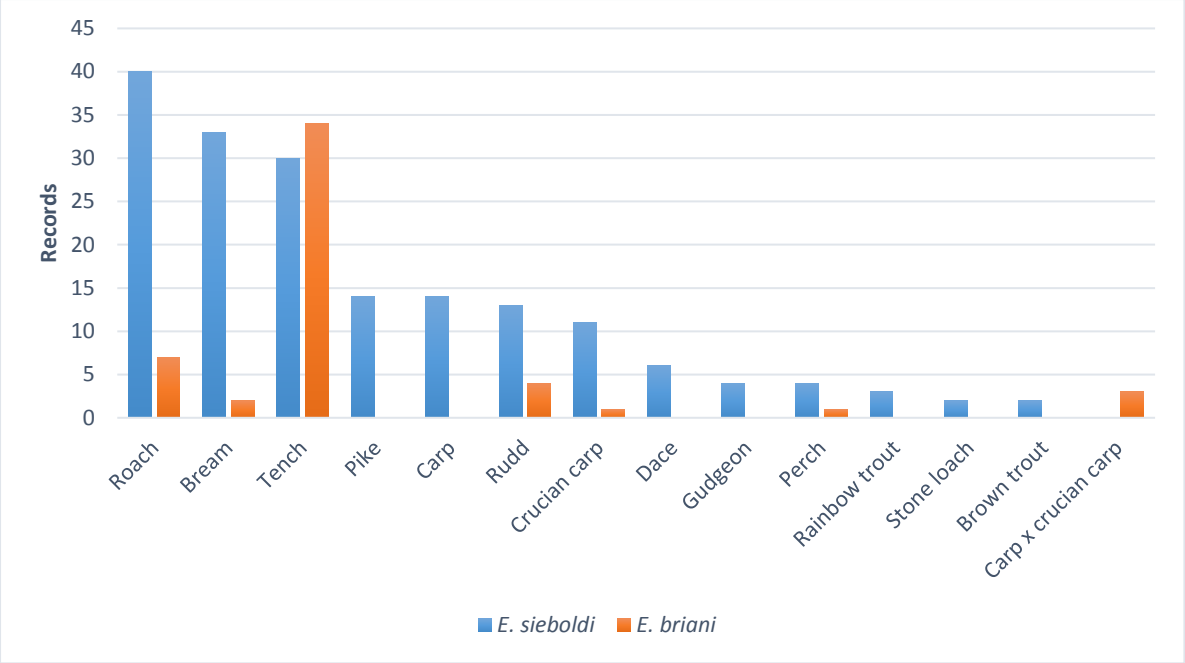

Figure 3.4 *Ergasilus sieboldi* and *Ergasilus briani* host records from *Aquatic Parasite Information*

Many data sources exclude details of the numbers of parasites present and how many fish were examined, more recent entries from independent sources provide this information but the number of records are limited. Data from sites stocked with coarse fish infected with *E. sieboldi* and *E. briani,* were extracted from *Aquatic Parasite Information* to compare the prevalence and mean intensity of infection.

Commercial sensitivity concerning Category 2 parasites restricts identification of the following fisheries infected with *E. sieboldi,* results are given in Figures 3.5 and 3.6:

Site A is a managed, mixed coarse fishery, over stocked with pike and bream

Site B mixed coarse fishery

Site C Convent lake, destocked to allow for re-development of the site

Site D mixed coarse fishery but stocked in excess of 1,000 kg per ha

Site E is a reservoir, with water quality issues arising from the large population of bream on the site.

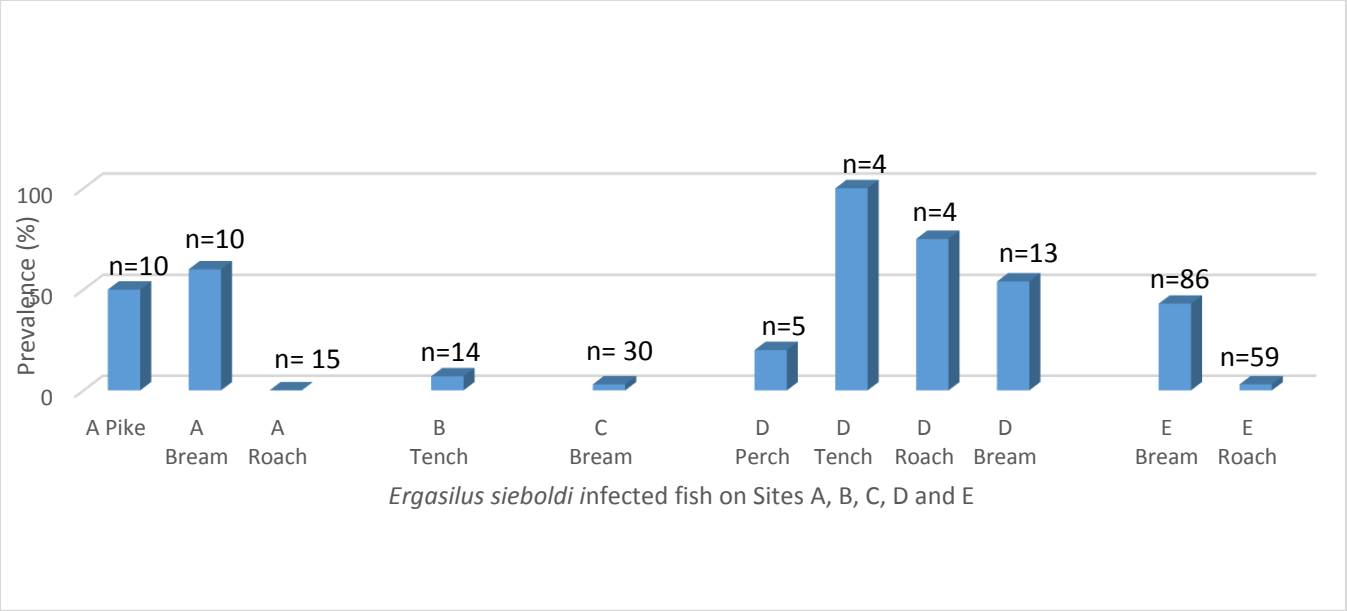

Figure 3.5 Prevalence of infection with *Ergasilus sieboldi* sites A to E, mixed coarse fisheries and Site E a reservoir, from *Aquatic Parasite Information* (n= the number of fish species present in the sample)

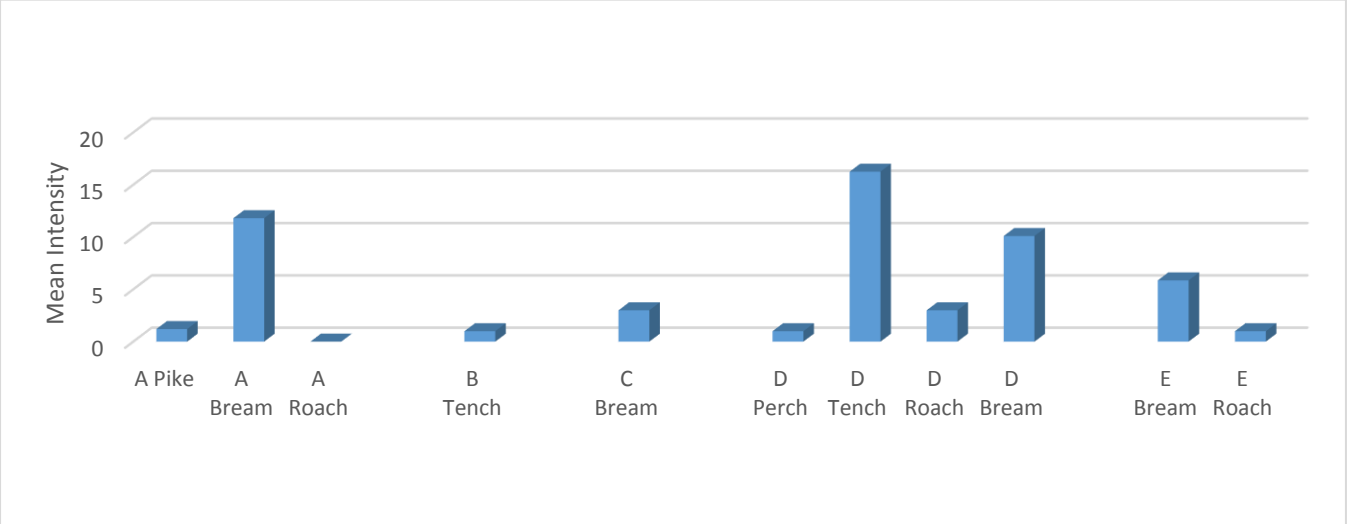

Figure 3.6 Mean intensity of *Ergasilus sieboldi* sites A to D, mixed coarse fisheries and Site E a reservoir, data from *Aquatic Parasite Information*

Brewster (2000; 2009; 2014) has previously expressed concern over the welfare of coarse fish on densely stocked fisheries. The data extracted from *Aquatic Parasite Information* (Figures 3.5 and 3.6) for those fisheries which have high stock levels show an increased prevalence and intensity of infection with *E. sieboldi* than fisheries with

moderate stocks. In the confines of a lake with large populations of fish, there is a greater probability of fish coming into contact with *E. sieboldi* and increasing the parasite population density, through greater host availability than in fisheries which contain a moderate population of fish.

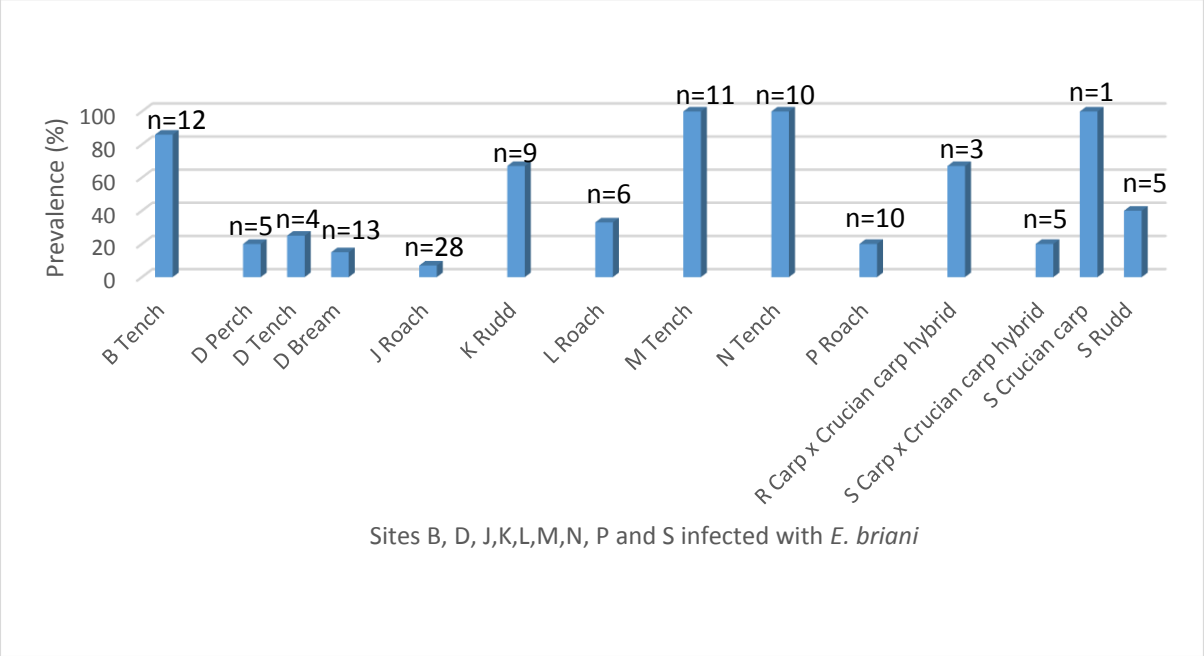

Figure 3.7 Prevalence of *Ergasilus briani* infection from sites B, D, J, K, L, M, N, P, R, and S mixed coarse fisheries, data from *Aquatic Parasite Information* (n= the number of fish in the sample)

The prevalence and intensity infection with *E. briani* on ten sites is given in Figures 3.7 and 3.8, sites B & D were also infected with *E. sieboldi*. The prevalence of *E. briani* infection on site B is greater than that of *E. sieboldi* whereas the prevalence of *E. briani* is less on site D than that of *E. sieboldi*, implying other factors influence the intensity of infection on these fisheries.

Site B mixed coarse fishery

Site D mixed coarse fishery but stocked in excess of 1,000 kg per ha

Site J managed mixed coarse fishery

Site K reservoir

Site L reservoir

Site M Fish farm

Site N Coarse fishery stocked in excess of 1,000kg per ha

Site P Coarse fishery stocked in excess of 1,000kg per ha

Site S Little Wake Pond, Epping Forest

Site J is exceptional, it is well managed and the populations of mixed coarse fish are routinely subject to de-stocking of small roach, a practice which would seem to reduce the prevalence of *E. briani.* Fish removed from this source water are moved to another water where this parasite also occurs (*pers. obs.).* Site K is termed a reservoir, although it is a redundant gravel pit with an area of approximately 1ha, which was transformed into a wildlife reserve in 2004 (www.writtle.ac.uk), the rudd were cropped in 2015 because the population of these fish had become excessive. The second reservoir, site L, is a potable water source, the site was free of any Category 2 parasites until the local area was subject to flooding in 2014 (pers. obs.). The origin of the fish from site M was given as a fish farm, however this given source is dubious. Sites N and P are fisheries with stock density in excess of 1,000kg per ha, lastly sites R and S are located in Epping Forest, both were small ponds, with a large, population of assorted species of fish and sampled as part of a survey of the waters in the Forest on behalf of the Corporation of London (pers. obs.). Those sites which support large populations of fish suggest there is a greater probability of fish coming into contact with *E. briani* and increasing the parasite population density.

In most instances, routine health examination of fish samples are curtailed if a fish is found to be infected with either *E. briani* or *E. sieboldi,* as the presence of a Category 2 parasite significantly reduces the commercial value of the stock. Hence, the total numbers of fish examined from samples identified with one of these parasites, tend to be low, which is reflected in Figures 3.5 & 3.7.

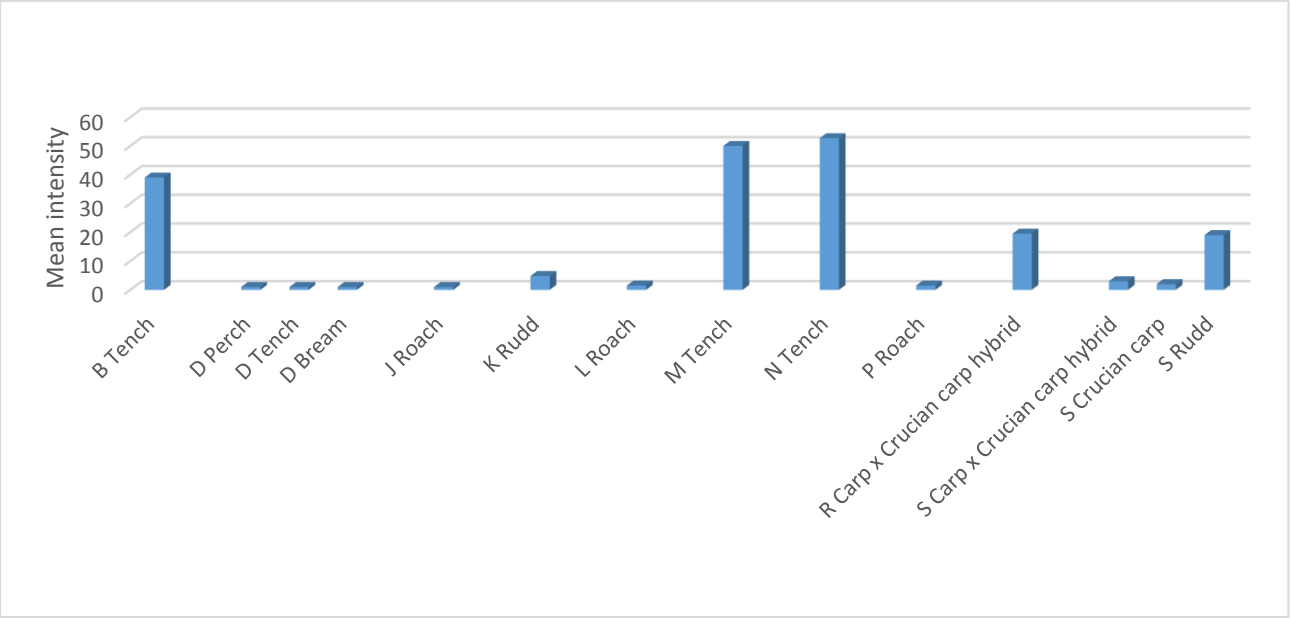

Figure 3.8 Mean intensity of *Ergasilus briani* on sites B, D, J, K, L, M, N, P, R, and S mixed coarse fisheries, data from *Aquatic Parasite Information*

The mean intensity of infection with *E. briani* would appear to suggest that tench are the preferred host for this parasite but comparison of the data for the prevalence of *E. sieboldi* and *E. briani* in fisheries B and D where these species are sympatric, shows conflicting results (Figures 3.6 & 3.8). With the exception of site D, in Figure 3.8, the mean intensity of *E. briani* is between 38 – 50 associated with tench, whereas for other species of fish, the mean intensity of infection varies between 1 – 18, from which it could be inferred that tench were the preferred host. Examination of the prevalence of infection in those fisheries where the two species are sympatric Figure 3.9, shows that on site B there is a greater prevalence of *E. briani* on tench, on site D the prevalence of both species is equal in association with perch but there is a greater

prevalence of *E. sieboldi* on tench and bream and no *E. briani* were found on the roach. If tench was the preferred host of *E. briani*, then a similar prevalence of infection on tench, bream and perch at site D would not be expected.

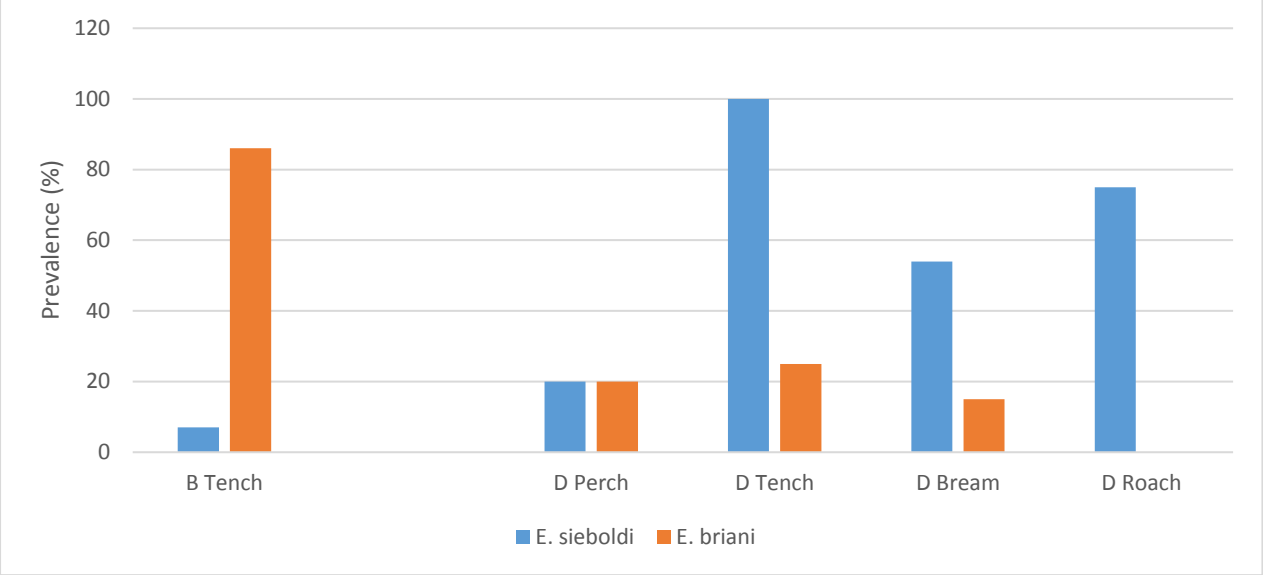

Figure 3.9 Prevalence of *Ergasilus sieboldi* and *Ergasilus briani* on sites B & D, data from *Aquatic Parasite Information*

Alston & Lewis (1994) indicated that host size affects susceptibility to infection finding *E. briani* most prevalent on bream of 8 cm fork length and *E. sieboldi* was more prevalent on fish greater than 16cm fork length. The size records in *Aquatic Parasite Information* are grouped according to the Environment Agency requirements for fish health examination, which are <5cm for fry; 5 – 14.99cm; 15 – 25cm and >25cm fork length. According to the database records *E. briani* occurs on fish species up to 25cm and *E. sieboldi* on fish species up to 70cm fork lengths. Comparison of the intensity of infection with *E. briani* and *E. sieboldi* host size is illustrated in Figure 3.10, showing *E. briani* has a greater affinity for small tench of 15 – 24cm, with just one specimen of *E. sieboldi* on a tench in this size range.

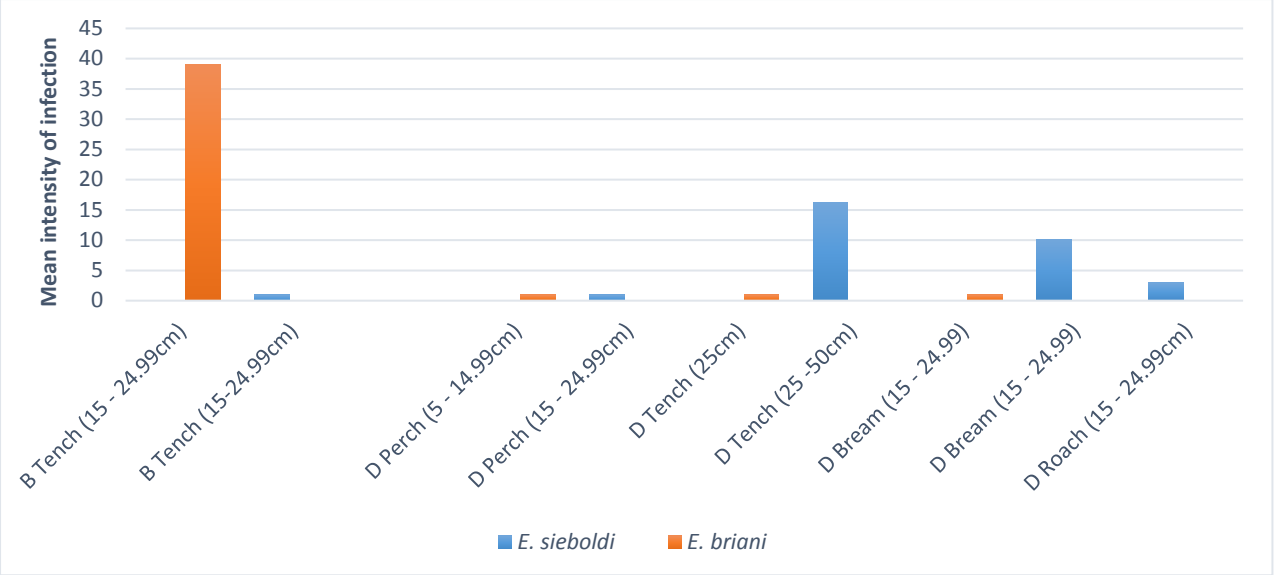

Figure 3.10 Mean intensity of infection of *Ergasilus sieboldi* and *Ergasilus briani* in association with host size on sites B & D, data from *Aquatic Parasite Information*

In site D, *E. briani* was associated with a perch of 5 – 14.99cm whereas *E. sieboldi* was found on the larger perch and showed a similar preference for the larger tench, however, all the bream in this sample were 15 – 24.99cm, which is within the preferred fish size range for *E. sieboldi.* Alston & Lewis (1994) postulated that *E. briani* may have difficulty attaching to the gills of larger fish, but these provide a bigger target for *E. sieboldi,* citing Gnadeberg (1948) and Abdelhalim (1990) who indicated that susceptibility to infection is dependent on primary and secondary lamellar size for effective attachment.

Ergasilus sieboldi is usually found attached to the external surface of the primary gill lamellae, where it causes injury through attachment to the tissues with the scimitar like first antennae and by browsing on the gill epithelium (Alston & Lewis 1994). On the basis of risk assessment and matrix analysis of this pathogenicity, environmental tolerance, range of water bodies and hosts Williams (2007) demonstrated that *E.sieboldi* continues to be a high risk parasite, retaining it on the Category 2 list.

Williams (2007) studied the pathological changes to the gill tissue associated with *E. briani* and concluded that injury arose from damage inflicted to the lamellae by the antennae and compression due to the body of the parasite pressing against the tissues, feeding induced haemorrhaging, erosion and compression of the epithelium. These pathologies were more evident in the smallest fish. Using the matrix analysis Williams (2007) concluded that *E. briani* posed little economical or ecological risk, however, this parasite is currently retained on the Environment Agency Category 2 list.

***Ergasilus gibbus* Nordmann 1832(Copepoda: Ergasilidae)**

Reference: Nordmann, A. von, (1832) *Mikrographische Beiträge zur Naturgeschichte der wirbellosen Thiere. First Part*. (Berlin) 118pp

Synonyms: None

Hosts: *Anguilla anguilla;* uncorroborated *Leuciscus leuciscus* (*Aquatic Parasite Information*)

Distribution: Cambridgeshire, Fermanagh, Mid-west Yorkshire, North Lincolnshire, North Somerset, South Devon, South Hampshire, West Suffolk (*Aquatic Parasite Information*)

Origin: North Sea and Baltic **(**Kabata, 2003)

Ergasilus gibbus has traditionally been regarded as a parasite of eels from the North Sea and Baltic coasts (Kabata, 1979) however, the first record for this ergasilid in the UK was from South Devon between 1966 – 1971, although later reported in 1973 (Canning *et al.* 1973). Kearn (2004) considers *E. gibbus* to be exclusively brackish, whereas McCarthy *et al.* (2009) found it to be a specialist parasite on the gills of European eels in Ireland. McCarthy *et al.* (2009) found *E. gibbus* infecting European eels in 10 out of the 19 freshwater rivers in studied suggesting the distribution is not

widespread. Annual records for *E. gibbus* extracted from *Aquatic Parasite Information* suggest this ergasilid has been infrequently identified infecting European eels in the UK (Figure 3.11). McCarthy *et al.* (2009) found the incidence of infection with *E. gibbus* was associated with larger eels which showed a preference for deeper water and suggested these were older fish, incurring a progressive accumulation of parasites.

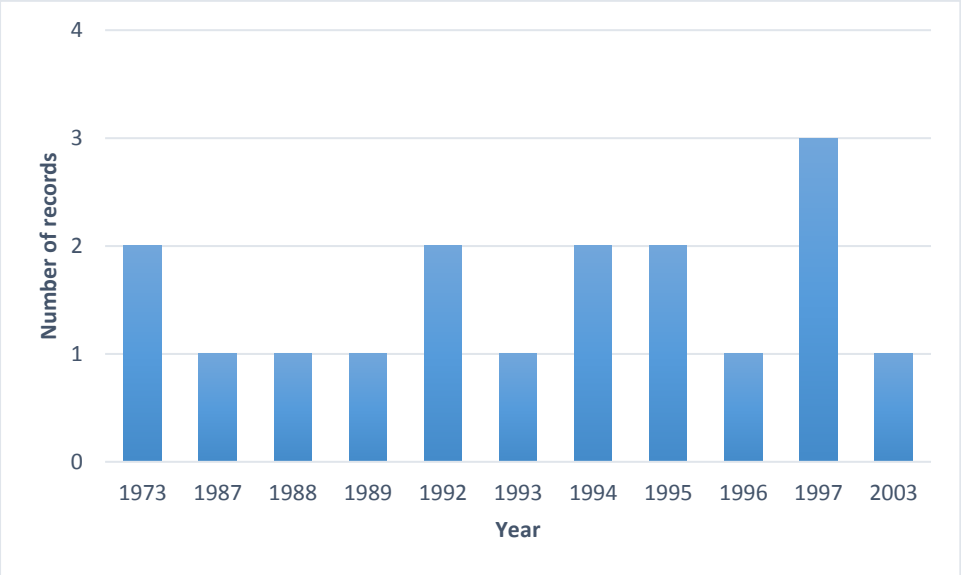

Figure 3.11 Records for the occurrence of *Ergasilus gibbus* 1973 – 2003, data from *Aquatic Parasite Information*

Saraiva (1996) recorded damage to the host gill tissue, associated with penetration of the antennae into the lamellae, with associated necrosis and hyperplasia, although the figures might suggest compression injury. Of note, although this author considered the ergasilids to be *E. gibbus*, there were differences in the 4th swimming leg which lead to uncertainty regarding the identification of the specimens.

Under the Eel Management Plans implemented in compliance with EC Regulation no. 11/2007, following the serious decline in populations of European eel, this species is

protected and further records for *E. gibbus* may only be received through research projects on the host species.

***Pomphorhynchus laevis (Zoega, 1776)* (Palaeacanthacephala; Pomphorhynchidae)**

Reference: Zoega in Müller, O. F. 1776 *Zoologiae Danicae prodromus, seu animalium Daniae et Norvegiae indigenarum characters, nomina, et synonyma imprimis popularium.* Havniae XXXII

Synonyms: *Echinorhynchus proteus*

Hosts: *Salmo salar; Salmo trutta; Onchorhynchus mykiss; Barbus barbus; Squalius cephalus; Leuciscus leuciscus; Rutilus rutilus; Gobio gobio; Cyprinus carpio; Gymnocephalus cernuus; Perca fluviatilis; Esox Lucius; Thymallus thymallus; Amblopytes rupestris; Barbatula barbatula; Phoxinus phoxinus; Anguilla anguilla; Alburnus alburnus; Abramis brama; Gasterosteus aculeatus; Platichthys flesus* (*Aquatic Parasite Information*)

Distribution: Argyllshire, Berkshire, Dorset, East Gloucestershire, East Kent, Herefordshire, Hertfordshire, London, Mid Perthshire, Middlesex, Montgomeryshire, North Devon, North Ebudes, North Hampshire, North Wiltshire, Oxfordshire, Shropshire (Salop), South Devon, South Essex, South Hampshire, West Invernesshire, West Ross & Cromarty (*Aquatic Parasite Information*)

Origin: Native

Popularly termed the 'yellow peril' by anglers because of the distinctive colour, *Pomphorhynchus laevis* is one of the Category 2 parasites occurring predominantly in lotic fish but also in the estuarine flounder, *Platichthys flesus*. The *Aquatic Parasite Information* records show a widespread distribution (Appendix 3) and occurrence of *P. laevis* (Figure 3.12). The increase in the number of records for the years 1988, 1993 and 1994 are due to the addition of data taken from published research projects (Lyndon & Kennedy, 2001; MacKenzie. 2002). Despite the number of records, *P. laevis* has a fragmented and localised distribution in the UK. This localised distribution has been hypothesised as due to the post glacial colonization of fish from mainland Europe, giving rise to a marine strain which colonized the Baltic and North Sea and estuaries of the latter (Kennedy *et al.*, 1989). The intermediate host of *P. laevis*

infecting barbel is a ubiquitous species of freshwater shrimp *Gammarus pulex* this host-parasite relationship is considered to be a relic of the post-glacial breakup of the Thames-Rhine basin. The subsequent fragmented distribution of *P. laevis* in the UK is a consequence of anthropogenic movements of infected barbel (Kennedy, 2006). Whilst many freshwater fish species will feed on *G. pulex*, the acanthocephalan *P. laevis* only becomes sexually mature in the preferred definitive hosts which are barbel, chub and rainbow trout (Brown *et al*., 1986; Kennedy, 2006) with brown trout a suitable host. In the absence of a preferred host, *P. laevis* will infect a variety of fish species but does not attain sexual maturity (Kennedy 2006). Kennedy (2006) also notes the presence of three strains of *P. laevis*, a marine strain which infects flounder, a freshwater strain and a third strain present in Ireland.

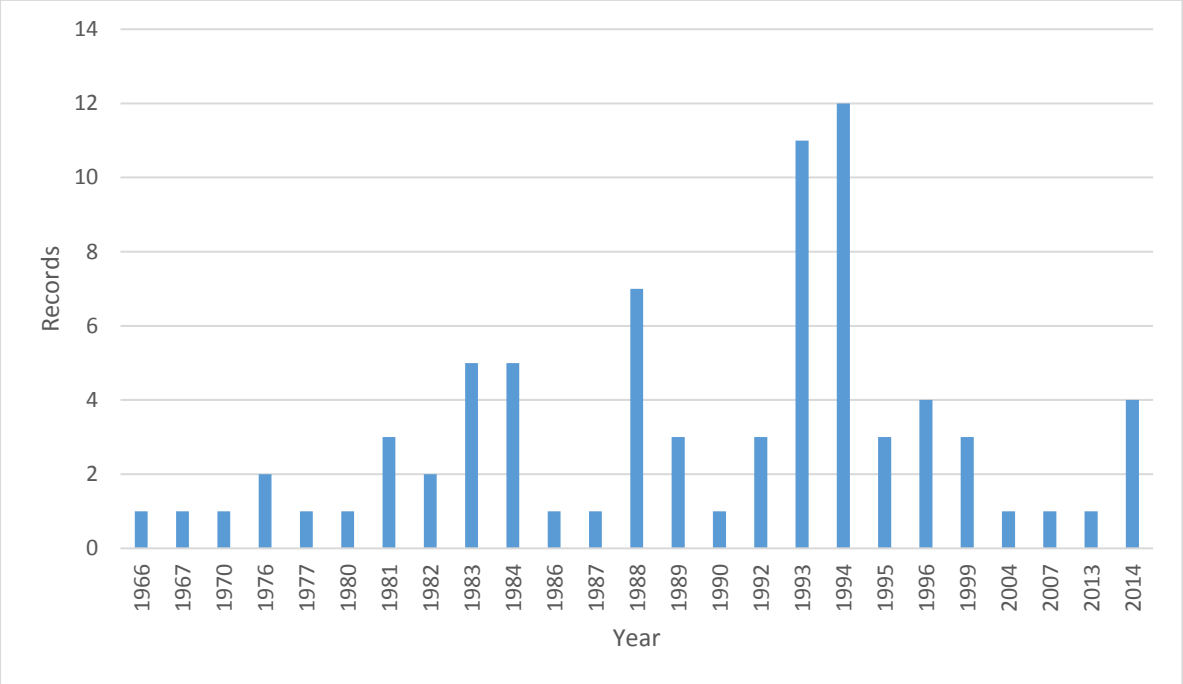

Figure 3.12 Records for *Pomphorhynchus laevis* in the UK from 1966 – 2014, data from *Aquatic Parasite Information*

According to Schäperclaus (1992), the proboscis of *P. laevis* penetrates the stratum compactum of the anterior fish intestine and posterior intestine, the sub-mucosa and may perforate other tissues such as the liver and pancreas.

***Anguillicoloides crassus* (Kuwahara, Niimi & Itagaki, 1974) (Dracunculoidea; Anguillicolidae**

Reference: Kuwahara, A. Niimi, A. & Itagaki, H. Studies of a nematode parasitic in the air bladder of the eel. 1. Description of *Anguillicola crassa* n. sp. (Philometridea; Anguillicolidae) *Japanese Journal of Parasitology* **23** (5): 275 – 279

Synonyms: None

Hosts: *Anguilla japonica* (natural host); *Anguilla anguilla* (*Aquatic Parasite Information*)

Paratenic hosts: *Gymnocephalus cernuus; Alburnus alburnus* (Pegg *et al.*, 2015*) Gasterosteus aculeatus* (R. Kirk pers. com.)

Distribution: Berkshire, Cambridgeshire, Dorset, East Sussex, East Kent, Hertfordshire, Fermanagh, East Kent, London, Mid-west and South Yorkshire, North and South Somerset, North and South Devon, South Essex, North Essex, South Hampshire, Surrey, North and South Lincolnshire, Mid-Perthshire, Glamorgan, Lancashire, Cumbria, Cheshire (*Aquatic Parasite Information*)

Origin: Non-native. Epidemiological history, Japan; original source of *A. crassus* as East Asia is open to debate (Lefebvre *et al.*, 2012[1])

The nematode *Anguillicoloides crassus* is an exotic parasite infecting the swimbladder of European eels, accidentally introduced to Europe in the 1980s with infected Japanese eel, *Anguilla japonica*, imported for either human consumption or re-stocking (De Charleroy *et al.*, 1990; Kirk 2003). The initial distribution of *A. crassus* in the UK was identified in 1987 as East Anglia, the Rivers Welland and Trent and the Thames near Tower Bridge matching the routes of infection with live eel movements in the UK (Kennedy & Fitch, 1990), although Kirk *et al.* (2002) have also suggested that infection may be transmitted by marine eels. Ab Aziz *et al.* (2012) have examined over 500 European eels from 27 river systems in England and Wales and have found the distribution of *A. crassus* to be widespread, as reflected by *Aquatic Parasite*

Information records. The life cycle of *A. crassus* is indirect, eels are infected by consuming copepods or ostracods containing J^3 larvae, or feeding on paratenic hosts, infected with the larval stage.

The number of records for *A. crassus* extracted from *Aquatic Parasite Information* for the years 1987 - 2016, is given in Figure 3.13. The records for the years 1998 to 2000 are data from Evans & Matthews (1999) and Evans, Matthews & McClintock (2001) research data on the spread of *A. crassus* through the Erne system in Northern Ireland, identifying a prevalence of infection of 9.9% and mean intensity of 6.7 of the European eels examined.

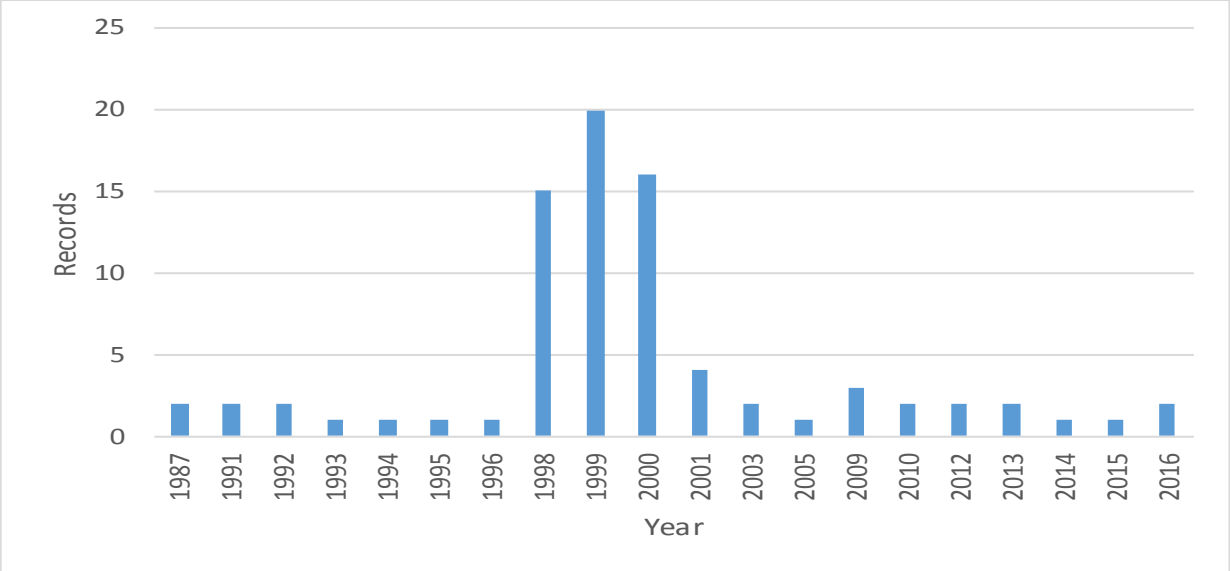

Figure 3.13 Records for *Anguillicoloides crassus* in the UK, data from *Aquatic Parasite Information*

In 2008 under EU Regulation No.1100/2007/EC, United Kingdom, Eel Management Plans (EMP) were introduced to ensure a minimum of 40% of the silver eel population can migrate in the absence of any anthropogenic interference, under these regulations and incorporating the European eel into the Wildlife and Countryside Act (1981), affords this species protection and requiring special permissions to take them from the

wild. European eels have rarely formed part of any routine fish health examination, recent data on the distribution of *A. crassus* is either from published research, or the Environment Agency have requested an examination prior to capture of land locked eels and their release into river systems in England.

The original source of *A. crassus* has been considered to be Japan and East Asia (e.g. Ashworth, 1994, Kirk, 2003) recently Lefebvre *et al.* (2012[1]) have questioned the authenticity of this geographical region as the native origin of this parasite as this area has a long history of intercontinental trade in all species of live eels. These authors are suggesting that *A. crassus* may have been an introduced species to East Asia, proposing a detailed study of the molecular genetics to resolve the phylogeography of the species (Lefebvre *et al.*, 2012[1])

Following ingestion, the J3 and J4 larvae migrate through the intestine and swimbladder causing tunnel like perforations and causing lesions as they feed in the pneumatic duct, *rete mirabile* and swimbladder (Lefebvre, Fazio & Crivelli, 2012). Adult and pre-adult *A. crassus* suck blood from the capillaries in the wall of the swimbladder where repeated feeding causes the formation of fibrous tissue and degeneration of the swimbladder and in heavy infections leading to the collapse and rupture of this organ (Lefebvre, Fazio & Crivelli, 2012).

Although *A. crassus* is included on the Environment Agency list of category 2 parasites, which restricts the movement of infected fish, the migratory nature of the European eel has contributed to the wide dissemination of this parasite in the UK.

Anguillicoloides crassus is also ubiquitous within the European eel populations of European countries (Lefebvre *et al.* 2012).

***Monobothrium wageneri* Nybelin 1922 (Caryophylidea; Caryophyllaeidae)**

Reference: Nybelin, O. 1922 Anatomische-systematische Studien über Pseudophyllideen *Kungliga Vetenskaps- och Vitterhets-Samhället i Göteburg Handlingar* **26**: 1 - 228

Synonyms: None

Hosts: *Tinca tinca* (*Aquatic Parasite Information*)

Origin: Non-native, first reported from Arno River, Pisa, Italy, later records are all from Eastern Europe (Gibson, 1993)

Table 3.2. *Aquatic Parasite Information* records of *Monobothrium wageneri* in the UK

Date	Reference	Location
01-01-98	NFL1998	London
01-01-98	NFL 8/98	London
07-07-92	1st record BMNH1992.7.24.1	London
27-05-92	1st record BMNH1992.7.12.1-2	Surrey
21-05-92	1st Record BMNH1992.6.5.7	Berkshire
01-02-92	1st record BMNH1992.6.5.3-6	North Hampshire

There are six entries in *Aquatic Parasite Information* for *Monobothrium wageneri,* four are taken from Gibson's (1993) initial published report of this exotic parasite of tench in the UK (Table 3.2). However, Williams *et al.* (2011) record it as also present in 10 stillwater fisheries in London and the south east, four in the midlands and one in Wales, localities are excluded from the publication on the basis of confidentiality. Kolar & Lodge (2001) predict biological invasions take place as a three step transition process of introduction, establishment and invasion. The distribution of this tapeworm suggests

the first of these criteria has been met but there are no further entries for this parasite in API, although there are 28 entries for parasites associated with tench, of which 19 are from the south east, in areas where this cestode was first identified. Once identified the National Rivers Authority (now the Environment Agency) placed *M. wageneri* on the Category 2 schedule and it is possible that imposed restrictions on the movement of all fish species from the affected sites has restricted the spread of the parasite. Scholz[2] *et al.* (2015) report that *M. wageneri* is a rare parasite, host specific for tench with a fragmented distribution in the Palaearctic.

During the 1990's many commercial fisheries and angling clubs began to increase the density of fish stocks in their lakes and still waters, with carp the preferred species, guaranteeing every angler a successful fishing session (Marlow, 1996; Wildgoose 1999), a continuing trend with 484,997 Environment Agency consented carp movements, compared with 49,370 consented movements for tench between January 2014 – January 2015 (Environment Agency 2015). Large populations of carp are detrimental to tench (Leonard, 2001; *pers. obs.*) which has led to an overall decline in tench numbers in the UK. Concomitant with the introduction of the exotic *M. wageneri*, carp were being excessively stocked on most fisheries, affecting tench populations and removing host availability. *Monobothrium wageneri* has an indirect life cycle, the intermediate host is an oligochaete worm, readily consumed by carp. The successful infection of any host requires the parasite to reach maturity and reproduce, Scholtz *et al.* (2012) refer to three host types and maturation of the parasite:

'required hosts', a definitive host, in which the parasite matures and reproduces

'suitable hosts' the parasite can attain sexual maturity but is usually present in low numbers

'unsuitable hosts' the parasite may infect the host but cannot mature

Monobothrium wageneri may have failed to establish because of the declining populations of the required host, tench and this cestode appears to be a specialist parasite which cannot establish in any other fish species.

Williams *et al.* (2011) describe *M. wageneri* as a significant pathogen of tench, as the scolex is deeply buried in the intestine and coupled with the host inflammatory response allows this cestode to form a strong attachment, with local haemorrhaging and occlusion of the intestine and justifying maintenance of this tapeworm on the Category 2 list.

***Schizocotyle acheilognathi* Yamaguti 1934 (Bothriocephalidea: Bothriocephalidae**

Reference: Yamaguti, S. 1934 *Japanese Journal of Zoology* **6**: 1 - 120

Synonyms: Originally described as *Bothriocephalus acheilognathi*

Hosts: *Cyprinus carpio, Ctenopharyngodon idella, Rutilus rutilus* (*Aquatic Parasite Information*)

Distribution: Berkshire, Buckinghamshire, Dorset, East Gloucestershire, East Norfolk, East Suffolk, East Sussex, Essex, Hertfordshire, Lincolnshire, London, Middlesex, North Essex, North Hampshire, Northamptonshire, Oxfordshire, Shropshire (Salop), South Devon, South Essex, South Hampshire, South Wiltshire, Surrey, West Gloucestershire, West Kent, West Norfolk, Yorkshire (*Aquatic Parasite Information*)

Origin: Non-native, East Asia (Scholz *et al.* 2012)

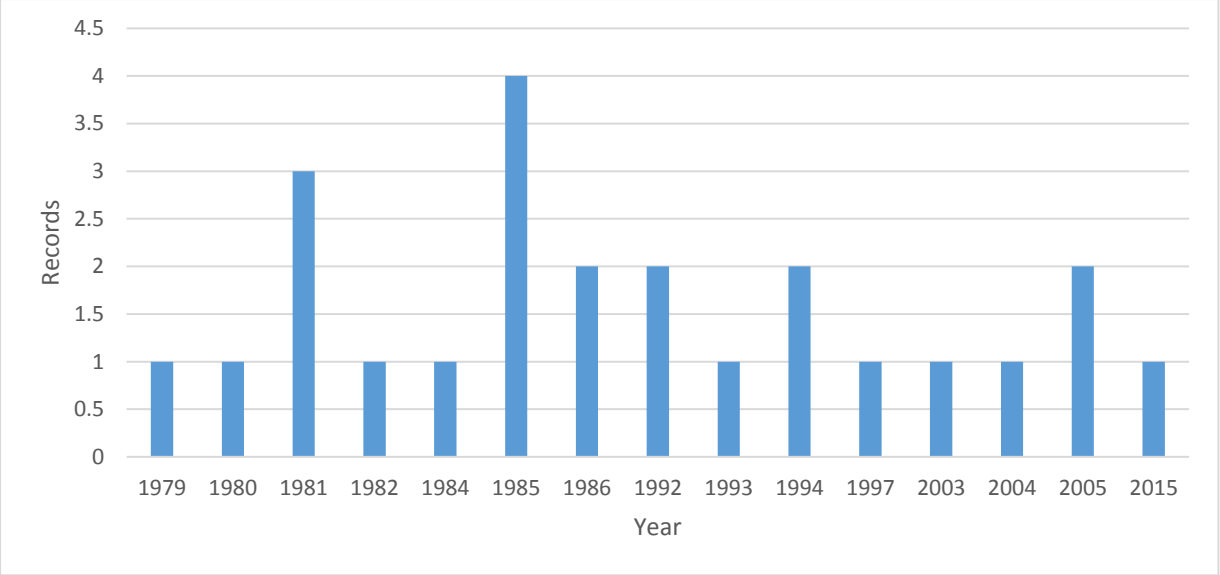

Figure 3.14 Annual records for *Schizocotyle acheilognathi,* data from *Aquatic Parasite Information*

Schizocotyle acheilognathi is native to China and Japan and hence its popular epithet the 'Asian tapeworm' and was most likely introduced to this country through importation of grass carp from China and ornamental carp, known as 'koi' from Japan for the ornamental trade. Andrews *et al.* (1981) published the first records of the occurrence of *S. acheilognathi* in Lincolnshire, Yorkshire and Essex, although *Aquatic Parasite Information* holds data from Thames Water Authority for the presence of this cestode infecting grass carp in Essex in 1979. Carp and grass carp are the preferred hosts for *S. acheilognathi*, but it will also infect other cyprinid species, Scholtz *et al.* (2012) indicate this tapeworm has been identified as parasitizing in excess of 200 species of fish from a number of orders and families.

The record of *S. acheilognathi* associated with roach appears to indicate this cyprinid is an unsuitable host as the cestodes were malformed and immature (pers. obs.; J.C. Chubb pers. com.). However, most recently Košuthova *et al.* (2015) reported this

species as both mature and causing mortalities in discus (*Symphysodon discus*). It is therefore quite surprising that *S. acheilognathi* was unable to establish in roach.

Schizocotyle acheilognathi is an important pathogen causing mortalities in fry and juvenile fish, their increased susceptibility most likely due to feeding on copepods, which are the intermediate host for this cestode. The intestinal lumen of the fish is generally occluded by both the intensity of infection and injury through attachment of the scolex, causing loss of the mucosa and an inflammatory response. Infection with *S. acheilognathi* affects the growth and condition of the fish (Britten *et al.*, 2011). Whilst *S. acheilognathi* causes significant pathology in carp fry and juveniles, this cestode can also be found infecting carp of between 1 – 5 kg (pers. obs.) possibly related to fish stock densities and food availability. The lumen of the intestine in these larger carp does not become occluded by *S. acheilognathi*, however infection with this cestode has been demonstrated to affect carbohydrate and protein metabolism and reduced enzyme activity (Scholtz *et al.*, 2012).

