Haplosporidium pinnae associated with mass mortality in endangered Pinna nobilis (Linnaeus 1758)fan mussels

Haplosporidium pinnae associated with mass mortality in endangered Pinna nobilis (Linnaeus 1758) fan mussels

Rossella Panarese[a], Perla Tedesco[b,1], Giovanni Chimienti[c,d,1], Maria Stefania Latrofa[a], Francesco Quaglio[e], Giuseppe Passantino[a], Canio Buonavoglia[a], Andrea Gustinelli[b], Angelo Tursi[c,d], Domenico Otranto[a,*]

[a] Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Str. prov. per Casamassima km 3, 70010 Valenzano, Bari, Italy

[b] Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum Università di Bologna, via Tolara di Sopra, 50, 40064 Ozzano dell'Emilia, Bologna, Italy

[c] Dipartimento di Biologia, Università degli Studi di Bari, via Orabona, 4, 70125 Bari, Italy

[d] CoNISMa, Piazzale Flaminio, 9, 00196 Roma, Italy

[e] Dipartimento di Biomedicina Comparata e Alimentazione, Università degli Studi di Padova, Polo di Agripolis, viale dell'Università, 16, 35020 Legnaro, Padova, Italy

Abstract

The fan mussel, *Pinna nobilis* (Linnaeus 1758), is an endemic bivalve of the Mediterranean basin, protected by international legislation as an endangered species. In the early summer of 2018, a mass mortality event (MME) of *P. nobilis* was recorded in the Gulf of Taranto (Southern Italy, Ionian Sea). Moribund specimens of *P. nobilis* were collected by scuba divers and processed by bacteriological, parasitological, histopathological and molecular analyses to investigate the causes of this MME. Different developmental stages (i.e., plasmodia, spores and sporocysts) of a presumptive haplosporidian parasite were observed during the histological analysis in the epithelium and in the lumen of the digestive tubules, where mature spores occurred either free or in sporocysts. The spores presented an operculum and an ovoid shape measuring 4.4 μm (±0.232) in length and 3.6 μm (±0.233) in width. BLAST analysis of an 18SrRNA sequence revealed a high nucleotide similarity (99%) with the reference sequence of *Haplosporidium pinnae* available in GenBank database. Phylogenetic analysis clustered the sequence of the pathogen in a paraphyletic clade with the reference sequence of *H. pinnae*, excluding other haplosporidians (i.e., *Bonamia* and *Minchinia* genera). Based on data reported, *H. pinnae* was the causative agent of MME in the populations of *P. nobilis* sampled in the Ionian Sea, where the conservation of this endangered species is heavily threatened by such a protozoan infection. Further investigations should contribute to knowledge about the life cycle of *H. pinnae* in order to reduce spread of the pathogen and to mitigate the burden of the disease where *P. nobilis* is facing the risk of extinction.

Graphical abstract

Keywords

Haplosporidium pinnae, Pinna nobilis, Mass mortality, Histology, Molecular analyses, 18SrRNA

1. Introduction

The fan mussel *Pinna nobilis* (Linnaeus 1758) is the largest saltwater bivalve in the Mediterranean Sea, where it is endemic and protected as endangered species (i.e., Annex II of the Barcelona Convention, SPA/BD Protocol 1995, and Annex IV of the EU Habitats Directive 2007) (Darriba, 2017, Vázquez-Luis et al., 2017). With a maximum reported age of 27 years, this filter-feeding mollusc usually settles on soft substrates (occasionally on hard ones) from 0.5 to 60 m depth, using byssal threads to anchor. It may reach up to 120 cm in height (Schultz and Huber, 2013, Basso et al., 2015). Populations of *P. nobilis* are distributed in many areas along the Italian coasts and are considered sensitive to anthropogenic and environmental threats, such as high levels of urbanization (Ladisa et al., 2010), urban discharges and freshwater agricultural inputs (Calace et al., 2008, Bellucci et al., 2016). One of the largest populations of *P. nobilis* known so far in Italy was present in the Gulf of Taranto, in the Mar Piccolo basin of the Ionian Sea (Centoducati et al., 2007, Tursi et al., 2018), despite this area being subject to severe anthropogenic impacts (Bracchi et al., 2016). In this area, a high survival rate and a low mortality (i.e., from a minimum of 0.1% up to 8.8%) have been recently observed in optimal conditions (Tursi et al., 2018). Protozoan infection by haplosporidan parasites has been recently implicated in a mass mortality event (MME) of *P. nobilis* occurring in the Spanish coast of the Western Mediterranean Sea (Darriba, 2017, Vázquez-Luis et al., 2017), with *Haplosporidium pinnae* nov. sp. identified as the causative agent of the still on-going MME in this area (Catanese et al., 2018). Haplosporidians are highly pathogenic for marine and freshwater invertebrates with high mortality rates caused, for example in different oyster species, by *Haplosporidium nelsoni*, *Bonamia ostreae* and *Bonamia exitiosa* (Engelsma et al., 2014). In particular, the sporulation of *H. pinnae* occurs in the digestive gland tubules, impairing food absorption and causing severe dysfunction and death of the host (Darriba, 2017, Vázquez-Luis et al., 2017, Catanese et al., 2018). In the early summer of 2018, a MME was recorded in *P. nobilis* populations in the Ionian Sea, with up to 100% mortality in 3 months. We investigated the causes of this sudden MME using bacteriological, parasitological, histopathological and molecular tools.

