A proteome study of *Myxobolus episquamalis* parasite of flathead mullet (*Mugil cephalus* Linné 1758) of Senegalese coasts

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Abstract

Myxozoa are endoparasites characterized by a simple cytoplasmic organization and multicellular spores containing polar capsules with extrusible polar filaments. Myxozoans have a complex life-cycle, typically alternating between teleost fish and invertebrate hosts, in which are formed myxospores and actinospores respectively. Both spores contain polar capsules, which are strikingly similar in their morphogenesis and mature structure to the nematocysts found in the phylum Cnidaria (Canning and Okamura 2004). Similarity with cnidarians was also supported by several phylogenetic molecular studies. In this context, we have performed the first large scale proteomic study of a Myxozoa, *Myxobolus episquamalis* which is a parasite of flathead mullet in Senegal. Comparisons with different databases combining different softwares confirm importance of cytoskeleton and central metabolism in the invasion process. The present study identified for the first time neuropeptides and analgesic toxins encoded by the *M. episquamalis*. It also highlighted long peptides presenting strong homologies with proteins of unknown function, a large fraction of them being identified from *Nematostella vectensis* proteome. Homologies with other lower Bilateria raise again the question of the position of Myxozoa at the transition between Cnidarian and Bilaterian.

Keywords: Myxozoa, *M. episquamalis*, Proteomics, Phmmer, Neuropeptide.

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Introduction

Myxozoans are parasites of poikilothermic vertebrates and invertebrates, especially fishes and count amongst the most known enigmatic organisms. Markiw and Wolf (1983, 1984) were the first discovering a tubificid annelids as alternate hosts for *Myxobolus cerebralis*. Later, it has been established that, there may be an obligatory or facultative invertebrate host in most, if not all of the life cycle of myxozoans. Next this two-stage life cycle was described in several marine species (Køie *et al,* 2004; 2007; 2008; 2013; Rangel *et al,* 2009; Karlsbakk and Køie, 2012). Vertebrates are considered as intermediate hosts since the sexual process (gametogony) has been observed during the actinospore phase in invertebrates (annelid oligochaete was described as definitive host for some species). A possible direct fish-to-fish transmission was demonstrated for some marine species (Diamant 1997; Redondo *et al* 2002; Yasuda *et al* 2002). In these later cases, the proliferative stages are responsible for the transmission of the disease, contradictorily to spores.

There are two classes of Myxozoans, the clades Myxosporea and Malacosporea. Their cell organization is typically eukaryotic with nucleus, endoplasmic reticulum, mitochondria and Golgi cisternae (Canning and Okamura, 2004).Their spores are made of three cell groups: valve cells, polar capsules and a sporoplasm generally with two nuclei, or two sporoplasms, each containing one nucleus. Their polar capsules, used to attach to a new host, are similar to the stinging organelles (nematocysts) of cnidarians. Structure similarities between the myxozoan polar capsules and nematocysts of cnidarians cnidocysts led to consider myxozoan to be aberrant cnidarians (Weill, 1938), a hypothesis further supported by ultrastructural studies (Lom and de Puytorac, 1965; Lom, 1969). First phylogenetic analysis of the SSU-rDNA allowed placing Myxozoa within the Metazoan (Smothers *et al,* 1994; Siddall *et al,* 1995). More recent phylogenomic analyses placed Myxozoa in Cnidaria (Holland *et al,* 2011; Jimenez-Guri *et al,* 2007; Nesnidal *et al,* 2013, Feng *et al,* 2014). Polar capsules differ from typical nematocysts of cnidarians in lacking chemo- and/or mechano-sensory structures and neural connections that modulate discharge (Jiménez-Guri *et al,* 2007).

