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A NEW SPECIES OF *HENNEGUYA* (MYXOZOA) IN THE BIG-EYED SCAD (*SELAR CRUMENOPHTHALMUS*) FROM HAWAII

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ABSTRACT: We describe a new myxozoan, *Henneguya akule* n. sp., infecting the carangid fish *Selar crumenophthalmus* in Hawaii. Spores were found only in the aortic bulb, characterized by elliptical capsule with 2 tails, and pyriform polar capsules that angled toward the anterior end of the spore. Polar filaments had 3–4 coils. Parasites were present in apparently healthy fishes and caused no evident gross pathology. On microscopy, parasites evinced a mild inflammatory response in the host characterized by accumulations of eosinophilic fibrillar material around spores and a mononuclear infiltrate in the adventitia of the bulbus arteriosus. Overall prevalence was 20%, and prevalence between 2001 and 2006 ranged from 12 to 27%, but did not differ significantly between years. In contrast, prevalence of infection was highest in south-central Oahu. There was no relationship between infection status and body condition or gender of fish, and infection was absent in the smallest and largest fishes. Phylogenetically, *H. akule* n. sp. is most closely related to other *Henneguya* species infecting the heart of marine fishes based on ribosomal DNA analysis. This is the first documentation of a myxozoan parasite in marine fishes from Hawaii.

The big-eyed scad (*Selar crumenophthalmus*), or akule, is an important fisheries resource for the state of Hawaii, with annual landings by commercial and artisanal fisheries ranging from 100 to 600 tons. In Hawaii, akule spawn from April through October and mature at ca. 25 cm (Clarke and Privitera, 1995). For such a commercially important fish, surprisingly little biological information exists. Even less data are available on factors such as disease that could affect populations. Developing some basic understanding of microorganisms that infect this species would seem critical, particularly when it is being considered as a potential candidate for aquaculture (Iwai et al., 1996). As part of a larger study monitoring big-eyed scad at sewer outfalls on Oahu for internal tumors, we discovered a myxozoan in the bulbus arteriosus. Our objective was to describe this parasite, its pathology, and prevalence.

MATERIALS AND METHODS

From 2001 to 2006, fish were collected using line and hook at depths ranging from 15 to 70 m throughout 5 sites on southern Oahu. Fish were killed with an overdose of MS-222 in seawater. Necropsies consisted of measuring total and fork length (0.5 cm) with a caliper, weighing (0.1 g) with an electric scale, and a complete external and internal examination. Sections of spleen, liver, cranial and caudal kidneys, swim bladder, brain, heart, skeletal muscle, gill, and gonad were excised and fixed in 10% neutral buffered formalin. For molecular analyses, sections of heart tissue were preserved in 95% ethanol. Tissues were processed for histopathology using routine methodology and stained with hematoxylin and eosin. Spores were dissected from formalin fixed tissues and measured as described (Lom and Arthur, 1989). For electron microscopy, tissues were fixed in Trump's fixative (McDowell and Trump, 1976), rinsed in 0.1 M Sorenson's phosphate buffer, and postfixed in 2% osmium tetroxide. Epoxy-embedded tissues were cut into 1-μm-thick toluidine blue stained sections. Ultra-thin sections were stained with uranyl acetate, poststained with lead citrate, and examined with a Zeiss EM 109 electron microscope.

The DNeasy Tissue Kit (Qiagen Inc., Valencia, California) was used to extract DNA from infected heart tissue. Overlapping regions of the small subunit (SSU) ribosomal DNA (rDNA) were amplified with PCR primers of Diamant et al. (2004), using Invitrogen Taq DNA polymerase (Invitrogen Co., Carlsbad, California). PCR products were gel-extracted using the QIAEX II Gel Extraction Kit (Qiagen). Direct sequencing was carried out with the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1, using the ABI PRISM® 3730 DNA Analyzer (Applied Biosystems, Foster City, California). Fragments were assembled with Contig Express (Invitrogen) and edited by visual inspection. The resulting 2058 nucleotide SSU sequence for *H. akule* n. sp. was deposited in GenBank (accession no. EU016076).

