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# Molecular phylogenetic analysis of the coccidian cephalopod parasites *Aggregata octopiana* and *Aggregata eberthi* (Apicomplexa: Aggregatidae) from the NE Atlantic coast using 18S rRNA sequences

Sheila Castellanos-Martínez[a], Marcos Pérez-Losada[b], Camino Gestal[a,*]

[a]*Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Eduardo Cabello 6, 36208 Vigo, Spain*
[b]*CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal*

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## Abstract

The coccidia genus *Aggregata* is responsible for intestinal coccidiosis in wild and cultivated cephalopods. Two coccidia species, *Aggregata octopiana*, (infecting the common octopus *Octopus vulgaris*), and *A. eberthi*, (infecting the cuttlefish *Sepia officinalis*), are identified in European waters. Extensive investigation of their morphology resulted in a redescription of *A. octopiana* in octopuses from the NE Atlantic Coast (NW Spain) thus clarifying confusing descriptions recorded in the past. The present study sequenced the 18S rRNA gene in *A. octopiana* and *A. eberthi* from the NE Atlantic coast in order to assess their taxonomic and phylogenetic status. Phylogenetic analyses revealed conspecific genetic differences (2.5%) in 18S rRNA sequences between *A. eberthi* from the Ria of Vigo (NW Spain) and the Adriatic Sea. Larger congeneric differences (15.9%) were observed between *A. octopiana* samples from the same two areas, which suggest the existence of two species. Based on previous morphological evidence, host specificity data, and new molecular phylogenetic analyses, we suggest that *A. octopiana* from the Ria of Vigo is the valid type species.
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***Keywords:*** *Aggregata octopiana*; *Aggregata eberthi*; Coccidia; *Octopus vulgaris*; *Sepia officinalis*; 18S rRNA

## Introduction

Coccidians are obligate intracellular parasites that cause severe injuries mainly in poultry and livestock (Levine 1985), but are also able to infect marine fishes and molluscs causing a detrimental effect on their physiological condition (Kent and Hedrick 1985; Lom and Dyková 1992). Cephalopods are specifically infected by coccidians of the genus *Aggregata* (Hochberg, 1990), which are heteroxenous parasites transmitted through the food web. Sexual stages (gamogony and sporogony) occur inside the digestive tract of the definitive cephalopod host, whereas asexual stages (merogony) can be found inside the digestive tract of the intermediate crustacean host (Hochberg 1990).

The genus *Aggregata* has a complex taxonomic history. It was first described by Lieberkuhn (1854) as a gregarine infecting *Sepia officinalis*. Schneider (1875) described a similar parasite infecting *Octopus vulgaris*, whereas the later genus was correctly classified as a coccidium (Schneider 1883). Then, the genus *Aggregata* was assigned by Frenzel (1885), who described merogonic stages of the parasite in *Portunus arcuatus.* Finally, the cephalopod coccidia were classified into the family Aggregatidae by Labbé (1899). The taxonomy of the *Aggregata* species has been controversial

*Corresponding author. Tel.: +34 986 231 930; fax: +34 986 292 762.
*E-mail address:* cgestal@iim.csic.es (C. Gestal).

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(Hochberg 1990), and confusing descriptions have been recorded in the past. The species *Aggregata octopiana* was first described by Schneider (1875) in *O. vulgaris* from the English Channel and Western Mediterranean Sea (Banyuls-sur-Mer, France), and redescribed in samples from the NE Atlantic Ocean (Gestal et al. 1999b). Comparative ultrastructural studies revealed that the taxon described by others as *Aggregata spinosa* in the same host and locations using light microscopy (Moroff 1908), was synonymous to *A. octopiana* (Gestal et al., 1999b). Consequently, 10 *Aggregata* species have been described to date (see Table 1), and three of them are found in European waters: (1) *A. eberthi*, which is the representative type-species of the genus *Aggregata* and infects the cuttlefish *S. officinalis* from the Mediterranean Sea, English Channel and NE Atlantic Ocean (Dobell 1925); (2) *A octopiana*, which infects the common octopus *O. vulgaris* and has been re-described in hosts from the NE Atlantic Ocean (Gestal et al. 1999b); and (3) *A. sagittata*, which infects the flying squid *Todarodes sagittatus* (Gestal et al., 2000).

