Vol. 110: 25–32, 2014
doi: 10.3354/dao02788

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published July 24

Contribution to DAO Special 7 'Microcell parasites of molluscs'

Review of *Mikrocytos* microcell parasites at the dawn of a new age of scientific discovery

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ABSTRACT: The genus *Mikrocytos* is traditionally known for *Mikrocytos mackini*, the microcell parasite that typically infects Pacific oysters along the west coast of North America. Multiple factors have conspired to create difficulty for scientific research on *Mikrocytos* parasites. These include their tiny cell size, infections that are often of light intensity, lack of suitable cell lines and techniques for *in vitro* culture, and the seasonal nature of infections. The extreme rate of molecular evolution in *Mikrocytos* stymied new species discovery and confounded attempts to resolve its phylogenetic position for many years. Fortunately, 2 recent landmark studies have paved the way forward for future research by drastically changing our understanding of the evolution and diversity of these parasites. No longer an orphan eukaryotic lineage, the phylogenetic placement of *Mikrocytos* has been confidently resolved within Rhizaria and as sister taxon to Haplosporidia. The genus has also found a taxonomic home within the newly-discovered order, Mikrocytida — a globally distributed lineage of parasites infecting a wide range of invertebrate hosts. Here we review available scientific information on *Mikrocytos* parasites including their evolution and diversity, host and geographic ranges, epizootiology, and detection of the regulated pathogen, *M. mackini.* We also make recommendations towards a consistent taxonomic framework for this genus by minimally suggesting the use of 18S rDNA sequence, host species information, and histopathological presentation in new species descriptions. This is timely given that we are likely embarking on a new era of scientific advancements, including species discovery, in this genus and its relatives.

KEY WORDS: *Mikrocytos mackini* · Mikrocytid · Mikrocytida · Protistan · Shellfish parasites · Taxonomy · Denman Island disease

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INTRODUCTION

Denman Island disease of Pacific oysters *Crassostrea gigas* was first reported by Quayle (1961) in Henry Bay on Denman Island, British Columbia, Canada, and decades later the responsible parasite was named *Mikrocytos mackini* (Farley et al. 1988). Also called mikrocytosis, the disease is characterized by focal green lesions within the mantle, body wall, labial palps or adductor muscle of Pacific oysters (Bower et al. 1994, Bower & Meyer 2004). Non-lethal cases of the disease can render oysters unmarketable due to the presence of green lesions, whereas heavy infections can cause high levels of morbidity (Bower & Meyer 2004, Bower et al. 2005). Although *M. mackini* was delisted by the World Organization for Animal Health (OIE) in 2007, the OIE maintains a reference laboratory for infection with *M. mackini*, and it remains a regulated disease in some countries. *M. mackini* is a reportable disease under Canada's National Aquatic Animal Health Program, is listed as an exotic disease in the European Union Directive 2006/88/EC, and is on Australia's National List of Reportable Diseases of Aquatic Animals.

Until now, *Mikrocytos* has been a stubbornly enigmatic subject for research. Scientific advances were

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Publisher: Inter-Research · www.int-res.com

hampered by its small cell size; the lack of knowledge of its evolutionary history, diversity, life cycle, and geographic and host ranges; an inability to culture the parasite; and by the lack of informative characters for taxonomy. Fortunately, significant recent breakthroughs on the evolution, diversity, and distribution of these parasites have significantly changed the landscape of *Mikrocytos* research and will greatly enhance our ability to detect and describe these parasites. Most notable are 2 recent studies that resolved the phylogenetic position of *Mikrocytos* and classified it within a newly-described parasite order, Mikrocytida (Burki et al. 2013, Hartikainen et al. 2014). As such, it seems timely to review the current scientific literature on *Mikrocytos*, including its evolution and diversity, host and geographic ranges, epizootiology, and detection.