The records of *S. acheilognathi* in the *Aquatic Parasite Information* database are given in Figure 3.14 which appears to show a sporadic occurrence of this parasite, however this tapeworm has been disseminated to every continent except Antarctica (Britton *et al*., 2011) and would seem to be very successful establishing in novel hosts, as well as the preferred hosts. *Schizocotyle acheilognathi* is a very distinctive parasite, with a heart shaped scolex (Figure 3.15) and presence of proglottids, which may have restricted the distribution as it is so readily identifiable. The annual records for the UK, (Figure 3.14) would suggest this ease of identification assists in regulating the dispersal of this non-native parasite.

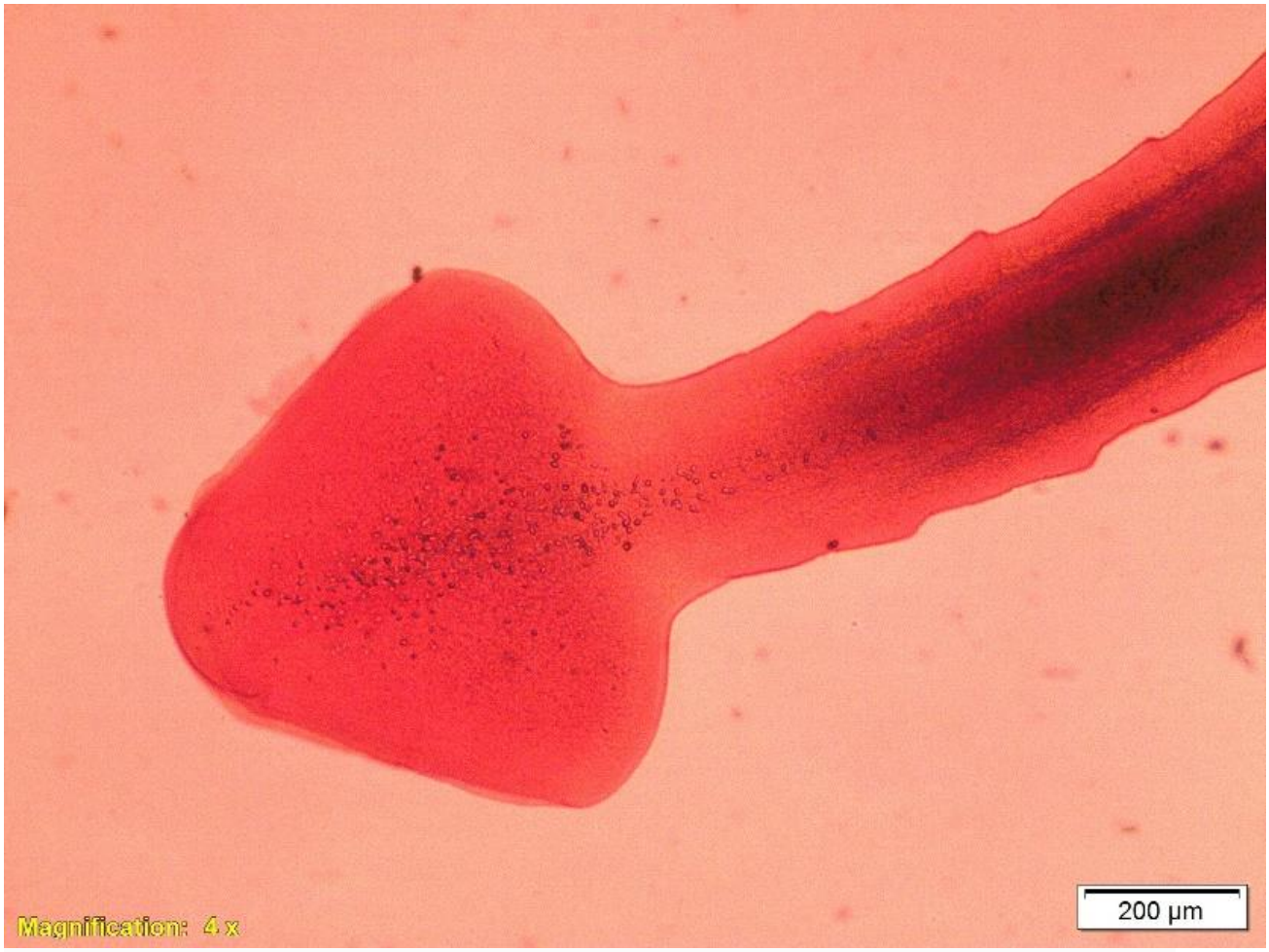

Figure 3.15 Characteristic scolex of *Schizocotyle acheilognathi (*Photograph B. Brewster)

***Lernaea cyprinacea* L. 1758 (Cyclopoidea; Lerneidae)**

Reference: Linnaeus, C. 1758 *Systema Naturae*

Synonyms: *Lernaeocera cyprinacea; Lernaeocera esocina; Lernaeocera gasterostei; Lernaea ranae; Lernaea carassii; ?Lernaea chackoensis*

Hosts: *Cyprinus carpio; Carassius auratus; Rutilus rutilus; Abramis brama; Leuciscus idus; Gasterosteus aculeatus* (API; Kabata, 2003)

Origin: Non-native, Eurasia (Kabata, 2003)

Table 3.3 *Aquatic Parasite Information* records of *Lernaea cyprinacea* in the UK

Date	Reference	Location
03-03-15	500/2015	West Sussex
01-01-74	Kennedy, C. 20	South Essex
01-01-74	Kennedy, C. 146	South Devon
01-01-74	Kennedy, C. 148	South Essex
01-08-67	Fryer, G 1st record L. cyprinacea (d)	Pembrokeshire
31-07-66	Fryer, G 1st record L. cyprinacea (b)	London
31-07-66	Fryer, G 1st record L. cyprinacea (a)	South Essex
31-07-66	Fryer, G 1st record L. cyprinacea (c)	Surrey

There are only a limited number of records for *L. cyprinacea* in the *Aquatic Parasite Information* database (Table 3.3). *Lernaea cyprinacea* is popularly termed 'anchor worm' as the first antennae form the attachment organ, which is shaped rather like a four pronged anchor that is embedded subcutaneously in the host. The Environment Agency regards *L. cyprinacea* as a novel parasite as it has a limited distribution in the UK. Hoole *et al.* (2001) describe *L. cyprinacea* as having a worldwide distribution, infecting more than 40 cyprinid species and other freshwater fish including salmonids, catfish and eels. According to Fryer (1982), the life cycle of *L. cyprinacea* is affected by temperature, taking five weeks to complete at 22^0C but between 5 – 6 months at 12^0C, below this temperature, reproduction ceases. In the localities where *L. cyprinacea* has been recorded it is possible the water temperatures do not reach an optimum for this crustacean parasite to become established in the UK.

Although the first copepodid stage is free-living, the following stages 2 – 5 are parasitic on the gills of the host, females mate at the copepodid stage 5, before finding a suitable

location on the host and metamorphosing to the adult (Fryer, 1982). The female *L. cyprinacea* pierces the skin and the attachment organ forms within the muscle and tissues, holding the anchor worm in place while enabling the parasite to feed on the surrounding muscle and blood, which leads to necrosis of the host tissues, often resulting in secondary infections and septicaemia (Hoole *et al.* 2001).

The free living stage of *L. cyprinacea* renders the parasite difficult to control once a site has become infected and coupled with the pathology associated with infection are reasons the Environment Agency retains this species on the Category 2 list.

***Pellucidhaptor pricei* Gussev & Strizhak 1972 (Monogenoidea; Dactylogyridae)**

Reference: Gussev, A. V. & Strizhak, O.I. 1972 *Parazitologiya* **6** (6): 555 - 557

Synonyms: None

Hosts: *Abramis brama* (API; Gussev 2010)

Origin: Non-native, Volga River, Russia; Lake Nevezhis, Lithuania (Gussev *et al*. 2010)

Table 3.4. *Aquatic Parasite Information* records of *Pellucidhaptor pricei* in the UK

Date	Reference	Location
01-01-05	EA 1	Leicestershire (with Rutland)
01-01-05	EA 2	West Sussex
24-08-03	1846 - 1995/2003	Middlesex

Pellucidhaptor pricei is host specific for common bream and the distribution is extremely localized (Table 3.4). In the UK, *P. pricei* has only been rarely located in the lateral line canal but in the original description, Gussev *et al.* (2010) found this parasite located in the nasal rosette. The Environment Agency, National Fisheries

Laboratory have been sampling the nasal rosette of bream routinely and it has yet to be found in this organ (C. Williams pers. com). Gussev (2010) regarded *P. pricei* as a very rare parasite but during routine screening of fish for movement consent, the standard practice is to remove and examine 12 lateral line scales and it is feasible this monogenean does have a wider distribution in the UK, which is being overlooked. On those sites where bream are known to be hosts, this monogenean can prove to be elusive and detection of *P. pricei* is achieved by the removal and examination of all the lateral line scales (pers. obs).

Very little is known about the ecology, life cycle, or pathogenicity, of *P. pricei*.

***Philometroides sanguineus* (Rudolphi, 1819) (Dracunculoidea; Philometridae)**

Reference: Entozoorum Synopsis cui Accedunt Mantesia Duplex et Indices Locupletissimi. Berolini. 811 pp

Synonyms: *Philometroides carassii; Philometra sanguinea; Philometra trilabiata*

Hosts: *Carassius carassius, Scardinius erythrophthalmus* (API; Andrews & Chubb, 1983)

Origin: Non-native, Sweden, Germany, Poland, Czechoslovakia, Hungary and countries of the former USSR, in addition to Asia (Moravec, 1971)

Table 3.5. Aquatic Parasite Iinformation records of *Philometroides sanguineus* in the UK

Date	Reference	Location
23-01-13	ACS 2013	Surrey
01-05-83	Andrews, C & Chubb, J.C. 1st record P. sanguinea	South-west Yorkshire

This nematode parasite is generally regarded as host specific for crucian carp and goldfish, however, Andrews & Chubb (1984) first record of *Philometroides sanguineus*

in the UK was identified as affecting rudd, in addition to crucian carp. Moravec (1971) doubts the authenticity of records of numerous cyprinid species as host to this parasite, considering them more likely to be either *Philometra ovata* or *P. abdominalis*. Although there are just two records currently held in *Aquatic Parasite Information*, Pegg *et al.* (2011) refer to infected crucian carp from five lakes in England and Williams *et al*. (2012) refer to eight stillwaters where *P. sanguineus* has been identified but locality details for all these sites have been withheld from publication in the interests of confidentiality (Table 3.5).

After the fish has ingested infected copepodids, *P. sanguineus* migrate through the intestinal wall to the serosa of the swimbladder, where the juveniles will mature and mate, after which the gravid females migrate through the body usually to the caudal fin (Schäperclaus, 1992). According to Schäperclaus (1992), the life cycle takes approximately a year, a more recent study by Williams *et al.* (2012) found mature male *P. sanguineus* on the kidney and serosal surface of the swimbladder throughout the year with females present from May to October. In the UK, the females have been found in the fins of crucian carp from September to May (Williams *et al.*, 2012) with the caudal fin the preferred site. In addition to the injuries and secondary infections affecting the fins, Schäperclaus (1992) described fish infected with *P. sanguineus* as having pathology associated with the swimbladder. Williams *et al.* (2012) found the gravid females caused distortion of affected fins, together with inflammation and swelling but notably the damage was influenced by the host size, with small fish suffering significant injury.

The native crucian carp is already under threat through habitat loss and hybridization with both goldfish (*Carassius auratus*) and common carp (Bolton *et al.* 1998; Wheeler, 2000) so is currently subject to conservation effort in regional Biodiversity Action Plans (Pegg *et al.*, 2011). Although the distribution of *P. sanguineus* would appear to be very limited, it is clear this parasite represents an additional threat to native crucian carp in the UK. *Philometroides sanguineus* is believed to have been introduced to the UK, with the importation and accidental release of infected ornamental goldfish.

Moravec (1971) records *P. sanguineus* from Sweden, Germany, Poland, Czechoslovakia, Hungary and countries of the former USSR, in addition to Asia.

***Tracheliastes* species (Maxillipoda: Lernaeopodidae)**

***Tracheliastes polycolpus* von Nordmann 1832**

Reference: Nordmann, A. von 1832 *Mikrographische Beiträge zur Naturgeschichte der wirbellosen Thiere. Heft* 2 I-XVIII 1 – 150 Reimer, Berlin

Synonyms: None

Hosts: *Leuciscus idus; Leuciscus leuciscus; Squalius cephalus* (*Aquatic Parasite Information*)

Origin: Non-native, Eurasia (Kabata, 2003)

Table 3.6. *Aquatic Parasite Information* records of *Tracheliastes polycolpus*

Date	Reference	Location
01-01-75	NHM 1975	North-east Yorkshire
01-01-65	Kennedy, C. 168	South-east Yorkshire
01-01-65	Kennedy, C. 170	North-east Yorkshire
01-01-64	Aubrook & Fryer 1st record non-captive fishE	North-east Yorkshire
01-01-63	Aubrook & Fryer 1st record non-captive fishB	Mid-west Yorkshire
01-01-63	Aubrook & Fryer 1st record non-captive fishF	North-east Yorkshire
01-01-62	Aubrook & Fryer 1st record non-captive fishD	North-east Yorkshire
01-01-61	Aubrook & Fryer 1st record non-captive fishA	Mid-west Yorkshire
01-01-61	Aubrook & Fryer 1st record non-captive fishC	South-east Yorkshire
01-01-33	Gurney, R 1st Record	Midlothian (Edinburgh)

***Tracheliastes maculatus* Kollar 1835**

Reference: Kollar, V. 1835 *Annals Wiener Museum* **1**: 81 - 92

Synonyms: *Tracheliastes fecundus*

Hosts: *Abramis brama* (API)

Origin: non-native, northern Europe and Eurasia (Fauna Europaea www.fauneur.org)

Table 3.7. *Aquatic Parasite Information* Record of *Tracheliastes maculatus*

Date	Reference	Location
17-01-90	Boxshall, G & Frear, A 1st record	Lancashire

The first record for *Tracheliastes polycolpus* is from ide in Edinburgh Zoo (Gurney, 1933), there are only three other records for this parasite in *Aquatic Parasite Information* from the Rivers Rye, Ouse and Derwent in 1965, where it was found on

the fins of dace and chub (Table 3.6). The only data entry for *T. maculatus* is from Lancashire, which is the first record of this species in the UK (Boxshall & Frear 1990) (Table 3.7). *Tracheliastes* species are generalist parasites whose hosts are cyprinids, notably dace, chub and bream (Environment Agency, 2007). Only the female is parasitic in both species, the bulla forms a firm attachment, usually to the pectoral, anal and dorsal fins, preferring the external surface of the fins, caudal and dorsal fins are apparently uninfected, injury is caused to the tissues through the rasping method of feeding (Loot *et al.* 2004*)*.

Tracheliastes species are found on freshwater fish in Eurasia and the specimens found in the UK are presumed to be accidental introductions through translocation of ornamental species. It would appear these species of parasite have been unable to establish in the UK.

Gyrodactylus salaris

Under current legislation, *Gyrodactylus salaris* is a List III disease, which is notifiable, indicating a legal obligation to notify the Government regulatory body Cefas, if there is a suspected incidence of the parasite, currently the UK is regarded as free of *G. salaris*, with an approved control and eradication programme for gyrodactylosis under EU Commission Decision 2004/453/EC. *Gyrodactylus salaris* is an alien introduction to the Atlantic region, being native to the Karelian part of Russia, Baltic areas of Finland and Sweden and is considered to have been introduced to Norway in the 1970's being found on a west coast farm on Atlantic salmon *Salmo salar* parr in 1975 and shortly after in the River Lakselva causing high mortalities (Mo, 1994; Olstad, 2013). Steinkjer (2013) considers *G. salaris* represents a major threat to Atlantic

salmon, as it is present in exceptionally high numbers on the fish, feeding on the epithelium. In the Norwegian rivers infection with *G. salaris* reduces the salmon parr density by 86% and adult salmon catch by 87% costing the Norwegian Government approximately 9 million €, annually in control measures (Steinkjer, 2013).

Kennedy's 1974 checklist included a record of *Gyrodactylus salaris* from brown trout in Loch Leven, Fifeshire and Kinross, a highly pathogenic, alien monogenean parasite, considered to have been introduced to Norway in 1975, although it had been previously identified on a Danish rainbow trout farm in 1972 (Olstad, 2013). The majority of introductions of *G. salaris* are a consequence of anthropogenic translocations of infected fish and given the first identification of *G. salaris* in Norway was in 1975, after the publication of Kennedy's checklist, the included Loch Leven record would appear to be dubious. Although migration of infected wild fish into freshwaters may occur, it seems unlikely that infected wild Atlantic salmon would have entered Scottish freshwaters at this time. Whilst it can neither be proven, nor disproven, that *G. salaris* was present in Loch Leven in the early 1970's, the record is doubtful and was not entered into *Aquatic Parasite Information*.

Anthropogenic mediated routes for introduction of invasive parasites

The distribution of parasite species is limited by the geographic span of the hosts, which have a preferred range associated with evolution, ecology and climate, leading to regional biotas, which creates our perception of native species and their associated parasites (Sax *et al.,* 2005). Improved methods of transportation of live fish have resulted in an increase of anthropogenic translocation of non-native species, from

Asia, South Africa, Israel and North America, primarily for the coldwater ornamental industry. Coarse fish, notably common carp, catfish and bream have been imported both legitimately and illegally, exotic tilapia and barramundi have been imported for aquaculture and more recently 'The Doctor Fish', *G. rufa*, was briefly fashionable in the beauty industry, imported as ornamental fish. Whilst legitimately imported fish for the ornamental industry and aquaculture must be from an approved source, certified to be free of List 1 and list 2 diseases (Aquatic Animal Health Guidance, 2014) there is no requirement to examine these fish for parasites. Aquaculture species are regarded as low risk imports as theoretically, they do not come into contact with native species (Aquatic Animal Imports Guide 2014). The recent fashion for the use of *G. rufa* in beauty therapy is a case in point, management of the fish was the responsibility of staff disinterested in the welfare of the fish and sick fish were released into open waters (J. Collins pers. com.). Whilst the source of the fish for this trade was the Far East (Wildgoose, 2012) it may be presumed that *G. rufa* released into the wild would not survive but similar releases of exotic fish such as *Leucaspius delineatus*, *Lepomis gibbosus* and *Pseudorasbora parva* have established breeding populations in the UK. The topmouth gudgeon, *P. parva* having introduced *Sphaerothecum destruens* a parasite which infects native cyprinids (Andreou *et al.* 2011).

Coldwater ornamental species are released either accidentally, such as during periods of flooding, or intentionally, either through ignorance on the part of the owner, seeking to give pets 'a better life' or fish which have outgrown aquaria and some anglers who willingly stock these imported fish (pers. obs). These ornamental fish have the potential to introduce a number of non-native parasites to naïve, native species of fish.

The market for large carp, exceeding 9kg has resulted in the illegal importation of coarse fish which are released into fisheries, or sold as 'English' fish for re-stocking purposes, providing the avenues for the release of non-native parasite species. Whilst anthropogenic fish translocations provide one mechanism for the dispersal of invasive, non-native parasites, Anderson *et al.* (2014) conducted an on-line survey, circulating a questionnaire regarding biosecurity and hygiene to 52 angling clubs, completed by 960 anglers. The survey revealed that 12% of the anglers neither cleaned or dried equipment between venues, which gives rise for the potential for the Category 2 parasites with free living stages of their life cycle, such as ergasilids, lernaeids and lernaeopodids, or even copepodids infected with procercoids of *Schizocotyle acheilognathi,* to be translocated on fishing kit. Significantly, Anderson *et al*. (2014) identified that 34% of the anglers questioned used live fish bait, mostly comprising roach, rudd, minnows, gudgeon and perch, whilst some of these baitfish were used on the same site from which they were caught, others used the bait fish at alternative venues and disturbingly, 7% of the anglers questioned, released the unused livebait into a different lake or river from the source in which they were originally caught.

It would appear there are multiple opportunities for non-native, invasive parasites to be translocated within the UK, through various unauthorized fish movements. Fisheries management practices operated by both angling clubs and commercial ventures can influence the ability of non-native parasites to become established or fail to establish within naïve fish hosts. High fish stock densities, of between 2.5 – 3,000kg per hectare (Brewster, 2014) are acceptable to many fishery managers, supplementary feeding is rarely offered, consequently, hungry fish tend to feed on fish eggs, larvae, fry, juvenile fish and aquatic macroinvertebrates, disturbing the ecology

allowing copepodids and other small zooplankton, including ergasilids to thrive coupled with an increased ability to find a host. *Schizocotyle acheilognathi* has been considered parasitic on fry and juvenile fish, rarely infecting carp over 500g (D.W. Pool pers.com), however, on densely stocked fisheries it is not uncommon to find carp in excess of 1kg host to this cestode, as microscopic zooplankton form the only food resource (pers. obs.).

Successful establishment of non-native parasite species invasions are dependent on the diversity and abundance of host populations, coupled with the local ecology and allowing some generalist parasites to infect more than one species (Holt *et al.*, 2003; Dunn & Hatcher, 2015) with the potential to outcompete native parasite species, although there is no evidence this has occurred in the UK. Dunn & Hatcher (2015) note that non-native, invasive parasites can drive changes in the host species but this is usually in conjunction with environmental change. Current attitudes and resulting policies towards fish stock densities on many fisheries are the drivers for aquatic environmental change, enabling the establishment of exotic parasites and providing the potential for novel combinations of host species.

The coarse fish industry as a whole, is very sensitive to the presence of Category 2 parasites with concerns for both business reputation and the financial implication that infected fish have a lower value. As a consequence of this sensitivity, there is probably under-reporting of many sites where fish are host to Category 2 parasites as there are no legal obligations to report the presence of these parasites.

3.4 Concluding remarks

Analysis of the records for Category 2 parasites held in the *Aquatic Parasite Information* database indicates the stored data can be used to monitor the annual history of non-native species. Species invasions are proposed to take place in a three step process, the initial introduction followed by establishment, where a non-native parasite can establish breeding populations, followed by dispersal and invasion (Kolar & Lodge, 2001). Based on the annual records held in *Aquatic Parasite Information* for parasites, analysis of the data for *Ergasilus sieboldi* and *E. briani,* these parasites have fulfilled Kolar & Lodge (2001) three requirements for introduction, establishment and invasion, whereas the introduction of *Monobothrium wageneri* has failed to become established. Such information can prove valuable in monitoring the ability of non-native parasite species to become established in the UK. In addition to the timeline of an introduction, the *Aquatic Parasite information* distribution records can monitor the dispersal and spread of a parasite species. Analysis of the records for *Ergasilus sieboldi* and *E. briani* demonstrate the data held in *Aquatic Parasite Information* can be used for the detailed investigation of individual, or groups of species.

Whilst the analysis of data held in *Aquatic Parasite Information* has concentrated on the Category 2 parasites in this study, the future analysis of other species of parasite not included here may reveal insight into the efficacy of legislation governing fish movements and current practices in fishery management, particularly with regard to stocking levels.

Chapter 4

Morphological and molecular study of species of *Dactylogyrus* (Monogenea; Dactylogyroidea) associated with coarse fish in the United Kingdom

4.1 Introduction

Popularly termed gill flukes, *Dactylogyrus* species are generally located on the primary gill lamellae of freshwater fish, although postlarvae may be found in the body mucus as they migrate towards the gills (Buchmann & Bresciani, 2006; pers. obs.). The majority of dactylogyrids are considered to be parasitic on the cyprinids (Kearn, 2004, Šimková, *et al*. 2004; Šimková & Morand, 2015) although they are occasionally encountered on other species of coarse fish such as perch, *Perca fluviatilis* and pike *Esox lucius* (Šimková, *et al*. 2004; Šimková & Morand, 2015, *pers. obs.*). Dawes (1947) was adamant that *Dactylogyrus* was not present in the UK. Subsequently there have been studies of dactylogyrids in the UK associated with bream, carp or freshwater fish from specific habitats (Anderson, 1971; Shillcock, 1972; Pool & Chubb, 1987; see also Discussion, p. 125 & 129) but few fish biologists in the UK routinely identify *Dactylogyrus* to species, because they are commonplace and regarded to be of low pathogenicity. In cyprinid aquaculture, dactylogyrids are regarded as significant pathogens, causing mortalities among juvenile fish up to 6cm length and impacting on fish production (Schaperclaus, 1991; Sommerville, 1998; Billard, 1999; Pillay & Kutty, 2005). Many UK fisheries are now densely stocked with up to 3,000kg of fish per hectare. Frequent capture due to low food availability results in physical damage to the mouth and buccal cavity, together with competition for other resources, notably dissolved oxygen, leads to stressed, weak fish (Brewster 2000, 2009, 2014). Heavily stocked fisheries with populations of weak fish provide an abundance of hosts,

increasing the potential for infection with *Dactylogyrus* species. In an immunoecological study of common carp (*Cyprinus carpio*) Rohlenová *et al.* (2011) found that in addition to abiotic factors, fish in poor condition had a higher incidence of infection with *Dactylogyrus* species.

In low numbers, the protease and glandular secretions produced by *Dactylogyrus* species invoke telangiectasis in the host, with local swelling which may entirely envelope the haptor, where they browse on the gill epithelium (Buchmann & Bresciani, 2006; *pers*. *obs.*). The host response to the chemical and mechanical damage caused by *Dactylogyrus* species is excess mucus secretion and lamellar hyperplasia, sometimes seen as elongated processes on the gill tips (Figure 4.1) leading to asphyxiation and osmoregulatory failure (Wootten, 1989; Schaperclaus, 1991; Gratzek, 1993; Stoskopf, 1993; Noga, 1999; Buchmann & Bresciani, 2006). There is also evidence the parasite is able to either suppress or evade the host immune system (Buchmann & Bresciani, 2006). More recently, Rastiannasab *et al.* (2015) have demonstrated changes in carp liver enzymes and kidney function in response to infection with *Dactylogyrus* and *Gyrodactylus* species.

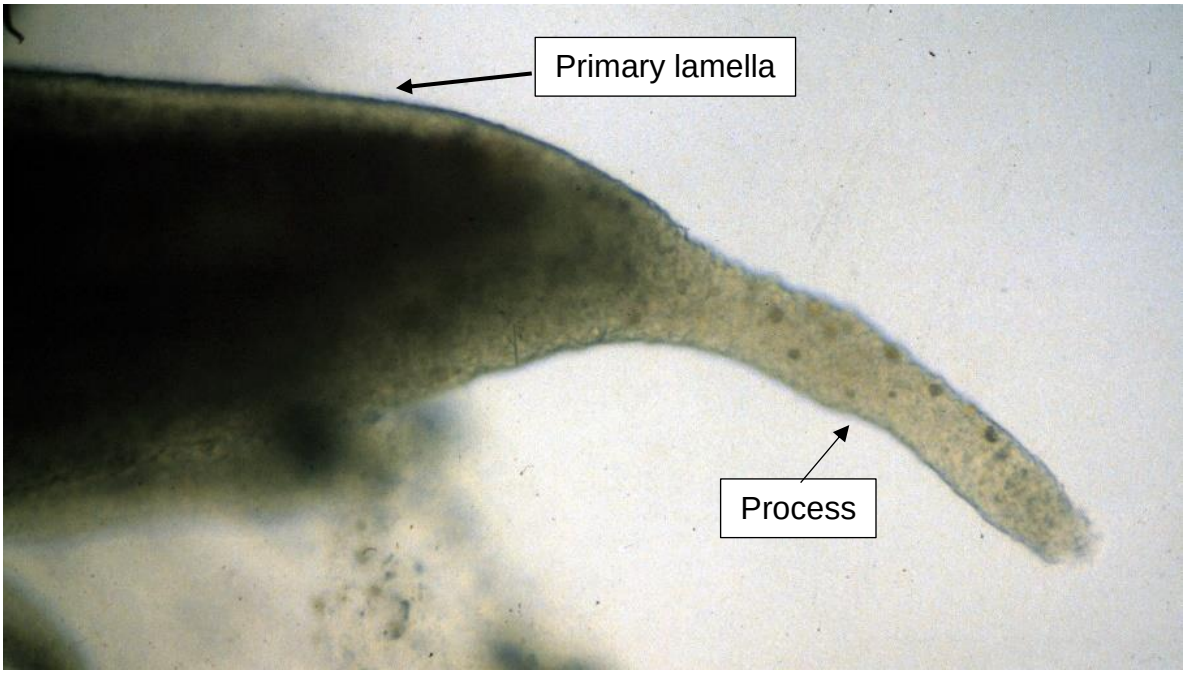

Figure 4.1 Primary gill lamella of carp with lamellar hyperplasia and elongated process associated with *Dactylogyrus* sp. infection. Photograph B Brewster

In the late 1980's mortalities of common carp occurred at a number of fisheries throughout the UK, termed Spring Carp Mortality Syndrome (SCMS). Clinical signs were respiratory distress, coupled with excess mucus on the body and gills and lamellar hyperplasia but the aetiology remains undetermined (Armitage *et al.* 2007). As this disease declined, it was closely followed in 1996 by an emerging disease of common carp, Koi Herpesvirus (KHV), which causes gill erosion, excess mucus production and acute mortalities (Haenan *et al.* 2004). Since the occurrence of these emerging diseases, the potential role for *Dactylogyrus* species causing fish mortalities has been overlooked (C. Williams *pers. com.*). Mortalities involving gill pathology is automatically sampled and screened for virology, but as noted by Rohlenová *et al.* (2011) *Dactylogyrus* species die rapidly after the fish host is *post mortem* so the potential role these monogeneans have either directly, through gill pathologies, or acting synergistically with any infectious agent is unnoticed. Generally, identification of dactylogyrids has a low priority, although 18 species of *Dactylogyrus* from the UK

are entered into *Aquatic Parasite Information* there are just 43 records for the last 60 years. Recognition of the role that dactylogyrids play in fish mortalities depends on accurate identification of these monogeneans, which traditionally has relied on the morphology of sclerotized marginal hooks, anchors and the copulatory organ, features which often prove difficult to visualize using the light microscope (e.g. Ling *et al*., 2016; Sharma *et al*., 2011). More recently molecular techniques such as the use of non-protein coding ribosomal Internal Transcribed Spacer (ITS) and partial 18s rDNA sequences (Šimkova *et al.*, 2004) have been employed to elucidate the identification and phylogeny of species of Dactylogyridae (Figure 4.2).

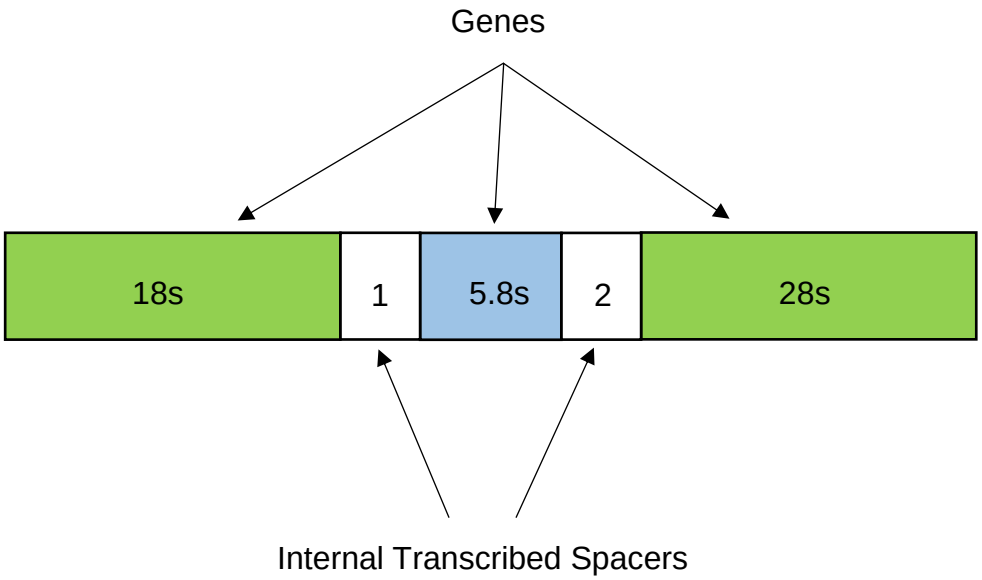

Figure 4.2 Relationship between the ribosomal Internal Transcribed Spacer (ITS1) and 18s gene used as markers to elucidate the phylogenetic relationships and identification of *Dactylogyrus* species

The routine examination of freshwater fish submitted for movement consent provided the opportunity to collect *Dactylogyrus* species for identification using traditional morphological methods, coupled with advances in molecular techniques for the identification of this group of monogeneans. This study initiates the identification of *Dactylogyrus* species associated with freshwater fish in the UK, using morphological

methods and molecular genetics. The aim of the research was to investigate *Dactylogyrus* species on UK freshwater fish and enable accurately identified records of species of the genus to be added to the *Aquatic Parasite Information* database.

4.2 Materials and methods

4. 2.1 Collection of dactylogyrids from UK freshwater fish

The fish submitted for movement consent were killed by submersion in an overdose of 2-phenoxyethanol, fish anaesthetic. All gill arches were removed from freshly killed common carp, *C. carpio* (n = 17)*;* bream, *Abramis brama* (n = 5); roach, *Rutilus* (n = 18); rudd, *Scardinius erythrophthalmus* (n = 4) and tench, *Tinca tinca* (n = 2) followed by examination under a stereomicroscope. Primary lamellae with attached *Dactylogyrus* species were removed and placed in 70% ethanol and subsequently the parasites were detached from the gill lamellae.

4. 2. 2 Preparation of *Dactylogyrus* specimens for morphological study

Dactylogyrus specimens taken from the fish are detailed in Table 4.1 and were either cleared in 10%, 50% 90%, 100% eugenol and permanent mounted in Numount (Canada Balsam substitute) or, stained with Semichon's Carmine for 1 - 2 minutes, de-stained in 1% and 5% acid alcohol, neutralized in alkaline alcohol, dehydrated in 90% and absolute alcohol, then cleared in 50% xylene/50% absolute alcohol and 100% xylene, followed by permanent mount in Numount (Canada balsam substitute, Brunel Microscopes), or examined as wet mounts. Examination of slide material was undertaken using an Olympus CX41 microscope and Olympus SC30 camera with Cellsens® software.

Identification of slide mounted *Dactylogyrus* species was undertaken using identification keys published by Gallo *et al*. (2010), based on morphological characters. Morphometric features used to identify *Dactylogyrus* specimens are based on the shape and size of sclerotized parts, comprising the anchors, marginal hooks, and copulatory organs, together with body length and width (Figure 4.3) The soft tissues of *Dactylogyrus* species are very delicate and preservation is often poor on permanent mount slide preparations as they continue to clear over a period of months (Strona *et al.*, 2013).

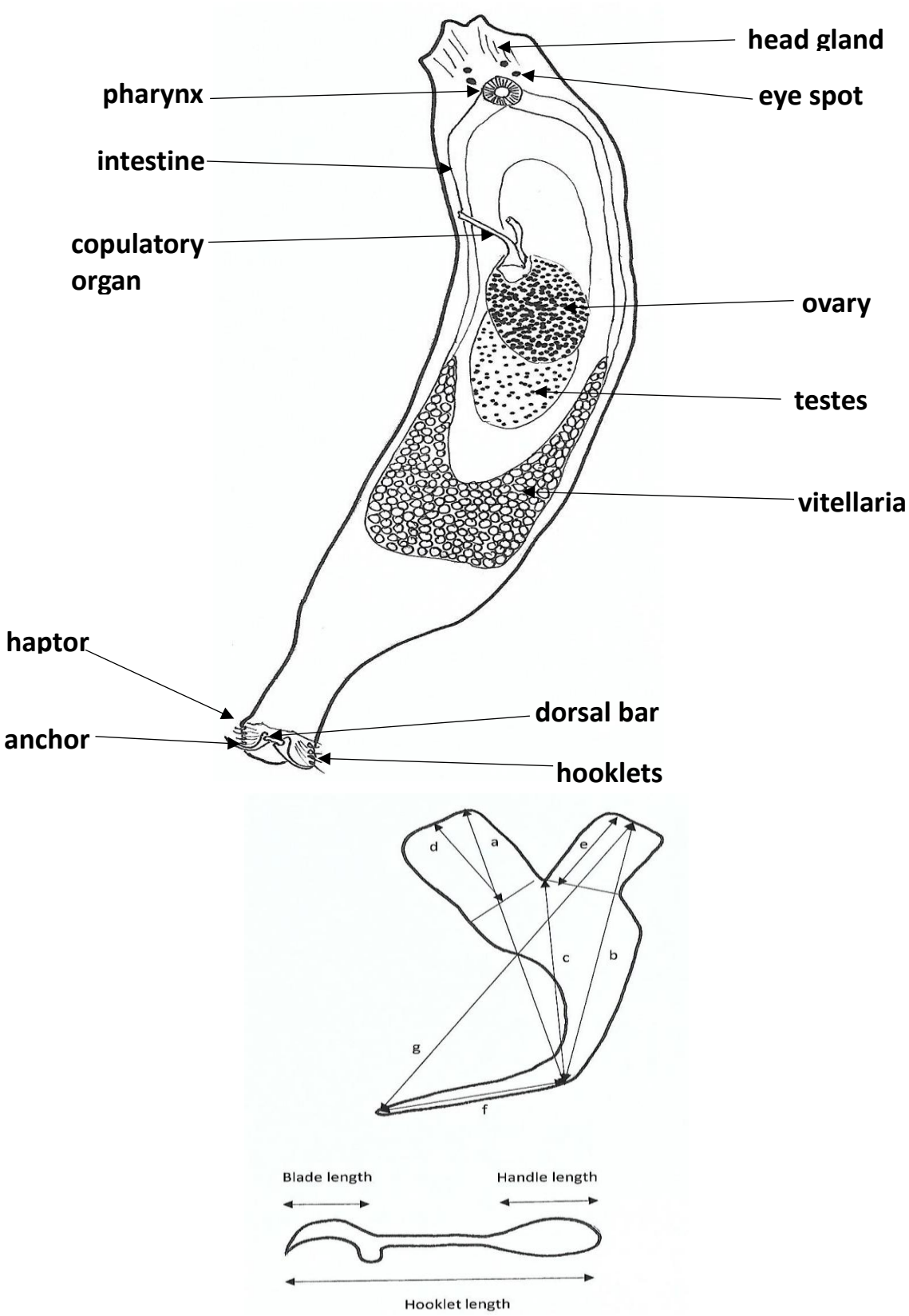

Figure 4.3 Diagram of *Dactylogyrus* species anchor and hooklet measurements (based on Galli *et al.*, 2010)

a – inner anchor length; b – outer anchor length; c - main part length; d – length of inner root; e – length of outer root; f – point length; g – inner root to point length

Table 4. 1 Fish hosts from which *Dactylogyrus* specimens were taken for morphology and molecular study; items in bold indicate successful extraction and sequencing of ITS1

Fish Species	*n*	Locality	Date	No. *Dactylogyrus*
Cyprinus carpio	1	Gresford Flash	11/09/2015	4
Cyprinus carpio	1	Homersfield, Norfolk	20/08/2015	7
Cyprinus carpio	1	Ponders End	10/06/2014	4
Cyprinus carpio	1	Cawood, Selby	11/05/2014	3
Cyprinus carpio	**2**	**Gresford Flash**	**06/05/2014**	**4**
Cyprinus carpio	2	Creeting Lakes, Creeting St Peter	01/03/2014	4
Cyprinus carpio	**1**	**Ingatestone**	**10/02/2014**	**1**
Cyprinus carpio	**1**	**Milton Hall**	**29/01/2014**	**3**
Cyprinus carpio	1	Broadwater Lake, Farncombe	08/12/2012	3
Cyprinus carpio	1	Willington Gravel Pits	14/09/2012	3
Cyprinus carpio	1	Hall Farm Reservoir, Woodham Mortimer	01/08/2012	2
Cyprinus carpio	1	Layer Pit, Layer de la Haye	30/07/2012	3
Cyprinus carpio	1	Bishopsdale Fisheries, Tenterden	25/07/2012	10
Cyprinus carpio	1	Creedy Main Lake	14/12/2011	3
Cyprinus carpio	1	Waltham Abbey	30/10/2011	3
Rutilus rutilus	1	Horton Kirby	06/03/2015	2
Rutilus rutilus	**4**	**Bradford on Tone**	**04/11/2014**	**4**
Rutilus rutilus	5	Digger Lakes, Cullompton	06/03/2014	5
Rutilus rutilus	2	Airfield Lakes, nr Diss	23/11/2013	2
Rutilus rutilus	1	Chafford Gorge Nature Park, Chafford Hundreds	24/10/2012	1
Rutilus rutilus	3	Newton Park	19/04/2012	3
Rutilus rutilus	1	Kenwick Park, Louth	14/02/2012	1
Rutilus rutilus	1	New Buildings Farm, Pease Pottage	07/06/2011	1
Abramis brama	2	Mawthorpe Pond	02/02/2012	6
Abramis brama	2	Ashby Park	05/01/2012	2
Abramis brama	1	Huntstrete, Bath	15/12/2015	1
Scardinius erythrophthalmus	**4**	**Alders**	**07/01/2015**	**4**
Tinca tinca	**2**	**Hollybush Pits, Farnborough**	**15/12/2014**	**9**

4. 2.3 DNA extraction and PCR amplification

Dactylogyrids were readily found on the gills of *C. carpio* but incidence of infection and numbers found on roach and rudd was exceptionally low. A total of eight *Dactylogyrus* species were found on the gills of two tench, of which two specimens were used for DNA extraction and sequencing, the remaining six were retained for morphological study. DNA was extracted from dactylogyrids from fish hosts (Table 4.1) and ITS1 was

successfully amplified and sequenced from some samples from carp, roach, rudd and tench. Extraction of DNA was undertaken using a DNeasy™ tissue kit (Qiagen), following the manufacturer's directions. The ITS1 region was amplified by PCR using primers S1 (5'-ATTCCGATAACGAACGAGACT-3') and the 18s rDNA fragments amplified using H7 (5'-GCTGCGTTCTTCATCGATACTCG-3') or IR8 (5'-GCTAGCTGCGTTCTTCATCGA-3') (Šimková *et al.,* 2004) PCR reactions were undertaken by combining 3.75 µl primer diluted to 10 µM, 12.5 µl DreamTaq® PCR MasterMix and 5 µl extracted DNA. The reaction was processed using the Veriti 96 well, thermal cycler PCR machine, in the following cycle, - 1 minute at 55^0C; 4 minutes at 95^0C; 35 cycles of 1 minute at 95^0C, 1 minute at 55^0C, 2 minutes at 70^0C, 1 minute at 70^0C and a final extension of 10 minute at 70^0C, or alternatively, 1 minute at 57^0C; 3 minutes at 94^0C; 40 cycles of 1 minute at 94^0C; 1 minute at 57^0C; 2 minutes at 72^0C and final extension of 10 minutes at 72^0C. Following DNA amplification, 5 µl of the resultant amplicons were visualised through electrophoresis on 1% agarose gels stained with GelRed (Bioline). The remaining 20 µl of positive amplicon samples were sequenced at the DNA Sequencing Facility of the Natural History Museum, London, using fluorescent dye terminator sequencing kits (Applied Biosystems™), these reactions were then run on an Applied Biosystems 3730XL automated sequencer.

4.2.4 Sequence assembly, initial comparison of species and phylogenetics

A total of eight ITS1 sequences were amplified from *Dactylogyrus* infecting carp, roach, rudd and tench. Sequences were manipulated and edited using BioEdit 7.2.5, then compared with other dactylogyrid ITS1 held in the GenBank® genetic sequence database, using the Basic Local Alignment Search Tool (BLASTn) (www.ncbi.nlm.nih.gov), for preliminary molecular identification.

For comparison, a further 23 European *Dactylogyrus* sequences published on Genbank® (http://www.ncbi.nlm.nih.gov//nuccore/?term=dactylogyrus) and representing species from related cyprinid hosts (Table 4..2) were aligned using MUSCLE sequence alignment tool (http://www.ebi.ac.uk), with the eight sequences successfully extracted. The Gblocks programme was used to remove any ambiguities in the sequences (http://molevol.cmima.csic.es/castresana/Gblocks.html) (Castresana, 2000).

Table 4.2. *Dactylogyrus* species, sequences acquired from GenBank®

Species	Host	Accession No.	Geographic Origin
Ancyrocephalus paradoxus outgroup	*Stizostedion lucioperca*	KF499079	Germany
Dactylogyrus crucifer	*Leuciscus idus*	AJ564122	Morava River, Czech Republic
Dactylogyrus crucifer	*Scardinius erythrophthalmus*	AJ564121	Morava River, Czech Republic
Dactylogyrus crucifer	*Rutilus rutilus*	AJ564120	Morava River, Czech Republic
Dactylogyrus difformis	*Scardinius erythrophthalmus*	AJ490160	Morava River, Czech Republic
Dactylogyrus amphibothrium	*Gymnocephalus cernuus*	AJ564110	Morava River, Czech Republic
Dactylogyrus vastator	*Cyprinus carpio*	AJ564159	Czech Republic
Dactylogyrus wunderi	*Abramis brama*	AJ564164	Morava River, Czech Republic
Dactylogyrus sphyrna	*Rutilus rutilus*	AJ564154	Czech Republic Morava River
Dactylogyrus similis	*Rutilus rutilus*	AJ564153	Czech Republic Morava River
Dactylogyrus rutili	*Rutilus rutilus*	AJ564152	Morava River, Czech Republic
Dactylogyrus nanus	*Rutilus rutilus*	AJ564145	Morava River, Czech Republic
Dactylogyrus intermedius	*Carassius auratus*	AJ564139	Morava River, Czech Republic
Dactylogyrus hemiamphibothrium	*Gymnocephalus cernuus*	AJ564137	Morava River, Czech Republic
Dactylogyrus folkmanovae	*Squalius cephalus*	AJ564134	Morava River, Czech Republic
Dactylogyrus fallax	*Rutilus rutilus*	AJ564131	Morava River, Czech Republic
Dactylogyrus sphyrna	*Blicca bjoerkna*	AJ564155	Morava River, Czech Republic
Dactylogyrus prostae	*Leuciscus idus*	AJ564148	Morava River, Czech Republic
Dactylogyrus nanoides	*Leuciscus cephalus*	AJ564144	Morava River, Czech Republic
Dactylogyrus inexpectatus	*Carassius auratus*	AJ564138	Morava River, Czech Republic
Dactylogyrus difformoides	*Scardinius erythrophthalmus*	AJ564124	Morava River, Czech Republic
Dactylogyrus vistula	*Leuciscus idus*	AJ564162	Morava River, Czech Republic
Dactylogyrus vastator	*Cyprinus carpio*	AJ564159	Morava River, Czech Republic

Phylogenetic analysis was undertaken utilising MEGA version 6 (Tamura *et al.,* 2013) with computation of neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) phylogenies on 30 nucleotide sequences. The NJ is a distance based

method, calculating a matrix of pairwise distance (p-distance) based on the number of nucleotide differences as an estimate of the evolutionary divergence (Pevsner, 2015; van den Peer, 2009). The NJ analysis of the *Dactylogyrus* sequences was undertaken based on the Jukes–Cantor method, clustering related taxa together as a percentage of a 500 replicate bootstrap test. Maximum parsimony is a character based analysis which assumes the minimum number of steps, or least number of changes in character or nucleotide states, to produce a tree inferring the minimum number of evolutionary changes (Pevsner 2015; Hall, 2011). The MP phylogeny was inferred from consensus of two trees obtained using the Subtree-Pruning-Regrafting (SPR) algorithm based on 500 bootstrap replicates. The ML trees are based on the statistical probability the aligned sequence data has resulted in a particular evolutionary configuration (Vandamme, 2009). Construction of the ML phylogenetic tree was based on the Kimura 2 model over a discrete Gamma distribution. The chosen model obtained the lowest Bayesian Information Criterion which created a phylogenetic tree based on the sequence data, substitution model and calculated on 500 bootstrap replicates.

The outgroup sequence for the *Dactylogyrus* sequences was *Ancyrocephalus paradoxus* (GenBank® KF499079).

4.2.5 Markers for species identification, inter- and intra-species molecular diversity

Single nucleotide polymorphisms are a useful measure for molecular diversity and may be markers for the identification of species and were used for comparison of the *Dactylogyrus* ITS1 sequences. Analysis of single nucleotide polymorphism was performed using DnaSP 5.10 (http://ub.esp/DnaSP) (Librado & Rozas, 2009), calculating the segregating sites (S), haplotypes nucleotides inherited together (H),

haplotype diversity, nucleotide diversity (π), average pairwise nucleotide differences (K).

The uncorrected pairwise distance was estimated using MEGA 6 with frequency of transitions (TS) and transversions (TV) for *Dactylogyrus* ITS1. Substitutional changes were estimated using DAMBE 5 (http://www.dambe.bio.uottawa.ca/dambe.asp) (Xia, 2013), substitution saturation leading to loss of the phylogenetic signal.

4.3 Results

4.3.1 Morphometric analysis of *Dactylogyrus* species from carp, bream, roach and tench

Identification is based on the size of the *Dactylogyrus* species, size and morphology of the haptor armament and copulatory organ (Gussev, Gerasev & Pugachev, 2010). Descriptions of anchor measurements are given in Figure 4.2. Descriptions are based on a total of 27 examples of *D. extensus* from five carp; a single specimen of *D. zandti* on each of four bream; two examples of *D. crucifer* from two roach and five specimens *D. tincae* from two tench.

4.3.2 Morphological description of *Dactylogyrus* species

Dactylogyrus extensus Müller & van Cleave, 1932 (Figures 4.4 – 4.5)

Body length 1032.7 – 1477.4µm; body width 144.0 – 197.9µm; inner anchor length 42.1 – 87.1µm; outer anchor length 41.2 – 78.7µm; main part length 40.5 – 67.9µm; length of inner root 10.0 – 27.8µm; length of outer root 7.2 – 19.1µm; point length 14.5 – 35.2µm; hooklet blade length 7.1 – 8.1µm; hooklet length 18.5 – 22.0µm; total length copulatory organ 44.8 – 98.0µm

The marginal hooks have a curved shape, with a small process at the base of each hook, the roots of the anchor are of a similar width, and the outer root is almost as long again as the inner root (Figure 4.4). The copulatory organ is distinctive, the outer tube is strongly curved forming a 'C' shape whereas the accessory piece is a slightly twisted tube (Figure 4.5).