Myxosporean are fish parasites. They are present in both freshwater and marine environments and have a significant incidence on wild and cultured stocks (Timi and Mackenzie, 2015). Few strong epizootic diseases have been described and associated with *Myxobolus pfeifferi*, responsible for the boil disease of barbels or *Myxobolus exiguus* which caused epidemic in flathead mullet of the northern sector of the Black Sea along the Crimean coast (Schulman, 1957). In addition, there are other serious pathogenic concerns with important commercial impact. Thus, in cultured salmonids, many cases of losses were due to *M. cerebralis* but also to *Tetracapsuloides bryosalmonae*, responsible for the mysterious disease called PKD, observed in USA and Europe. Besides, carp Eurasia infection caused by other parasites (such as *Sphaerospora arenicola, Sphaerospora molnari, Myxobolus eucephalicus, Thelohanellus nikolskii, Thelohanellus kitauei* or *Chloromyxum cristatum*) may also cause serious damages in fish farms. *Myxobolus episquamalis* Egusa, Maeno and Sorimachi, 1990 was found for the first time in flathead mullet (*Mugil cephalus* Linneaus 1758) in Senegal. It was previously described in *M. cephalus* from Israel, Portugal, Japan and Tunisia. The infected specimens of flathead gray mullet (*Mugil cephalus*) present whitish myxosporidian cystic masses on their bodies, measuring approximately 5-7 mm. The cystic

masses are distributed over the entire body and cover 10 to 70% of the body. They are located on the distal part of the scales and also affected the fins causing malformations. The cyst mass is ovoid, containing numerous spores. The spore is pyriform in front view and each valve presents 7 to 9 markings along the suture wall. The polar capsules are pyriform and extending half of the spore length and formed 5 or 6 coils inside of the polar capsule. Finally, the sporoplasm is binucleate, occupying the posterior part of the spore.

Up to now, very few molecular investigations were dedicated to *M. episquamalis*. Thus we performed a proteomic study to identify M. episquamalis cyst expressed proteins. To achieve this goal. Thus, in this study, we performed a proteomics study to address identifications of proteins expressed in *M. episquamalis* cysts. To reach this objective, peptide sequencing was combined to computational methods to identify major functions associated to this stage of development of the parasite.

Materials and methods

Biological material

Mugil cephalus Linnaeus1758 is a eurythermal and euryhaline fish having a wide geographical range. It hosts many parasites including *Myxobolus episquamalis* which belongs to the phylum Myxozoa and parasitizes apical part of the scales where it induces the formation of cysts (Fig.1). The scales of parasitized fishes were removed and ground with cysts in a mortar with PBS. The homogenate was centrifuged for ten minutes at 2000 tr/mn. The pellet were distributed into 1.5ml tubes and stored at 4°C.

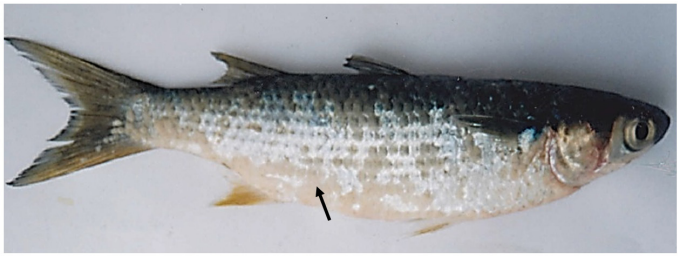

Figure 1. Flathead mullet (*Mugil cephalus*) presenting numerous cysts on its scales.

These cysts contain mature spores of the myxosporean parasite *Myxobolus episquamalis.* Microscopic cysts were measured between 150 and 400 microns and form a cystic mass of around 6-7 mm length. Arrow indicates cystic mass containing microscopic cysts.

Protein extraction

The proteins of the spores of *Myxobolus episquamalis* were extracted in 1ml of a lysis buffer containing 7M urea, 2M thiourea, 4% CHAPS, 2% DTT and10mM Tris. To allow this, spores were sonicated 5 times using a microtip probe. This initial treatment was followed by 5 freezing-thawing cycles at -80°C and 37°C, respectively. The resulting material was centrifuged at 20800g for 30 min at 4°C. The pellets were stored at – 20°C.

Two-dimensional gel electrophoresis (2-DE)