For phylogenetic analysis, the SSU rDNA sequence of *H. akule* n.

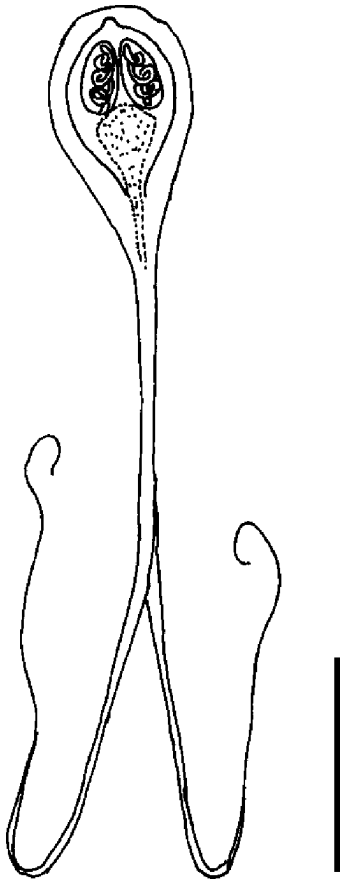

FIGURE 1. Line drawing of *H. akule* n. sp. (bar = 10 μm).

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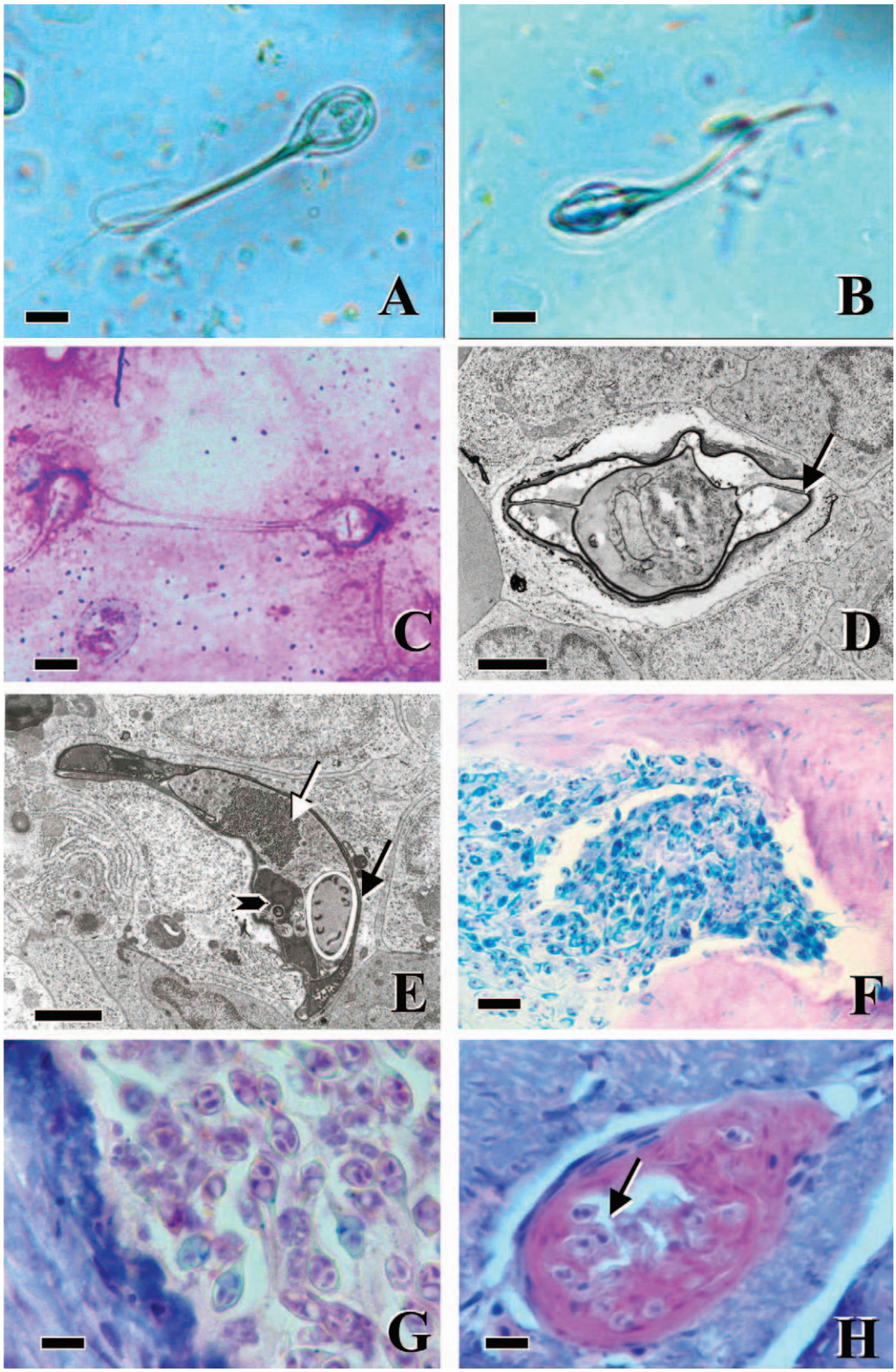