Dactylogyrus extensus is one of the larger species of dactylogyrid and is found on the gills of carp in varying numbers, where it is readily visible under a stereomicroscope at 10x magnification. The site of attachment of *D. extensus* to the gills, is usually swollen, with local haemorrhaging and frequently the haptor is encapsulated in the host tissue (Figure 4.6)

Although generally regarded as a specialist parasite restricted to a single host, *D. extensus* has also been recorded from *Misgurnus fossilis* (Gussev, Gerasev & Pugachev, 2010). *Dactylogyrus extensus* is distributed throughout Europe, Asia and North America (Gussev, Gerasev & Pugachev, 2010) but was first recorded in the UK by Pool & Chubb in 1987.

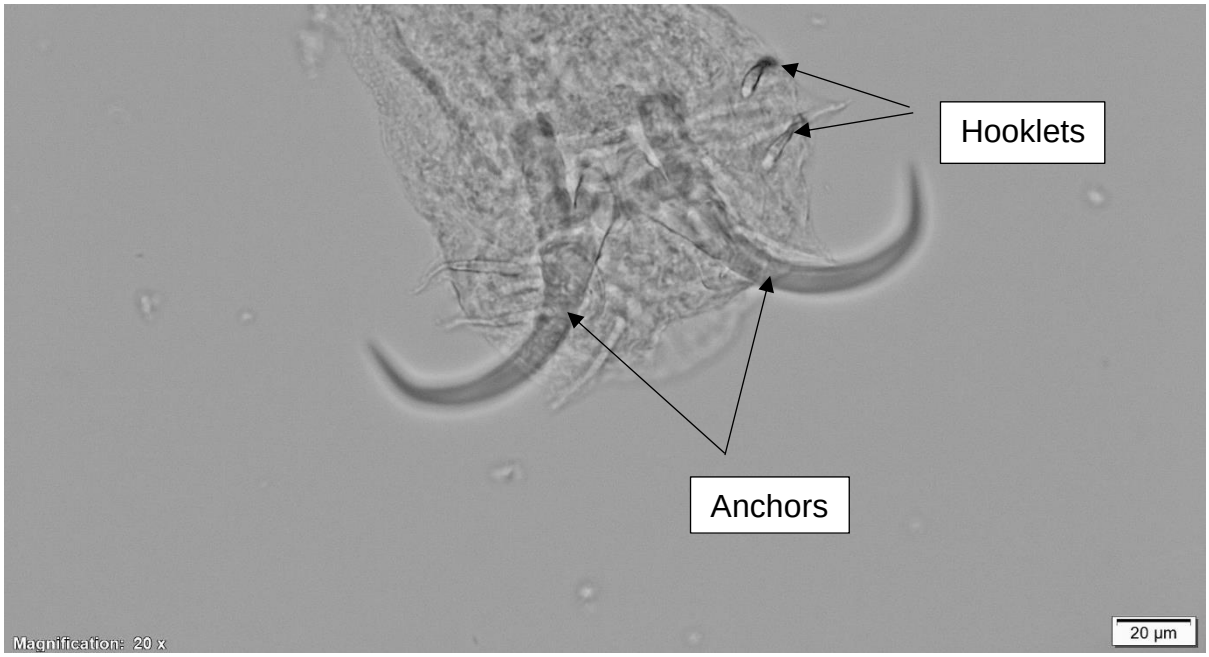

Figure 4.4 *Dactylogyrus extensus* haptor (Photograph B. Brewster)

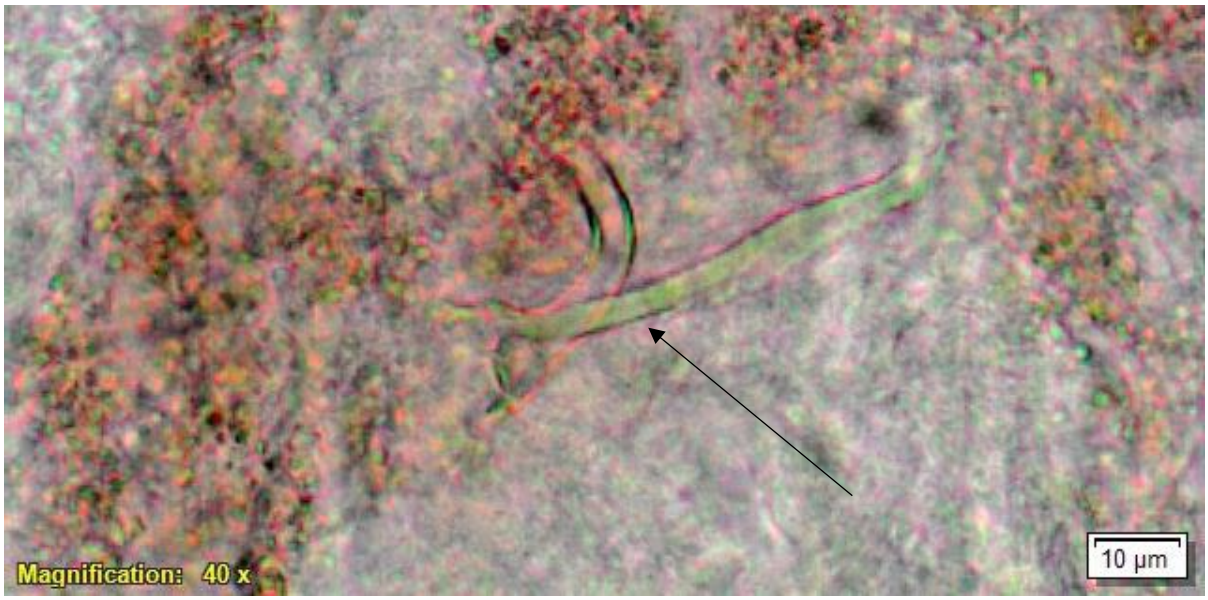

Figure 4.5 *Dactylogyrus extensus* copulatory organ (arrowed) (Photograph B. Brewster)

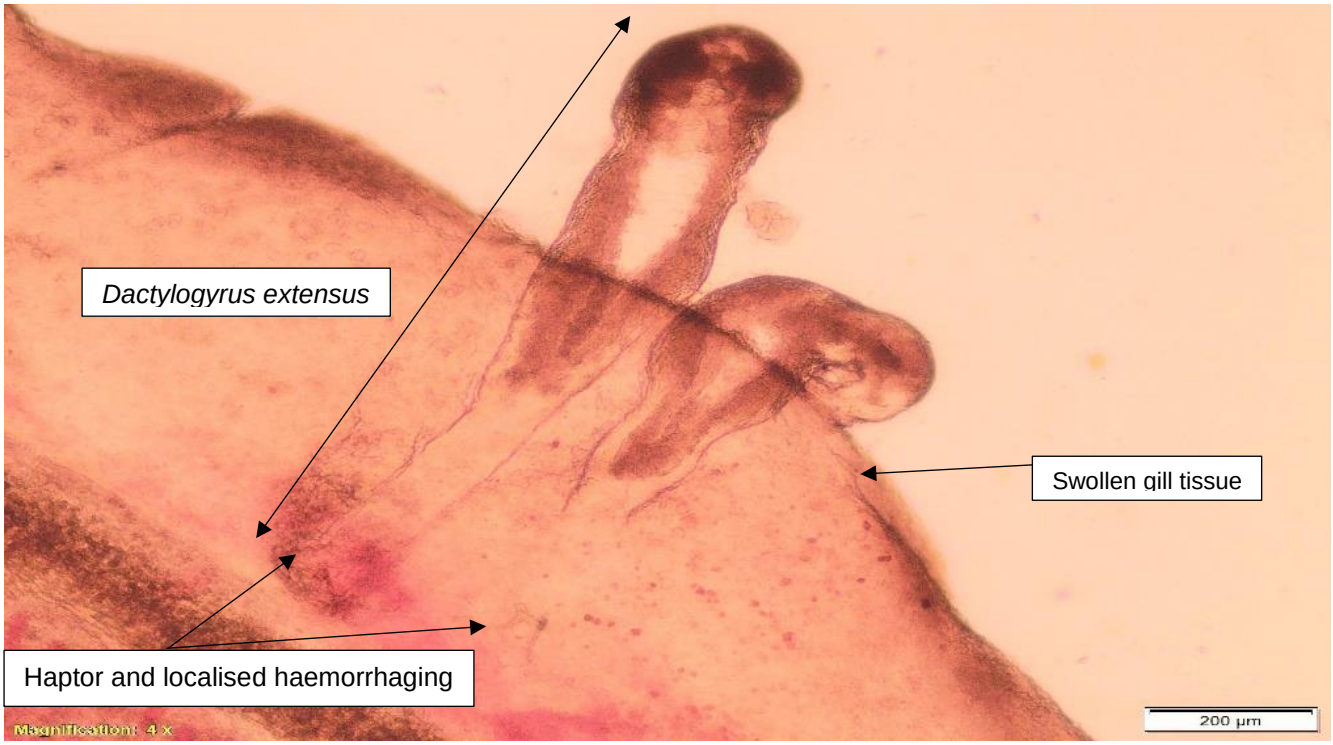

Figure 4.6 Two *Dactylogyrus extensus* attached to carp gill, with swelling and haemorrhaging associated with attachment (Photograph: B. Brewster)

***Dactylogyrus zandti* Bychowsky, 1931 (Figures 4.7 – 4.8)**

Body length 294.9- 407.9µm; body width 39.3 – 80.4µm; inner anchor length 50.6 – 56.4µm; outer anchor length 45.7 – 50.0µm; main part length 41.1 - 44.2µm; length of inner root 21.0µm; length of outer root 11.5µm; point length 17.1µm; hooklet blade length 10.8µm; hooklet length 23.3µm total length copulatory organ 20.9 – 56.0µm

Dactylogyrus zandti is a small dactylogyrid found on the gills of bream. Two specimens have been stained and mounted but their orientation is poor proving difficult to discern the diagnostic features, however, it is possible to determine the sickle shaped anchors but the copulatory organ is difficult to visualise. This is a specialist parasite of bream according to Gussev, Gerasev & Pugachev (2010) with the same distribution as the host in England, Wales and European drainages (Kottelat & Freyhof, 2007). There is frequently only a single *D. zandti* found on the bream gills (pers. obs.) and any pathology associated with infection is unresolved.

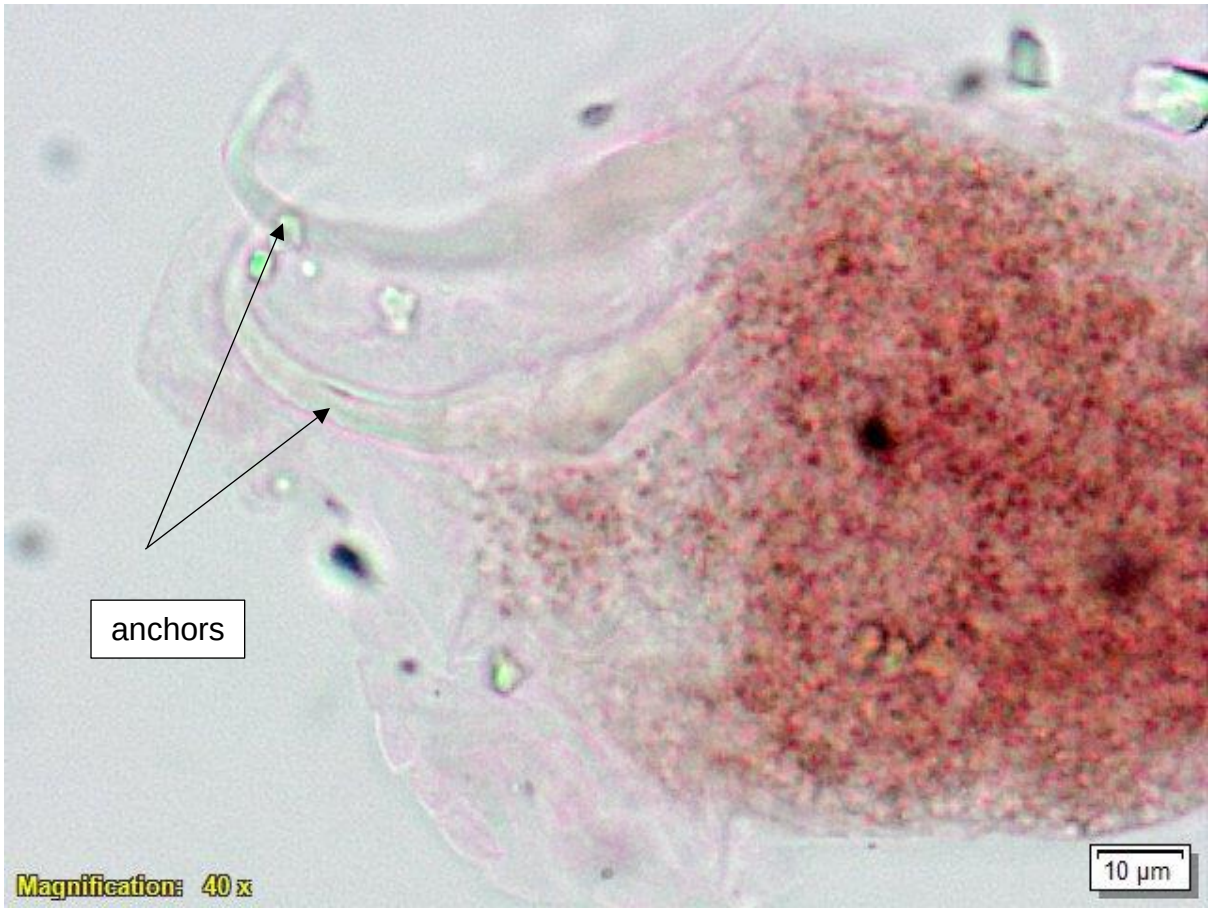

Figure 4.7 *Dactylogyrus zandti* haptor (Photograph B Brewster)

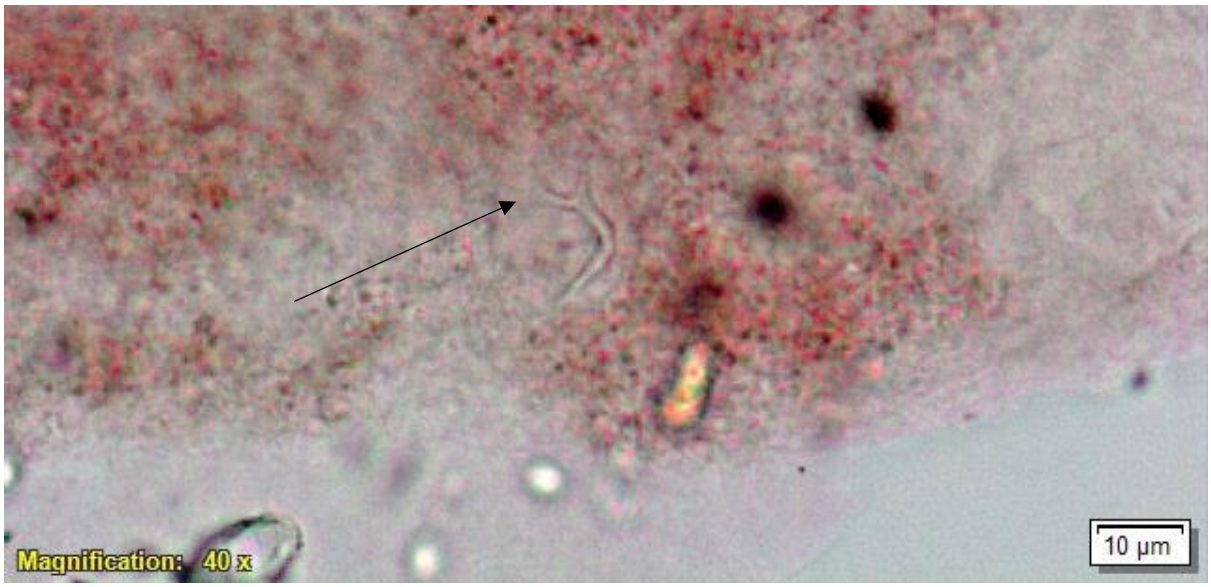

Figure 4.8 *Dactylogyrus zandti* copulatory organ (arrowed)(Photograph B. Brewster)

***Dactylogyrus crucifer* Wagener 1857 (Figures 4.9 – 4.10)**

Body length 224.0 – 361.8µm; body width 54.3 – 76.1µm; inner anchor length 25.1 – 46.2µm; outer anchor length 31.8 – 37.0µm; main part length 27.6 – 32.5µm; length of inner root 9.6 – 14.5µm; length of outer root 5.3 – 7.8µm; point length 13.5 – 22.1µm; hooklet blade length 6.1 – 14.0µm; hooklet length 23.1 – 30.9µm; hooklet base 12.3 – 22.6µm; total length copulatory organ 40.5 –59.5µm

The anchor point is long and strongly tapered; the marginal hooks have a blade like process on the dorsal surface, the inner root of the anchor is roughly between two to three times the size of the outer root, both with blunt edges, the bar is hour glass shaped, the basal part of the copulatory organ is expanded to form an oval shape with a strongly curved copulatory tube, the accessory copulatory organ has a comma shaped hook anteriorly.

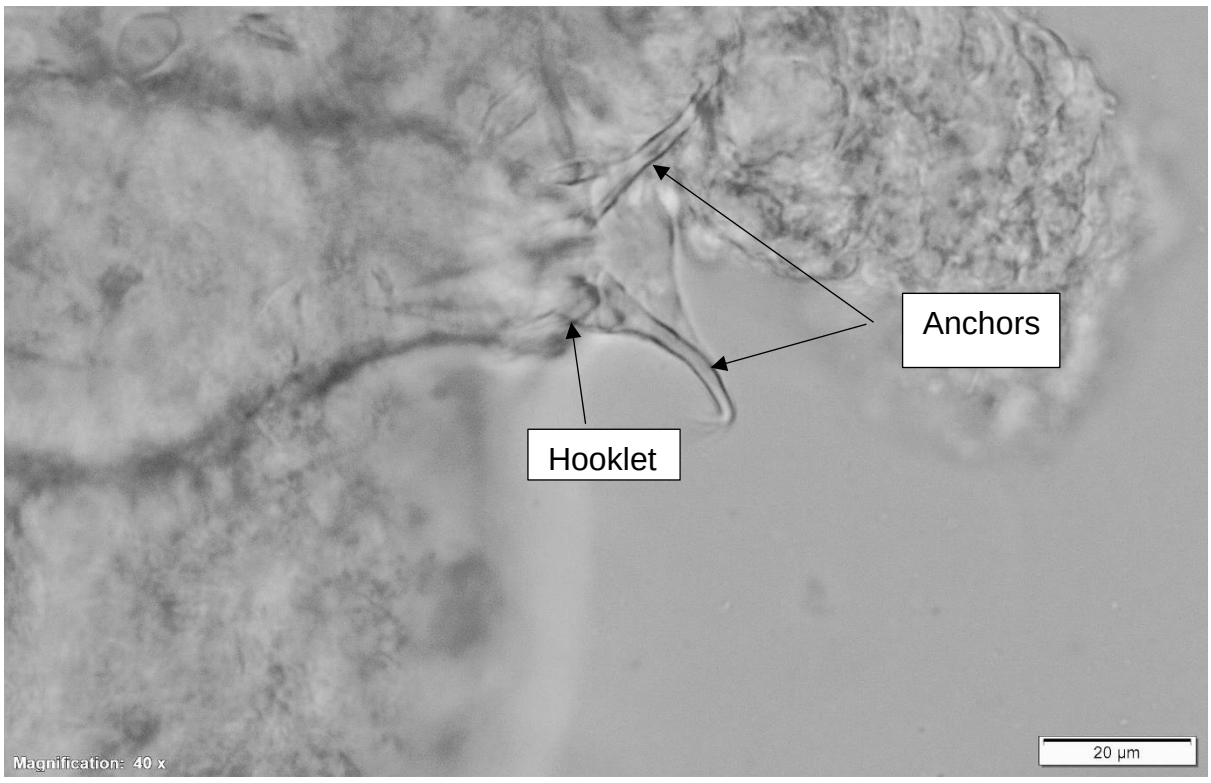

Figure 4.9 *Dactylogyrus crucifer* haptor (Photograph B. Brewster)

Dactylogyrus crucifer is a small dactylogyrid which is a specialist parasite of roach, it is found occasionally on this fish host and does not appear to cause any significant pathology.

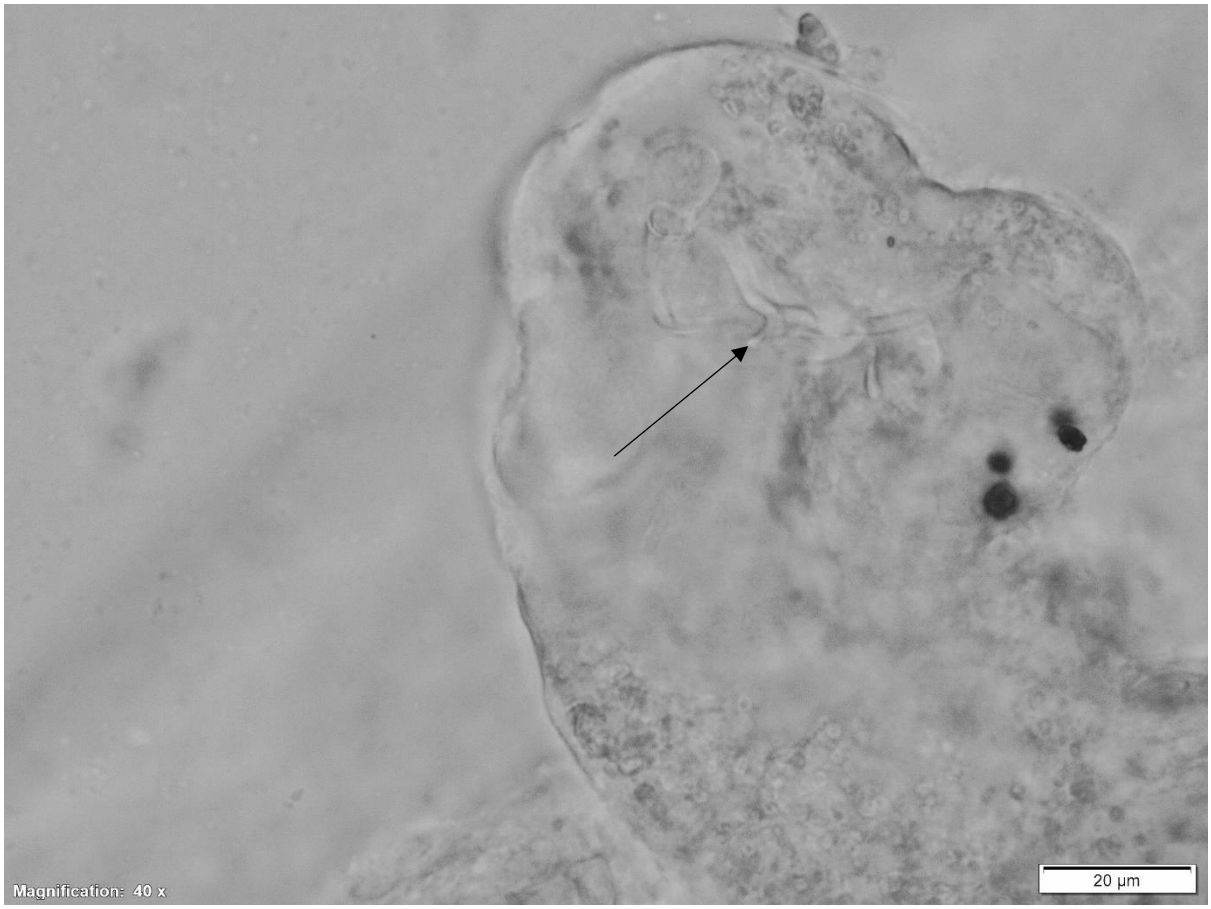

Figure 4.10 *Dactylogyrus crucifer* copulatory organ (arrowed) (Photograph B. Brewster)

***Dactylogyrus tincae* Gussev, 1965 (Figures 4.11 – 4.12)**

Body length 931.6 – 1398.3µm; body width 118.4 – 218.7µm; inner anchor length 53.7 – 56.4µm; outer anchor length 44.2 – 50.1µm; main part length 39.7 – 46.1µm; length of inner root 17.5 – 19.7µm; length of outer root 10.2 – 13.0µm; point length 19.1 – 21.3µm; hooklet blade length 10.5µm; hooklet length 23.4µm total length copulatory organ 53.4 –72.7µm

The ventral bar is oblong with rough edges, the marginal hooks are robust and the copulatory tube is strongly sickle shaped, the accessory piece is curved with a 'c' shape supporting the copulatory tube. The combined copulatory tube and accessory piece have a pincer like appearance.

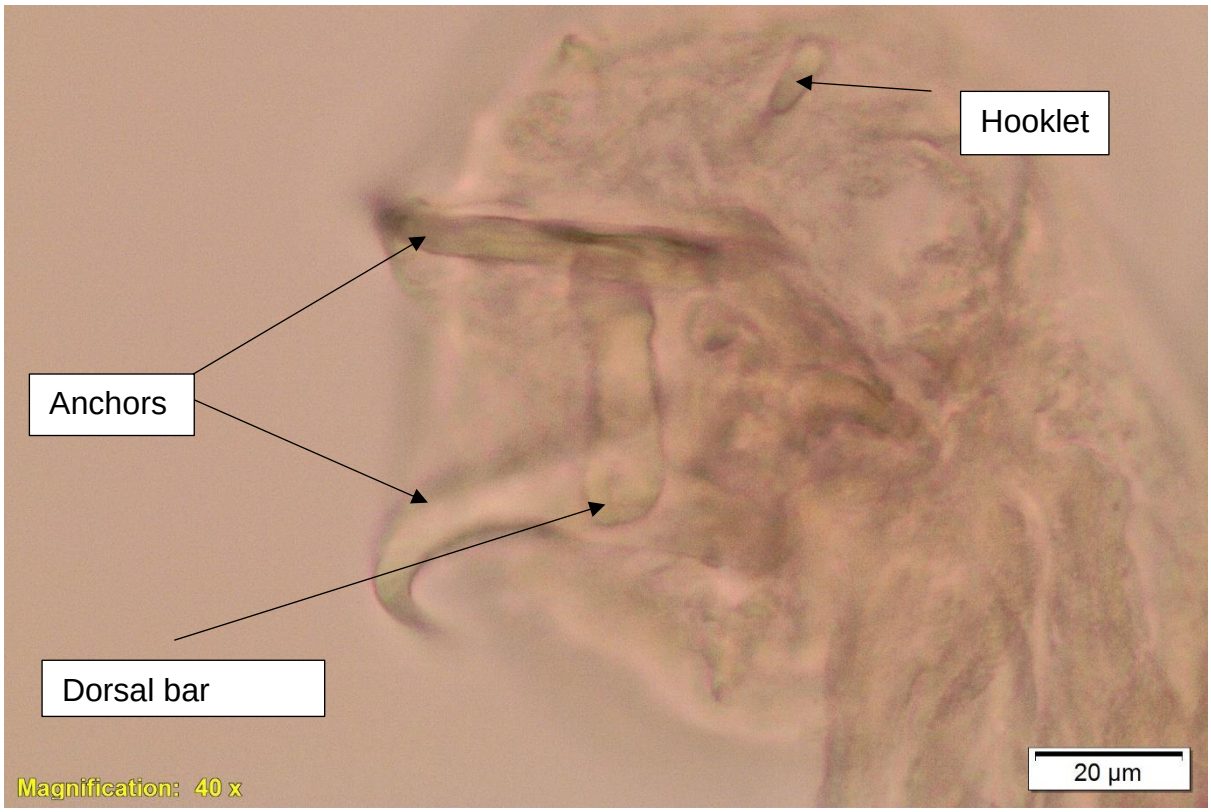

Figure 4.11 *Dactylogyrus tincae* haptor (Photograph B. Brewster)

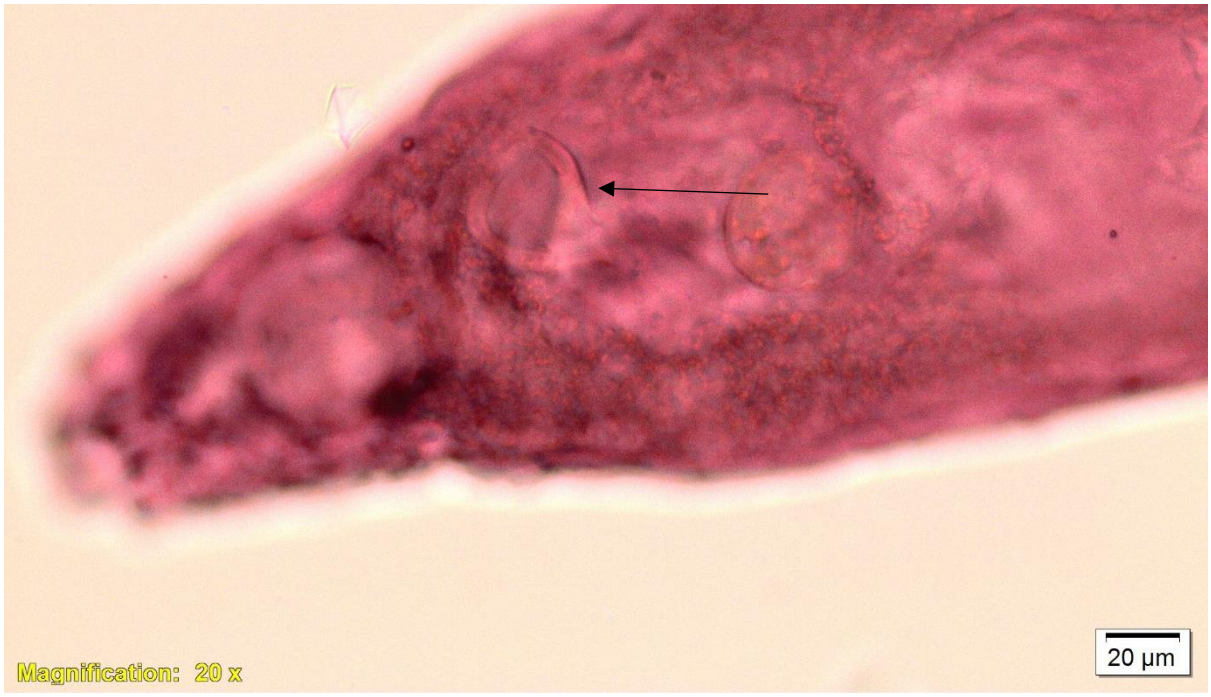

Figure 4.12 *Dactylogyrus tincae* copulatory organ (arrowed) (Photograph B. Brewster)

Although the BLAST analysis of the sequences from this dactylogyrid suggested the species were either *D. amphibothrium* or *D. hemiamphibothrium,* using Galli *et al*. (2010) keys to identification based on morphological characters, the specimens from tench were identified as *D. tincae*. One of the largest dactylogyrids, *D. tincae* is a specialist parasite of tench but the distribution is confined to the Danube and Elbe Rivers (Gussev, Gerasev & Pugachev, 2010), although Galli *et al*. (2007) found this dactylogyrid infecting tench in Italy. A total of seven *D. tincae* were found on two *T. tinca* but this is the first occasion on which any dactylogyrids have been found during routine examination of this fish species (pers. obs.). Svobodova & Kolarova 2004 report clinical infections of *D. tincae* cause haemorrhaging and necrosis of gill tissue in tench, which may result in mortalities.

4.3.3 Initial identification of sequences using BLASTn

BLASTn analysis of the sequences identified the dactylogyrids from carp similar to *D. extensus*, with between 91 – 94% shared identity. Sequences extracted from dactylogyrids parasitizing roach had a shared identity with *D. crucifer*, and the sequences extracted from dactylogyrids from rudd had a shared identity with *D. difformis.* The *Dactylogyrus* sequences from tench produced two possible identities *D. amphibothrium* and *D. hemibothrium* from the BLASTn analysis, however, based on morphological characters the species were identified as *D. tincae*. There are no representative sequences for *D. tincae* currently in the GenBank® sequence database. The results are given in Table 4.3.

Table 4.3 *Dactylogyrus* ITS1 sequence identity analysis using BLASTn

Reference	BLASTn result	BLASTn % shared identity
CC1 Carp, Gresford Flash	*D. extensus*	92
CC3 Carp, Milton Hall	*D. extensus*	94
CC4 Carp, Ingatestone	*D. extensus*	91
RRA Roach, Bradford on Tone	*D. crucifer*	97
SC1 Rudd, Alders	*D. difformis*	93
SC2 Rudd, Alders	*D. difformis*	87
T1A Tench, Hollybush Pits	*D. amphibothrium* *D. hemiamphibothrium*	86 85
T1B Tench Hollybush Pits	*D. amphibothrium* *D. hemiamphibothrium*	84 83

4.3.4 Phylogenetic reconstruction

The number of *Dactylogyrus* species ITS1 and reference sequences were sufficient for phylogenetic analysis enabling clades of related species to form and for identification of species. Phylogenetic trees NJ, MP and ML were constructed for the dactylogyrid ITS1 sequences extracted from the UK species, together with those downloaded from GenBank®, resulting in the trees given in Figures 4.13– 4.15. The resulting phylogenetic analysis produced incongruent trees, many branches have less than 70% bootstrap support, implying the branching order is uncertain and therefore not representative of evolutionary relationships. Although the phylogenetic trees were incongruent, the following sequences consistently formed clades:

Clade A: *D. difformis, D. difformoides, D. prostae, D. nanoides, D. folkmanovae, D. rutilus* and *D. nanus*

Clade B: *D. crucifer*

Clade C: *D. vistulae, D. similis, D. sphyrna*

Clade C^1: *D. fallax* (NJ tree)

Clade D: *D. amphibothrium, D. hemiamphibothrium*

Clade E: *D. tincae*

Clade F: *D. intermedius, D. vastator, D. inexpectatus*

Clade D. *D. extensus*

In the NJ tree, the sequence for *D. fallax* (C^1) forms a clade with the *D.* crucifer (B) sequences, whereas in the MP and ML trees this sequence is consistently grouped with *D. vistulae, D. similis* and *D. sphyrna* (C). With the exception of the sequences

comprising clade A, which is consistently sited at the top of all three phylogenetic trees, all other clades are variously located on different branches.

In clade A *D. difformis* and *D. difformoides* sequences and *D. rutili* and *D. nanus* consistently form branches with a high bootstrap value in all trees indicating these may be phylogenetically related. Sequences on other branches have low bootstrap values and clustered within clade A, this would suggest some similarity between the sequences but may not infer any phylogenetic relationship. Clade B comprises sequences of *D. crucifer* which are clustered together in all phylogenetic trees, with high bootstrap values suggesting they are representative of this species. Within clade C, *D. vistulae, D. similis* and *D. sphyrna* cluster together, whilst *D. fallax* is clustered with clade C in the MP and ML trees, but in the NJ tree it is clustered with group B, this differing topology suggests the position is unresolved. *Dactylogyrus amphibothrium* and *D. hemiamphibothrium* in clade D form a natural group, clustering together with a high bootstrap value in all trees. The two sequences extracted from *Dactylogyrus* parasitizing tench included in clade E share the same branch in all trees, indicating they represent a single species, identified using morphological characters as *D. tincae.* The sequences from *D. extensus* comprising clade G, also share the same branch, with the implication these are representative of one species. Group G consistently forms a cluster with clade F, comprising *D. intermedius, D. vastator* and *D. inexpectatus*.

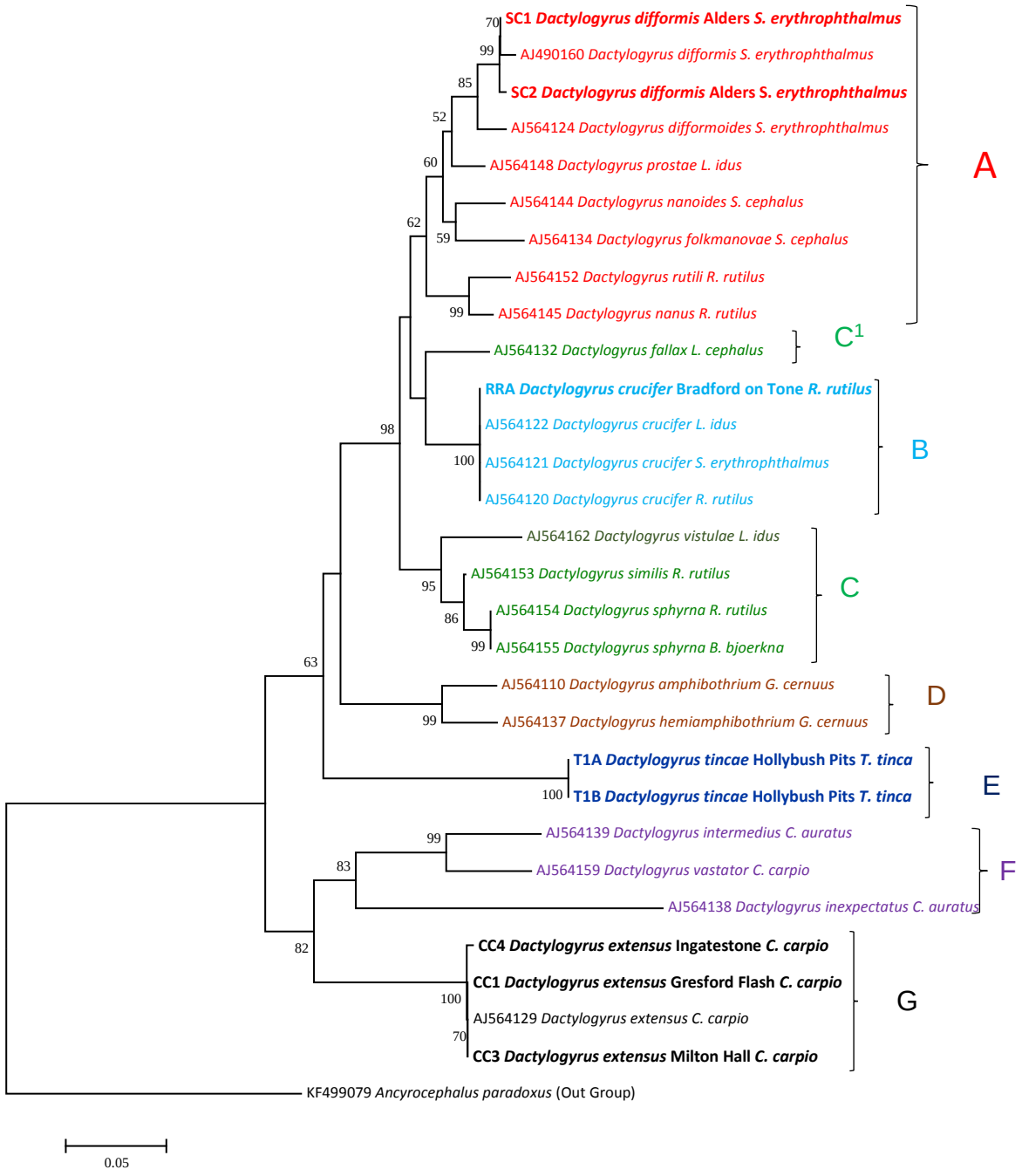

Figure 4.13 The phylogenetic reconstruction of the ITS1 sequences using the Neighbour-Joining method The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. Items in bold are sequences extracted as part of this study

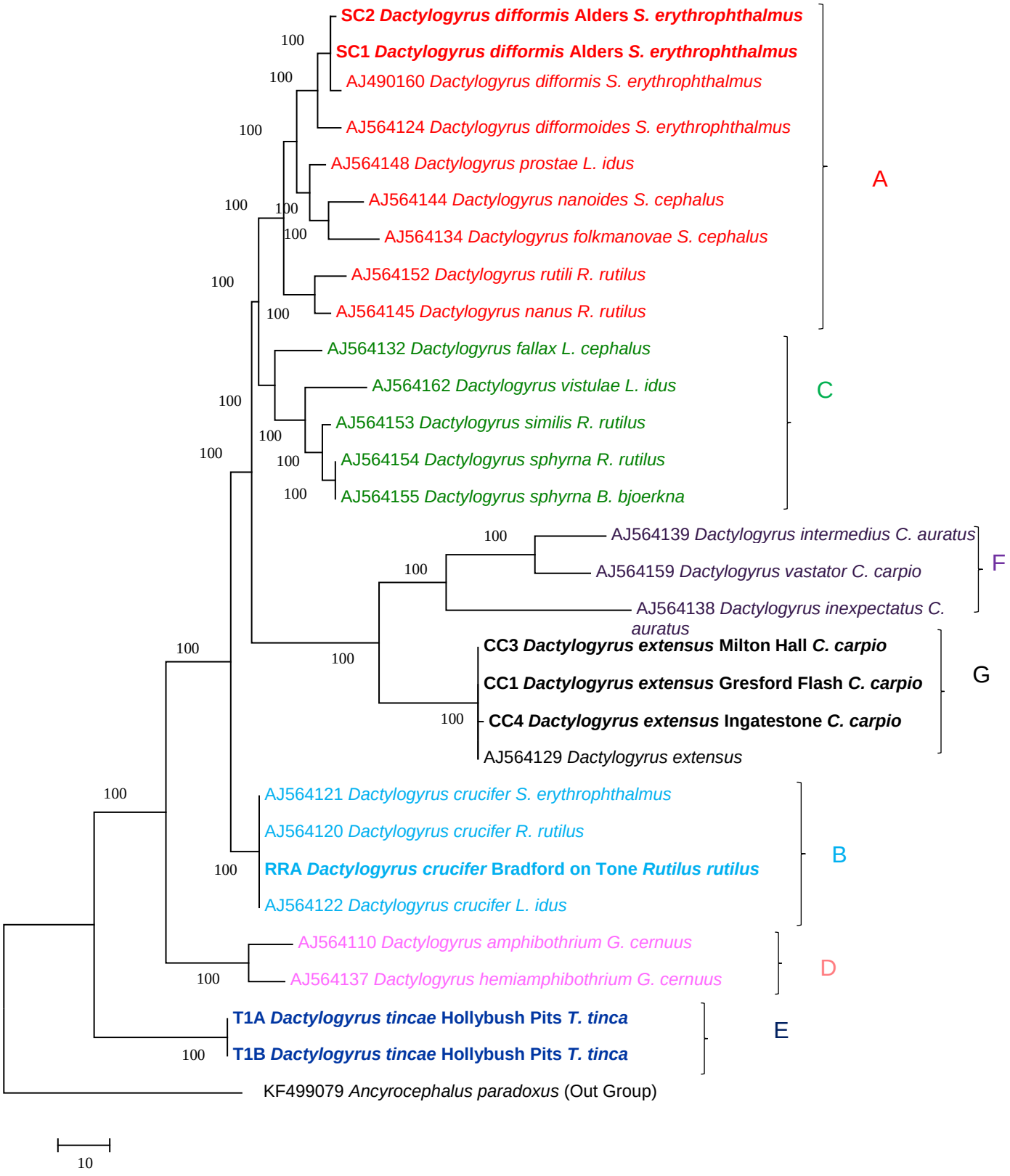

Figure 4.14 Phylogenetic reconstruction of species of the ITS1 for the genus *Dactylogyrus* using a character based Maximum Parsimony method, the percentage of replicate trees in which the associated species clustered together are shown next to the branches and based on a 500 replicate bootstrap test. Items in bold were sequenced as part of this study

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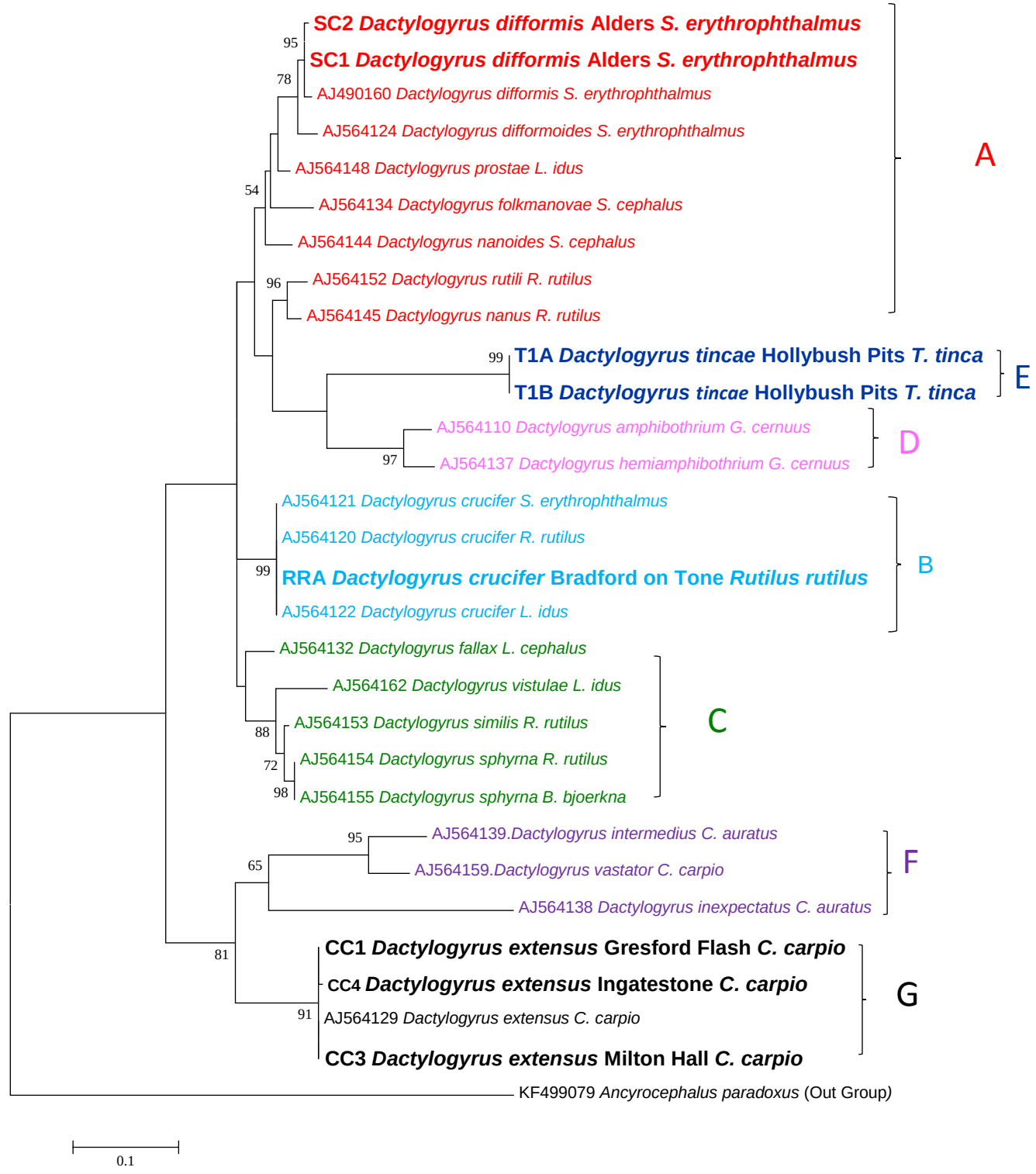


Figure 4.15 Maximum Likelihood method based on the Kimura 2 + Gamma parameter model. The percentage of tre Phylogenetic reconstruction of the *Dactylogyrus* ITS1 sequences using a character based es in which associated species clustered together, based on a 500 replicate bootstrap, is shown next to the branches. Branch lengths are measured in number of substitutions per site. Items in bold are sequences extracted as part of this study

4.3.5 Diversity and phylogenetic power of ITS1 in resolving *Dactylogyrus* taxonomy

Single nucleotide polymorphisms are different nucleotides found at a particular locus on a genome within a population and which may be markers for the differentiation of species. ITS1 is non protein coding, with the consequence that mutations have no effect on biochemistry or physiology and accumulated single nucleotide polymorphisms can be effective markers for intraspecific differentiation. *Dactylogyrus* ITS1 single nucleotide polymorphism was assessed using DnaSP 5.10 (Librado & Rozas, 2009). The nucleotide diversity (π) is a computation of the average number of nucleotides in the sequences which differ. Results of single nucleotide polymorphism are given in Figure 4.16, the peaks are the numbers of variable sites in the sequences which show the greatest variability between nucleotide positions 563 – 1076 indicating the ITS1 sequences have numerous single nucleotide polymorphisms, which can be used as markers for differentiation of *Dactylogyrus* species.

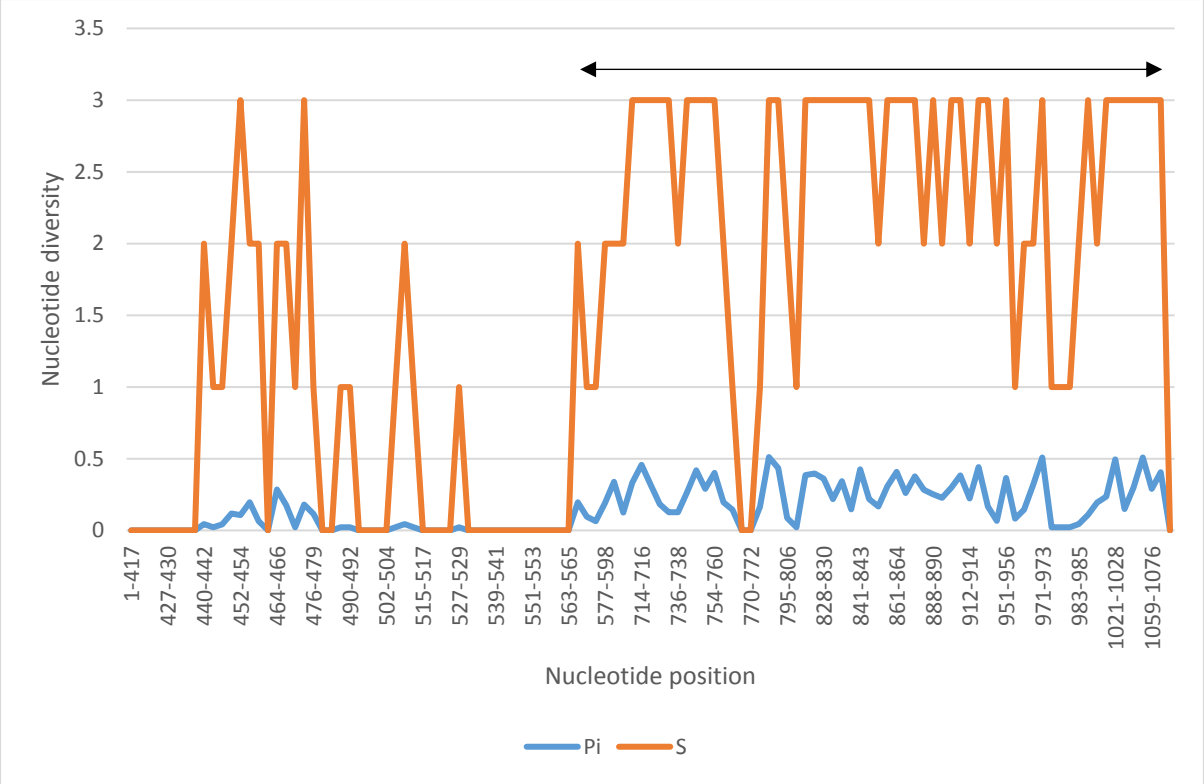


Figure 4.16 *Dactylogyrus* ITS1 nucleotide diversity and nucleotide variable sites, nucleotide positions with the greatest diversity indicated by an arrow.