For all experiments, two biological replicates were obtained and the corresponding two-dimensional gels made in duplicate. Protein concentrations were evaluated using the Bradford protein assay (Bio-Rad) with BSA used as standard. For the first dimension (isoelectric focusing, IEF), 50 µg of proteins (final volume 350 µL) were loaded onto immobilized pH gradients (ImmobilineDryStrip pH 3-10, 18 cm; GE Healthcare). IEF was performed using an IEF-CELL (Bio-Rad) as follows: active rehydration for 1h at 350 V, 1h at 750V, 1h at 1500V and final focusing for 28h at 3500 V. The strips containing focused proteins were then equilibrated for 10 min in a buffer containing 6 M urea, 30% glycerol, 2% SDS,50 mMTris-HCl (pH 6.8) with 5 mM dithiothreitol, and then for an additional 10 min in the same solution except that DTT was replaced by 25 mM iodoacetamide. Thereafter, the second-dimension gel separation was ensured by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) electrophoresis using a 12.5 % polyacrylamide resolving gel (width 16 cm, length 20 cm, thickness 0.75 cm). SDS-PAGE was carried out using Protean II Xi vertical systems (Bio-Rad) according to the protocol described by the manufacturer. Migrations were performed at 10 mA per gel in the stacking gel and 20 mA in the running gel. Finally, proteins were revealed using silver staining and the gels were scanned using a dedicated imager (ProXpress, Perkin-Elmer). Protein patterns were analyzed using the ProgenesisSameSpotssoftware (v3.0; Nonlinear Dynamics).

Analysis by Mass Spectrometry

Spots were excised from the polyacrylamide gel and sliced into small pieces. Excised fragments were then washed several times and dried in a SpeedVac centrifuge for 15 minutes. Trypsin digestion of protein spots was performed overnight at 37°C by using a dedicated automate (MultiPROBE II, Perkin Elmer) with an enzyme/protein ratio of 1/20 (w/w). The gel fragments were then incubated several times for 15 min in water/acetonitrile mixtures acidified with 0.1% TFA, to allow peptide extraction from the gel pieces. After lyophilization, the peptide extracts were suspended in 10 µL of 0.2% formic acid (FA).

The resulting samples were analyzed with an LTQ Orbitrap mass spectrometer Velos (Thermo Scientific) equipped with a nano-ESI and coupled to a nano-chromatography system (Easy-nLC II, Thermo Scientific). After loading the sample on an enrichment column, separation was achieved on a C18 column (NikkyoTechnos, Japan) using a linear gradient of 15% to 45% of B buffer in 30 min (buffer A: H_2O/0.1% FA; buffer B: CH_3CN/0.1% FA)

The mass spectrometer was operated in positive mode with a capillary voltage set at 1.5 kV and the source temperature set at 200 ° C. The samples were analyzed by HCD fragmentation using a method referenced "top20". The initial MS scan was recorded in the Orbitrap analyzer with a resolution of 60000 on a mass range m/z 400-1800 (mono-charged ions were not selected for fragmentation and dynamic exclusion was applied).

Label Free

For these analysis, the spore proteins were obtained in the same manner as described above. Then, 50µg of the whole protein extract were concentrated within the stacking gel of SDS-PAGE experiment. After a short period of migration (< 1 h), the revealed protein band was cut and digested with 2 µg of trypsin. After solvent evaporation, the whole

peptide pool was suspended in aqueous solution of 0.1% formic acid prior to analysis by mass spectrometry. MS settings were similar to those described previously except the gradient duration which was increased to 120 min.

Protein identification

For protein identification, raw data files were processed either using Proteome Discoverer (V1.3, Thermo Scientific) or Peaks (V6.0, Bioinformatic Solutions, Canada) for de novo sequencing of fragmentation data. To achieve these identifications, parameters used included 2 missed cleavages, variable modifications of cysteine (carbamidomethylation) and methionine (oxidation). The tolerance of the precursor ion mass was set to10.0 ppm and that of fragment ions to 0.05 Da. To validate protein identification, the protein had to be characterized by at least 2 peptides with individual ion fragmentation score higher than 53 (default value of Mascot).

Sequence comparison

Mascot and Peaks analysis was used to identify protein homologues and there by infer functional annotation. Secondly HMMER was used to query sequences, using phmmer web interface at EBI (http://www.ebi.ac.uk/Tools/hmmer/). We choose to compare our peptides of length over 11 amino-acids (1497 sequences) with the Uniprot data base (http://www.uniprot.org/). Best homologues were kept for functional and taxonomic analysis.

Results

Protein identification.

Two-dimensional gel electrophoresis revealed the presence of a large panel of proteins distributed all over the gel (Fig. 2). On the pH gradient (range from 3 to 10), we noticed that high molecular weight proteins had some trouble getting out of the staking gel. Added to this is the presence of spots of varying size and intensity between replicates.