FIGURE 2. Spores of *Henneguya akule* n. sp. (**A**) Wet mount dorso-ventral view, bar = 7 μm. (**B**) Wet mount side view, bar = 7 μm. (**C**) Giemsa-stained impression smear, bar = 6 μm. (**D**) Electron micrograph *en face* view, note suture of bivalvular spore (arrow), bar = 1 μm. (**E**) Electron micrograph side view; note polar filaments within the polar capsule (black arrow), glycogen granules (white arrow), and sporoplasm (arrowhead), bar = 1 μm. (**F**) Aggregate of spores in aortic bulb (Giemsa), bar = 50 μm. (**G**) Closeup of section F (hematoxylin and eosin), bar = 10 μm. (**H**) Nidus of eosinophilic debris surrounding individual spores (arrow) in aortic bulb (H&E), bar = 25 μm.

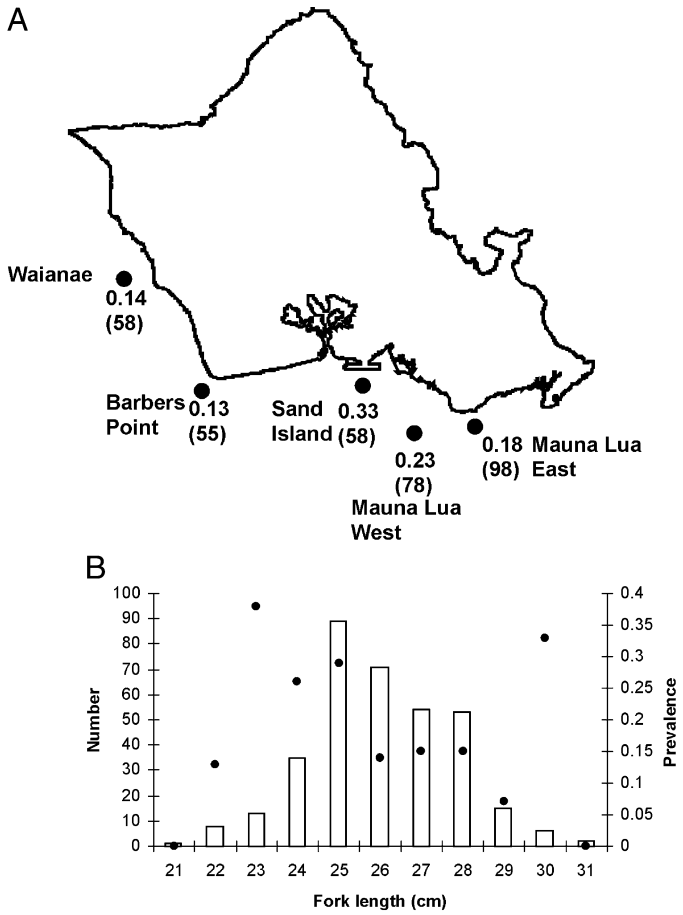

FIGURE 3. (**A**) Map of Oahu (Hawaii) with sites sampled for prevalence of *Henneguya akule* n. sp. Numbers indicate prevalence, and numbers in parentheses are sample size for each area. (**B**) Prevalence (dots, right axis) of *H. akule* versus frequency histogram of fork length (bars, left axis).

sp. was aligned to SSU sequences from other species in the relevant subclade based on the phylogenies of Easy et al. (2005), Yokoyama et al. (2005), Fiala (2006), and our preliminary analyses of which incorporated sequences recently deposited in GenBank. The marine myxozoans *Unicapsula* sp. and *Kudoa alliaria* were used to root the tree. Nucleic acid sequence alignments were conducted in ClustalX (Thompson et al., 1997) using default settings and edited by eye. Phylogenetic analyses using parsimony and maximum likelihood algorithms were conducted in PAUP*4.01 (Swofford, 1998). Bayesian analyses (BA) was conducted using MrBayes v. 3.0 (Ronquist and Huelsenbeck, 2003). Parsimony analyses (MP) were conducted using a heuristic search algorithm with 10 random additions of sequences and tree bisection-reconnection (TBR) branch swapping. Bootstrap values were calculated