2. Materials and methods

2.1. Sampling collection and processing

Samples were collected in the Mar Piccolo basin (T: ±25 °C; salinity: ±37ppt), a coastal marine ecosystem with lagoon features (Gulf of Taranto, Southern Italy, Central Mediterranean Sea; Fig. 1). The seafloor is dominated by soft sediment, from mud to mixed sand, locally colonised by benthic communities consisting mainly of filter- and suspension-feeders and seaweeds (Matarrese et al., 2004, Mastrototaro et al., 2008). These communities coexist with a suite of anthropogenic impacts, including high level of urbanization, heavy industries, intense maritime traffic, as well as mussel and fish farms (Bracchi et al., 2016). According to recent monitoring programs carried out in this area (Tursi et al., 2018), two sampling sites with the highest density of *P. nobilis* were selected (Fig. 1). Specimens of *P. nobilis* were collected in July 2018, from 3 to 8 m depth, by scuba divers in the two sampling sites. Specimens of *P. nobilis* presented generic symptoms of a disease condition (i.e., slow response to mechanical stimuli, opened valves and high presence of mucous secretions). The sampling of 10 moribund specimens, collected from a subpopulation of 7107 *P. nobilis*, was carried out under the permission of the Italian Ministry for Environment, Land and Sea Protection, based on the agreement between the Special Commissioner for Urgent Intervention for Remediation, Environmental Enhancement and Upgrading of Taranto and the University of Bari Aldo Moro (no. 1890, 16/06/2016). Total length and weight of specimens were recorded and a macroscopic examination was conducted to evaluate the external aspect of the specimens, their nutritional state and internal organs, the gross alterations of valves as well as the presence of macroscopic lesions. Samples of hemolymph (2 ml from each specimen) were taken from the anterior adductor muscle and from the heart with a sterile syringe and plated on different culture media (TSA + 2%NaCl, Blood Agar, TCBS e FMM). Fresh smears of hemolymph were stained with May-Grunwald Giemsa and Hemacolor®. The remaining hemolymph (frozen at −20 °C) and the digestive glands (fixed in ethanol 70%) were used for molecular analyses.

2.2. Histopathological studies

Portions of digestive gland, mantle, gills, gonads and muscle of fan mussels were preserved in buffered formalin 10% for histological analyses. Samples were dehydrated in an increasing ethanol gradient, embedded in paraffin wax, sectioned at 3–4 μm with a rotary microtome, and stained with Hematoxylin and Eosin, following standard methods (Culling et al., 1985).

2.3. Molecular analyses

Genomic DNA was extracted from digestive glands that had been chopped by sterile scissors and washed twice (15 min) with sterile distilled water (800 μl), and from hemolymph samples (100 μl), using DNEasy Blood & Tissue kit and QIAampDNA Minikit (Qiagen, Germany), respectively. Pathogen DNA was screened by standard PCR (PCR) using generic primers targeting the 18SrRNA region for *Haplosporidium* spp. (~350 bp) and *Bonamia* spp. (~573 bp) and a specific pair of primers for *H. nelsoni* (~300 bp), as previously described (Cochennec et al., 2000, Renault et al., 2000). PCR products were examined on 2% agarose gels stained with GelRed (VWR International

PBI, Milano, Italy) and visualised on a GelLogic 100 gel documentation system (Kodak, New York, USA). The amplicons were purified and sequenced in both directions with the same primers used for PCR, employing the Big Dye Terminator v.3.1 chemistry in a 3130 genetic analyser (Applied Biosystems, California, USA). Sequences were aligned using the ClustalW program (Larkin et al., 2007) and compared with those available in GenBank by Basic Local Alignment Search Tool (BLAST-http://blast.ncbi.nlm.nih.gov.ezproxy.unibo.it/Blast.cgi). The evolutionary history was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). A discrete Gamma distribution was used to model evolutionary rate differences among sites. Evolutionary analyses were tested on 4000 Bootstrap replications, using MEGA6 software (Tamura et al., 2013). The phylogenetic analysis was run by using 18SrRNA sequences of species belonging to Haplosporidia order available in GenBank. *Mikrocytos mackini* (AN:HM563060) and *Marteilia cochillia* (AN: KF278722) were used as outgroups.

