Aggregata Bathytherma SP. NOV. (APICOMPLEXA: COCCIDEA: AGGREGATIDAE), A NEW COCCIDIAN PARASITE ASSOCIATED WITH A DEEP-SEA HYDROTHERMAL VENT OCTOPUS

C. Gestal[1]*, S. Pascual[1], F.G. Hochberg[2]

[1]Instituto de Investigaciones Marinas. Consejo Superior de Investigaciones Científicas. Eduardo Cabello 6, 36208 Vigo, Spain

[2]Department of Invertebrate Zoology. Santa Barbara Museum of Natural History. 2559 Puesta del Sol, Santa Barbara, CA 93105, USA

*Instituto de Investigaciones Marinas
Consejo Superior de Investigaciones Científicas
Eduardo Cabello 6
36208 Vigo, Spain
Tf. +34 986 231930 Ext. 285
e-mail: cgestal@iim.csic.es

ABSTRACT: *Aggregata bathytherma* sp. nov. is described from the digestive tract of *Vulcanoctopus hydrothermalis*, a deep-sea octopus recently discovered associated with hydrothermal vents in the northeast Pacific Ocean. Oocysts typically are spherical in shape, sometimes irregular, 163-356 µm in length, and 219-313 µm in width. Each oocyst contains from 50 to over 200 sporocysts. Sporocyst measure 27-32 µm in longest diameter. The cyst wall is smooth and 1 µm thick. Each sporocyst typically contains 14-17 sporozoites, 49 µm in length. Histological lesions associated with the presence of *A. bathytherma* include rupture of the basal membrane and detachment of the epithelial cells. In heavily infected areas most of the tissue of the host digestive tract is replaced by parasites. *Aggregata bathytherma* is the first *Aggregata* species described from a host that lives in association with hydrothermal vents, and the third species of *Aggregata* from eastern North Pacific waters.

KEY WORDS: *Aggregata bathytherma*, *Vulcanoctopus hydrothermalis*, coccidian parasite, deep-sea hydrothermal vent

INTRODUCTION

Species within the genus *Aggregata* are intracellular coccidian parasites with a two-host life cycle. Sexual stages, gamogony and sporogony, occur in the digestive tracts of cephalopods, the definitive host, and asexual stages, merogony, in the digestive tracts of crustaceans, the intermediate hosts (Dobell, 1925; Gestal et al., 2002a). The union of a microgamete and a macrogamete in the digestive tract of cephalopods produces a zygote, which become an early sporont with numerous nuclei. Individual nuclei with accompanying cytoplasm later bud off, forming uninucleated spherical sporoblasts. The development of sporoblast into sporocysts is characterized by an increase in the number of nuclei and further partitioning of nuclei and cytoplasm forming sporozoites. After formation and maturation, sporocysts containing infective sporozoites leave the host with the faces. In the sea sporocysts must be ingested by a suitable crustacean intermediate host to continue their life cycle. Sporocysts pass to the digestive tract of the crustacean where they hatch as a result of the action of stomach acid and release sporozoites. Sporozoites migrate through the midgut epithelium to the submucous connective tissue, where they grow and become meronts. Finally, meronts generate merozoites by schizogony in the same way as the sporozoites develop in the cephalopod host (Hochberg, 1990; Gestal et al., 2002a). Experimental infections have concluded that coccidians of the genus *Aggregata* show a high degree of specificity in the definitive host, however, lower specificity was observed in the intermediate crustacean host (Gestal et al., 2002a).

To date coccidian infections have not been reported from deep-sea invertebrates. All previous records are known from shallow-water cephalopod and pelagic-benthic crustaceans (Hochberg, 1990). In contrast haematozoans have been described in deep-sea demersal fishes in the Atlantic Ocean (Khan *et al.*, 1992), and haemogregarines infecting blood cells of *Zeus capensis* from deep waters of South Africa (Smit & Davies, 2006). A diversity of myxosporidians have been reported from deep-water fishes, especially macrourids, in both the Atlantic and Pacific (Yoshino & Moser, 1974; Threlfall & Khan, 1990; Lom & Dyková, 1992). With regard to crustaceans, five named and numerous unnamed species of *Aggregata* are known to occur in benthic and pelagic crustacean hosts (see Hochberg, 1990; Théodoridès & Desportes, 1975). However, no data exist on the presence of coccidian parasites in deep-sea crustaceans.

