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Ichthyosporidium weissii n. sp. (Microsporidia) Infecting the Arrow Goby (*Clevelandia ios*)

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ABSTRACT. Gonadal infections by a novel microsporidium were discovered in 34% (13/38) of arrow gobies, *Clevelandia ios,* sampled over a 3-yr period from Morro Bay Marina in Morro Bay, California. Gonadal tumors had been reported in arrow gobies from this geographic area. The infected gonads, found primarily in females, typically appeared grossly as large, white-gray firm and lobulated masses. Histological examination revealed large, multilobate xenomas within the ovaries and no evidence of neoplasia. Typical of the genus *Ichthyosporidium*, the large xenomas were filled with developmental stages and pleomorphic spores. Wet mount preparations showed two general spore types: microspores with mean length of 6.2 (7.0–4.9, SD = 0.6, $N = 20$) μm and mean width of 4.3 (5.3–2.9, SD = 0.8) μm; and less numerous macrospores with mean length of 8.5 (10.1–7.1, SD = 1.0, $N = 10$) μm and mean width of 5.5 (6.2–4.8, SD = 0.5) μm. Transmission electron microscopy demonstrated stages consistent with the genus and 35–50 turns of the polar filament. Small subunit rDNA gene sequence analysis revealed that the parasite from arrow gobies was most closely related to, but distinct from *Ichthyosporidium* sp. based on sequences available in GenBank. We conclude that this microsporidium represents a new species of *Ichthyosporidium,* the first species of this genus described from a member of the family Gobiidae and from the Pacific Ocean.

Key Words. Electron microscopy, gonads, neoplasia, new species, parasite, phylogeny.

THE first description of an *Ichthyosporidium* species was by Thélohan (1895) who described in the corkwing wrasse *Crenilabrus melops,* the presence of large parasitic masses that occupied most of the abdominal cavity and appeared to originate from the renal connective tissue. Considering the parasite to belong to the Microsporidia, Thélohan (1895) named the parasite *Glugea gigantea* but did not describe developmental stages of the parasite. The genus *Ichthyosporidium* was later erected by Caullery and Mesnil (1905) who described two named species: *Ichthyosporidium gasterophilum*, which was later reassigned to the genus *Ichthyophonus* by Sprague (1965), and *Ichthyosporidium phymogenes,* with both genera causing parasitic infections in marine fish of the family Labridae. They noted the close similarity between *I. phymogenes* and *G. gigantea* described by Thélohan (1895) and observed that they were found in the same fish host, *C. melops*. These parasites were also considered to be haplosporidians based on the resemblance of the sporoblasts to developmental stages of haplosporidians. Numerous later authors also considered these parasites to be members of the Haplosporidia (Kudo 1966).

Swellengrebel (1911) described developmental stages of a parasite from *C. melops*, which he noted as likely being the same as that described by Thélohan (1895), but placed it within the genus *Pleistophora* due to the formation of a pansporoblast. Believing this organism to be a microsporidium, he was unable to germinate spores to demonstrate the presence of a polar filament. Later, Sprague and Vernick (1968) demonstrated the presence of polar filaments in spores of this microsporidium from Swellengrebel's material by the use of electron microscopy and the periodic acid-Schiff (PAS) stain by light microscopy, firmly placing these parasites within the Microsporidia. Sprague and Vernick (1968) also noted that the organism in question, *Pleistophora gigantea,* was synonymous with *Ichthyosporidium giganteum*.

Infections by members of the genus *Ichthyosporidium* are characterized by the formation of large, multilobate xenomas in infected host tissue. These xenomas are distinguished from the cell hypertrophy-type xenoma formed by species in the genus *Glugea* in that they appear to be produced by the coalescence of multiple infected, hypertrophic cells, forming a syncytial-type xenoma. This type of xenoma does not have host nuclei in the periphery, is relatively devoid of host organelles, and contains a "fibro-granular layer" with no distinct inner boundary. It is limited on the outer surface by microvillus-like projections (Canning et al. 1986; Lom and Dyková 2005). We found similar xenomas in the gonads of arrow gobies, *Clevelandia ios*, collected from one site in Morro Bay, California. These xenomas were initially thought to be gonadal neoplasms. However, we concluded that they are actually the result of infection by a novel species of the genus *Ichthyosporidium*. This finding represents the first description of *Ichthyosporidium* in a member of the family Gobiidae and also the first described from the Pacific Ocean.

MATERIALS AND METHODS

Samples, collection, and histological examination. A total of 1,115 arrow gobies were collected from Morro Bay, California, over a 3-yr period, 2007–2009. Thirty-eight of these fish from one site, Morro Bay Marina, were examined by histology. We also examined 60 arrow gobies collected from this same site in November 2009. Fish were acclimated in the laboratory for 2 wk and held for up to 60 d further at California Polytechnic State University, San Luis Obispo. Fish were