3. Results

The average length of *P. nobilis* processed was 30 cm (SD: ±1.58) and the average weight was 212 g (±5.48). Several epibionts were attached to the valves, such as the polychaete *Eulalia ornata* (Saint-Joseph, 1888), unidentified bryozoans, the common limpet *Patella vulgata* (Linnaeus, 1758), the crab *Tumidotheres maculatus* (Say, 1818) and the starfish *Asterina pancerii* (Gasco, 1876). On macroscopic observation the specimens of *P. nobilis* appeared emaciated, but no other gross alterations were observed. A large watery vesicle was found in the visceral mass of one specimen from Site 2. In all the specimens, the gills were collapsed and appeared pale brownish in color, while the digestive glands were darker and softer than expected for healthy specimens. Fresh preparations of digestive gland showed the presence of mature spores occurring either free or in sporocysts (Fig. 2). Spores were ovoid with visible operculum and measured 4.4 µm (±0.232) in length × 3.6 µm (±0.233) (mean values calculated on n = 50 individuals). Culturing on TCBS, MacConkey agar and *Aeromonas* agar base to detect *Vibrio* spp., *Escherichia coli* and *Aeromonas* spp., respectively, was negative. Histopathological analyses showed diffused degenerative lesions in the presence of different developmental stages of a haplosporidan parasite in the epithelium and in the lumen of digestive tubules (Fig. 3A). Some larger sporocysts were protruding in the lumen of the tubules causing atrophy in the surrounding cells as a consequence of compression. In some tubules the epithelium was completely detached leaving only the basal lamina (Fig. 3B). Detached cells appeared in coagulative necrosis. Spherical binucleate stages were also observed within the epithelium of digestive tubules (Fig. 3C). In the interstitial space between tubules, an intense inflammatory response characterized by the presence of hemocyte infiltration was observed. Parasitic stages were present in the lumen of the intestine (Fig. 3D, arrow) associated with necrotic sloughing of digestive cells with loss of the cilia, although they were absent in the intestinal mucosa. The same response was observed in the mantle tissue, along with the presence of uninucleate developmental stages inside host hemocytes (Fig. 3E). Numerous brown cells were observed in the connective tissue (Fig. 3F) around the digestive gland and in the lumen of digestive tubules, as well as in the mantle, gills and gonads. No other parasites were observed. All digestive glands and hemolymph samples scored positive in PCR using degenerate primers for *Haplosporidium* spp., resulting in amplicons of the expected size (~350 bp). No amplification was obtained using primers for *H. nelsoni* and *Bonamia* spp. The BLAST analysis of the 18SrRNA

sequences of all the specimens tested revealed highest nucleotide identity, 99%, with the reference sequence of *H. pinnae* in the GenBank database (AN: LC338065). The molecular identity was confirmed by clustering of the 18SrRNA sequence obtained with that of *H. pinnae* reference strain, supported by high bootstrap value (99%, Fig. 4). The *H. pinnae* clade clustered in a paraphyletic group, appearing distinct from the *Bonamia/Minchinia* clade and from the clade containing most of the other *Haplosporidium* species (Fig. 4). The sequence was deposited in GenBank under accession number MK163629.

