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Ultrastructure and small-subunit ribosomal DNA sequence of *Henneguya lesteri* n. sp. (Myxosporea), a parasite of sand whiting *Sillago analis* (Sillaginidae) from the coast of Queensland, Australia

Sascha L. Hallett[1], Ariel Diamant[2,*]

[1]Department of Microbiology and Parasitology, The University of Queensland, Brisbane, Queensland 4072, Australia
[2]The National Center for Mariculture, Israel Oceanographic and Limnological Research Institute, PO Box 1212, Eilat 88112, Israel

ABSTRACT: *Henneguya lesteri* n. sp. (Myxosporea) is described from sand whiting, *Sillago analis*, from the southern Queensland coast of Australia. *H. lesteri* displays a preference for the pseudobranchs and is typically positioned along the afferent blood vessels, displacing the adjoining lamellae and disrupting their normal array. The plasmodia appeared as whitish-hyaline, elliptical cysts (mean dimensions 230 × 410 µm) attached to the oral mucosa lining of the hyoid arch on the inner surface of the operculum. Infections of the gills were also found, in which the plasmodia were spherical, averaged 240 × 240 µm in size and were located on the inner hemibranch margin. The parasites lodged in the gill filament crypts and generated a mild hyperplastic response of the branchial epithelium. In histological sections, the plasmodium wall and adjoining ectoplasm appeared as a finely granulated, weakly eosinophilic layer. Ultrastructurally, this section of the host-parasite interface contained an intricate complex of pinocytotic channels. *H. lesteri* is polysporic, disporoblastic and pansporoblast forming. Sporogenesis is asynchronous, with the earliest developmental stages aligned predominantly along the plasmodium periphery, and maturing sporoblasts and spores toward the center. Ultrastructural details of sporoblast and spore development are in agreement with previously described myxosporeans. The mature spore is drop-shaped, length (mean) 9.1 µm, width 4.7 µm, thickness 2.5 µm, and comprises 2 polar capsules positioned closely together, a binucleated sporoplasm and a caudal process of 12.6 µm. The polar capsules are elongated, 3.2 × 1.6 µm, with 4 turns of the polar filament. Mean length of the everted filament is 23.2 µm. Few studies have analyzed the 18S gene of marine Myxosporea. In fact, *H. lesteri* is the first marine species of *Henneguya* to be characterized at the molecular level: we determined 1966 bp of the small-subunit (18S) rDNA. The results indicated that differences between this and the hitherto studied freshwater *Henneguya* species are greater than differences among the freshwater *Henneguya* species.

KEY WORDS: Marine Myxosporea · Pseudobranchs · Gills · Ultrastructure · 18S rDNA gene sequence

INTRODUCTION

The majority of species included in the genus *Henneguya* Thelohan (1892) parasitize freshwater fishes (Lom & Dyková 1992). *Henneguya* species are important fish pathogens, documented from both freshwater and marine habitats, with the gills often being the most heavily infected organ (Azevedo & Matos 1995). The available information on the genus does not include any study on the ultrastructure or molecular biology of a marine *Henneguya*.

In Australia, 2 species have been described from freshwater fishes: *Henneguya australis* Johnston & Bancroft 1918 from *Macquaria ambigua* (Richardson 1845) and *H. gracilis* Bancroft & Johnston 1918 from *Scortum hillii* (Castelnau 1877). Six additional re-

*Corresponding author. E-mail: diamant@agri.huji.ac.il

corded *Henneguya* spp. from Australia are 4 unidentified species from freshwater fish (O'Donoghue & Adlard 2000) and 2 from coastal marine fish: *Henneguya* spp. from yellowfin bream *Acanthopagrus australis* (Gunther 1859) in Queensland (Roubal 1994) and grey mullet *Mugil cephalus* Linnaeus 1758 in New South Wales (Lom et al. 1992).

The present study describes a new species, *Henneguya lesteri* n. sp., from the pseudobranch and gills of sand whiting *Sillago analis* from a mangrove mud flat and a sandy beach at Moreton Bay, Queensland, Australia.