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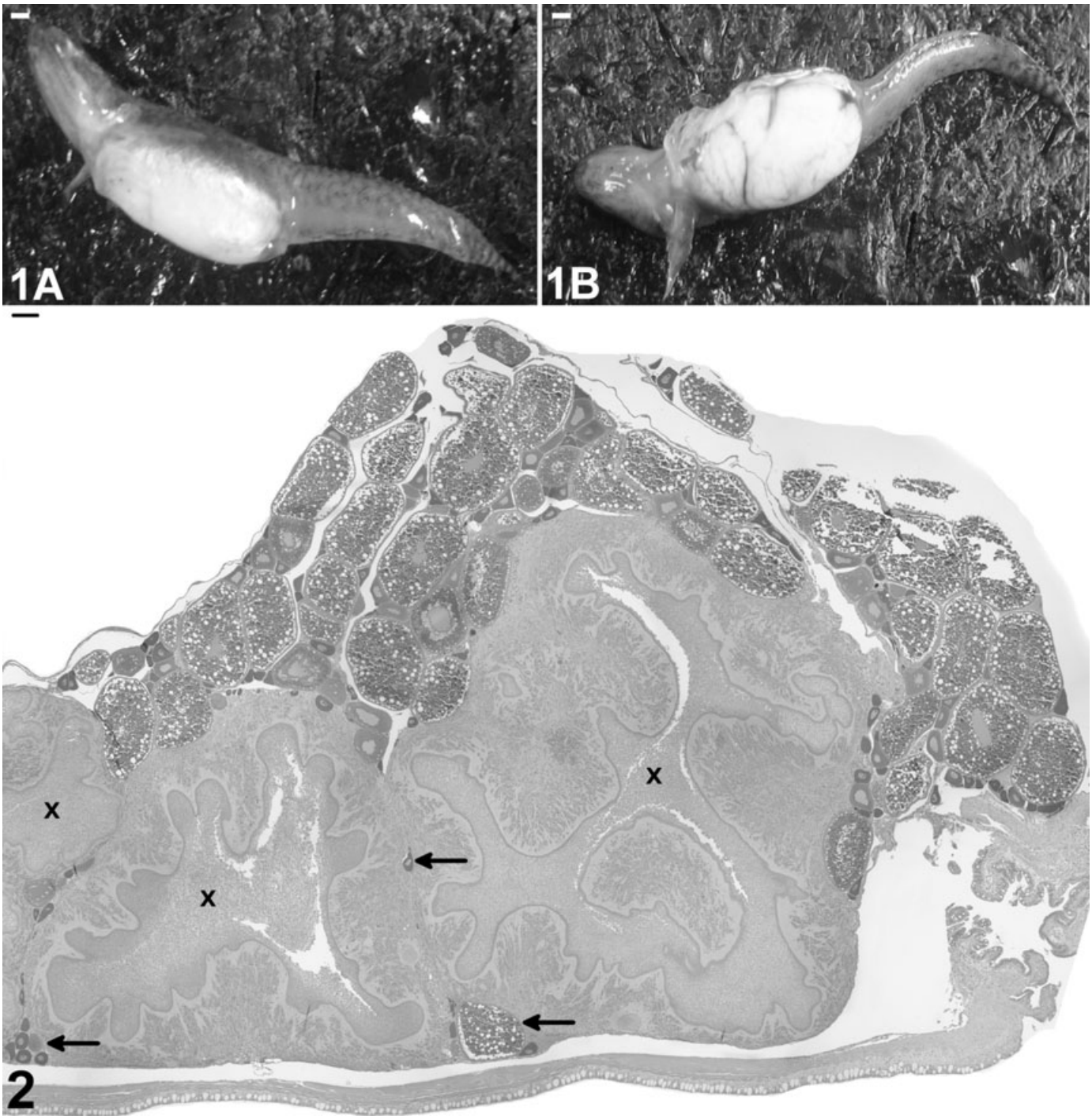

Fig. **1–2.** Macroscopic and low-magnification microscopic images of gonadal lesion in female arrow goby, *Clevelandia ios*. Figure 1 courtesy of Sarah Johnson, California Polytechnic State University, San Luis Obispo, California. **1(A).** Female arrow goby, *C. ios*, with grossly distended abdomen. Scale bar = 1 mm. **1(B).** The same goby with the skin removed from the abdomen. Scale bar = 1 mm. **2.** Hematoxylin and eosin (H&E)-stained histological section of an ovary infected with *Ichthyosporidium weissii* n. sp. Large, multilobate xenomas (X) can be seen with developing ovarian follicles interspersed (arrows) and surrounding the infected area. Scale bar = 100 μm.

euthanized and fixed in 10% (v/v) neutral buffered formalin. After fixation, fish were processed for histology, stained with hematoxylin and eosin (H&E), and examined by light microscopy.

Three additional female fish were collected from Morro Bay Marina in 2011. Ovarian tissue from two infected female fish was examined by wet mount and 30 microsporidian spores total, 20 microspores and 10 macrospores, were measured using SPOT Advanced imaging software version 4.0.9 (Diagnostic Instruments, Sterling Heights, MI). One fish was fixed in Dietrich's fixative and decalcified using CalExII (Fisher Scientific, Fair Lawn, NJ). Fixed and decalcified tissues were pro-

