J. Parasitol., 91(1), 2005, pp. 152–157

GLUGEA VINCENTIAE N. SP. (MICROSPORIDIA: GLUGEIDAE) INFECTING THE AUSTRALIAN MARINE FISH *VINCENTIA CONSPERSA* (TELEOSTEI: APOGONIDAE)

A. Vagelli, A. Paramá*, M. L. Sanmartín*, and J. Leiro*†
New Jersey State Aquarium, 1 Riverside Drive, Camden, New Jersey 08103. *e-mail: mpleiro@usc.es*

ABSTRACT: A parasite of the marine fish *Vincentia conspersa* was examined by light microscopy and transmission electron microscopy. This parasite develops in the subcutaneous tissue of the body and fins, forming spherical xenomas about 1–2 mm in diameter surrounded by a layer of amorphous material. The observed characteristics of the new parasite are in line with those of the other *Glugea* species; merogony takes place in the outer zone of the cytoplasm of the host cell, sporogony takes place in sporophorous vesicles, and mature spores are located in the central part of the xenoma. Meronts were cylindrical uninucleate or occasionally triradiate multinucleate, with plasmodia in direct contact with the host cytoplasm. Sporogonic plasmodia divided by multiple cleavage to produce sporoblast mother cells, which after binary fission became sporoblasts. Two types of spores were recognized, both uninucleate, i.e., ovoid or slightly ovoid microspores with a mean size of 5.1 × 2.2 μm and much less frequent as elongated oval macrospores with a mean size of 8.9 × 3.1 μm. The polar tube has between 12 and 14 coils arranged in 1, 2, or 3 layers. Taken together, these characteristics suggest that this microsporidian infecting *V. conspersa* is a new species of *Glugea*, which we have named *Glugea vincentiae*.

Microsporidians are an unusual group of obligatory intracellular eukaryotic parasites that infect a variety of animal hosts (Canning and Lom, 1986), including almost all invertebrate phyla (fundamentally arthropods), all 5 classes of vertebrates (including bony fishes), and even some protozoans (Lom, 2002). Until quite recently, microsporidians were recognized as a separate protozoan phylum; currently, however, based on molecular phylogeny and various morphological features, microsporidians are included in the Kingdom Fungi (Keeling and Fast, 2002; Lom, 2002; Lom and Nilsen, 2003). In marine habitats, microsporidians are an important cause of disease and have significant economic effects on natural fish stocks and cultured fishes (Dyková, 1995). To date, 156 microsporidian species from 14 genera have been recorded from fish hosts (Lom and Nilsen, 2003). Of these genera, *Glugea* is among the largest, with species, some still insufficiently described, infecting a wide range of fishes from marine, euryhaline, and freshwater habitats (Lom, 2002). The apogonid (cardinal fish) *Vincentia conspersa* Klunzinger, 1872, is a marine species with a restricted geographic range, cited from southern Australia (Melbourne coast, possibly in the Spencer Gulf a little further west), Tasmania (northeast and southeast coasts), and Flinders Island off northern Tasmania. It is 1 of the few apogonid fishes found in temperate waters; the great majority of apogonids (more than 250 species) are found in warm waters. To date, no microsporidian parasite has been described from apogonids. In this article, we present a morphological and ultrastructural study of a microsporidian parasite infecting *V. conspersa*. Following detailed morphological studies with light and electron microscopy, we describe this microsporidian as a new species.

MATERIALS AND METHODS

Fifteen specimens of *V. conspersa* Klunzinger, 1872 (Teleostei, Apogonidae), were acquired from an authorized collector in February 2003 and maintained together in a 1,200-L recirculating system at the New Jersey State Aquarium (Camden, New Jersey). A specimen of *V. conspersa* with several conspicuous subcutaneous cysts was obtained, and consultations with the collector indicated that the specimen had already been infected at the time of capture. All fishes had been captured in Port Phillip Bay, southern Australia. For analysis, the infected specimen was anesthetized with MS-222 (Sigma, St. Louis, Missouri), and xenomas were removed for fresh examination and histological study. The fish was then returned to its tank. Processing of samples for light microscopy and transmission electron microscopy was done as described previously (Leiro et al., 1996, 1999). In brief, samples of tissue from the infected fish were fixed in 2.5% glutaraldehyde, buffered to pH 7.2 with sodium cacodylate buffer, postfixed in 1% osmium tetroxide, prestained in saturated aqueous uranyl acetate, dehydrated through a graded acetone series, and embedded in Spurr's resin. Semithin sections were cut on an ultratome (Reichert-Jung, Ultracut, Austria) and stained with 1% toluidine blue for light microscopic examination. Ultrathin sections were stained with alcoholic uranyl acetate lead citrate and viewed in a Philips CM12 (Philips, Eindhoven, Netherlands) electron microscope at an accelerating voltage of 80 kV. For statistical analysis, means were compared by unpaired Student's 2-tailed *t*-tests. *P*-values of less than 0.05 were considered significant.