Few years ago, González, Guerra, Pascual & Briand (1998), described a new genus and species of a deep-sea octopus named *Vulcanoctopus hydrothermalis*. The species inhabits depths ranging from 2500-2700 m where it lives in close association with hydrothermal vents on the East Pacific Rise. This paper presents morphological and morphometric characteristics on the sporogonial stages of a new species of coccidia parasite of the genus *Aggregata* found in this hydrothermal octopus. This is the third species of *Aggregata* to be described from the northeastern Pacific Ocean.

MATERIALS AND METHODS

Samples of *Vulcanoctopus hydrothermalis* were caught during several dives of the deep-sea manned submersible *Alvin* at the “Genesis” site on the East Pacific Rise at 12°48.68’N, 103°56.39’W. The octopod hosts were collected by a robotic arm grab at depths ranging from 2595-2635 m. Host specimens were collected near a high temperature hydrothermal vent. Octopus specimens were frozen immediately following capture. In the laboratory they were fixed in 10% formalin and later transferred and preserved in 70% ethanol. Dorsal mantle length (DML), total body weight (BW), sex, and stage of maturation were recorded for each octopus examined (see Table 1).

Aggregata oocysts were obtained from the digestive tracts, mainly caecum and intestine, of infected octopus previously deposited at the Santa Barbara Museum of Natural History. Squash preparations were examined by excising white cysts containing sporocysts and crushing them between two microslides. Sporogonial stages were measured under 100x magnification with an oil immersion objective using a calibrated ocular micrometer. All measurements are in micrometers (μm) unless otherwise indicated.

Paraffin embedded tissue which contained *Aggregata* was sectioned at 4 μm and stained with hematoxylin and eosin following standard procedures (Culling et al., 1985).

RESULTS

***Aggregata bathytherma* sp. nov.**

(Fig.1A-I)

Description

Material examined: Based on the examination of 5 infected octopus (Table 1).

Oocysts: Shape typically spherical, sometimes irregular; sporocyst numbers range from 50 to over 200. Lengths range from 163-245.6-356; widths 219-255.0-313 (n=10, 3 hosts).

Sporocysts: Mature sporocysts large; shape subspherical to subovoid; surface smooth with thick wall (1 μm). Lengths range from 27-28.7-32; widths 24-27.9-32 (n=30, 3 hosts). Giant sporocysts not observed.

Sporozoites: Number of sporozoites typically 14-17 per sporocysts (n=5, measured in 1 octopus); curled in spiral within sporocyst. Isolated sporozoites uniform in size, length 49, width 5 (n=5, measured in 1 octopus).

Taxonomic summary

Type Specimens (Syntypes): Histological sections of host digestive tracts containing mature sporocysts deposited in the Santa Barbara Museum of Natural History: SBMNH 345335 (2 microslides) and SBMNH 345347 (2 microslides).

Type Locality: Northeastern Pacific Ocean, East Pacific Rise, “Genesis” site, 12°48.68’N, 103°56.39’W, 2595-2635 m.

Other Localities: Additional material examined in this study was collected in the region of the type locality and to the south at 9°50.33’N, 104°17.48’W. Cephalopod hosts were collected at depths ranging from 2512-2635 m.

Symbiotype: *Vulcanoctopus hydrothermalis* González, Guerra, Pascual & Briand, 1998 (González et al., 1998)(Mollusca: Cephalopoda: Octopodidae).

Symbiotype: Mature male, 37 mm ML; SBMNH 142882.

Additional Host Vouchers: See Table 1.

Additional Host Species: None.

Prevalence: The infection was confirmed in all five hosts examined (see Table 1).

Site of infection: Sporogonial stages (sporoblasts and sporocysts containing sporozoites) present in the intestine, spiral caecum, and other non-cuticularized regions of the host digestive tract.

Etymology: The specific name is derived from the Greek word *bathytherma* meaning “deep heat” in reference to the host’s association with deep-sea hydrothermal vents.

Histopathology

Marked distension of the infected tissue area (intestine and caecum) due to the development of the sporogonic stages was seen, causing rupture of the basal membrane and the detachment of the epithelial cells (Fig. 1A, C, E). In heavily infected areas most of the infected host tissue was replaced by parasites, resulting in the loss of the digestive tract epithelium and destruction of the tissue organ architecture (Fig. 1G).