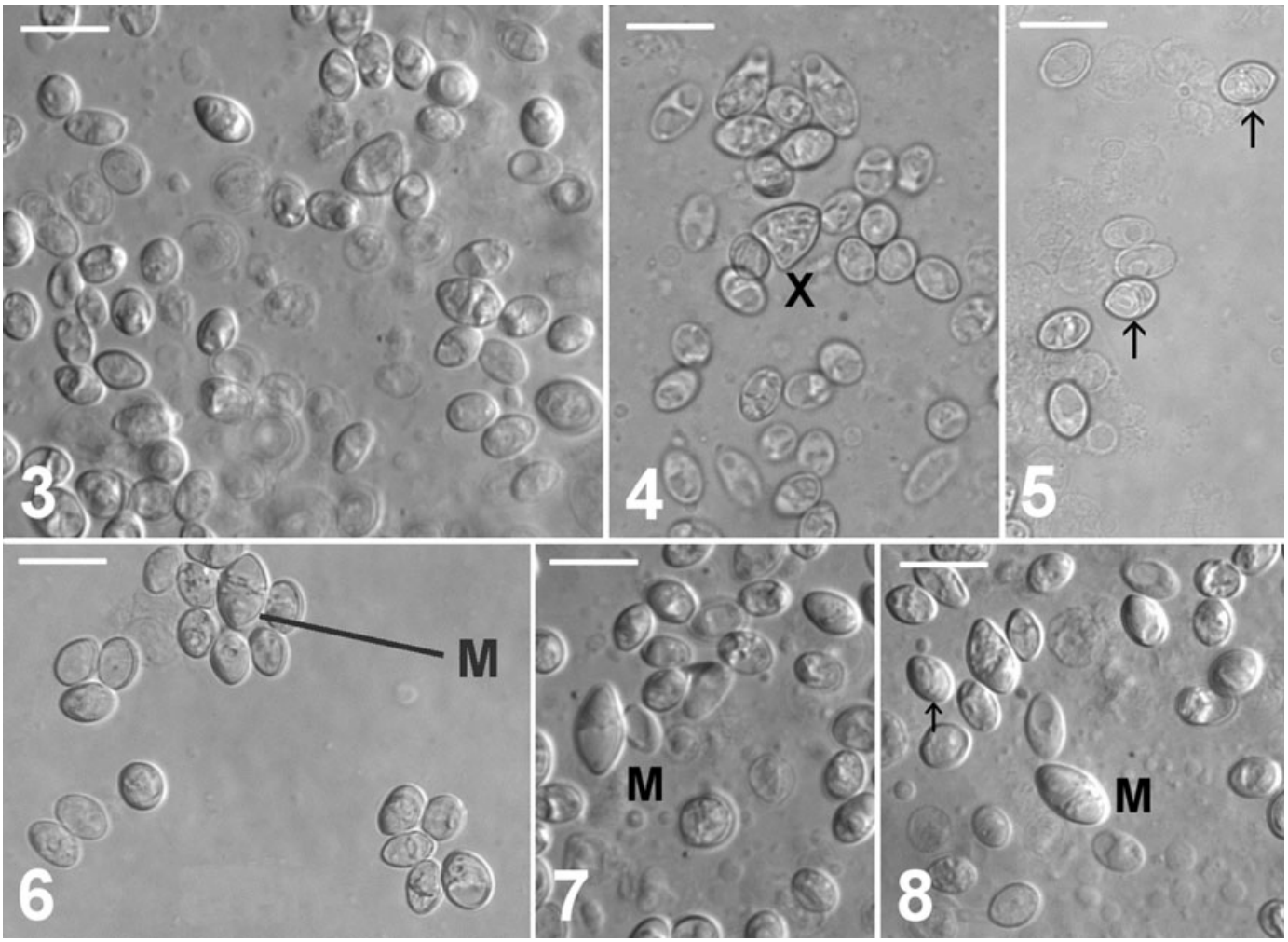

Fig. **3–8.** Light micrographs of spores of *Ichthyosporidium weissii* n. sp. obtained from wet mount preparations of ovarian tissue of an infected female arrow goby, *Clevelandia ios*. **3.** Spores viewed by Nomarski phase interference. Scale bar = 10 μm. **4.** Pleiomorphic spores with an aberrant, triangular-shaped spore (X). Scale bar = 10 μm. **5.** Spores containing visible coiled polar filaments (arrows). Scale bar = 10 μm. **6.** Numerous microspores with a single macrospore present (M). Nomarski phase interference. Scale bar = 10 μm. **7.** Several pleiomorphic spores with a single macrospore (M). Nomarski phase interference. Scale bar = 10 μm. **8.** One microspore containing a visible coiled polar filament (arrow) with a single macrospore (M). Viewed by Nomarski phase interference. Scale bar = 10 μm.

cessed for histology. Sections were cut at 5 μm and stained with H&E, PAS, and Luna (Luna 1968) stains.

Ultrastructure. Ovarian tissue from two additional infected female arrow gobies with grossly distended abdomens were cut into approximately 2–3 mm sections and placed in fixative; 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer at 4 °C overnight. Tissues were removed and post-fixed in 1% (w/v) osmium tetroxide in 0.2 M sodium cacodylate buffer for 1 h prior to embedding in epoxy resin. Thick sections were cut at 0.5 μm and stained with toluidine blue. Ultrathin sections (i.e. 30–40 nm) were cut and stained for 1.5 h in 5% (w/v) aqueous uranyl acetate solution and then stained with lead citrate. Transmission electron microscopy was performed using either a Philips CM12 scanning transmission electron microscope (Philips, Eindhoven, the Netherlands) or a FEI Tecnai 12 transmission electron microscope (Phillips, Hillsboro, OR).

DNA extraction, PCR amplification, and sequencing of small subunit ribosomal RNA (SSU rDNA) gene. Ovarian tissue from one female fish displaying gross gonadal lesions and microsporidian spores present as seen by wet mount was removed and the DNA extracted using the QIAgen Blood and Tissue Extraction kit (QIAgen, Valencia, CA) according to the manufacturer's protocol. A second microsporidium resembling *Kabatana* sp. was found in the skeletal muscle of 7 of the 60 fish collected at the Marina site, held in the laboratory at San Luis Obispo, and examined by light microscopy. Hence, skeletal muscle from a separate fish that showed the presence of this microspordium was also collected and DNA was extracted using the above method.

PCR was performed using the general microsporidian ribosomal primers 18F (5′-GAAAATTACCGGAGCCTGAA GTC-3′) and 580r (5′-GGTCCGTGTTTCAAGACGG-3′) to amplify the 5′-region of the SSU rDNA gene, ITS1 region, and partial large-subunit ribosomal DNA gene segments. All reactions were performed in 50-μl volumes using the Platinum PCR Supermix (Invitrogen, Carlsbad, CA), 0.9 mmol of each primer, and 5 μl of each DNA extraction. Amplifications were performed on a Peltier 200 thermocycler (MJ Research, Watertown, MA) with an initial denaturation at 94 °C for 2 min, 40 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min with a final extension at 72 °C for 10 min. The

