Opinion
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Policy, phylogeny, and the parasite

Grant D. Stentiford[1,2*], Stephen W. Feist[2], David M. Stone[2], Edmund J. Peeler[2], and David Bass[3]

[1] European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries, and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset DT4 8UB, UK
[2] Aquatic Pests and Pathogens Group, Cefas, Weymouth Laboratory, Dorset DT4 8UB, UK
[3] Division of Genomics and Microbial Diversity, Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

Animal diseases gain political attention by their inclusion on lists of global bodies such as those of the World Organisation for Animal Health (OIE). Inclusion requires national governments to report outbreaks promptly but may lead to trading restrictions between nations in an attempt to limit spread. Detection therefore has consequences that may have direct impact from farm to state levels. We consider here current approaches to discriminating listed parasites from related but unlisted counterparts. We outline necessary drivers for the discrimination of important taxa and how these may be influenced by national policies. Further, we propose a set of 'best practice' measures, broadly based upon current taxonomic philosophies for protists and metazoans, that should be applied when defining taxa for listing as notifiable.

Parasites on lists

The OIE (http://www.oie.int/) maintains a dynamic list of notifiable diseases of terrestrial and aquatic animals. The list corresponds to the major domesticated terrestrial animal groups, and includes diseases caused by bovine, ovine, porcine, equine, swine, avian, lagenmorph, and aparine pathogens as well as by those of commercially important aquatic hosts including amphibian, piscine, molluscan, and crustacean taxa [1]. Detection of any of these diseases in farmed or wild animals by any of the 178 OIE member countries (http://www.oie.int/index.php?L=3&id=103) should be communicated to the OIE via the official state veterinary services of that country. Thereafter, a formalised sequence of events may culminate in trading restrictions of live animals and commodity products from infected to non-infected farms, zones, or even whole countries. In addition, member countries are expected to document the official controls used during the disease outbreak. The major aim of the process is to limit the spread and subsequent impact of important pathogens for which treatment strategies are either not available or are not feasible. The list includes viral, bacterial, fungal, protistan, and metazoan pathogens, all of which are afforded specific chapters in the regularly updated OIE *Manual of Diagnostic Tests*, the designation of experts who provide a global reference point for the disease and maintain appropriate test materials for its diagnosis. Currently, there are 116 diseases listed by the OIE [1]. Of these, 30 are caused by eukaryotic (fungal, oomycete, protistan, and metazoan) parasites (Table 1). The principles that underlie the diagnosis and phylogeny of these parasite taxa, and the implications for their detection, form the focus of this opinion article.

Identifying the culprits

Accurate definition of taxa is not simply aimed at expanding our appreciation of organism diversity. Rather, it is fundamentally applied both to ecological research and, in particular, to the production of inventories of biodiversity, behind which political force may be applied for the conservation of specific taxa over time and space. In this article we propose an additional significant role for taxonomy in underpinning the legislative frameworks that are necessary to limit the spread of animal and plant disease-causing agents via international trade. Enforcement of policy, be it for the purposes of protecting biodiversity or in a disease control setting, therefore has a fundamental need for specific and robust discrimination of the target organism. In this context, and in reference to those parasites listed by the OIE (Table 1), definition of the 'species' provides the most commonly accepted means by which this is likely to be applied.

However, despite major advances in diagnostic methodologies over the past two centuries, a consensus definition of 'species' remains a notoriously difficult concept [2]. Nowhere is this more apparent than in the hyper-diverse protists where so-called 'alpha taxonomy' at the level of 'species' is 'fraught with uncertainty and disagreement' [3]. Increasing recognition of convergent traits across the spectrum of known protists, and of other parasite groups (e.g., the Microsporidia), underlies a growing disparity between taxonomic systems built solely on morphological traits and those where molecular marker data are utilised [3,4]. Recent philosophical reviews by taxonomists working on organism groups from across the 'web of life' conclude that, because consistent definition of the species is elusive, a case-by-case approach should be applied; those defining new taxa do so first by outlining the basis for their definition and subsequently by providing details of the methods used for the delineation, for instance, of morphology or a marker gene [3]. However, this apparently workable solution, in which the 'species' is ultimately defined according

Corresponding author: Stentiford, G.D. (grant.stentiford@cefas.co.uk).
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Twitter: @grantstent

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Table 1. OIE listed parasite taxa and parasitic diseases in terrestrial and aquatic animal hosts[a]