The fact that *M. mackini* is a regulated pathogen creates a need for confident species delineations and specific diagnostic tests, both of which have historically been lacking. Until recently (Abbott et al. 2014, this DAO Special; Hartikainen et al. 2014), reports of new *Mikrocytos* parasites have not lead to new species descriptions due to the challenges described above. This review of *Mikrocytos* highlights the fact that there is now enough information available for the naming of new species. This has been synthesized into taxonomic recommendations for this genus.

Prevalence of *M. mackini*

Long term annual survey data collected from Denman Island between 1960 and 1985 found that *M. Mackini* prevalence among Pacific oysters varied from 11–39% (Quayle 1988). First estimates of mortality rates induced by the disease in beach-cultured Pacific oysters ranged from 17% (1.2 m tide level) to 53% (0.3 m tide level) and averaged 34% across the 0–5 m tide range (Quayle 1961, as cited in Bower 1988). The implementation of management practices that include not growing oysters in the lower intertidal zone (<0.5 m) and harvesting stocks when they are less than 3 yr old, seems to have minimised the impact of the disease on commercial production within its enzootic range (Bower & McGladdery 2003; and see Bower 1988).

Geographic and host ranges of *M. mackini*

For many years, *M. mackini* was only known to occur in Pacific oysters *C. gigas*, but laboratory and field challenges later determined that *Crassostrea viginica*, *Ostrea edulis* and *Ostrea lurida* were also susceptible, perhaps to a greater degree than *C. gigas* (Bower et al. 1997). In contrast, laboratory challenges showed that 2 commercially important clam species, Manila clam *Ruditapes philippinarum* and geoduck clam *Panope abrupta,* were resistant to infection by this parasite (Meyer et al. 2008, Bower et al. 2005) although Ramilo et al. (2014, this DAO Special) recently detected a divergent *Mikrocytos* parasite in Manila clams. The known geographic range of *M. mackini* was initially limited to southern British Columbia and it was also confirmed in *C. gigas* from northern areas of Washington State in May 2002 (R. A. Elston pers. comm.). *M. mackini* was recently discovered in Kumamoto oysters *Crassostrea sikamea* from Humboldt Bay, California (Elston et al. 2012); however, associated mortalities have only been reported from British Columbia. Although infections with *M. mackini* have only been confirmed in oyster hosts from the west coast of North America, Hartikainen et al. (2014) detected its DNA sequence in a sample of mixed copepod species collected from the South Atlantic.

Epizootiology of *M. mackini*

Field and laboratory studies have shown that infection with *M. mackini* can be transmitted directly between oysters (Quayle 1961, Bower et al. 1997); however, the annual life cycle of *M. mackini* remains a mystery. Hine et al. (2001) identified 3 cell types based on ultrastructural characteristics and proposed a developmental cycle within host tissues. However, there remains no evidence of an alternate or spore-like stage that would facilitate protection and existence of the parasite outside the host. In terms of host age and susceptibility, disease exposure experiments by Bower et al. (1997) showed that Pacific oysters less than 2 yr old were less affected by the disease. Although juvenile oysters are susceptible to infection with *M. mackini,* they do not typically show clinical signs of the disease until the following spring (Bower & Meyer 2004).

The initial description of *M. mackini* noted a seasonal pattern to prevalence of gross signs of disease (Farley et al. 1988). Indeed, *M. mackini* infections in British Columbia are only detectable by histology between January and June, with associated disease and mortalities occurring only between March and May (Bower 1988, Quayle 1988). Expression of the disease is linked to water temperature; laboratory

experiments determined that oysters exposed to the disease must be held in cool water (≤10°C) for a prolonged period (≥3 mo) for the disease to develop (Hervio et al. 1996, Bower et al. 1997). Temperatures above 15°C were found to prevent disease development but did not eliminate the parasite: oysters held at 17°C for 3 mo following exposure to *M. mackini* still developed infections when returned to cold water (Bower et al. 1997). Interestingly, despite the recent discovery of *M. mackini* infecting *C. sikamea* in California from a much lower latitude (~41°N) than its already known range in British Columbia and Washington (≥48°N), average winter surface seawater temperatures at that location over a 7 yr period were close to those required (≤10°C) for the development of clinical infections (between 10.2°C and 11.5°C; Elston et al. 2012).

