J. Parasitol., 86(5), 2000, p. 1107–1113

TWO NEW *AGGREGATA* SPECIES (APICOMPLEXA: AGGREGATIDAE) INFECTING *OCTOPUS TEHUELCHUS* AND *ENTEROCTOPUS MEGALOCYATHUS* (MOLLUSCA: OCTOPODIDAE) IN PATAGONIA, ARGENTINA

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ABSTRACT: During a long-term study carried out between 1981 and 1996 on the biological and fishery aspects of octopuses inhabiting the Gulfs of San Matías, San José, and Nuevo, Patagonia, Argentina, 2 new species of *Aggregata* (Apicomplexa: Aggregatidae) were found in the digestive tracts of *Octopus tehuelchus* d'Orbigny, 1834 (prevalence 72%) and *Enteroctopus megalocyathus* (Gould, 1852) (prevalence 77%). Both species can be distinguished from other congeners on the basis of their hosts, the diameters of sporocysts, and number and length of sporozoites. Despite overlap in the distributions of the two hosts species in the area covered in this study, both *Aggregata* new species exhibited high host specificity.

In the eimeriorin Aggregatidae Labbé, 1899, several species have heteroxenous life cycles, with merogonic development occurring in 1 host, and gamogonic and sporogonic development in another (Desser et al., 1998). The 2-host life cycle of *Aggregata* Frenzel, 1885 was elucidated by Léger and Duboscq (1906) as cited in Hochberg (1983) and Dobell (1925) for *A. eberthi* (Labbé, 1895) Léger and Dubosq (1906); it was experimentally confirmed by Porchet et al. (1981). Merogony is known to occur in the crab *Macropipus* (*Portunus*) *depurator* Linnaeus, whereas gamogony and sporogony occur in the cephalopod, *Sepia officinalis* Linnaeus, 1758 (Théodorides, 1965; Porchet-Henneré and Richard, 1971; Vivares, 1972,1973; Levine, 1988). Of the 7 genera occurring in marine invertebrates, *Aggregata* is the best known and is the type genus for the family (Levine, 1988; Poynton et al., 1992).

During a long-term study on the biological and fishery aspects of octopuses from Argentinean coasts (Ré, 1998a, 1998b), species of *Aggregata* were observed. Sardella and Ré (1990) reported the gamogonic and sporogonic development of *Aggregata* sp. in the cecum, proximal intestine, and crop of *Octopus tehuelchus* d'Orbigny, 1834 from Puerto Lobos (San Matías Gulf, 42°S/65°04′W) and sporocysts of *Aggregata* sp. in the digestive tract of the octopodids *Eledone massyae* Voss, 1964 and *Enteroctopus megalocyathus* (Gould, 1852). Recently, Sardella and Martorelli (1997) described the merogony of *Aggregata* spp. from crustaceans other than brachiurans; that is, the natantians *Pleoticus muelleri* (Bate, 1888) and *Artemesia longinaris* Bate, 1888 from San Jorge Gulf (45°47′S/65°50′W) and Rawson Port (43°15′S/65°05′W).

The aim of the present paper is to describe the gamogonic and sporogonic stages of 2 new species of coccidians from 2 species of cephalopods (Mollusca: Octopodidae) inhabiting the north Patagonian coasts of Argentina.

MATERIALS AND METHODS

A total of 1,977 specimens of *O. tehuelchus* were caught between 1983 and 1996 in the Gulfs of San Matías, San José, and Nuevo; 121 *Enteroctopus megalocyathus* were collected between 1981 and 1995 in the Gulfs of San José and Nuevo, between 42° and 42°47′S/64°27′S and 65°04'W, Chubut Province, Argentina (Fig. 1). All specimens were examined for coccidians.

Each host was dissected and the digestive tract (crop, cecum, and intestine for *O. tehuelchus* and only intestine for *E. megalocyathus*) was examined. Those octopuses with white cysts in the gut were considered as infected. Subsamples were fixed in formalin; the white cysts were excised, squashed by cover glass pressure, and observed under a light microscope.

For histological observations, the digestive tracts of 70 *O. tehuelchus* and 19 *E. megalocyathus* were fixed in 10% formalin or Bouins, embedded in paraffin, sectioned, and stained with hematoxylin–eosin.

Gamogonic and sporogonic stages of the 2 species of *Aggregata* were described and measured from hematoxylin-and-eosin-stained slides, with the exception of isolated sporozoites, which were measured from squashed cysts from formalin-fixed material.