4. Discussion

We used molecular analysis to identify *H. pinnae* as the agent of the MME in *P. nobilis* populations in the investigated area of Ionian Sea, and observed haplosporidia in pathological lesions in the host. The presence of *H. pinnae* in examined *P. nobilis* with no other pathogens present, the observed lesions in the digestive gland and the absence of inflammatory nodular lesions typical of micobacteria indicate that this MME is due to this protozoan infection as observed in the Western Coast of Mediterranean Sea in Spain (Darriba, 2017, Vázquez-Luis et al., 2017, Catanese et al., 2018, Carella et al., 2019). Histological analysis showed that the presence of *H. pinnae* in all specimens was associated with heavy lesions of the digestive gland structure and severe tubular necrosis. The spores developed in the epithelium of the digestive gland and appeared to be released in the lumen of the gland's tubules, reaching the intestine of the host for elimination into the environment. In addition, the presence of different stages of sporulation of the protozoa (Hine and Thorne, 2002) in the digestive gland confirmed *Haplosporidium* sp. as the agent of the lesions in the examined specimens of *P. nobilis* (Catanese et al., 2018). Similar pathological conditions of the digestive gland have also been associated with the sporulation of *H. nelsoni* and *Haplosporidium tuxtlensis* in eastern oyster *Crassostrea virginica* (Gmelin, 1791) and the striped false limpet *Siphonaria pectinata* (Linnaeus, 1758) (Couch et al., 1966, Vea and Siddall, 2011). Before the MME in Spain, haplosporidan parasites were detected infecting species of bivalves, gastropods, crustacean, worms, ascidians and even hyperparasite trematode larvae (Burreson and Ford, 2004, Arzul and Carnegie, 2015), but never in a member of the Pinnoidea Superfamily. The spreading of this parasite into non-endemic areas is still unknown, but it may be argued that the outbreak spread from Spain, being transported in the summer marine currents (Fernández et al., 2005). Nonetheless, it cannot be ruled out that anthropic activity, such as maritime transport, ballast waters and trade of living bivalves may have enhanced dispersal of the protozoa. Dynamics of haplosporidians in their hosts suggest that these parasites could be seasonal, depending on environmental parameters such as temperature and salinity (Darriba, 2017).

First evidence of unexpected mortality of *P. nobilis* (40% of the individuals) in the study area was observed during the summer 2017, followed by a low mortality period during the winter (unpublished data) and by the drastic decline of the population in the following summer of 2018. Based on our observations, environmental conditions such as warm temperatures may be an important driver for the development of *H. pinnae*, suggesting that the impact of global warming could enhance the spreading of this parasite all over the Mediterranean Sea. Control of spread is difficult due to the lack of an adaptive immune system of the host and the rapid death of infected individuals, resulting in up to 100% mortality in a few months. Furthermore, the administration of treatment is impossible to carry out because of the potential impact on the marine ecosystem, as

well as the restrictions by European legislation (Guardiola et al., 2012). Therefore, resettlement of *P. nobilis* populations at the end of the MME seems to be the only option available to mitigate the on-going local extinction of this protected species.

Some aspects of the life cycle of *H. pinnae* remain unknown, including the potential of an intermediate host, the role of other definitive hosts, such as *Pinna rudis* (Linnaeus, 1758) or *Atrina* spp., and the persistence of infective spores in the environment. Further studies are needed to improve knowledge about the life cycle of *H. pinnae* in order to mitigate the ongoing disease and plan proper repopulation strategies for *P. nobilis* in areas where the MME caused the extinction of the species.

Acknowledgements

The study was funded through a Collaboration agreement between the Special Commissioner for urgent intervention for remediation, environmental enhancement and upgrading of Taranto (Dr. Vera Corbelli) and the University of Bari Aldo Moro.

References

Arzul and Carnegie, 2015
I. Arzul, R.B. Carnegie
New perspective on the haplosporidian parasites of molluscs
J. Invertebr. Pathol., 131 (2015), pp. 32-42, 10.1016/j.jip.2015.07.014

Basso et al., 2015
L. Basso, M. Vázquez-Luis, J.R. Gracía-March, S. Deudero, E. Álvarez, N. Vicente, C.M. Duarte, I.E. Hendriks
Chapter three – the Pen Shell, *Pinna nobilis*: a review of population status and recommended research priorities in the Mediterranean Sea
Adv. Mar. Biol., 71 (2015), pp. 109-160, 10.1016/bs.amb.2015.06.002

Bellucci et al., 2016
L.G. Bellucci, D. Cassin, S. Giuliani, M. Botter, R. Zonta
Sediment pollution and dynamic in the Mar Piccolo of Taranto (Southern Italy): insights from bottom sediment traps and surficial sediments
Mar. Pollut. Bull., 23 (2016), pp. 12554-12565, 10.1007/s11356-016-6738-6

Bracchi et al., 2016
V.A. Bracchi, F. Marchese, A. Savini, G. Chimienti, F. Mastrototaro, C. Tessarolo, F. Cardone, A. Tursi, C. Corselli
Seafloor integrity of the Mar Piccolo Basin (Southern Italy): quantifying anthropogenic impact
J. Maps. (2016), 10.1080/17445 647.2016.11529 20

Burreson and Ford, 2004
E.M. Burreson, S.E. Ford
A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease)

