Motility, morphology and phylogeny of the plasmodial worm, *Ceratomyxa vermiformis* n. sp. (Cnidaria: Myxozoa: Myxosporea)

E. A. ADRIANO1,2* and B. OKAMURA2

1 Departamento de Ciências Biológicas, Universidade Federal de São Paulo (UNIFESP), Diadema, SP, Brazil
2 Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

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SUMMARY

The Myxozoa demonstrate extensive morphological simplification and miniaturization relative to their free-living cnidian ancestors. This is particularly pronounced in the highly derived myxosporeans, which develop as plasmodia and pseudoplasmodia. To date, motility in these stages has been linked with membrane deformation (e.g. as pseudopodia and mobile folds). Here we illustrate a motile, elongate plasmodium that undergoes coordinated undulatory locomotion, revealing remarkable convergence to a functional worm at the cellular level. Ultrastructural and confocal analyses of these plasmodia identify a highly differentiated external layer containing an actin-rich network, long tubular mitochondria, abundant microtubules, a secreted glycocalyx layer, and an internal region where sporogony occurs and which contains homogeneously distributed granular/fibrillar material. We consider how some of these features may support motility. We also describe the species based on spore morphology and SSU rDNA sequence data, undertake molecular phylogenetic analysis to place it within an early-diverging clade of the ceratomyxids, and evaluate the resultant implications for classification (validity of the genus *Meglitschia*) and for inferring early host environments (freshwater) of ceratomyxids.

Key words: ultrastructure, mitochondrial distribution, vermiform morphology, SSU rDNA, confocal microscopy, freshwater fish hosts, Amazonia.

INTRODUCTION

Myxozoa are microscopic cnidarians that have undergone extensive morphological simplification and miniaturization as adaptations to parasitism (Okamura et al. 2015). They have complex life cycles involving primarily aquatic vertebrate and invertebrate hosts and are comprised two classes: the Malacosporea Canning, Curry, Feist, Longshaw & Okamura, 2000 and the Myxosporea Bütschli, 1881. There are some 2400 described species distributed in 64 genera. The great majority are myxosporeans (Fiala et al. 2015a).

Malacosporeans have retained certain primitive features, including muscles in active myxoworm stages produced in some species (e.g. *Buddenbrockia plumatella*), and epithelia in both myxoworms and the sac-like stages produced in non-motile species. Myxosporeans have lost such tissues and are highly derived. Their trophic stages generally consist of multinucleate plasmodia with many spores or uninucleate pseudoplasmodia that produce one or two spores (Canning and Okamura, 2004).

Motility in myxozoans has been observed in different stages in both malacosporeans and myxospor-ear. M. r. Some malacosporeans produce myxoworms whose movement is supported by four sets of muscles whose cells are orientated at 12° with respect to the longitudinal axis of the worm (Gruhl and Okamura, 2012). Muscle contraction results in helical swimming. Motility in myxosporeans is achieved at the cellular level *via* amoeboid movement and ‘dancing’ (also referred to as twitching) (see Feist et al. 2015 for review). The former involves extensions of the cell membrane, often as pseudopodia or filopodia, and there is direct evidence for the involvement of actin (Alama-Bermejo et al. 2012). Dancing is observed in blood stages of sphaerosporids and is proposed to be achieved by a mobile fold of the plasmalemma that acts like an undulating membrane (Lom et al. 1983).

During a survey of fish parasites in rivers of the Amazon basin, Brazil, we observed a myxosporean with unusually shaped, worm-like plasmodia in the gallbladder of *Colossoma macropomum* (Cuvier, 1816), a serrasalmid fish of great importance to both the local fish market and Brazilian aquaculture (Goudinho and Carvalho, 1982; MPA, 2012). Here we describe the morphology and motility of this remarkable myxosporean using light, confocal, transmission and scanning electron microscopy and movements captured by video. We also undertake molecular phylogenetic analysis to determine the relationships of this bizarre species to other myxospor-ear. M. r. Some malacosporeans produce myxoworms whose movement is supported by four sets of muscles whose cells are orientated at 12° with respect to the longitudinal axis of the worm (Gruhl and Okamura, 2012). Muscle contraction results in helical swimming. Motility in myxosporeans is achieved at the cellular level *via* amoeboid movement and ‘dancing’ (also referred to as twitching) (see Feist et al. 2015 for review). The former involves extensions of the cell membrane, often as pseudopodia or filopodia, and there is direct evidence for the involvement of actin (Alama-Bermejo et al. 2012). Dancing is observed in blood stages of sphaerosporids and is proposed to be achieved by a mobile fold of the plasmalemma that acts like an undulating membrane (Lom et al. 1983).