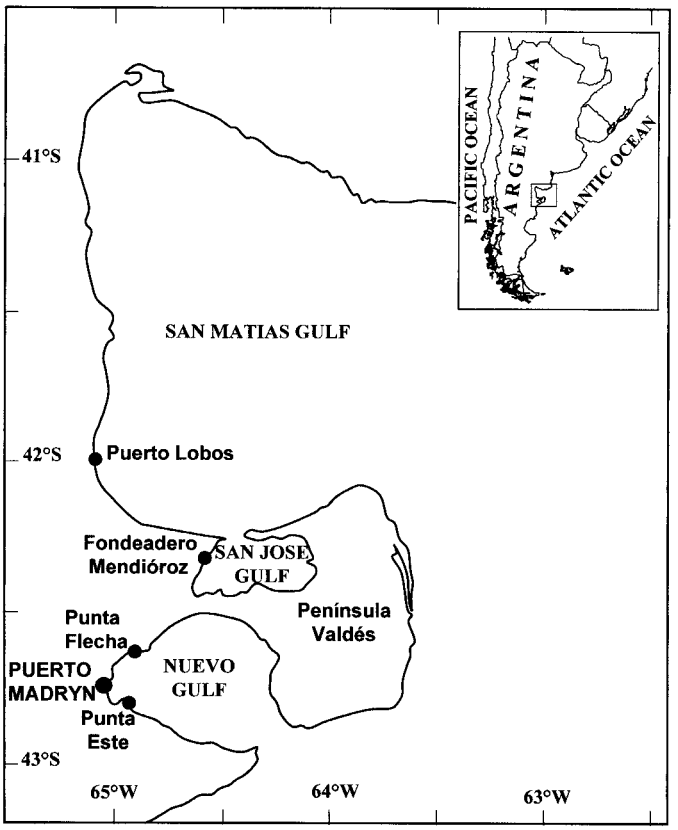

FIGURE 1. Map showing the study area.

Received 29 September 1999; revised 13 March 2000; accepted 13 March 2000.

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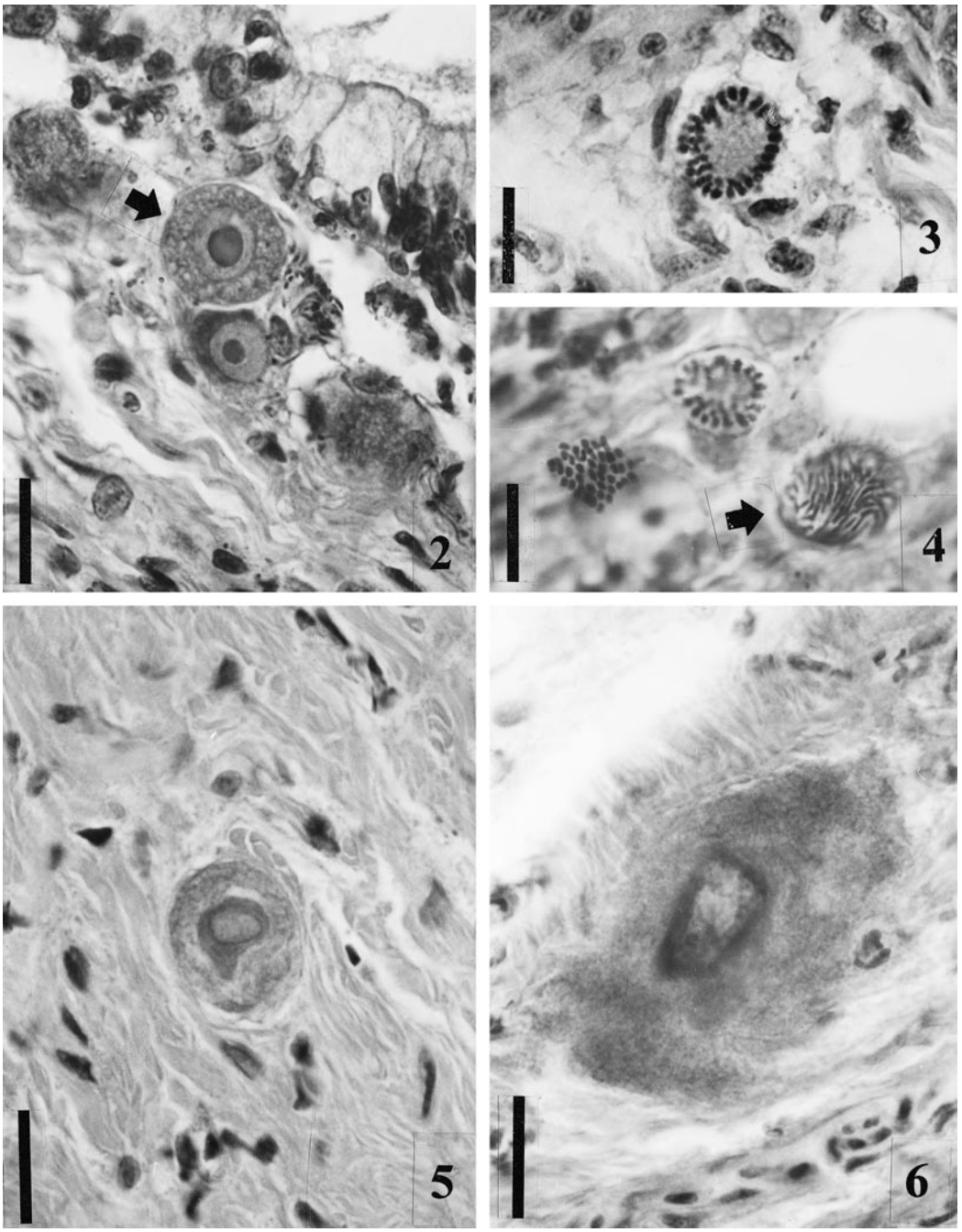