Aquat. Living Resour., 17 (2004), pp. 499-517, 10.1051/alr:2004056

Calace et al., 2008

N. Calace, N. Cardellicchio, S. Ciardullo, B.M. Petronio, M. Pietrantonio, M. Pietroletti

Metal distribution in marine sediments of the Mar Piccolo in Taranto (Ionic Sea, Southern Italy)

Toxicol. Environ. Chem., 90 (2008), pp. 549-564, 10.1080/02772240701570420

Carella et al., 2019

F. Carella, S. Aceto, F. Pollaro, A. Miccio, C. Iaria, N. Carrasco, P. Prado, G. De Vico

A mycobacterial disease is associated with the silent mass mortality of the pen shell *Pinna nobilis* along the Tyrrhenian coastline of Italy

Sci. Rep., 9 (2019), p. 2725, 10.1038/s41598-018-37217-y

Catanese et al., 2018

G. Catanese, A. Grau, J.M. Valencia, J.R. Garcia-March, E. Alvarez, M. Vázquez-Luis, S. Deudero, S. Darriba, M.J. Carballal, A. Villalba

***Haplosporidium pinnae* sp. nov., a haplosporidan parasite associated with mass mortalities of the fan mussel, *Pinna nobilis*, in the Western Mediterranean Sea**

J. Inv. Pathol., 157 (2018), pp. 9-24, 10.1016/j.jip.2018.07.006

Centoducati et al., 2007

G. Centoducati, E. Tarsitano, A. Bottalico, M. Marvulli, O.R. Lai, G. Crescenzo

Monitoring of the endangered *Pinna nobilis* Linné, 1758 in the Mar Grande of Taranto (Ionian Sea, Italy)

Environ. Monit. Assess., 131 (2007), p. 339, 10.1007/s10661-006-9479-z

Cochennec et al., 2000

N. Cochennec, F. Le Roux, F. Berthe, A. Gerard

Detection of *Bonamia ostreae* based on small subunit ribosomal probe

J. Invertebr. Pathol., 76 (2000), pp. 26-32, 10.1006/jipa.2000.4939

Couch et al., 1966

J.A. Couch, C.A. Farley, A. Rosenfield

Sporulation of *Minchinia nelsoni* (Haplosporida, Haplospordiidae) in *Crassostrea virginica* (Gmelin)

Science, 153 (1966), pp. 1529-1531, 10.1126/science.153.3743.1529

Culling et al., 1985

C.F.A. Culling, R.T. Allison, W.T. Barr

Cellular Pathology Techniques

(fourth ed.), Butterworths, London (1985)

Darriba, 2017

S. Darriba

First haplosporidan parasite reported infecting a member of the Superfamily Pinnoidea (*Pinna noblis*) during a mortality event in Alicante (Spain, Western Mediterranean)

J. Inv. Pathol., 148 (2017), pp. 14-19, 10.1016/j.jip.2017.05.006

Engelsma et al., 2014

M.Y. Engelsma, S.C. Culloty, S.A. Lynch, I. Arzul, R.B. Carnegie

***Bonamia* parasites: a rapidly changing perspective on a genus of important mollusc pathogens**

Dis. Aquat. Org., 110 (2014), pp. 5-23, 10.3354/dao02741

Fernández et al., 2005

V. Fernández, D.E. Dietrich, R.L. Haney, J. Tintoré

Mesoscale, seasonal and interannual variability in the Mediterranean Sea using a numerical ocean model

Prog. Oceanogr., 66 (2005), pp. 321-340, 10.1016/j.pocean.2004.07.010

Guardiola et al., 2012

F.A. Guardiola, A. Cuesta, J. Meseguer, A.M. Esteban

Risks of Using Antifouling Biocides in Aquaculture

Int. J. Mol. Sci., 13 (2012), pp. 1541-1560, 10.3390/ijms13021541

Hine and Thorne, 2002

P.M. Hine, T. Thorne

***Haplosporidium* sp. (Alveolata: Haplosporidia) associated with mortalities among rock oysters *Saccostrea cucculata* in north Western Australia**

Dis. Aquat. Org., 51 (2002), pp. 123-133, 10.3354/dao051123

Kimura, 1980

M. Kimura

A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence

J. Mol. Evol., 16 (1980), pp. 111-120, 10.1007/BF01731581

Ladisa et al., 2010

G. Ladisa, M. Todorovic, G. Trisorio Liuzzi

Assessment of desertification in semi-arid Mediterranean environments: the case study of Apulia region (Southern Italy)