Host group	Disease or infecting organism as listed	Agent(s)	Taxonomic affinity	Group-level taxonomy	Group-level taxonomic references
Multi-terrestrial species	Echinococcosis	*Echincoccus* spp.	Cestoda	Whole SSU and D1-D3 region of LSU	[21,22]
Multi-terrestrial species	Trichinellosis	*Trichinella* spp.	Nematoda	SSU	[23,24]
Multi-terrestrial species	Leishmaniosis	*Leishmania* spp.	Kinetoplastida	SSU, Concatenated sequence phylogeny	[17,25]
Multi-terrestrial species	Trypanosomosis	*Trypanosoma evansi*	Kinetoplastida	SSU, Concatenated sequence phylogeny	[17,25,26]
Bees	Acaripisosis	*Acarapis woodi*	Acari: Tarsonemidae	SSU, COI	[27,28]
Bees	Nosemosis	*Nosema apis, N. ceranae*	Microsporidia	SSU	[29,30]
Bees	Small Hive Beetle Infestation	*Aethina turrida*	Coleoptera	None reported	[1]
Bees	*Tropilaelaps* infestation	*Tropilaelaps* spp.	Acari: Laelapidae	SSU, COI	[27,28,31]
Bees	Varroosis	*Varroa destructor*	Acari: Varroidiae	SSU, COI	[1,27,28]
Bovine	Babesiosis	*Babesia bovis* and other spp.	Apicomplexa	Concatenated sequence phylogeny	[18,32]
Bovine	Cysticercosis	*Taenia* spp.	Cestoda	ITS2, Whole SSU and D1–D3 region of LSU	[22,33,34]
Bovine	Dermatophilosis	*Amblyomma variegatum*	Acari: Ixodida	SSU, COI	[1,27,28]
Bovine	Theileriosis	*Theileria* spp.	Apicomplexa	SSU and other rRNA genes; Concatenated sequence phylogeny	[18,35,36]
Bovine	Trichomonosis	*Tritrichomonas foetus*	Trichomonida	SSU, ITS, 5.8S	[37–40]
Bovine	Trypanosomosis	*Trypanosoma* spp.	Kinetoplastida	SSU, ITS1, RFLP; Concatenated sequence phylogeny	[17,25,41]
Equine	Dourine	*Trypanosoma equiperdum*	Kinetoplastida	'maxi-circles'; VSG RoTod 1.2; Concatenated sequence phylogeny	[17,25,42]
Equine	Equine Piroplasmosis	*Theileria equi, Babesia caballi*	Apicomplexa	SSU; Concatenated sequence phylogeny	[18,43]
Equine	Mange	Various Acarid mites	Acari: Astigmata and Acari: Prostigmata	SSU, COI	[27,28,44]
Equine	Cryptosporidiosis	18 spp. and 40 genotypes of *Cryptosporidium*	Apicomplexa	COWP, CTRAP1/2, HSP70, Actin; Concatenated sequence phylogeny	[18,45,46]
Equine	Toxoplasmosis	*Toxoplasma gondii*	Apicomplexa	B1 repetitive sequence, P30(SAG1) gene, SSU; Concatenated sequence phylogeny	[18,47,48]
Amphibia	*Batrachochytrium dendrobatidis*	*Batrachochytrium dendrobatidis*	Chytridiomycota	SSU, 5.8S, IGS, ITS1, and ITS2	[49,50]
Crustacea	Crayfish plague	*Aphanomyces astaci*	Oomycetida	SSU, LSU, ITS, COI; RAPD PCR	[51,52]
Osteichthys	Epizootic Ulcerative Syndrome	*Aphanomyces invadens*	Oomycetida	SSU, LSU, ITS, COI	[52,53]
Osteichthys	*Gyrodactylus salaris*	*Gyrodactylus salaris*	Platyhelminthes	COI, ITS; Concatenated sequence phylogeny	[16,54–56]
Mollusca	*Bonamia exitiosa*	*Bonamia exitiosa*	Haplosporidia	SSU (+RFLP)	[8,57]
Mollusca	*Bonamia ostraea*	*Bonamia ostraea*	Haplosporidia	SSU (+RFLP)	[8,58,59]
Mollusca	*Marteilia refringens*	*Marteilia refringens*	Paramyxida	SSU, ITS1, IGS	[60,61]
Mollusca	*Perkinsus marinus*	*Perkinsus marinus*	Perkinsida	SSU, ITS (+RFLP); nuclear-encoded spliced leader (SL) RNA; mt genes, intron prevalence; Concatenated sequence phylogeny	[19,62,63]
Mollusca	*Perkinsus olseni*	*Perkinsus olseni*	Perkinsida	SSU, ITS (+RFLP); nuclear-encoded spliced leader (SL) RNA; mt genes, intron prevalence; Concatenated sequence phylogeny	[19,62,63]
Mollusca	*Mikrocytos mackini*	*Mikrocytos mackini*	Mikrocytida	SSU, ITS1, 5.8S, ITS2; Concatenated sequence phylogeny	[20,64–66]

[a]World Organisation for Animal Health, 2012.
Abbreviations: World Organisation for Animal Health (OIE); small subunit rRNA (SSU); large subunit rRNA (LSU); cytochrome oxidase (COI); internal transcribed spacer (ITS) regions 1 (ITS1) and 2 (ITS2); 5.8S region of rRNA (5.8S); intergenic spacer region of rRNA (IGS); restriction fragment length polymorphism (RFLP); variant surface glycoprotein (VSG); *Cryptosporidium* oocyst wall protein (COWP); thrombospondin-related adhesive protein (CTRAP 1/2); heat shock protein (HSP70); surface antigen 1 (SAG1).

Box 1. Policy and the parasite: the case of *Gyrodactylus salaris*

Gyrodactylus salaris is a highly pathogenic monogenean parasite of Atlantic salmon (*Salmo salar*) (Figure I). It is listed by the OIE and in other regional legislation. Discrimination of *G. salaris* from non-pathogenic co-generics (*G. thymalli*, *G. teuchis*, and *G. bohemicus*) living on salmonid fishes has traditionally been based on the morphology of the 'marginal hooks' responsible for attachment to the host [67]. However, these hard parts display morphological plasticity depending on the age and species of host fish [68,69] and on the location of host attachment [70–72]. Consequently, taxonomy based on morphology alone demands considerable experience and is prone to error. Molecular tools developed to assist with species identification have inadvertently complicated matters because small subunit rRNA and internal transcribed spacer (ITS) sequence data are identical for the listed and pathogenic *G. salaris* as well as for its sister species *G. thymalli* which infects grayling. From the wealth of sequence data now available, it appears that strains of *G. salaris* can in fact survive and reproduce on several salmonid hosts including rainbow trout (*Oncorhynchus mykiss*), Arctic charr (*Salvelinus alpinus*), North American brook trout (*Salvelinus fontinalis*), North American lake trout (*Salvelinus namaycush*), brown trout (*Salmo trutta*), and grayling (*Thymallus thymallus*); all cases infection results in no significant pathological outcome. For the purposes of risk management it is argued that salmon-adapted *G. salaris* strain (*G. salaris sensu stricto*) should be listed separately from all other strains, thereby allowing protection against trade-related movements of salmon-adapted strains to countries where other potentially non-pathogenic strains are known to exist. In the spirit of the current opinion article, to implement such control measures requires robust discrimination between taxa (and subtypes thereof) that cause disease and their less-pathogenic relatives. Analysis of other (protein-coding) gene sequences (cytochrome oxidase subunit I, COI) has revealed considerably higher levels of diversity than depicted using rRNA genes and spacers or other ribosomal gene markers, allowing some discrimination between *G. salaris* and *G. thymalli*. With a few exceptions, the clades of *G. salaris* and *G. thymalli* generally correspond well to host preferences and/or the geographical distribution of the parasites. However, within *G. salaris*, COI barcodes do not correlate strictly with the virulence of strains for salmon and, as a result, the current phylogenetic approaches are not sufficiently robust to support the listing of individual clades of *G. salaris* [73].