FIGURES 2–6. Gamogony of *Aggregata valdessensis* n. sp. **2.** Young microgamont (arrow). **3.** Advanced microgamont. **4.** Microgamont with microgametes (arrow). **5.** Young macrogamete. **6.** Mature macrogamete (fertilized?). Scale bars = 30 μm.

Measurements are given in micrometers, with the mean followed by range in parentheses.

DESCRIPTION

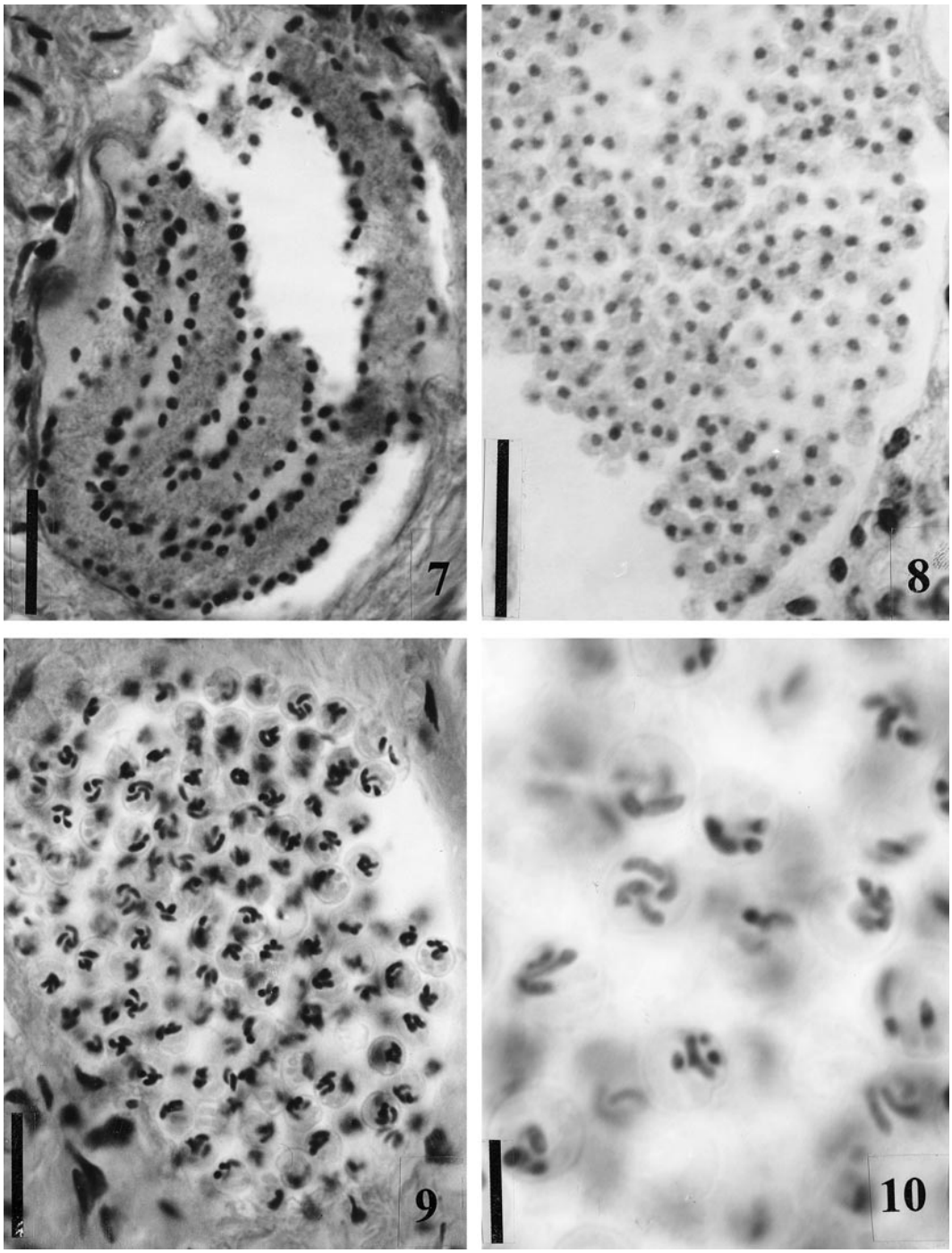
Aggregata valdessensis n. sp.
(Figs. 2-10)