P. Zdruli, M. Pagliai, S. Kapur, A. Faz Cano (Eds.), Land Degradation and Desertification: Assessment, Mitigation and Remediation, Springer, New York (2010), pp. 493-516

Larkin et al., 2007

M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, D.G. Higgins

Clustal W and Clustal X version 2.0

Bioinformatics, 23 (2007), pp. 2947-2948, 10.1093/bioinformatics/btm404

Mastrototaro et al., 2008

F. Mastrototaro, A. Giove, G. D'Onghia, A. Tursi, A. Matarrese, M.V. Gadaleta

Benthic diversity of the soft bottoms in a semi-enclosed basin of the Mediterranean Sea

J. Mar. Biol. Assoc., UK, 88 (2008), pp. 247-252, 10.1017/S0025315408000726

Matarrese et al., 2004

A. Matarrese, F. Mastrototaro, G. D'Onghia, P. Maiorano, A. Tursi

Mapping of the benthic communities in the Taranto seas using side-scan Sonar and an underwater video camera

Chem. Ecol., 20 (2004), pp. 377-386, 10.1080/02757540410001727981

Renault et al., 2000

T. Renault, N.A. Stokes, B. Chollet, N. Cochennec, F. Berthe, E.M. Burreson

Haplosporidiosis in the Pacific oyster, *Crassostrea gigas*, from the French Atlantic coast

Dis. Aquat. Org., 42 (2000), pp. 207-214, 10.3354/dao042207

Schultz and Huber, 2013
P.W. Schultz, M. Huber
Revision of the worldwide Recent Pinnidae and some remarks on fossil European Pinnidae
Acta Conch., 13 (2013), pp. 1-164
Tamura et al., 2013
K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar
MEGA6: molecular evolutionary genetics analysis version 6.0
Mol. Biol. Evol., 30 (2013), pp. 2725-2729, 10.1093/molbev/mst197
Tursi et al., 2018
A. Tursi, V. Corbelli, G. Cipriano, G. Capasso, R. Velardo, G. Chimienti
Mega-litter and remediation: the case of Mar Piccolo of Taranto (Ionian Sea). Rend. Lincei Sci. Fis. Nat. (2018), pp. 1-8, 10.1007/s12210-018-0738-z
Vázquez-Luis et al., 2017
M. Vázquez-Luis, E. Álvarez, A. Barrajón, J.R. García-March, A. Grau, I.E. Hendriks, S. Jimenez, D. Kersting, D. Moreno, M. Perez, J.M. Ruiz, J. Sánchez, A. Villalba, S. Deudero
SOS *Pinna nobilis*: A Mass Mortality Event in Western Mediterranean Sea
Front. Mar. Sci., 4 (2017), p. 220, 10.3389/fmars.2017.00220
Vea and Siddall, 2011
I.M. Vea, M.E. Siddall
Scanning electron microscopy and molecular characterization of a new *Haplosporidium* species (Haplosporidia), a parasite of the marine gastropod *Siphonaria pectinata* (Mollusca: Gastropoda: Siphonariidae) in the Gulf of Mexico
J. Parasitol., 97 (2011), pp. 1062-1066, 10.1645/GE-2850.1

[1] Both authors equally contributed and share the second position.

Fig. 1. Map of the Mar Piccolo of Taranto (Ionian Sea, Southern Italy) indicating the two sampling sites (dots).

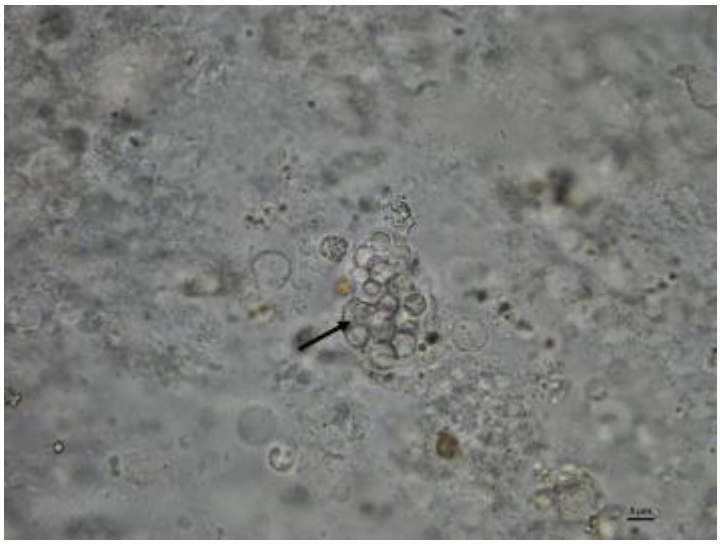

Fig. 2. Spores and sporocysts (arrow) of *Haplosporidium pinnae* in fresh preparation of a digestive gland.