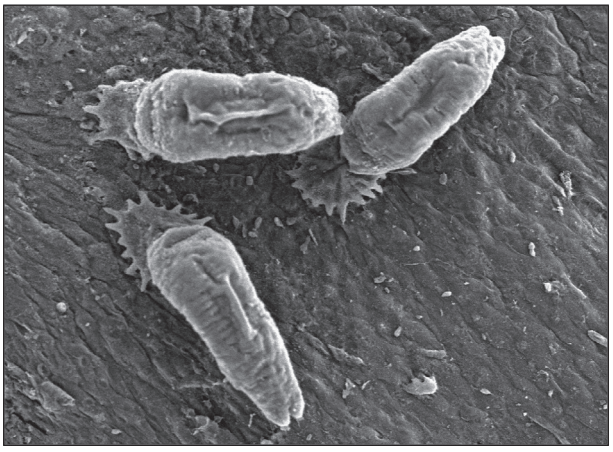

Figure I. *Gyrodactylus salaris* infection of Atlantic salmon (*Salmo salar*). Attachment of the parasite to the skin and gills can lead to serious pathology. Control programmes for the eradication of the parasite from national waterways include the use of system-wide biocides and associated native fish breeding programmes for restocking. The considerable financial and ecological implications for control have led to a political focus on *G. salaris* and its congenerics. We propose that current phylogenetic frameworks for the gyrodactylids fall short of allowing efficient protection against its inadvertent trading in farmed salmonid hosts.

to those criteria outlined by individual researchers working towards a consensus for each organismal group, creates considerable intellectual and political space to challenge the proposed taxonomy. Although taxonomic decisions will never be beyond scientific debate, this process can clearly pose problems when these same taxa (species) are written into legislative frameworks, and whereby their detection leads to consequences for the trading of their hosts. For this reason, and owing to the significant political issues that arise from detection (Box 1), it appears appropriate that a defined set of best practice measures should be applied consistently to the taxonomic classification and diagnosis of those pathogens afforded promotion to such lists.

Are approaches to listing consistent?

The political basis for the listing and de-listing of a particular disease by the OIE is well established. A proposal for listing by one or more member countries leads to a vote for agreement (or not) for inclusion by the remaining members [5]. The broad criteria for listing are also well established, focussing on the potential consequences of introduction for wild or farmed animals or humans, the likelihood of spread, and the availability of robust diagnostic methods for detection [5]. The latter criterion becomes the focus in the aforementioned *Manual of Diagnostic Tests* [1]. A case definition should also be produced in which it is stipulated how the disease/agent is clearly identified and is distinguished from other agents/pathologies. Although the emphasis on listing and subsequent user focus are on application of a validated diagnostic test, less emphasis is placed upon the wider phylogenetic framework in which this test may operate and, similarly, upon the accepted robustness of the taxonomy of the group in which the listed parasite resides. Let us focus on this issue.

One inconsistency highlighted in Table 1 relates to resolution. Some listed diseases, such as *Perkinsus marinus* infection of molluscs, are associated with infection by a single parasite species. Others, for instance, cryptosporidiosis in horses, are associated with infection by genetically distinct organisms (multiple species) within the genus *Cryptosporidium*. Other examples abound, such as the splitting of parasite lineages that cause very similar diseases in the same hosts (e.g., *Bonamia exitiosa* and *Bonamia ostreae* are listed as separate disease-causing agents in molluscs) that conflicts with the joint grouping of multiple species of the genus *Theileria* that cause a single listed disease, theileriosis, in cattle.

Some of these inconsistencies relate again to problems in defining 'cut-off' points for one species from another, with the added consideration of whether the species of interest causes disease, or not, in a given host group. The inconsistency has clear parallels in the wider field of taxonomy where, owing to difficulties in defining 'species', particularly in microscopic organisms, some authors have proposed grouping them into operational taxonomic units (OTUs), usually based upon sequence similarity [6,7].

Although this does seem to offer a useful solution when generating evidence for the existence of novel diversity in specific organism groups, for example, several novel clades/OTUs for the Haplosporidia have been detected from lineage-specific environmental sampling of small ribosomal subunit (SSU) sequence variants [8], it seems less appropriate for defining specific parasites of concern to global animal health. In this respect a general movement away from the listing of diseases as being caused by multiple parasite 'species', such as nosemosis in bees caused by *N. apis* and *N. ceranae*, to specific listing of diseases caused by single parasite 'species', for example, *Mikrocytos mackini* in molluscs, or even subtypes thereof, is the future preference. Although the association of a given disease with a specific parasite taxon would remove inconsistencies in the list, it does necessitate improved rigour in the definition of those parasites to be listed and, importantly, of the phylogenetic framework in which they exist. In this way why one species (or genotype) is listed, whereas a sister species (or another genotype) is not, will need to be justified. Presumably this will be on the basis of disease-causing capacity in important internationally traded hosts, and the rigour in which the diagnostic test can be shown to be appropriate in discriminating the agent from related non-listed – and non disease-causing – taxa.