Table 1. Primer sequences

Forward	
18e	5′ CTG GTT GAT TCT GCC AGT 3′
MYX1f	GTG AGA CTG CGG ACG GCT CAG
ACT1f	GGC AGC AGG CGC GCA AAT TAC CCA A
ACT2f	CCT GGT CCG AAC ATC CGA AGG ATA C
ACT3f	CAT GGA ACG AAC AAT
Reverse	
18g	CGG TAC TAG CGA CGG GCG GTG TG
MYX4r	CTG ACA GAT CAC TCC ACG AAC
ACT1r	AAT TTC ACC TCT CGC TGC CA
ACT1fr	TTG GGT AAT TTG CGC GCC TGC TGC C

MATERIALS AND METHODS

Collection of material. Twenty-three sand whiting *Sillago analis* Whitley 1943 were collected from 2 coastal sites of Moreton Bay during 2 sampling periods in January and July 1999. The fish were transported live in seawater to the laboratory at the Pinjarra Hills Veterinary Farm, University of Queensland, placed in a recirculating seawater system and examined within 3 d of capture. The fish were anaesthetized with benzocaine, and branchial and internal organs were thoroughly examined for the presence of myxosporeans. Blood smears were air dried and stained with Hemacolor (Merck, Australia Pty Ltd, Kilsyth, Victoria). For measurements of fresh spores, wet smears made from squashed plasmodia were measured and drawn under a phase contrast microscope with the aid of camera lucida, according to the guidelines of Lom & Arthur (1989). For additional observations, smears were air dried and stained with Giemsa. Measurements of plasmodia were carried out on formalin preserved gills and pseudobranchs. Samples of infected gill and pseudobranch were fixed and processed for paraffin histology and transmission electron microscopy as previously described (Diamant & Paperna 1992).

Extraction, purification and precipitation of DNA. Spores within plasmodia fixed in absolute ethanol were washed over 2 nights in 500 µl 1× Tris-EDTA (TE) and re-suspended in 500 µl 1× TE. The plasmodia were crushed, 15 µl of 20 mg ml^{-1} proteinase K was added and the sample was incubated at 37°C for 1 h. Then 140 µl of 5 M NaCl followed by 100 µl Cetyltrimethylammonium Bromide buffer (CTAB) was added and the sample was incubated for a further 10 min at 65°C. The extracted DNA was purified with 2 applications of chloroform/phenol and precipitated with (0.6 v) 100% isopropanol and cold 70% ethanol. The dry pellet was re-suspended in 30 µl nuclease-free water and stored at 4°C.

Amplification of DNA. The small-subunit ribosomal DNA gene cluster (18S rDNA) was targeted for amplification using PCR. A combination of primers that we (MYX1f, Act1f, Act2f, Act3f, MYX4r, ACT1r and ACT1fr) and other groups (18e and 18g) have used with myxozoans (S. L. Hallett unpubl. data, Andree et al. 1997, Bartholomew et al. 1997, Hallett 1998) were employed in PCRs and sequencing reactions (Table 1, Fig. 1). Twenty microliter volumes containing 2 µl 10× reaction buffer, 50 mmol $MgCl_2$, 4 nmol deoxynucleotide triphosphate (dNTP), 10 pmol of each primer, 2 µl DNA template and nuclease-free water were overlaid with 30 µl mineral oil. Samples were given a hot start. They were denatured at 95°C for 2 min and 1 unit of Taq DNA polymerase was added under oil. Amplifications were run in a FTS-320 Fast Thermal Sequencer (Corbett Research, Sydney). Thirty cycles of template denaturation (95°C for 1 min), primer annealing (55°C for 30 s) and chain extension (72°C for 1 min) were followed by a single cycle of template denaturation (95°C for 1 min), primer annealing (55°C for 30 s) and chain extension (72°C for 7 min) and finished at 4°C.

Visualization of PCR products. PCR products were combined with gel dye (0.25% bromophenol blue, 30% glycerol) and electrophoresed on a 0.9% agarose gel (agarose, deionized water, 1× Tris-Acetate (TAE) buffer and ethidium bromide) alongside a molecular weight standard (100 bp DNA ladder, Gibco BRL, Sydney) and visualized with a UV transilluminator. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN Pty Ltd, Clifton Hill, Victoria) and run against a mass standard ladder on an agarose gel to estimate the concentration of DNA.