DISCUSSION

At present nine named and several more unnamed species of *Aggregata* have been reported in the literature to occur in cephalopod hosts (Hochberg, 1990; Poynton et al., 1992; Sardella & Re, 1988; Gestal et al., 1999; Gestal et al., 2000; Gestal et al., 2005). Additionally, five named and numerous unnamed species of *Aggregata* are known to occur in benthic and pelagic crustacean hosts (Hochberg, 1990; Thédoridès & Desportes, 1975). Of these, only two species previously have been described from octopuses in the northeastern Pacific Ocean by Poynton et al. (1992), namely: *A. dobelli* in *Enteroctopus dofleini* (Wülker, 1910); and *A. millerorum* in *Octopus bimaculoides* Pickford & McConnaughey, 1949. Furthermore, two other *Aggregata* species have been described in nerito-oceanic ommastrephid squids, namely: *A. sagittata* in *Todarodes sagittatus* (Lamarck, 1798) from the northeastern Atlantic Ocean (Gestal et al., 2000); and *A. andresi* in *Martialia hyadesi* Rochebrune & Mabille, 1889 from the southwestern Atlantic Ocean, at the Antarctic Polar Front Zone (Gestal et al., 2005).

Traditionally, diagnostic characters among species of *Aggregata* include phenotypic aspects related to the sporocyst structure (shape, size and thickness of the outer surface wall), number and size of sporozoites contained within the sporocysts, as well as data on host-specificity (Table 2). *Aggregata bathytherma* sp. nov. can be distinguished from all other known species in the genus by: 1) larger sporocyst size; 2) larger size and larger number of sporozites in each sporocyst; and 3) thick, smooth sporocyst wall. The later character could be an adaptation to the greater depth (*i.e.*, higher hydrostatic pressure) to which *V. hydrothermalis* is exposed. This character previously has been reported for *A. sagitattus* infecting nerito-oceanic ommastrephid squids from the NE Atlantic (Gestal et al., 2005).

The histopathological analysis is coincident with the previously described for other *Aggregata* species. The damage depends upon the intensity of infection, as it is proportional to the degree of destruction of host cells (Gestal et al., 2002b). Similarly than observed in *O. vulgaris* infected by *A. octopiana*, the destructive effect of this parasite deduced by histopathological analysis may impair on gastrointestinal functions, including the correct absorption of nutrients (Gestal et al., 2002c), and may have weakened the octopuses, making them more vulnerable to other biotic and abiotic effects.

The description of a new *Aggregata* species for the first time in a deep-sea cephalopod host suggests a broader habitat and distributional range than previously expected not only for the genus *Aggregata*, but also for any coccidian parasite. With regard to food habits, and determining potential intermediate host, very little information is available. Rocha et al. (2002) indicated that *V. hydroythermalis* likely feed on hydrothermal vent crabs, *Bythograea thermydron* Williams, 1980. Voight (2005) reported that remains of the bathypelagic amphipod *Halice hesmonectes* Martin, France & Van Dover, 1993 were found in the gut of the octopod. Other authors have suggested that this octopus may also feed on deep-sea anomuran crabs. At present no deep-sea crustaceans have been examined for the presence of *Aggregata*.

Although traditional identification and characterization of *Aggregata* species has relied primarily on differences in morphological features well standardized such as size and shape of life-cycle stages and host specificity (Hochberg, 1990), molecular techniques provide alternative methods for taxonomic studies and are important tools in solving the problems of species delimitation. The only molecular reference up to date is referred to the nucleotide analysis of the small rDNA subunit of *Aggregata* species infecting the coastal cephalopods *O. vulgaris* and *Sepia officinalis* (Kopecná et al., 2006). Therefore, molecular sequence analysis of small and large nuclear rDNA subunits or even internal transcribed spacers (ITS) should be carried out to ascertain the taxonomic status of *Aggregata* species within cephalopods, supporting their current classification using morphological characters, to confirm their taxonomic affiliation within the genus and to validate conservative, robust phenotypic characters useful as diagnostic tools.

Acknowledgments. We are indebted to James J. Childress (University of California, Santa Barbara, California) and Richard A. Lutz (Rutgers University, New Brunswick, New Jersey) for collecting the octopod host specimens while on expedition to the East Pacific Rise. Brad A. Seibel (University Rhode Island, Kingston, Rhode Island), Michael Sweeney (National Museum of Natural History,

Smithsonian Institution, Washington, D.C.), and Janet R. Voight and Jon Slapsinsky (Field Museum, Chicago, Illinois) facilitated our study by providing donations or loans of octopus host specimens.

LITERATURE CITED

Culling CFA, Allison RT, Barr WT (1985) Cellular Pathology Technique. Butterworth & Co., London.