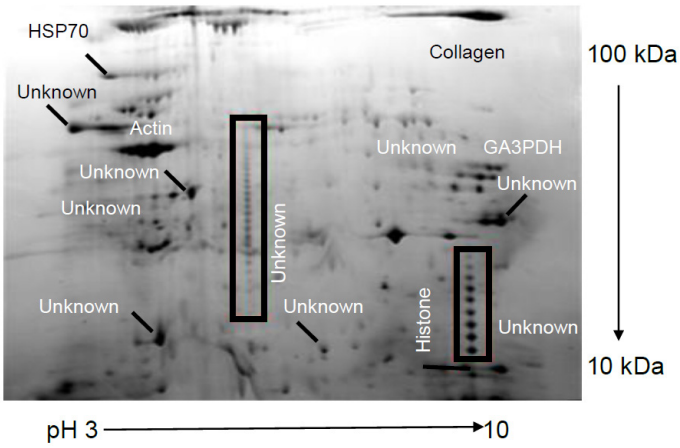

Figure 2. Two dimension electrophoresis gel of protein extracts from *Myxobolus episquamalis*.

Protein spots that were excised and analyzed by mass spectrometry are highlighted. Unfortunately, probably due to lack of genomic sequence available, many excised spots remain unannotated.

A set of 21 reliable spots were extracted from gel and submitted to trypsic digestion. Mass spectrometry analysis and Mascot searches provide annotation for only few proteins with strong background of contaminant (among which keratin and trypsin). Positive annotation was kept for proteins that were correctly identified with at least two peptides. This combination of analysis enabled the unambiguous identification of actin, collagen, histone, heat shock protein 70 (HSP70), glyceraldehyde-3 phosphate deshydrogenase (GA3PDH) and triosephosphate isomerase.

These proteins have been conserved during evolution. It was not possible to distinguish whether they were corresponding to *M. episquamalis* proteins or host contamination as no genome data were available for both organisms. The total protein extracts were analyzed using shotgun analysis for whole proteome analysis of *M. episquamalis* proteins. A set of 3121 peptides were kept as significant after a filtering stage based on the peptide score threshold application using Peaks. Peptides above the threshold did not yield much significant hit allowing a very low FDR (Fig. 3A).

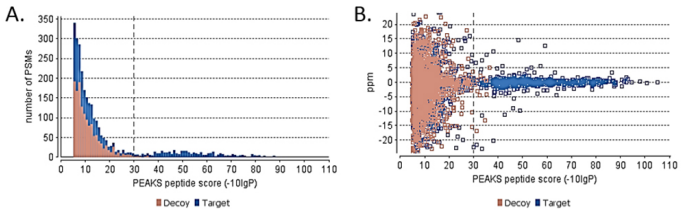

Figure 3. Validation of proteins identification.

A. The total number of peptide sequence match (PSM) was plotted against the peptide probability score (true database in blue and decoy database in pink). This allowed to estimate a probality score of 30 to propose a low FDR while maintening the highest level of protein identifications. **B.** The peptide score was also plotted against the difference of mass (in ppm). This graph highlighted different distributions of Dm depending on the database used for identification searches.

This was supported by the fact that average Δmass (difference between observed and calculated masses) was much lower for true positives than for hits obtained when searching against the decoy database (Fig. 3B). About 350 peptides at the 3121 full set of filtered peptides had homologies with788 proteins present in the database. Many peptides match with several proteins but in most cases they were orthologues. Reciprocally, 196 proteins of 788 homologues were identified with at least two peptides. Altogether, PEAKS analysis defines a non-redundant set of 254 proteins matching with the 3121 peptides that were initially selected.

Functional analysis of Myxobolus episquamalis peptides.

Functional analysis was performed using the 254 protein groups characterized after shot-gun mass spectrometry analysis and compared with a second analysis that was performed only on 1497 peptides that were longer than10 residues from the initial set of 3121 peptides. These 1497 peptides were compared with the Uniprot database using Phmmer online server. We obtained 352 significant homologies. The level of proteins with unknown function was of 37.5% and 78.5% after Peaks and Phmmer analysis respectively (Fig. 4A and 4B).

The low percentage of homologues of known function with Phmmer could result from the composition of the Uniprot data base which contains a large proportion of peptides from recently sequenced and automatically annotated genome. On the opposite, the Peaks database was composed of peptides that were considered to be relevant for mass-spectrometry

analysis. Most of them are selected among proteins from well annotated genomes.