Received 27 April 2004; revised 14 June 2004; accepted 14 June 2004.

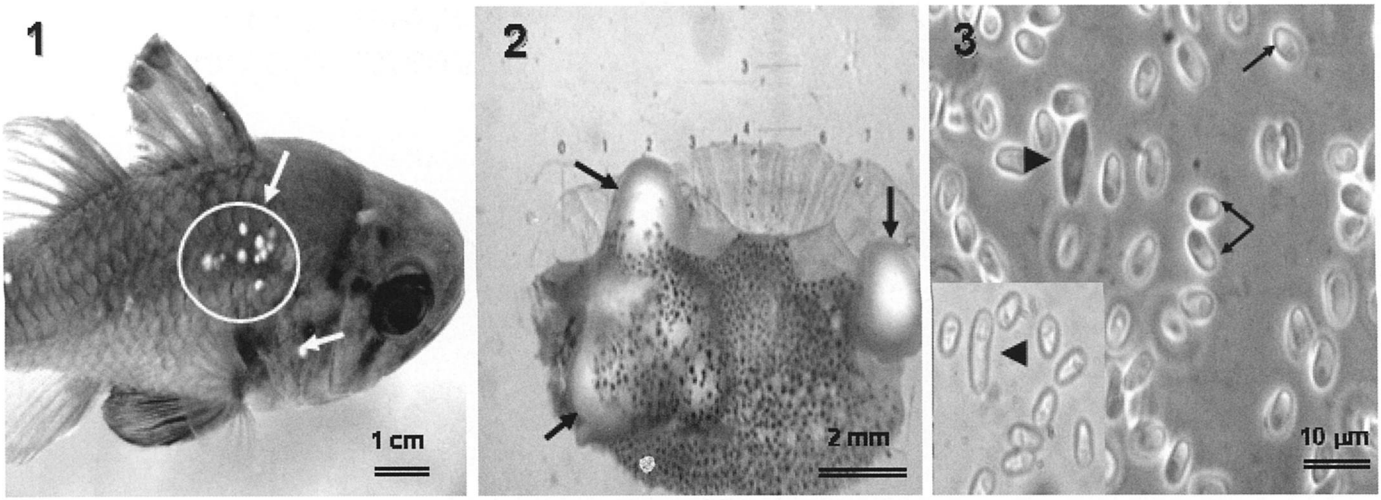
* Laboratorio de Parasitología, Instituto de Investigación y Análisis Alimentarios, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain.
† To whom correspondence should be addressed.

DESCRIPTION

Glugea vincentiae n. sp.
(Figs. 1–14)

Macroscopically, infection appeared as opaque white spherical subcutaneous cysts of 1–2 mm in diameter (Figs. 1, 2), distributed widely over the body surface, as well as on ventral part of mandible and caudal and dorsal fins.

In fresh smears, 2 spore types present; majority type (about 99% of spores) ovoid or slightly ovoid microspores, with mean dimensions of 5.1 × 2.2 μm (range, 4.5–6.0 × 2.0–2.7 μm; n = 26; Fig. 3); minority type (about 1% of spores) elongated oval macrospores, with mean dimensions of 8.9 × 3.1 μm (range, 7.5–12 × 2.0–4.0 μm; n = 10; Figs. 3, 4). Semithin sections of mature xenomas showed typical stratified *Glugea* organization, with developmental stages located peripherally and mature spores located centrally (Fig. 4). Capsule a wall of amorphous material about 30 μm thick (Fig. 4). Outermost zone of cytoplasm in mature xenomas parasite free, with elements of host cell. Abundant rounded vesicles and plasmalemma showing pinocytotic activity (Fig. 4). Hypertrophic host nucleus fragmented into many irregular parts located peripherally (Fig. 4). Merogonic stages occupy adjacent zone, some incrusted among numerous branched host nuclei, whereas sporogonic stages located in interior of xenoma (Fig. 4). Some meronts are rounded uninucleate cells of about 5-μm diameter, whereas oth-



FIGURES 1–3. Light micrographs showing infections by *Glugea vincentiae*. **1.** Xenomas in the subcutaneous tissue of *Vincentia conspersa*. **2.** Group of oval or spherical white xenomas. **3.** Fresh spores showing posterior vacuoles (arrows), including a macrospore (arrowhead).

ers are cylindrical multinucleate structures about 40 μm long (Fig. 4). In some cases, cylindrical meronts show triradiate cross section (Fig. 4).