MIKROCYTOS EVOLUTION AND DIVERSITY

Denman Island disease was first reported in 1961 (Quayle 1961). When Farley et al. (1988) later described *M. mackini*, they also named *M. roughleyi* infecting the Sydney rock oyster *Saccostrea commercialis*, but this latter description was refuted and renamed *Bonamia roughleyi* (Cochennec-Laureau et al. 2003). New findings indicate that *Bonamia roughleyi* may not be a valid taxonomic entity, and that the identity of this parasite remains unknown (Carnegie et al. 2014). Only once it became possible to sequence 18S rDNA for *M. mackini* (Carnegie et al. 2003) did findings of other *Mikrocytos* parasites begin to emerge. None were formally described due to the paucity of informative taxonomic characters and the absence of a resolved phylogeny at the time. Attempts to determine the phylogenetic position of *Mikrocytos* using both ultrastructural (Hine et al. 2001) and molecular (18S sequence; Carnegie et al. 2003) data were unsuccessful. *Mikrocytos* remained a phylogenetic orphan with no known relatives until very recently.

Phylogenetic position and taxonomic classification

Early studies based on cell ultrastructure and 18S sequences both refuted the possibility of a close relationship between *M. mackini* and the only other known group of tiny intracellular parasites affecting ostreid hosts — the haplosporidian genus *Bonamia* (Hine et al. 2001, Carnegie et al. 2003). Given that protistan diversity is exceedingly high relative to multicellular eukaryotic groups (see Pawlowski et al. 2012 and references therein), it always made intuitive sense that *Mikrocytos* relatives existed and were awaiting discovery and that *M. mackini* was not the only extant representative of its lineage. Regardless, it took 25 yr since its initial description (Farley et al. 1988) and the advent of next-generation sequencing methods for researchers to finally solve the conundrum surrounding the evolutionary origins of *Mikrocytos*.

Burki et al. (2013) sequenced the transcriptome of *M. mackini* and used a phylogenomic data set comprised of 119 genes to robustly resolve its position within the eukaryotic supergroup Rhizaria (see Burki & Keeling 2014 for introductory review on Rhizaria; see Pawlowski 2013 for review on eukaryotic evolution). Shortly thereafter, Hartikainen et al. (2014) solved the remaining piece to the evolutionary puzzle by further resolving the phylogenetic position of *Mikrocytos* within Rhizaria as sister to Haplosporidia. Substantial new diversity within this lineage across a wide geographic and host range was also discovered. They found genetic evidence of 10 distinct lineages (including *Mikrocytos*) that together represent a unique, hitherto unknown, global parasite radiation, and collectively placed them within a new taxonomic order: Mikrocytida (Hartikainen et al. 2014). Mikrocytids were sequenced from across a striking range of hosts, geography, and habitat types: they were detected in 4 continents and in both hemispheres, associated with hosts from 3 invertebrate phyla (Arthropoda, Annelida, and Mollusca), and from across freshwater, brackish, and marine environments (Hartikainen et al. 2014). They formally described *Mikrocytos mimicus* infecting Pacific oysters from north Norfolk, UK, and *Paramikrocytos canceri* infecting edible crabs *Cancer parugus*. *Paramikrocytos* is a mikrocytid lineage that is morphologically distinct from the *Mikrocytos* one, most notably due to the development of unicellular stages into plasmodia (Hartikainen et al. 2014).

It appears that the diverse and geographically widespread lineage comprising mikrocytid parasites remained undiscovered for so long because of its striking evolutionary rate (see commentary by Abbott 2014). Findings by Burki et al. (2013) clearly established *Mikrocytos* to be one of the fastest-evolving eukaryotes known. Hartikainen et al. (2014) then confirmed that mikrocytid sequences were indeed completely absent from publicly available next-generation sequencing data sets; they are so divergent from all other known eukaryotic lineages that they eluded amplification using 'universal' 18S primers.