The results measuring sequence polymorphism and divergence between the aligned sequences, are given in Table 4.4, where: S = variable sites, π = nucleotide diversity, K = average number of nucleotide differences, indels = insertion and deletion of nucleotides. Parsimony informative sites are those where there are two different types of nucleotide, which occur at least twice in the sequences and monomorphic sites have the same nucleotide. The table shows the nucleotide diversity, measuring the average number of nucleotide differences between the sequences the average nucleotide differences and parsimony informative sites indicating that ITS1 is a good marker for differentiation of *Dactylogyrus* species.

Table 4.4 *Dactylogyrus* species ITS1 sequence analysis

Dactylogyrus species ITS1 sequences	Analysis
No. Sequences	30
Alignment length	1511
Total sites (excl gaps and missing data)	342
Alignment gaps or missing data	1169
S	184
Total no mutations	294
π + SD	0.15435 ± 0.01616
K	52.79
p-distance	0.1547
Parsimony Informative	134
Monomorphic sites	158
Indels	1169

Results of computation of the uncorrected p-distance are given in Figure 4.16 measuring transitions that are purine↔purine or pyrimidine↔pyrimidine mutations, or transversions, involving purine↔pyrimidine changes.

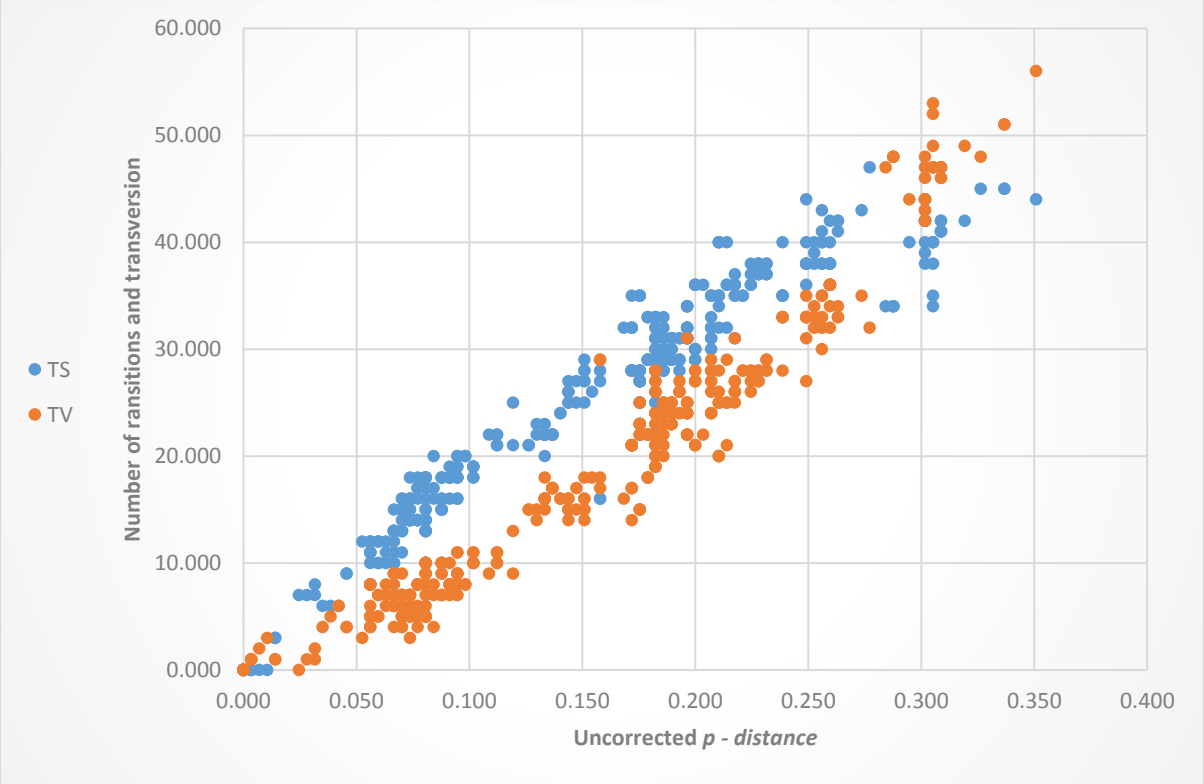


Figure 4.17 ITS1 uncorrected p-distance showing nucleotide transition and transversion substitution of *Dactylogyrus* species and out group *Ancyrocephalus paradoxus* (TS = transitions; TV = transversions)

Transitions are considered to occur more frequently than transversions, but repeated substitutions at a locus result in saturation and as a consequence the phylogenetic signal is lost. The p-distance measures the genetic difference between the sequences but is 'uncorrected' as it cannot take into account multiple substitutions at the same site. The number of transitions for the ITS1 sequences, show an upward trend which start to plateau but at a p-distance of 0.250, fall below the number of transversions (Figure 4.17). This pattern of transitions falling below transversions with increasing p-distance indicates the transitions are reaching substitution saturation as a result of which the phylogenetic signal has been lost.

Further analysis of ITS1 substitution saturation was undertaken using DAMBE 5 (http://www.dambe.bio.uottawa.ca/dambe.asp) (Xia *et al*., 2013), which assesses saturation by comparing the Index of Substitution Saturation (ISS) with a calculated Index of Substitution Saturation (ISSc), results are given in Table 4.5.

Table 4.5 DAMBE analysis of ITS1 *Dactylogyrus* sequences

	Symmetrical tree	Asymmetrical tree
Proportion invariant sites	0.1891	0.1891
Mean H	2.1625	
Standard Error	0.0509	
Hmax	1.8924	
Iss	1.1427	
Iss.c	0.7832	0.5640
T	7.0612	11.3650
DF	1169	1169
Prob (two tailed)	0	9
95% Lower Limit	1.0428	1.0428
95% Upper Limit	1.2426	1.2426

The DAMBE analysis of the *Dactylogyrus* ITS1 sequences produced an ISS value 1.1427 which was considerably greater than the ISSc value of 0.7832. Where the ISS value exceeds the ISSc value, the implication is there is substitution saturation of the nucleotide sequences and they are of poor utility for phylogenetic analysis.

4.4 Discussion

Until the increase in the popularity of the sport of freshwater fishing, cyprinids were regarded of low economic value (Brewster, 2000, 2009, 2014). In 2009, the Environment Agency estimated the coarse fish industry to be valued at over £1 billion annually (www.gov.uk/government/uploads) thus coarse fishing is a vibrant economy, with fish stocks of high value. In the event of outbreaks of fish disease, abiotic factors are usually considered together with viral screening (www.gov.uk/guidance/report-

serious-fish-or-shellfish-diseases), but as noted by Rohlenová *et al.* (2011) *Dactylogyrus* species are quite delicate and may die when the host becomes moribund so the role these monogeneans may play in fish mortalities is overlooked. Rohlenová *et al*. (2011) took blood samples from common carp and found that whilst temperature influenced numbers of *Dactylogyrus* species infecting the fish, these parasites caused immunosuppression in the host fish and whilst not directly causing disease, have the potential to render the fish susceptible to other infectious agents. Rastiannasab *et al*. (2015) also sampled blood from carp infected with *Dactylogyrus* and *Gyrodactylus* and found alteration in the function of liver enzymes, leading to the potential for liver and kidney dysfunction. Whilst these studies have been carried out on a single fish species, it would seem that *Dactylogyrus* species have the potential to influence outbreaks of serious disease in fish and should not be overlooked as part of a fish disease investigation process.

Traditionally, *Dactylogyrus* species have been identified using morphological characters associated with the sclerotized copulatory organ, anchors and hooks but these can prove difficult to visualize due to the orientation of the specimen on the slide, obscuring features which are important for identification and affecting the accuracy of measurements of the copulatory organ, anchors and hooks. Methods used for preserving and staining also affect the dactylogyrids, often causing desiccation and distorting the soft tissues which affects the accuracy of measurements. Preservation in ethanol causes *Dactylogyrus* specimens to become very delicate and shrivelling on exposure to air (pers. obs.), a factor which may have resulted in the failure to extract and amplify DNA because the parasites were not immersed in the extraction reagents.

Morphological examination of *Dactylogyrus* species infecting carp, roach, tench and bream resulted in the tentative identification of four species of dactylogyrid associated with these fish. Using morphological characters, *Dactylogyrus extensus* was identified from carp hosts, where the parasite was observed to be causing gill pathology (Figures 4.1 & 4.6). The dactylogyrids which were sequenced, clustered together as *Dactylogyrus extensus* (Group G, Figures 4.13 – 4.15). The *D. extensus* sequences were taken from the same hosts and localities as the *D. extensus* identified on morphological characters. The combination of molecular and morphological characteristics confirms the identification of *Dactylogyrus extensus* parasitizing carp from Gresford Flash, Ingatestone and Milton Hall in this study. Pool & Chubb first recorded the presence of *D. extensus* in 1987 and considered the species as a probable introduction to the UK. Gibson *et al.* (1996) gave the Nearctic Region as the origin of this species but also stated it has been recorded in the Palearctic, China and South East Asia. *Aquatic Parasite Information* database holds just two records for *D. extensus*, one of which is Pool & Chubb's original publication.

In both morphological and molecular work *D. crucifer* was identified from roach from Bradford on Tone, Somerset, (Figures 4.9 – 4.10 & 4.13 – 4.15, clade B). Šimková *et al.* (2001) found an increasing prevalence of *D. crucifer* in roach in two localities in the Morava river, Czech Republic finding an increase in parasite numbers from April onwards as water temperatures rose and declining in the autumn. Selver *et al.* (2009) investigating helminth parasites of roach in the Kocadere stream, Bursa, Turkey, also found some variation in the numbers of *D. crucifer* infecting roach, most were found from February onwards and peaking in abundance in April. The discrepancy in seasonality may be explained by *D. crucifer* preferring an optimum temperature. The

nearest city to the Morava river locality is Brno, where air temperatures are 10 – 20^{0}C April – August, but air temperatures in Bursa, Turkey are 10 – 20^{0}C during February – April (https://weather-and-climate.com). This data relating to temperature given by Šimková *et al.* (2001) and Selver *et al.* (2009) suggest *D. crucifer* has a preferred temperature range of 10 – 20^{0}C. *Dactylogyrus crucifer* used as part of this study were usually present in low numbers from lacustrine roach, submitted for movement consent during the winter, from November to March. Based on the work of Šimková *et al.* (2001) and Selver *et al.* (2009) *D. crucifer* may have a greater epidemiological impact in the UK when water temperatures are 10 – 20^{0}C, which is overlooked. Coincidentally, the coarse fish industry avoid translocating roach at temperatures above 12^{0}C because of the mortalities this incurs and possibly *D. crucifer* is a contributing factor. Mierzejewska *et al.* (2006) also consider host size plays a role and larger fish carry a greater parasite burden of *Dactylogyrus* species. The relationship between numbers of *D. crucifer* as a function of temperature, stock density or host length is potential for further study in the UK.

Šimková & Morand (2015) consider *D. crucifer* to be a generalist parasite associated with unrelated host species, whereas Gussev *et al.* (2010) regard this species as a specialist parasite of roach and its subspecies, with a distribution in the Palaearctic (Gussev, Gerasev & Pugachev, 2010; Kottelat & Freyhof, 2007). Gibson *et al.* (1996) in their catalogue of *Dactylogyrus* species have records of *D. crucifer* infecting bream, silver bream, rudd, common carp, pike, dace, bleak and vimba bream. Data in API shows records dating to 1965, with roach the only host. Identification of the *D. crucifer* in these records would have been based on morphological characters, with the associated difficulties of positive identification. Use of the ITS1 as a marker for the

molecular identification of *Dactylogyrus* species associated with these species of coarse fish would resolve the host specificity of *D. crucifer.*

The dactylogyrid species associated with tench is of interest as this family of monogeneans does not appear to have been previously reported as present in the UK. BLASTn analysis of the ITS1 sequences indicated the *Dactylogyrus* sequences from the tench host to have a shared identity with *D. amphibothrium* and *D. hemiamphibothrium* (Table 4.3). The NJ, MP and ML analyses comparing sequences from *D. amphibothrium* (GenBank®: AJ564110) and *D. hemiamphibothrium* (GenBank®: AJ564137), consistently placed these dactylogyrid sequences from the tench host on a separate branch (clade E, Figures 4.13 – 4.15). Using identification keys (Galli *et al.*, 2010) these dactylogyrids were identified as *D. tincae*, for which there are no reference sequences published on Genbank®. This is the first time that *D. tincae* has been sequenced.

Gibson *et al.* (1996) list *D. tincae* as native to the Palearctic Region but a literature search indicates little information is available on the distribution of this dactylogyrid species in Europe. In a study of Monogenea infecting fish in Óswin Lake, a shallow, eutrophic water, Mierzejewska *et al.* (2006) found low numbers of *D. tincae* on the tench. Galli *et al.* (2007) report this species of dactylogyrid to be an alien introduction to Italy. In this study, the locality source of the host tench was one of a number of former gravel pits with a myriad of interconnecting waterways. *Dactylogyrus tincae* is most likely an alien species which has been introduced to the UK through the translocation of tench from Europe.

Dactylogyrus zandti is a specialist parasite of *A. brama.* Whilst some specimens were available for morphology, extraction and amplification of DNA was unsuccessful. Turgut, *et al.,*(2006) indicate that *D. zandti* prefers the proximal section of the hemibranch, however, the three specimens identified here were found on the distal, outer hemibranch, usually of gill arches two or three of the bream. Galli *et al.* (2007) identified *D. zandti* in Italy and regard it as an alien species originating from Central Europe, whereas Gibson *et al.* (1996) describe the distribution more generally as the Palaearctic Region, with hosts bream and silver bream. Given the Palaearctic distribution of *D. zandti* and the host distribution in the UK, it is quite possible this dactylogyrid is native but owing to the small size is readily overlooked. *Aquatic Parasite Information* holds no records for *D. zandti.*

In view of the problem of desiccation which had affected the successful extraction of DNA from dactylogyrids only four specimens of *Dactylogyrus* from the gills of rudd, were successfully sequenced but regrettably, none were available for morphological examination. BLASTn analysis of the sequences indicated a shared identity with *Dactylogyrus difformis* Wagener, 1857 and the NJ, MP and ML trees formed a cluster with *D. difformis* and *D. difformoides* from the Czech Republic (clade A, Figures 4.13 – 4.15). Gibson *et al.* (1996) indicated *D. difformis* is of Palearctic origin, with hosts including rudd, roach, common bream, silver bream, dace and carp. However, Galli *et al.* (2010) assert this species is a specialist parasite of rudd and similarly, Šimkova & Morand (2015) are also of the opinion this is a specialist parasite. Turgut et *al.* (2006) identified this species of dactylogyrid from rudd giving a generalized locality of Humberside. *Dactylogyrus difformis* is most likely to be a native parasite.

The results of phylogenetic analysis of ITS1 sequences from *Dactylogyrus* species were ambivalent, the topology varied between the constructed NJ, MP and ML trees which Šimková *et al*. (2004) had also encountered. The analysis of transitions and transversions in the ITS1 sequences using DAMBE 5 (http://www.dambe.bio.uottawa.ca/dambe.asp) and p-distance method demonstrates that substitution saturation has been reached. Blanco-Costa *et al.* (2016) stated that all genes mutate over time but some such as ITS1 may undergo rapid evolution as the unconstrained mutations have little or no impact on the cell biochemistry. These multiple substitutions at the same loci, resulting in substitution saturation mean the ITS1 marker is of little value in reconstructing the phylogeny of *Dactylogyrus.*

The analysis of single nucleotide polymorphism of the sequences using DnaSP (Librado & Rozas, 2009) showed the nucleotide diversity (π), average nucleotide differences and parsimony informative sites and demonstrated the sequences to be heterogeneous. The heterogeneity of ITS1 indicates it is a good marker for the differentiation of *Dactylogyrus* species. The results obtained here using *Dactylogyrus* ITS1 sequences, support the view of Blanco-Costa *et al.* (2016) that ITS1 is a potentially useful marker for intraspecific variation and low level taxonomy. The ITS1 sequences analysed here are regarded as positive identification of the *Dactylogyrus* species.

4.5 Concluding remarks

It is apparent the dactylogyrid fauna parasitizing freshwater fish in the UK has been neglected, largely owing to the difficulty of identification based on morphological characters. In the last 30 years there have been significant changes in fishery management, from overstocking to legitimately and illegally stocked fish from within

the UK and Europe but with common carp dominating in popularity (Brewster, 2000; 2009; 2014). These changes in fishery management are impacting on the structure and diversity of freshwater fish populations which also affects their associated parasites. Studies have shown that dactylogyrids may not be as benign as previously considered, affecting the immune system and liver and kidney function (Rohlenova *et al.* 2011; Rastiannasab, *et al.* 2015). Currently *Dactylogyrus* species are overlooked as part of investigation into outbreaks of fish disease, based on the fragile nature of these parasites but mostly because these monogeneans are notoriously difficult to identify using morphological characters. The use of ITS1 has shown this molecular marker can be utilized for identification of these monogeneans and in the future can be used to produce a comprehensive catalogue of *Dactylogyrus* species present in the UK.

Chapter 5

Cestodes of freshwater fish in the UK

5. 1. Introduction

Morphology is the current method employed for identifying species of cestode but since Chubb *et al.* (1987) the number of species of tapeworm infecting freshwater fish in the UK has increased through the release of non-native fish. Identification of cestodes has proved to be an increasing challenge because of the morphological similarity between species such as *Caryophyllaeus laticeps* and *Khawia sinensis* the latter a non-native parasite, first reported in 1986 (Chubb & Yeomans, 1986). The exotic cestode *K. japonensis,* is already present in Europe (Oros *et al*., 2015) and may already be in the UK, but is of unknown pathogenicity to native fish and is morphologically similar to both *K. sinensis* and *C. laticeps*. The shape of the scolex has traditionally been used as a feature for identifying cestodes, however, the scolex is subject to distortion in formalin fixed specimens (Oros *et al.*, 2010). The anterior limit of the testes and vitelline follicles have been used to compliment scolex shape as additional characters in confirming the identity of Caryophyllidea, but Oros *et al*. (2010) also found some variability in these characters. Using scolex morphology, testes size and distance from the vitelline follicles, Hanzelová *et al.* (2015) identified five different morphotypes of *C. laticeps* associated with different hosts. Morphological characters currently remain the most readily available tool for identifying tapeworms, however accuracy of identification may depend on experience, given that identification keys make no allowance for phenotypic variability (Oros, *et al.*, 2010; pers. obs.).

Such morphological similarity between species of cestode can lead to potential pathogens being overlooked and the introduction of novel or exotic cestodes represents a potential risk to native freshwater fish as the pathogenicity and host specificity is unknown. The emergence of genomics has resulted in the ability to easily extract, amplify and sequence DNA from cestodes, which have been collected during routine screening of fish for movement consent. The DNA sequences extracted from the cestodes may then be compared with other tapeworm sequences which are deposited in the Genbank® database. Although morphological studies on fish cestodes have been carried out in the UK (e.g. Andrews & Chubb, 1984; Chubb *et al.,* 1987) this is the first time the combination of morphology and genomics have been applied to the study of the cestodes associated with freshwater fish in the UK. The aim of the research was to describe the morphological features of commonly encountered cestodes and investigate a molecular marker which can be used to differentiate the species of tapeworm found in UK freshwater fish.

5.2. Materials and methods

5. 2. 1. Collection of cestodes from UK freshwater fish

The fish submitted for movement consent were killed by submersion in an overdose of 2-phenoxyethanol, fish anaesthetic. As part of the dissection procedure, the fish abdominal wall was removed, the intestine cut at the anus and gently teased away from other soft tissues, then for cyprinids, cutting it open along the length to the pharynx. In non-cyprinid species the intestine, stomach and pyloric caeca were also dissected. For fish less than 15cm the digestive tract was removed and dissected under a stereomicroscope. Cestodes found in the intestine were removed and placed

either into hot phosphate buffered saline, before fixing in formalin for histological preparation, or placed directly into 70% ethanol for molecular work.

5. 2. 2. Preparation of cestodes for morphological study

Formalin preserved cestodes used for morphology (Table 5.1) were stained in Langeron's carmine for between 3 – 5 minutes, depending on size, rinsed in 70% ethanol, placed in 5% acid alcohol to destain for 2 – 3 minutes; transferred to 80% ethanol for 10 minutes; the cestodes were then sandwiched between squares of filter paper impregnated with 96% ethanol, leaving the scolex of large cestodes exposed, a cover slip was placed on the uppermost paper, with a light weight to flatten the specimen and the container topped up with 96% ethanol. Flattening the specimen took between 1 to 12 hours depending on size, after flattening the specimens were transferred to absolute alcohol for 10 minutes, before clearing in 10%, 50%, 90% and 100% clove oil or eugenol for 10 minutes in each solution. After clearing specimens were mounted in Numount (Brunel Microscopes, Canada Balsam substitute). Examination of slide material was undertaken using an Olympus CX41 microscope and captured using an Olympus SC30 camera with Cellsens® software.

Table 5.1. Fish hosts from which cestodes were taken for morphology and molecular study; items in bold indicate successful extraction and sequencing of Cox 1 and r28s rDNA

	n			n
Caryophyllaeides fennica	**3**	**Babylon Fish Farm, Hawkenbury**	**22/04/2014**	**3**
Caryophyllaeides fennica	**1**	**Blithfield Reservoir**	**03/05/2014**	**1**
Caryophyllaeides fennica	**2**	**Mill Pond**	**28/03/2014**	**2**
Caryophyllaeides fennica	**1**	**Wades Marsh, Haslemere**	**19/03/2014**	**1**
Caryophyllaeides fennica	3	Ashby Park Lincolnshire	04/06/2014	3
Caryophyllaeides fennica	**1**	**Riverfield Fish Farm, Marden**	**24/10/2008**	**1**
Caryophyllaeides fennica	1	Water Lane Fish Farm, Burton Bradstock	01/11/2014	1
Caryophyllaeides fennica	1	Water Lane Fish Farm, Burton Bradstock	01/11/2014	1
Caryophyllaeus laticeps	**1**	**Mill Pond**	**28/03/2014**	**2**
Caryophyllaeus laticeps	**2**	**Blithfield Reservoir**	**03/05/2014**	**5**
Caryophyllaeus laticeps	**2**	**Blithfield Reservoir**	**08/05/2014**	**4**
Caryophyllaeus laticeps	**2**	**QMR**	**23/05/2014**	**2**
Khawia sinensis	**2**	**Iheart, Cawood, Selby**	**11/05/2014**	**2**
Khawia sinensis	**2**	**Gresford Flash**	**06/05/2014**	**2**
Khawia sinensis	2	Earsby Farm, Spilsby	16/12/2014	2
Khawia sinensis	3	Hall Farm Reservoir, Woodham Mortimer	01/11/2014	3
Khawia sinensis	2	Greenhalgh, Preston	14/11/2015	2
Proteocephalus percae	**1**	**Earsby Farm, Spilsby**	**16/12/2014**	**1**
Schyzocotyle acheilognathi	1	Environment Agency -10/079/11		1
Schyzocotyle acheilognathi	1	Rye Meads STW, Hoddesdon	22/11/2015	1
***Eubothrium* sp.**	**1**	**Environment Agency – 10/079**		**1**
***Hepatoxylon* sp.**	**1**	**Environment Agency - 12/003**		**1**
Monobothrium wageneri	1	Environment Agency		1

5. 2. 3. DNA extraction and PCR amplification

The fish hosts from which cestodes were removed and the numbers used to extract DNA are given in Table 5.1. Extraction of DNA was undertaken using a Qiagen DNeasy™ kit, following the manufacturer's instructions. The mitochondrial COX1 and cellular ribosomal small sub-unit r28s rDNA were amplified by polymerase chain reaction (PCR), using primers COX1: Forward, CFCYT2 (ACTAAGTCCTTTTCAAAA); Reverse, CRCYT2 (CCAAAAACCAAAACAT) and r28s: Forward, LSU (TACGTCGACCCGCTGAAY); Reverse, 1500R GCTATCCTGAGGGAAACTTCG) using the Veriti 96 well thermal cycler PCR machine, in the following cycle, - 1 minute at 50^0C; 5 minutes at 94^0C; 30 cycles of 1 minute at 94^0C, 1 minute at 50^0C, 2 minutes at 72^0C, and a final extension of 10 minute at 72^0C. Following DNA amplification, 5µl of the resultant amplicons were visualised through electrophoresis on 1% agarose gels

stained with GelRed (Bioline). Not all extractions were successful, thus, only positive 20µl of amplicon samples were submitted for sequencing at the DNA Sequencing Facility of the Natural History Museum, London, using fluorescent dye terminator sequencing kits (Applied Biosystems™), these reactions were then run on an Applied Biosystems 3730KL automated sequencer.

5. 2. 4. Assembly of Caryophyllidea and Bothriocephalidea cox1 and r28s rDNA fragments, molecular identification of species and phylogenetic analysis

Fifteen cox1 and 12 r28s Caryophyllidea nucleotide sequences were successfully extracted and amplified. Despite several attempts it was not possible to generate cox1, Bothriocephalidea PCR products. Three Bothriocephalidea r28s nucleotide sequences, EA 12/003 EA10/079 and Pp Earsby Farm, Spilsby were successfully extracted and amplified. The Caryophyllidea cox1 and r28s and Bothriocephalidea r28s sequences were manipulated and edited utilizing BioEdit 7.2.5, then compared with other cox 1 and r28s cestode sequences held in the GenBank® genetic sequence database, using the Basic Local Alignment Search Tool (BLASTn) (www.ncbi.nlm.nih.gov) for preliminary, molecular identification of species.

For comparison, a further 27 cox 1, 39 r28s Caryophyllidea and 42 Bothriocephalidea sequences published on GenBank® (www.ncbi.nlm.nih.gov/nuccore/) (Tables 5.2 - 5.4), were accessed and aligned with the extracted sequences using MUSCLE sequence alignment tool (http://www.ebi.ac.uk). The Gblocks programme was used to remove any ambiguities in the Caryophyllidea r28s sequences (http://molevol.cmima.csic.es/castresana/Gblocks.html) (Castresana, 2000). The

selection of the Bothriocephalidea r28s reference sequences was based on those analysed by Brabec *et al* (2015).

Table 5.2. Caryophyllidea cox 1 sequences downloaded from GenBank®

Species	Host	Accession No.	Geographic Origin
Diphyllobothrium latum (Out Group)	*Perca fluviatilis*	GU997614	Switzerland
Caryophyllaeus brachycollis	*Barbus meridionalis*	JQ034064	Slovakia
Caryophyllaeides fennica	*Rutilus rutilus*	JQ034062	Slovakia
Caryophyllaeides fennica	*Leuciscus leuciscus*	JQ034059	Finland
Caryophyllaeides fennica	*Leuciscus leuciscus*	JQ034057	Finland
Caryophyllaeides fennica	*Leuciscus leuciscus*	JQ034052	Finland
Caryophyllaeus laticeps	*Rutilus rutilus*	AF286911	Switzerland
Caryophyllaeus laticeps	*Abramis brama*	JQ034070	Slovakia
Caryophyllaeus laticeps	*Abramis sapa*	JQ034077	Slovakia
Caryophyllaeus laticeps	*Abramis brama*	JQ034071	Slovakia
Caryophyllaeus laticeps	*Cyprinus carpio*	JQ034068	Slovakia
Caryophyllaeus laticeps	*Cyprinus carpio*	JQ034067	Slovakia
Caryophyllaeus laticeps	*Cyprinus carpio*	JQ034066	Slovakia
Glaridacris catostomi	*Catostomid catfish*	JQ034088	USA
Glaridacris commersoni	*Catostomus commersoni*	JQ034090	USA
Hunterella nodulosa	*Catostomus commersoni*	JQ034091	USA
Hunterella nodulosa	*Catostomus commersoni*	JQ034094	USA
Hunterella nodulosa	*Catostomus commersoni*	JQ034095	USA
Hunterella nodulosa	*Catostomus commersoni*	JQ034093	USA
Hunterella nodulosa	*Catostomus commersoni*	JQ034092	USA
Khawia japonensis	*Cyprinus carpio*	JN004225	Japan
Khawia sinensis	*Cyprinus carpio*	JN004232	China
Khawia sinensis	*Cyprinus carpio*	JN004231	Japan
Khawia sinensis	*Cyprinus carpio*	JN004228	Slovakia
Promonobothrium hunteri	*Catostomus commersoni*	JQ034110	USA
Promonobothrium hunteri	*Catostomus commersoni*	JQ034109	USA
Wenyonia virilis	*Synodontis schall*	JQ034111	Sudan

Table 5.3. Caryophyllidea r28s sequences downloaded from GenBank®

Species	Host	Accession No.	Geographic Region
Diphyllobothrium latum (Out Group)	*Gymnocephalus cernuus*	DQ925326	Russia
Archigetes sieboldii	*Gnathopogon elongatus*	EU343736	Japan
Caryophyllaeus brachycollis	*Barbus meridionalis*	JQ034120	Slovakia
Caryophyllaeides fennica	*Leuciscus leuciscus*	JQ034118	Finland
Caryophyllaeus laticeps	*Rutilus rutilus*	AY157180	Switzerland
Caryophyllaeus laticeps	*Abramis sapa*	JQ034122	Slovakia
Caryophyllaeus laticeps	*Cyprinus carpio*	JQ034121	Slovakia
Caryophyllaeus laticeps	*Abramis brama*	JQ034123	Slovakia
Glaridacris catostomi	*Catostomus commersoni*	JQ034126	USA
Hunterella nodulosa	*Catostomus commersoni*	JQ034127	USA
Hunterella nodulosa	*Catostomus commersoni*	AF286912	USA
Khawia baltica	*Tinca tinca*	JN004266	Czech Republic
Khawia japonensis	*Cyprinus carpio*	JN004258	Japan
Khawia parva	*Carassius auratus*	JN004267	Russia
Khawia rossittensis	*Carassius auratus*	JN004260	Slovakia
Khawia rossittensis	*Carassius auratus*	JN004259	Japan
Khawia saurogobii	*Saurogobio dabryi*	JN004262	China
Khawia sinensis	*Cyprinus carpio*	JN004264	Japan
Khawia sinensis	*Cyprinus carpio*	JN004265	China
Khawia sinensis	*Cyprinus carpio*	EU343740	United Kingdom
Monobothrium wageneri	*Tinca tinca*	KM507586	USA*
Promonobothrium hunteri	*Hypentelium nigricans*	KM507583	USA
Promonobothrium hunteri	*Catostomus commersoni*	JQ034131	USA
Promonobothrium ingens	*Carpiodes cyprinus*	KM507582	USA
Promonobothrium minytremi	*Minytrema melanops*	KM507585	United Kingdom**
Promonobothrium ulmeri	*Minytrema melanops*	KM507584	USA
Wenyonia acuminata	*Synodontis acanthomias*	HQ848519	Democratic Republic of Congo
Wenyonia minuta	*Synodontis schall*	HQ848518	Kenya
Wenyonia minuta	*Synodontis frontosa*	HQ848507	Sudan
Wenyonia minuta	*Synodontis schall*	HQ848508	Kenya
Wenyonia minuta	*Synodontis schall*	HQ848503	Sudan
Wenyonia youdeoweii	*Synodontis schall*	HQ848496	Sudan
Wenyonia virilis	*Synodontis schall*	HQ848522	Kenya
Wenyonia virilis	*Synodontis schall*	HQ848517	Kenya
Wenyonia virilis	*Synodontis frontosa*	HQ848521	Kenya
Wenyonia virilis	*Synodontis schall*	HQ848516	Sudan
Wenyonia virilis	*Synodontis schall*	HQ848515	Sudan
Wenyonia virilis	*Synodontis geledensis*	HQ848520	Kenya
Wenyonia virilis	*Synodontis schall*	JQ034131	Sudan

* The locality for *Monobothrium wageneri* , Accession Number KM507586 is published on Genbank® as the USA, Scholz *et al.* (2015) give the geographic location as the UK. According to Hoffmann (1999) *M. wageneri* is not present in the USA.
** The locality for *Promonobothrium minytremi* Accession Number KM507585 is published on Genbank® as the UK, Scholz *et al.* (2015) give the geographic location as the USA, a species parasitic on catostomid catfish which are native to North America

Whilst there was a large number of caryophyllid, r28s reference sequences available on Genbank®, there was only a single sequence available for *C. fennica* but in combination with the cox 1 sequences this was considered adequate for identification of this species.

Table 5.4. Bothriocephalidea r28s sequences downloaded from GenBank®

Parasite	Host	Accession No.	Geographic location
Caryophyllaeus laticeps (Out Group)	*Abramis brama*	JQ034070	Slovakia
Eubothrium tulipae	*Ptychocheilus oregonensis*	KR780904	USA
Abothrium gadi	*Gadus morhua*	AF286945	UK
Anantrum tortum	*Synodus foetens*	KR780883	USA
Bathybothrium rectangulum	*Barbus*	DQ925321	Czech Republic
Bothriocephalus australis	*Platycephalus aurimaculatus*	KR780886	Australia
Bothriocephalus celineae	*Cephalopholis aurantia x spiloparaea*	KR780921	New Caledonia
Bothriocephalus cf carangis	*Uraspis uraspis*	KR780888	Indonesia
Bothriocephalus claviceps	*Anguilla anguilla*	DQ925323	Czech Republic
Bothriocephalus cuspidatus	*Sander vitrius*	KR780908	USA
Bothriocephalus manubriformis	*Istiophorus platypterus*	KR780887	Maldives
Bothriocephalus scorpii	*Myxocephalus scorpius*	AF286942	UK
Bothriocephalus timii	*Cottoperca gobio*	KR780885	Argentina
Clestobothrium crassiceps	*Merluccius merluccius*	KR780884	UK, North Sea
Clestobothrium cristinae	*Merluccius hubbsi*	KR780901	Argentina
Clestobothrium splendidum	*Merluccius australis*	KR780920	Argentina
Diphyllobothrium latum	*Gymnocephalus cernuus*	DQ925326	Russia
Eubothrium crassum	*Salmo salar*	KR780880	Scotland
Eubothrium fragile	*Alosa fallax*	KR780899	UK
Eubothrium rugosum	*Lota lota*	KR780914	Russia
Eubothrium salvelini	*Salvelinus alpinus*	KR780916	Scotland
Hepatoxylon trichiuri	*Taractes rubescens*	FJ572943	Indonesia
Ichthybothrium ichthybori	*Ichthyborus besse*	JQ811837	Sudan
Kirstenella gordoni	*Heterobranchus bidorsalis*	JQ811838	Ethiopia
Marsipometra hastata	*Polyodon spathula*	AY584867	USA
Marsipometra parva	*Polyodon spathula*	KR780909	USA
Oncodiscus sauridae	*Saurida tumbil*	KR780893	Indonesia
Parabothrium bulbiferum	*Pollachius pollachius*	KR780915	Norway
Petrocephalus ganapattii	*Saurida tumbil*	KR780892	Indonesia
Polyonchobothrium polypteri	*Polypterus senegalensis*	JQ811836	Sudan
Proteocephalus fluviatilis	*Micropterus dolmieu*	KP729390	Japan
Proteocephalus percae	*Perca fluviatilis*	JQ639166	Germany
Proteocephalus pinguis	*Esox lucius*	KP729395	USA
Ptychobothrium belones	*Strongylura leiura*	DQ925333	Pacific Ocean
Schyzocotyle acheilognathi	*Homo sapiens*	HM367067	France
Schyzocotyle nayarensis	*Barilius sp.*	KR780922	India
Senga lucknowensis	*Mastacembalus armatus*	KR780891	Viet Nam
Senga magna	*Siniperca chuatsi*	KR780913	Russia
Senga visakhapatnamensis	*Channa punctata*	KR780890	India
Tetracampos ciliotheca	*Clarias gariepinus*	JQ811835	Ethiopia
Triaenophorus crassus	*Coregonus lavaretus*	DQ925334	Germany
Triaenophorus nodulosus	*Esox lucius*	KR780879	Scotland
Triaenphorus stizostedionis	*Sander vitreus*	KR780900	USA

Phylogenetic analysis was undertaken using MEGA version 6 (Tamura *et al.*) with computation of neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) on 49 cox 1 and 48 r28s Caryophyllidea nucleotide sequences and 45 r28s Bothriocephalidea nucleotide sequences. The distance based NJ analysis of all

cestode sequences was based on the Jukes-Cantor method using a 500 replicate bootstrap test, clustering related taxa together as a percentage of the replicates.

Character based phylogenetic trees were constructed using MP which analyses the data for the minimum number of character based changes in a particular position to create the best fit, trees of the sequences were achieved using the Subtree-Pruning-Regrafting (SPR) algorithm calculated on 500 bootstrap replicates. Maximum Likelihood analysis is based on the likelihood of those character states occurring in that particular evolutionary configuration in Mega 6, models used are given in Table 5.5.

Table 5.5 Models used in MEGA 6 ML analysis

Marker	Model
Caryophyllidea cox 1	Hasegawa-Kishino-Yano over a discrete Gamma distribution
Caryophyllidea r28s	model Kimura-2 parameter over a discrete Gamma distribution
Bothriocephalidea r28s	model Hasegawa-Kishino-Yano over a discrete Gamma distribution

The models used in the ML analysis, obtained the lowest Bayesian Information Criterion, which creates a phylogenetic tree based on the sequence data and chosen substitution model (Hall, 2011) calculated on 500 bootstrap replicates.

The outgroup sequence for Caryophyllidea cox 1 and r28s was *Diphyllobothrium latum* (GenBank® GU997614) and for r28s Bothriocephalidea, *Caryophyllaeus laticeps* (GenBank® JQ034123).

5. 2. 5. Markers for species identification, inter- and intra-species molecular diversity

Single nucleotide polymorphisms are a locus on the genome where the nucleotide sequences vary between species, forming a useful measure for molecular diversity and species identification and were used for comparison of Caryophyllidea Cox 1 and r28s and Bothriocephalidea r28s. Analysis of sequence polymorphism was performed using DnaSP 5.10 (http://ub.esp/DnaSP) (Librado & Rozas, 2009), calculating the segregating sites (S), nucleotide diversity (π), average pairwise nucleotide differences (K). The uncorrected pairwise distance was estimated using MEGA 6 with frequency of transitions (TS) and transversions (TV) for Caryophyllidea Cox 1 and r28s and Bothriocephalidea r28s. Substitutional changes were estimated using DAMBE 5 (http://www.dambe.bio.uottawa.ca/dambe.asp) (Xia, 2013) because substitution saturation of the sequences results in a poor phylogenetic signal.

5. 3. Results

5. 3. 1. Morphology of cestodes identified from UK species of freshwater fish

Since Chubb *et al.* (1987) produced keys to species of cestode infecting freshwater fish in the UK, the number of species has increased as a consequence of anthropomorphic fish translocations. The literature assisting identification is quite scattered and may confusingly include species not yet present in the UK (Scholz *et*

al., 2001; Oros, *et al.*, 2010; Scholz *et al.,* 2011). Morphology of cestodes found infecting freshwater fish during routine examination for movement consent are described here but this is an incomplete representation of species found in the UK, as this majority of work is largely conducted on species of cyprinid, which are the most popular with anglers.

5. 3. 2 Caryophyllidea morphology

The Caryophyllidea are characterized by a fusiform monozoic body, tapering posteriorly, scolices with a simple morphology but variable shape, inner longitudinal muscle well developed, single male and female genitalia, testes ovoid in shape, cortical or medullary and reaching the anterior part of the small cirrus sac, which is at the posterior end of the body; irregularly shaped vitelline follicles may also be cortical or medullary, surrounding the testes, laterally and medially and posterior to the ovary (Mackiewicz, 1994; Williams & Jones, 1994; Oros *et al.*, 2010, Hanzolová *et al.*, 2015).

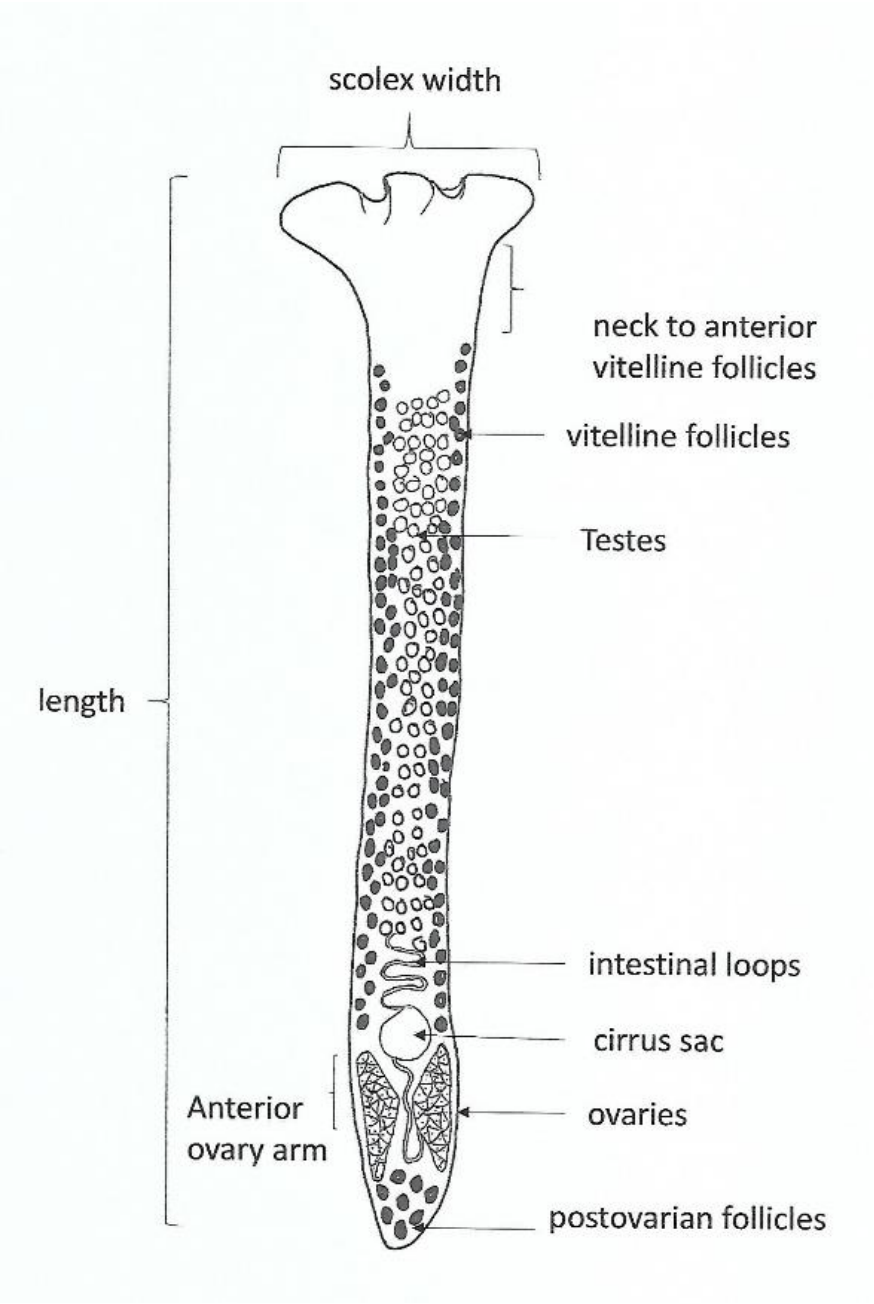
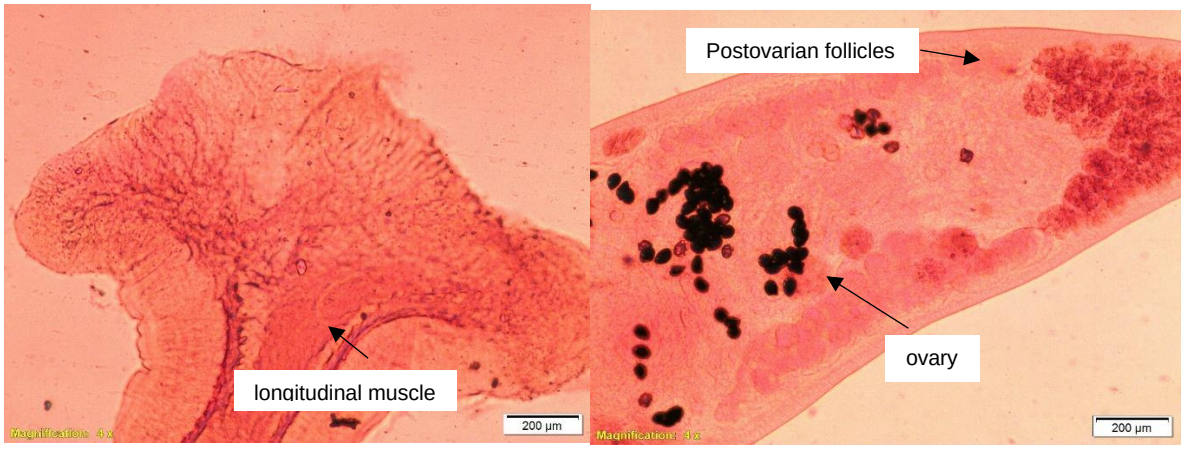
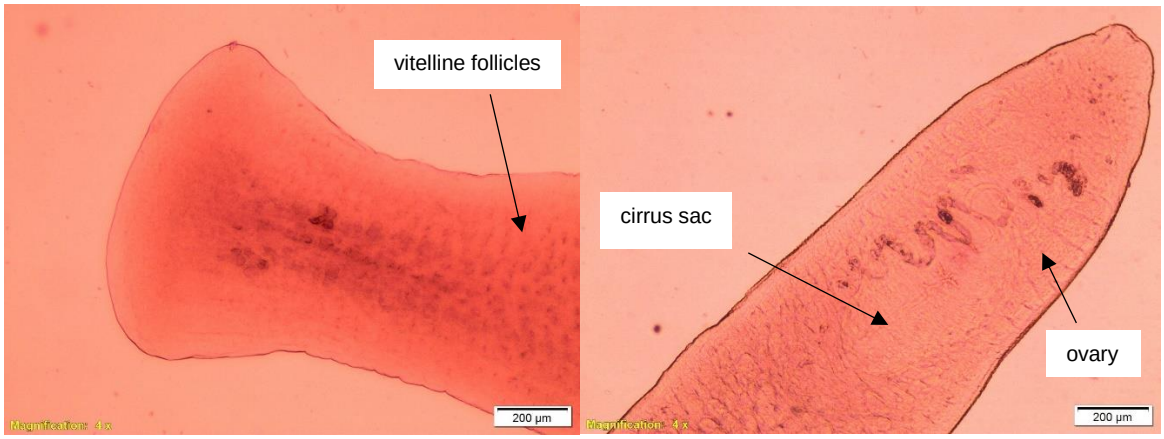


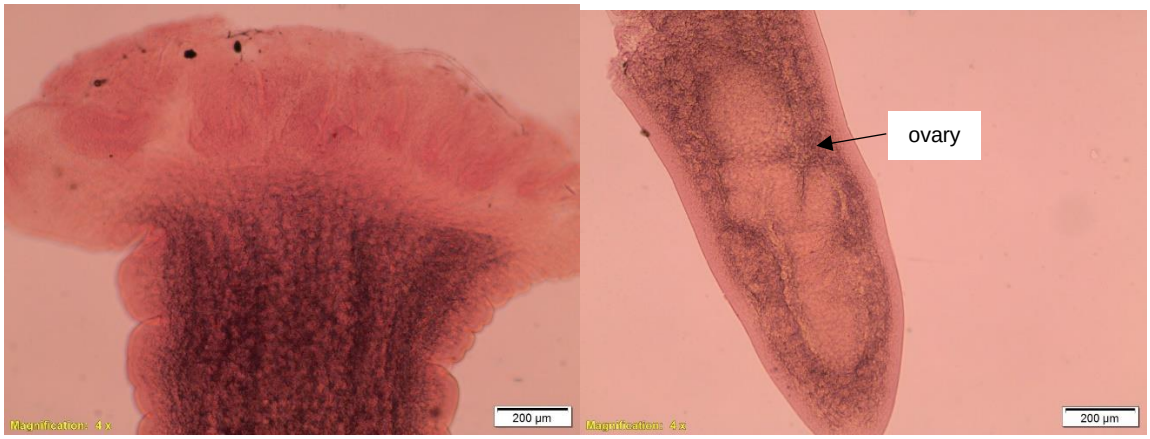
Figure 5.1 Diagram of Caryophyllidea cestode and measurements. The width of the cestode was made at the widest part of the body (B. Brewster)



Caryophyllaeus laticeps



Caryophyllaeus fennica scolex - although the scolex appears wider than the body this is an artefact of preservation



Khawia sinensis

Figure 5.2 Caryophyllidea scolices and posterior body, morphological features used for identification of species (Photographs B. Brewster)

***Caryophyllaeus laticeps* (Pallas, 1878) (Figure 5.2)**

Length (mm) 10.57 – 17.82; width (mm) 1.01 – 1.69; scolex width (mm) 1.21 – 2.27 neck width (µm) 440 – 930; anterior ovary arm (µm) 346.12 – 521.39; neck to anterior vitelline follicles (mm) 0.7 – 2.0

The body is fusiform, and is very slightly widest in the posterior third; the scolex is wider than the body and the margins are finely scalloped; the neck is long; the inner longitudinal muscle is well developed; anteriorly, the vitelline follicles originate at the base of the neck and the testes below them but both are combined in the medullary; the cirrus sac is round; the ovaries are 'H' shape and post ovarian follicles are small.