Gamogony (Figs. 2–6)

Young microgamont (Fig. 2) oval, cytoplasm with inclusions, central nucleus surrounded by a pale homogenous area. Advanced microgamont (Fig. 3) with numerous peripheral condensed nuclei. Mature microgametes (Fig. 4) with condensed nuclei and flagella. Young macrogamete (Fig. 5) irregular in shape, similar in size to young microgamont, without the pale area around nucleus mentioned above. Mature macrogamete (fertilized?) (Fig. 6) enlarged, irregular to polygonal in section, darkly stained, with spongy cytoplasm.

Sporogony (Figs. 7–10)

Sporont (Fig. 7) with deeply folded cytoplasm, and numerous nuclei in peripheral cytoplasm. Uninucleate sporoblasts (Fig. 8), with spherical to subspherical cytoplasm, forming a mass filling the oocyst. Young



FIGURES 7–10. Sporogony of *Aggregata valdessensis* n. sp. **7.** Sporont showing folding. **8.** Oocyst with uninucleate sporoblasts. **9.** Mature sporocysts with 4 sporozoites. **10.** Detail of mature sporocysts. Scale bars 7–9 = 30 μm; 10 = 10 μm.

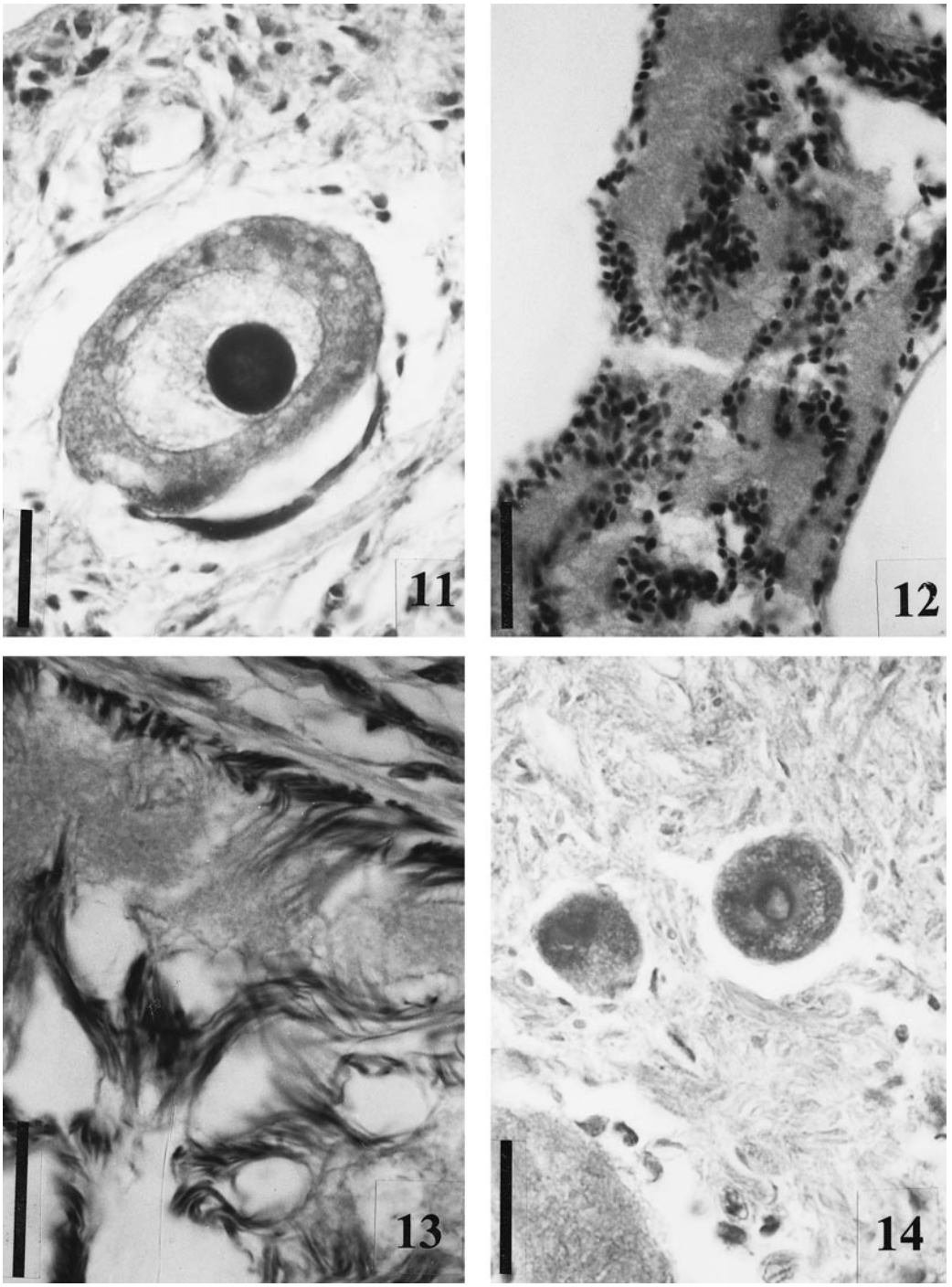
sporocysts with capsule, with 1 to 4 nuclei. Smooth mature sporocysts containing 4 sporozoites (Figs. 9, 10).