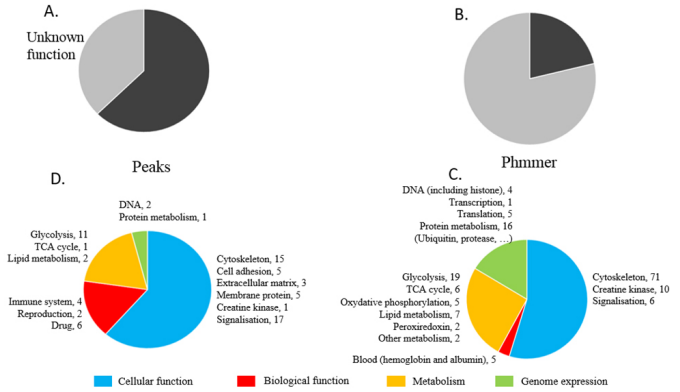

Figure 4. Distribution of main functions associated with protein homologues of *Myxobolus episquemalis* peptides identified by label-free mass spectrometry analysis.

A. Frequency of proteins with known function after Peaks *de novo* sequencing. **A total of 254 non redundant protein groups** were characterized after Peaks analysis.

B. Frequency of proteins with known function after Phmmer analysis. A total of 352 peptides displayed significant level of homology (e-value < 0.01) against the Uniprot database using phmmer software.

C. Major functional categories of **the 159 proteins homologues with known annotation identified by Peaks**. Number of hits per function is given after the coma.

D. Major functional categories of the 75 proteins with known annotation identified by phmmer analysis. Number of hits per function is given after the coma.

Functional annotation associated to proteins was classified into four groups: cellular functions, biological functions, metabolism and genome expression (Fig. 4B and 4C). Peaks analysis gave 159 protein homologues with known function and Phmmer 75 homologues with known function. They correspond to major functions found in eukaryotes. Only 17 peptides have been found in common between Peaks and Phmmer analysis. Analysis at functional level revealed high redundancy for some proteins such as Myosin, actin or glyceraldehyde-3-phosphate dehydrogenase. Redundancy was higher for Peaks analysis. Protein functions that were in common between both methods represented about one third from the analyses (Peaks: 61/159; Phmmer: 24/75). The most redundant protein functions in common with both sets of homologues were cytoskleton proteins (actin, myosin, nebulin and troponin) and enzymes of the glycolysis, (glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase and phosphoglucomutase). Ubiquitin was also found in common in analysis as well as creatine kinase and calmodulin, two enzymes sufficiently present in muscles. More specific proteins and enzymes were identified after either the Peaks or Phmmer analysis. The Peaks analysis identified a large number of cytosketon enzymes and a large proportion of enzymes of the central metabolism (Fig. 4C). Keratin contamination was less observed than during 2DE analysis. The same analysis also identified hemoglobin and albumin. Possible contamination with host tissue cannot be excluded even if homologues among lower Metazoan were also found for most common proteins 'e.g. actin, tubulin, calmodulin, elongation factor EF1alpha or histone H2A). Phmmer analysis revealed less homology with muscle proteins as demonstrated by the composition of cytoskeleton function which included mainly actin and myosin.

Comparison to the Uniprot data base provided more information about proteins from *M. episquamalis,* including cellular periphery and adhesion: a protein from the adhesive plaque matrix, a putative adhesin/hemaglutinin found in bacteria, a mucin protein with matches in worm eukaryotes. Homology with the transmembrane protease serine 9 could be related with maturation of the extracellular matrix but could also be considered as a host-interacting factor. Functions related to signaling were more heterogeneous. In addition, homology was found with creatine kinase from sea anemone (1 peptide) and calmodulin from oyster (3 peptides).

Taxonomic analysis of Myxobolus episquamalis peptides

The distribution of organisms presenting protein homologues provided interesting insight about the taxonomic position of *M. episquamalis*. Analysis was performed on the full set of 788 homologues and synthetic constructs were removed. We observed that 664 of the 769 remaining homologues were originating from metazoan (Fig. 5A). Indeed Peaks database was built to promote peptide matching and composition is therefore strongly based for well characterized peptides. Nevertheless, two matches with a virus polyprotein were intriguing. The Phmmer analysis was performed against Uniprot database which could be considered as unbiased even though no myxozoan genome has been sequenced so far.

