USE OF MOLECULAR TOOLS FOR THE IDENTIFICATION OF CRYPTIC *CERTAOMYXA* SPECIES INFECTING THE GALLBLADDER OF THE BOGUE *BOOPS BOOPS* IN TUNISIAN COASTS

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Abstract
During a study on fish parasites in the Gulf of Gabès 'GG' (SE Tunisia), two *Ceratomyxa* (Myxozoa: Bivalvulida) parasites were identified using molecular techniques in the gallbladder of *Boops boops* (Sparidae) : *C. pallida* Thélohan, 1894, a poorly described parasite infecting *B. boops* gallbladder and *C. gabesiensis*, a new cryptic species in GG. As *C. gabesisnes* was never observed solely, it was firstly considered as a morphotype of *C. pallida* which was encountered as a single infection or in association with *C. gabesiensis*. Monthly surveys showed the occurrence of the two parasites throughout the year with a mean prevalence of 23% and 64% for *C. gabesiensis* and *C. pallida*, respectively. Molecular analysis using the SSU rDNA partial sequence, allowed us to distinguish the two *Ceratomyxa* species

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The bogue, *Boops boops* (Linnaeus, 1758) is a small benthopelagic sparidae species distributed in the Mediterranean, Eastern Atlantic and rarely in the Black Sea. It has an important commercial value in various countries including Tunisia. Studies on *Ceratomyxa* parasites infesting the gallbladder of *B. boops* are very ancient and limited to the single record of *C. pallida* in the Mediterranean off France, Croatia and Monaco [1]. The present work was conducted in this regard. One hundred and eighty fresh bogues were collected between July 2013 and August 2014 from the GG, SE Tunisia. Gallbladders were removed and examined for the presence of coelozoic Myxozoan parasites under a Leica DM1000 microscope equipped with a digital camera (Leica D-LUX3). Mature spores and vegetative stages were photographed and were morphometrically identified following the guidelines adopted by Lom and Arthur [2]. DNA was extracted from gallbladders filled with mature spores and preserved in absolute ethanol. A partial sequence of the SSU rDNA was amplified by PCR using the MyxospecF 18R primers [3]. Gallbladders with unique types of myxospores were directly sequenced using the same primers as used for the PCR. For gallbladders with two morphological types of myxospores, we inserted the purified PCR products into the pGEM®-T Easy Vector system (Promega) and cloned them into competent XL1-Blue *Escherichia coli* cells. From each cloned PCR product, 2 positive clones were selected for sequencing in both directions using the universal M13 primers. Obtained sequences were used for molecular comparison and phylogenetic analysis. Based on the infected host species and shell valve measurements; we considered that the thicker *Ceratomyxa* species could be identical to *C. pallida*. The measurements of the thickness of the shell valves (28.5 ± 2.45 (26-33) µm) and length (6.0 ± 0.12 (5-8) µm) are in the range of previously-published values [1]. The second morphotype occurs less frequently in the same host species and had mature spores with a relatively small size compared to those of *C. pallida* (Figure 1).

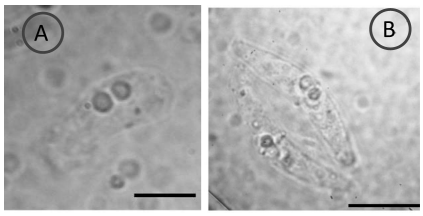

Fig. 1. *Ceratomyxa gabesiensis* n. sp (A) and *Ceratomyxa pallida* (B) from the glalblader of *Boops boops.* Scale-bars = 5 µm (A) and = 10 µm (B)

This morphotype is different compared to *C. pallida*, and was then considered a potential new species. This latter species is characterized by its thinner and asymmetrical shell valves. Phylogenetic analysis using the Maximum Likelihood (ML) and Bayesian inference methods yielded grouping of *C. gabesiensis, C. pallida*, *C. tunisiensis* and *C. leatherjacketi* in the same clade with the highest statistical support (Figure 2).

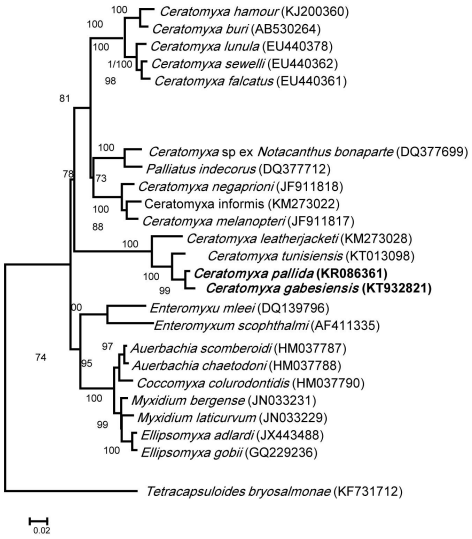

Fig. 2. Phylogenetic tree of *Ceratomyxa* spp, resulting from maximum likelihood analysis using the SSU rRNA dataset showing the position of *C. gabesiensis* n. sp. and *C. pallida*

The obtained results confirmed the necessity of the use of molecular tools for an accurate identification of *Ceratomyxa* parasites up to the species level, particularly in cases of co-infection. The new described parasite (*C. gabesiensis*) is the second species of the genus *Ceratomyxa* recorded and described in the Gulf of Gabes, after *C. tunisiensis* [3].

References

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