Oocysts spherical to irregularly shaped, with numerous sporocysts. Measurements based upon 16 oocysts (5 octopuses): 250 ± 75 μm long by 169 ± 39 μm wide.

Mature sporocysts large, ovoid to spherical; surface smooth; within a single oocyst, all sporocysts at approximately the same stage of development. Measurements based upon 19 sporocysts (8 oocysts, 6 octopuses): 9.12 ± 0.36 μm long by 8.5 ± 4.6 μm wide. In some oocysts, a small number of giant sporocysts, containing 8 sporozoites, was observed; measurements from 5 sporocysts (2 oocysts, 1 host): 11.82 ± 0.4 μm long by 11.01 ± 0.4 μm wide.

Sporozoites vermiform, commonly 4 per sporocyst, twisted inside sporocyst, with nucleus at 1 pole. Measurements based upon 23 sporozoites (10 oocysts, 2 hosts): 16.73 ± 1.09 μm long by 2.65 ± 0.29 μm wide; nucleus approximately more than one-third length of sporozoite and located near its posterior end (20 sporozoites, 5 oocysts, 2 hosts): 7.24 ± 1.5 μm long by 2.65 ± 0.1 μm wide.

Sporulation endogenous. Sporocysts fully sporulated in host.



FIGURES 11–14. Gamogony of *Aggregata patagonica* n. sp. **11.** Young microgamont. **12.** Advanced microgamont. **13.** Microgametes. **14.** Young macrogamete. Scale bars = 30 μm.

Taxonomic summary

Type host: Octopus tehuelchus d'Orbigny, 1834.

Type locality: Puerto Lobos, San Matías Gulf (42°S/65°04′W), Chubut Province, Argentina. Depth of capture: 0–20 m.

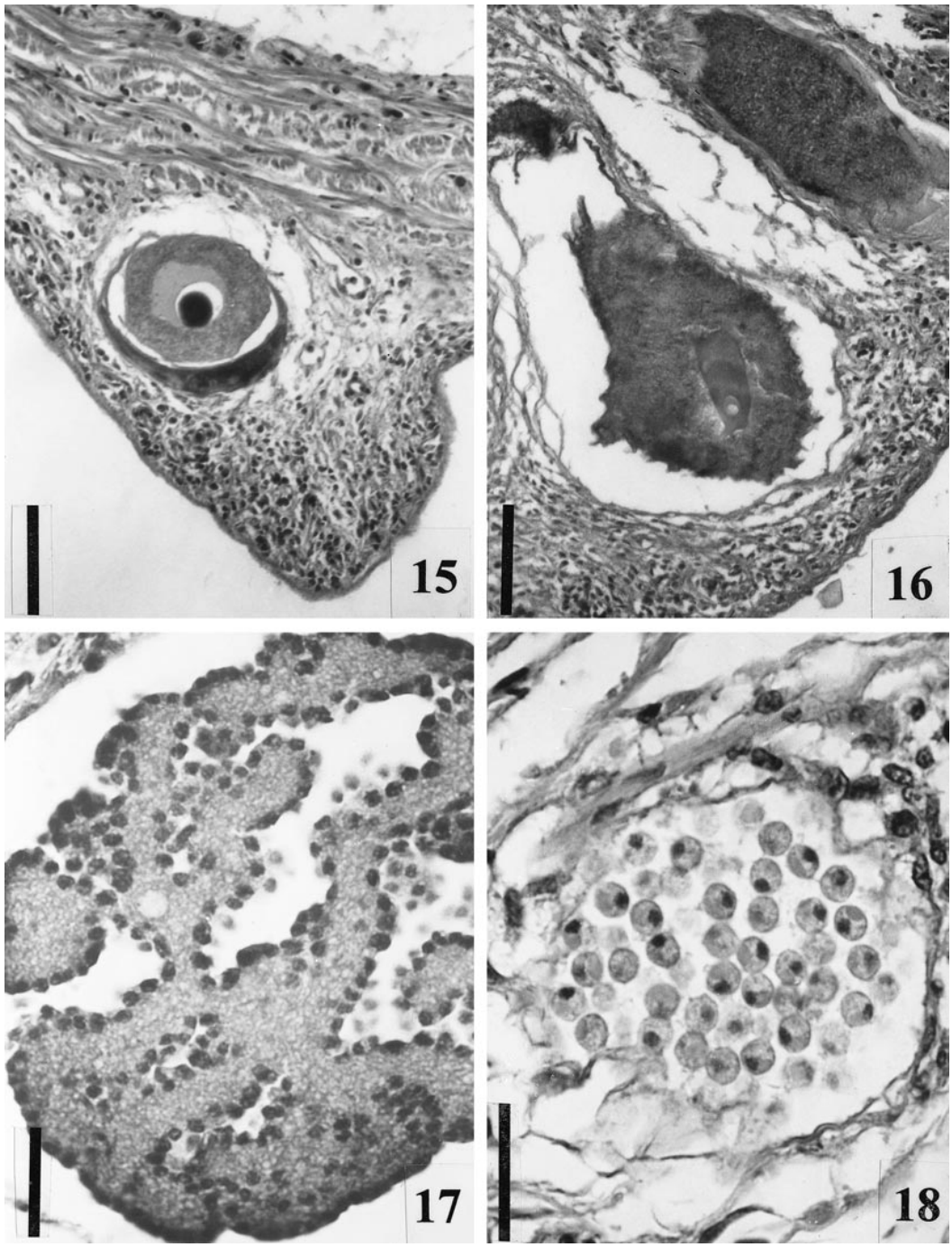
Other localities: Fondeadero Mendióroz, San José Gulf (42°14′S/64°27′W), Punta Flecha, Nuevo Gulf (42°38′S/64°58′W), Chubut Province, Argentina. Depth of capture: 0–20 m.