By keeping only the best homologies (homologue with lower E-value) for each peptide, only a small proportion of metazoans were retained during Peaks analysis (Fig. 5B). No function was associated to bacterial homologues. Fungi proteins with known function were related to signalization (2 peptides) and lipid metabolism (one peptide). Two peptides were similar to surface antigens of protists. Three peptides displayed homologies with mimiviridae proteins which are known pathogens of protists and lower metazoan.

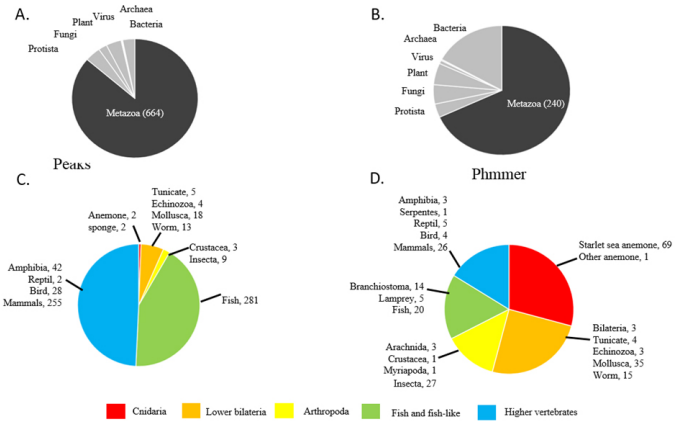

Figure 5. Taxonomic distribution of protein homologues of *Myxobolus episquemalis* peptides identified by mass spectrometry analysis.

A.Frequency of proteins of metazoan origin after Peaks analysis and comparison with other major kingdom or branches of living organisms. **A total of 769 natural protein homologues** were characterized.

B. Frequency of proteins of metazoan origin after Phmmer analysis and comparison with other major kingdom or branches of living organisms. A total of 352 peptides had significant level of homology (e-value < 0.01).

C. Distribution of proteins identified by Peaks analysis among major groups of metazoan. Number of hits per groups is given after the coma.

D. Distribution of proteins identified by Phmmer analysis among major groups of metazoan. Number of hits per groups is given after the coma.

Phmmer analysis shed original light on *M. episquamalis* peptide composition (Fig. 5D). Almost 30%o homology search results were cnidarian proteins of *Nematostella vectensis* (Starlet sea anemone). These results are in agreement with the taxonomic position of Myxozoa among cnidarian. The second larger organism providing homology results was the mollusk *Lottia gigantean* whose genome has been sequenced. More generally, Phmmer analysis provided homologues in a wide range of organism, including animals which are often described at the transition between groups such as Amphoxius, Lamprey or Coelacanth. Despite the availability of the tunicate *Ciona intestinalis* genome, we observed little homology with this organism.

Discussion

Myxosporidians are major fish parasites causing outbreaks with serious economic impact. The objective of this work was to provide an insight on the inventory of proteins in *M.episquamalis* by mass spectrometry. To reach this goal, we used two approaches, namely 2-DE and shotgun mass spectrometry, coupled to sequence homology searches after *de novo* sequencing. To get high quality results, samples were analyzed by a high resolution mass spectrometer associated to a very good mass accuracy (~ 1ppm). One major problem was related to the presence of chitin in the spore of Myxozoa as evidenced by the work of Julius *et al* (1993). The later demonstrated resistance of spores to alkaline hydrolysis, which strongly complicated the extraction of proteins from spores of *M.episquamalis*. Evidence of contamination with fish proteins was provided by the distribution of protein homologues. The Peaks analysis was more responsive to this contamination. Major homologues were found with the most common protein in the animal cell such as actin or enzymes of the glycolysis. Presence of Myosin provides also evidence of contamination with muscle cell. Taxonomic analysis of Peaks homologues confirmed the host contamination as87.5% of the proteins were part of the phylum Metazoans. The contamination with animal proteins was also found with Phmmer analysis where 68% of the homologues had homologies with Metazoan peptides. Nevertheless, both functional and taxonomic analysis confirmed that relevant homologies were found with *M. episquamalis* peptides. Even some of the homologies with animal proteins could be the result of *M. episquamalis* peptides matches with nearly identical residues of highly conserved proteins. The confusion of the analysis may result from the fact that the genome of the flathead mullet and of a Myxozoa is not available.