Hosts: Roach, bream, crucian carp, dace, chub, carp (*Aquatic Parasite Information*)

Comments: Bream were host to all specimens of *C. laticeps* identified here. Hanzelová *et al.* (2015) state bream as the type-host but that all cyprinids are potential hosts for this species of caryophyllid and identified five different host morphotypes:-

morphotype 1 associated with a number of cyprinid species, including bream; morphotype 2 *Vimba melanops, V. vimba*

morphotype 3 *C. carpio*

morphotype 4 *Chondrostomus nasus*

morphotype 5 *Abramis brama, Ballerus sapa*

The *C. laticeps* examined from bream did not conform to one particular morphotype described by Hanzelová *et al.* 2015, the scoleces most closely resembled those illustrated for morphotype 4, the length of the neck and position of the anterior vitelline

follicles and testes to morphotype 1 and the posterior body to morphotype 1. Based on comparison of measurements the body length of UK specimens is within the range of morphotype 3, body width morphotype 4, scolex width morphotype 1, neck width morphotypes 1 and 3, neck to anterior vitelline follicles morphotype 4. These discrepancies in the measurements between the morphotypes identified by Hanzelová *et al.* (2015) indicate there is greater morphological variation in the specimens examined here, possibly associated with host size as noted by Chubb (1982) for *Caryophyllaeides fennica.*

Caryophyllaeus laticeps causes compression of the host intestinal epithelium, with larger worms causing cellular damage and rupturing the brush border (Karanis & Taraschewski, 1993). Pathology associated with *C. laticeps* is most significant in common bream, where large numbers of the parasite are attached to the intestine (Karanis & Taraschewski, 1993, Williams & Jones, 1994) Schaperclaus (1992) reports heavy infections with this cestode caused carp mortalities by occluding the intestine.

***Caryophyllaeides fennica* (Scheider, 1902) (Figures 5.2)**

Length (mm) 1.62 – 13.89; width (mm) 0.14 – 1.58; neck to anterior vitelline follicles (mm) 0.4 – 1.2

The body is cylindrical, tapered posteriorly, the scolex is blunt, undifferentiated and the same width as the anterior body; the inner longitudinal muscle is poorly developed; the testes are medullary, originating with the vitelline follicles just posterior to the scolex; uterine coils are compressed; the ovary is shaped like an inverted ‘A’.

Hosts: Barbel, chub, roach, dace, gudgeon, minnow, bream, rudd (*Aquatic Parasite Information*)

Comments: Chubb (1982) identified five stages of maturation in *C. fennica* host roach from Llyn Tegg i) genitalia absent in the smallest worms of 1 – 4 mm; ii) genitalia appearing in worms of 3 – 4mm; iii) genitalia developed iv) eggs being produced, 6 – 10mm; v) eggs present >10mm, the immature worms were found March, May, June, September and October and size of mature worms varied with host size. The specimens of *C. fennica* examined here were at stages i) genitalia absent and v) mature worms in June and in April, October and November, which concurs with Chubb (1982) that seasonality is absent in this cestode in the UK.

Ellenby & Smith (1996) report that as the incidence of infection of *C. fennica* in roach is low, this species causes little harm and there is no significant pathology.

***Khawia sinensis* Hsü 1935 (Figure 5.2)**

Length (mm) 5.82 – 48.9; width (mm) 0.54 – 2.26; scolex width (mm) 0.64 – 2.72; neck width (mm) 0.54 – 1.41; neck to anterior vitelline follicles (mm) 0.7 – 7.5

A large cestode with a cylindrical body, tapering posteriorly, the scolex is well developed with strongly scalloped margins; the vitelline follicles originate posterior to the neck, the testes are medullary and originate below the vitelline follicles; testes and vitelline follicles are combined in the medulla; cirrus sac is oval; a few vitelline follicles associated with the uterine loops; the ovary is ‘H’ shaped, although the posterior lobes may just touch each other; post ovarian vitelline follicles present.

Hosts: Common carp, ghost carp, carp x crucian carp hybrids, tench (*Aquatic Parasite Information*)

Comments: Small specimens are very similar to *C. laticeps* in appearance, although the inner longitudinal muscle is not as well developed in *K sinensis* and the two species differ in the shape of the ovaries.

5. 3. 3. Bothriocephalidea morphology

These are segmented cestodes with very diversely shaped scolices and associated with both marine and freshwater fish species. *Proteocephalus percae*, *Eubothrium* sp. and *Hepatoxylon* sp. used in this study comprised single specimens which were prepared for molecular work.

***Schyzocotyle acheilognathi* (Yamaguti, 1934) (Figure 3.15)**

Scolex length (µm) 646.99; scolex widest point (µm) 708.43; bothria (µm) 484.93; proglottid length (µm) 270.13; proglottid width (µm) 496.28

A large, segmented cestode, with a characteristic, heart-shaped scolex with two deep

Hosts: carp, grass carp, roach, crucian carp, goldfish (*Aquatic Parasite Information*)

Comments: *Schyzocotyle acheilognathi* is a non-native cestode, introduced from Asia and which has been established in the UK for over 30 years and is euryxenous, Scholtz *et al.* (2012) notes that it has been reported from approximately 200 species of freshwater fish. Other species of non-native cestode such as *K. sinensis* and *Atractolytocestus huronensis* which are parasites of carp, have become established and been widely disseminated in the UK, whereas the distribution of *S. acheilognathi* has remained localized. The morphology of *S. acheilognathi* is very characteristic,

whereas differentiation of caryophyllids can prove difficult. It is most likely the distinctive features of *S. acheilognathi* have resulted in ease of identification and successful imposition of movement restrictions on fish populations which are host to this tapeworm.

5. 3. 4. Sequence assembly and initial comparison of species and phylogenetics based on Caryophyllidea cox 1, r28s and Bothriocephalidea r28s

A preliminary comparison of the extracted and amplified sequences was undertaken using BLASTn analysis, which identifies similar nucleotide sequences held in the Genbank® genetic sequence database. The result of the BLASTn analysis of Caryophyllidae cox 1 sequences corresponded with the initial identification of the specimens using morphological characters (Table 5.6). The BLASTn analysis of the Caryophyllidea r28s sequences for *Caryophyllaeus laticeps*, *Caryophyllaeides fennica* and *Khawia sinensis* 16 and 17 Iheart (carp farm), Selby, corresponded to identifications using morphological characters (Table 5.7). Sequence reference '13 Riverfield FF' was identified as *Caryophyllaeus fimbriceps* based on morphological characters but the cox 1 sequence BLASTn analysis identified this cestode as *Caryophyllaeides fennica*. The r28s '13 Riverfield FF' sequence was very short, such small nucleotide sequences can be matched to a variety of organisms and the BLASTn analysis gave a 76% comparison with *Khawia parva* which is unlikely, therefore this sequence was excluded from the phylogenetic analysis. Three other r28s sequences produced some unusual results from BLASTn analysis; the sequence labelled '18 Gresford Flash' produced a comparison with *Tetracampos ciliotheca* which is a cestode but included in the Bothriocephalidae (Brabec *et al.* 2015) which are

segmented tapeworms, whereas the specimen from a carp at Gresford Flash was morphologically an unsegmented, typical caryophyllid. The corresponding Gresford Flash cox 1 sequence of this cestode produced a BLASTn analysis of 85% comparison with *Khawia sinensis.* The '18 Gresford Flash' r28s sequence was retained, although should have been excluded from the phylogenetic analysis. Sequence reference 'EA Mw' was a cestode donated by the Environment Agency, identified as *Monobothrium wageneri*, however, the BLASTn analysis resulted in an 87% similarity with *Dactylogyrus extensus*, a monogenean. This r28s sequence of *M. wageneri* was extremely short and difficult to match with nucleotide sequences held on GenBank®, using BLASTn, regrettably no DNA was visualized for the cox 1 amplicon for EA Mw. Although *M. wageneri* is of interest because it is an exotic cestode introduced to the UK, the sequence was eliminated from further phylogenetic analysis, because of the short length, which would have given unreliable results.

Table 5.6. Cestode cox 1 sequence identity using BLASTn analysis

Reference COX 1	BLASTn result	BLASTn shared identity
a Blithfield bream	*Caryophyllaeus laticeps*	99%
b Blithfield bream	*Caryophyllaeus laticeps*	99%
c Blithfield bream	*Caryophyllaeus laticeps*	99%
f Mill Pond bream	*Caryophyllaeus laticeps*	97%
g QMR bream	*Caryophyllaeus laticeps*	98%
e Mill Pond Bream	*Caryophyllaeus laticeps*	91%
h Babylon FF roach	*Caryophyllaeides fennica*	88%
j Blithfield roach	*Caryophyllaeides fennica*	94%
k Mill Pond roach	*Caryophyllaeides fennica*	89%
l Wades Marsh roach	*Caryophyllaeides fennica*	93%
o Riverfeld FF chub	*Caryophyllaeides fennica*	94%
p Iheart, Selby carp	*Khawia sinensis*	93%
q Iheart, Selby carp	*Khawia sinensis*	88%
r Gresford Flash carp	*Khawia sinensis*	85%
s Gresford Flash carp	*Khawia sinensis*	87%

Table 5.7. Cestode r28s sequence identity using BLASTn analysis; extracted sequences in bold are Bothriocephalidea

Reference r28s	BLASTn result	BLASTn shared identity
5 Mill Pond bream	*Caryophyllaeus laticeps*	99%
6 Mill pond bream	*Caryophyllaeus laticeps*	96%
8 Babylon FF roach	*Caryophyllaeides fennica*	97%
9 Babylon FF roach	*Caryophyllaeides fennica*	99%
10 Blithfield roach	*Caryophyllaeides fennica*	97%
11 Mill Pond roach	*Caryophyllaeides fennica*	99%
12 Wades Marsh roach	*Caryophyllaeides fennica*	97%
13 Riverfield FF chub	*Khawia parva*	76%
16 Iheart, Selby carp	*Khawia sinensis*	97%
17 Iheart, Selby, carp	*Khawia sinensis*	97%
18 Gresford Flash carp	*Tetracampos ciliotheca*	91%
20 Gresford Flash carp	*Khawia sinensis*	99%
EA Mw	*Dactylogyrus extensus*	87%
EA -12/003	***Hepatoxylon trichiuri***	**92%**
EA 10/079	***Eubothrium crassum***	**89%**
Pp Earsby Farm, Spilsby	***Proteocephalus pinguis***	**83%**

Extraction and amplification of DNA from EA 12/003 EA10/079 and Pp Earsby Farm, Spilsby was successful and BLASTn analysis of the resulting r28s sequences was undertaken (Table 5.7). The r28s sequence for sample reference, EA 12/003 produced a 92% comparison with *Hepatoxylon trichiuri* (Trypanoryncha), a marine cestode whose plerocercoids have been reported from a variety of different teleosts, including Atlantic salmon, elasmobranchs and the giant squid *Architeuthis dux* (Pippy, & Aldrich, 1969; Waterman & Sin, 1991; Mladineo, 2006). BLASTn analysis of the r28s sequence for EA 10/0079 resulted in an 89% comparison with *Eubothrium crassum*. The sample 'Pp Earsby Farm, Spilsby' was identified using morphological characters as *Proteocephalus percae*, however, BLASTn analysis produced an 83% comparison with *P. pinguis*, a proteocephalid from North America whose hosts include *Esox lucius* and *E. reticulatus* (www.eol.org).

5.3.5 Caryophyllidea phylogeny

The number of Caryophyllidea cox 1, r28s and reference sequences were sufficient for phylogenetic analysis. Phylogenetic trees NJ, MP and ML were constructed for Caryophyllidea cox 1 and r28s sequences extracted from the UK specimens, together with those downloaded from GenBank®, resulting trees are given in Figures 5.3 - 5.8. The resulting phylogenetic analysis for the Caryophyllidea cox 1 sequences produced trees congruent for MP and ML, although both were incongruent with the NJ tree. None of the trees produced from the Caryophyllidea r28s sequences were congruent. Both the Caryophyllidea cox 1 and r28s formed sequences consistently grouped together (Table 5.8), although there were slight differences in the arrangement between the cox1 and r28s clades, shown below in bold.

Table 5.8. Clades formed in phylogenetic analysis of Caryophyllidea cox 1 and r28s

Clade	cox 1	r28s
A	*Caryophyllaeus laticeps, C. brachycollis, Khawia baltica*	*Caryophyllaeus laticeps, C. brachycollis, Khawia baltica*
B	*Promonobothrium hunteri,* ***Glaridacris catostomi****, Wenyonia virilis, Hunterella nodulosa*	*Promonobothrium ingens, Promonobothrium ulmeri, Promonobothrium hunteri, Promonobothrium minytrema, Hunterella nodulosa*
C	*Khawia rossitensis K. parva* and *K. japonensis*	*Khawia rossitensis K. parva* and *K. japonensis*
D	*Caryophyllaeides fennica*	*Caryophyllaeides fennica,* ***Glaridacris catostomi***
E	*Khawia sinensis* and *K. saurogobii*	*Khawia sinensis* and *K. saurogobii*

The clade comprising *Wenyonia* species form a discreet group of African species, representative of a separate biogeographic region from the Palaearctic

Caryophyllidea. Although all phylogenetic trees for Caryophyllidea cox 1 and r28s sequences are incongruent with those given by Scholz *et al.* (2015) in their ML tree, the five clades identified here and the *Wenyonia* species clade are in agreement with the tree produced by these authors.

The r28s sequence '*Khawia sinensis* (18) Gresford Flash' and cox 1 '*Khawia sinensis* (r) Gresford Flash', are from the same extraction solution. The BLAST analysis indicated this r28s sequence was similar to a sequence for *Tetracampos ciliotheca* a bothriocephalid cestode. The morphology of the specimen was typical of Caryophyllidea and had been previously identified as *Khawia sinensis* on the basis of morphological characters. The molecular sequence in question was short, making comparative alignments difficult, on this basis, the branch representing the r28s sequence, '*Khawia sinensis* (18) Gresford Flash', was disregarded.

The position of *Monobothrium wageneri* is unresolved in all Caryophyllidea r28s phylogenetic trees.

5.3.6 Bothriocephalidea phylogeny

The reference and three successfully extracted Bothriocephalidea sequences were sufficient for phylogenetic analysis. Phylogenetic trees NJ, MP and ML were constructed for the Bothriocephalidea r28s sequences extracted from the UK specimens, together with those downloaded from GenBank®. Phylogenetic trees NJ, MP and ML were constructed for the bothriocephalid sequences (Figures 5.9 – 5.11), whilst the trees are incongruent, four clades were repeated:

Clade A including representative species of Bothriocephalidae

Clade B which includes representative species of Hepatoxylidae, Diphyllobothriidae and Proteocephalidae

Clade C Triaenophoridae

Clade D the genus *Eubothrium*, included in the family Triaenophoridae

The position of the species *Parabothrium bulbiferum* and *Abothrium gadi* are unresolved in all three trees.

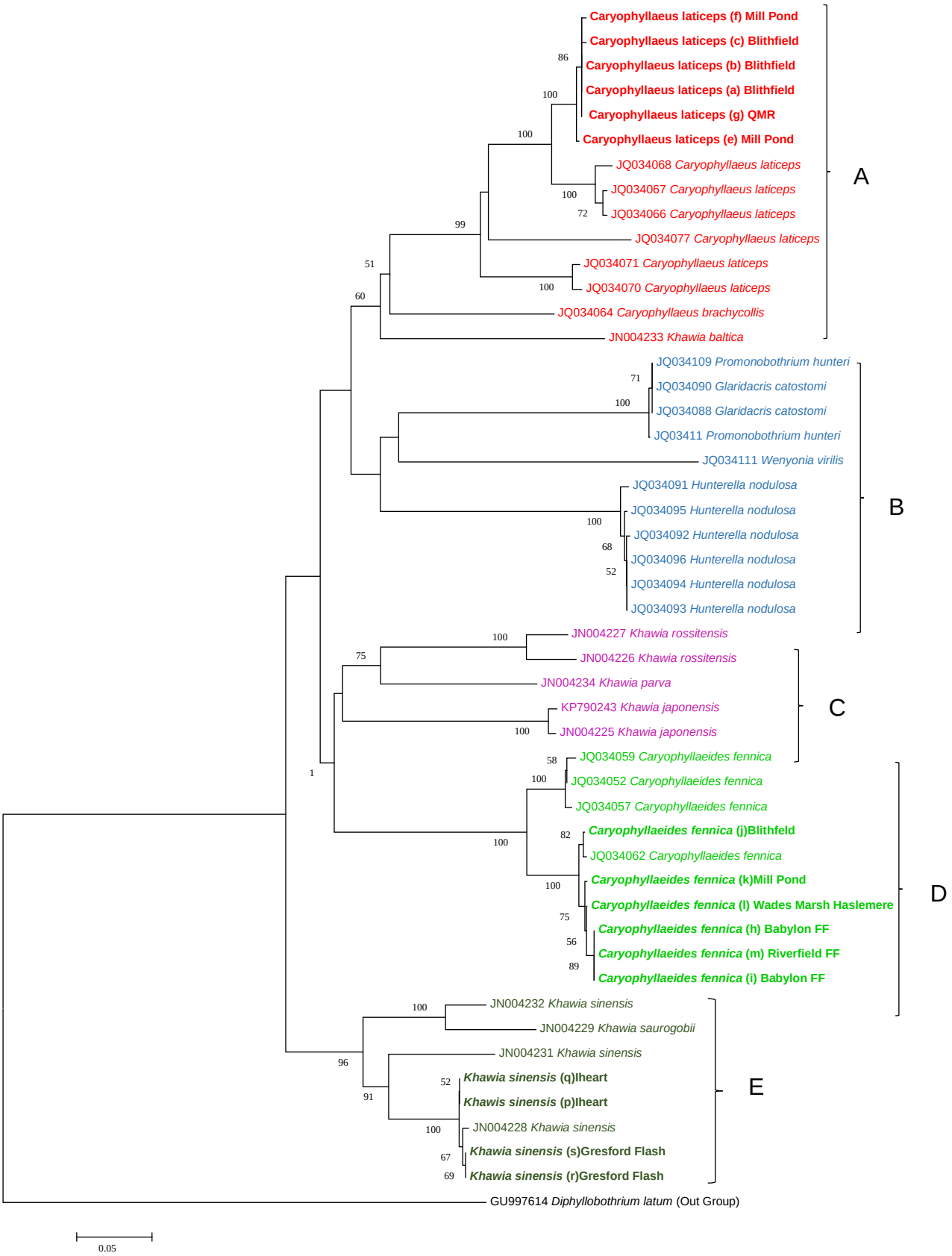


Figure 5.3 The phylogenetic reconstruction of the Caryophyllidea cox 1, was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Items in bold were sequences extracted as part of this study.

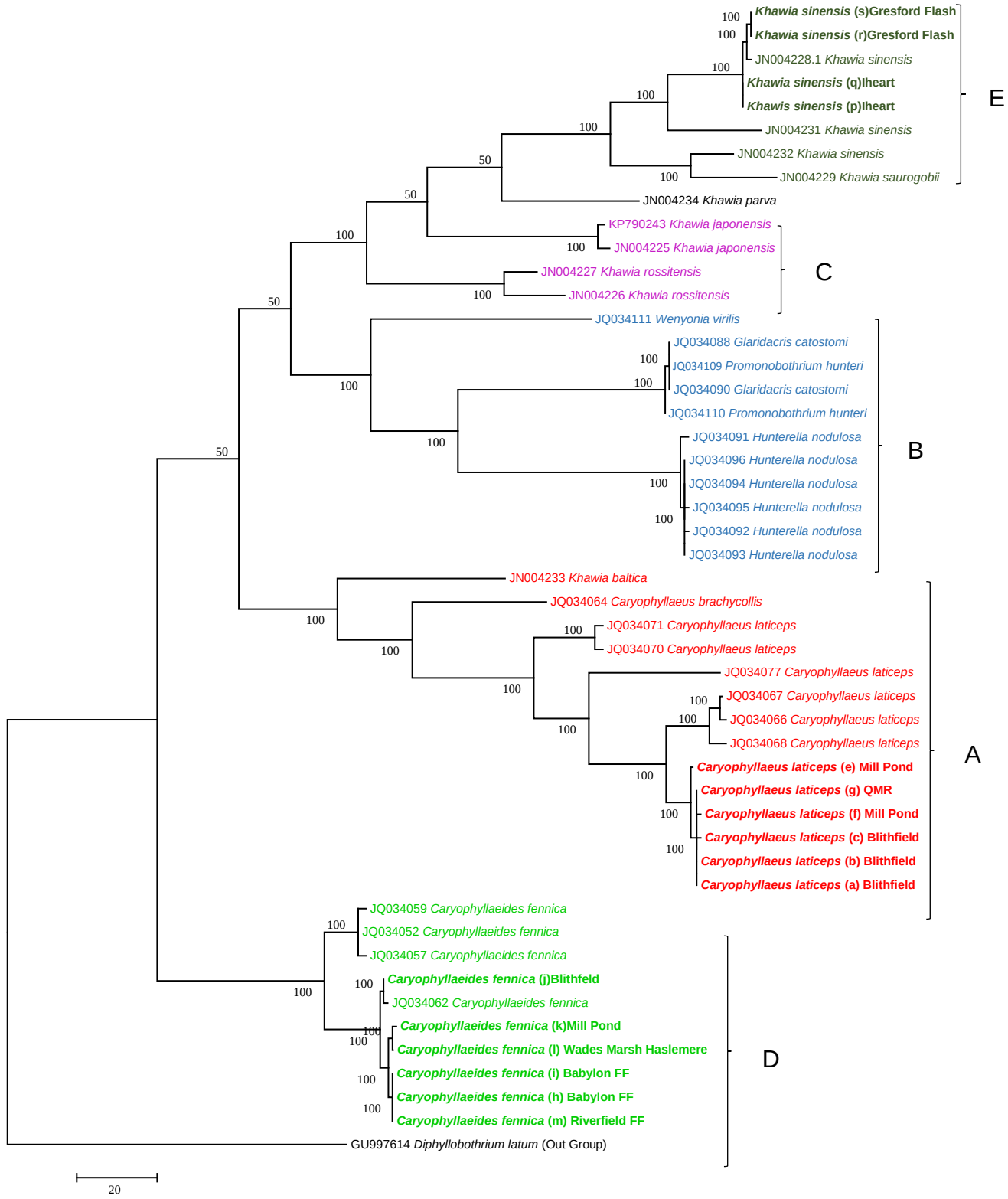


Figure 5.4 Phylogenetic reconstruction of Caryophyllidea cox 1, inferred using the Maximum Parsimony method. The consensus tree inferred from 2 most parsimonious trees is shown. Branches corresponding to partitions reproduced in less than 50% trees are collapsed. The percentage of parsimonious trees in which the associated taxa clustered together are shown next to the branches. Items in bold were sequenced as part of this study.

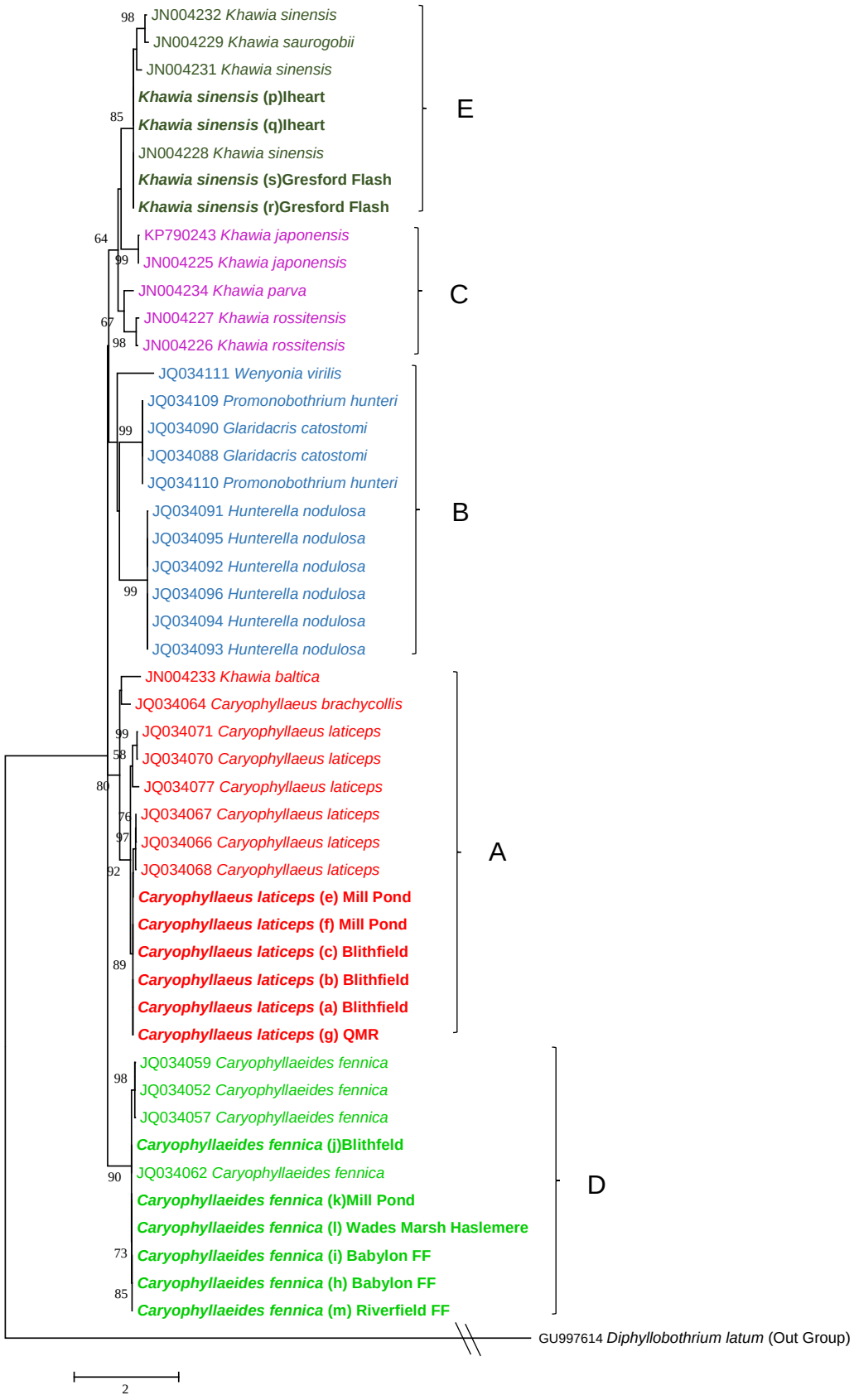


Figure 5.5 The phylogenetic reconstruction of Caryophyllidea cox 1 was inferred using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.3040)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Items in bold were sequenced as part of this study.

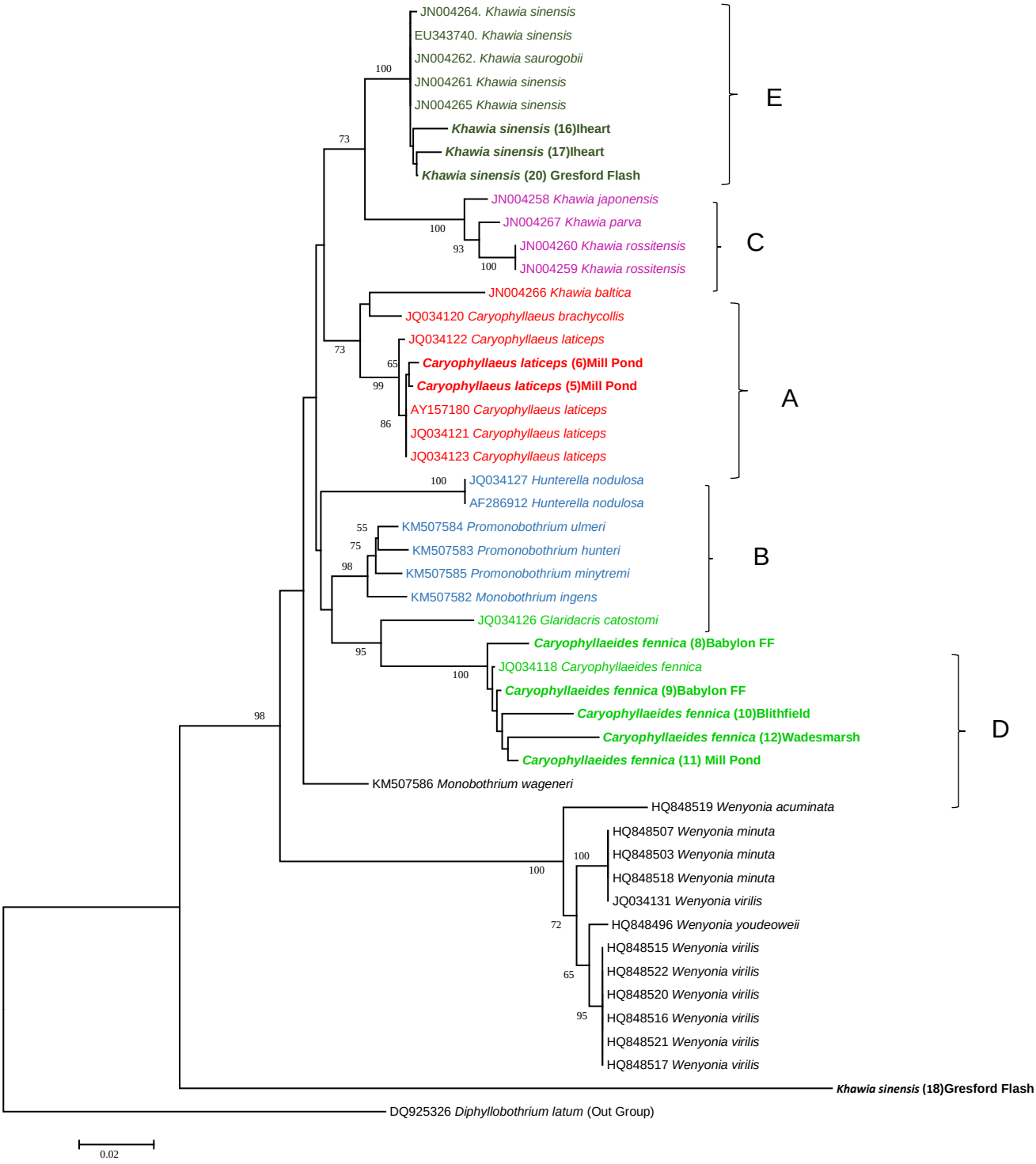


Figure 5.6 The phylogenetic reconstruction of Caryophyllidea r28s inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Items in bold were sequences extracted as part of this study.

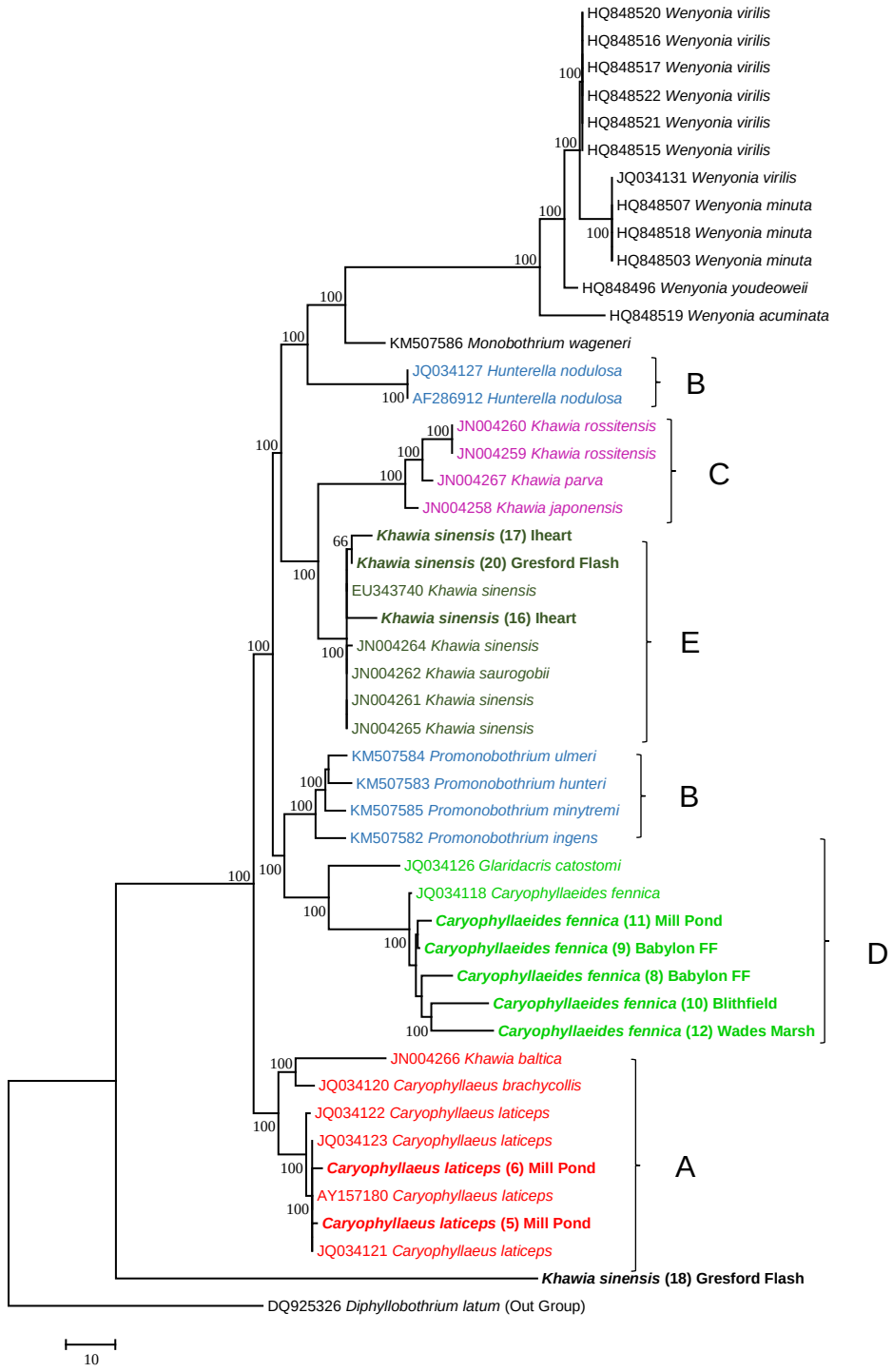


Figure 5.7 The phylogenetic reconstruction of Caryophyllidea r28s was inferred using the Maximum Parsimony method. The consensus tree inferred from 3 most parsimonious trees is shown. The percentage of parsimonious trees in which the associated taxa clustered together are shown next to the branches. The tree is drawn to scale, with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. Items in bold were sequences extracted as part of this study.

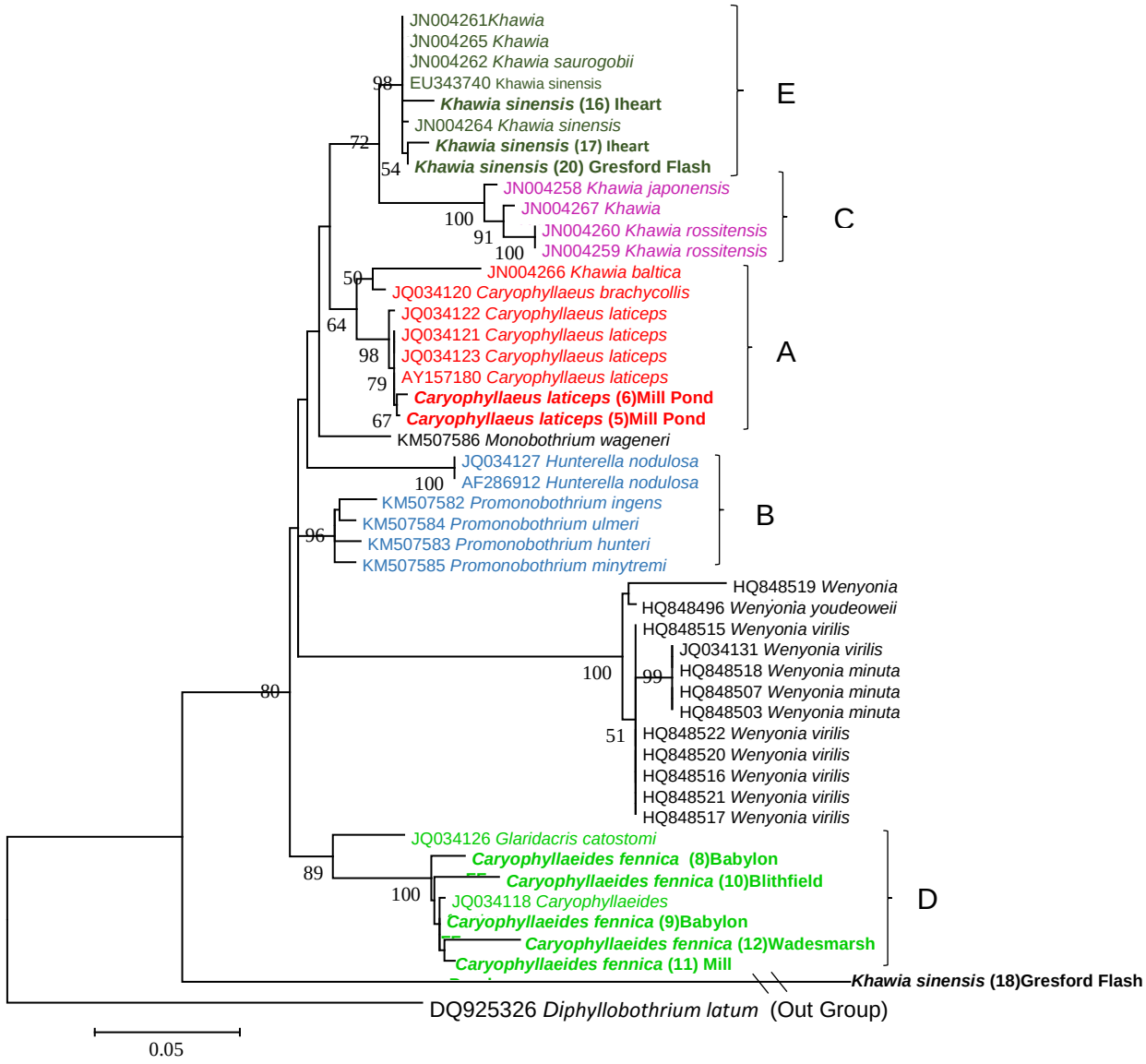


Figure 5.8 The phylogenetic reconstruction of the Caryophyllidea r28s was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Items in bold were sequences extracted as part of this study.

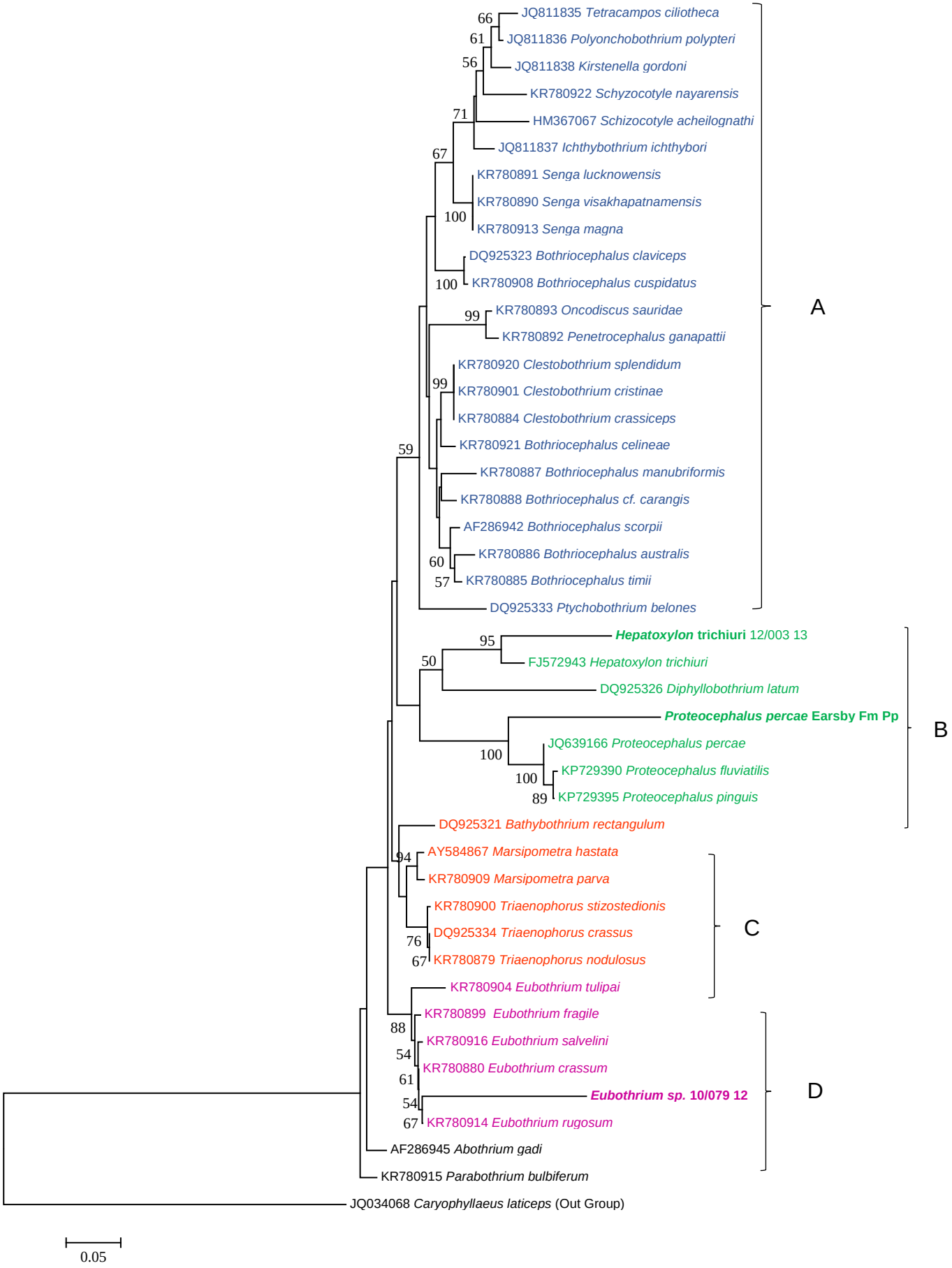


Figure 5.9 Phylogenetic reconstruction of Bothriocephalidea r28s inferred using the Neighbor-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Items in bold are sequences extracted as part of this study. Sequences in bold were extracted as part of this study.

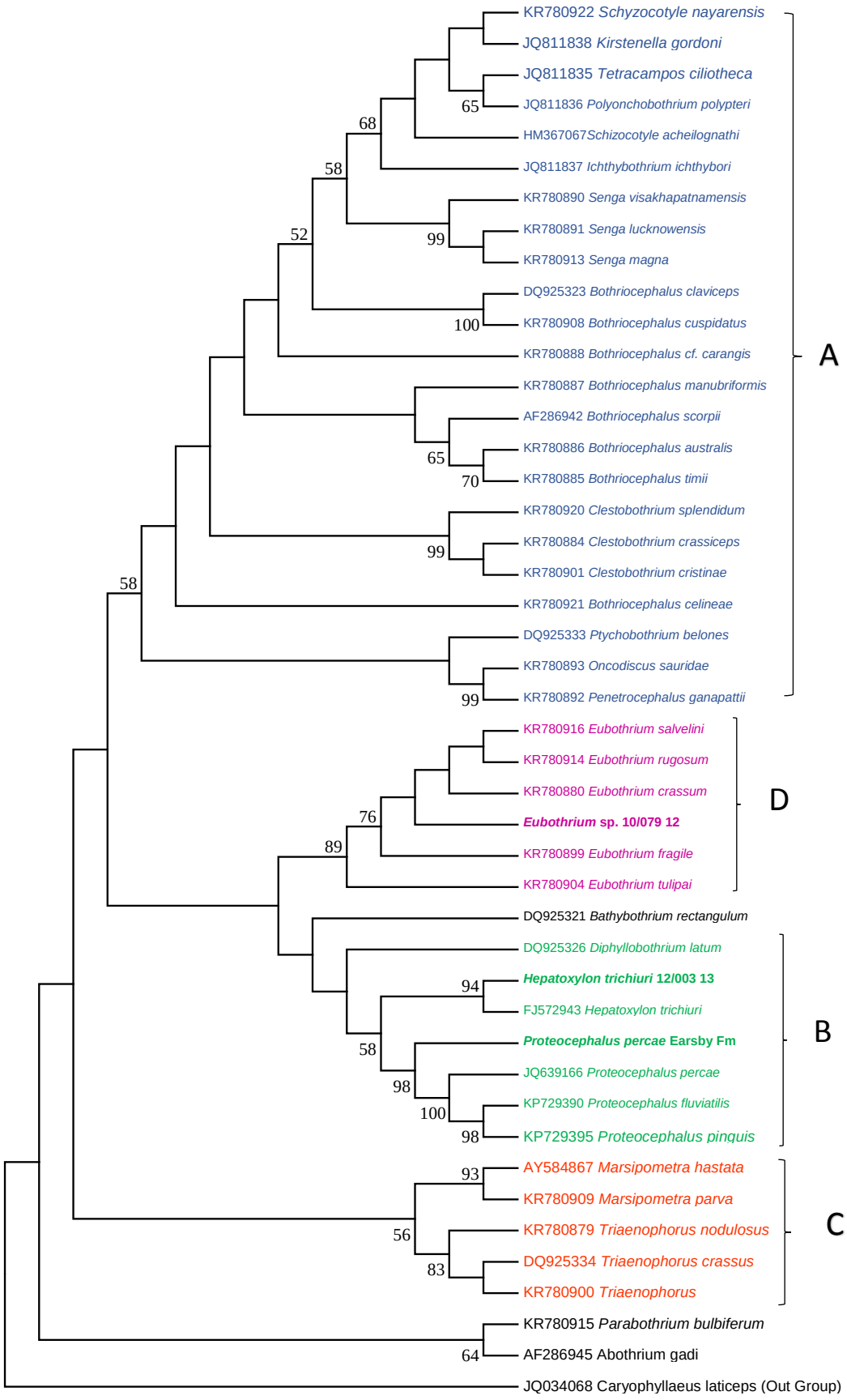


Figure 5.10 Phylogenetic reconstruction of r28s Bothriocephalidea inferred using the Maximum Parsimony method. The most parsimonious tree with length = 491 is shown. The tree is drawn to scale, with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. Sequences in bold were extracted as part of this study.

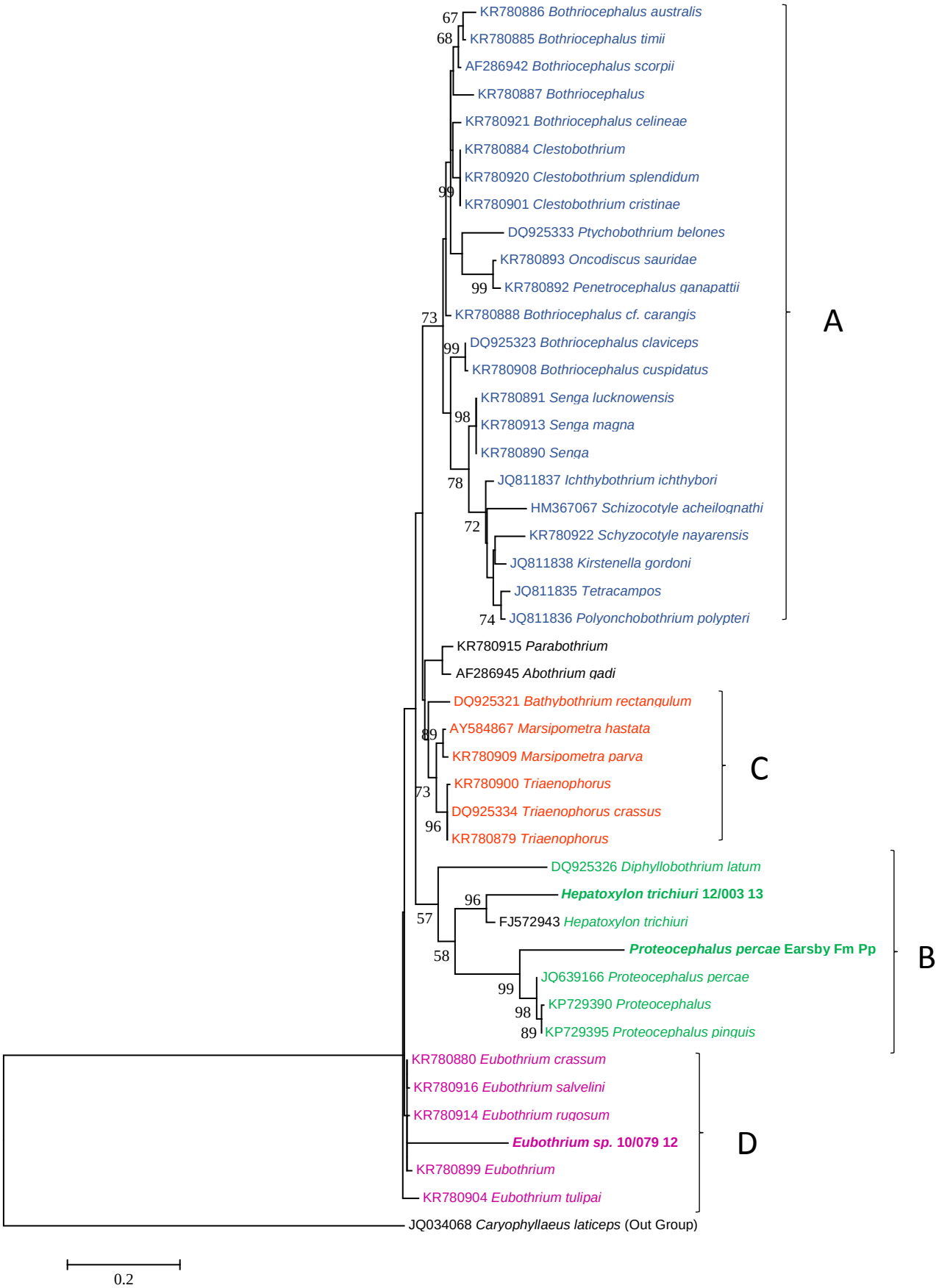


Figure 5.11 The phylogenetic reconstruction of the Bothriocephalidea was inferred by using the Maximum Likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Sequences in bold were extracted as part of this study.