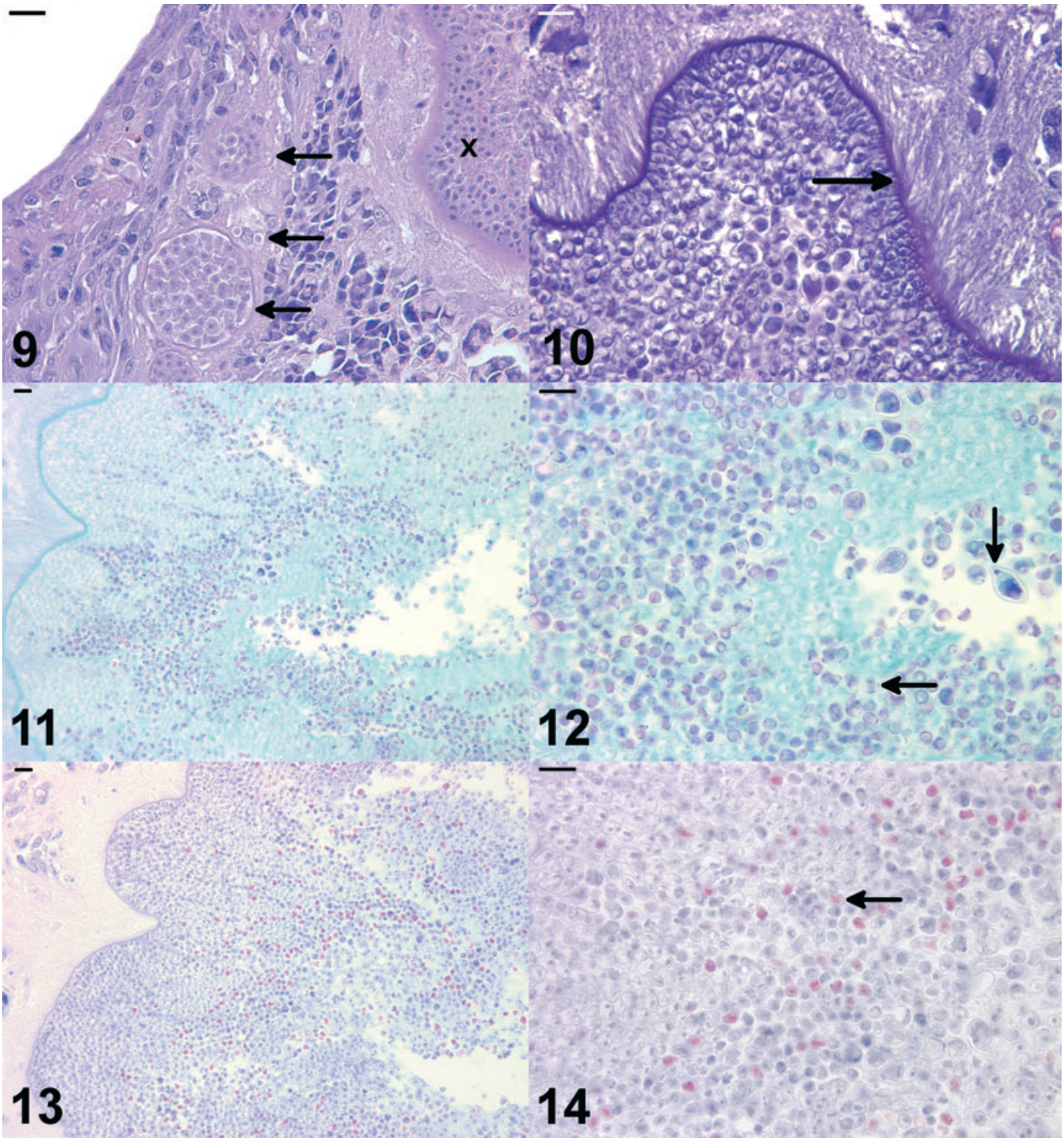

Fig. **9–14.** Histological sections of gonadal tissue from female arrow gobies, *Clevelandia ios*, infected with the microsporidium, *Ichthyosporidium weissii* n. sp. **9.** Hematoxylin and eosin (H&E) stained section with several cyst-like foci of infection (arrows). The margin of a mature xenoma (X) is also visible. Scale bar = 10 μm. **10.** H&E stained section of the periphery of a large, multilobate xenoma. Note the fibrous capsule (arrow) surrounding the xenoma, which is filled with proliferative and spore stages of the parasite. Scale bar = 10 μm. **11.** Periodic acid-Schiff (PAS)-stained section of ovarian xenoma with mature spores showing PAS-positive structures. Scale bar = 10 μm. **12.** PAS-stained section at higher magnification with arrows indicating examples of PAS-positive polar structures. Scale bar = 10 μm. **13.** Luna-stained section of the same xenoma as in Fig. 11. Mature spores stain red while presporogonic stages of the parasite appear blue. Scale bar = 10 μm. **14.** High magnification of Luna-stained section of xenoma. Mature spores (arrow) stain red. Scale bar = 10 μm.