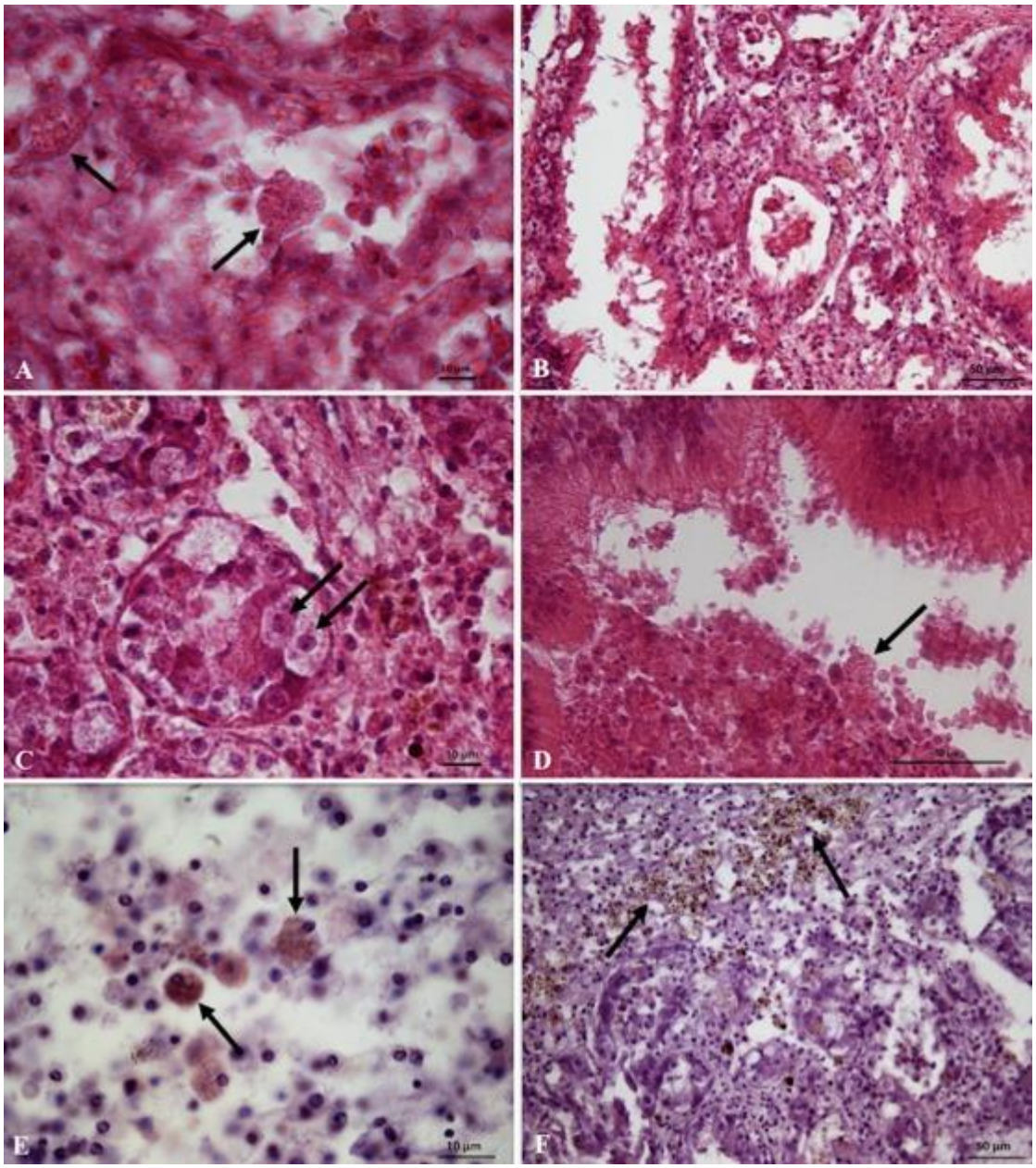

Fig. 3. (A) Histological section of a digestive gland showing detachment of epithelial cells and *Haplosporidium pinnae* spores in the epithelium and lumen of digestive tubules (arrows); (B) histological section of digestive gland showing detachment from the basal lamina; (C) spherical binucleate stages of *H. pinnae* in the epithelial cells of digestive tubules (arrows); (D) histological section of intestine showing the presence of parasitic stages in the intestinal lumen (arrow) and necrotic cells; (E) developmental stages of *H. pinnae* (arrows) in the cytoplasm of haemocytes in the mantle; (F) numerous brown cells (arrows) in the connective tissue around the digestive gland and in the lumen of digestive tubules.