Wall of the xenoma formed by an amorphous material covering the plasmalemma, which shows pinocytotic activity and may be thrown into low irregular projections, giving rise to numerous pinocytotic vesicles lining the periphery of the xenoma (Fig. 5).

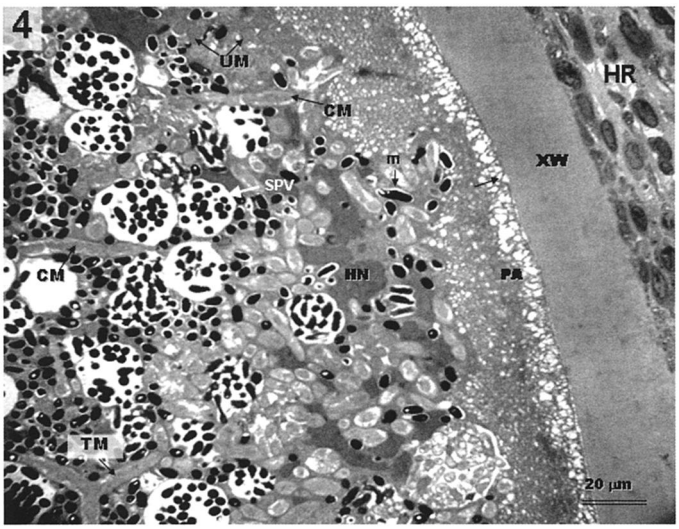


FIGURE 4. Semithin section of the peripheral part of a mature xenoma of the microsporidian infecting *Vincentia conspersa*, showing the stratified organization typical of *Glugea* species. The xenome wall (XW; thickness, ~30 μm) is composed of an amorphous substance covering the host-cell plasmalemma with invaginations (arrow) into the cytoplasm. The xenome wall shows marked host tissue reaction (HR). The outer peripheral zone is free of parasites and shows increased pinocytotic activity (PA). More internally, the cytoplasm contains host-cell elements, such as irregular nucleus fragments (HN), as well as rounded uninucleate meronts (UM), cylindrical multinucleate meronts (CM, length, ~40 μm), and occasional triradiate cylindrical multinucleate meronts (TM). Further toward the center of the xenoma, SPV (diameter, ~25 μm) are seen. In this zone, spores (UM, CM, and TM; also occasional macrospores, m) are more abundant than in the more peripheral zone.

As noted, various types of meront were detected, i.e., rounded uninucleate, cylindrical plurinucleate, and cylindrical plurinucleate triradiate cross section, in direct contact with host-cell cytoplasm (Fig. 4). Meronts delimited by simple plasmalemma, bounded by electron-dense surface coat closely pressed to flat cisternae of host-cell smooth endoplasmic reticulum, and containing cytoplasm with free ribosomes and rough endoplasmic reticulum (RER) (Figs. 6–10). Spindle plaques common on merogonic nuclear envelope (Figs. 7, 8), indicating prolific reproduction. Merogonic plasmodia divide by constriction along their length (Fig. 9), ultimately producing uninucleate meronts (Fig. 10).

Transition from merogony to sporogony identifiable by presence of spaced ''crossbars'' running to sporont surface, giving a septate desmosome appearance by loss of electron-dense surface coat, dispersal of reticulum endoplasmic cisternae, and formation of sporophorous vesicles (SPV) (Fig. 11). Multinucleate sporogonial plasmodia divide into sporoblasts by multiple cleavage to produce sporoblast mother cells (Fig. 11) containing a system of well-developed RER cisternae that may subsequently serve for polar tube formation, and by division of the sporoblast mother cells, probably by binary fission, give 2 uninucleate sporoblasts that subsequently give rise to spores (Fig. 12). Spores (microspores or macrospores; see above) are slightly ovoid to elongated ovoid and uninucleate, with an electron-dense exospore and a thick electron-lucent endospore (Figs. 13, 14). Polar filament isofilar, with 12–14 turns generally arranged in a single layer, although sometimes in 2 or 3 layers (Figs. 13, 14).