The importance of being able to specify and diagnose particular taxa is highlighted by our increasing awareness of the vast genetic diversity of microorganisms that are yet to be described and characterised. Specimen/culture-independent environmental sequencing studies are revealing high levels of 'hidden' diversity of microbial taxa, particularly microscopic eukaryotes, bacteria, and viruses [8–11]. These studies demonstrate that known parasitic lineages may have many, often close, relatives about which we have very little information, even regarding whether they are parasitic or free-living. This information is essential for circumscribing the genetic signatures of parasites of concern, whether or not their nearest known relatives are parasitic and whether their parasitic nature is similar to or different from their characterised relatives, if accurate identification and monitoring are to be achieved. Because this cryptic diversity will take years to reveal, and may increase the lineage richness of many groups by an order of magnitude or more, careful and conservative molecular definition of listed parasites is required now as proof against future discoveries.

Where policy meets phylogenetics

Far from being a criticism of the considerable international efforts that have so far been applied to highlight globally important parasite groups, and their negative effects on farmed and wild hosts, it is timely to consider how emerging tools in the ever-evolving field of taxonomy can be utilised to the benefit of global bio- and food-security. Building upon the concept of defining new taxa by first outlining the driver for the definition [3], we propose that 'biology' rather than 'politic' must drive the need (Figure 1). In the context of this paper, the biological driver is the propensity of the parasite to cause disease in hosts of concern relative to closely related, and potentially non-disease-causing, taxa. A recent virological example is the change in listing by the OIE of infectious salmon anaemia (ISA). The pathogenic ('deleted') strain is listed separately from the ancestral non-pathogenic form (designated 'highly polymorphic region 0', HPR0). However, HPR0 remains listed for reasons of risk management, and evidence suggests that the deleted strain may arise from HPR0 [12]. In these instances research should focus on the provision of an appropriate resolution of test data to allow discrimination of the disease-causing taxon of concern from related forms. The 'biological driver' concept contrasts with that of the 'policy driver' in which research focuses on provision of supporting evidence, often at ever-increasing molecular resolution, to support a claim for absence (freedom) of a particular parasite of concern from a geographical boundary or host group therein (Figure 1). Indeed, the OIE makes it very clear that the presence of the listed parasite is reportable irrespective of the presence of clinical disease. In these instances, because the politically driven taxonomic outcome may bear little biological relevance in terms of disease-causing potential, it may result in active facilitation of the transboundary trading of the parasite from zones of endemicity.

Best practice and way forward

There is clear potential for conflict in the accurate definition of novel or existing parasite taxa. It is too simplistic to propose that ecological or morphological traits are unusable in the face of data emerging from novel molecular technologies. Similarly, underutilisation of molecular phylogenetic data when attempting to define novel taxa may lead to an inadequate appreciation of natural diversity. Taxonomy is a moving target that is largely driven by technological advances. Given aforementioned difficulties in defining specific taxonomic units, the anthropogenic construct of the 'species', it is therefore important that we do not 'trade-off' available technologies against one another but instead understand their specific limitations in assisting the process of definition. In this respect, an appreciation of the potential for 'plastic' or convergent morphological traits in parasite taxa [3,4] should be considered alongside our understanding of potential for intraspecific variation in ribosomal gene or spacer sequences utilised for taxonomic purposes [13,14], and of finer-scale whole-genome variations between individuals of the same species [15]. The issue of 'cut-offs' between taxa therefore emerges once again – how different must a morphological/molecular trait be to consider that a taxon is distinct from another? As we have described, this question is unlikely to be answered by considering a single source of data – either phenotypic or molecular. More likely, by employing the somewhat philosophical approach outlined by Boenigk and colleagues [3], we should first consider the basis for our definition and then adopt an appropriate set of markers to distinguish unambiguously between the biologically distinct lineages. We suggest that such support should utilise a 'weight of evidence' approach, in which all relevant data sources are employed, rather than relying on a single line of evidence. What does this mean in the context of listed parasites?

Given the commercial and political ramifications of detecting a listed disease (Box 1), the keystone to improving the resolution of listing is the availability of robust