Species diversity

There are several instances of divergent *Mikrocytos* parasites having been detected in bivalves before the recent description of Mikrocytida. In all cases except one (see Abbott et al. 2014), these parasites were not taxonomically described. Gagné et al. (2008) detected *Mikrocytos* parasites in 4.5% of *Ostrea edulis* from Atlantic Canada whose 18S sequence at the variable region 4 (V4; GenBank acc. no. DQ237912) was 88% identical to *M. mackini* (HM563060). *Mikrocytos* parasites with the same 18S-V4 sequence as that found by Gagné et al. (2008) were reported infecting 4% of *C. gigas* sampled from the Yellow Sea, China (Wang et al. 2010; data not in GenBank). Abbott et al. (2011) reported a genetically divergent '*Mikrocytos* sp.-BC' (HM563061) in a single juvenile *C. gigas* from within the endemic zone of *M. mackini* in British Columbia, Canada. They later detected the same species in *O. lurida* in the same geographic region and named it *M. boweri* (KF297352, KF297353; Abbott et al. 2014). Interestingly, its 18S-V4 sequence was again identical to that of the parasites reported by Gagné et al. (2008) and Wang et al. (2010), and a sequence from this same lineage was found by Hartikainen et al. (2014) in an environmental sample from the UK. New *Mikrocytos* parasites that are more divergent (~20%) from *M. mackini* at 18S-V4 have recently been detected in clam hosts *Donax trunculus* and *Ruditapes philippinarum* following disease investigations in France (data not in GenBank) and Spain (KF548044–KF548052), respectively (Garcia et al. 2012, Ramilo et al. 2014).

Mikrocytos is not truly amitochondriate

Burki et al. (2013) sequenced part of the transcriptome of *M. mackini* and refuted earlier suggestions that it is truly amitochondriate (Hine et al. 2001) despite its lack of canonical mitochondria. Evidence based on the discovery of 4 mitochondrion-derived genes in *M. mackini* indicates that it harbours a yet-to-be described, double-bounded, reduced mitochondrion-related organelle (MRO). Eukaryotic lineages that lack typical mitochondria were originally thought to be primitive to mitochondriate lineages; however, this theory has been disproved as it is now clear that all eukaryotic cells retain some portion of traditional mitochondrial functioning (reviewed by Embley & Martin 2006). The presence of an MRO in *M. mackini* may result from reductive evolution, an adaptive process whereby cell structure and function become simplified in response to extreme specialization to parasitic or anaerobic life (reviewed by Hjort et al. 2010).

DETECTION

Seasonality of *Mikrocytos* infections

As described earlier, *M. mackini* requires a prepatent period at ≤10°C in order to cause disease (Hervio et al. 1996, Bower et al. 1997). In its usual host Pacific oysters in its endemic zone it is normally detectable only in spring months (Bower 1988, Quayle 1988); therefore surveillance testing needs to be done at the appropriate time of year. Possible temperature dependence of pathology induced by other *Mikrocytos* parasites can be speculatively inferred from what is known about winter water temperatures in the areas where they were found. Disease caused by *M. mimicus* occurred at high latitude (53°N) and was discovered after a long period of cool water temperatures consistent with those needed for *M. mackini* infections (Hartikainen et al. 2014, supplementary info). The rare, light, *Mikrocytos* sp. infections detected in Atlantic Canada and China were from a high latitude zone where winter water temperatures below 10°C are normal (Gagné et al. 2008, Wang et al. 2010). The *Mikrocytos*-like parasites found in Spain (~42°N) occurred where water temperatures range down to 10°C (Ramilo et al. 2014) and the *Mikrocytos* sp. found in France (Garcia et al. 2012) caused summer mortalities in a high latitude area (over 47°N; C. Garcia pers. comm.) where sufficiently cold winter water temperatures are presumably plausible. *M. boweri* was detected within the normal clinical period for *M. mackini* in British Columbia (Abbott et al. 2014). Taken together, these findings suggest that the temperature dependence of *M. mackini* pathology occurs across the genus, such that clinical infections induced by *Mikrocytos* species are generally dependent on cool water temperatures.