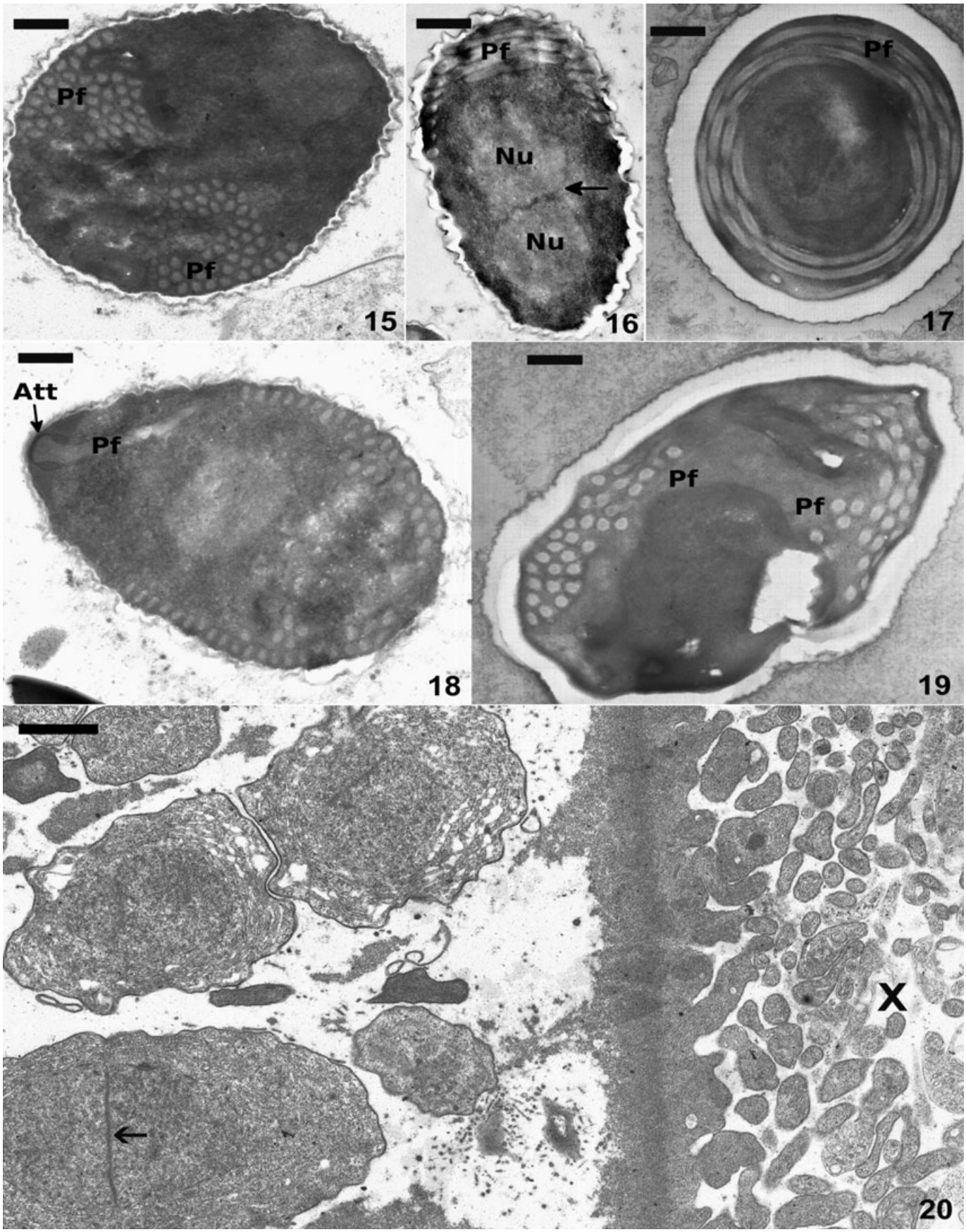
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Fig. **15–20.** Transmission electron microscopy of spores and developmental stages of *Ichthyosporidium weissii* n. sp. **15.** Parasagittal view with approximately 50 turns of the polar filament (Pf) visible. Scale bar = 0.5 μm. **16.** Parasagittal section of a spore with a diplokaryotic nucleus (Nu) visible in the anterior portion (arrow = division between diplokarya). The coiled polar filament (Pf) is visible at one end of the spore. Scale bar = 0.5 μm. **17.** Transverse section of a spore with the polar filament (Pf) arranged in four distinct rows. Scale bar = 0.5 μm. **18.** Mature spore showing the manubroid portion of the polar filament (Pf) and the anterior attachment complex (Att). Scale bar = 0.5 μm. **19.** Spore with visible cross sections of the isofilar polar filament (Pf). Scale bar = 0.5 μm. **20.** Transmission electron microscopy of the margin of a xenoma with sporonts. Arrow = division between diplokarya. X = xenoma wall with numerous projections. Scale bar = 1 μm.

resulting PCR product was both sequenced directly and cloned into TOPO TA Cloning vectors (Invitrogen). Three clones of the gonadal microsporidium and one from the muscle parasite were sequenced in both directions using primers flanking the inserted sequence. The primers 530f (5′-GTGCCAGCAGCCGCGG-3′), 1047r (5′-AACGGCCATG CACCAC-3′), and 1061f (5′-CACCAGGTTGATTCTGCC-3′) were used to sequence internal portions of the cloned SSU rDNA gene. All DNA analyzed in the study was sequenced on an ABI Prism® 3730 Genetic Analyzer with the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

Phylogenetic analyses. The sequences were compared with those in the National Center for Biotechnology Information's GenBank database using the BLASTN program (Altschul et al. 1990). Multiple sequence alignment was performed using CLUSTALW in the software package MEGA 5 (Tamura et al. 2011). Poorly aligned or ambiguous regions of the alignment were removed using the Gblocks program version 0.91b (Castresana 2000) on the webserver available at http://molevol.cmima.csic.es/castresana/Gblocks_server.html. The results of the multiple sequence alignment were analyzed using the jModeltest program version 0.1.1 (Posada 2008) to determine the most likely model of nucleotide substitution. Based on the results of the jModeltest analysis, phylogenetic reconstruction using the maximum likelihood method was performed using PhyML (Guindon et al. 2010) on the webserver at http://www.atgc-montpellier.fr/phyml/ using the generalized time-reversible model with gamma-distributed rate variation among sites (GTR+G). The analysis was run using 500 bootstrap replicates to test the robustness of the resulting tree. Bayesian analysis was performed using the software MrBayes version 3.1.2 also using the GTR model with a gamma-distributed rate variation across sites. The analysis was run for 1,000,000 replicates and sampled every 1,000 generations.

A second multiple sequence alignment was performed using a region from nucleotides 507 to 867 of the 16S SSU rRNA gene sequence obtained from the arrow goby ovarian tissue, the same region from *Ichthyosporidium* sp. (GenBank accession number L39110), and sequence from *I. giganteum* (GenBank accession number L13293). This alignment was used to analyze the pairwise distance (*p*-distance) between the three sequences using the MEGA 5 software.

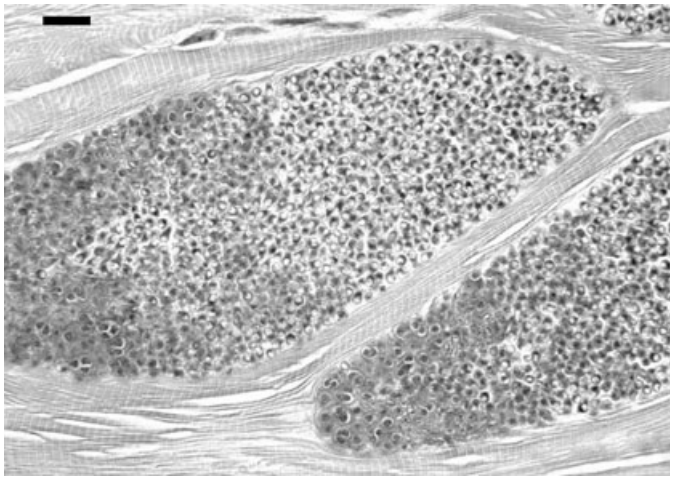

Fig. **21.** Hematoxylin and eosin-stained histological section of skeletal muscle of an arrow goby, *Clevelandia ios*, infected with *Kabatana* sp., a myocyte-infecting microsporidian. Scale bar = 10 μm.