Dobell CC (1925) The life history and chromosome cycle of *Aggregata eberthi*. Parasitol 17: 1-139

Gestal C, Pascual S, Corral L, Azevedo C (1999) Ultrastructural aspects of the sproogony of *Aggregata octopiana* (Apicomplexa, Aggregatidae) a coccidian parasite of *Octopus vulgaris* (Mollusca, Cephalopoda) from NE Atlantic coast. Europ J Protistol 35: 417-425

Gestal C, Guerra A, Pascual S, Azevedo C (2002a) On the life cycle of *Aggregata eberthi* and observation on *Aggregata octopiana* (Apicomplexa, Aggregatidae) from Galicia (NE Atlantic). Europ J Protistol 37: 427-435

Gestal C, Abollo E, Pascual S (2002b) Observations on associated histopathology with *Aggregata octopiana* infection (Protista: Apicomplexa) in *Octopus vulgaris*. Dis Aquat Org 50: 45-49

Gestal C, Páez de la Cadena M, Pascual S (2002c) Malabsorption syndrome observed in the common octopus *Octopus vulgaris* infected with *Aggregata octopiana* (Protista: Apicomplexa). Dis Aquat Org 51: 61-65

Gestal C, Guerra A, Abollo E, Pascual S (2000) *Aggregata sagittata* n. sp. (Apicomplexa: Aggregatidae), a coccidian parasite from the European flying squid *Todarodes sagitatus* (Mollusca: Cephalopoda). Syst Parasitol 47: 203-206

Gestal C, Nigmatullin ChM., Hochberg FG, Guerra A, Pascual S (2005) *Aggregata andresi* n. sp. (Apicomplexa: Aggregatidae) from the ommastrephid squid *Martialia hyadesi* in the SW Atlantic Ocean and some general remarks on *Aggregata* spp. in cephalopod hosts. Syst Parasitol 60: 65-73

González AF, Guerra A, Pascual S, Briand P (1998) *Vulcanoctopus hydrothermali*s gen. et sp. nov. (Mollusca, Cephalopoda): an octopod from a deep sea hydrotermal vent. Cah Biol Mar 39: 169–184

Hochberg FG (1990) Diseases of Mollusca: Cephalopoda. Diseases caused by protistans and metazoans. In: Kinne O (ed) Diseases of Marine Animals. Vol. III. Cephalopoda to Urochordata. Biologisches Anstalt Helgoland, Hamburg, Germany, p 47-227

Khan RA, Threlfall W, Whitty WS (1992) Hematozoa of deep-sea demersal fish of the northwestern Atlantic ocean. Can J Zool 70: 1926-1933

Kopecná J, Jirku M, Miroslav O, Tokarec YS, Lukes J, Modrý D (2006) Phylogenetic analysis of coccidian parasites from invertebrates: search for missing links. Protist 157: 173-183

Labbé A (1895) Sur le noyau et la division nucléaire chez les Benedenia. C R Acad Sci Paris, CXX. 381 pp.

Lom J, Dyková I (1992) (ed) Protozoan parasites of fishes. In: Developments in Aquaculture and Fisheries Science. Vol. 26. Elsevier, London, p 1-315

Narasimhamurti CC (1979) The Eimeriid *Aggregata kudoi* n. sp. from *Sepia eliptica*. Angew Parasitol 20: 154-158

Poynton SL, Reimschuessel R, Stoskopf MK (1992) *Aggregata dobelli* n. sp. and *Aggregata millerorum* n. sp. (Apicomplexa: Aggregatidae) from two species of octopus (Mollusca: Octopodidae) from the eastern North Pacific Ocean. J Parasitol 39(1): 248-256

Rocha F, González AF, Segonzac M, Guerra A (2002) Behavioural observations of the cephalopod *Vulcanoctopus hydrothermalis*. Cah Biol Mar 43: 299-302

Sardella NH, Re ME (1988) Parasitosis por coccidios del genero *Aggregata* en pulpos costeros patagonicos. I. *Aggregata* sp. en *Octopus tehuelchus* d'Orbigny. Physis, (Buenos Aires) (Sec. A) 46(111): 51-60

Sardella NH, Ré ME, Timi JT (2000) Two new *Aggregata species* (Apicomplexa: Aggregatidae) infecting *Octopus tehuelchus* and *Enteroctopus megalocyatus* (Mollusca: Octopodidae) in Patagonia, Argentina. J Parasitol 86(5): 1107-1113

Smit NJ, Davies AJ (2006) *Desseria zei* sp. nov. (Adeleorina: Haemogregarinidae) infecting *Zeus capensis* from deep waters off the south and west coasts of South Africa. J Mar Biol Ass UK 86 : 1477-1480.