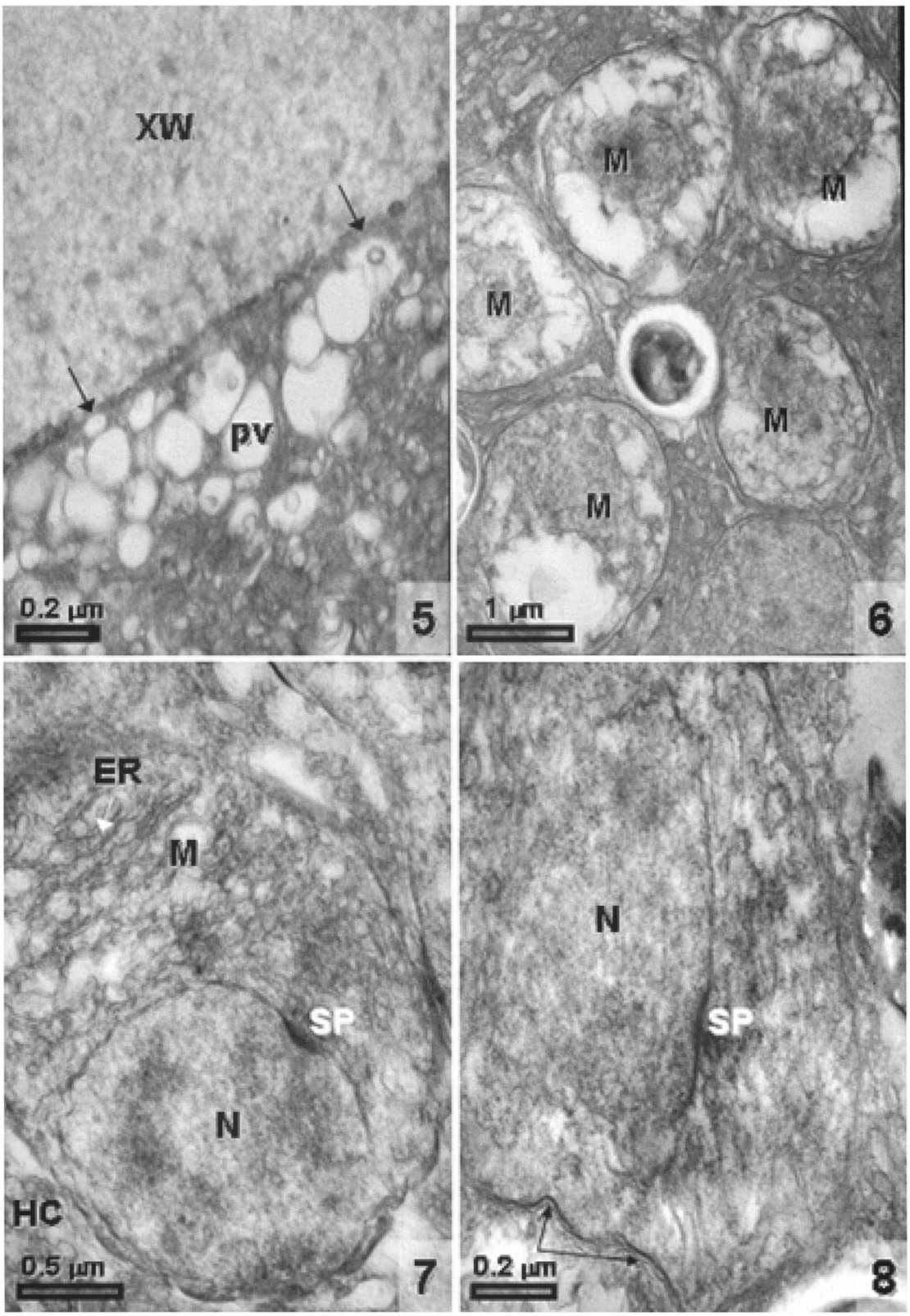
Taxonomic summary

Type host: Vincentia conspersa Klunzinger, 1872 (Teleostei, Apogonidae).