The combination of 2-DE and shotgun analyses identified proteins that were classified into five groups: cellular function, biological function, metabolism, genome expression and proteins of undetermined function, this latter comprising the majority of proteins. One of the main reason for this relies to the fact that the closest organisms that has been sequenced remain poorly annotated. Strong homologies with the *Nematostella vectensis* starlet sea anemone and *Lottia gigantean* genome do not help us to address a complete map of the pathways. Strong homologies with functions found in bilateria raised again the origin of the Myxozoa and their position in the evolution as it was questioned in the past by the discovery of *Hox* genes in Myxozoa genome (Anderson *et al* 1998). The identification of peptides matching with neuropeptides and analgesic toxins offers new output on the interaction between Myxozoa and their host. It is the first time that pathogenic factors are described in Myxozoa. A major output of peptide annotations was the presence of a large proportion of records within microorganism including bacteria, archaea, protists, fungi and virus (112 proteins 352 Phmmer homologues) About one third of these organisms were marine microbes such as *Cyanobacteria, Oceanospirillales, Algae, Perkinsus* and *Stramenopiles*, which revealed the composition of the microbiome associated with the host scale and *M. episquamalis* cysts The presence of Mimivirus peptides in the protein set may suggested that Myxozoans may be infected by specific viruses. Among identified proteins were actin, tropomyosin, myosin, tubulin, troponin, also proposed in others works (Hartigan *et al* 2016; Gema *et al,* 2012; Uspenskaya and Raikova 2004). Indeed, the presence of F-actin is consistent with previous investigations that have highlighted for the first time cellular components and mechanisms involved in the motility of Myxozoans, through a study dedicated to *Ceratomyxa puntazzi* (Gema *et al,* 2012). Their work based on a morphological study (ultra-structural and three-dimensional structure) revealed the role of filamentous actin in the different developmental stages of *C. puntazzi*. In addition, Monteiro *et al* (2002) characterized the presence of muscle blocks in the Myxozoan worm *Buddenbrokia*. Uspenskaya and Raikova (2004) demonstrated the role of F-actin and β-tubulin cytoskeletal proteins in the anchoring mechanism of myxosporidians during the infestation phase.

The presence of collagen was shown by 2-DE analysis but not confirmed after shotgun analysis. Jason *et al,* (2011) reported the presence of a gene encoding a mini-collagen called Tb-Ncol-1 in a *Tetracapsuloides bryosalmonae*. Interestingly, a similar protein had been identified in nematocysts of cnidarians (Ulrike *et al,* 2002). More recently, transcriptomic analysis revealed the presence of several genes encoding nemato-galectins and mini-collagens in myxozoan and cnidarian genomes (Shpirer *et al* 2014).

It has been demonstrated some phylogenetic relationship between Cnidarians and Myxozoa, or nematocysts, which are special cells of cnidarians containing a polar filament whose primary role is the defense and capture of prey which could further confirm the mechanical role of myxosporidians polar filament in the spore attachment to host cells. Noteworthy, a polyclonal antibody (pAbMPPF) was generated against the polar filament of the myxozoan *Myxobolus pendula* (Ringuette *et al,* 2011). In western blot analysis, many bands located at 35, 40, 42, 55, 70, 100, and 200 kDa were revealed in *M. pendula* while a major band located at 40 kDa and 3 minor bands at 48, 70, and 130 kDa were found in *M. bartai*. The proteins corresponding to pAbMPPF reactive spots possess glycine-rich hydrophobic domains with sequence similarity to spinalin (Ringuette *et al,* 2011). Spinalin is a 24-kDa glycine- and histidine-rich protein in spines of Hydra nematocysts, with regions presenting homology to loricrins and keratins. Unfortunately, these proteins were not identified during this analysis. The presence of the polar tube was also described in microsporidia. Proteins from the polar tube of

Encephalitozoon cuniculi were already described (Delbac *et al,* 1998; Delbac *et al,* 2001; Peuvel *et al,* 2002) but there were no record from *M. episquamalis* peptides. Understanding of the molecular organization and function of the polar tube that is conserved from protist to cnidarian remains a challenge for future studies.

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Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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