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Table 1. Sites and periods of collection of *Colossoma macropomum* in the Amazon Basin, the number of fish examined, their sizes (total length), and the number parasited by *Ceratomyxa vermiformis* sp. n. Most fish were immature (≤35 cm; Costa et al. 2001). Size ranges are provided when relevant for distinguishing immature and mature fish.

| Locality                        | Latitude/longitude | Period of collection | No. of fish examined | Fish size (and n-value) | No. of fish infected (and prevalence) |
|---------------------------------|--------------------|----------------------|----------------------|-------------------------|--------------------------------------|
| Tapajós River, Santarém/Pará State | 0°20′03″-46″;S; 54°52″W | October 2014          | 10                    | 29–42 cm (7); 62–69 cm (3) | 5 (50%)                             |
|                                |                    | March 2015            | 18                    | 16–53 cm                  | 0                                    |
|                                |                    | January 2016          | 8                     | 23–30 cm (5); 69–74 cm (3) | 0                                    |
| Amazon River, Laranjal do Jari/Amapá State | 01°08′17″-24″;S; 51°48″W | September 2015        | 1                     | 29 cm                    | 1 (100%)                            |
| Solimões River, Manacapuru/Amazonas State | 03°03′-46″;S; 61°09″W | December 2015         | 16                    | 28–40 cm                  | 0                                    |

also used to describe this new vermiform-like myxosporean species.

**MATERIAL AND METHODS**

**Collection of material and morphological analysis**

Fifty-three wild *C. macropomum* specimens were collected from the Tapajós, Amazon and Solimões Rivers in Brazil (Table 1). The catches were authorized by the Brazilian Ministry of the Environment (SISBIO no 44268-4) and fish were transported live to a make-shift field laboratory on the shores of the river, where they were euthanized. The methodology of the present study was approved by the ethics research committee of Federal University of São Paulo (CEUA N 92090802140) in accordance with Brazilian law (Federal Law No. 11794, dated 8 October 2008). All organs and body fluids were examined for myxosporeans and representative material was then collected for morphological and molecular studies (see below). In addition, smears containing free myxospores were air-dried, stained with Giemsa solution and placed in mounting media on permanent slides. Type specimens were deposited in the collections of the Museum of Zoology ‘Adão José Cardoso’, of State University of Campinas (UNICAMP), Brazil. Morphological and morphometric analyses of myxospores based on Lom and Arthur (1989) and following Gunter et al. (2004) (with some modification; see Supplementary File Fig. S1) were performed at the Federal University of São Paulo using a computer equipped with AxioVision 4.1 image capture software coupled to an Axioplan 2 Zeiss microscope.

**DNA isolation, sequencing and phylogenetic analysis**

Bile from gallbladders infected by worm-like plasmodia was preserved in absolute ethanol for molecular analysis. DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, USA), in accordance with the manufacturer’s instructions. The concentration of the DNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). Polymerase chain reactions (PCRs) were carried out in 25 µL reaction volumes using 100 ng of extracted DNA, 5× Go Taq Flexi Buffer (Promega Madison, WI, USA), 10 mM dNTP mix, 25 mM MgCl₂, 5 mM for each primer ERIB1-ERIB10 (Barta et al. 1997), and 1× Go Taq G2 Flexi DNA polymerase (Promega Madison, WI, USA). The amplification was performed in an Eppendorf AG 22331 Hamburg Thermocycler (Eppendorf, Hamburg, Germany) using a touchdown PCR method (Korbie and Mattick, 2008), with initial denaturation at 94 °C for 30 s, followed by nine cycles at 94 °C for 30 s, 75 °C (−1 °C/cycle) for 90 s, 72 °C for 45 s, 36 cycles at 94 °C for 30 s, 65 °C for 40 s, 72 °C for 45 s, and then final elongation at 72 °C for 5 min. PCR products were subjected to electrophoresis in 1·0% agarose gel (BioAmerica, Miami, FL, USA) in TBE buffer (0·045 M Tris-borate, 0·001 M EDTA, pH 8·0), stained with ethidium bromide, and then analysed with a FLA-3000 scanner (Fuji Photo Film, Tokyo, Japan). SSU rDNA was amplified using the primers ERIB1 and ERIB10 (Barta et al. 1997), MYXGEN4f (Diamant et al. 2004) and a specifically designed primer CERATBr (5′-AGAATTTCACCTCTCGCCATC-3′). The sequencing was performed using the BigDye® Terminator v3·1 cycle sequencing kit (Life Technologies Carlsbad, CA, USA) according to the manufacturer’s protocol, adapting the reaction end volume to 5 µl in an ABI 3500 DNA sequencing analyser (Applied Biosystems, Foster City, CA, USA) and using the polymer POP-7 (Life Technologies Carlsbad, CA, USA).