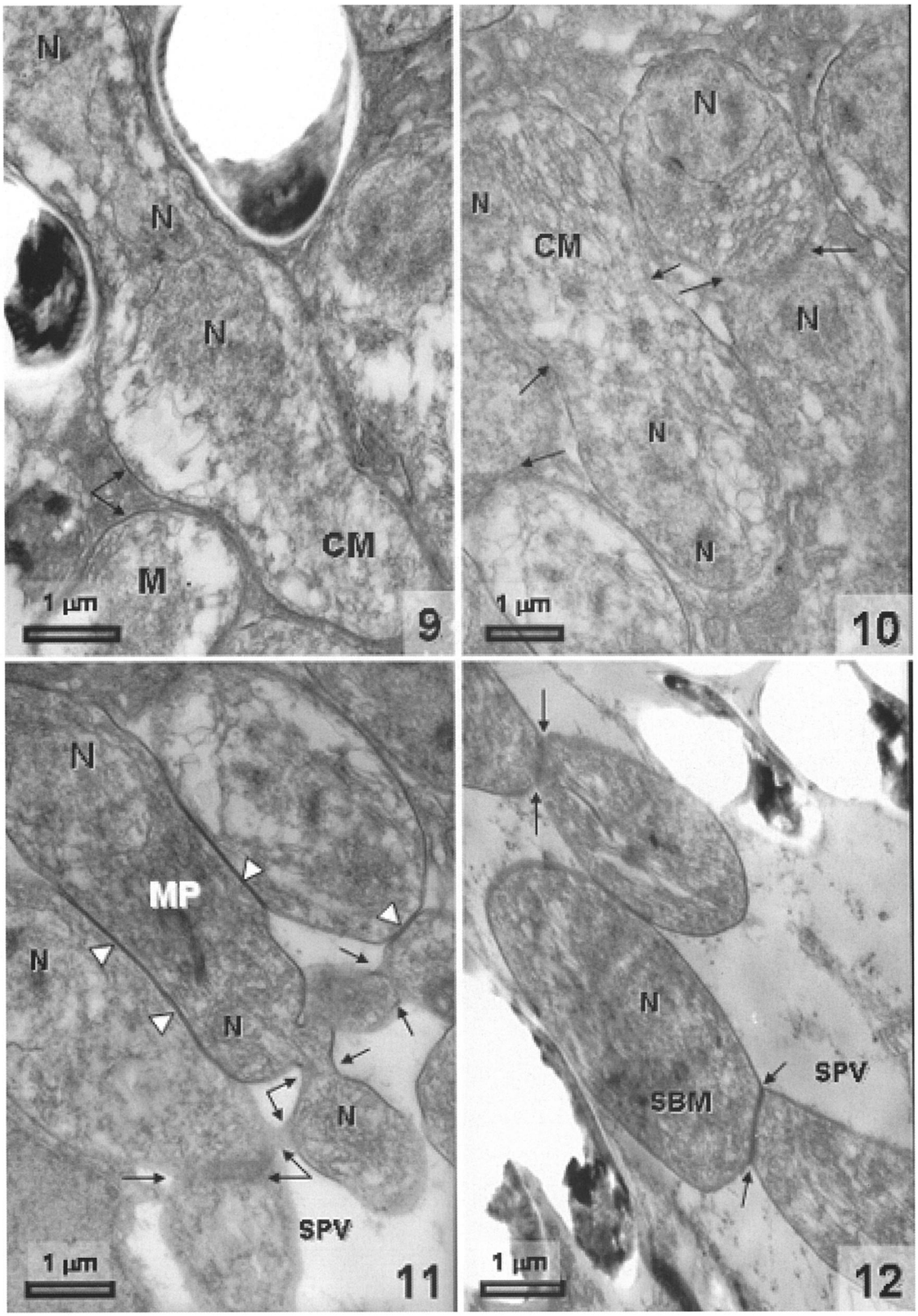
Type locality: Port Phillip Bay, Southern Australia, Pacific Ocean.

Localization in the host: Subcutaneous tissues.