Gross signs

Macroscopic signs of infection with *Mikrocytos* have thus far only been observed with *M. mackini* and *M. mimicus* infecting Pacific oysters, and are characterized by focal green lesions within the mantle, labial palps and/or adductor muscle. However, lesions alone cannot be used to confirm that a *Mikro-*

cytos microcell is the causative agent (cf. Bower et al. 1994, Bower & Meyer 2004). Contrary to the initial description indicating that 'microcells were never found outside of abscesses' (Farley et al. 1988, p. 583), we now know that infections can be detected in the absence of gross signs. Photographic images of gross lesions caused by *M. mackini* in Pacific oysters are in Bower & Meyer (2004).

Histopathology

Traditional detection methods for *M. mackini* have relied on histopathology, although confident species identification of the parasite is not possible by light microscopy, and light infections may be missed (Cochennec-Laureau et al. 2003). Indeed, the small size and intracellular habit of *M. mackini* combined with its faint staining characteristics when using routine histological techniques often make its microscopic detection difficult (Meyer et al. 2005). Due in part to their small cell size (2 to 4 µm diameter), distinguishing among *Mikrocytos* species and confidently separating them from *Bonamia* microcells is not possible by light microscopy.

M. mackini normally occurs within vesicular connective tissue (VCT) cells adjacent to focal lesions caused by hemocytic infiltration. The histopathological presentation of infection with other *Mikrocytos* parasites is thus far consistent with that known for *M. mackini* (Abbott et al. 2014, Hartikainen et al. 2014, Ramilo et al. 2014). In contrast, *Bonamia* infections are usually systemic and typically infect host haemocytes (Carnegie & Cochennec-Laureau 2004), though not much weight should be given to location of the parasite within the host, since *M. mackini* infects many other tissues in *C. gigas* including adductor muscle, digestive gland, gonad, gut, heart, gills, and kidney (Meyer et al. 2005). Detections of infection with other *Mikrocytos* spp. do not always report macroscopic lesions nor the usual histological presentation of focal hemocyte infiltration in the VCT (Gagné et al. 2008, Wang et al. 2010), which may be because they were sub-clinical infections.

In-situ hybridization

The first *in situ* hybridization method developed for detection of *M. mackini* was a fluorescent *in situ* hybridization (FISH) technique which used fluorescently-labelled probes designed to hybridize to 18S rDNA (Carnegie et al. 2003). Later, Meyer et al. (2005) used digoxigenin-labelling of one of the DNA probes designed by Carnegie et al. (2003) and found this *in situ* hybridization method (DIG-ISH) allowed for easy detection of *M. mackini* at low magnification with much higher sensitivity than routine histology. A brief description of FISH and how it can inform studies on marine protistan diversity is provided by Caron et al. (2012).

PCR/qPCR

The first DNA sequence generated for *Mikrocytos* was 1457 bp of the 18S rRNA gene of *M. mackini* (Carnegie et al. 2003). A 544 bp fragment therein became the target of the first PCR assay for specific detection of this parasite that, not surprisingly, offered significantly higher sensitivity than standard histopathogy (Carnegie et al. 2003). This was a big breakthrough, given the usefulness of PCR tests for routine testing and surveillance, but the *M. mackini* PCR assay was found to cross-react with other *Mikrocytos* spp. (e.g. Gagné et al. 2008, Wang et al. 2010, Abbott et al. 2011) and *M. boweri* (authors' unpubl. data).

Given that species diversity of *Mikrocytos* is poorly characterized and that the existing PCR assay (Carnegie et al. 2003) is not specific to *M. mackini*, a new molecular diagnostic tool is needed for routine regulatory testing. A specific molecular assay for detection of *M. mackini* is not yet available, but a TaqMan qPCR assay targeting ITS2-28S rDNA of this parasite developed by Lowe et al. (2012) is currently in the late stages of full diagnostic validation and so should become available in the near future.