A standard nucleotide–nucleotide Basic Local Alignment Search Tool (BLAST) (blastn) search was conducted (Altschul et al. 1997). The sequences of all *Ceratomyxa* species available in GenBank plus...
**Myxodavisia bulani** KM273030 and **Palliatus indecorus** DQ377712 were aligned by ClustalW (Thompson *et al.* 1997) using the BioEdit program (Hall, 1999). Phylogenetic analysis was conducted using maximum likelihood (ML) in PhyML software (Guindon *et al.* 2010), with NNI search, automatic model selection by SMS (Smart Model Selection), under a GTR + G + I substitution model (with six categories), equilibrium frequencies optimized, transition/transversion ratio estimated, proportion of invariable sites fixed (0·097) and Gamma shape parameter fixed (0·398). To avoid the long branch attraction (LBA) effect (Anderson and Swoford, 2004), Maximum Parsimony (MP) analysis (with complete deletion) was conducted using MEGA7 software (Kumar *et al.* 2016) on another alignment excluding six long-branching Ceratomyxa species. For comparative purposes, ML analysis was also performed using this same dataset, under a GTR + G + I substitution model (with six categories), equilibrium frequencies optimized, proportion of invariant sites estimated (0·186) and Gamma shape parameter estimated (0·449). Bootstrap analyses (1000 replicates) were employed to assess the relative robustness of internal branches. The malacosporeans *Tetracapsuloides bryosalmonae* and *B. plumatellae* were used as an outgroup in both phylogenetic analyses.

**Electron and confocal microscopy**

For transmission electron microscopy, plasmodia were fixed for at least 12 h in 2·5% glutaraldehyde with 0·1 M cacodylate buffer (pH 7·4), washed in the same buffer and post-fixed with osmium tetroxide (OsO₄), all procedures being performed at 4 °C. After dehydration in an ascending ethanol series, the samples were embedded in EMBed 812 resin (Electron Microscopy Sciences, Hatfield, PA, USA). Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined using a LEO 906 transmission electron microscope operating at 60 kV. For scanning electron microscopy, infected bile fixed at 10% formalin in 0·1 M phosphate-buffered saline (PBS) was left for 1 h on a polysine pre-treated round coverslip. Coverslips were then washed in the same buffer, dehydrated in ethanol, critical-point-dried, mounted on stubs, covered with metallic gold, and examined in a Zeiss Ultra Plus scanning electron microscope at 5 kV. For confocal analyses, infected bile fixed at 10% formalin in 0·1 M PBS was left for 30 min. on polysine pre-treated slides. The samples were rinsed three times in PBS and then permeabilized with PBS containing 0·1% Triton X-100 for 1 h. Specimens were then stained with Alexa Fluor® 488 Phalloidin (Invitrogen Eugene, OR, USA) at 0·001 mg mL⁻¹ for 4 h and with 4’,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich Saint Louis, MO, USA) at 0·004 mg mL⁻¹ for 10 min. The samples were rinsed in PBS and mounted in 90% glycerol, 10% PBS, 0·5% 1,4-diazabicyclo[2·2·2]octane mounting medium. They were examined using a Nikon A1 Confocal Microscope.

**RESULTS**

**Infections in fish**

Motile plasmodia of a new myxosporean species were observed in the gallbladders of six of the 53 *C. macropomum* specimens examined. Five of 36 fish examined from the Tapajós River were infected. The single fish examined from the Amazon River was also infected (Table 1). Prevalences of infection were variable and their estimation compromised by low sample sizes; however, data from the Tapajós River suggest that infection prevalences may vary over time (Table 1).