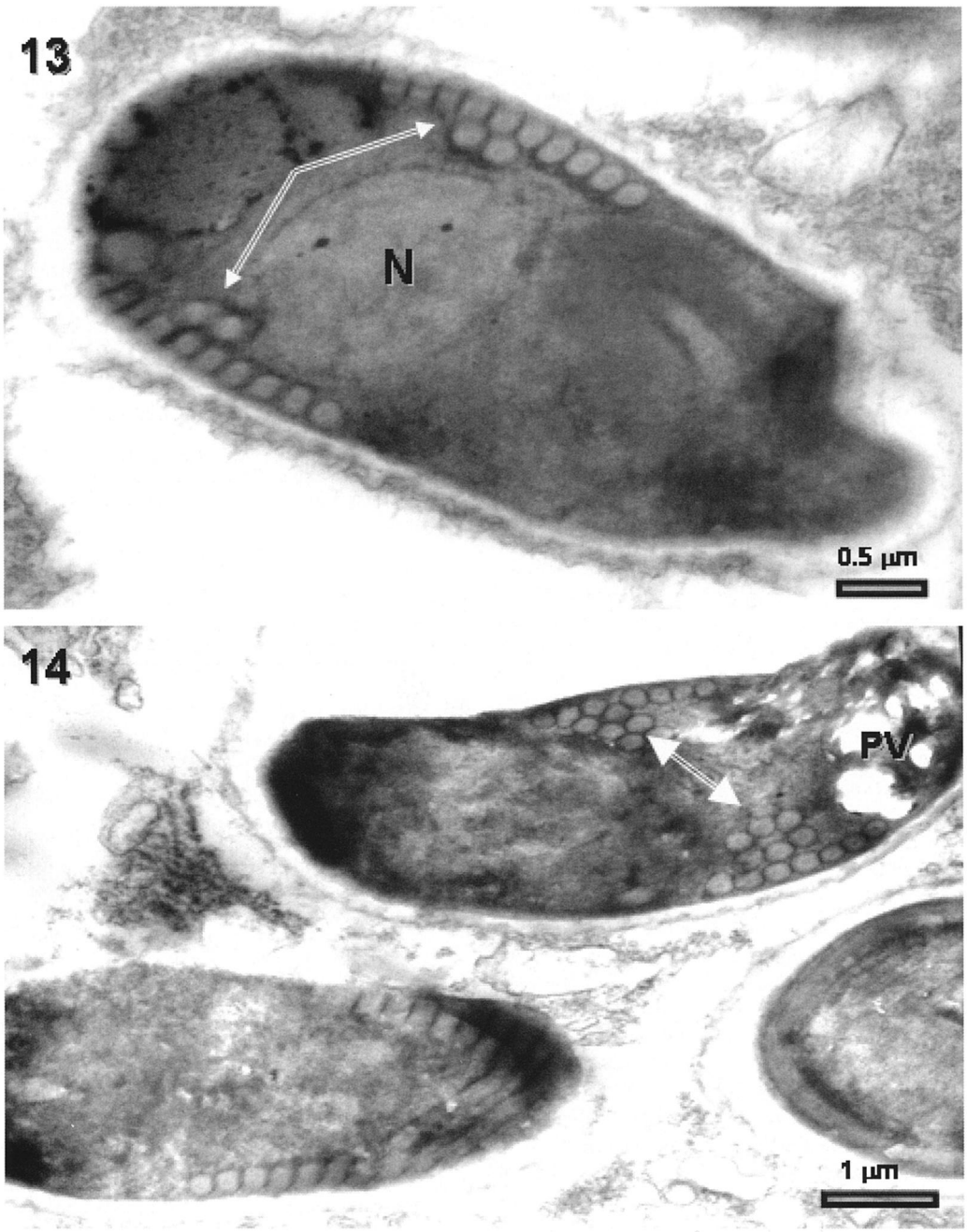
Prevalence: From a total of 15 acquired specimens of *V. conspersa*, 1 (6.6%) showed signs of infection on arrival. In this individual, 15 xenomas were detected. During the next 12



FIGURES 5–8. Ultrastructure of xenomas and meronts (M) of *Glugea vicentiae*. **5.** Electron micrograph of xenoma margin showing the amorphous capsule (XW), invaginations from plasmalemma of host cells, and abundant pinocytic vesicles (pv). **6.** Group of uninucleate meronts (M). **7.** Meront (M) in direct contact with the host-cell cytoplasm (HC), showing spindle plaque (SP), endoplasmic reticulum (ER), and nucleus (N). **8.** Meront showing a spindle plaque (SP) and nucleus (N); the meront surface (arrows) bears an electron-dense deposit and is surrounded by cisternae of host ER.



FIGURES 9–12. **9.** Multinucleate cylindrical meront (CM) with plasma membrane bounded by an electron-dense surface coat closely adpressed to a flat cisterna of the host-cell smooth endoplasmic reticulum (arrows). **10.** Multinucleate cylindrical meronts (CM) in the process of plasmotomy: constrictions (arrows) delimit 2 new meronts. **11.** Multinucleate cylindrical sporogonial plasmodium (MP) showing a septate junction (arrowheads) and dividing by multiple cleavage (arrows) to produce sporoblast mother cells. **12.** Sporoblast mother cells (SBM) dividing by binary fission to give sporoblasts within the SPV.