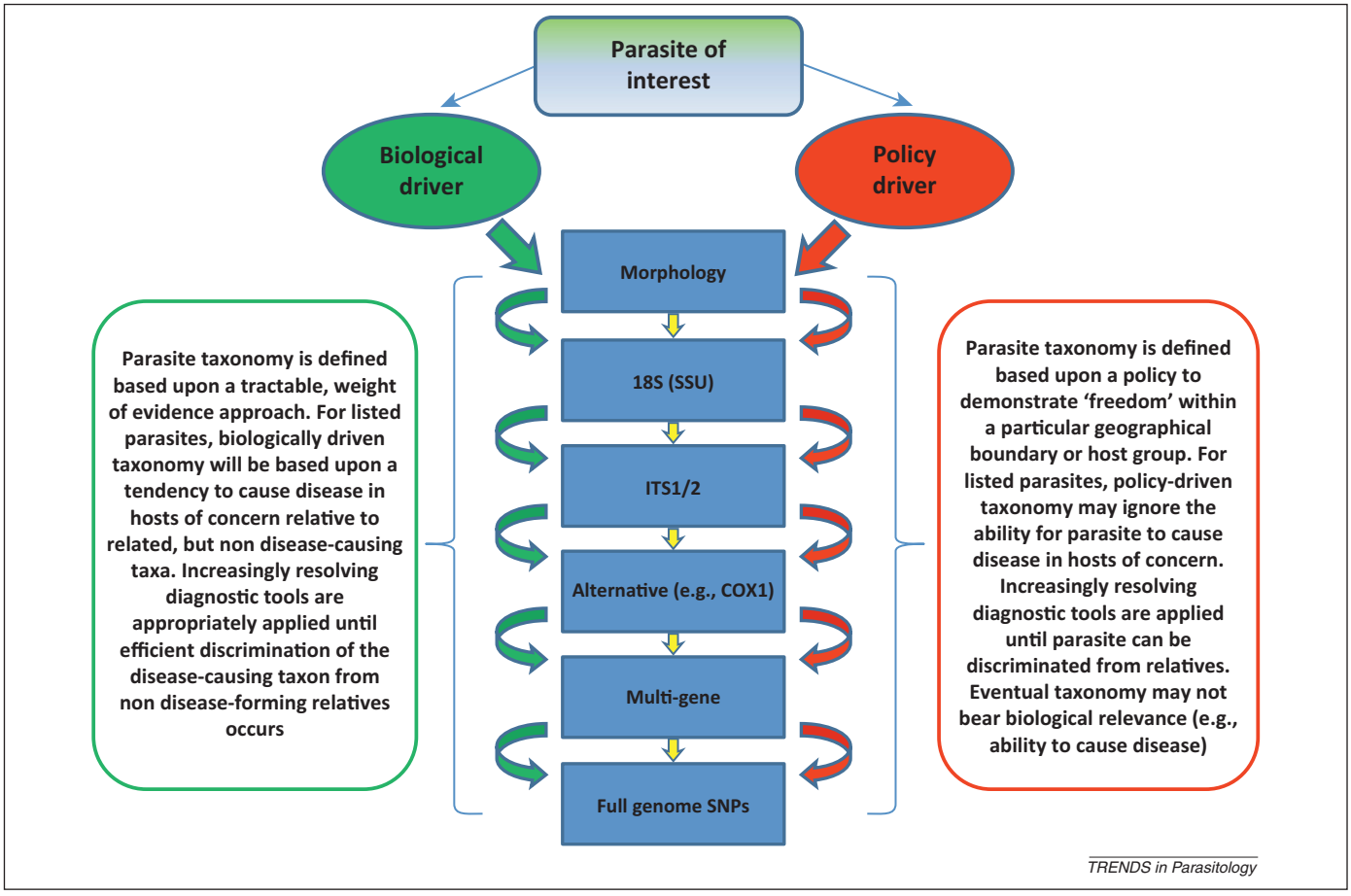

Figure 1. The 'diagnostic cascade'. The driver to circumscribe a particular parasite taxon may be driven by 'biological' or 'political' reasoning. In the case of important parasites of traded animal hosts we propose that a biological driver, in which discrimination occurs based on the ability of the agent to lead to disease in the host(s) of concern, should be present. In this case, increasingly sensitive diagnostic tests should be applied until the disease-causing pathogen can be discriminated from its non disease-causing relatives. Although the suite of markers applied may be the same when the driver is political, here there may be an aim to discriminate the taxon of concern regardless of biological outcome in the host. As such, increasingly sensitive diagnostic tests are applied until the taxon can be distinguished. In these cases, discrimination may not bear biological relevance in the context of disease. Abbreviations: COI, cytochrome oxidase 1; ITS1, internal transcribed spacer; SNP, single-nucleotide polymorphism; SSU, small ribosomal subunit;.

sequence-based phylogenetic frameworks for these taxa and their relatives. Currently, the taxonomy of listed parasites spans the eukaryotes, including representatives from the Cestoda (*Echinococcus*, *Taenia solium*), Nematoda (*Trichinella*), Platyhelminthes (*Gyrodactylus salaris*), Acarida (e.g., *Varroa*), Trichomonida (*Tritrichomonas foetus*), Kinetoplastida (e.g., *Trypanosoma*), Apicomplexa (e.g., *Toxoplasma*), Chytridiomycota (*Batrachochytrium dendrobatidis*), Oomycetida (e.g., *Aphanomyces astaci*), Microsporidia (e.g., *Nosema apis*), Haplosporidia (e.g., *Bonamia exitiosa*), Paramyxida (*Marteilia refringens*), Perkinsozoa (e.g., *Perkinsus marinus*), and the Mikrocytida (*Mikrocytos mackini*). Perhaps not surprisingly, the status of the taxonomy of these groups is not consistently represented in the available literature. However, some general trends are observed. For most groups, traditional taxonomic frameworks, largely built upon morphological observations, are being significantly challenged by an increasingly replete molecular phylogenetic dataset. The transition is well illustrated for groups such as the Platyhelminthes and the Kinetoplastida where traditional morphology-based approaches to taxonomy have been superseded by numerous phases of molecular phylogenetics – from those based upon the use of ribosomal genes (e.g., SSU), through multi-gene concatenated approaches, to genome-wide analyses [16,17]. Currently, taxonomic frameworks, generally built upon ribosomal genes or spacer sequences, are available for the majority of eukaryote groups in which listed diseases are placed. In numerous cases, for instance the Kinetoplastida, Apicomplexa, Perkinsozoa, and Mikrocytida, robust phylogenies based upon concatenated sequences of tens or hundreds of genes are available, although each is represented by limited taxon sampling [17–20]. Given widespread consensus that morphological traits in many eukaryotic parasite lineages are not only plastic but also convergent [3], taxonomic frameworks built upon multigene concatenated sequence data, if not upon more highly pan-genomic datasets, will likely become the standard. In the context of the current paper, the generation of such deep knowledge for important (listed) parasite groups and their diseases, which have potential to impact upon food security and wildlife health, must be given top priority. In Figure 2 we propose a basic framework for assessing data quality in support of listing a given parasite taxon. The framework is driven by first considering the basis for the proposed listing (Figure 1). In most cases the key driver will be a definition of the biological basis for the separation of the proposed parasite taxon; for example,