TAXONOMY

Stentiford et al. (2014) highlight the important role taxonomy plays in supporting global biosecurity: it underpins the legislative frameworks that enable measures aimed at minimizing the spread of regulated diseases to be implemented. Enforcement of policies aimed at protecting food security, biodiversity, and wildlife health requires that regulated disease-causing organisms be specifically and robustly defined, which typically involves their designation as 'species' (Stentiford et al. 2014). Accurate and accepted species names are also vital for regulated pathogens, because they provide a direct link to all available knowledge of the pathogen and the disease they cause that can then be used to make

informed management and regulatory decisions (Cai et al. 2011).

Species concepts are notoriously hotly debated and are particularly challenging for protists (see Boenigk et al. 2012). Parasitologists have traditionally combined information on phenotype/morphology, life-cycles, and host-range specificities to make evolutionary hypotheses for protistan parasites (Sogin & Silberman 1998). However, traditional morphological approaches to protist taxonomy are often encumbered by morphological conservation or multiple life stages with different morphologies, high incidences of asexuality, very small cell sizes, and evolutionary convergence (Boenigk et al. 2012, Caron et al. 2012). Further, morphologically-based taxonomies are often challenged by molecular phylogenies. Indeed, DNA sequences can greatly expand the number of informative taxonomic characters, are not dependent on life stage, and can lead to molecular species identification methods that alleviate the need for specific expertise once the initial species description is available. Particularly for microbes but also for other eukaryotic groups with few or conserved morphological features, rDNA sequences have been heavily relied upon to assess diversity in natural assemblages, including in the marine environment, and to delimit species (Pace 1997, Moon-van der Staay et al. 2001, Countway et al. 2005, Caron et al. 2009, 2012).

Until now, taxonomic descriptions for new *Mikrocytos* parasites were hindered by a lack of informative characters and its unresolved phylogenetic position; however, it is important to properly characterize them given that *M. mackini* is a regulated pathogen. Perhaps other *Mikrocytos* or, more broadly, other mikrocytid species will also become of regulatory significance once more is learned about the diseases they cause. The incorporation of morphological information into a taxonomic framework for *Mikrocytos* is precluded by their small cell size and lack of distinguishing characters. Previous ultrastructural examination of *M. mackini* revealed relatively few organelles or distinguishing characteristics (Hine et al. 2001), and cell ultrastructure of *M. mimicus* (Hartikainen et al. 2014) was found to be highly similar to that of *M. mackini*.

Thus far, 18S seems to be a useful marker for phylogeny of mikrocytids and can therefore help inform taxonomy. Hartikainen et al. (2014) used 480 bp of 18S (variable regions V5 to V7) to resolve phylogenetic relationships within mikroctyids, and to support the description of *P. canceri* and *M. mimicus*. They reported 79% sequence similarity between *M. mackini* and *M. mimicus* at this region. Sequence similarity observed between *M. mackini* and *M. boweri* at 1265 aligned bases of 18S was 91%, with no variation observed within *M. mackini* or *M. boweri* (i.e. among isolates within each species; Abbott et al. 2011, 2014). Available data indicate that ITS regions evolve too quickly in *Mikrocytos* for reliable alignment at the genus level, thus precluding its general use for resolving stable species-level phylogenetic relationships (cf. Abbott et al. 2011, 2014, Ramilo et al. 2014). However, its high levels of variation may prove useful for discriminating strain level differences, and elucidating underlying ecological correlates, as well as for verifying close taxonomic relationships between 'sister' species.