**Taxonomic summary and description**

Phylum: Cnidaria Verrill, 1865

Class: Myxosporea Bütschli, 1881

Order: Bivalvulida Shulman, 1959

Family: Ceratomyxidae Dolfin, 1899

Genus: Ceratomyxa Thélohan, 1892

Species: *Ceratomyxa vermiformis* sp. n.

Type host: The fish *C. macropomum* Cuvier, 1818 (Teleostei: Serrasalmidae).

Location in host: Gallbladder (plasmodia with or without mature spores swimming actively in bile).
Type locality: Tapajós River (Municipality of Santarem, PA), Amazon Basin, Brazil.
Type material: Syntypes – air-dried, stained with Giemsa solution and mounted in mounting medium on permanent slides (accession numbers Zuec Myx 54 and 55).
Etymology: The specific name is based on the form and associated movements of the plasmodia, unprecedented observations for myxosporeans.

Movement and morphology

Plasmodia have an elongate form and showed coordinated, worm-like undulations reminiscent of nematode sinusoidal locomotion (Fig. 1 and Videos S1 and S2). The alternating bending movements result in translocation through the bile as can be seen in the Supplementary Material (Video S1).

The plasmodia are characterized by a highly developed cytoplasm that is clearly segregated into an external layer and an internal region. The external layer ranges from about 200–600 nm in thickness (Fig. 2A), has an actin-rich cytoskeleton (Fig. 3) and is bounded by an external membrane that is covered by a secreted glycocalyx-like layer (Fig. 2B). Tubular mitochondria, microtubules, rough endoplasmic reticulum and granular material that may represent ribosomes and/or glycogen are abundant in the external layer (Figs 2 and 4). The elongate mitochondria demonstrate an unusually regular distribution and orientation. Cross-sectional views reveal that the mitochondria are spaced at regular intervals around the periphery of the plasmodia (Fig. 2A), while longitudinal sections demonstrate that their long axis is orientated in parallel with the long axis of the plasmodia (Fig. 4).

The external surface of the plasmodium appears to generally display a series of bulges or ridges that occur as regular transverse bands (Figs 3–6). In certain longitudinal/oblique sections these appear to become highly exaggerated to form a corrugated or peaked surface (Figs 4 and 5) that extends only partially around the circumference of the plasmodia (Fig. 6). SEM suggests this corrugated surface may extend along much of the length of the plasmodia (Fig. 6).

The internal region of the plasmodia contains homogeneously distributed granular/fibrillar material. Compared with the external layer, it is less electron-dense and lacks organelles (Figs 2 and 5). Developing spores are present, but are associated with the external layer at all stages of development (Figs 2 and 5). Sporogony is asynchronous and plasmodia contain early sporogenic stages and immature and mature myxospores (Figs 2, 3, 5, 7 and 8). Mature plasmodia containing myxospores had a mean length of 442 µm (s.d. = 44·9, range = 379–520 µm, n = 19) and a mean width of 22·1 µm (s.d. = 2·6, range = 18–26 µm, n = 19) (Fig. 8). One
end of the plasmodium is blunt, while the opposite end is very thin at its extremity (Figs 3 and 8). Early sporogonic stages are concentrated in the blunt end, specified here as the anterior pole. TEM and confocal microscopy revealed that numerous cells are present in this anterior end (Figs 2, 3 and 5) and that there is a gradient in maturation of spore developmental stages, with progressively older stages appearing towards the posterior, thin end (the posterior pole) of the plasmodium (Figs 3, 7 and 8). These observations suggest that the cells in the anterior end represent a growth centre.

The spores are strongly arcuate (Fig. 7 and Fig. S1) with a mean length of 4.5 µm (s.d. = 0.2, range = 4.2–4.8 µm, n = 28), a mean thickness of 8.4 µm (s.d. = 0.4, range = 7.9–9.3 µm, n = 28) and a posterior angle of 30–2° (s.d. = 6.6, range = 22–43°, n = 18). The two elongated valves resemble appendages that are of unequal size and become tapered approximately halfway along their lengths. The mean length of the larger valve = 23.7 µm (s.d. = 0.7, range = 22.1–24.3 µm, n = 28) and that of the smaller valve = 21.9 µm (s.d. = 0.8, range = 20.6–23 µm, n = 23). The two polar capsules are spherical and of equal size with a mean diameter of 2.7 µm (s.d. = 0.1 µm, range = 2.5–2.9 µm, n = 28). The polar filament undergoes three to four turns oblique to the longitudinal axis of the polar capsule and the binucleated sporoplasm occupies the wider region of the spore (Fig. 7 and Fig. S1).