with 100 replicates using a heuristic search algorithm with simple sequence addition and TBR branch swapping. An optimal evolutionary model (GTR+G+I) for the alignment was determined using Modeltest 3.06 (Posada and Crandall, 1998). Maximum likelihood (ML) analysis employed a heuristic search algorithm with random sequence addition and TBR branch swapping. Bootstrap confidence values were calculated with 100 repetitions. Tree construction using BA was run with 4 simultaneous Monte Carlo chains for 10^6 generations, sampling every 100 generations, with a burn-in of 100 trees.

Body condition indices (weight/fork length^3) were calculated (Petrushevski and Kogteva, 1954). Data were tested for normality and equal variance. A chi square analysis was performed to evaluate relationship between parasitism and sex, parasitism and year of collection, and parasitism and location of collection. Student's *t*-test was used to evaluate difference in body condition index between parasitized and parasite-free fish (Daniel, 1987). Alpha for all comparisons was 0.05.

DESCRIPTION

Henneguya akule n. sp.

Spores ellipsoid with long bifurcated tails (Figs. 1, 2A–E). Morphometrics (μm) based on measurements of 50 spores from aortic bulb of 2 akule. Spore length (mean ± SD, range), 12.1 ± 0.8, 10–14; spore width, 7.4 ± 0.7, 5–9; spore total length, 40.8 ± 5.2, 29–52; spore thickness, 5.3 ± 0.6, 3–7; polar capsule width, 1.4 ± 0.5, 1–2; polar capsule length, 3.4 ± 1, 2–6. Polar filament had 3–4 coils. Plasmodia pleiomorphic ranging from 0.01 to 0.7 mm long (Figs. 2F–H).

Taxonomic summary

Type host: Selar crumenophthalmus (Bloch 1793) (Carangidae), big-eyed scad or akule.

Site in host: Bulbus arteriosus.

Type locality: Southern Oahu: Barbers Point (21.15°N, 158.00°W).

Other localities: Southern Oahu: Mauna Lua Bay (21.15°N, 157.45°W), Waianae coast (21.30°N, 158.15°W), Sand Island (21.18°N, 157.54°W).