Understanding cephalopod pathogens is particularly relevant to the worldwide aquaculture of octopus species, which has to satisfy the global demand of cephalopods for human consumption (Iglesias et al. 2004; Domingues et al. 2007; Solorzano et al. 2009). The coccidian *A. octopiana* is known to cause heavy infections in the digestive tract of *O. vulgaris* (Pascual et al., 1996). Gamogonic and sporogonic stages cause the host's digestive tissue to rupture (Gestal et al. 2002a). Malabsorption syndrome is a secondary effect of high infection rates, reducing the growth and condition of infected octopuses (Gestal et al. 2002b) and negatively impacting octopus culture (Gestal et al. 2007). Moreover, food sanitary regulations forbid commercialization of parasitized fishery and aquaculture products. Hence, despite *Aggregata* spp. not being zoonotic parasites, the octopus will be withdrawn from human consumption circulation as soon as oocysts are detected in muscle (Peñalver et al. 2008).

Due to the increasing importance of coccidian diseases, particularly those caused by *Aggregata* species, the use of highly sensitive molecular methods for parasite diagnosis becomes crucial. Furthermore, molecular approaches are also useful to characterize parasites, complementing morphological descriptions, and phylogenetic classification (Jirků et al. 2009; Rueckert et al. 2011). The species *A. octopiana* and *A. eberthi* have been identified and characterized in the NE Atlantic coast according to morphological characters and host specificity (Gestal et al. 1999b, 2002c; Gestal and Pascual 2002). In contrast, very little is known about their molecular classification and phylogenetic position, which could confirm their taxonomic affiliation within the genus and validate conservative and robust phenotypic characters used for species diagnosis. Kopečná et al. (2006) generated the first 18S rRNA sequences for *A. octopiana* and *A. eberthi* from Croatia (Adriatic Sea); however, the phylogenetic position of both coccidians remained unresolved.

In this study, we generated new 18S rRNA nucleotide sequences for *A. octopiana* and *A. eberthi* from the

**Table 1.** *Aggregata* species recorded from cephalopod hosts. Length and width measurements are given as ranges (– denotes no data available).

| *Aggregata* species | Host | Locality (ocean/sea) | Sporocysts | | | Sporozoites | | References |
|---|---|---|---|---|---|---|---|---|
| | | | Length | Width | Cyst wall | *n*° | Length | |
| *Octopiana* | *O. vulgaris* | NE Atlantic, W Mediterranean | 11–15 | 11–15 | Spiny | 8 | 16–24 | Gestal et al. (1999b), Schneider (1875) |
| *Aggregata* sp. | *O. vulgaris* | E Mediterranean (Adriatic Sea) | – | – | – | 4–5, 8 | – | Mladineo and Jozić (2005), Mladineo and Bočina (2007) |
| *Dobelli* | *Enteroctopus dofleini* | NE Pacific | 18–31 | 15–27 | Smooth | 9–22 | 18–23 | Poynton et al. (1992) |
| *Millerorum* | *O. bimaculoides* | NE Pacific | 12–20 | 11–17 | Smooth | 8–10 | 18–31 | Poynton et al. (1992) |
| *Patagonica* | *E. megalocyatus* | SW Atlantic | 13 | 12 | Smooth | 8 | 18 | Sardella et al. (2000) |
| *Valdesensis* | *O. tehuelchus* | SW Atlantic | 10 | 10 | – | 4–8 | 17 | Sardella et al. (2000) |
| *Bathytherma* | *Vulcanoctopus hydrothermalis* | NE Pacific | 27–32 | 24–32 | Smooth; thick | 14–17 | 49 | Gestal et al. (2010) |
| *Sagittata* | *T. sagittatus* | NE Atlantic | 17 | 15 | Smooth; thick | 4–8 | 12 | Gestal et al. (2000) |
| *Andresi* | *Martialia hyadesi* | SW Atlantic | 9.7 | 8.2 | Smooth; thick | 3 | 16–20 | Gestal et al. (2005a,b) |
| *Eberthi* | *S. officinalis* | NE Atlantic, W Mediterranean | 8–9 | – | Smooth | 3 | 15–17 | Labbé (1895) |
| *Kudoi* | *S. elliptica* | NW Indian | 9–14 | – | Smooth | 6–12 | 16–18 | Narasimhamurti (1979) |