**Phylogenetic analysis**

A total of 1,601 bases of SSU rDNA was generated from sequencing of this worm-like myxosporean (GenBank Accession No. KX278420) and molecular phylogenetic analysis performed with ML reveal that it is sister to Ceratomyxa amazonensis Mathews et al. 2016 (Fig. 9). Further molecular phylogenetic analyses performed on a dataset excluding the long-branching Ceratomyxa species and using both MP and ML approaches were consistent with this result (Fig. S2). These two species in turn group with Ceratomyxa leatherjacketi Fiala et al. 2015b and Ceratomyxa tunisiensis Thabet et al. 2016, forming a lineage with the early diverging M. bulani Fiala et al. 2015b. This Myxodavisia/Ceratomyxa clade is sister to the remaining Ceratomyxa clade (+P. indecorus). The two species of the genus Ceratonova Atkinson et al. 2014 cluster together in a separate clade to this large Ceratomyxa lineage (Fig. 9 and Fig. S2).

**Remarks**

The highly arcuate spores of C. vermiformis sp. n. with its long and thin valves resembling appendages are similar to those of Meglitischia mylei Azevedo et al. 2011, a parasite reported from the serrasalmid fish Myleus rubripinnis of the Amazon basin. However, M. mylei exhibits a larger number of polar filament turns and smaller sizes of polar capsules and spores than those exhibited by C. vermiformis sp. n. In addition, the valves of C. vermiformis sp. n. are of unequal sizes whereas they are of a similar size in M. mylei (Azevedo et al. 2011) (for detailed comparison see Table S1). Based in these morphological differences we propose the erection of a new species and assign it to the genus
Ceratomyxa, a decision based on molecular phylogenetic data and an earlier suggestion that the basic spore architecture of Meglitschia insolita (Meglitsch, 1960) (first described as Ceratomyxa insolita) supports assignment to Ceratomyxa (Meglitsch, 1960) as detailed in the discussion section.

**DISCUSSION**

Fine structure and development in relation to movement

The extraordinary movement displayed by plasmodia of *C. vermiformis* sp. n., as demonstrated in
our videos, is associated with particular features that may support or result from motility and movement. These features variously include: (a) an actin-rich network distributed throughout the cytoskeleton of the plasmodia; (b) the regularly arranged, extremely elongate mitochondria orientated along the longitudinal axis of the worm-like plasmodia, in a peripheral position near the plasmodial membrane; (c) a glycocalyx-like layer secreted externally; (d) regions of the plasmodial surface that demonstrate regular corrugations; (e) microtubules that may function in positioning and contribute to the cytoskeleton (Cooper, 2003; Feist et al. 2015); and (f) segregation to form an electron dense, organelle-rich external layer and an internal region with sporogonic stages but depauperate in organelles. As outlined below, these features provide initial insights on how *C. vermiformis* sp. n. has achieved convergence to a worm-like form at the cellular level.