FIGURES 13–14. **13.** Ultrastructure of a uninucleate (N) spore showing the polar tube coiled in 2 layers (double-headed arrows). **14.** Microphotograph of a spore showing the polar tube coiled in 3 layers (arrows) and the posterior vacuole (PV).

mo, 2 more specimens (13.3%) developed xenomas, although these were less abundant (2 and 3) and smaller than those from the originally infected specimen.

Xenomas: Whitish, oval or spherical, about 1–2 mm in diameter; wall 30 μm thick, formed by an amorphous material covering the cell plasmalemma.

Development: Nuclei isolated throughout the life cycle. Meronts rounded uninucleate or cylindrical multinucleate (occasionally triradiate), with electron-dense coat on plasmalemma, surrounded by cisternae of host endoplasmic reticulum, dividing by plasmotomy or by multiple fission. Sporogonial plasmodia divide by multiple cleavage and produce sporoblast mother cells that divide by binary fission to produce sporoblasts. Two uninucleate spore types, including a majority type (microspores) that is ovoid or slightly ovoid, with a mean size 5.1 × 2.2 μm, and a minority type (macrospores) that is elongated oval, with a mean size 8.9 × 3.1 μm. The polar tube is isofilar and makes 12–14 turns arranged in 1, 2, or 3 layers.

Type specimens: Syntypes deposited in the Museo Nacional de Ciencias Naturales, Madrid (MNCN 36.02/4). Specimens embedded in Spurr's resin are in the collection of J.L.

DISCUSSION

Our morphological data for this microsporidian infecting the cardinal fish *V. conspersa* basically coincide with those for other species of *Glugea*. However, a number of important differences suggest that this microsporidian should be considered a new species. According to Lom (2002), 3 basic characters are required for including a given species of microsporidia within *Glugea*: (1) xenoma covered by wall made up of sloughed-off layers of cell coat, (2) host-cell nucleus divided into peripheral fragments, and (3) spores developed within SPV of parasite origin in the peripheral part of the xenoma. From the ultrastructural data obtained in this study, the microsporidian infecting *V. conspersa* meets criteria (2) and (3) but not (1): specifically and as can be seen from both light and electron micrographs, the xenoma wall has a thick amorphous cell coat similar to that produced by species of *Loma* (Loubés et al., 1984; Lom and Pekkarinen, 1999) and does not have the stratified appearance typical of *Glugea* (Canning et al., 1982). Other typical characteristics of *Glugea* spp. shown by our specimens from *V. conspersa* include (1) the presence of unpaired nuclei throughout development; (2) cylindrical meronts with plasmalemma bounded by an electron-dense surface coat, adpressed to host ER cisternae; (3) sporogonial plasmodia that divide by multiple cleavage to produce sporoblast mother cells, which in turn divide by binary fission to produce sporoblasts; (4) highly branched hypertrophic host-cell nucleus fragments located at the periphery of the xenoma; and (5) parasite developmental stages stratified in xenoma, with earlier stages located peripherally and mature stages located centrally (Lom, 2002). However, the microsporidian from *V. conspersa* does not show other characters likewise typical of *Glugea*, such as monomorphic spores with isofilar polar tube coiled in a single layer. The species *Glugea pimephales* has been reported to have occasional spores double the size of normal spores (6.7–8.1 × 2.4–3.4 μm vs. 4.5–6.0 × 2.5–3.0 μm) (Morrison et al., 1985). Calculations with the data reported by the latter investigators indicate that the mean length of the larger spores is about 1.4-fold that of the smaller spores, and comparison of the 2 means by Student's 2-tailed *t*-tests indicates a significant difference ($P < 0.0002$); this suggests that these are 2 different spore types, rather than extremes of a continuous size distribution. In our specimens, the mean length of the larger spores is about 1.7-fold that of the smaller spores, and comparison of the 2 means again indicates a significant difference ($P < 0.0001$). This again suggests the existence of 2 clearly defined spore types.

In *Pleistophora*, the existence of species with 2 types of spores is widely accepted. In *Pleistophora typicalis*, for example, microspores have a mean size of 4.4 × 2.3 μm and macrospores have a mean size of 7.5 × 3.0 μm (Canning and Nicholas, 1980), i.e., a 1.7-fold difference in mean length, the same as for the *Glugea* species described in this study. Most *Glugea* species infecting marine fishes have a single spore type with mean length of about 5 μm, and only *Glugea fennica* and, occasionally, *G. pimephales* have spores as long as 7 μm (Lom and Laird, 1976; Morrison et al., 1985), smaller than the macrospores described in this study, which have a mean length of about 9 μm and maximum length of up to 12 μm.