Schneider A (1875) Note sur le sporospermies oviformes du poulpe. Archs Zool Exp Gén 4 : 11-14

Théodoridès J, Desportes I (1975) Sporozoaires d'invertébrés pélagiques de Villefranche-sur-Mer (étude descriptive et faunistique). Protistologica 1: 205-220

Threlfall W, Khan RA (1990) Myxozoa of deep-sea fishes in the northwestern Atlantic. J Parasitol 76(2): 288-290

Voight JR (2005) Hydrothermal vent octopuses of *Vulcanoctopus hydrothermalis* feed on bathypelagic amphipods of *Halice hesmonectes*. J Mar Biol Assoc UK 85: 985-988

Yoshino TP, Moser M (1974) Myxosporida (Protozoa) in macrourid fishes (*Coryphaenoides* spp) of the northeastern Pacific. J Parasitol 60(4): 655-659

FIGURE AND TABLE LEGENDS

Fig. 1. *Aggregata bathytherma* sp. nov.: Histological sections of intestine and caecum of *Vulcanoctopus hydrothermalis* infected by parasite. (A) Oocyst containing sporoblast with sporozoites in formation. Arrow shows rupture of infected tissue from development of the coccidian detachment and loss of epithelial cells. (B) Detail of the sporoblast development and division to form sporozoites. (C, E) Caecum villous infected with parasite showing oocysts containing sporocysts and destruction of the tissue organ architecture by replacement by parasites. Arrows show distension and rupture of the basal membrane and loss of digestive tract epithelium. (D) Oocyst containing sporocysts with mature sporozoites inside. (F) Detail of mature sporocysts containing inside between 14-17 sporozoites. G. Heavy infected intestinal area showing replacement of the infected host tissue by parasites, resulting in the loss of the digestive tract epithelium and destruction of the tissue organ architecture. (H) Detail of sporozoites inside the sporocyst. Transversal section. I. Detail of sporozoites inside the sporocyst. Longitudinal section. Scale bars: A, C, D, E, G = 30 µm; B, E, F, G, H, I = 15 µm

Table 1. *Vulcanoctopus hydrothermalis* specimens examined for the presence of *Aggregata bathytherma* sp. n. Host octopus ordered by mantle length (ML) and body weight (BW). # = mantle length distorted. * = host symbiotype

Table 2. Comparative data on morphology and morphometry of *Aggregata* species based on sporogonial stages. n: mean length or width measurement of sporocysts

Table 1.

Sex	**Maturity**	**ML (mm)**	**BW (g)**	***Aggregata***	**Host Repository & Catalog No.**
female	mature	38	16.6	++	SBMNH 142880
*male	mature	37	18.6	++	SBMNH 142882
male	mature	52#	20.6	++	FMNH 27864
male	mature	45	21.8	++	SBMNH 142881
male	mature	53	30.6	++	USNM 885672

Table 2.

Aggregata species	Host	Locality (Ocean/Sea	Sporocysts			Sporozoites		References
			length	width	cyst wall	no	length	
A. bathytherma sp. n.	*Vulcanoctopus hydrothermalis*	NE Pacific	27-28.7-32	24-27.9-32	smooth; thick	14-17	49	Present paper
	Ommastrephidae							
A. andresi	*Martialia hyadesi*	SW Atlantic	9.7	8.2	smooth; thick	3	16-20	Gestal et al., 2005
A. sagittata	*Todarodes sagittatus*	NE Atlantic	17	15	smooth; thick	4-8	12	Gestal et al., 2000
	Sepiidae							
A. eberthi	*Sepia officinalis*	NE Atlantic & W Mediterranean	8-9	-	smooth	3	15-17	Labbé, 1895
A. kudoi	*S. elliptica*	NW Indian	9-12-14	-	smooth	6-12	16-18	Narasimhamurti, 1979
	Octopodidae							
A. octopiana	*Octopus vulgaris*	NE Atlantic & W Mediterranean	11-15	11-15	spiny	8	16-20-24	Schneider, 1875 Gestal et al., 1999
A. dobelli	*Enteroctopus dofleini*	NE Pacific	18-23-31	15-21-27	smooth	9-22	18-21-23	Poynton et al., 1992
A. millerorum	*O. bimaculoides*	NE Pacific	12-16-20	11-14-17	smooth	8-10	18-24-31	Poynton et al., 1992
A. patagonica	*E. megalocyatus*	SW Atlantic	13	12	smooth	8	18	Sardella et al., 2000
A. valdesensis	*O. tehuelchus*	SW Atlantic	10	10	-	4-8	17	Sardella et al., 2000