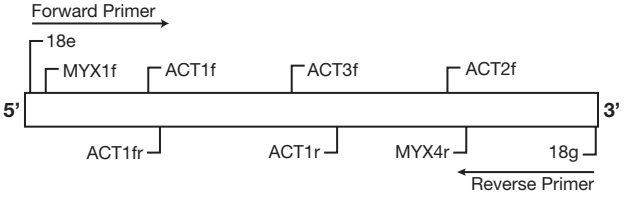

Fig. 1. Map of primers on 18S small-subunit RNA showing approximate annealing sites

Sequencing of PCR products. Products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Melbourne) and 10 µl half terminator reactions were run on a MINICYCLER (MJ Research Inc., Watertown, MA, USA). Ready Reaction Mix (containing terminators labeled with dichlor, dNTPs, AmpliTaq-FS DNA polymerase, $MgCl_2$ and Tris-HCl buffer, pH 9.0; Applied Biosystems) was combined with primer (3.2 pmol), DNA template (30 to 45 ng) and nuclease-free water and run at 96°C for 30 s (template denaturation), 50°C for 15 s (primer annealing) and 60°C for 4 min (chain extension) for 25 cycles. The resultant DNA product was precipitated using 3 M sodium acetate at pH 4.6 and cold 100 % ethanol. The sample was centrifuged at 14 000 × *g* for 20 min, and the pellet was rinsed with cold 70 % ethanol and air-dried overnight.

Auto-sequencing in an ABI 373A DNA sequencer was done at the sequencing unit of the Molecular Biology Facility, Faculty of Science and Technology, Griffith University, Queensland.

Sequence alignments. Nine segments of 18S sequence were generated with the various forward and reverse primers. These were imported into Sequence Navigator (Applied Biosystems) and aligned manually to produce a single 1966 bp sequence. Ambiguous bases were clarified using corresponding ABI chromatograms. The sequence was lodged in GenBank, Accession Number AF306794.

Partial to complete 18S rDNA sequences for 5 *Henneguya* species (all freshwater) were available from the GenBank database through the Australian National Genomic Information System (ANGIS): *Henneguya* sp., U13826; *H. doori*, U37549; *H. zschokkei*, U13827; *H. salminicola*, AF03-1411; and *H. exilis*, AF021881. Sequences were also available for 4 marine actinosporeans: *Sphaeractinomyxon ersei*, AF306790; *Tetraspora discoidea*, AF306793; *Endocapsa rosulata*, AF306791; and *Triactinomyxon* sp., AF306792. The marine *H. lesteri* was aligned separately with these 2 data sets in Sequence Navigator and the sequences were truncated for homology. These edited sequences were aligned using the Clustal V computer program (Des Higgins Molecular Biology Laboratory, Germany; Higgins et al. 1992) and MacClade 3.05 (Maddison & Maddison, see http://phylogeny.arizona.edu/macclade/macclade.html). A base similarity matrix was constructed using PAUP3.1.1.

RESULTS

Degree of infection

Nine out of 23 sand whiting were infected, displaying pseudobranchial or gill plasmodia (cysts). In the January sampling, the precise locations of the plasmodia (pseudobranchs or gill arches) were not noted. However, in the July sampling, 3 out of 15 fish had pseudobranchial infections only, and 1 had a gill infection as well. All infections were bilateral. The plasmodia contained drop-shaped spores with 2 unequal, smooth valves, each tapering off into a slender caudal projection forming a tail, placing this myxosporean parasite in the genus *Henneguya* (Fig. 2). No developmental stages were found in any of the blood smears.

Spore

The mature spore is drop shaped in valvular view and bi-convex in sutural view (Fig. 3). The anterior margin is blunt, tapering off into a point at the opposite posterior end. The valves are smooth and equal in size. The polar capsules are positioned close together and are equal in size, ellipsoid and elongated, about twice as long as wide with 4 turns of the filament. The spore is broadest just behind the posterior end region of the polar capsules. In the spore cavity immediately posterior to the polar capsules is a bi-nucleated sporoplasm containing a spherical polysaccharide inclusion. The caudal process is composed of 2 fused filaments that