5. 3. 7 Diversity and phylogenetic power of cox1 and r28s in resolving Caryophyllidea and Bothriocephalidea taxonomy

Analysis of single nucleotide polymorphism was undertaken using DnaSP 5.10 (Librado & Rozas, 2009), measuring the sequence polymorphism and divergence between species. Sequences with large numbers of variable sites in the sequences would indicate the cox1 and r28s markers are suitable for identifying species of Caryophyllidea and Bothriocephalidea. Results of single nucleotide polymorphism analysis are given in Figures 5.12 – 5.14, the peaks are the numbers of variable sites in the sequences.

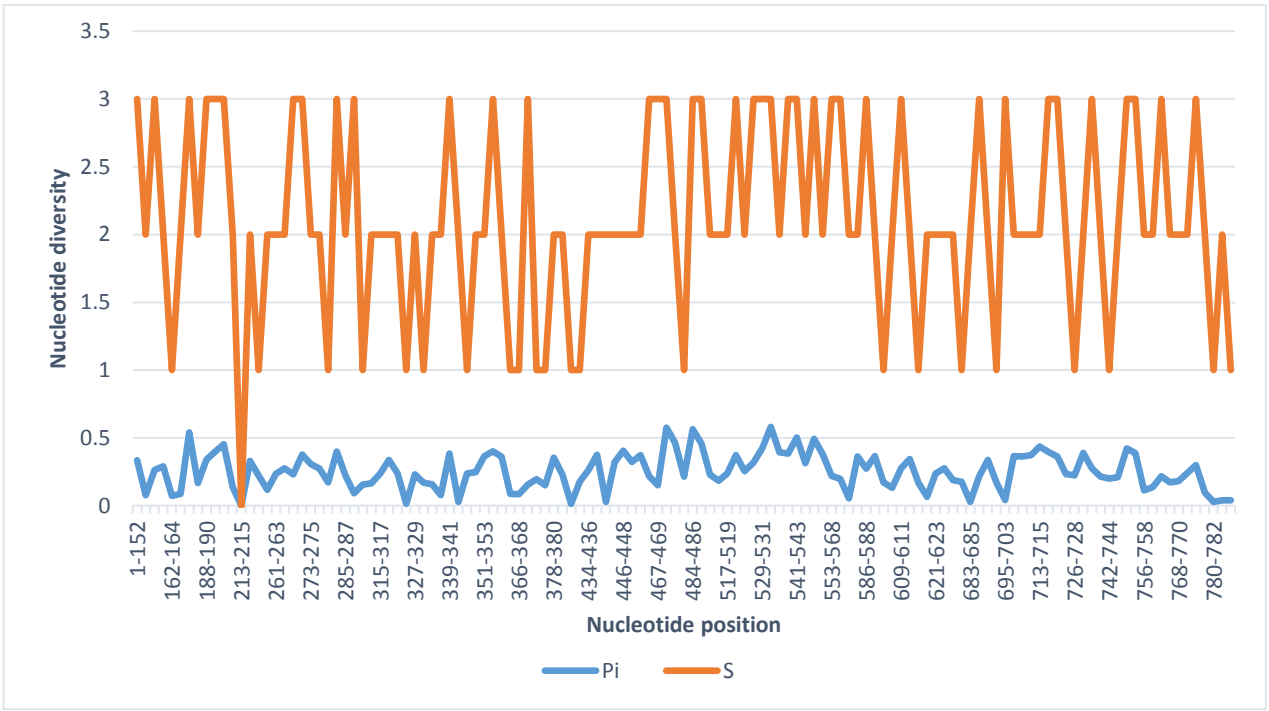


Figure 5.12 Caryophyllidea cox 1 sequences, nucleotide diversity (Pi) and nucleotide variable sites (S)

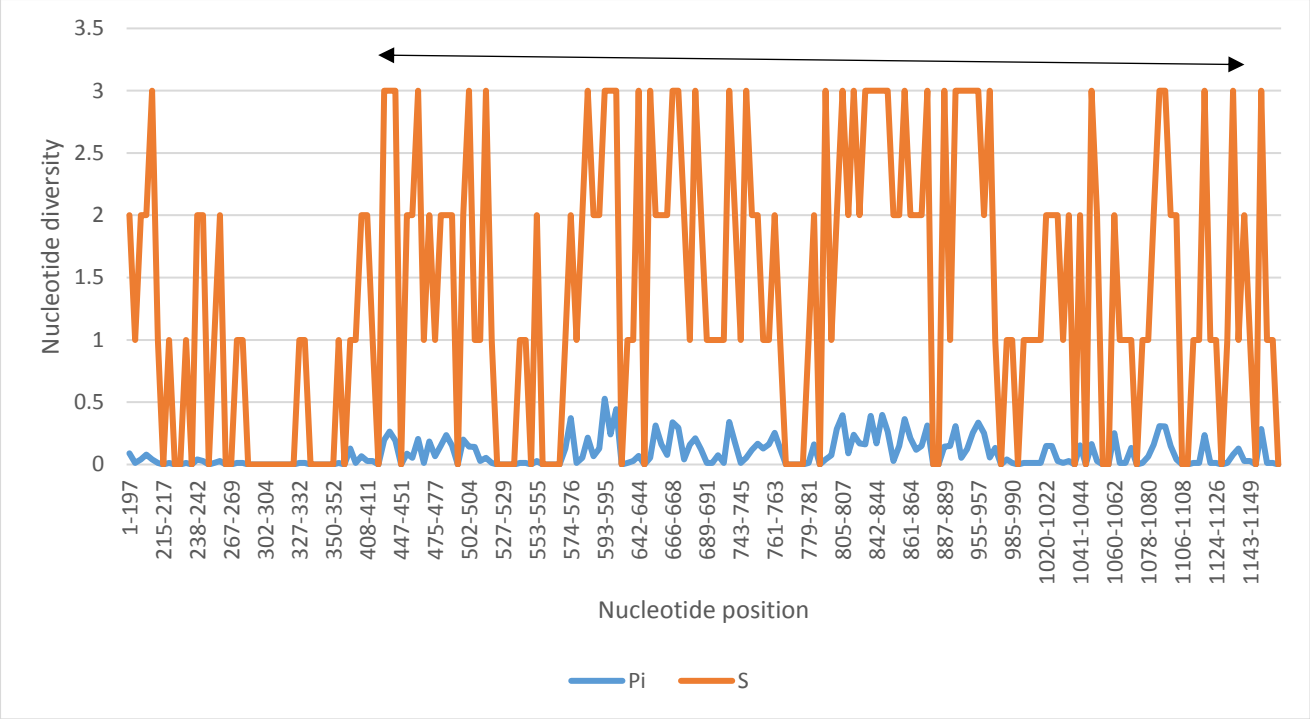


Figure 5.13 Caryophyllidea r28s sequences, nucleotide diversity (Pi) and variable nucleotide sites (S), area of greatest diversity arrowed

The Caryophyllidea cox 1 sequences show great variability in nucleotide diversity and variable nucleotide site as evidenced in Figure 5.12, by the number and height of the peaks, the r28s sequences showed the greatest variability from nucleotide sites 408 – 1322, Figure 5.13 (arrowed). The results of analysis of the nucleotide diversity and number of variable sites in the sequences indicate that cox 1 and r28s are suitable markers for identifying species of Caryophyllidea.

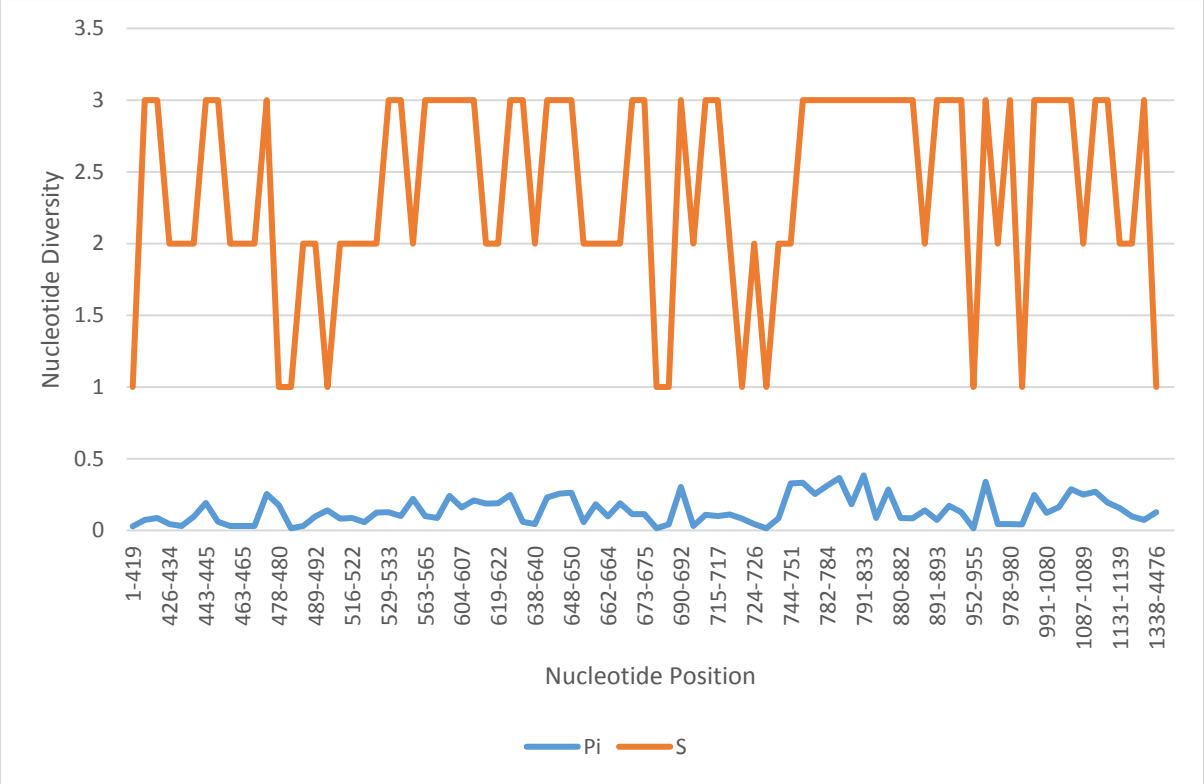


Figure 5.14. Bothriocephalidea r28s nucleotide diversity (Pi) and variable sites (S)

There is both nucleotide diversity and variable sites shown in Figure 5.14 for the Bothriocephalidea but there is less fluctuation in the number peaks and lower variability in the height which allows for differentiation of species. However, the r28s may not be the most effective marker and it is possible cox1 may prove to be preferable for identification of bothriocephalid species.

The results of further DnaSP (Librado & Rozas, 2009) analysis for Caryophyllidea and Bothriocephalidea are given in Table 5.9. The data for the Caryophyllidea in this table compliments the graphs, cox1 sequences show greater nucleotide variability and divergence than the r28s sequences. On the basis of these results the cox1 marker is better than the r28s marker for the identification of species of Caryophyllidea. DnaSP analysis for Bothriocephalidea shows fewer variable and parsimony informative sites than for caryophyllid r28s, but the nucleotide diversity is greater. This

further supports the view that r28s can be used for identification of Bothriocephalidea, but cox1 may be the better genetic marker.

Table 5.9. Single polymorphic nucleotide analysis of cestode sequences

DnaSP analysis	Caryophyllidea Cox 1	Caryophyllidea r28s	Bothriocephalidea r28s
No. Nucleotides	1322	1093	1672
No. sequences	49	48	45
s (variable sites)	268	281	254
π	0.25362	0.0888	0.13912
π ± SD	0.25362±0.00977	0.0888±0.013	0.13912±0.01775
K (average no. nucleotide differences)	96.37415	54.25443	35.33534
p-distance	0.253811	0.088467104	-
Parsimony informative	191	159	121
variable nucleotides	0.2452	2.1256	-

The NJ analysis is a distance method, analysing pairwise distance between the sequences calculating a matrix, from which it is possible to establish evolutionary divergence based on the ratio of transitions and transversions which have taken place in the Caryophyllidea cox 1 and r28s and Bothriocephalidea sequences (Figures 5.15 - 5.17). A graph displaying a linear increase of transitions and transversions indicates that substitution saturation has not been approached, however as saturation is approached the substitution rate plateaus or falls beneath the transversion rate (Page & Holmes, 1998). Figure 5.15 shows the transition rate of Caryophyllidea cox 1 sequences are beginning to plateau, inferring that substitution saturation has been reached, implying the sequences are of poor value for phylogenetic analysis.

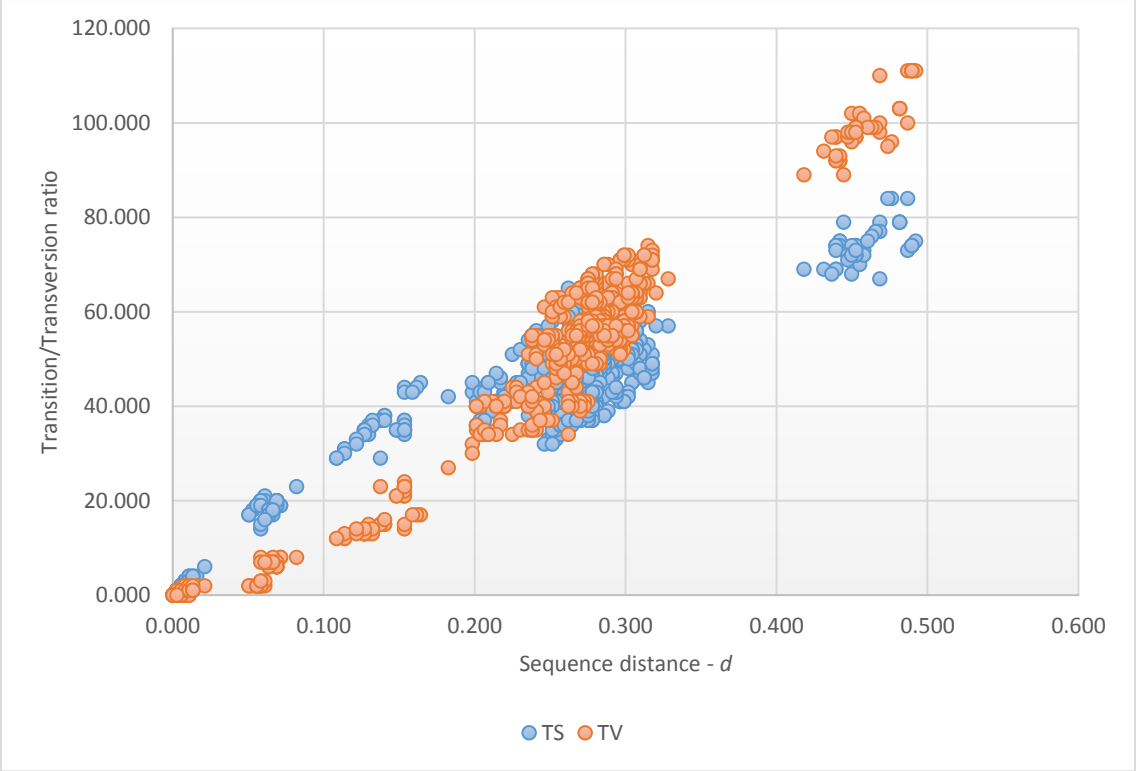


Figure 5.15 Caryophyllidea cox 1 uncorrected p-distance showing nucleotide transition and transversion substitution (TS = transitions; TV = transversions)

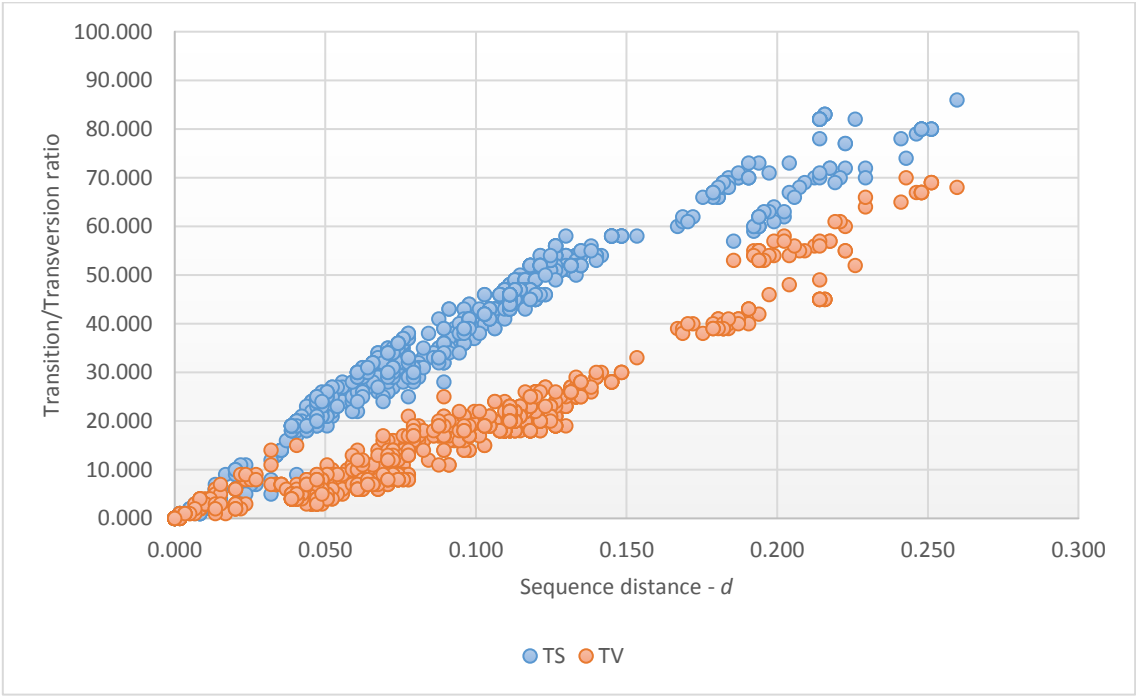


Figure 5.16 Caryophyllidea r28s uncorrected p-distance showing nucleotide transition and transversion substitution (TS = transitions; TV = transversions)

Figure 5.16 for the uncorrected p-distance Caryophyllidea r28s shows an increasing trend of transitions and transversions, which should indicate there is little substitution saturation and therefore the sequences should have a good phylogenetic signal but this is contradicted by the incongruent phylogenetic trees. The Caryophyllidea r28s marker is of questionable value in establishing evolutionary relationships.

Analysis of the uncorrected p-distance for the Bothriocephalidea r28s sequences are shown in Figure 5.17

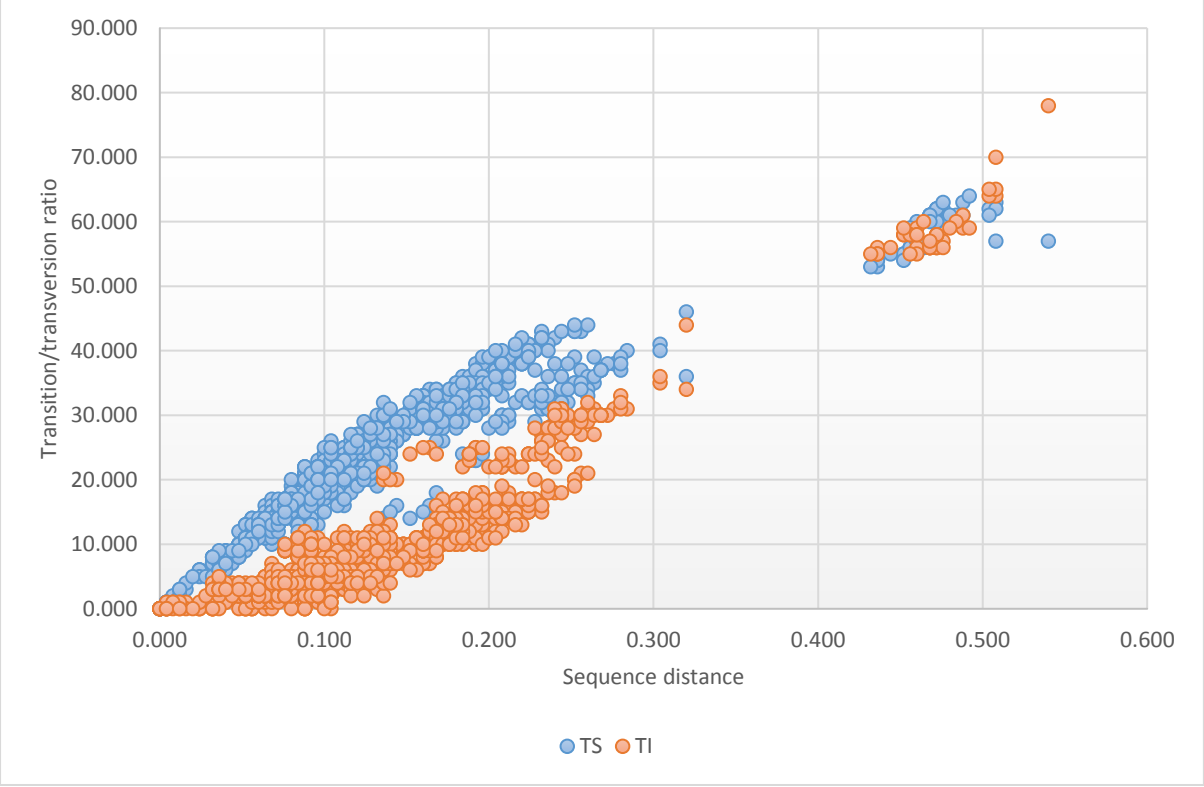


Figure 5.17 Bothriocephalidea r28s sequences uncorrected p-distance showing nucleotide transition and transversion substitution (TS = transitions; TI = transversions)

The trend for the Bothriocephalidea r28s sequence transitions is shown to be plateauing in Figure 5.17, which infers that substitution saturation is occurring with

accompanying loss of the phylogenetic signal and therefore of little value for inferring evolutionary relationships.

Further analysis of substitution saturation of cox 1 and r 28s Caryophyllidea and Bothriocephalidea r28s sequences was undertaken using DAMBE (Xia et al. 2003; Xia & Lemey, 2009; Xia, 2013). Where the index of substitution saturation (Iss) exceeds the calculated values for symmetrical (Iss.cSym) and asymmetric (Iss.cAsym) tree topology it is indicated there is substitution saturation and the sequences are of no phylogenetic value (Xia et al. 2003; Xia & Lemey, 2009; Xia, 2013).

Table 5.10 DAMBE analysis of Caryophyllidea cox1 and r28s substitution saturation

Caryophyllidea DAMBE Test	**cox 1**	**r28s**
Proportion invariate sites	0.13	0.2545
Mean H	1.6643	0.5429
Standard error	0.052	0.0169
Hmax	1.8152	1.8579
Iss	0.9168	0.2894
Iss.c	0.7588	0.7609
T	3.0399	27.9709
DF	742	656
Prob (Two tailed)	0.0025	0
95% lower limit	0.8148	0.2563
95% upper limit	1.0189	0.3225

The results of DAMBE analysis of Caryophyllidea cox1 have an Iss value which exceeds the Iss.c (Table 5.10), indicating there is substantial substitution saturation and the sequences are of little value for phylogenetic analysis. The r28s sequences are more promising with an Iss < Iss.c which indicates little saturation and compliments the p-distance result, however, the narrative accompanying the analysis described the sequences as poor and of no use for phylogenetic interpretation (Xia et al. 2003; Xia & Lemey, 2009; Xia, 2013).

Analysis of the Bothriocephalidea r28s sequences (Table 5.11) with DAMBE (Xia et al. 2003; Xia & Lemey, 2009; Xia, 2013) show Iss > Iss.c for all Operational Taxonomic Units (OTU) which indicate there is substitution saturation in this marker and it is of no value for phylogenetic analysis.

Table 5.11 DAMBE analysis of Bothriocephalidea r28s substitution saturation

NumOTU	Iss	Iss.cSym	Iss.cAsym
4	1.13	0.85	0.841
8	1.338	0.845	0.764
16	1.591	0.83	0.677
32	1.905	0.81	0.562

5.4. Discussion

5. 4. 1. Caryophyllidea

Identification of the Caryophyllidea based on morphological characters is subjective, even with the assistance of comprehensive keys (Chubb *et al.*, 1987; Oros *et al.,* 2010; Scholz *et al.*, 2011). In the UK, the Caryophyllidea is represented by *Caryophyllaeides fennica, Caryophyllaeus laticeps, Khawia sinensis* and *Atractolyocestus* species. The morphology of *C. fennica* is distinctive with the blunt scolex which is undifferentiated from the body. The euryxenous species *Caryophyllaeus laticeps* and *Khawia sinensis,* are very similar in appearance, both infect common carp and are difficult to differentiate especially with evidence of phenotypic plasticity and numerous morphotypes in the Caryophyllidea (Bazsalovicsová *et al.*, 2014; Barčák *et al.,* 2014. Both species are widespread in the UK (*Aquatic Parasite Information*) and whilst *K. sinensis* was initially regarded by the Environment Agency as a potential pathogen of carp, the rapid distribution of this species was probably aided by the similarity of

morphological characters shared with *C. laticeps.* Histological preparations indicate the inner longitudinal muscle is well developed in *C. laticeps* and the scolex is finely scalloped compared with *K. sinensis,* however, Bazsalovicsová *et al.* (2014) point out that scolex morphology is variable and its reliability as a useful character for identification is doubtful.

The analysis of single nucleotide polymorphisms indicate that whilst both cox1 and r28s markers can be used to identify species of Caryophyllidea, the cox 1 marker is preferable for differentiating species as there is a greater inter-specific diversity. The use of the cox1 marker for identification of Caryophyllidea will be of value in establishing the identity and distribution of these cestodes infecting freshwater fish in the UK.

The use of Caryophyllidea cox 1 for estimates of evolutionary divergence proved unreliable owing to substitution saturation of the sequences, which corroborates the work of Brabec *et al.* (2012). Although substitution saturation had not been approached in the Caryophyllidea r28s, the DAMBE analysis indicated the sequences were of no value for estimating the evolutionary divergence. In addition, there was incongruence between phylogenetic trees for each genetic marker in terms of the position of the clades. However, in all phylogenetic trees cox 1 and r28s sequences form consistent species clades (A) *Caryophyllaeus laticeps, C.brachycollis* and *Khawia baltica* ; (B) *Promonobothrium hunteri*, *Hunteralla nodulosa,* and *Glaridacris catostomi*; (C) *Khawia japonensis, K. rossitensis* and *K. parva*; (D) *Caryophyllaeides fennica* and (E) *Khawia sinensis* and *K. saurogobii*. In the Cox 1 sequences the African *Wenyonia virilis* forms a sister group to *P. hunter* and *G. catostomi* and in the r28s sequences a separate clade. Given the African origin of the *Wenyonia s*pecies it is understandable these form a separate clade.

Clade A. All the sequences of C*. laticeps* from the UK were parasites of common bream and with the exception of the r28s MP tree, formed a single clade. Of the comparative material downloaded from GenBank®, sequences, hosts of JQ034066, JQ034067 and JQ034068 were common carp; JQ 034070 and JQ034071 common bream and JQ034077 white bream, which supports Hanzelová *et al.* (2015) identification of *C. laticeps* morphotypes associated with different hosts. The host for GenBank® *C. laticeps* sequences JQ 034070 and JQ034071 was common bream from Slovakia and these formed a separate clade from the UK specimens of *C. laticeps* for which the host was also common bream. Although Hanzelová *et al.* (2015) recorded only 'morphotype 1' infecting common bream, the formation of a separate clade in the molecular sequences suggests a genetic divergence of UK morphotypes from the European species.

Within Clade A, all trees indicate *C. brachycollis* and *Khawia baltica* sequences form a sister group to *C. laticeps*, a topology also shown in the ML tree of Scholz *et al.* (2015). This grouping of *K. baltica* with *C. laticeps* and *C. brachycollis* based on molecular studies is in conflict with the morphological work. Based on morphology, *K. baltica* is included in the Lytocestidae, whereas *C. laticeps* and *C. brachycollis* are included in the Caryophyllidae. The consistency of molecular studies in placing *K. baltica* in the same clade as *C. laticeps* and *C. brachycollis* suggests it would be appropriate to redefine the morphological characters of Lytocestidae and Caryophyllidae. Given the morphological plasticity of the genus *Caryophyllaeus* (Barčák *et al.* 2014; Bazsalovicsová *et al.,* 2014; Hanzelová, 2015), *Khawia baltica* is most likely a member of the genus *Caryophyllaeus.*

With reference to Clade B, Scholtz *et al.* (2015) revised the genus *Monobothrium* by assigning all Nearctic species to the resurrected genus *Promonobothrium*, on the

basis all were found in this region, parasitizing catostomids. The ML phylogenetic tree produced by these authors shows *Glaridacris catostomi* as a sister taxon to *Promonobothrium* species. In the cox 1 sequences, the Nearctic species *G. catostomi* formed a clade with *Promonobothrium hunteri*, which infers these sequences represent a single species. The ML tree given by Scholz *et al.* (2015) shows *G. catostomi* forming a sister clade to *P. hunteri*. The phylogenetic trees produced here for the r28s sequences showed a completely different relationship for *G. catostomi,* consistently forming a sister group to the Palaearctic species, *Caryophyllaeides fennica.* The topology of *G. catostomi* which is a Nearctic species forming a sister group to *C. fennica*, in the Caryophyllidea r28s sequences is considered to be incorrect.

Clade C comprising *Khawia japonensis, K. parva* and *K. rossitensis* form a monophyletic clade in all of the constructed phylogenetic trees. This clade C forms a sister clade with E *Khawia sinensis* and *K. saurogobii*, in the MP and ML trees, but in the NJ phylogenetic tree they form the sister group to clade D *Caryophyllaeides fennica.* The most taxonomically considered relationship of the three species comprising clade C is with all other species of *Khawia,* comprising clade E. *Khawia sinensis* is a species of Eurasian origin, presumed to have been introduced to Europe with imports of ornamental fish (Scholtz *et al*. 2011), *K. japonensis, K. parva* and *K. rossitensis* are also of Eurasian origin and hence form sister taxa. Scholtz *et al*. (2011) state that *K. japonensis* and *K. sinensis* are not closely related and affirms that *K. japonensis, K. parva* and *K. rossitensis* are phylogenetically related. Therefore with respect to the topology of Clade C, the cox 1 NJ phylogenetic tree is incorrect.

Caryophyllaeides fennica, Clade D, is most frequently found parasitizing roach in the UK but hosts also include bream, dace and chub (*Aquatic Parasite Information*). The

host of the *C. fennica* sequences in this study were all roach, included in the cox 1 phylogenetic trees is Genbank® sequence JQ034062 also from roach. In both the cox 1 and r28s phylogenetic trees the *C. fennica* parasitizing roach form a sister group to GenBank® sequences JQ034059, JQ034052, JQ034057 and JQ034118, for which the host species was dace. Although Hanzelová *et al.* (2015) described polymorphism of *Caryophyllaeus laticeps* associated with different fish species, there is an indication from the sequences analysed here, *C. fennica* may also demonstrate genetic and molecular polymorphism associated with different hosts.

The UK sequences of *K. sinensis* form Clade E, together with GenBank® sequence JN004228 *K. sinensis* from carp, whose geographic location is Slovakia, suggesting a close link, possibly through translocation of infected carp. Certainly the *K. sinensis* sequences from Europe form a sister clade to the sequence of this species from Japan, which indicates a link to the ornamental trade through importation of infected ornamental carp, known as koi. European and Japanese sequences of *K. sinensis* form a sister group to the Chinese *K. sinensis* and *K. saurogobii.*

Regrettably the preparation of *Monobothrium wageneri* resulted in extracted r28s sequences which were too short for phylogenetic analysis. However, a GenBank sequence KM507586 of *M. wagneri* was used in the analysis of the r28s sequences and this produced the same result as Scholtz *et al.* (2015) of an inconclusive topology.

5. 4. 2 Freshwater Bothriocephalidea

Brabec *et al.* (2015) revised the Bothriocephalidea on the basis of molecular data, producing a single ML phylogeny by concatenating a four-gene dataset. The sequences selected from GenBank® in this study included freshwater species of cestode analysed by Brabec *et al*., (2015). However the phylogenetic trees produced

from the Bothriocephalidea r28s sequences were incongruent. The uncorrected p-distance indicated the Bothriocephalidea r28s showed some indication of substitution saturation. Further DAMBE analysis showed significant substitution saturation and the sequences were of little use for phylogenetic analysis. Whilst the phylogenetic analysis of the Bothriocephalidea was unsuccessful, there was some consistency in the formation of clades, matching the topology of the ML tree produced by Brabec *et al.* (2015).

Clade A represents a diverse polyphyletic group, consistent with the results of Brabec et al. (2015). Clade B comprises *Diphyllobothrium latum*, *Proteocephalus percae* and *Hepatoxylon trichiuri* species which are taxonomically divergent. The definitive hosts of trypanorhynchids are sharks and rays, the specimen *Hepatoxylon trichiuri* EA12/003/13 was from an Atlantic salmon migrating into freshwater. Fish infected with *H. trichiuri* come from a range of geographic areas and sea-depth (Mladino, 2006). *Diphyllobothrium latum* is usually found as a plerocercoid in the musculature of freshwater fish, with mammals the definitive host, whereas the definitive hosts of *Proteocephalus* species are freshwater fish. The formation of these taxa as Clade B seems an unlikely combination and the relationship in all trees is certainly distant, however, sequencing of additional representatives of these three families may prove of interest.

Proteocephalus percae from Earsby Farm was identified using scolex morphology (Scholz, Drábek & Hanzelová, 1998), the topology of these species in all of the trees would indicate some divergence of this UK species from the European species of *P. percae.*

Clades C and D are taxa representative of the Bothriocephalidea and these clades corroborate the ML tree in Brabec *et al.* (2015). Atlantic salmon was the host of *Eubothrium* sp. 10/079 12, the sequence extracted was sufficient to confirm the genus, however, identification of the species is unclear and the relationship to sequences of other *Eubothrium* species may simply be due to a poor sequence.

Analysis of single nucleotide polymorphisms for the Bothriocephalidea indicated that r28s can be used for the identification of species in this group based on the nucleotide diversity and the sequences clustered in consistent species clades. Analysis showed that cox1 was a better marker for identification of Caryophyllidea, but there were issues with extraction of cox1 sequences for the Bothriocephalidea and therefore this marker could not be validated. r28s was not suitable for phylogenetic analysis as indicated by DAMBE and incongruent phylogenetic trees.

The Proteocephalidae is difficult to identify based on morphological characters and the number of described species is questionable (Škeříková *et al.,* 2001). It would be particularly useful to have an effective genetic marker for this family, use of which would remove ambiguity in the identification of proteocephalid species present in the UK.

5.4.3 Concluding remarks

Caryophyllaeides fennica has a distinctive morphology and can be readily identified, but other species of Caryophyllidea are not as easily distinguished. Morphologically, none of the UK specimens of *Caryophyllaeus laticeps* conformed to the five morphotypes described by Hanzelová *et al.* (2015). Such morphological plasticity indicates that key features used to identify these tapeworms show considerable variability. Where variability in a key morphological feature used for identification

occurs, there is potential for overlap with other species and the opportunity for misidentification. The various morphotypes of *C. laticeps* may be confused with either *Khawia sinensis* or possibly the potentially introduced *K. japonensis.* The illicit trade in freshwater fish from Europe can potentially introduce one or more of the different morphotypes of *C. laticeps* which may prove more pathogenic than the native morphotype. A combined morphological and molecular approach is the most suitable approach for identification of species. The present study has shown that cox1 is suitable for identification of caryophyllid species, but this marker could not be tested for the bothriocephalids. Both markers, however, were not suitable for phylogenetic analysis.

Within the bothriocephalids, *Schyzocotyle acheilognathi* is highly pathogenic and has been reported from 200 species of fish (Scholz *et al.,* 2011). Although the hosts in the UK are thought to be restricted to carp and grass carp, it has also been recorded from a roach (*Aquatic Parasite Information*). Should other freshwater UK fish become host to *S. acheilognathi* the use of molecular techniques would confirm the identification and allow the Environment Agency to introduce movement restriction of infected fish.

Chapter 6

Identification of *Atractolytocestus* (Cestoda: Caryophyllidea: Lytocestidae) species parasitizing common carp (*Cyprinus carpio*) in the United Kingdom

6.1 Introduction

Atractolytocestus species are monozoic, caryophyllidean intestinal tapeworms of carp. Their life cycle is thought to involve aquatic annelids (Oligochaeta) as intermediate hosts, although only the life cycle of *Atractolytocestus sagittatus* has been studied (Oros *et al*., 2011). *Tubifex and Limnodrilus* species were shown to ingest the eggs of *A. sagittatus* which release a six-hooked onchosphere in the intestine. These larvae penetrate into the body cavity and develop into plercocercoids. Carp are then infected by predation on the intermediate hosts (Demshin and Dvoryadkin, 1981).

Morphological similarity within the genus *Atractolytocestus* has led to taxonomic confusion. *Atractolytocestus huronensis* Anthony (1958) can be distinguished from its congeners by a low number of testes (up to 66 recorded pers. com. R. Kirk) compared to numerous testes (>100, in some specimens several hundred) in *A. sagittatus* (Kulahovskaya and Akhmerov, 1965) and *A. tenuicollis* (Li, 1964) Xi *et al.,* 2009 (Scholz *et al.,* 2001; Králová-Hromodová *et al.*, 2013). The testes commence posterior to the first vitelline follicles in *A. huronensis* (Anthony, 1958) and *A. tenuicollis* (Králová-Hromodová *et al.*, 2013) but anterior to the first vitelline follicles in *A. sagittatus* (Scholz *et al*., 2001). It should be noted that the number of testes is difficult to quantify accurately without serial sectioning owing to the obstruction of medullary testes by extensive cortical vitellaria (Kirk *et al*., in prep.).

All three species possess a mobile bulboacuminate scolex, but differ in the length of the neck. Kulakovskaya and Akhmerov (1965) thought *A. huronensis* and *A. sagittatus* were sufficiently different to erect a new genus, *Markevitschia* to accommodate *M.*

sagittata, later Mackiewicz (1994) synonymized *Markevitschia* with *Atractolytocestus*. *Atractolytocestus tenuicollis* was originally described as *Khawia tenuicollis* (Li, 1964) but was transferred to the genus *Atractolytocestus* by Xi *et al.,* (2009). The three species have since been validated using molecular analysis of ITS1, ITS2 and cox1 (Králová-Hromodová *et al.*, 2010; Bazsalovicsová *et al.*, 2011; Bazsalovicsová *et al.*, 2012; Králová-Hromodová *et al.*, 2013).

The first report of *A. huronensis* in the UK, and indeed Europe, was in 1993 from a carp in a stillwater fishery in Wales (Chubb *et al.*, 1996). This monozoic tapeworm was initially considered to be native to North America having originally been described parasitizing carp in the Huron River in 1950, followed by reports of infected carp from other States and territories (Anthony, 1958; Hensley & Nahhas, 1975; Hoffman, 1999; Oros *et al*., 2004). Following the introduction to the UK, *A. huronensis* has been widely disseminated through carp movements, particularly in south-east England and the Midlands, with a more restricted distribution within Wales and the north and south-west of England. The distribution of *A. huronensis* reflects the areas where most carp fisheries are located and correspondingly where detection will arise from routine fish health checks (Kirk *et al.*, in prep.) *Atractolytocestus* huronensis was first detected in mainland Europe in 2001 in pond farms in Hungary (Majoros *et al*., 2003) and over the next nine years was reported from the Czech Republic, Slovakia, Croatia, Germany and Romania onwards (Bazsalovicsová *et al.,* 2011) through river systems and the anthropogenic translocation and re-stocking of carp (Králová-Hromodová *et al.*, 2013). Although there are regular anthropogenic translocations of carp from eastern Europe to France for aquaculture and re-stocking fishing lakes (D. Midgeley pers. com.) there

are no reports of the incidence of *A. huronensis* in this part of western Europe. Most recently, Scholz *et al*. (2015) have reported *A. huronensis* infecting carp in the Limpopo and Mpumalanga Provinces of South Africa, associated with the global trade and transport of cultured fish. The native origin of *A. huronensis* is now considered to be Asia (Oros *et al*., (2004). The presence in South Africa indicates this parasite of carp has been successfully established in four continents: Asia, North America, Europe and Africa (Scholz *et al.,* 2015). By comparison, *A. sagittatus* appeared to have a more restricted Eurasian distribution in Russia (Kulakovskaya and Akhmerov, 1965) and Japan (Scholz *et al*., 2001). The report from China by Xi *et al.* (2009) may be erroneous (R. Kirk pers. com). *Atractolytocestus tenuicollis* has only been recorded from China and Inner Mongolia (Li, 1964). However, reporting bias may operate where veterinary inspections are less frequent.

There is a concern that there will be further spread of *A. huronensis* to countries without regular veterinary inspection of imported fish stocks through the ornamental industry and other routes (Oros *et al*., 2011). The tapeworm can cause local pathology to the intestinal epithelium due to the deep penetration of the scolex between the intestinal folds into the lamina propria and submucosa (Majoros *et al*., 2003; Williams, 2007). This causes local atrophy, disruption, erosion and necrosis of epithelial cells (Majoros *et al*., 2003; Williams, 2007; Gjurčavič *et al*., 2012). However, no carp mortalities have been attributed to *A. huronensis* so it is considered to be low risk in Europe.

During a routine fish health examination of fish from a stillwater in Pease Pottage, West Sussex, a monozoic tapeworm was found in the intestine of carp, which showed the morphological characters of the genus, but appeared to differ morphologically from both *A. huronensis* and *A. sagittatus. Atractolytocestus sagittatus* has not been recorded as present in the UK and therefore can be considerd a non-native parasite of unknown pathogenicity to carp, so a positive identification of this tapeworm was a matter of importance. The effect of the introduction of a non-native parasite on both the host and potentially novel hosts is unpredictable and evaluation of their threat to native fish remains a vital incentive in fish parasitology. Establishing this *Atractolytocestus* species as an introduction to the parasite fauna of freshwater fish in the UK would enable the Environment Agency to place it on the Category 2 list of parasites, restricting the movement of infected fish.

The aim of this study was to identify the unknown species of *Atractolytocestus* from West Sussex. In addition to traditional methods of histology and scanning electron microscopy to study morphological features of the *Atractolytocestus* species, a molecular approach was also undertaken. Mitochondrial cox1 was selected as the molecular marker because intra-individual sequence diversity in ITS1 and ITS2 ribosomal spacers is known to occur in *A. huronensis* due to nucleotide polymorphisms and varying numbers of repeats resulting in different lengths of ITS variants (Bazsalovicsová *et al*., 2012).

6.2 Materials and methods

The *Atractolytocestus* species was found in carp which originated from a stillwater in Pease Pottage, West Sussex, the comparative specimens of *A. huronensis* were from

Hall Farm Reservoir, Woodham Mortimer, Essex and Lee Valley Regional Park, Waltham Abbey, Essex. All *Atractolytocestus* spp. found in the intestine were removed and placed either into hot phosphate buffered saline, before fixing in formalin for histological preparation, or placed directly into 70% ethanol for molecular work.

6.2.1 Histological preparation of *Atractolytocestus* species

Formalin preserved *Atractolytocesus* were stained in Langeron's carmine for between 3 – 5 minutes, depending on size, rinsed in 70% ethanol, placed in 5% acid alcohol to destain for 2 – 3 minutes; transferred to 80% ethanol for 10 minutes; the cestodes were then sandwiched between squares of filter paper impregnated with 96% ethanol, leaving the scolex of large cestodes exposed, a cover slip was placed on the uppermost paper, with a light weight to flatten the specimen and the container topped up with 96% ethanol. Flattening the specimen took between 1 to 12 hours depending on size, after flattening the specimens were transferred to absolute alcohol for 10 minutes, before clearing in 10%, 50%, 90% and 100% clove oil or eugenol for 10 minutes in each solution. After clearing specimens were mounted in Canada Balsam or Numount (Brunel Microscopes, Canada Balsam substitute). Examination of slide material was digitally captured using a Nikon Eclipse 80i microscope with a Nikon camera and NIS Elements BR3® software and an Olympus CX41 microscope, Olympus camera and Cellsens® software, respectively.

6.2.2. Preparation of *Atractolytocestus* for scanning electron microscopy

The formalin preserved *Atractolytocestus* were washed in Sorenson's phosphate buffer for two hours, followed by dehydration in 10%, 25%, 50%, 75%, 95% ethanol,

allowing 10 minutes for each stage, followed by a final three, 10 minute changes of 100% ethanol. The cestodes were adhered to E.M. stubs using tape, with the head of the worm overlapping the stub, followed by gold sputter coating in an SC7640 Polaron. Examination of the *Atractolytocestus* was undertaken using a Zeiss Evo 50 scanning electron microscope.

6.2.3 DNA extraction and PCR amplification

Extraction of DNA was undertaken using a Qiagen DNeasy™ kit, following the manufacturer's instructions. Only the primer for cox1 was used in this study as the ribosomal ITS2 of *Atractolytocestus huronensis* is triploid (Bazsalovicsová *et al.* 2011; Bazsalovicsová *et al.,* 2012; Králová-Hromodová *et al.* 2013). The cox1 was amplified by polymerase chain reaction (PCR), using primers COX1: Forward, CFCYT2 (5'-ACTAAGTCCTTTTCAAAA - 3'); Reverse, CRCYT2 (5'- CCAAAAACCAAAACAT – 3') using the Veriti 96 well thermal cycler PCR machine, in the following cycle, - 1 minute at 50^0C; 5 minutes at 94^0C; 30 cycles of 1 minute at 94^0C, 1 minute at 50^0C, 2 minutes at 72^0C, and a final extension of 10 minute at 72^0C. Following DNA amplification, 5µl of the resultant amplicons were visualised through electrophoresis on 1% agarose gels stained with GelRed (Bioline). Samples were submitted for sequencing at the DNA Sequencing Facility of the Natural History Museum, London, using fluorescent dye terminator sequencing kits (Applied Biosystems™),these reactions were then run on an Applied Biosystems 3730KL automated sequencer.

6.2.4 Assembly of *Atractolytocestus* cox1, molecular identification of species and phylogenetic analysis

The successfully extracted and amplified sequences were manipulated and edited utilizing BioEdit 7.2.5, then compared with other *Atractolytocestus* cox1 sequences held in the GenBank® genetic sequence database, using the Basic Local Alignment Search Tool (BLASTn) (www.ncbi.nlm.nih.gov) for preliminary, molecular identification of species. For comparison, a further 12 *Atractolytocestus* and 5 Caryophyllidea species cox1, sequences published on GenBank® (www.ncbi.nlm.nih.gov/nuccore/) were downloaded (Table 6.1)

Table 6.1 Cox1 sequences downloaded from GenBank®

Species	Accession No.	Geographic Origin
Atractolytocestus huronensis	HM480478	Romania
Atractolytocestus huronensis	HM480477	Croatia
Atractolytocestus huronensis	HM480476	Hungary
Atractolytocestus huronensis	HM480475	Slovakia
Atractolytocestus huronensis	HM480474	UK
Atractolytocestus huronensis Isolate C	JQ034053	Hungary
Atractolytocestus sagittatus	JF424669	Japan
Atractolytocestus tenuicollis Isolate 1	KC834609	China
Atractolytocestus tenuicollis Isolate 2	KC834610	China
Atractolytocestus tenuicollis Isolate 3	KC834611	China
Atractolytocestus tenuicollis Isolate 4	KC834612	China
Atractolytocestus tenuicollis Isolate 5	KC834613	China
Caryophyllaeides fennica	KF051101	Bulgaria
Caryophyllaeus laticeps	KF051127	Russia
Khawia japonensis	JN004225	Japan
Khawia sinensis	(s) Gresford Flash	UK

Phylogenetic analysis was undertaken using MEGA version 6 (Tamura *et al.*, 2013) with computation of 18 cox1 sequences for maximum likelihood (ML) using the lowest Bayesian Information Criterion model, Hasegawa-Kishino-Yano + Gamma + Invariable, calculated on 500 bootstrap replicates.

6.2.5 Intra-species molecular diversity

The uncorrected pairwise distance was estimated using MEGA 6 (Tamura *et al.*, 2013) to compare the frequency of transitions (TS) and transversions (TV), and synonymous and non-synonymous mutations between species of *Atractolytocestus*.

6.3 Results

6.3.1 Morphology of *Atractolytocestus huronensis* in comparison to *Atractolytocestus* sp. from Pease Pottage, West Sussex

The morphological characters used to differentiate the Caryophyllidea are the shape of the scolex, the positions of the testes and vitelline follicles, features described here for *A. huronensis* from Hall Farm Reservoir, Woodham Mortimer, Essex and Lee Valley Regional Park, Waltham Abbey, Essex and the specimens from West Sussex.

Atractolytocestus huronensis (Figures 6.1A & 6.2A)

Length (mm) 10.64 – 11.79; Width (mm) 1.07 – 1.38; Scolex width (mm) 1.3 – 2.27; base of scolex to vitelline follicles 950 µm

Atractolytocestus huronensis is a relatively small caryophyllaeid, compared with species such as *Caryophyllaeus laticeps* or *Khawia sinensis.* The scolex is bulboacuminate shape on a narrow neck but comparison of Figure 6.1A, shows a striking similarity with Figure 1, of *A. sagittatus* in Oros *et al.* (2010). The vitelline follicles commence at the base of the neck and the testes are situated posterior to the first vitelline follicles. The scanning electron micrograph of the scolex of *A. huronensis* (Figure 6.2A) quite clearly illustrates the bulboacuminate scolex on a narrow neck.