Specimen deposited: Hapantotypes (giemsa-stained slide, hematoxylin, and eosin sections) deposited at the U.S. Department of Agriculture National Parasite Collection in Beltsville, Maryland (USNPC no. 099994.00 and USNPC no. 099995.00). Small subunit ribosomal DNA sequence deposited in GenBank (EU016076).

Prevalence and relative intensity: A total of 347 fish was examined histologically for the presence of *Henneguya akule* n. sp.; overall prevalence of infection was 20%. Prevalence in males (15%, n = 140) versus females (22%, n = 205) (2 fish were of unknown sex) did not differ significantly. Annual prevalence ranged from 12 to 27% between 2001 and 2006 and monthly prevalence during January–June ranged from 5 to 20% and 18 to 32% from July to December. Prevalence between year and month did not differ significantly. In contrast, prevalence between sites differed significantly ($\chi^2 = 9.671$, $P < 0.05$), with the highest prevalence concentrated in south-central Oahu (Fig. 3A). Prevalence was lowest in the smallest and largest size classes (Fig. 3B). Body condition of infected fishes did not differ significantly from non-infected fishes.

Etymology: Akule refers to the Hawaiian language name for big-eyed scad.

Remarks

The parasite seen here is consistent with species of *Henneguya* based on ellipsoid spores with a suture separating biconvex valves having long caudal projections (Lom and Dyková, 1992). *Henneguya* species infect mainly freshwater fishes and parasitic stages of this parasite are mostly found in the gills, skin, kidneys, musculoskeletal system, or gastrointestinal tract (Eiras, 2002); however, *Henneguya* sp. in the bulbus arteriosus are relatively less common (Yokoyama et al., 2005).

Despite the advent of molecular tools to examine the phylogeny of the Myxosporea, considerable uncertainty continues to exist on exactly how to classify species. Although predominantly freshwater species, marine representatives of these genera clustered together in early analyses (Kent et al., 2001), suggestive of a single marine invasion from freshwater. Increased taxon sampling in later studies (Fiala, 2006) placed freshwater and marine species within the same clade, indicating multiple reversals to and from the marine environment. Based on analyses of SSU rDNA (Kent et al., 2001; Fiala, 2006), *Henneguya* and *Myxobolus* form a polyphyletic clade comprised of both marine and freshwater species. Kent et al. (2001) further concluded that taxa seemed to cluster more by development and tissue location than by spore morphology. The importance of tissue specificity in myxozoan evolution has been reinforced by subsequent phylogenetic studies (Blaylock et al., 2004; Eszterbauer, 2004; Whipps et al., 2004; Fiala, 2006; Burger et al., 2007). Consistent with these findings, *H. akule* n. sp. was phylogenetically most similar to *H. lateolabracis* and *H. pagri*, both parasites of the bulbus arteriosus of marine fishes (Fig. 4). As in previous analyses (Easy et al., 2005; Yokoyama et al., 2005; Fiala, 2006), much of the remaining tree topology was unstable as indicated by numerous polytomies and low bootstrap support (Fig. 4). Bayesian anal-