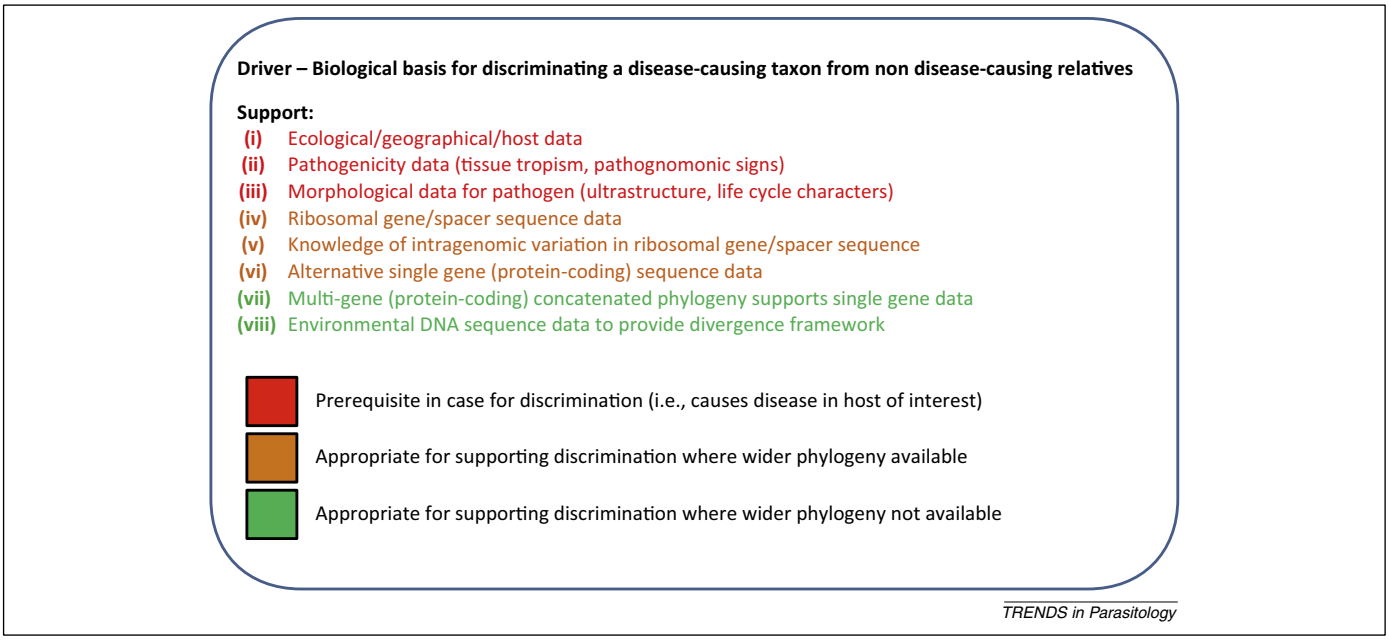

Figure 2. Best practice guidelines to support discrimination of listed parasite taxa.

infection leads to disease in host(s) of concern whilst closely related parasite taxa do not lead to disease in hosts of concern. Subsequently, consideration of data pertaining to pathology and ultrastructure, and phylogenies built upon single genes (e.g., ribosomal) or multiple genes (including protein coding genes), can be used to provide scientific support for separation. Best practice uses all such diagnostic data pertaining to the parasite taxon of interest and its known relatives. In addition, inclusion of environmental diversity data for unknown relatives will provide crucial information relating to the potential for false negatives in surveillance programmes or outbreaks.

Concluding remarks

The political and economic implications of detecting a listed parasite can be considerable. Although detection relies on the application of accurate diagnostic testing, the approaches to detection, and the molecular 'resolution' of such, differ considerably among the listed taxa. In this opinion article we propose that a robust phylogenetic framework should underpin the listing of parasite taxa and, further, that environmental sampling can augment the wider taxonomic framework in which listed parasite taxa reside. Importantly, biological data rather than political concerns should drive the process. Until now, researchers have studied the taxonomy of parasites for ecological or evolutionary reasons. We suggest here that taxonomy has an additional role to play in supporting local, national, regional, and global biosecurity initiatives by providing robust classification schemes for important parasitic diseases of food animals (Box 2). In this respect we foresee an emergence of high-resolution parasite taxonomy as a requisite tool for sustainable globalised trading of foodstuffs under the wider food security agenda. As such, this opinion article is not intended to criticise the basis for listing of important parasite taxa, or the status of current lists, but rather to propose that taxonomic rigour should be consistently applied to the classification of listed parasites for the benefit of global biosecurity and sustainable food production.

Box 2. Outstanding questions

- Should best practice measures be included as a prerequisite for the listing of parasitic disease?
- Should environmental sampling be utilised as standard to augment phylogenetic frameworks, and cases for freedom, for the listed parasite groups in aquatic and terrestrial systems?
- Should listing be more dynamic – that is, responding to emergence of genotypes and possibly being updated on an annual basis?
- Should listing be based on grouping (to disease) or splitting (to parasite taxa)?
- Is sufficient investment in place for robust taxonomy of listed parasites?

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References

1 World Organisation for Animal Health (2012) *Manual of Diagnostic Tests for Aquatic Animals 2012*, World Organisation for Animal Health
2 Hey, J. (2001) The mind of the species problem. *Trends Ecol. Evol.* 16, 326–329
3 Boenigk, J. *et al.* (2012) Concepts in protistology: species definitions and boundaries. *Eur. J. Protistol.* 48, 96–102
4 Stentiford, G.D. *et al.* (2013) Microsporidia: diverse, dynamic and emergent pathogens in aquatic systems. *Trends Parasitol.* 29, 567–578