Atractolytocestus species from West Sussex (Figures 6.1B – 6.2B)

Scolex length; 309.5µm scolex width 1300µm; base of scolex to vitelline follicles 125µm

The scolex is broad and acuminate, with grooves, the neck is absent so the scolex is only slightly differentiated from the body by its width at the base. The anterior vitelline follicles are situated immediately behind the scolex and the testes are posterior to the vitellaria. The scanning electron micrograph (Figure 6.2B) shows the acumimate scolex in detail, the grooves are deep, extending to the base.

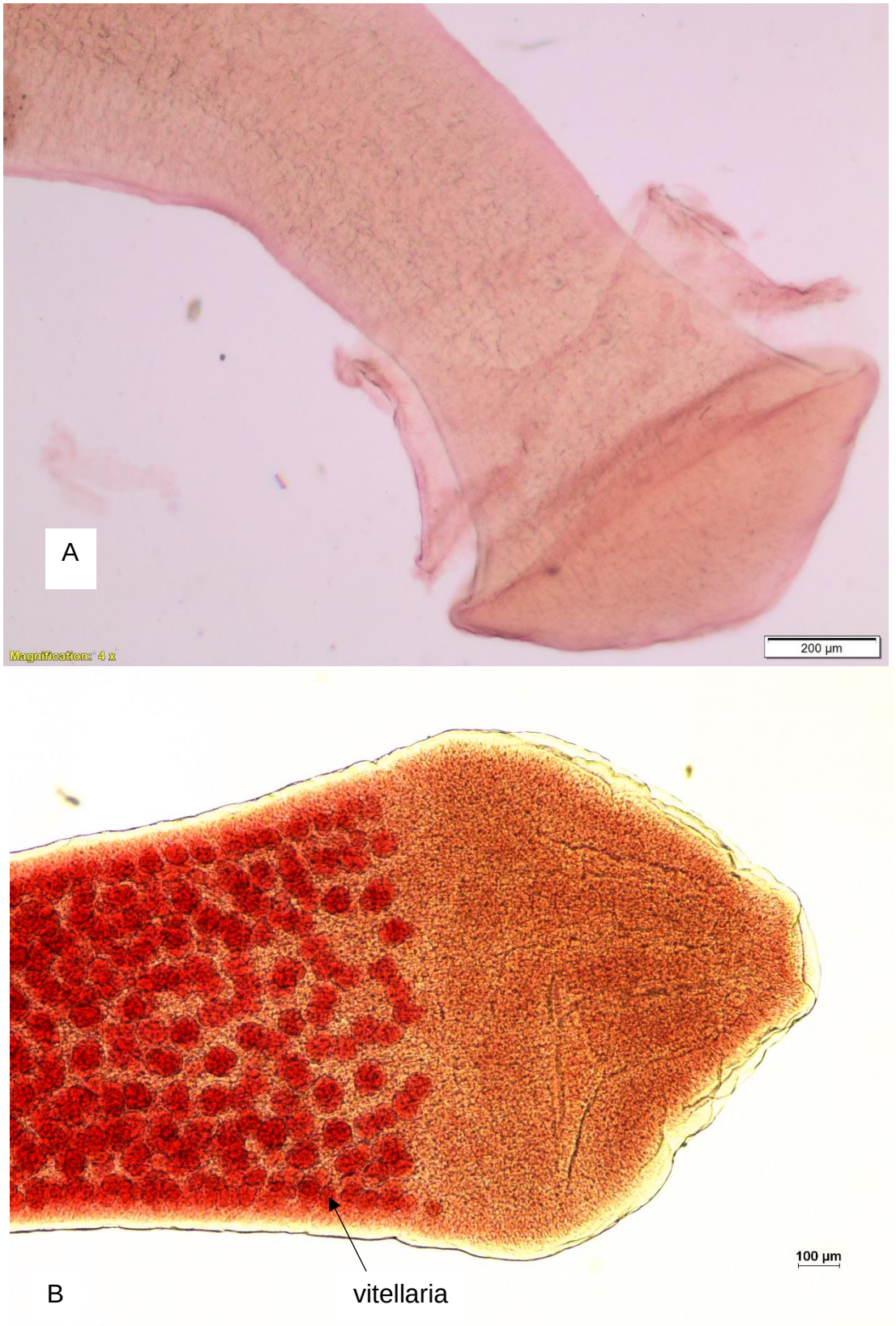


Figure 6.1 A. *Atractolytocestus huronensis* with bulboacuminate scolex and long neck B. *Atractolytocestus* from West Sussex, acuminate scolex, with vitellaria immediately behind the scolex

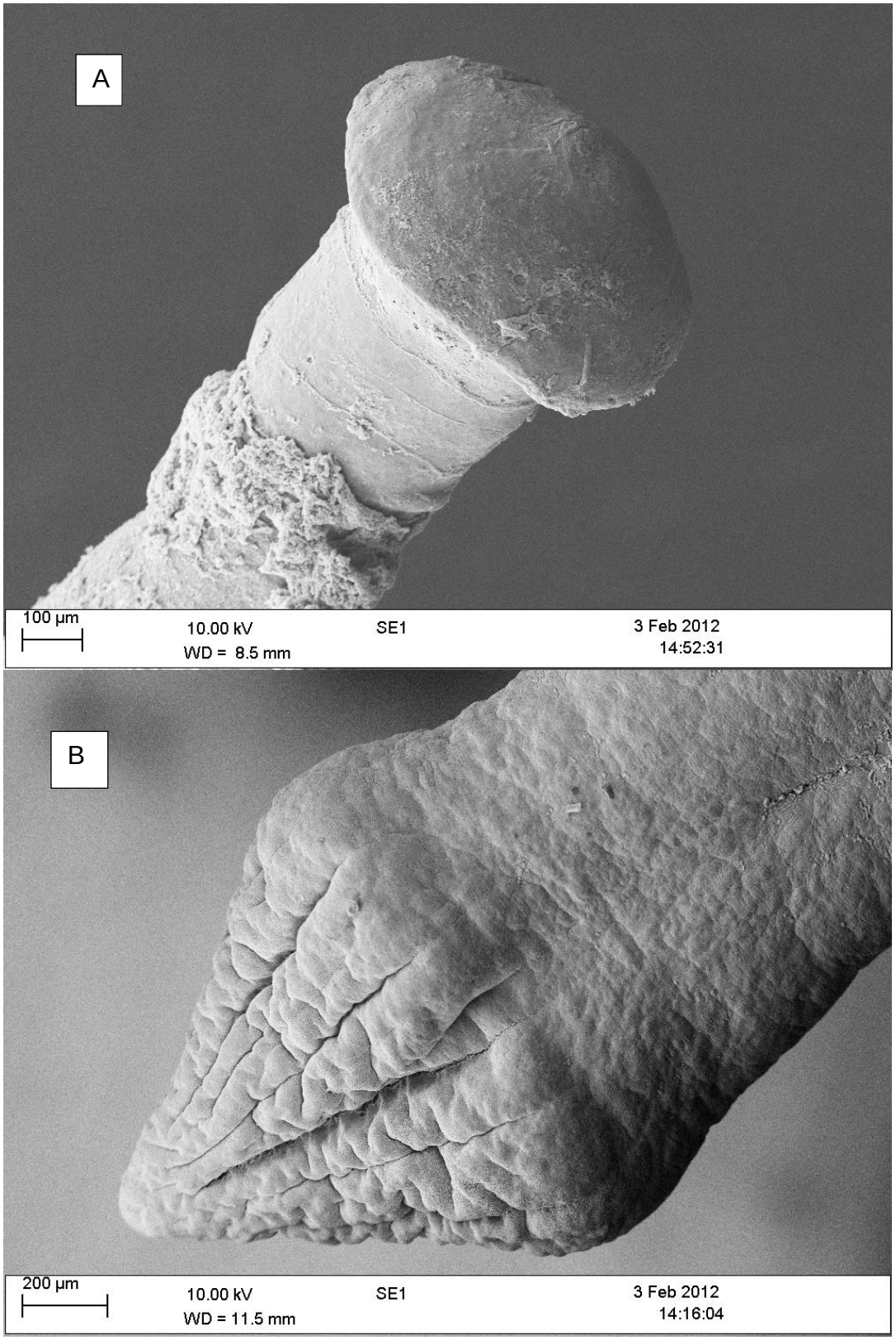


Figure 6.2

A. Scanning electron micrograph *Atractolytocestus huronensis* scolex

B. Scanning electron micrograph of the scolex of *Atractolytocestus* from West Sussex

The differences in morphology between *A. huronensis* and the specimen from West Sussex are very distinctive and summarized in Table 6.2.

Table 6.2. Summary of morphological differences between *A. huronensis* and the West Sussex specimens

Character	***A. huronensis***	**West Sussex specimens**
Scolex	Bulboacuminate	Acuminate
Scolex grooves	Absent	Present
Neck	Present	Absent
Vitellaria	Posterior to the neck	Immediately beneath the scolex

6.3.2 Initial comparison of *Atractolytocestus* sequences and phylogenetics based on cox1

Preliminary comparison of the *Atractolytocestus* sequences from West Sussex was undertaken using BLASTn analysis which identifies similar nucleotide sequences held in the Genbank® genetic sequence database. The result of the BLASTn analysis showed all of the sequences from West Sussex sequences bore a 78% shared identification with *A. huronensis* from the UK.

A maximum likelihood phylogenetic tree was constructed using MEGA 6 (Tamura *et al.* 2013) for the West Sussex sequences, labelled as SL1 CFCYT2; SLG7-G-CFCYCT2; SL-H7-H-CFCYT2, together with the 20 sequences obtained from GenBank®. The ML tree (Figure 6.3) shows that *Atractolytocestus* species are monophyletic. *Atractolytocestus huronensis* forms a separate clade which is a sister group to the *A. tenuicollis* clade. The West Sussex sequences form a sister clade to *A. sagittatus*. Other Caryophyllidea included in the Maximum Likelihood analysis are unresolved indicating they share few similarities with the *Atractolytocestus* sequences.

6.3.4 Intraspecific molecular variance of *Atractolytocestus* cox1 sequences

Applying the uncorrected p-distance which analyses the number of transitions and transversions and then comparing the differences between the sequences, showed substantial variation between the *Atractolytocestus* cox 1 (Figure 6.4). The comprisons made:

West Sussex *v.* West Sussex (Asp *v.* Asp)

A. tenuicollis v. A. tenuicollis (At *v.* At)

A. huronensis v. A. huronensis (Ah *v.* Ah)

A. sagittatus v. A.sagitattus (As v As)

A. sagittatus v. West Sussex (As *v.* Asp)

West Sussex *v. A. tenuicollis* (Asp *v.* At)

West Sussex *v. A. huronensis* (Asp *v.* Ah)

A. sagittatus v. A. huronensis (As *v.* Ah)

A. sagittatus v. A. tenuicollis (As *v.* At)

A, huronensis v. A. tenuicollis (Ah *v.* At)

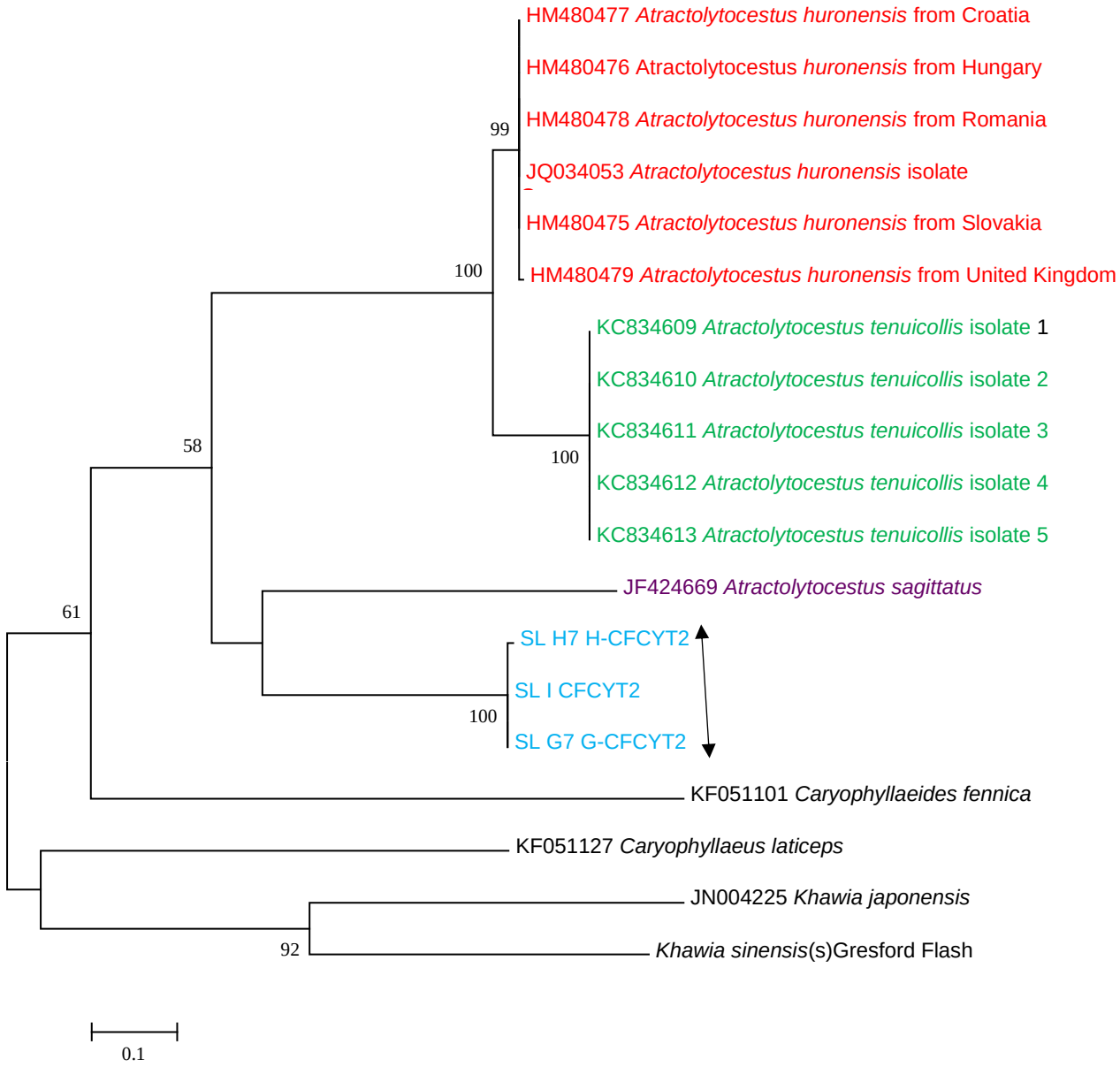


Figure 6.3 The phylogenetic reconstruction of *Atractolytocestus* species together with other species of Caryophyllidea using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.4283)). The rate variation model allowed for some sites to be evolutionarily invariable. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

West Sussex sequences arrowed

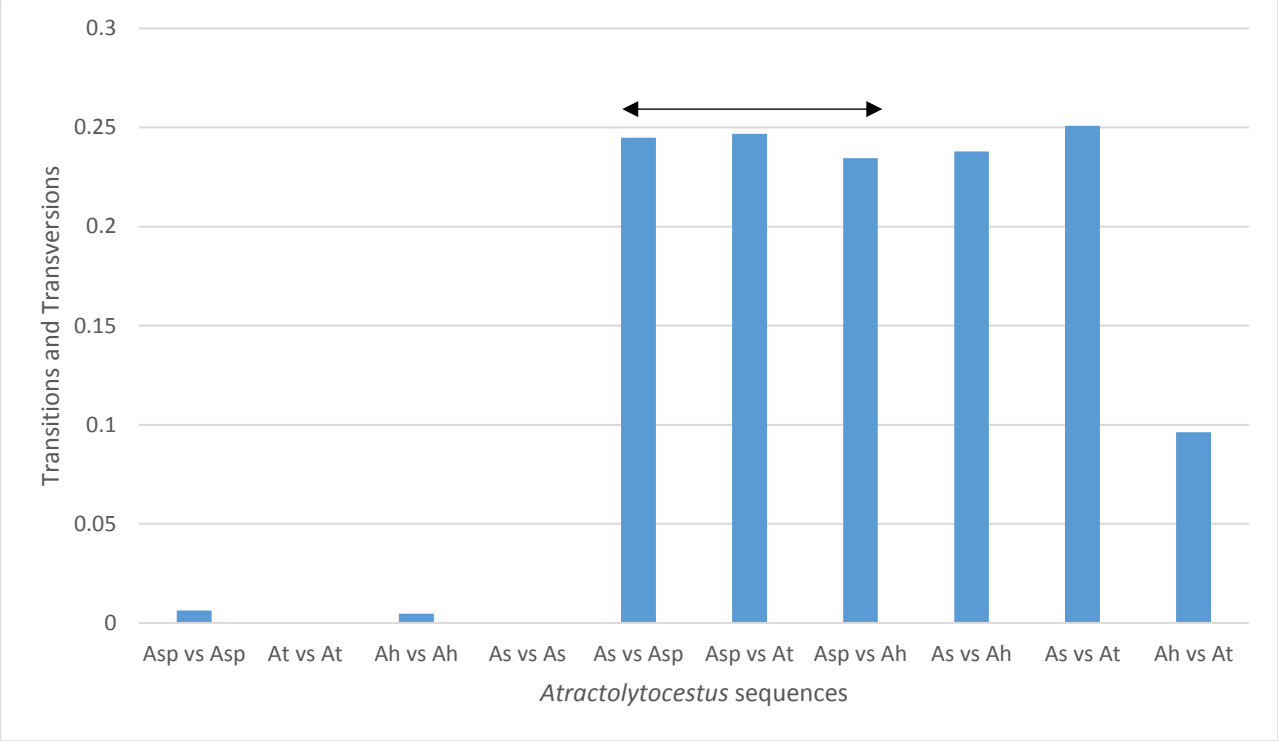


Figure 6.4 Comparison of transitions and transversions between *Atractolytocestus* cox1 sequences. Differences between the West Sussex and other *Atractolytocestus* species arrowed. Key: As – *A. sagittatus;* Asp – West Sussex sequence; At – *A. tenuicollis*; Ah – *A. huronensis*

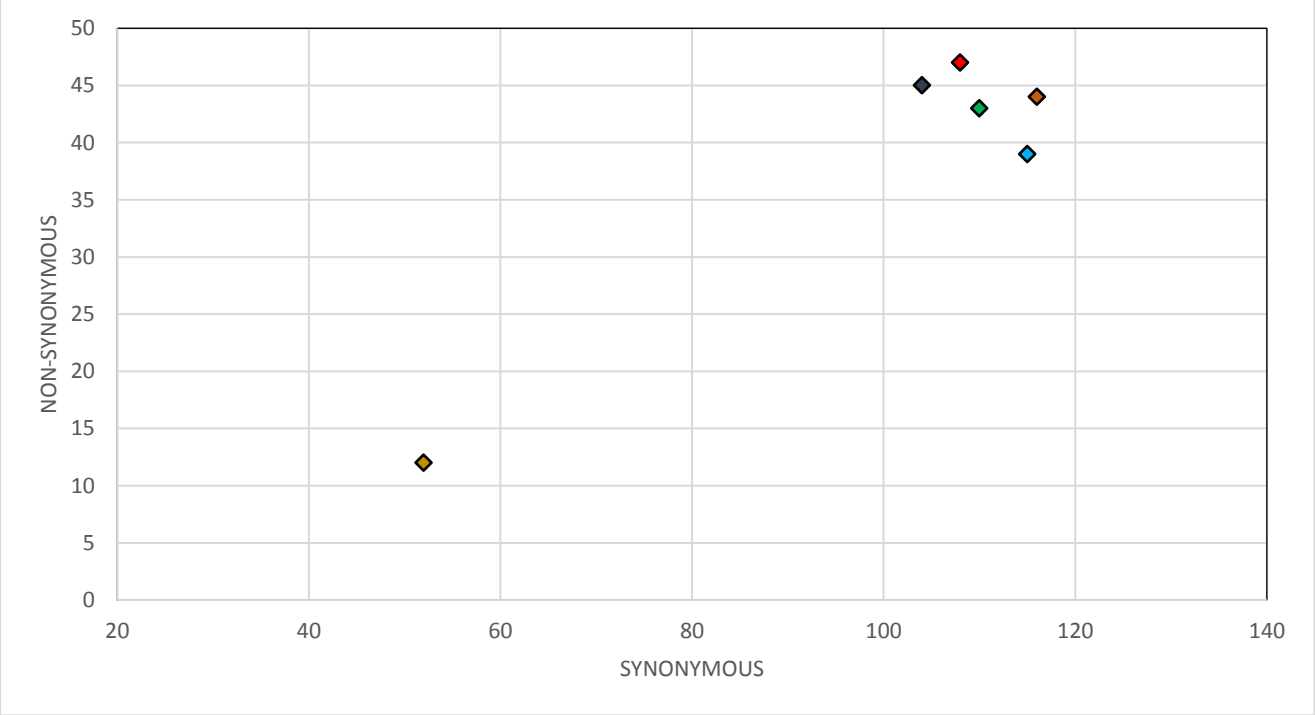


Figure 6.5 *Atractolytocestus* cox1 sequences, synonymous and non-synonymous mutations: Key: Ah = *A.huronensis*; At = *A.* tenuicollis; As = *A.* sagittatus; Asp = West Sussex sequence. Yellow - Ah *v*. At; blue - As *v*. At; green – Asp *v*. Ah; brown – Asp *v*. At; purple – As *v.* Ah; red As *v*. At

Comparison between the transitions and transversions in the cox1 sequences representing the same species, that is *A. sagittatus v. A. sagittatus;* West Sussex *v.* West Sussex; *A. tenuicollis v. A. tenuicollis; A. huronensis v. A. huronensis* showed either none or very small differences (Figure 6.4. Asp *v.* Asp and Ah *v.* Ah). Where there is very little or no variation in the number of transitions and transversions this indicates there is slight intraspecific variation in the cox1 sequences, and the sequences represent one species. When the *Atractolytocesus* cox1 sequences are compared with sequences representing the other species, the numbers of transitions and transversions increase to between 0.23-0.25 (Figure 6.4). The differences in numbers of transitions and transversions are indicative of genetic variation between the sequences. Comparison of the West Sussex sequences with *A. huronensis, A. sagittatus* and *A. tenuicollis* showed large numbers of differences in the transitions and transversions (Figure 6.4, arrowed). For example, the number of transitions and transversions differences between *A. sagittatus* (As) and West Sussex sequences (Asp) is 0.24 (Figure 6.4). Similarly, the number of differences in transitions and transversions between the other sequences is between 0.23 and 0.25. The exception is *A. huronensis* (Ah) and *A. tenuicollis* (At) with less than 0.1 transitions and transversions, which suggests these sequences are genetically similar. The number of differences in transitions and transversions indicate there is genetic variation between the sequences and that *A. sagittatus*, *A. tenuicollis, A. huronensis* and the West Sussex *Atractolytocestus* are separate species.

Synonymous mutations occur more frequently than non-synonymous mutations (Bromham, 2016) but the numbers of these mutations can be used to test genetic variability. Comparison of the synonymous and non-synonymous mutations shows the *Atractolytocestus* cox1 sequences segregating, indicating there is genetic variation in these sequences (Figure 6.5). Each of the plots in Figure 6.5 represents the sequences of *Atractolytocestus* which are seen to be separating according to species. *Atractolytocestus huronensis* and *A. tenuicollis* cox1 sequences have the least number of differences with 52 synonymous and 12 non-synonymous mutations (Figure 6.5), indicating these sequences have some genetic similarity. The cox1 sequences of the other *Atractolytocestus* species have significant differences of between 104 – 106 synonymous and 39 – 47 non-synonymous mutations. The most divergent number of synonymous and non-synonymous mutations are found in *A. sagittatus*, *A. tenuicollis* and the West Sussex *Atractolytocestus* sequence (Figure 6.5). These differences in the numbers of synonymous and non-synonymous mutations demonstrate significant genetic variability and are further evidence of intraspecific variation in the *Atractolytocestus* cox1 sequences and suggesting that the West Sussex sequence represents an undescribed species of this genus.

6.4 Discussion

Conflict between morphological and genetic data is a frequently reported problem in the study of taxonomy and systematics in the Caryophyllidea (Scholz *et al*., 2011; Králová-Hromodová *et al.,* 2013). In this study, it was observed that *Atractolytocestus* sp. from West sussex were morphologically distinct from *A. huronensis* The only morphological characteristic in common was that the testes commenced posterior to

the vitelline follicles. This feature distinguished *Atractolytocestus* sp. from *A. sagittatus* in which as many as 20 – 60 testes can be anterior to the first vitelline follicles (Scholz *et al*., 2001). However, the number of testes in *Atractolytocestus* sp. was estimated to be lower than *A. sagittatus* or *A. tenuicollis* although serial sectioning was not carried out due to a limited number of specimens.

The ML phylogenetic analysis indicates that *A. sagittatus* and the West Sussex specimens are more closely related to each other than other species of *Atractolytocestus,* corroborating the work of Králová-Hromodová *et al.* (2013). They are not the same species however, as they are on separate branches of the phylogenetic reconstruction, which indicates there is genetic variation between them. Pairwise analysis of the sequences, comparing the number of transitions and transversions and the number of differences in synonymous and non-synonymous mutations, demonstrates that the West Sussex *Atractolytocestus* specimens differ genetically from other described *Atractolytocestus* species (Figures 6.4 – 6.5). The molecular data therefore supports the hypothesis that the West Sussex *Atractolytocestus* specimens represent a new species.

Atractolytocestus huronensis was the first species to be described for the genus and was thought initially to be native to North America (Anthony, 1958) subsequently the origin of this carp-specific tapeworm species has been considered to be Asia since carp is also of Asian origin (Oros *et al*., 2011). The origin of the European and USA populations are not clear. *Atractolytocestus huronensis* may have been introduced to mainland Europe from Asia with human assisted and natural movements of carp.

Dekay (1842) reported that carp imported from France had been released into the Hudson River to create a commercial fishery and from 1877 the American Fish and Fisheries Commission began a programme of importing carp from Germany, stocking them into every State and territory to address the exploitation and decline of North American native fish stocks (http://nas.er.usgs.gov accessed May 2016). Both carp and *A. huronensis* are regarded as non-native introductions to North America but it seems possible this parasite was translocated from Germany along with its host in the 19th century.

During the 1980s and mid-1990s the hobby of keeping coldwater ornamental fish was at its height in the UK and goldfish and koi were imported to this country from North America (Brewster *et al*. 2007; pers. obs.). In view of the genetic similarity between UK and North American *A. huronensis* (Bazsalovicsová *et al.* 2011) there is a possibility the introduction of this non-native cestode into the UK occurred as a result of the ornamental trade in koi from North America. Introduction from mainland Europe may have occurred as a second invasion event.

Królová-Hromodová *et al.* (2013) have hypothesized that the triploid *A. huronensis* is a result of hybridization from a common ancestor and the closest potential common ancestor is diploid *A. tenuicollis*. *Atractolytocestus sagittatus* was originally described from infected carp from the Amur Basin in the Primorsk Region of Russia (Kuakovskaya and Akhmerov, 1965) which may indicate a Eurasian origin for *Atractolytocestus*, rather than the current view of a Far Eastern origin, although *A. tenuicollis* was originally described from Lake Wulasuhai, Inner Mongolia (Li, 1964).

Could the presence of *A. huronensis* in Europe have been overlooked but the increase in carp aquaculture allowed the prevalence and intensity of infection of this tapeworm to increase? The triploid (3n = 24) and supposedly parthenogenetic nature of *A. huronensis* has been supported by karyological (Králová-Hromodová *et al.*, 2010) and ultrastructural studies (Bruňanská *et al.*, 2011). It is surprising that triploid *A. huronensis* has exhibited better colonizing abilities than its diploid ancestor, but anthropogenic forces may have favoured dissemination.

The difference in the position of the West Sussex *Atractolytocestus* sequences in the ML phylogenetic tree, the pairwise analyses comparing transitions and transversions, synonymous and non-synonymous mutations, indicate this is a valid species. The West Sussex lake which is home to the host carp, is a long established site, which may owe its origins as an 18th century flight pond, for shooting wildfowl, but seems to be the only site in the UK where carp are host to this *Atractolytocestus* species (pers. obs.). The origin of the carp is unknown and apparently there have been no known introductions for over 20 years (D. Minnet pers. com.), the lake is situated in an isolated wood, surrounded by farmland and approximately 1km from the nearest road, therefore it is extremely unlikely that any ornamental fish have been released on to the site. It is possible this population of *Atractolytocestus* have undergone allopatric speciation.

The appearance of a non-native parasite represents a potential hazard as the establishment and dispersal amongst native fish populations and ability to infect novel hosts is unpredictable. The West Sussex *Atractolytocestus* were found in large numbers in the host carp intestine but there was no obvious associated pathology.

When the carp were translocated and held in tanks, the *Atractolytocestus* were shed into the water, suggesting there is a very loose attachment to the intestine. It is worth noting the host carp were all in excess of 2kg and it is possible the West Sussex *Atractolytocestus* had difficulty attaching to the intestinal epithelium.

6.5 Concluding remarks

The morphology of the *Atractolytocestus* species is conspicuously different from *A. huronensis*, the only other species of the genus considered to be present in the UK. The molecular studies indicate the West Sussex specimens are genetically different. The differences in the transitions and transversions and synonymous and non-synonymous mutations between the sequences from the West Sussex specimens and all other species of *Atractolytocestus* indicate the former to be genetically divergent and representative of a new species.

Chapter 7

Summary

1. Overview

Kennedy (1974) published a checklist of parasites associated with freshwater fish in the UK but this has never been updated and the parasite fauna has changed over the last forty years as a consequence of the introduction of non-native species. Whilst the importation of freshwater fish for aquaculture, recreational fishing and the ornamental industry are licenced by Cefas under the Aquatic Animal Health Regulations (http://www.legislation.gov.uk/uksi/2009/463/pdfs/uksi_20090463_en.pdf) and imported fish from Europe and third world countries must be certified free of List 1 and List 2 diseases, there is no screening for parasites. The increasing trend for fish production and global trade in fish and fish products is resulting in the introduction of non-native freshwater fish parasites. For example, *Sphaerothecum destruens,* a parasite of the topmouth gudgeon, (*Pseudorasbora parva*) has been introduced to the UK and whilst apparently causing little pathology to its native host, caused mortalities in experimentally infected carp, bream and roach, therefore representing a serious threat to native fish species (Andreou *et al*., 2012). Apart from legitimate freshwater fish imports, there remains an illicit trade in fish from continental Europe, which are released into lakes and stillwaters throughout England and Wales, with great potential for introducing non-native parasites. The consequences of the introduction of a non-native parasite on the host and potential novel hosts is unpredictable and evaluation of their threat to native fish remains a vital incentive for the study of fish parasitology. The scientific study of freshwater fish parasitology in the UK has been in steady decline for a number of years, as freshwater fish are perceived to be of low economic value even though the coarse fish industry supports 37,000 people in fulltime employment,

with in excess of £1.15 billion contribution to the UK economy annually (Environment Agency, 2009).

Checklists are a useful source of information concerning the distribution of native and non-native parasites of freshwater fish and also for research projects into parasite, parasite-host relationships, comparative data and identification guides (Poulin, 2016) but only if the information is contemporary. The information relating to checklists of fish parasites when published in journals is often rapidly superseded and these published works cannot be updated quickly, if at all. The concept of organizing data into an interactive, electronic, updateable database incorporating information on parasite taxonomy, associated hosts and distribution in the UK, was realized through the design and construction of a relational database, *Aquatic Parasite Information* (Appendix 1) which was populated with published and unpublished data on freshwater fish parasites and their hosts.

Interrogation of the data in *Aquatic Parasite Information* has generated a checklist of freshwater fish parasites in the UK (Appendix 2) and shown disproportionate records for metazoan parasites, compared with unicellular species, indicating a bias towards larger and easily identifiable parasites. Scholz & Choudhury (2014) found data representing the number of species of unicellular parasites infecting freshwater fish in Europe lacking, but found the metazoa are well represented in this region. One metazoan group which is exceptionally poorly represented in the records of *Aquatic Parasite Information* is the Dactylogyridae, which are difficult to identify based on morphological characters and overlooked because these monogeneans have been considered as benign and causing little, if any, pathology to infected fish. Rohlenova

et al. (2011) and Rastiannasab *et al.* (2015) have demonstrated that dactylogyrids affect the immune system, kidney and liver function in carp and may play a significant role in host susceptibility to disease and may influence morbidity. Although carp are the subject of these studies, other *Dactylogyrus* species may also be implicated in mortalities associated with other fish species, for example roach, are temperature sensitive over 10^0, which meets the preferred temperature range 10 – 20^0C of *D. crucifer* (Šimková *et al.,* 2001; Selver *et al*., 2009). Accurate identification of species is critical if dactylogyrids are implicated in fish disease or mortalities. *Dactylogyrus* species commonly encountered parasitizing UK freshwater fish proved extremely difficult to identify using morphology, so molecular techniques were employed to assist in the identification of a number of *Dactylogyrus* species from UK fish hosts. The single nucleotide polymorphisms demonstrate intraspecific genomic variability proving the ITS1 to be a useful marker and that molecular techniques are more reliable than morphology for the identification of *Dactylogyrus* species.

Since Chubb *et al*. (1987) published their monograph on the cestodes parasitic in British and Irish freshwater fish, the number of species of non-native tapeworm infecting fish has increased (Appendix 2). Whilst not every species of tapeworm infecting freshwater fish in the UK was available, the application of molecular techniques was employed as a potential means of identification. Two molecular markers were used for the study of the caryophyllids, the mitochondrial cox1 gene and the ribosomal r28s, whereas only the r28s marker was used for the bothriocephalids. The results showed the cox1 was genetically variable and a useful marker for the identification of the caryophyllid cestodes, the r28s marker showed less genetic variability but still proved to be capable of differentiating species of caryphyllid and bothriocephalid. The study indicated a preference for the use of the cox1 marker for

the identification of the cestodes. The increase in the global translocation of freshwater fish and fish products is resulting in the introduction of non-native species of cestode and the use of molecular markers will be invaluable as an identification tool for cryptic species such as *Khawia japonensis* which is potentially already in the UK.

Whilst molecular techniques can provide an identification of the parasites associated with freshwater fish, morphological characters should not be underestimated as these may still give an initial indication of parasite family or genus. During a routine fish health examination of carp from West Sussex, a species of *Atractolytocestus* was found which differed in morphology from *A. huronensis*, the only other representative of this genus in the UK. Morphological techniques using histology and scanning electron microscopy indicated the West Sussex species is quite distinct from *A. huronensis*. Applying molecular techniques using the cox1 marker to compare the genetic variation in *A. huronensis, A. tenuicollis* and *A. sagittatus* indicated the West Sussex specimens were not conspecific with any of these three species and therefore may represent a new species.

In the last 30 years angling has increased in popularity and is now regarded as the fifth most popular pastime (http://www.notsoboringlife.com accessed May 2016), with coarse fishing attracting an annual attendance of 26.4 million anglers on lakes or still waters (Environment Agency, 2009). All varieties of carp are the most sought after species of fish on lakes and stillwater fisheries. The demand from within the coarse fish industry for carp over 9kg and other large growing species such as wels catfsh, has led to a continuing illicit trade in freshwater fish from mainland Europe. The

parasite burden of these illegally imported fish is unknown as is the overall health status. Ornamental coldwater fish such as koi (ornamental carp), goldfish, orfe and grass carp have also been released into native habitats (Copp *et al.,* 2010) either through ignorance, intentionally or flooding. As a consequence of these illegal fish movements, non-native parasites have been introduced to the UK. Many of these non-native parasites are regarded by the Environment Agency as Category 2, defined as either of significant disease potential, or exotic parasites of unknown pathogenicity and distribution. Mining the data in *Aquatic Parasite Information* for Category 2 parasites revealed only one native species on this list, *Pomphorhynchus laevis*, the original distribution being regarded as a consequence of the post glacial dispersal of freshwater fish (Kennedy *et al.*, 1989; Kennedy, 2006). The records for Category 2 parasites extracted from *Aquatic Parasite Information* also indicated that whilst some species of non-native parasite such as the ergasilids were both established and invasive, other parasites of freshwater fish were declining through what appears to be the consequence of anthropogenic activities.

One consistent theme arising from interrogation of data held in *Aquatic Parasite Information* is the impact current fishery management policies have with regard to the stocking of fish into lakes and stillwaters and the potential effect this has for altering the parasite fauna of freshwater fish. Many lakes or stillwaters are heavily stocked with fish, often in the region of 3,000kg per ha fish (Brewster, 2000, 2009, 2014), a stock density of fish which is more appropriate to aquaculture than a poorly managed fishery. On many of these fisheries there is an issue concerning the supplementary feeding of fish, as angling club or society members have great concerns that feeding the fish will lower the opportunities for fishing and numbers of fish caught (pers. obs.).

Fishing is also a fair weather sport, with more anglers pursuing the hobby in the warmer summer months. Wet summers, cool autumns and cold winters, result in few anglers populating the banks, giving rise to malnourished or starving fish (pers. obs.). Consequently the fish in these densely populated lakes or stillwaters consume all the aquatic macroinvertebrates including those which are intermediate hosts to fish parasites. The decline in *S. inermis* is most likely a consequence of these fishery management stocking policies, where large populations of carp have consumed all lymnaeid snails, the intermediate host of the blood fluke. Fish stocking and fishery management policies are also becoming drivers in the freshwater fish parasite fauna and distribution of parasites in the UK.

The realization of *Aquatic Parasite Information* has enabled an updatable checklist for the distribution of parasites of freshwater fish in the UK. Recording and monitoring the distribution of parasites and their fish hosts is of significant value for evaluating the establishment and dispersal of non-native parasites which have been introduced and the impact these may have on both hosts and native parasites. It is also easy to lose focus on the native parasites of freshwater fish and the impact that factors such as fishery management can impose on their distribution. By virtue of the fact that *Aquatic Parasite Information* can be constantly updated provides a means of monitoring the anthropogenic influence on fish parasites.

Interrogation of the records contained in the database has identified that some parasite species are poorly represented either because there is a paucity of data, such as the unicellular parasites, or because they are difficult to identify such as *Dactylogyrus*

species or some of the cestodes infecting freshwater fish in the UK. The employment of molecular techniques has demonstrated that ITS1 markers differentiate *Dactylogyrus* species and cox1 is the preferred marker for the identification of cestodes, parasitizing freshwater fish in the UK. The cox1 and r28s markers proved to be of little value for phylogenetic analysis of the Caryophyllidea and Bothriocephalidea, but are useful for the identification of cestode species. The cox1 marker was a more effective marker for identification of the Caryophyllidea then the r28s but use of the cox1 marker for identification of species of Bothricephalidea requires further investigation. Whilst molecular techniques provide an accurate method of identifying certain groups of parasite, morphology remains a useful tool for the preliminary identification of many parasite groups.

Appendix 1

Database Software Design

The initial stage in the design of the database was the identification of 'entities', component elements representing the main focus for collation and storage of data. Convention requires an entity is described by a noun, those entities forming the foundation of this database were 'Parasite Species' and 'Fish Species', other entities were then identified, relating to the classification and taxonomy of the parasites, host nomenclature, author, references, target organ, location of the fish species, or sample and administrative details.

The entities were linked by one of three possible types of relationship:

a) 'One to one', is an entity which has a single entry, related to a second entity that also has just a single entry related to the entry in the first entity. A 'one to one' relationship is represented by a single line connecting the two entities. For example, the entity 'Sample', refers to a sample of fish, which has a one to one relationship with the entity 'Location', because any fish sample can only originate from a single location (Figure 1a).

Figure 1a. Example of a 'one to one' relationship, indicated by a line connecting a fish 'sample' with location as the fish can only have been collected from one source

b) 'One to many', defined as an entity related to a second entity which includes more than one related data entry but this second entity can only be related to the first entity by a single entry. 'One to many' relationships are indicated in the conceptual database design by a line originating from an entity 'one' and ending

in a tricorn (Opell, 2009) to indicate ‘many’. An example of ‘one to many’ is the entity ‘Fish Species, which may be host to more than one Parasite Species (Figure 2a).

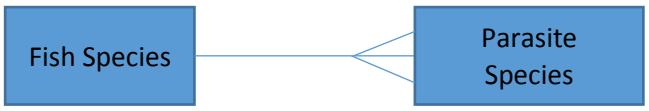


Figure 2a. Example of a ‘one to many’ relationship, one fish may be host to a number of parasites, where ‘one’ is indicated by a single line and ‘many’ by a tricorn (Oppel, 2009)

c) ‘Many to many’, which is represented by a line with a tricorn (Opell, 2009) at both ends connects two entities, where data in one entity has related multiple data in a second entity, which also has multiple data related to the first entity, for example there may be many ‘Parasitespecies’ infecting more than one ‘Organ’ in a single fish (Figure 3a).

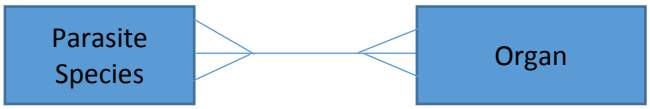


Figure 3a. ‘Many to many’ relationship, a parasite may be found in more than one fish organ and a fish organ may contain more than one species of parasite, with ‘many indicated by a tricorn (Opell, 2009)

The defined entities became the subject of the database tables, which were then populated with fields, displayed as columns, which effectively describe the data contained in each table (Unsworth, 2007). Following the entry of fields into the tables, ‘primary keys’ were identified, each table has only one primary key which specified a

unique field which formed the basis for creating relationships between the tables (Hernandez, 2013) and depicted by a key icon in Microsoft Access® (Figure 4a)

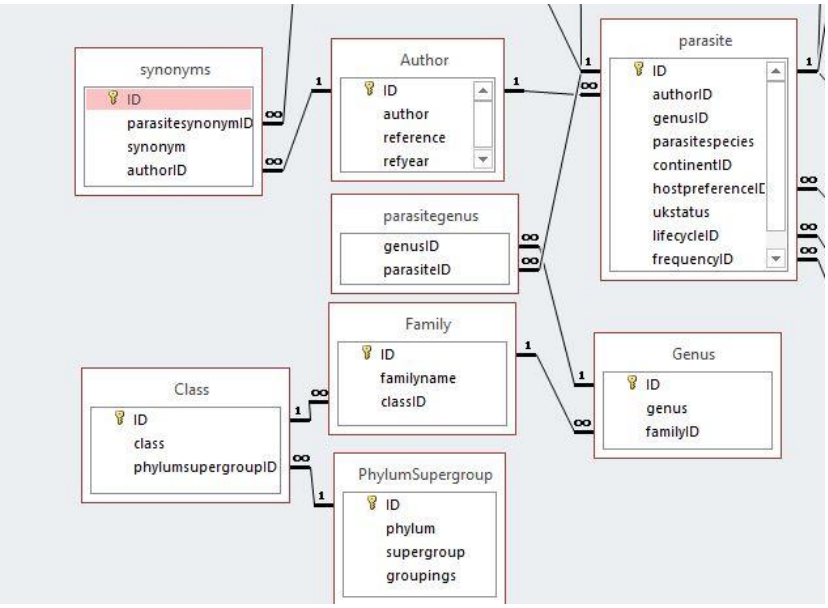


Figure 4a. Primary keys relating tables in the relationship diagram

Focussing on the table 'Author' in Figure 4a, it may be seen the primary key is a single entry related to many entries in both 'AuthorID' in the 'Parasite' and 'Synonyms' tables. Relationships are defined using the same criteria as for the entities of 'one to one', and 'one to many', using the figure '1', for 'one' a single entry and ∞ for 'many' related entries (Figure 4a). Where a table containing a primary key is connected to another table containing a field with the same name as the primary key, the pair are termed a 'common field' but the similarly named field in the second table is then termed the 'foreign key' (Figure 5a).

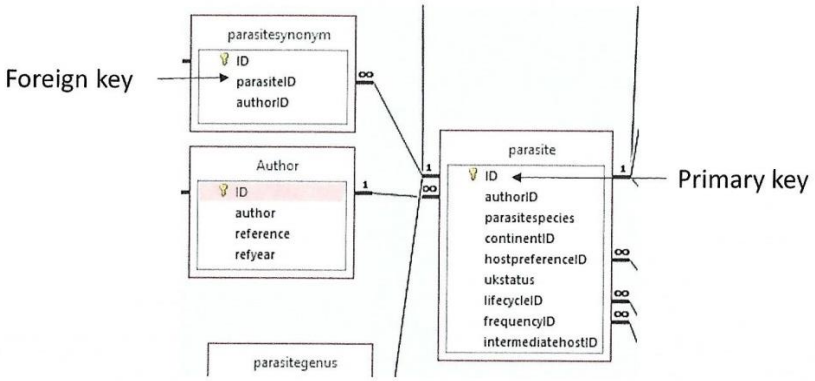


Figure 5a. Example of a common field, parasite is the primary key and 'ParasiteID' is the foreign key, which serves as the parasitesynonym primary key. 'ID' is Integral Definition, combining data from residing in different sources

The foreign key bears the same name as the primary key with the same field specification and takes values from the primary key to which it refers (Hernandez, 2013).

The completed relationship diagram varied from the conceptual, entity design based on the identification of primary keys and then establishing the relationships between the tables (Figure 6a). Once the relationship diagram was deemed to be satisfactory, the tables were populated with 'fields'.

Data was entered into the database through the use of data entry forms which were created in the Microsoft Access® software from the tables, the forms also incorporated a query for fish species and their common names, enabling information stored in more than one table to be located and populated. The data entry forms enabled data extrapolated from routine screening of fish health for movement consents, and published literature for synonyms, authors and references to be added methodically and populate the database.