Boenigk et al. (2012) suggest that protistan species can be justifiably delineated in a variety ways, and so recommend a case-by-case approach whereby multiple lines of evidence are applied within a robust phylogenetic framework to develop a consistent and clearly communicated taxonomic strategy for a particular protistan group. As a starting point for establishing a robust taxonomic framework for *Mikrocytos*, we suggest that a reasonable minimum data standard for new species descriptions is the inclusion of 18S-rDNA sequence data, host information, and histopathological presentation. The inclusion of electron microscopy information in taxonomic descriptions of *Mikrocytos* is considered here to be highly desirable; hence it is important that attempts to obtain this be made. However, it is not pragmatic to recommend this as essential for new species descriptions because it is generally not feasible when the prevalence and intensity of infections are low, which has been true thus far for most new *Mikrocytos* discoveries (e.g. Gagné et al. 2008, Wang et al. 2010, Abbott et al. 2011, 2014; but see Garcia et al. 2012). Further, although it does enable morphologically-based discrimination between *Bonamia* and *Mikrocytos*, electron microscopy is unlikely to be useful for distinguishing among *Mikrocytos* species. The recent taxonomic descriptions of 3 new mikrocytid species all included the 3 data types we are recommending here as a reasonable minimum requirement (*P. canceri* and *M. mimicus*: Hartikainen et al. 2014; *M. boweri*: Abbott et al. 2014).

RECOMMENDED METHODS FOR RDNA SEQUENCING

Hartikainen et al. (2014) provide a nested PCR method for sequencing ~500 bp of 18S (variable

regions V5 to V7) in mikrocytids, which should be useful for new species discoveries and phylogenetics. Abbott et al. (2011) developed methods for sequencing a longer fragment of 18S in *Mikrocytos* spp., which have not been tested outside the genus. They published several primers that we tested here (using their published methods exactly) on *M. mackini*, *M. boweri*, and *Mikrocytos* sp. from France (reported by Garcia et al. 2012) for the purpose of elucidating which primers were most effective across all 3 species tested. For PCR and sequencing of ≥1000 bp of 18S, results lead us to recommend either forward primer Mm18SF1 or Mm18S_120F paired with reverse primer Mm18S1403R (authors' unpubl. data); however, note that Ramilo et al. (2014) successfully used Mm18SF1/Mm18S_1450R for 18S sequencing of Spanish *Mikrocytos*. For PCR and sequencing of complete ITS1-5.8S-ITS2 in *Mikrocytos*, we recommend Mm18S_1435F/pro28SR (authors' unpubl. data). These primers also worked successfully on the Spanish *Mikrocytos* (Ramilo et al. 2014).

CONCLUSIONS

As reviewed here, despite *Mikrocytos* parasites having historically been elusive subjects for scientific research, new molecular data are paving the way forward and have led to significant new insights about this genus and its relatives. The recent elucidation of its phylogenetic position as sister taxon to Haplosporidia solves a long-standing puzzle, and will now allow researchers to approach existing knowledge gaps within an evolutionary context. With the taxonomic placement of *Mikrocytos* within a newly-described lineage of parasites with a broad geographic and host range (Mikrocytida; Hartikainen et al. 2014), we are likely embarking on a new era of species discovery that will revolutionize our understanding of the diversity and distribution of these parasites. Improved detection methods for the regulated pathogen *M. mackini* are needed, given that other mikrocytids are being discovered; effective species identification capabilities are vital to support measures aimed at minimizing anthropogenic spread of regulated disease agents. It is our hope that recommendations made here towards establishing a standard taxonomic approach for delineating and describing new *Mikrocytos* species will support continuing scientific advancements about this enigmatic and fascinating genus.

Acknowledgements. The authors thank Celine Garcia for providing samples for primer testing of *Mikrocytos* sp. from France, and Geoff Lowe for conducting these tests. Helpful ideas on how to approach species descriptions for *Mikrocytos* were provided by Scott R. Gilmore, and Stewart Johnson provided helpful feedback on manuscript drafts.

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Editorial responsibility: Marc Engelsma, Lelystad, The Netherlands

Submitted: October 21, 2013; Accepted: July 7, 2014
Proofs received from author(s): July 17, 2014