5 Lightner, D.V. (2012) Global transboundary disease politics: the OIE perspective. *J. Invert. Pathol.* 110, 184–187
6 Green, J.L. *et al.* (2004) Spatial scaling of microbial eukaryote diversity. *Nature* 432, 747–750
7 Schloss, P.D. and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.* 71, 1501–1506
8 Hartikainen, H. *et al.* (2013) Lineage-specific molecular probing reveals high novel haplosporidian parasite diversity and ecological partitioning in coastal waters. *ISME J.* 8, 177–186
9 Jones, M.D.M. *et al.* (2011) Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* 474, 200–203
10 Maslov, D.A. *et al.* (2013) Diversity and phylogeny of insect trypanosomatids: all that is hidden shall be revealed. *Trends Parasitol.* 29, 43–52
11 Cook, S. *et al.* (2013) Novel virus discovery and genome reconstruction from field RNA samples reveals highly divergent viruses in dipteran hosts. *PLOS ONE* 8, e80720
12 Lyngstad, T.M. *et al.* (2012) Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming. *Dis. Aquat. Organ.* 101, 197–206
13 van Herwerden, L. *et al.* (1999) Intra- and interindividual variation in ITS1 of *Paragonimus westermani* (Trematoda: Digenea) and related species: implications for phylogenetic studies. *Mol. Phylogenet. Evol.* 12, 67–73
14 Harris, D.J. and Crandall, K.A. (2000) Intragenomic variation within ITS1 and ITS2 of freshwater crayfishes (Decapoda: Cambaridae): implications for phylogenetic and microsatellite studies. *Mol. Biol. Evol.* 17, 284–291
15 Emerson, K.J. *et al.* (2010) Resolving postglacial phylogeography using high-throughput sequencing. *Proc. Natl. Acad. Sci. U.S.A.* 107, 16196–16200
16 Riutort, M. *et al.* (2012) Evolutionary history of the Tricladida and the Platyhelminthes: an up-to-date phylogenetic and systematic account. *Int. J. Dev. Biol.* 56, 5–17
17 Deschemps, P. *et al.* (2011) Phylogenomic analysis of kinetoplastids supports that trypanosomatids arose from within bodonids. *Mol. Biol. Evol.* 28, 53–58
18 Kuo, C-H. *et al.* (2008) The apicomplexan whole-genome phylogeny: an analysis of incongruence among gene trees. *Mol. Biol. Evol.* 25, 2689–2698
19 Zhang, H. *et al.* (2011) Spliced leader RNAs, mitochondrial gene frameshifts and multi-protein phylogeny expand support for the genus *Perkinsus* as a unique group of alveolates. *PLOS ONE* 6, e19933
20 Burki, F. *et al.* (2013) Phylogenomics of the intracellular parasite *Mikrocytos mackini* reveals evidence for a mitosome in Rhizaria. *Curr. Biol.* 23, 1541–1547
21 Dinkel, A. *et al.* (1998) Detection of *Echinococcus multilocularis* in the definitive host: coprodiagnosis by PCR as an alternative to necropsy. *J. Clin. Microbiol.* 36, 1871–1876
22 Brabec, J. *et al.* (2006) Paraphyly of the Pseudophyllidea (Platyhelminthes: Cestoda): circumscription of monophyletic clades based on phylogenetic analysis of ribosomal RNA. *Int. J. Parasitol.* 36, 1535–1541
23 Pozio, E. and La Rosa, G. (2003) PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. *Methods Mol. Biol.* 216, 299–309
24 Holtermann, M. *et al.* (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution towards crown clades. *Mol. Biol. Evol.* 23, 1792–1800
25 Maslov, D.A. *et al.* (2001) Phylogeny of the Kinetoplastida: taxonomic problems and insights into the evolution of parasitism. *Mem. Inst. Oswaldo Cruz* 96, 397–402
26 Moser, D.R. *et al.* (1989) Detection of *Trypanosoma congolense* and *Trypanosoma brucei* subspecies by DNA amplification using the polymerase chain reaction. *Parasitology* 99, 57–66
27 Cruickshank, R.H. (2002) Molecular markers for the phylogenetics of mites and ticks. *Syst. Appl. Acarol.* 7, 3–14
28 Dabert, M. *et al.* (2010) Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long-branch attraction artifacts. *Mol. Phylogenet. Evol.* 56, 222–241
29 Fries, I. *et al.* (2013) Standard methods for *Nosema* research. In *The COLOSS BEEBOOK: Volume II: Standard Methods for Apis mellifera Pest and Pathogen Research* ((Dietemann, V. *et al.*, eds), In International Bee Research Association
30 Vossbrinck, C.R. and Debrunner-Vossbrinck, B.A. (2005) Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. *Folia Parasitol. (Praha.)* 52, 131–142
31 Anderson, D.L. and Morgan, M.J. (2007) Genetic and morphological variation of bee parasitic *Tropilaelaps* mites (Acari: Laelapidae): new and re-defined species. *Exp. Appl. Acarol.* 43, 1–24
32 Buling, A. *et al.* (2007) A quantitative PCR assay for the detection and quantification of *Babesia bovis* and *B. bigemina*. *Vet. Parasitol.* 147, 16–25
33 Gasser, R. and Chilton, N.B. (1995) Characterisation of taeniid cestode species by PCR RFLP of ITS2 ribosomal DNA. *Acta Trop.* 59, 31–40
34 Gonzalez, L.M. *et al.* (2004) Differential diagnosis of *Taenia saginata* and *Taenia saginata asiatica* taeniasis through PCR. *Diagn. Microbiol. Infect. Dis.* 49, 183–188
35 Allsopp, B.A. *et al.* (1993) Discrimination between six species of *Theileria* using oligonucleotide probes which detect small subunit ribosomal RNA sequences. *Parasitology* 107, 157–165
36 Bishop, R.P. *et al.* (1995) *Theileria*: improved species discrimination using oligonucleotides derived from large subunit ribosomal RNA sequences. *Exp. Parasitol.* 80, 107–115
37 Gookin, J.L. *et al.* (2002) Single tube nested PCR for detection of *Tritrichomonas foetus* in feline feces. *J. Clin. Microbiol.* 40, 4126–4130
38 Grahn, R.A. *et al.* (2005) An improved molecular assay for *Tritrichomonas foetus*. *Vet. Parasitol.* 127, 33–41
39 Kleina, P. *et al.* (2004) Molecular phylogeny of Trichomonadidae family inferred from ITS-1, 5.8S rRNA and ITS-2 sequences. *Int. J. Parasitol.* 34, 963–970
40 Lun, Z-R. *et al.* (2004) Are *Tritrichomonas foetus* and *Tritrichomonas suis* synonyms? *Trends Parasitol.* 21, 122–125
41 Desquesnes, M. and Davila, A.M.R. (2002) Applications of PCR-based tools for detection and identification of animal trypanosomes; a review and perspectives. *Vet. Parasitol.* 109, 213–231
42 Li, F.J. *et al.* (2007) PCR approach for the detection of *Trypanosoma brucei* and *T. equiperdum* and their differentiation from *T. evansi* based on maxicircle kinetoplast DNA. *Mol. Cell. Probes* 21, 1–7
43 Alhassan, A. *et al.* (2005) Development of a single-round and multiplex PCR method for the simultaneous detection of *Babesia caballi* and *Babesia equi* in horse blood. *Vet. Parasitol.* 129, 43–49
44 Bezold, G. *et al.* (2001) Hidden scabies: diagnosis by polymerase chain reaction. *Br. J. Dermatol.* 144, 614–618
45 Xiao, L. *et al.* (2004) *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clin. Microbiol. Rev.* 17, 72–97
46 Xiao, L. and Ryan, U.M. (2008) Molecular epidemiology. In *Cryptosporidium and Cryptosporidiosis* (2nd edn) (Fayer, R. and Xiao, L., eds), pp. 387–410, CRC Press and IWA Publishing
47 Burg, J.L. *et al.* (1989) Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *J. Clin. Microbiol.* 27, 1787–1792
48 Savva, D. *et al.* (1990) Polymerase chain reaction for detection of *Toxoplasma gondii*. *J. Med. Microbiol.* 32, 25–31
49 Hyatt, A.D. *et al.* (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Organ.* 73, 175–192
50 James, T.Y. *et al.* (2006) A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). *Mycologia* 98, 860–871
51 Tuffs, S. and Oidtmann, B. (2011) A comparative study of molecular diagnostic methods designed to detect the crayfish plague pathogen, *Aphanomyces astaci*. *Vet. Microbiol.* 153, 343–353
52 Beakes, G.W. and Sekimoto, S. (2009) The evolutionary phylogeny of oomycetes – insights gained from studies of holocarpic parasites of algae and invertebrate. In *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools* (Lamour, K. and Kamoun, S., eds), pp. 1–24, John Wiley and Sons
53 Phadee, P. *et al.* (2004) A PCR method for the detection of *Aphanomyces piscicida*. *Fish Pathol.* 39, 25–31
54 Cunningham, C.O. (1997) Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea: Gyrodactylidae) ribosomal RNA genes. *J. Parasitol.* 83, 215–219
55 Olstad, K. *et al.* (2007) Host-based identification is not supported by morphometrics in natural populations of *Gyrodactylus salaris* and G. *thymalli* (Platyhelminthes, Monogenea). *Parasitology* 134, 2041–2052