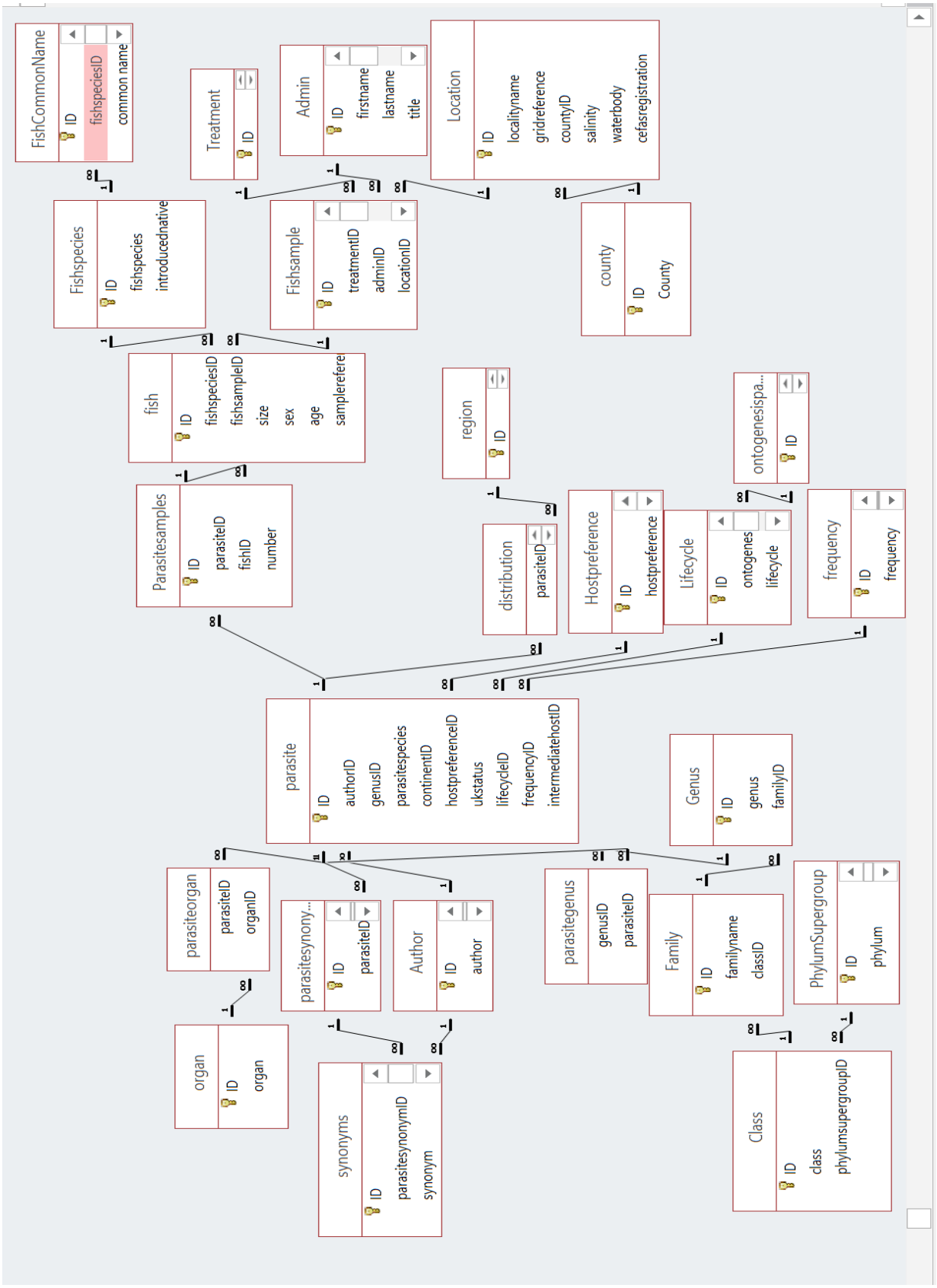


Figure 6a. Finalized Relationship Diagram on which *Aquatic Parasite Information* was constructed using Microsoft Access®

Appendix 2. Fish hosts and associated parasites

Abramis brama	***Anguilla anguilla***
Ichthyophthirius multifilis	*Epieimeria anguillae*
Chilodonella cyprini	*Ichthyophthirius multifilis*
Ichthyobodo necator	*Ichthyobodo necator*
Myxobolus mülleri	*Trypanosoma granulosum*
Acanthocephalus lucii	*Myxidium giardi*
Pomphorhynchus laevis	*Myxobolus dermatobius*
Dactylogyrus auriculatus	*Neoechinorhynchus rutili*
Dactylogyrus crucifer	*Acanthocephalus anguillae*
Dactylogyrus wunderi	*Acanthocephalus clavula*
Pellucidhaptor pricei	*Acanthocephalus lucii*
Gyrodactylus elegans	*Echinorhynchus truttae*
Paradiplozoon homoion	*Pomphorhynchus laevis*
Diplozoon paradoxum	*Pseudodactylogyrus anguillae*
Eudiplozoon nipponicum	*Pseudodactylogyrus bini*
Diphyllobothrium latum	*Discocotyle sagittata*
Ligula intestinalis	*Proteocephalus macrocephalus*
Caryophyllaeides fennica	*Ligula intestinalis*
Caryophyllaeus laticeps	*Schistocephalus solidus*
Valipora campylancristata	*Bothriocephalus claviceps*
Allocreadium isoporum	*Crepidostomum farionis*
Anodonta cygnea	*Crepidostomum metoecus*
Argulus coregoni	*Triaenophorus nodulosus*
Argulus foliaceus	*Cyathocephalus truncatus*
Asymphylodora kubanicum	*Deropristis inflata*
Diplostomum spathaceum	*Diplostomum spathaceum*
Posthodiplostomum cuticola	*Phyllodistomum simile*
Tylodelphys clavata	*Lecithochirium gravidum*
Echinochasmus perfoliatus	*Sphaerostoma bramae*
Sphaerostoma bramae	*Anguillicoloides crassus*
Ichthyocotylurus variegatus	*Argulus foliaceus*
Ergasilus briani	*Camallanus lacustris*
Ergasilus sieboldi	*Contracaecum aduncum*
Neoergasilus japonicus	*Paraquimperia tenerrima*
Paraergasilus longidigitus	*Raphidascaris acus*
Lernaea cyprinacea	*Raphidascaris cristata*
Tracheliastes maculatus	*Truttaedacnitis truttae*
Piscicola geometra	*Spinitectus inermis*
	Piscicola geometra
Alburnus alburnus	*Ergasilus gibbus*
Ichthyophthirius multifilis	*Ergasilus sieboldi*
Acanthocephalus, anguillae	
Pomphorhynchus laevis	
Argulus foliaceus	
Amblopites rupestris	
laevis, Pomphorhynchus	

Barbatula barbatula	***Carassius carassius***
Pomphorhynchus laevis	*Apiosoma piscicola*
Gyrodactylus pavlovskyi	*Ichthyophthirius multifilis*
Gyrodactylus sedelnikowi	*Chilodonella cyprini*
Triaenophorus nodulosus	*Ichthyobodo necator*
Ergasilus sieboldi	*Trypanoplasma borelli*
	Trypanoplasma keisselitzi
Barbus barbus	*Dactylogyrus anchoratus*
Ichthyophthirius multifilis	*Paradiplozoon homoion*
Chilodonella cyprini	*Diplozoon paradoxum*
Ichthyobodo necator	*Schizocotyle acheilognathi*
Sphaerospora dykovae	*Caryophyllaeus laticeps*
Neoechinorhynchus rutili	*Valipora campylancristata*
Acanthocephalus anguillae	*Sanguinicola inermis*
Pomphorhynchus laevis	*Diplostomum spathaceum*
Gyrodactylus laevis	*Posthodiplostomum cuticola*
Bathybothrium rectangulum	*Tylodelphys clavata*
Caryophyllaeus fimbriceps	*Philometroides sanguinea*
Diplostomum spathaceum	*Piscicola geometra*
Posthodiplostomum cuticola	*Anodonta cygnea*
Ergasilus sieboldi	*Ergasilus briani*
Argulus foliaceus	*Ergasilus sieboldi*
Piscicola geometra	*Neoergasilus japonicus*
	Paraergasilus longidigitus
Blicca bjoerkna	*Argulus foliaceus*
Paradiplozoon homoion	*Lernaea cyprinacea*
Diplostomum spathaceum	
Posthodiplostomum cuticola	***Cobitis taenia***
	Triaenophorus nodulosus
Carassius auratus	*Ichthyocotylurus variegatus*
Apiosoma piscicola	*Nicolla gallica*
Trichodina acuta	
Chilodonella cyprini	***Coregonus clupeoides***
Schizocotyle acheilognathi	*Henneguya tegidiensis*
Sanguinicola inermis	
Diplostomum spathaceum	***Coregonus lavaretus***
Tylodelphys clavata	*Acanthocephalus anguillae*
Argulus foliaceus	*Ichthyocotylurus erraticus*
Lernaea cyprinacea	
	Coregonus pennantii
	Acanthocephalus clavula
	Diphyllobothrium ditremum
	Diphyllobothrium dendriticum
	Phyllodistomum folium
	Coregonus pollan
	Proteocephalus pollanicola
	Ichthyocotylurus variegatus

Cottus gobio	***Cyprinus carpio x Carassius auratus hybrid***
Chilodonella cyprini	*Diplostomum spathaceum*
Gyrodactylus rogatensis	
Ichthyocotylurus variegatus	***Cyprinus carpio x C. auratus/C.carassius F1 hybrids***
Nicolla gallica	*Diplostomum spathaceum*
Lernaea cyprinacea	*Piscicola geometra*
Ctenopharyngodon idella	***Cyprinus carpio x Carassius carassius hybrid***
Chilodonella cyprini	*Apiosoma piscicola*
Schizocotyle acheilognathi	*Chilodonella cyprini*
Triaenophorus nodulosus	*Ichthyophthirius multifilis*
Piscicola geometra	*Khawia sinensis*
Argulus foliaceus	*Sanguinicola inermis*
Ergasilus sieboldi	*Diplostomum spathaceum*
	Echinochasmus perfoliatus
Cyprinus carpio	*Piscicola geometra*
Eimeria rutili	*Ergasilus briani*
Apiosoma piscicola	*Argulus foliaceus*
Ichthyophthirius multifilis	*Salmincola edwardsii*
Chilodonella cyprini	
Chilodonella hexasticha	
Ichthyobodo necator	
Zschokkella cyprini	
Sphaerospora dykovae	
Neoechinorhynchus rutili	
Acanthocephalus anguillae	
Acanthocephalus lucii	
Pomphorhynchus laevis	
Dactylogyrus anchoratus,	
Dactylogyrus extensus	
Dactylogyrus vastator	
Paradiplozoon homoion	
Eudiplozoon nipponicum,	
Diplozoon paradoxum,	
Schizocotyle acheilognathi	
Caryophyllaeus laticeps	
Atractolytocestus huronensis	
Khawia sinensis	
Sanguinicola inermis	
Diplostomum spathaceum	
Tylodelphys clavata	
Piscicola geometra	
Anodonta cygnea	
Ergasilus briani	
Ergasilus sieboldi	
Neoergasilus japonicus	
Paraergasilus longidigitus	
Argulus foliaceus	
Lernaea cyprinacea	

Esox lucius	***Gasterosteus aculeatus***
Ichthyophthirius multifilis	*Ichthyophthirius multifilis*
Ichthyobodo necator	*Ichthyobodo necator*
Chilodonella cyprini	*Trichodina domerguei*
Glugea luciopercae	*Trichodina pediculus*
Chloromyxum esocinum	*Trichodina reticulata*
Myxidium lieberkühni	*Trichodina tenuidens*
Henneguya oviperda	*Glugea luciopercae*
Henneguya psorospermica	*Dermocystidium gasterostei*
Myxobolus volgensis	*Sphaerospora elegans*
Acanthocephalus clavula	*Acanthocephalus clavula*
Acanthocephalus lucii	*Pomphorhynchus laevis*
Pomphorhynchus laevis	*Gyrodactylus arcuatus*
Tetraonchus menonteron	*Gyrodactylus pungitii*
Gyrodactylus lucii	*Gyrodactylus rarus,*
Proteocephalus percae	*Proteocephalus fillicollis*
Diphyllobothrium latum	*Diphyllobothrium norvegicum*
Triaenophorus nodulosus	*Diphyllobothrium dendriticum*
Cyathocephalus truncatus	*Schistocephalus solidus*
Azygia lucii	*Diplostomum gasterostei*
Bucephalus polymorphus	*Tylodelphys clavata*
Diplostomum spathaceum	*Phyllodistomum folium*
Tylodelphys clavata	*Raphidascaris cristata*
Phyllodistomum folium	*Thersitina gasterostei*
Ichthyocotylurus variegatus,	*Argulus foliaceus*
Camallanus lacustris	*Lernaea cyprinacea*
Raphidascaris acus	
Raphidascaris cristata	***Gobio gobio***
Spinitectus inermis	*Goussia metchnikovi*
Piscicola geometra	*Ichthyophthirius multifilis*
Anodonta cygnea,	*Chilodonella cyprini*
Ergasilus sieboldi	*Ichthyobodo necator,*
Neoergasilus japonicus	*Myxobolus cyprini*
Paraergasilus longidigitus	*Sphaerospora dykovae*
Argulus coregoni	*Pomphorhynchus laevis.*
Argulus foliaceus	*Dactylogyrus gobii*
	Ligula intestinalis
	Caryophyllaeides fennica
	Paradiplozoon homoion
	Diplozoon paradoxum
	Diplostomum spathaceum
	Tylodelphys clavata
	Ergasilus briani
	Ergasilus sieboldi
	Neoergasilus japonicus
	Camallanus lacustris,
	Piscicola geometra
	Anodonta cygnea
	Argulus foliaceus

Gymnocephalus cernuus	***Leuciscus leuciscus***
Glugea luciopercae	*Ichthyophthirius multifilis*
Dactylogyrus amphibothrium	*Chilodonella cyprini*
Pomphorhynchus laevis	*Ichthyobodo necator*
Proteocephalus percae	*Henneguya zschokkei*
Allocreadium isoporum	*Myxobolus mülleri*
Tylodelphys clavata	*Myxobolus volgensis*
Tylodelphys podicipina	*Acanthocephalus anguillae*
Camallanus lacustris	*Pomphorhynchus laevis*
Anguillicoloides crassus	*Dactylogyrus cordus*
	Dactylogyrus vistulae
Lampetra fluviatilis	*Dactylogyrus tuba*
Diplostomum petromyzi-fluviatilis	*Proteocephalus torulosus*
	Caryophyllaeus fimbriceps
Leucaspius delineatus	*Caryophyllaeus laticeps*
Myxidium rhodei	*Caryophyllaeides fennica*
Sphaerothecum destruens	*Allocreadium isoporum*
Diplozoon paradoxum	*Bucephalus polymorphus*
Diplostomum spathaceum	*Diplostomum spathaceum*
Ergasilus briani	*Posthodiplostomum cuticola*
Neoergasilus japonicus	*Tylodelphys clavata*
	Sphaerostoma bramae
Leuciscus idus	***Raphidascaris acus***
Diplostomum spathaceum	*Argulus coregoni*
Lernaea cyprinacea	*Argulus foliaceus*
Tracheliastes polycolpus	*Ergasilus sieboldi*
	Ergasilus briani
	Neoergasilus japonicus
	Tracheliastes polycolpus
	Thersitina gasterostei
	Onchorhynchus mykiss
	Ichthyophthirius multifilis
	Trichodina acuta
	Trichodina nigra
	Ichthyobodo necator
	Acanthocephalus lucii
	Discocotyle sagittata
	Pomphorhynchus laevis
	Proteocephalus percae
	Diphyllobothrium dendriticum
	Triaenophorus nodulosus
	Tylodelphys clavata
	Tylodelphys podicipina
	Cystidicola farionis
	Piscicola geometra
	Argulus foliaceus
	Ergasilus sieboldi
	Lernaea cyprinacea

Perca fluviatilis	***Phoxinus phoxinus***
Ichthyophthirius multifilis	*Trichodina acuta*
Chilodonella cyprini	*Trichodina intermedia*
Ichthyobodo necator	*Chloromyxum phoxini*
Trypanosoma percae	*Pomphorhynchus laevis*
Glugea luciopercae	*Dactylogyrus phoxini*
Henneguya psorospermica	*Gyrodactylus aphyae,*
Myxobolus mülleri	*Gyrodactylus laevis*
Acanthocephalus anguillae	*Gyrodactylus limneus*
Acanthocephalus clavula	*Gyrodactylus macronychus*
Acanthocephalus lucii	*Gyrodactylus medius*
Pomphorhynchus laevis	*Gyrodactylus minimus*
Ancyrocephalus paradoxus	*Caryophyllaeides fennica*
Ancyrocephalus percae	*Allocreadium isoporum*
Proteocephalus filicollis	*Macrolecithus papilliger*
Proteocephalus percae	*Rhipidocotyle illense*
Triaenophorus nodulosus	*Diplostomum phoxini*
Bunodera lucioperca	*Posthodiplostomum cuticola*
Bucephalus polymorphus	*Phyllodistomum folium*
Rhipidocotyle illense	*Sphaerostoma bramae*
Diplostomum gasterostei	*Rhabdochona denudata*
Diplostomum spathaceum	*Raphidascaris cristata,*
Tylodelphys clavata	
Tylodelphys podicipina	***Platichthys flesus***
Ichthyocotylurus cucullus	*Pomphorhynchus laevis*
Ichthyocotylurus pileatus	
Ichthyocotylurus variegatus	***Pungitius pungitius***
Camallanus lacustris	*Trichodina domerguei*
Raphidascaris acus	*Trichodina tenuidens*
Raphidascaris cristata	*Dermocystidium gasterostei*
Truttaedacnitis truttae	*Gyrodactylus pungitii*
Piscicola geometra	*Gyrodactylus rarus*
Anodonta cygnea	*Proteocephalus filicollis*
Ergasilus briani	*Thersitina gasterostei*
Ergasilus sieboldi	
Neoergasilus japonicus	
Argulus foliaceus	
Salmincola percarum	

Rutilus rutilus	***Rutilus rutilus* continued**
Eimeria rutili	*Philometra rischta*
Ichthyophthirius multifilis	*Piscicola geometra*
Trichodina polycirra	*Anodonta cygnea*
Trichodina urinaria	*Argulus coregoni*
Chilodonella cyprini	*Argulus foliaceus*
Ichthyobodo necator	*Ergasilus briani*
Trypanoplasma borelli	*Ergasilus sieboldi*
Trypanoplasma keisselitzi	*Neoergasilus japonicus*
Pleistophora longifilis	*Paraergasilus longidigitus*
Myxidium rhodei	*Lernaea cyprinacea*
Myxobolus artus	
Myxobolus mülleri	***Rutilus rutilus x Abramis brama***
Myxobolus pseudodispar	*Myxidium rhodei*
Myxobolus volgensis	*Diplostomum spathaceum*
Sphaerospora dykovae	*Tylodelphys clavata*
Acanthocephalus anguillae	*Ergasilus sieboldi*
Acanthocephalus clavula	
Acanthocephalus lucii	***Salmo salar***
Pomphorhynchus laevis	*Chloromyxumn truttae*
Dactylogyrus crucifer	*Henneguya zschokkei*
Dactylogyrus nanus	*Myxidium truttae*
Dactylogyrus similis	*Myxobolus arcticus*
Dactylogyrus sphyrna,	*Myxobolus neurobius*
Dactylogyrus suecicus	*Sphaerospora truttae*
Gyrodactylus elegans	*Neoechinorhynchus rutili*
Gyrodactylus medius	*Acanthocephalus lucii*
Paradiplozoon homoion	*Echinorhynchus truttae*
Diplozoon paradoxum	*Pomphorhynchus laevis*
Proteocephalus torulosus	*Discocotyle sagittata*
Schizocotyle acheilognathi	*Gyrodactylus derjavini*
Ligula intestinalis	*Diphyllobothrium dendriticum*
Biacetabulum appendiculatum	*Diphyllobothrium ditremum*
Caryophyllaeides fennica	*Eubothrium salvelini*
Caryophyllaeus laticeps	*Cyathocephalus truncatus*
Paradilepis scolecina	*Phyllodistomum folium*
Allocreadium isoporum	*Phyllodistomum simile*
Aspidogaster limacoides	*Crepidostomum farionis*
Bucephalus polymorphus	*Ichthyocotylurus erraticus*
Diplostomum spathaceum	*Rhabdochona oncorhynchi*
Hysteromorpha triloba	*Raphidascaris cristata*
Posthodiplostomum cuticola	*Truttaedacnitis truttae*
Tylodelphys clavata	*Cystidicola farionis*
Echinochasmus perfoliatus	*Cystidicoloides tenuissima*
Asymphylodora kubanicum	*Margaritifera margaritifera*
Sphaerostoma bramae	*Argulus foliaceus*
Ichthyocotylurus variegatus	*Salmincola salmoneus*
Camallanus lacustris	
Raphidascaris acus	
Philometra ovata	

Salmo trutta	***Scardinius erythrophthalmus***
Eimeria rutili	*Ichthyophthirius multifilis*
Trichodina acuta	*Chilodonella cyprini*
Trichodina nigra	*Ichthyobodo necator*
Chilodonella cyprini	*Myxidium rhodei*
Ichthyobodo necator	*Myxidium scardini*
Octomitus truttae	*Paradiplozoon homoion*
Glugea luciopercae	*Caryophyllaeides fennica*
Chloromyxum truttae	*Valipora campylancristata*
Myxidium truttae	*Posthodiplostomum cuticola*
Myxobolus neurobius	*Diplostomum spathaceum*
Neoechinorhynchus rutili	*Tylodelphys clavata*
Acanthocephalus clavula	*Echinochasmus perfoliatus*
Acanthocephalus lucii	*Philometra ovata*
Gyrodactylus truttae	*Philometroides sanguinea*
Discocotyle sagittata	*Piscicola geometra*
Echinorhynchus truttae	*Anodonta cygnea*
Pomphorhynchus laevis	*Argulus foliaceus*
Proteocephalus neglectus	*Argulus japonicus*
Proteocephalus percae	*Ergasilus briani*
Diphyllobothrium dendriticum	*Ergasilus sieboldi*
Diphyllobothrium ditremum	*Neoergasilus japonicus*
Diphyllobothrium latum	*Paraergasilus longidigitus*
Diphyllobothrium norvegicum	
Cyathocephalus truncatus	***Silurus glanis***
Bunodera lucioperca	*Chilodonella cyprini*
Tylodelphys clavata	*Thaparocleidus vistulensis*
Phyllodistomum folium	*Glanitaenia osculata*
Phyllodistomum simile	*Diplostomum spathaceum*
Apatemon gracilis	*Piscicola geometra*
Ichthyocotylurus erraticus	*Argulus foliaceus*
Raphidascaris acus	
Raphidascaris cristata	
Cystidicola farionis	
Piscicola geometra	
Argulus coregoni	
Argulus foliaceus	
Ergasilus sieboldi	
Salmincola salmoneus	
Lernaea cyprinacea	
Salvelinus alpinus	
Diphyllobothrium dendriticum	
Eubothrium salvelini	
Phyllodistomum folium	
Salvelinus fontinalis	
Phyllodistomum folium	
Lernaea cyprinacea	

Squalius cephalus	***Tinca tinca***
Ichthyophthirius multifilis	*Apiosoma piscicola*
Chilodonella cyprini	*Ichthyophthirius multifilis*
Ichthyobodo necator	*Chilodonella cyprini*
Myxobolus macrocapsularis	*Ichthyobodo necator*
Myxobolus mülleri	*Trypanoplasma borelli*
Myxobolus volgensis	*Trypanoplasma keisselitzi*
Acanthocephalus anguillae	*Acanthocephalus anguillae*
Acanthocephalus lucii	*Acanthocephalus lucii*
Pomphorhynchus laevis	*Dactylogyrus tincae*
Dactylogyrus nanus	*Monobothrium wageneri*
Dactylogyrus prostae	*Khawia sinensis*
Proteocephalus torulosus	*Valipora campylancristata*
Ligula intestinalis	*Neogryporhynchus cheilancristrotus*
Caryophyllaeus laticeps	*Sanguinicola armata*
Caryophyllaeides fennica	*Sanguinicola inermis*
Allocreadium isoporum	*Rhipidocotyle illense*
Diplostomum spathaceum	*Diplostomum spathaceum*
Posthodiplostomum cuticola	*Posthodiplostomum cuticola*
Tylodelphys clavata	*Tylodelphys clavata*
Sphaerostoma bramae	*Asymphylodora tincae*
Raphidascaris acus	*Skrjabillanus tincae*
Piscicola geometra	*Piscicola geometra*
Anodonta cygnea	*Anodonta cygnea*
Argulus appendiculosus	*Argulus foliaceus*
Argulus foliaceus	*Ergasilus briani*
Ergasilus sieboldi	*Ergasilus sieboldi*
Thersitina gasterostei	*Neoergasilus japonicus*
Tracheliastes polycolpus	*Lernaea cyprinacea*
Thymallus thymallus	
Ichthyobodo necator	
Acanthocephalus clavula,	
Pomphorhynchus laevis	
Tetraonchus borealis	
Diphyllobothrium dendriticum	
Diphyllobothrium ditremum	
Allocreadium transversale	
Bunodera lucioperca	
Tylodelphys clavata	
Phyllodistomum folium	
Asymphylodora tincae	
Argulus coregoni	

Appendix 3. Freshwater fish parasite distribution in the UK

Apicomplexa	***Trichodina domerguei***
Eimeria rutili	Caernarvonshire
Hertfordshire	East Norfolk
London	South Essex
Staffordshire	Stirlingshire
Warwickshire	West Invernesshire
Epieimeria anguillae	***Trichodina intermedia***
North Lincolnshire	Caithness
	Merionethshire
Goussia metchnikovi	Mid Perthshire
Hertfordshire	Renfrewshire
Middlesex	
South Essex	***Trichodina nigra***
	Clyde Isles
Ciliophora	Dumfriesshire
Apiosoma piscicola	Mid Perthshire
South Essex	Stirlingshire
South Somerset	West Perthshire (with Clackmannan)
West Norfolk	
	Trichodina pediculus
Ichthyophthirius multifilis	Stirlingshire
Derbyshire	
East Kent	***Trichodina polycirra***
East Suffolk	East Suffolk
Hertfordshire	Hertfordshire
Isle of Wight	
Leicestershire (with Rutland)	***Trichodina reticulata***
London	South-west Yorkshire
Montgomeryshire	
North Lincolnshire	***Trichodina tenuidens***
North Somerset	Caernarvonshire
Northamptonshire	South Essex
Nottinghamshire	Stirlingshire
Shropshire (Salop)	
South Devon	***Trichodina urinaria***
South Essex	East Suffolk
South-west Yorkshire	North Lincolnshire
Staffordshire	
Surrey	
Warwickshire	
West Kent	
Worcestershire	
Trichodina acuta	
Clyde Isles	
Dumfriesshire	
Renfrewshire	
Stirlingshire	
West Perthshire (with Clackmannan)	

Ciliophora continued	***Trypanosoma granulosum***
Chilodonella cyprini	London
Derbyshire	South Devon
East Kent	South Hampshire
East Suffolk	
Lancashire	***Trypanosoma percae***
Leicestershire (with Rutland)	South Devon
Montgomeryshire	South Essex
North Somerset	
Northamptonshire	**Retortamonada**
Nottinghamshire	***Octomitus truttae***
Shropshire (Salop)	Fifeshire (with Kinross)
South Essex	
Staffordshire	**Microsporidia**
Surrey	***Glugea anomala***
Warwickshire	East Norfolk
West Gloucestershire	Mid-west Yorkshire
West Kent	North Somerset
West Norfolk	South Essex
Worcestershire	Tyrone
	Westmorland
Chilodonella hexasticha	
Surrey	***Glugea luciopercae***
	Cheshire
Euglenozoa	Fifeshire (with Kinross)
Ichthyobodo necator	South Essex
Berkshire	
Derbyshire	***Pleistophora longifilis***
East Gloucestershire	Hertfordshire
East Suffolk	
Leicestershire (with Rutland)	**Myxozoa**
Montgomeryshire	***Dermocystidium gasterostei***
North Lincolnshire	East Norfolk
Northamptonshire	Surrey
Nottinghamshire	
Shropshire (Salop)	***Sphaerothecum destruens***
South Essex	South Hampshire
South Somerset	South Somerset
Staffordshire	
Warwickshire	***Chloromyxum esocinum***
West Gloucestershire	Fifeshire (with Kinross)
Worcestershire	
	Chloromyxum phoxini
Trypanoplasma borelli	Westmorland
Berkshire	
South Devon	
Trypanoplasma keisselitzi	
Berkshire	

Myxozoa continued	*Myxidium truttae*
Chloromyxum truttae,	Angus (Forfar)
Angus (Forfar)	Argyllshire
Argyllshire	Fifeshire (with Kinross)
Berwickshire	North Ebudes
East Perthshire	South Aberdeenshire
Fifeshire (with Kinross)	West Invernesshire
North Ebudes	West Ross & Cromarty
West Invernesshire	West Sutherland
West Ross & Cromarty	Wigtownshire
West Sutherland	
Wigtownshire	***Zschokkella cyprini***
	Hertfordshire
Myxidium giardi	
North Devon	***Henneguya oviperda***
North-east Yorkshire	Cheshire
	Radnorshire
Myxidium lieberkühni	
Cheshire	***Henneguya psorospermica***
	Cheshire
Myxidium rhodei	London
Berkshire	Merionethshire
Dorset	Radnorshire
East Kent	
East Suffolk	***Henneguya tegidiensis***
Herefordshire	Merionethshire
Hertfordshire	
Lancashire	***Henneguya zschokkei***
London	Argyllshire
North Lincolnshire	Radnorshire
Northamptonshire	West Sutherland
Nottinghamshire	Wigtownshire
Oxfordshire	
South Essex	***Myxobolus arcticus***
South Lincolnshire	West Invernesshire
South Somerset	West Ross & Cromarty
South-east Yorkshire	West Sutherland
Surrey	Wigtownshire
West Kent	
West Norfolk	***Myxobolus artus***
Worcestershire	Radnorshire
Myxidium scardini	***Myxobolus cyprini***
Westmorland	Hertfordshire
	Myxobolus dermatobius
	North Devon
	North-east Yorkshire

Myxozoa continued	Acanthocephala
Myxobolus macrocapsularis	***Neoechinorhynchus rutili***
Radnorshire	Angus (Forfar)
	Carmarthenshire
Myxobolus mülleri	Derbyshire
Hertfordshire	East Perthshire
London	London
Merionethshire	North Lincolnshire
Radnorshire	Nottinghamshire
South Essex	South-east Yorkshire
	West Invernesshire
Myxobolus neurobius	West Sutherland
Angus (Forfar)	Wigtownshire
Argyllshire	
Fifeshire (with Kinross)	***Acanthocephalus anguillae***
North Ebudes	Derbyshire
West Invernesshire	Dunbartonshire
West Ross & Cromarty	Fermanagh
West Sutherland	Leicestershire (with Rutland)
Wigtownshire	London
	North Lincolnshire
Myxobolus pseudodispar	South-east Yorkshire
Hertfordshire	South-west Yorkshire
London	Warwickshire
Myxobolus volgensis	***Acanthocephalus clavula***
North Lincolnshire	Anglesey
South Essex	Caernarvonshire
	Denbighshire
Sphaerospora dykovae	Fermanagh
Cambridgeshire	Lancashire
East Kent	Merionethshire
East Sussex	North Lincolnshire
Hertfordshire	North Somerset
London	Nottinghamshire
North Essex	Nottinghamshire
North Lincolnshire	South Devon
Northamptonshire	South-east Yorkshire
Surrey	
West Kent	
West Norfolk	
Sphaerospora elegans	
Fifeshire (with Kinross)	
Sphaerospora truttae	
Argyllshire	
West Ross & Cromarty	

Acanthocephala continued	***Pomphorhynchus laevis***
Acanthocephalus lucii	Argyllshire
Angus (Forfar)	Berkshire
Cheshire	Dorset
Derbyshire	East Gloucestershire
East Gloucestershire	East Kent
Fermanagh	Herefordshire
Hertfordshire	Hertfordshire
London	Kent
Middlesex	London
North Lincolnshire	Mid Perthshire
Northamptonshire	Middlesex
Nottinghamshire	Montgomeryshire
Shropshire (Salop)	North Devon
South Devon	North Ebudes
South Essex	North Hampshire
South-east Yorkshire	North Wiltshire
Staffordshire	Oxfordshire
Surrey	Shropshire (Salop)
Warwickshire	South Devon
West Invernesshire	South Essex
West Kent	South Hampshire
West Ross & Cromarty	West Invernesshire
West Sutherland	West Ross & Cromarty
Worcestershire	West Sutherland
	Worcestershire
	Platyhelminthes
	Dactylogyrus amphibothrium
	East Norfolk
	Lancashire
	London
	South Essex
	Dactylogyrus anchoratus
	North Lincolnshire
	South Essex
	Dactylogyrus auriculatus
	East Norfolk
	Dactylogyrus cordus
	Radnorshire
	South Essex

Platyhelminthes continued	***Dactylogyrus vistulae***
Dactylogyrus crucifer	Radnorshire
Cheshire	South Essex
East Norfolk	
London	***Dactylogyrus wunderi***
Radnorshire	Cheshire
South Essex	
	Pellucidhaptor pricei
Dactylogyrus extensus	Leicestershire (with Rutland)
Denbighshire	Middlesex
Lancashire	West Sussex
Dactylogyrus gobii	***Pseudodactylogyrus anguillae***
South Essex	Fermanagh
	South Devon
Dactylogyrus nanus	
Radnorshire	***Pseudodactylogyrus bini***
	Fermanagh
Dactylogyrus phoxini	London
South Essex	
	Ancyrocephalus paradoxus
Dactylogyrus prostae	Merionethshire
Radnorshire	
	Ancyrocephalus percae
Dactylogyrus similis	Merionethshire
Cheshire	
	Thaparocleidus vistulensis
Dactylogyrus sphyrna	Essex
Cheshire	Hampshire
East Norfolk	Kent
Middlesex	Staffordshire
Radnorshire	
South Essex	***Tetraonchus borealis***
	Radnorshire
Dactylogyrus suecicus	
Cheshire	***Tetraonchus monenteron***
	Cheshire
Dactylogyrus tincae	East Norfolk
North Lincolnshire	Fifeshire (with Kinross)
Surrey	Lancashire
Berkshire	Merionethshire
	Montgomeryshire
Dactylogyrus tuba	Radnorshire
Radnorshire	South Devon
	South Essex
Dactylogyrus vastator	South Hampshire
Lancashire	Warwickshire
	Westmorland

Platyhelminthes continued	*Gyrodactylus macronychus*
Gyrodactylus aphyae	Buckinghamshire
Buckinghamshire	Caernarvonshire
Caernarvonshire	Dorset
Dorset	Hertfordshire
Hertfordshire	South Devon
South Devon	West Sussex
West Sussex	Westmorland
Westmorland	Westmorland with North Lancashire
Westmorland with North Lancashire	
Worcestershire	***Gyrodactylus medius***
	Midlothian (Edinburgh)
Gyrodactylus arcuatus	South Essex
Caernarvonshire	Westmorland
Gyrodactylus derjavini	***Gyrodactylus minimus***
Orkney Islands	Buckinghamshire
Shetland Islands (Zetland)	West Sussex
Gyrodactylus elegans	***Gyrodactylus pavlovskyi***
South Essex	Buckinghamshire
	Dorset
Gyrodactylus laevis	Hertfordshire
Buckinghamshire	Shropshire (Salop)
Caernarvonshire	West Sussex
Dorset	Worcestershire
Hertfordshire	
South Hampshire	***Gyrodactylus pungitii***
West Sussex	Caernarvonshire
	Cheshire
Gyrodactylus limneus	East Kent
Berkshire	Hertfordshire
Buckinghamshire	North Lincolnshire
Caernarvonshire	Nottinghamshire
Dorset	South Lincolnshire
Hertfordshire	West Sussex
South Devon	
West Sussex	***Gyrodactylus rarus***
Westmorland	East Kent
Westmorland with North Lancashire	Hertfordshire
Worcestershire	South Essex
	South-west Yorkshire
Gyrodactylus lucii	West Sussex
Fifeshire (with Kinross)	
	Gyrodactylus rogatensis
	Hertfordshire
	Mid-west Yorkshire
	West Sussex

Platyhelminthes continued	*Discocotyle sagittata*
Gyrodactylus sedelnikowi	Angus (Forfar)
Buckinghamshire	Argyllshire
Dorset	East Perthshire
Hertfordshire	Fermanagh
Mid-west Yorkshire	Fifeshire (with Kinross)
Shropshire (Salop)	Montgomeryshire
West Sussex	North Ebudes
Worcestershire	North Lincolnshire
	West Invernesshire
Gyrodactylus truttae	West Ross & Cromarty
Hertfordshire	West Sutherland
	Wigtownshire
Paradiplozoon homoion	
Buckinghamshire	***Glanitaenia osculata***
Cheshire	South-east Yorkshire
Denbighshire	
East Suffolk	***Proteocephalus filicollis***
Hertfordshire	Caernarvonshire
Huntingdonshire	Cardiganshire
North Lincolnshire	Cheshire
North Somerset	Durham
North-east Yorkshire	Lanarkshire
South Lancashire	Lancashire
South-east Yorkshire	North Somerset
Staffordshire	South Essex
Surrey	South-west Yorkshire
	Warwickshire
Eudiplozoon nipponicum	
Buckinghamshire	***Proteocephalus macrocephalus***
Denbighshire	Caernarvonshire
North Devon	Cheshire
North Somerset	Fermanagh
Northamptonshire	North Lincolnshire
North-east Yorkshire	Nottinghamshire
South Essex	South Devon
Surrey	South Essex
West Suffolk	South-east Yorkshire
	Westmorland
Diplozoon paradoxum	
Derbyshire	***Proteocephalus neglectus***
Dorset	Merionethshire
North-east Yorkshire	
Nottinghamshire	***Proteocephalus percae***
Staffordshire	Cheshire
Surrey	Leicestershire (with Rutland)
Warwickshire	London
West Cornwall (with Scilly)	South Essex

Platyhelminthes continued	***Diphyllobothrium ditremum***
Proteocephalus pollanicola	Argyllshire
Tyrone	Denbighshire
	Merionethshire
Proteocephalus torulosus	North Ebudes
Dorset	West Invernesshire
Radnorshire	West Ross & Cromarty
South Essex	West Sutherland
South Hampshire	Westmorland
Schizocotyle acheilognathi	***Diphyllobothrium latum***
Berkshire	North Somerset
Buckinghamshire	South Essex
Dorset	Westmorland
East Gloucestershire	
East Norfolk	***Diphyllobothrium norvegicum***
East Suffolk	Mid-west Yorkshire
East Sussex	
Essex	***Ligula intestinalis***
Hertfordshire	Berkshire
Lincolnshire	Derbyshire
London	East Kent
Middlesex	Hertfordshire
North Essex	Lancashire
North Hampshire	Leicestershire (with Rutland)
Northamptonshire	London
Oxfordshire	Middlesex
Shropshire (Salop)	North Lincolnshire
South Devon	Nottinghamshire
South Essex	Oxfordshire
South Hampshire	South Essex
South Wiltshire	South Wiltshire
Surrey	South-east Yorkshire
West Gloucestershire	South-west Yorkshire
West Kent	Staffordshire
West Norfolk	Warwickshire
Yorkshire	West Kent
	Worcestershire
Diphyllobothrium dendriticum	
Caernarvonshire	***Schistocephalus solidus***
Denbighshire	Derbyshire
East Perthshire	Nottinghamshire
Fifeshire (with Kinross)	South Essex
Merionethshire	South-east Yorkshire
Pembrokeshire	
South Essex	***Bathybothrium rectangulum***
Westmorland	North-east Yorkshire

Platyhelminthes continued	*Cyathocephalus truncatus*
Bothriocephalus claviceps	Buckinghamshire
Anglesey	Caernarvonshire
Caernarvonshire	East Perthshire
Carmarthenshire	Fifeshire (with Kinross)
Cheshire	Flintshire
Fermanagh	Mid Perthshire
London	South Hampshire
Merionethshire	
North Lincolnshire	***Biacetabulum appendiculatum***
Nottinghamshire	South Essex
Pembrokeshire	
South Devon	***Caryophyllaeus fimbriceps***
South Essex	South Hampshire
South Hampshire	South Lincolnshire
South Lincolnshire	
South-east Yorkshire	***Caryophyllaeus laticeps***
Westmorland	Cheshire
Worcestershire	Dorset
	East Kent
Eubothrium salvelini	Hertfordshire
West Sutherland	Lancashire
Westmorland	London
	Middlesex
Triaenophorus nodulosus	North Devon
Cheshire	North Essex
Derbyshire	North-east Yorkshire
Dunbartonshire	Radnorshire
East Gloucestershire	South Devon
Hertfordshire	South Essex
Lancashire	South Hampshire
London	South Lincolnshire
Merionethshire	South-east Yorkshire
Montgomeryshire	Surrey
North Essex	
North Lincolnshire	***Monobothrium wageneri***
North Somerset	Berkshire
Northamptonshire	London
Nottinghamshire	North Hampshire
Radnorshire	Surrey
South Essex	
South Hampshire	
South Lincolnshire	
Staffordshire	
Surrey	
Warwickshire	
West Kent	
Westmorland	

Platyhelminthes continued	*Khawia sinensis*
Atractolytocestus huronensis	Bedfordshire
Buckinghamshire	Berkshire
Cambridgeshire	Cambridgeshire
East Norfolk	Dorset
East Sussex	East Kent
Hertfordshire	East Suffolk
Lancashire	Flintshire
London	Hertfordshire
North Essex	London
Northamptonshire	Middlesex
Radnorshire	North Essex
South Essex	North Lincolnshire
South Somerset	Northamptonshire
Staffordshire	Nottinghamshire
Surrey	Oxfordshire
West Cornwall (with Scilly)	South Devon
West Kent	South Essex
West Norfolk	South Lincolnshire
West Sussex	South Somerset
Worcestershire	South-east Yorkshire
	Surrey
Caryophyllaeides fennica	West Kent
Berkshire	West Norfolk
Cheshire	
Dunbartonshire	***Neogryporhynchus cheilancristrotus***
East Suffolk	Berkshire
Lancashire	
London	***Paradilepis scolecina***
Merionethshire	Surrey
Middlesex	
North Devon	***Valipora campylancristata***
North Lincolnshire	Hertfordshire
Northamptonshire	North Essex
Pembrokeshire	Northamptonshire
Radnorshire	
South Devon	***Allocreadium isoporum***
South Essex	Caernarvonshire
South Hampshire	Cambridgeshire
South Lincolnshire	Cheshire
South Somerset	Lancashire
South-west Yorkshire	North Essex
Stirlingshire	North Lincolnshire
Surrey	Radnorshire
West Kent	South Essex
Worcestershire	South Hampshire
	Allocreadium transversale
	Radnorshire

Platyhelminthes continued	*Sanguinicola inermis*
Bunodera lucioperca	Berkshire
Cheshire	Buckinghamshire
Derbyshire	Derbyshire
Dunbartonshire	Dorset
London	East Gloucestershire
Merionethshire	East Suffolk
South Lincolnshire	Hertfordshire
Staffordshire	Leicestershire (with Rutland)
	London
Crepidostomum farionis	North Essex
Angus (Forfar)	North Lincolnshire
Argyllshire	Northamptonshire
Carmarthenshire	Nottinghamshire
Fifeshire (with Kinross)	Shropshire (Salop)
North Ebudes	South Essex
West Invernesshire	South-west Yorkshire
West Ross & Cromarty	Staffordshire
West Sutherland	Surrey
Wigtownshire	Warwickshire
	West Kent
Crepidostomum metoecus	West Sussex
Carmarthenshire	Worcestershire
Macrolecithus papilliger	***Aspidogaster limacoides***
Cardiganshire	East Norfolk
West Perthshire (with Clackmannan)	
Westmorland	***Azygia lucii***
	Hertfordshire
Sanguinicola armata	South Essex
West Kent	Warwickshire
	Bucephalus polymorphus
	South Lincolnshire
	Warwickshire
	Rhipidocotyle illense
	London
	North Lincolnshire
	South Essex
	Deropristis inflata
	North Lincolnshire
	Westmorland

Platyhelminthes continued	*Diplostomum spathaceum*
Diplostomum gasterostei	Bedfordshire
Fifeshire (with Kinross)	Berkshire
Huntingdonshire	Buckinghamshire
Lanarkshire	Cambridgeshire
Merionethshire	Cheshire
North Somerset	Derbyshire
South-west Yorkshire	Dorset
	East Kent
Diplostomum petromyzi-fluviatilis	East Suffolk
North-west Yorkshire	East Sussex
	Hertfordshire
Diplostomum phoxini	Lancashire
Argyllshire	Lincolnshire
Cardiganshire	London
Dunbartonshire	Merionethshire
Fifeshire (with Kinross)	Middlesex
Lanarkshire	North Devon
Midlothian (Edinburgh)	North Essex
South Essex	North Lincolnshire
West Perthshire (with Clackmannan)	North Somerset
	Northamptonshire
	Nottinghamshire
	Oxfordshire
	South Devon
	South Essex
	South Hampshire
	South Lincolnshire
	South Wiltshire
	South-east Yorkshire
	South-west Yorkshire
	Staffordshire
	Surrey
	West Kent
	West Norfolk
	West Sussex
	Worcestershire
	Hysteromorpha triloba
	East Sussex
	North Lincolnshire
	Surrey

Platyhelminthes continued	*Tylodelphys clavata*
Posthodiplostomum cuticola	Cambridgeshire
Cheshire	Cheshire
Derbyshire	East Kent
Dorset	East Suffolk
East Kent	Hertfordshire
East Sussex	Lancashire
Hertfordshire	London
Lancashire	Middlesex
Leicestershire (with Rutland)	North Devon
London	North Essex
Middlesex	North Lincolnshire
North Essex	Northamptonshire
North Somerset	North-west Yorkshire
Northamptonshire	Oxfordshire
Nottinghamshire	South Essex
South Essex	South Lincolnshire
South Hampshire	South Somerset
South Lancashire	South Wiltshire
South Lincolnshire	South-west Yorkshire
South-west Yorkshire	Staffordshire
Staffordshire	Surrey
Surrey	West Kent
Warwickshire	West Lancashire
West Gloucestershire	West Norfolk
West Kent	Worcestershire
West Norfolk	
Worcestershire	***Tylodelphys podicipina***
	London
	South Essex
	Echinochasmus perfoliatus
	Cheshire
	Derbyshire
	East Kent
	East Suffolk
	Hertfordshire
	Lincolnshire
	London
	Middlesex
	North Essex
	Northamptonshire
	Nottinghamshire
	South Essex
	South Lincolnshire
	South Wiltshire
	Staffordshire
	West Kent
	Worcestershire

Platyhelminthes continued	*Ichthyocotylurus erraticus*
Phyllodistomum folium	Angus (Forfar)
Argyllshire	Dunbartonshire
Caernarvonshire	Fifeshire (with Kinross)
Cambridgeshire	South Essex
Hertfordshire	West Invernesshire
Lancashire	West Ross & Cromarty
London	West Sutherland
Merionethshire	Wigtownshire
Montgomeryshire	
South Essex	***Ichthyocotylurus pileatus***
South-west Yorkshire	London
West Invernesshire	
West Sutherland	***Ichthyocotylurus variegatus***
Westmorland	London
	Middlesex
Phyllodistomum simile	South Essex
Pembrokeshire	Tyrone
Lecithochirium gravidum	
Pembrokeshire	**Nematoda**
	Camallanus lacustris
Asymphylodora kubanicum	Cheshire
Cheshire	Fermanagh
	Hertfordshire
Asymphylodora tincae	Lancashire
East Perthshire	Leicestershire (with Rutland)
London	London
	Merionethshire
Sphaerostoma bramae	Oxfordshire
Cambridgeshire	South Essex
Cheshire	South Lincolnshire
Dorset	Westmorland
Lancashire	
Leicestershire (with Rutland)	***Contracaecum aduncum***
North Essex	North Lincolnshire
North Lincolnshire	South-east Yorkshire
Radnorshire	
South Essex	***Paraquimperia tenerrima***
South Hampshire	Anglesey
Tyrone	Caernarvonshire
	Carmarthenshire
Nicolla gallica	Lancashire
South Hampshire	Merionethshire
	North Lincolnshire
Apatemon gracilis	Nottinghamshire
East Perthshire	Pembrokeshire
	South Devon
Ichthyocotylurus cucullus	South-east Yorkshire
Fifeshire (with Kinross)	West Lothian (Linlithgow)

Nematoda continued	*Anguillicoloides crassus*
Rhabdochona denudata	Berkshire
Westmorland	Cambridgeshire
	Dorset
Rhabdochona oncorhynchi	East Sussex
East Perthshire	East Kent
South Aberdeenshire	Fermanagh
	Hertfordshire
Raphidascaris acus	London
Caernarvonshire	Mid-west Yorkshire
Carmarthenshire	North Essex
Fermanagh	North Lincolnshire
Fifeshire (with Kinross)	North Somerset
Merionethshire	South Devon
North Lincolnshire	South Essex
Nottinghamshire	South Hampshire
Radnorshire	Surrey
South-east Yorkshire	Mid-Perthshire
Westmorland	Glamorgan
Worcestershire	Lancashire
Raphidascaris cristata	***Philometra ovata***
Caernarvonshire	North Lincolnshire
Denbighshire	South Essex
Lancashire	South-east Yorkshire
Merionethshire	Staffordshire
Montgomeryshire	
Pembrokeshire	***Philometra rischta***
	Cheshire
Truttaedacnitis truttae	
Angus (Forfar)	***Philometroides sanguinea***
Argyllshire	South-west Yorkshire
Caernarvonshire	Surrey
Fermanagh	
Fifeshire (with Kinross)	***Skrjabillanus tincae***
Merionethshire	Berkshire
South Hampshire	North Lincolnshire
West Invernesshire	
West Ross & Cromarty	***Cystidicola farionis***
West Sutherland	Angus (Forfar)
Wigtownshire	Argyllshire
	East Perthshire
	Fifeshire (with Kinross)
	North Lincolnshire

Nematoda continued	Mollusca
Cystidicoloides tenuissima	***Anodonta cygnea***
Angus (Forfar)	Derbyshire
Argyllshire	East Kent
North Ebudes	East Sussex
West Invernesshire	Lancashire
West Ross & Cromarty	London
West Sutherland	North Essex
Wigtownshire	North Lincolnshire
	Northamptonshire
Spinitectus inermis	South Essex
Merionethshire	South-east Yorkshire
Radnorshire	Surrey
West Lothian (Linlithgow)	West Kent
	Margaritifera margaritifera
Annelida	Argyllshire
Piscicola geometra	West Invernesshire
Berkshire	West Ross & Cromarty
Buckinghamshire	
Cambridgeshire	
Derbyshire	**Arthropoda**
Dorset	***Ergasilus briani***
East Kent	Berkshire
East Suffolk	Buckinghamshire
East Sussex	Dorset
Hertfordshire	East Gloucestershire
Lancashire	Hertfordshire
Leicestershire (with Rutland)	North Devon
Lincolnshire	North Hampshire
London	North Lincolnshire
Middlesex	Northamptonshire
North Essex	Nottinghamshire
North Hampshire	Oxfordshire
North Lincolnshire	South Essex
Northamptonshire	South Hampshire
Nottinghamshire	South Lancashire
South Essex	South Lincolnshire
South Lincolnshire	South Somerset
South-east Yorkshire	South-west Yorkshire
South-west Yorkshire	Surrey
Staffordshire	West Kent
Surrey	West Suffolk
Warwickshire	
West Gloucestershire	
West Kent	
West Norfolk	
Worcestershire	

Arthropoda	*Neoergasilus japonicus*
Ergasilus gibbus	Dorset
Cambridgeshire	East Sussex
Fermanagh	North Hampshire
Mid-west Yorkshire	Northamptonshire
North Lincolnshire	Nottinghamshire
North Somerset	Oxfordshire
South Devon	South Essex
South Hampshire	South-west Yorkshire
West Suffolk	Surrey
	West Sussex
Ergasilus sieboldi	
Berkshire	***Paraergasilus longidigitus***
Buckinghamshire	East Sussex
Cambridgeshire	Hertfordshire
Derbyshire	North Essex
East Gloucestershire	Oxfordshire
East Suffolk	Surrey
East Sussex	West Kent
Flintshire	West Norfolk
Hertfordshire	
Huntingdonshire	***Thersitina gasterostei***
London	Argyllshire
Middlesex	East Norfolk
Mid-west Yorkshire	Mid-west Yorkshire
North Essex	Outer Hebrides
North Hampshire	South Essex
North Lincolnshire	West Perthshire (with Clackmannan)
North-east Yorkshire	
Nottinghamshire	***Argulus appendiculosus***
South Essex	Dorset
South-east Yorkshire	
South-west Yorkshire	***Argulus coregoni***
Staffordshire	Cumberland
Surrey	Denbighshire
Warwickshire	Derbyshire
West Kent	East Norfolk
West Lancashire	Lanarkshire
West Sussex	Merionethshire
	Oxfordshire
	Shropshire (Salop)
	South Wiltshire

Arthropoda continued	*Lernaea cyprinacea*
Argulus foliaceus	Cambridgeshire
Bedfordshire	London
Cambridgeshire	Midlothian (Edinburgh)
Derbyshire	Pembrokeshire
Dorset	South Devon
East Kent	South Essex
East Suffolk	Surrey
East Sussex	West Sussex
Flintshire	
Herefordshire	***Salmincola edwardsii***
Hertfordshire	East Kent
Isle of Wight	
Leicestershire (with Rutland)	***Salmincola percarum***
Lincolnshire	Cheshire
London	Middlesex
Middlesex	North Essex
Mid-west Yorkshire	South Essex
Montgomeryshire	West Perthshire (with Clackmannan)
North Essex	
North Hampshire	***Salmincola salmoneus***
North Lincolnshire	Argyllshire
Northamptonshire	Mid Perthshire
Nottinghamshire	North-east Yorkshire
Oxfordshire	West Perthshire (with Clackmannan)
Shropshire (Salop)	
South Essex	***Tracheliastes maculatus***
South Lincolnshire	Lancashire
South Wiltshire	
South-east Yorkshire	***Tracheliastes polycolpus***
Staffordshire	Midlothian (Edinburgh)
Surrey	Mid-west Yorkshire
Warwickshire	North-east Yorkshire
West Gloucestershire	South-east Yorkshire
West Kent	
West Norfolk	
West Sussex	
Worcestershire	
Argulus japonicus	
West Sussex	

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