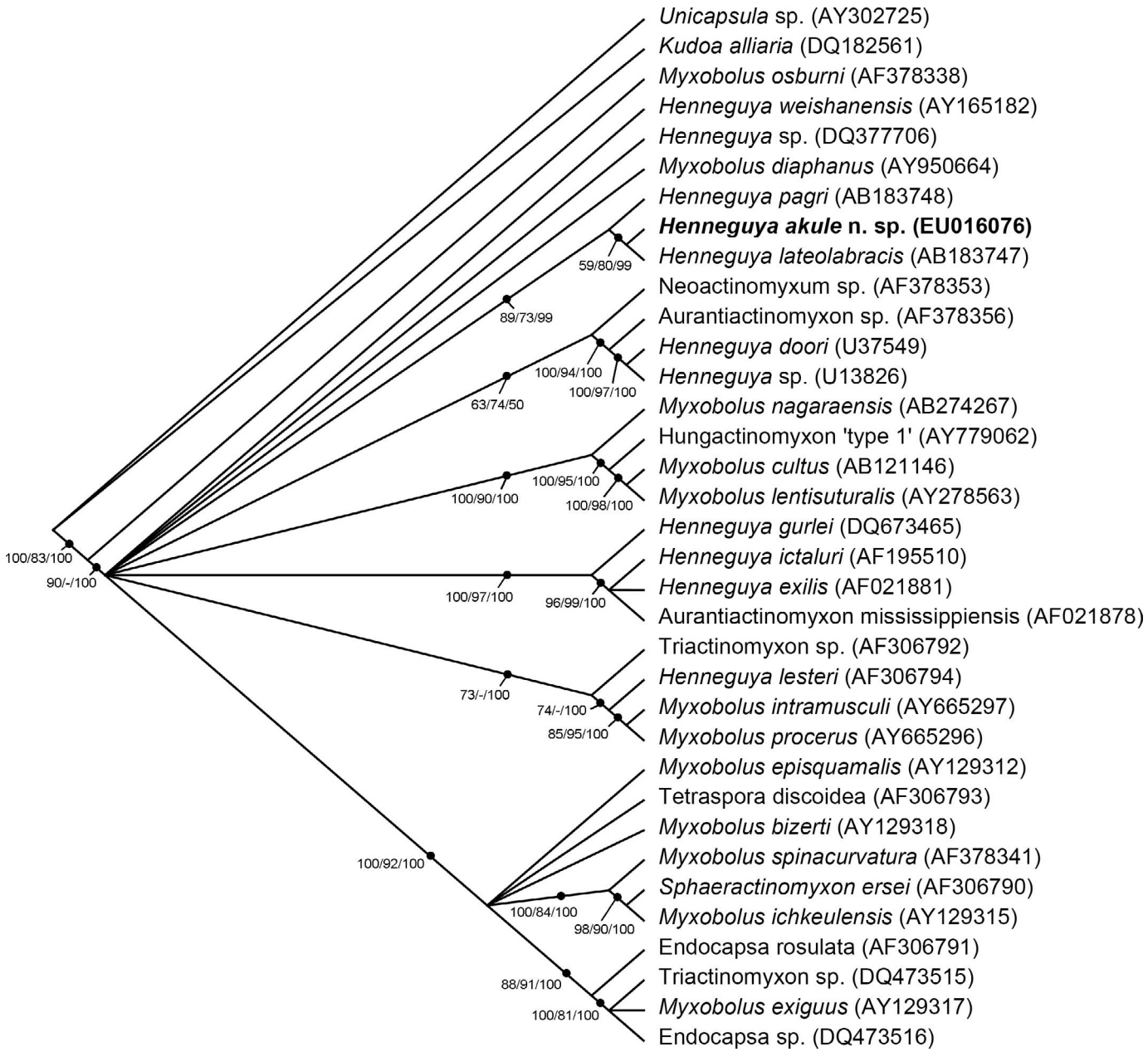

FIGURE 4. Phylogenetic analysis of small subunit ribosomal DNA of *Henneguya akule* n. sp. Strict consensus of the 3 most parsimonious trees generated by maximum parsimony. In-group taxa were selected to represent an entire myxobolid subclade supported by previous analyses (Easy et al., 2005; Fiala, 2006) including relevant BLAST matches and sequences derived from unclassified actinospore stages. *Unicapsula* sp. and *Kudoa alliaria* were used to root the tree. GenBank accession numbers are shown adjacent to names. Numbers on branches indicate bootstrap support resulting from maximum parsimony, bootstrap support from maximum likelihood, and clade confidence values from Bayesian analysis. Values below 50 are not shown.

yses yielded fewer polytomies (tree not shown) and gave higher clade confidence values, but these tend to be overestimates relative to bootstrap support values from parsimony analysis (Simmons et al., 2004). Thus, we chose to present the more conservative maximum parsimony consensus tree that illustrates these instabilities. Regardless, the marine *Henneguya* species from the heart consistently clustered (Fig. 4).