Site of infection and pathology: Macroscopically, oocysts are observed as pearly white cysts, located in cecum and proximal intestine (in crop of 2 specimens). Nuclei and cytoplasms of host cells were hypertrophied. Parasites were observed in epithelium, sub- and muscularis mucosa and serosa, most frequently in submucosa. In heavy infections, parasites replaced host tissues, with deformed villosities.

Prevalence: Seventy-two percent (1,427 of 1,977) of *O. tehuelchus* examined were infected.

Type specimens: Syntypes (2 glass slides of stained tissues sections) deposited in Colección Zoología Invertebrados del Museo de La Plata (Protistas), Argentina. Coll. no. 005 and 006.

Etymology: The specific name refers to the geographic area (Península Valdés) where parasites were found.



FIGURES 15–18. Gamogony and sporogony of *Aggregata patagonica* n. sp. **15.** Mature macrogamete. **16.** Mature macrogamete (fertilized?). **17.** Sporont showing extensive folding. **18.** Oocyst with uninucleate sporoblasts. Scale bars 15–16 = 100 μm; 17–18 = 30 μm.

***Aggregata patagonica* n. sp.**
(Figs. 11–23)

Gamogony (Figs. 11–16)

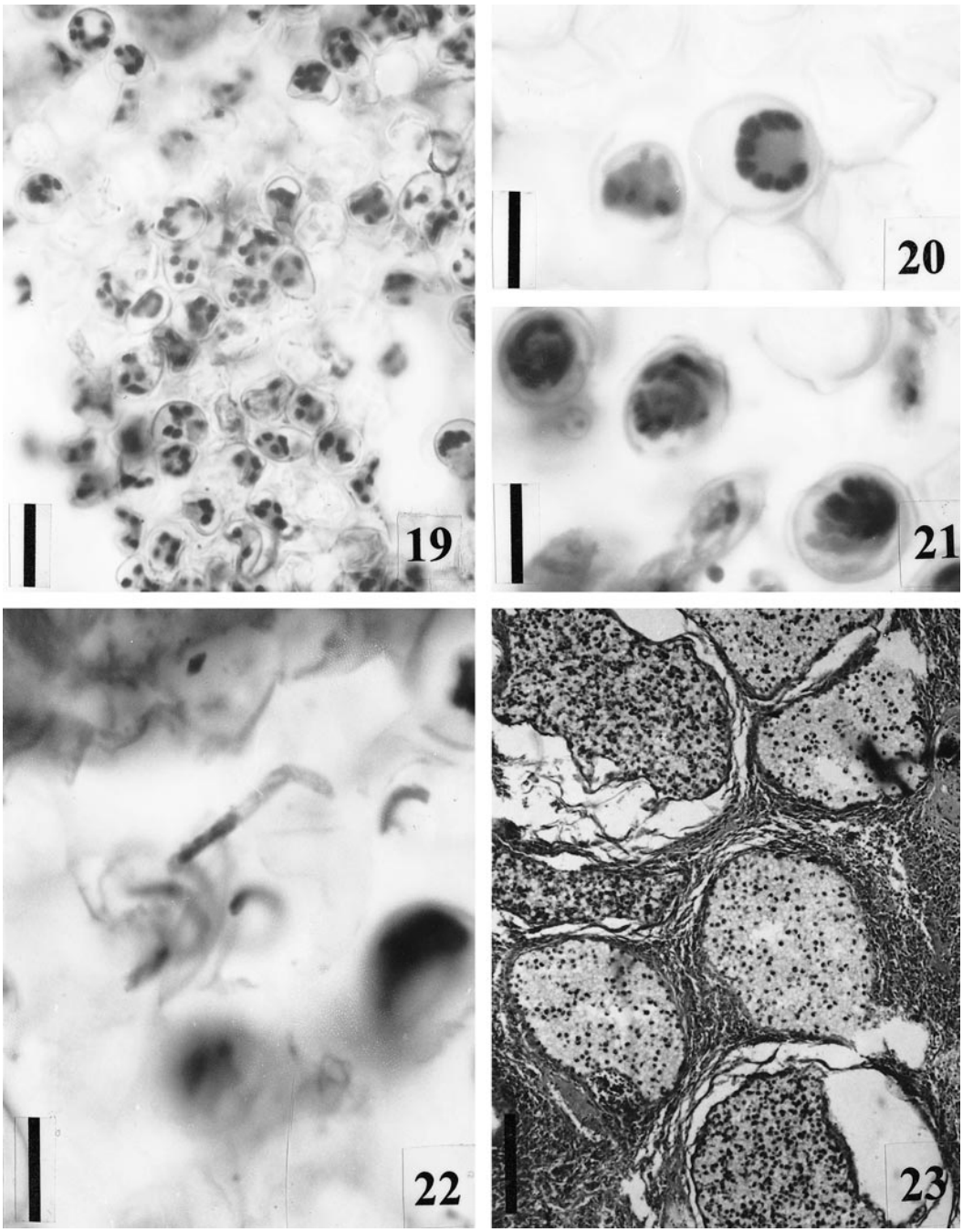
Young microgamont (Fig. 11) oval, cytoplasm with inclusions, central nucleus surrounded by a pale homogenous area. Advanced microgamonts (Fig. 12), cytoplasm highly folded, with numerous peripheral nuclei. Mature microgametes (Fig. 13) with condensed nuclei and flagella. Young macrogamete (Fig. 14), irregular in shape, similar in size to young microgamont, without the pale area around nuclei. Mature macrogamete (Figs. 15, 16) (fertilized?) irregular to polygonal in section, dark-stained and with spongy cytoplasm.