The cuticle-like extracellular secretion and components of the external layer may contribute to hardening or strengthening of the plasmodial wall as is suggested for the external and internal secretions of valve cells in spores (see Gruhl and Okamura, 2015 for review). Our combined morphological investigations provide evidence that regions of the wall are highly corrugated (e.g. Figs 5 and 7). It is possible that the corrugations may result from squeezing and shortening during bending that accentuates the ridged surface of the wall—in which case these would be transient developments. An alternate scenario is that the markedly corrugated regions are permanent features and perhaps serve to increase surface area (e.g. for absorption or to facilitate
Further study is required to resolve this issue. The peripheral deployment of microtubules, actin and mitochondria results in a highly consolidated cytoskeleton in the external region. The positioning of the elongate mitochondria around the circumference of the plasmodia may be linked with the distribution of actin, which could influence mitochondrial function (Anesti and Scorrano, 2006), for example by shaping, tethering or moving the elongate mitochondria. An amoeboid motility resulting from filipodia has been noted in some Ceratomyxa species infecting the gallbladder (Cho et al. 2004; Alama-Bermejo et al. 2012), and it is proposed that this motility provides a means of avoiding premature release of immature forms with the bile (Alama-Bermejo et al. 2012). A similar function may be attributed to the motility of C. vermiformis n. sp. Alternatively, swimming may enable the plasmodia to pass through the bile duct into the intestinal tract. In either case, it is striking that very different modes of motility have evolved convergently in cnidarians at the cellular level. It is notable that certain features associated with bending and contractile movements in protists are also displayed by C. vermiformis n. sp., suggesting convergence of form and function at the cellular level. Thus, regularly situated mitochondria are observed to line up in the cortex of ciliates – in this case below a filamentous sheet that is believed to achieve localized bending (Hausmann et al. 2003).
analyses using three genes. *Ceratomyxa leatherjacketi* and *M. bulani*, in turn, were revealed to form a basal subclade within *Ceratomyxa*. This basal subclade also now incorporates the newly described *C. tuni-siensis* and *C. amazonensis* (Mathews et al. 2016) and here we show that *C. vermiformis* sp. n. groups as sister to *C. amazonensis*. It is notable that fish parasitized by members of the early diverging *Ceratomyxa/Myxodavisia* subclade are associated with freshwater environments. Within this subclade, the early diverging *M. bulani* is a parasite of the amphidromous fish *Megalps cyprinoides* and *C. tuni-siensis* has been reported infecting *Caranx rhonchus*, which inhabits brackish-water lagoons and estuaries. *C. amazonensis* and *C. vermiformis* sp. n. parasitize, respectively, *S. discus* and *C. macropomum*, which live exclusively in freshwater environments (Froese and Pauly, 2009). Another parasite of the gallbladder of a serrasalmid fish from the Amazon River (*M. myle; Azevedo et al. 2011*) is also likely to be a member of this clade (see below discussion on *Meglitschia*). Although the bootstrap support in our MP analysis is low, the strong support observed in both ML analyses suggests that infection of hosts associated with freshwater environments may have been primitive for ceratomyxids. This would imply a subsequent extensive radiation of ceratomyxids in hosts inhabiting fully marine environments.

**A freshwater ancestral environment of ceratomyxids?**

Recently Fiala et al. (2015b) identified the *Ceratomyxa* clade as basal to all other marine myxosporean lineages based on molecular phylogenetic

The validity of Genus *Meglitschia*

Zhao et al. (2008) alluded to the general resemblance of *Myxodavisia* and *Ceratomyxa* myxospores and we further point out the similarity of myxospores of *C. vermiformis* sp. n. to those described for the genus *Meglitschia* Kovaleva (1988). Kovaleva erected this genus to harbor a species originally described as *C. insolita* (Meglitsch, 1960; Kovaleva, 1988). In the original description, Meglitsch (1960) argued that although the arcuate spores and large, elongated polar capsules differentiated *C. insolita* (which forms amorphous plasmodia) from other *Ceratomyxa* species described at the time, the basic spore architecture supported assignment to *Ceratomyxa*. Our combined molecular and morphological analyses support Meglitsch’s original premise that some *Ceratomyxa* species produce highly arcuate myxospores with elongated and tapered valves. Thus, the minor morphological differences used to create *Meglitschia* appear to be insignificant, suggesting that the genus is not supported. Unfortunately, there are no molecular data available for *Meglitschia* species to help to resolve this issue.

**Concluding remarks**

The Myxozoa demonstrate how metazoans have evolved to become endoparasites by miniaturization and morphological simplification as descendants of...
free-living cnidarian ancestors. The highly derived Myxosporea have taken this to the extreme, having effectively converged with parasitic protists to exploit hosts at the cellular level. Here we show that such miniaturization can nevertheless be accompanied by innovations that may promote coordinated movements as plasmodial worms. However, the basis for such movement at the cellular level in *C. vermiformis* n. sp. remains to be revealed. Whether the remarkable swimming demonstrated by *C. vermiformis* sp. n. is unique, remains unknown as myxozoan diversity is poorly sampled. Further research is likely to reveal new insights on how myxozoans have evolved abilities to move through and to maintain their positions within their host environments thus illustrating the extraordinary plasticity in lifestyles that can be supported by the cnidarian bauplan.

**SUPPLEMENTARY MATERIAL**

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182016001852.

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