Comparing the morphology of spores of *Henneguya* species located in the heart of fish, *H. akule* n. sp. was most similar in shape and size to its phylogenetic sister species. *Henneguya akule* n. sp. spores were longer and thinner than those of *H. lateolabracis* from Chinese sea bass in the western Pacific (Yokoyama et al., 2003), with narrower polar capsules and shorter caudal appendages. We recognize that our spore measurements are from tissues fixed in 10% formalin, and thus measurements may be slightly different than those made from fresh material (Parker and Warner, 1970). However, we believe this makes little difference for 3 reasons: (1) the size of *H. akule* is larger than other species to which it was compared; (2) the shrinkage due to formalin is smaller than the detectable limits of precision for our measurements (ocular and stage micrometer on microscope); and (3) 10% formalin is deemed as acceptable for morphometrics of Myxosporea (Lom and Arthur, 1989).

Unlike infections with *H. lateolabracis*, we did not find any morphologic stages of *H. akule* n. sp. in the gills of akule (347 fish ex-

amined). *Henneguya akule* n. sp. has caudal filaments similar to those seen in *H. pagri* (Yokoyama et al., 2005) (Figs. 1, 2A–B), but these are considerably shorter and adopt a tortuous appearance; *H. akule* also has a longer spore. Other *Henneguya* species that have been documented from the heart of fish (reviewed in Yokoyama et al., 2005) can be differentiated from *H. akule* n. sp. in having much larger spores, or, in the case of *Henneguya otolithi*, much longer caudal appendages. Given its location in the host (bulbus arteriosus only), its morphology, and the geographic isolation of the parasite's host (Hawaii), we believe the naming of *H. akule* n. sp. as a new species is justified. Furthermore, DNA sequence data support distinction of *H. akule* n. sp. from its morphologically similar relatives. Despite morphological similarities, the genetic similarity of the SSU rDNA of *H. akule* n. sp. to *H. lateolabracis* and *H. pagri* was 88.7 and 87.2 percent, respectively, which is well in excess of the 1–2% distance that might be considered intraspecific variation for myxozoans (Whipps and Diggles, 2006).

Host response to the parasite was minimal, and gross lesions were not present. On microscopy, host response was mild and characterized by accumulations of eosinophilic fibrillar material around spores (Fig. 2H) and occasional mononuclear infiltrates in the adventitia (seen in 17% of infected fish). Unlike Meyers et al. (1977), we did not observe rodlet cells as a component of the inflammatory response to *H. akule* n. sp. It appears that pathological changes due to infection with *Henneguya* sp. are more severe in cultured fish (Yokoyama et al., 2003, 2005) where, presumably, opportunities for infection are greater than for wild fish. Like Ganapati (1941) and Meyers et al. (1977), parasites were absent in very young and very old fishes, but prevalence was relatively constant for intervening sizes. Mild pathology and constant prevalence with age suggests this parasite may have little measurable detrimental effect on the host.

Although there appeared to be an increase in prevalence from June–December, this was not significant and contrasted with seasonality observed in infections by *H. pagri*, which had a peak prevalence in August, and *H. otolithi*, which had a peak prevalence in March. Overall prevalence of infection of akule with *H. akule* n. sp. was generally lower than that reported in other studies (Ganapati, 1941; Meyer et al., 1977) and similar to that reported for *H. lateolabracis* (Yokoyama et al., 2003) and *H. sebasta* in selected species of rock fish (Moser and Love, 1975). Few studies have examined geographic variation of infection with marine *Henneguya* species; the high prevalence of infection in fishes from south-central Oahu was intriguing. This region is adjacent to the highest urban concentration (Honolulu) and is close to Pearl Harbor, suggesting that certain physiographic aspects of these areas may be contributing to high prevalence there. Explaining this higher prevalence in south-central Oahu will require more detailed epizootiologic studies and further knowledge about the life cycle of *H. akule* n. sp.

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