Sporogony (Figs. 17–23)

Sporont (Fig. 17) with deeply folded cytoplasm, with numerous nuclei on the surface. Uninucleate sporoblasts (Fig. 18) with spherical to subspherical cytoplasm, forming a mass filling the oocyst. Young sporocysts (Figs. 19, 20) with capsule, with 1, 4, or 8 nuclei. Mature sporocysts (Fig. 21), with smooth capsules, containing 8 developed sporozoites (Fig. 22).

Oocysts spherical to irregularly shaped, containing numerous sporocysts. Measurements based upon 18 oocysts (7 octopuses): 519 ± 133 μm long by 346 ± 84 μm wide.

Mature sporocysts large, spherical to ovoid; surface smooth; within



FIGURES 19–23. Sporogony of *Aggregata patagonica* n. sp. **19.** Oocyst with young sporocysts, with 4 nuclei. **20.** Octonucleate sporocyst. **21.** Mature sporocysts with sporozoites. **22.** Free sporozoites. **23.** Heavily infected intestine of *Enteroctopus megalocyathus* containing parasites at different developmental stages. Scale bars 19 = 20 μm; 20–22 = 10 μm; 23 = 200 μm.

a single oocyst, all sporocysts at approximately the same stage of development. Measurements based upon 78 sporocysts (11 oocysts, 7 octopuses): 12.60 ± 0.86 μm long by 11.70 ± 1.01 μm wide.

Sporozoites vermiform, commonly 8 per sporocyst, twisted inside sporocyst with the nucleus at 1 pole. Measurements based upon 16 sporocysts (3 oocysts, 2 octopuses): isolated sporozoites, 17.60 ± 0.71 μm long by 1.50 ± 0.16 μm wide; nucleus approximately one-third length of sporozoite (6.70 ± 0.53 μm long) and located near its posterior end.

Sporulation endogenous. Sporocysts fully sporulated in host.

Taxonomic summary

Type host: Enteroctopus megalocyathus (Gould, 1852).