RESULTS

Prevalence. A total of 5.7% (64/1115) of the fish from the overall collection exhibited prominently distended abdomens at gross examination (Fig. 1A). Upon dissection, large, elongate, whitish lesions in the gonadal region were observed (Fig. 1B). Histological examination of 38 fish from a single site, Morro Bay Marina, revealed the presence of a microsporidian with a prevalence of 34.2% (13/38), with all affected fish being female. The overall prevalence of infection of a subsequent sampling of arrow gobies for a laboratory experiment in 2009 from the same site was 15% (9/60), with one single male being affected.

Light microscopy/histology. Refractile spores with indistinct posterior vacuoles were seen in wet mounts prepared from infected ovarian tissue (Fig. 3–8). Spores were ovoid to pyriform. They were very pleomorphic, but generally consisted of two sizes, with numerous microspores (Fig. 3–8) with a mean length of 6.2 (7.0–4.9, SD = 0.6, $N = 20$) μm and a mean width of 4.3 (5.3–2.9, SD = 0.8) μm and occasional macrospores (Fig. 6–8) with a mean length of 8.5 (10.1–7.1, SD = 1.0, $N = 10$) μm and a mean width of 5.5 (6.2–4.8, SD = 0.5) μm.

Histological examination of infected fish (Fig. 2, 9–14) showed the parasite to be confined to cells of the ovarian stromal tissue in females and the stromal tissue of the testes in the single infected male observed. Parasite-containing lesions of two types were observed: large, multilobate xenomas surrounded by a common fibrous capsule (Fig. 2) with apparently normal ovarian tissue and developing oocytes interspersed, and smaller rounded cyst-like lesions, appearing to be single, infected and hypertrophic cells with a less defined laminated capsule (Fig. 9). In some sections, numerous smaller foci were observed proximal to larger, lobular xenomas, suggesting the possibility of autoinfection (Fig. 9).

Newly developing small xenomas contained only proliferative stages with no apparent spores (Fig. 9), whereas other, more mature xenomas, contained spores dispersed throughout the xenoma and intermixed with large numbers of presporogonic forms (Fig. 10–14). Sections stained with PAS showed xenomas with centrally located spores containing PAS-positive structures that appeared to be polar filaments and apical polar caps (Fig. 11, 12). Few, presumably more mature spores, located toward the center of the xenomas, stained brick red with the Luna stain (Fig. 13, 14).

Ultrastructure. Transmission electron microscopy (TEM) of infected ovaries showed that spores (Fig. 15–19) and other

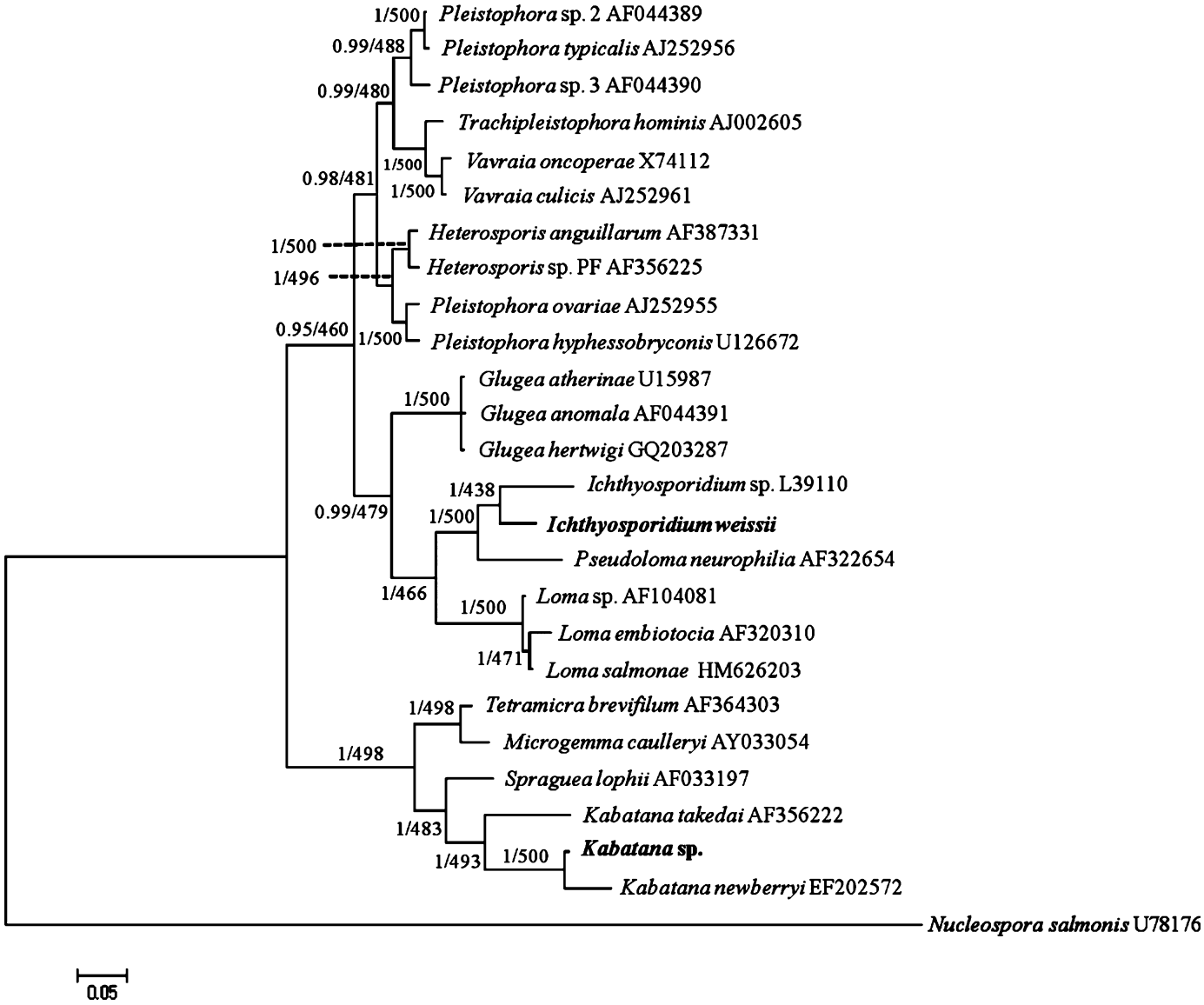

Fig. **22.** Phylogenetic tree of microsporidian small subunit (SSU) rRNA gene sequences inferred by Bayesian analysis (BI), showing the placement of *Ichthyosporidium weissii* n. sp. (in bold). The placement of *Kabatana* sp. (in bold) sequenced from the skeletal muscle of an arrow goby is also shown. Numbers at nodes represent BI posterior probability support and bootstrap values (out of 500 replicates) from maximum-likelihood analysis, respectively.