Species in some genera (*Pleistophora*, *Ovipleistophora*, and *Heterosporis*) produce micro- and macrospores that differ in the size and number of polar tube turns (Lom and Nilsen, 2003). For example, in spores of microsporidians of *Pleistophora* spp., the polar tube is arranged in 1 layer (microspores) or 2 or 3 layers (macrospores) (Canning and Nicholas, 1980). The spores of the microsporidian infecting *V. conspersa* clearly show a polar tube arranged in various layers, a character not reported previously in any other *Glugea* species.

In view of the observed differences between the microsporidian infecting *V. conspersa* and other microsporidians described from marine fishes, we consider that this microsporidian constitutes a new species, which we hereby denominate as *G. vincentiae*.

ACKNOWLEDGMENTS

This study was supported by grants PGIDIT02RMA23701PR from the Xunta de Galicia, Spain, and AGL2003-04644 from the Comisión Interministerial de Ciencia y Tecnología, Spain.

LITERATURE CITED

CANNING, E. U., AND J. LOM. 1986. The microsporidia of vertebrates. Academic Press, Inc., New York, 289 p.

———, ———, AND J. P. NICHOLAS. 1982. Genus *Glugea* Thélohan 1891 (Phylum Microspora): Redescription of the type species *Glugea anomala* (Moniez 1887) and recognition of its sporogonic development within sporophorous vesicles (pansporoblastic membranes). Protistologica **18:** 193–210.

———, AND J. P. NICHOLAS. 1980. Genus *Pleistophora* (Phylum Microspora): Redescription of the type species, *Pleistophora typicalis* Gurley, 1893 and ultrastructural characterization of the genus. Journal of Fish Diseases **3:** 317–328.

DYKOVÁ, I. 1995. Phylum microspora. *In* Fish diseases and disorders, Vol. I. Protozoan and metazoan infections, P. T. K. Woo (ed.). CAB International, Wallingford, U.K., p. 149–179.

KEELING, P. J., AND N. FAST. 2002. Microsporidia: Biology and evolution of highly reduced intracellular parasites. Annual Review of Microbiology **56:** 93–116.

LEIRO, J., M. ORTEGA, R. IGLESIAS, J. ESTÉVEZ, AND M. L. SANMARTÍN. 1996. *Pleistophora finisterrensis* n. sp., a microspordian parasite of blue whiting *Micromesistius poutassou*. Systematic Parasitology **34:** 163–170.

———, A. PARAMÁ, M. ORTEGA, M. T. SANTAMARINA, AND ———. 1999. Redescription of *Glugea caullery*, a microsporidian parasite of greater sand-eel, *Hyperoplus lanceolatus* (Le Sauvage), (Teleostei: Ammodytidae), as *Microgemma caulleryi* comb. nov. Journal of Fish Diseases **22:** 101–110.

LOM, J. 2002. A catalogue of described genera and species of microsporidians parasite in fish. Systematic Parasitology 53: 81–99.

———, AND M. LAIRD. 1976. Parasitic protozoa from marine and euryhaline fish of Newfoundland and New Brunswick II. Microsporidia. Transactions of the American Microscopical Society **95:** 569–580.

———, AND F. NILSEN. 2003. Fish microsporidia: Fine structural diversity and phylogeny. International Journal for Parasitology **33:** 107–127.

———, AND M. PEKKARINEN. 1999. Ultrastructural observations on *Loma acerinae* (Jírovec, 1930) comb. nov. (Phylum Microsporidia). Acta Protozoologica **38:** 61–74.

LOUBÉS, C., J. MAURAND, C. GASC, I. DE BURON, AND J. BARRAL. 1984. Ultrastructural study of *Loma dimorpha* n. sp., a microsporidian parasite of fishes Gobiidae from the Languedoc. Protistologica **20:** 579–589.

MORRISON, C., G. L. HOFFMAN, AND V. SPRAGUE. 1985. *Glugea pimephales* Fantham, Porter and Richardson, 1941, n. comb. (Microsporidia, Glugeidae) in the fathead minnow, *Pimephales promelas*. Canadian Journal of Zoology **63:** 380–391.