56 Harris, P.D. *et al.* (1999) Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. *J. Parasitol.* 85, 188–191
57 Cochennec, N. *et al.* (2003) Revisiting *Mikrocytos roughleyi* taxonomic affiliation points to the genus *Bonamia* (Haplosporidia). *Dis. Aquat. Organ.* 54, 209–217
58 Carnegie, R. *et al.* (2000) Development of a PCR assay for detection of the oyster pathogen *Bonamia ostreae* and support for its inclusion in the Haplosporidia. *Dis. Aquat. Organ.* 42, 199–206
59 Cochennec, N. *et al.* (2000) Detection of *Bonamia ostreae* based on small subunit ribosomal probe. *J. Invertebr. Pathol.* 76, 26–32
60 Le Roux, F. *et al.* (2001) Molecular evidence for the existence of two species of *Marteilia* in Europe. *J. Eukaryot. Microbiol.* 48, 449–454
61 Lopez-Flores, I. *et al.* (2004) The molecular diagnosis of *Marteilia refringens* and differentiation between *Marteilia* strains infecting oysters and mussels based on the rDNA IGS sequence. *Parasitology* 129, 411–419
62 Abollo, E. *et al.* (2006) Differential diagnosis of *Perkinsus* species by polymerase chain reaction-restriction fragment length polymorphism assay. *Mol. Cell. Probes* 20, 323–329
63 Mangot, J-F. *et al.* (2010) Perkinsozoa, a well-known marine protozoan flagellate parasite group, newly identified in lacustrine systems: a review. *Hydrobiologia* 659, 37–48
64 Carnegie, R.B. *et al.* (2003) Molecular detection of the oyster parasite *Mikrocytos mackini* and a preliminary phylogenetic analysis. *Dis. Aquat. Organ.* 54, 219–227
65 Abbott, C.L. *et al.* (2011) Sequence homogeneity of internal transcribed spacer rDNA in *Mikrocytos mackini* and detection of *Mikrocytos* sp. in a new location. *Dis. Aquat. Organ.* 93, 243–250
66 Hartikainen, H. *et al.* (2014) Mikrocytids are a broadly distributed and divergent radiation of parasites in aquatic invertebrates. *Curr. Biol.* 24, 807–812
67 Malmberg, G. (1970) The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). *Ark. Zool.* 23, 1–235
68 Ergens, R. (1965) Die Morphogenese der chitinoiden Teile des Haptors bei Gyrodactylus tincae (Malmberg, 1956) Malmberg, 1964 (Monogenoidea) und ihre morphologish-metrische Variabilitat. *Z. f. Parasitenkunde* 26, 173–184
69 Ergens, R. (1983) *Gyrodactylus* from Eurasian freshwater Salmonidae and Thymallidae. *Folia Parasitol.* 30, 15–26
70 Mo, T.A. (1993) Seasonal variations of the opisthaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *S. salar* L. parr in the River Sandvikselva, Norway. *Syst. Parasitol.* 26, 225–231
71 Geets, A. *et al.* (1999) Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus cf. arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. *Parasitology* 119, 27–40
72 Appleby, C. (1996) Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea, Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. *Syst. Parasitol.* 33, 199–207
73 Hansen, H. *et al.* (2007) DNA taxonomy and barcoding of monogenean parasites: lessons from *Gyrodactylus*. *Trends Parasitol.* 23, 363–367