developmental stages (Fig. 20) were diplokaryotic. Sporonts with diplokaryotic nuclei were dispersed throughout the xenomas. The xenoma wall was distinct and dense, and the outer layer consisted of numerous, long, finger-projections (Fig. 20). Mature spores were dense, contained 36–50 turns of the isofilar polar filament arranged in four to five rows, and had a prominent endospore wall (Fig. 15–19).

***Kabatana* sp.** Subsequent sampling from the same site of 60 arrow gobies, which were held in the laboratory for experimentation, revealed the presence of another microsporidium, resembling a *Kabatana* sp. It was observed in the skeletal muscle of seven fish. These parasites did not form xenomas, but rather were limited to development within the sarcoplasm of skeletal muscle cells (Fig. 21).

Molecular phylogeny. The total length of amplified ribosomal DNA was 1,839 bp and has been deposited in the NCBI GenBank database as accession number JQ062988. The top three hits returned by the BLASTN search of the NCBI nonredundant database were *Ichthyosporidium* sp., *Pseudoloma neurophilia,* and *Loma embiotocia* with percent identities of 91.7%, 90.6%, and 89.7%, respectively. Phylogenetic analysis using both maximum likelihood and Bayesian methods place the new microsporidium in a clade with *Ichthyosporidium* sp., and sister to *P. neurophilia* (Fig. 22). Pairwise comparison of the sequence from arrow goby with the partial sequence (375 bp) obtained from *I. giganteum* (NCBI accession number L13293) from *Leiostomus xanthurus* and that of *Ichthyosporidium* sp. from the same host also showed 10.1% (*p*-distance) and 10.2% nucleotide difference, respectively, within this region of the gene. In contrast, the sequence of *Ichthyosporidium* sp. and *I. giganteum* had 0.8% (*p*-distance) nucleotide difference in this region of the gene. The sequence obtained from the microspo-

ridium from the skeletal muscle has been deposited in the NCBI GenBank database as accession number JQ062989. It is similar to, but distinct from, other species of the genus *Kabatana*, with 2.8% (*p*-distance) nucleotide difference between that and the SSU rDNA gene sequence of *K. newberryi*.

DISCUSSION

Ichthyosporidium giganteum has been described from *C. melops* along the Atlantic coast of France, *Crenilabrum ocellatus* in the Black Sea, *Ctenolabrus rupestris* off the Atlantic coast of Portugal (Casal and Azevedo 1995), and *L. xanthurus* along the Atlantic coast of the United States (Schwartz 1963). The only other described species of *Ichthyosporidium, Ichthyosporidium hertwigi,* has been described from the gills of *Crenilabrus tinca* on the Crimean coast of the Black Sea (Swarczewsky 1914). To our knowledge, this is the first description of a species of *Ichthyosporidium* from the Pacific ocean.

The morphology and histological appearance of the microsporidium observed in the arrow goby *C. ios* is consistent with that previously described for *Ichthyosporidium* species with regard to the formation of large, irregular, multilobate xenomas surrounded by a thick fibrous capsule and the presence of diplokaryotic, developmental and mature spore stages. *Ichthyosporidium giganteum* infections have been described as being localized to the subcutaneous connective tissue, especially fibroblasts, of the anterior abdomen (Sprague and Hussey 1980), while the single description of *I. hertwigi* describes the site of infection as being in the connective tissue of the gills (Swarczewsky 1914). The microsporidium described here appears to infect connective tissue cells of the ovigerous stroma, and rarely the intratesticular stroma. The xenoma formed by *I. giganteum* has been described as having two types. (1) A cyst-like structure consisting of a single infected, hypertrophic cell. Proliferating, merogonic stages of the parasite are exclusively present with an absence of spore stages. (2) A lobular, large and irregularly shaped xenoma with proliferating stages and mature spores present. This multicystic lesion may be formed by the coalescence of several foci of infection to form a syncytium (Sprague and Hussey 1980). In the present study, xenomas resembling both of these types were observed in arrow gobies infected with *Ichthyosporidium weissii* n. sp. While the original description of *Ichthyosporidium* sp. by Schwartz (1963) described the spores as being uniform in shape and 7 × 4 μm, Sprague (1966) noted the presence of a few larger macrospores in one cyst in a subsequent description from the same set of slides. Sprague and Hussey (1980) tentatively considered the *Ichthyosporidium* sp. described by Schwartz (1963) and *I. giganteum* to belong to the same species based on their morphological features. They did not explicitly describe the spore morphology of *I. giganteum*, instead referring to the previous description given by Sprague and Vernick (1974). However, the presence of macrospores was not noted in this latter description. We speculate that this could be due to the very low number of macrospores produced by *I. giganteum*. The PAS-positive structures within spores of *I. weissii*, presumably the polar filament and a mass at the anterior end of the spore, seen in several mature spores are also consistent with the findings of Sprague (1966). Peterson et al. (2011) demonstrated that the Luna stain, which stains chitin, is useful for demonstrating mature spores of microsporidia. This stain was very useful for distinguishing mature spores in histological sections of these infected arrow gobies, given that they are quite pleomorphic, in contrast to the uniformly sized spores described for *I. giganteum*.