Type locality: Puerto Madryn, Nuevo Gulf, Chubut Province, Argentina (42°46′S/65°02′W). Depth of capture: 0–20 m.

Other localities: Fondeadero Mendióroz, San José Gulf (42°14′S/64°27′W), Punta Flecha, Nuevo Gulf (42°38′S/64°58′W), Punta Este, Nuevo Gulf (42°47′S/64°57′W), Chubut Province, Argentina. Depth of capture: 0–20 m.

Site of infection and pathology: Macroscopically, oocysts are observed as pearly white cysts. Gamogony and sporogony in the intestine;

parasites in the esophagus and cecum not sampled. Parasites most frequently observed in submucosa, but also in muscularis and serosa. Parasites at different stages of development occupy adjacent cysts. Nuclei and cytoplasm of parasitized cells hypertrophied. In heavy infections, parasites replaced host tissues, with deformed villosities (Fig. 23).

Prevalence: Seventy-seven percent (94 of 121) of *E. megalocyathus* examined were infected.

Type specimens: Syntypes (2 glass slides of stained tissues sections) deposited in Colección Zoología Invertebrados del Museo de La Plata (Protistas), Argentina. Coll. no. 007 and 008.

Etymology: The specific name refers to the Argentinean coastal area of the present known distribution of *E. megalocyathus.*

DISCUSSION

At present, sporocysts of 9 species of *Aggregata* have been well described and measured (Poynton et al., 1992). Species of *Aggregata* are distinguished on the basis of host, diameter of sporocysts, and number and length of sporozoites (Hochberg, 1983, 1990). Following these criteria, coccidians previously reported as *Aggregata* spp. from *O. tehuelchus* and *E. megalocyathus* by Sardella and Ré (1990) are described as 2 new species. In this sense,. *A. valdessensis* is distinguished from *A. patagonica* by the smaller size of both sporocysts and sporozoites, the smaller number of sporozoites per sporocyst, and the host species. In addition to the morphometric differences in the sporogonic stages between the 2 new species, microgamete development is also different. In *A. patagonica,* microgamonts are enlarged and folded, whereas these features were not observed in *A. valdessensis.* The presence of occasional giant sporocysts, such as those found in *A. valdessensis,* has been reported for other species of *Aggregata* (Poynton et al., 1992).

Sporocyst size and sporozoite length of *A. valdessensis* resemble those of *A. eberthi* Dobell, 1925, a parasite of *Sepia officinalis* and *A. kudoi* Narasimhamurti, 1979, a parasite of *S. elliptica* Hoyle, 1885 (Poynton et al., 1992); nevertheless, the new species differs mainly by the host (an octopodid instead of a decapod) and by the presence of 4 sporozoites inside each sporocyst (3 and 6, respectively, were reported for the previously mentioned species).

Sporocyst size range of *A. patagonica* partially overlaps those of *A. octopiana* Moroff, 1908, a parasite of *O. vulgaris* Cuvier, 1797 and *A. millerorum* Poynton, Reimschuessel, and Stoskopf, 1992, a parasite of *O. bimaculoides* Pickford and McConnaughey, 1949 (Poynton et al., 1992), the latter with a similar number of sporozoites per sporocyst. However, *A. millerorum* has longer sporozoites (21 μm) than *A. patagonica* and *A. octopiana* has 9 to 16 sporozoites per sporocyst.

A. valdessensis and *A. patagonica* were common, with high prevalences in both *O. tehuelchus* and in *E. megalocyathus,* respectively. Despite overlap in the distributions of the 2 host species in the area covered in this study, and their similarity in feeding behaviour (Ré, 1998 a; Ré and Gómez Simes, 1992), they harbor different species of *Aggregata.* This is consistent with the concept of high host specificity of the species of *Aggregata* in cephalopods (Hochberg, 1990).

Merogonic phases of unidentified species of *Aggregata* were found at high prevalences in the shrimps *Pleoticus muelleri* and *Artemesia longinaris,* collected from Patagonian coasts (San Jorge Gulf and Rawson Port, respectively) (Sardella and Martorelli, 1997); these crustaceans also are found in San Matías, San José, and Nuevo Gulfs, and may be intermediate hosts in the life cycles of *A. valdessensis* or *A. patagonica,* or both.

ACKNOWLEDGMENTS

We thank all the staff at the Centro Nacional Patagónico that contributed to the present study. J. Berón, M. Díaz, J. Rúa, divers M. Medina, H. García, A. Sosa, and R. Vera, and fisherman J. Signorelli mainly helped with field samples; J. Berón, L. Kuba, and M. Monsalve assisted with laboratory work and L. Kuba and N. de Bustos prepared slides. Furthermore, Centro Nacional Patagónico provided the necessary infrastructure. This study was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET, 0516/98) and by grant 006/91 from BID-CONICET (1992–1997).

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