Detection of *Bonamia* sp., infecting flat oysters, *Ostrea puelchana* (d'Orbigny, 1842) in San Matias Gulf (NW Patagonia, Argentina) based on 18S Small sub-unit and ITS Segment sequence

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Introduction

The Argentinean native flat oyster, *Ostrea puelchana* d'Orbigny, is found on natural beds in the temperate waters of South America, the southern limit of its distribution being the San Matias Gulf (Figure 1). Oyster culture in Argentina has until recently remained undeveloped, but a market for the product is emerging. Trial cultures were conducted in the past with foreign species including *Crassostrea virginica* and *Ostrea edulis,* which always gave negative results. Commercial native flat oyster culture has been initiated in San Antonio Bay in March 1995 and was promising until an abnormal mortality occurred in 1996. A preliminary diagnosis revealed the presence of *Bonamia*–like cells in some individuals by histology (Kroeck and Montes, 2005). The parasite was reported again on oysters collected during an epidemiological survey between 1996 and 2001 from natural beds and culture areas (Kroeck et al., 2004).

Bonamia spp. are the causative agents of bonamiosis in flat oysters and has led to high mortalities around the world. Two of them have been well characterized and are listed at the Office International des Epizooties (OIE): *B. ostreae* reported in Europe and North America and *B. exitiosa* reported in New Zealand. Acclimatization tests in French waters demonstrated the susceptibility of the Argentinean oyster to infection with *Bonamia ostreae* (Pascual *et al.*, 1991). Regarding these results and the recent characterization of new *Bonamia* isolates very closed to *Bonamia exitiosa* in Chile (Arzul, personnal communication) and North Carolina (Burreson *et al.*, 2004) , the Argentinean isolate species is questionned. In order to compare it with well-known *Bonamia* species, the 18S small sub-unit and the ITS1 segment were cloned and sequenced.

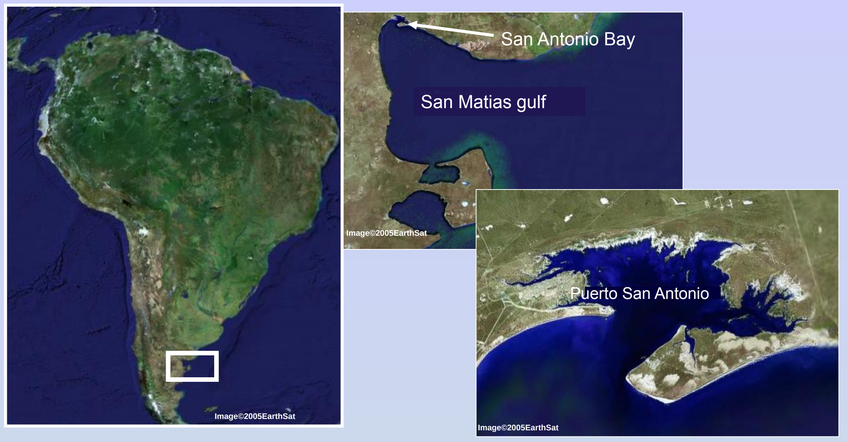

Figure 1 – Geographical localization of *Ostrea puelchana* production in Argentina.

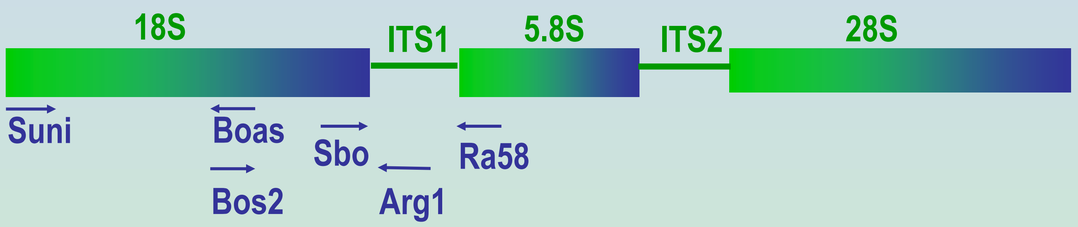

Figure 2 – Set of primers used for the amplification of the 18S small sub unit gene, ITS1 segment and part of the 5.8S small sub unit gene.

Material and Methods

- Two 2-2,5 years old oysters sampled in August 2002 in San Antonio Bay (Figure 1) during an epidemiological survey and detected positive by histology and PCR were selected for sequencing.
- Amplification of the 18S small sub-unit and ITS1 segment was done using a set of primers (Suni-Boas; Sbo-Ra58 and Bos2-Arg1, Figure 2) and PCR conditions presented by Cochennec et al. 2000.
- All PCR products were cloned using the TA cloning kit (Invitrogen) following Manufacturer's instructions. Two clones were selected for each PCR products for sequencing using the Big Dye V3 sequencing kit (Applied Biosystem) and plasmidic and specific primers.
- Sequences were aligned using Clustawl algorithm and phylogenetic analysis was performed using a Neighbour Joining approach and Kimura's 2-parameter distances.