There are two reports describing the ultrastructure of *I. giganteum* spores (Casal and Azevedo 1995; Sprague and Vernick 1974). Spore measurements varied considerably between these two descriptions with Sprague and Vernick (1974) reporting average dimensions of 5.9 × 4 μm and Casal and Azevedo (1995) reporting average dimensions of 7.26 × 5.16 μm. In addition, Sprague and Vernick (1974) described the spores as being uniform in size, in contrast to the spores seen in the present study. The number of coils of the polar filament has been used as a criterion in taxonomy for many microsporidia. However, this character appears to be quite variable for members of the genus *Ichthyosporidium*. Sprague et al. (1992) and Casal and Azevedo (1995) reported approximately 32 and 43 turns in 4–5 rows, respectively, for *I. giganteum*, and we found that the number of coils observed in *I. weissii* n. sp. ranged from 35 to well over 50. The number of coils of the polar filament is much greater than what has been reported for most other microsporidia, and the variability observed in the spores in our study and that reported in descriptions of *I. giganteum* suggests that this characteristic is not suitable for taxonomic differentiation of species within the genus *Ichthyosporidium*. The ultrastructure of *I. weissii* n. sp. was also consistent with the previous reports on *Ichthyosporidium*, with the presence of diplokarya in both spores and presporogonic stages. Moreover, the xenoma wall was also similar to that of *I. giganteum*, with distinctive, long finger-like projections extending outward. The genus description of *Ichthyosporidium* includes tetrasporogonic development. Careful examination of wet mount material, histological sections, and images obtained by electron microscopy did not allow us to confirm this character. However, the mature spores do not appear to occur in aggregates, particularly well demonstrated with the Luna stain, which only stains positive with mature spores (Fig. 13, 14).

Phylogenetic analyses of the SSU rDNA gene sequence obtained from this organism placed it in the genus *Ichthyosporidium* Caullery & Mesnil, 1905. The most complete rDNA sequence of an *Ichthyosporidium* species available in GenBank before the present study was provided for *Ichthyosporidium* sp. (i.e. GenBank accession number L39110) from the fish host *L. xanthurus* (Baker et al. 1995). A partial SSU rDNA gene sequence of *I. giganteum* (i.e. 404 bp, GenBank accession number L13293), also obtained from *L. xanthurus*, is available (Vossbrinck et al. 1993). Pairwise alignment and analysis of the three putative *Ichthyosporidium* species suggests that the sequences of both *Ichthyosporidium* sp. and *I. giganteum* are related to *I. weissii* n. sp. at the genus level, and cluster together in a monophyletic clade. The two sequences from *L. xanthurus* were almost identical over corresponding regions, and hence presumably both represent *I. giganteum*. However, it would be very useful to obtain sequence from the type host, *C. rupestris*. The closest relative to this clade is *P. neurophilia*, a microsporidium of the zebrafish *Danio rerio*. These two genera share few similarities in development or spore structure (Cali et al. 2011).

Casal and Azevedo (1995) described *C. rupestris* infected with *I. giganteum* as having grossly visible abdominal swelling, similar to that seen in *C. ios* infected with *I. weissii* n. sp., in the present study. They noted that these prominent lesions grossly resembled tumors and this similarity likely contributed to the initial misdiagnosis of the lesions found in the arrow gobies as resembling ovarian neoplasms. Sprague (1969) used infections in *L. xanthurus* by *Ichthyosporidium* sp. as an example to describe the formation of "tumors" by microsporidia. Various other infectious agents, particularly protists, cause macroscopic lesions suggestive of neoplasia (Harshbarger

1984), demonstrating that diagnosis of neoplasia in wild fishes must include proper histological interpretations.

The microsporidium observed in the skeletal muscle of several of these fish appears most closely related to the genus *Kabatana* based on phylogenetic analysis of the SSU rDNA gene sequence. The microsporidium, *K. newberryi,* infecting the skeletal muscle of another member of the family Gobiidae, the tidewater goby *Eucyclogobius newberryi*, has been described from fish collected in Big Lagoon, Humboldt County, California (McGourty et al. 2007), located considerably north of Morro Bay. The difference in host and SSU rDNA gene sequence between *K. newberryi* and the microsporidium found in the skeletal muscle of arrow gobies described herein suggests this is a novel species of *Kabatana*. We elected not to assign a species name to this organism, as we did not have ultrastructural data.

Phylogenetic analysis, the presence of a diplokaryotic nucleus in both developmental and spore stages of the parasite, and the formation of a multilobate, syncytial xenoma support the placement of this microsporidium in the genus *Ichthyosporidium*. Based on the geographic location, host species, and differences in the SSU rDNA between this and the other two sequences of *Ichthyosporidium* available in the GenBank database, we conclude that this microsporidium represents a novel species of the genus *Ichthyosporidium*.

TAXONOMIC SUMMARY

Phylum Microsporidia Balbiani, 1882

Family Ichthyosporidiidae Sprague, Becnel & Hazard, 1992

Genus *Ichthyosporidium* Caullery & Mesnil, 1905

Ichthyosporidium weissii n. sp.

Diagnosis. With characters of the genus. Meronts, sporonts, and spores; diplokaryotic, developing within large xenomas. Spores, pleomorphic, ovoid to pyriform. Microspores: length 6.2 (7.0–4.9) μm, width 4.3 (5.3–2.9) μm. Macrospores: length 8.5 (10.1–7.1) μm, width 5.5 (6.2–4.8) μm.

Type locality. Morro Bay Marina, California (35°20.7′N, 120°50.7′W)

Type host. Arrow goby *Clevelandia ios* (Teleostei, Gobiidae).

Site of infection. Cytoplasm of connective tissue cells of the gonadal stroma in male and female fish.

Prevalence. Thirty-four percent of female fish (n = 38) from one sampling site in type locality were affected.

Deposition of type material. Slides of histological sections from whole fish were deposited in the collections of the Queensland Museum, Brisbane, Australia. (Nos. G465498, G465499, G465500).

Gene sequence. Sequence of the SSU rRNA gene, ITS, and partial large-subunit rRNA gene was deposited as GenBank Accession JQ062988.

Etymology. The species is named after the prominent protistologist, Dr. Louis Weiss, USA.

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