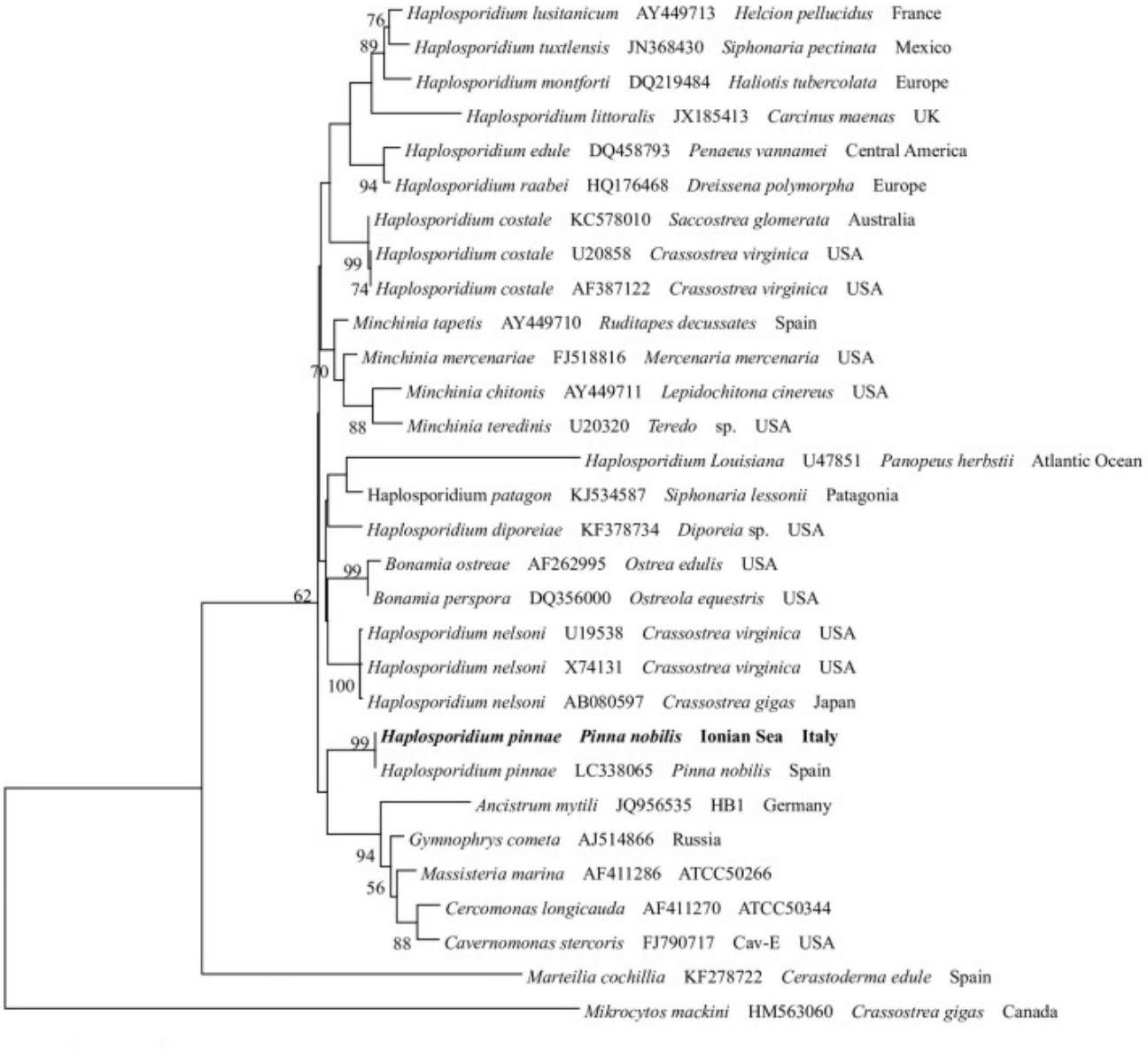

Fig. 4. Maximum likelihood tree based on 18SrRNA sequences of *H. pinnae* generated with those of other haplosporidans parasite available from GenBank. Bootstrap values are based on 4000 replicates and only bootstraps >50% are indicated. Accession number, host and country of haplosporidians, *Mikrocytos mackini* and *Marteilia cochillia* 18SrRNA sequences used as outgroups are reported.