Results and discussion

- A consensus sequence could be obtained from both clones from the two selected samples for each of the three amplified segments: 994 bp for Suni-Boas; 959 bp for Bos2-Arg1; 474 bp for Sbo-Ra58.
- Assembling these segments allowed to obtain a 1998 bp sequence including the complete sequences of the 18S and the ITS1 (1797 bp and 143 bp respectively) and partial sequence of the 5.8S (58 bp).
- The Argentinean isolate shows 99.86 % of homology with the sequence of the isolate detected in North America in *Crassostrea ariakensis.*
- The comparison of the 18S sequence of the Argentinean isolate with other characterized *Bonamia* species is presented in Table I. The Argentinean isolate appears very closed to the Chilean isolate, *Bonamia roughleyi* as well as *B. exitiosa* and different fom *B. ostreae* (Figure 3).

	B. argent	*B.chilean*	*B. roughl*	*B. exitio*	*B. ostr*	*M.tapet*	*M. teredi*	*M. chiton*	*H. costa*	*H. pickfor*	*H. lusitani*	*H. nelsoni*	*H. louisia*	*U. cresce*	*C. longica*
B.chilean	0,0053	0													
B. roughleyi	0,0095	0,0085	0												
B. exitiosa	0,0162	0,0106	0,0084	0											
B. ostreae	0,0642	0,0632	0,0609	0,0528	0										
M.tapetis	0,1435	0,1389	0,1402	0,1449	0,1516	0									
M. teredinis	0,1804	0,1756	0,1836	0,1881	0,1988	0,1507	0								
M. chitonis	0,1922	0,1874	0,1901	0,1946	0,2016	0,1443	0,1116	0							
H. costale	0,1722	0,1674	0,1701	0,1747	0,1767	0,1631	0,2114	0,2153	0						
H. pickfordi	0,2453	0,2445	0,2504	0,25461	0,2665	0,2367	0,2658	0,2949	0,2180	0					
H. lusitanicum	0,2936	0,2015	0,2015	0,2059	0,2124	0,1921	0,2251	0,2419	0,1670	0,1526	0				
H. nelsoni	0,2123	0,2101	0,2074	0,2119	0,2099	0,2032	0,2183	0,2177	0,2115	0,2824	0,2334	0			
H. louisiana	0,3607	0,3584	0,3621	0,3576	0,3578	0,3647	0,4009	0,3837	0,3644	0,3999	0,3652	0,3448	0		
U. crescens	0,2641	0,2574	0,2620	0,2676	0,2607	0,2561	0,2929	0,2880	0,2822	0,3257	0,2788	0,2781	0,3778	0	
C. longicauda	0,3207	0,3215	0,3199	0,3206	0,3211	0,3278	0,3408	0,3392	0,3301	0,3917	0,3581	0,3325	0,4737	0,3118	0

Table I – Pairwise sequence divergences for 1047 nucleotide fragments of the SSU rDNA gene.

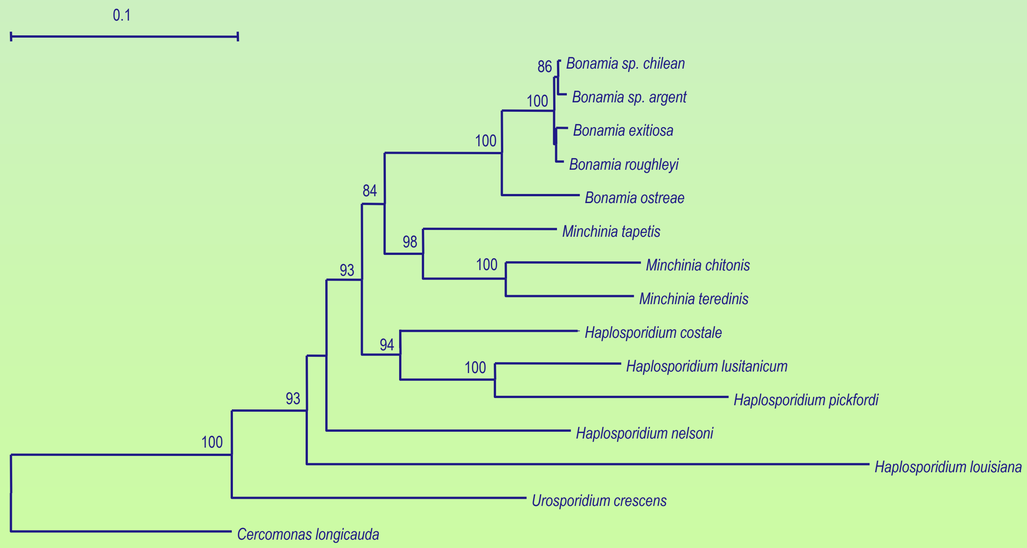

Figure 3 - Neighbour Joining tree resulting from sequence divergence of a 1047 nucleotide SSU rDNA fragment according to Kimura's model with 2 parameters for different haplosporidian parasites. *Cercomonas longicauda* was used as an outgroup.

Conclusion

These results confirm that the Argentinean parasite belongs to the *Bonamia* genus like it was suspected on the basis of histological characteristics. This new isolate appears very similar to the other southern hemisphere *Bonamia.* However, regarding the polymorphism, particularly on the ITS1 segment, it is proposed to treat the Argentinean species as a different species or more probably sub species until more studies by electron microscopy are made to determine the correct taxonomy.

References

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