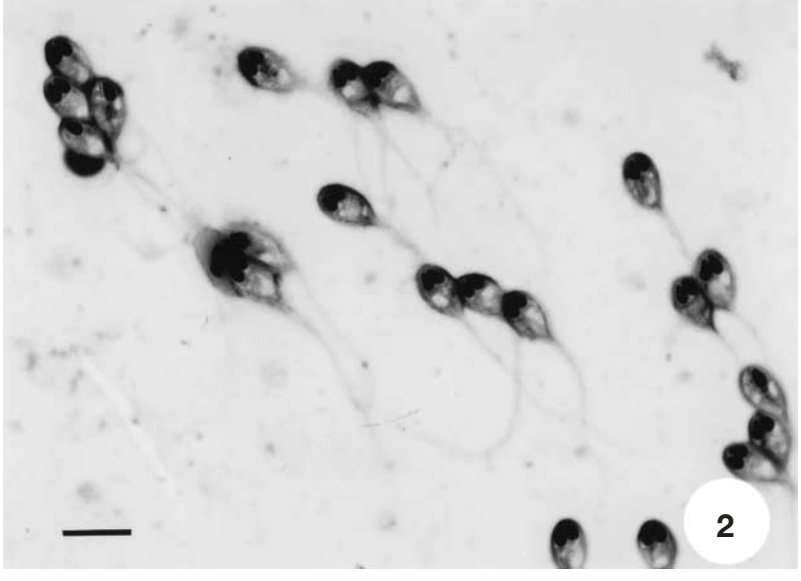

Fig. 2. Smear of air dried spores of *Henneguya lesteri* n. sp. from the pseudobranch of *Sillago analis* (Giemsa stain, scale bar = 10 µm)

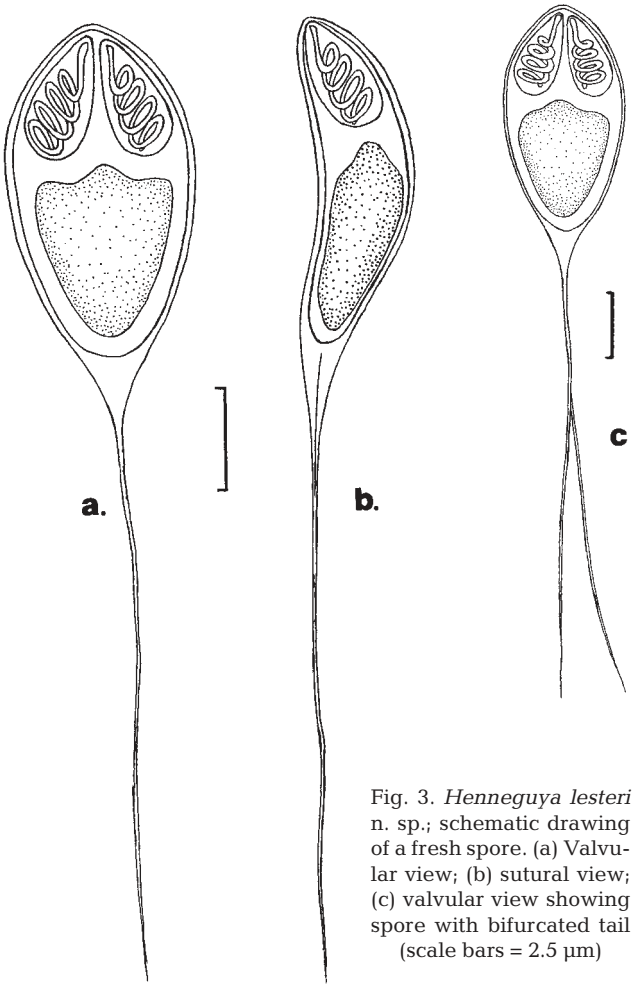

Fig. 3. *Henneguya lesteri* n. sp.; schematic drawing of a fresh spore. (a) Valvular view; (b) sutural view; (c) valvular view showing spore with bifurcated tail (scale bars = 2.5 µm)

gradually taper off, rarely with slight bifurcation at the terminal end. Measurement ranges (means) (n = 20, in micrometers) are as follows: spore length 8.0 to 10.4 (9.1), width 4.0 to 5.0 (4.7), thickness 2.4 to 2.6 (2.5), caudal process 11.2 to 16 (12.6), polar capsule length 3.2 to 3.5 (3.2), polar capsule width 1.3 to 1.6 (1.6) and everted filament length 14.7 to 26.8 (23.2).

Vegetative forms

Pseudobranch

Plasmodia were observed underlying the pseudobranch, attached to the mucosa lining of the hyoid arch on the inner surface of the operculum and the adjoining lamellae. The plasmodia, sub-spherical or elongated in shape, appeared as whitish-hyaline trophozoites, the outer margin displaying an indistinct zone of ectoplasm and measuring 200 to 270 µm × 210 to 550 µm (mean 230 × 410) (n = 7). In the plasmodium, development was polysporic, and sporogenesis was asynchronous, disporoblastic and within a pansporoblast. The earliest developmental stages were located along the peripheral ectoplasm, with advanced stages further inwards. *In situ*, the plasmodia were partly covered by the adjoining pseudobranchial filaments. The plasmodium was firm and its envelope was relatively durable, which readily enabled its intact dissection from host tissue. In histological sections, the plasmodia were typically positioned along the afferent pseudo-

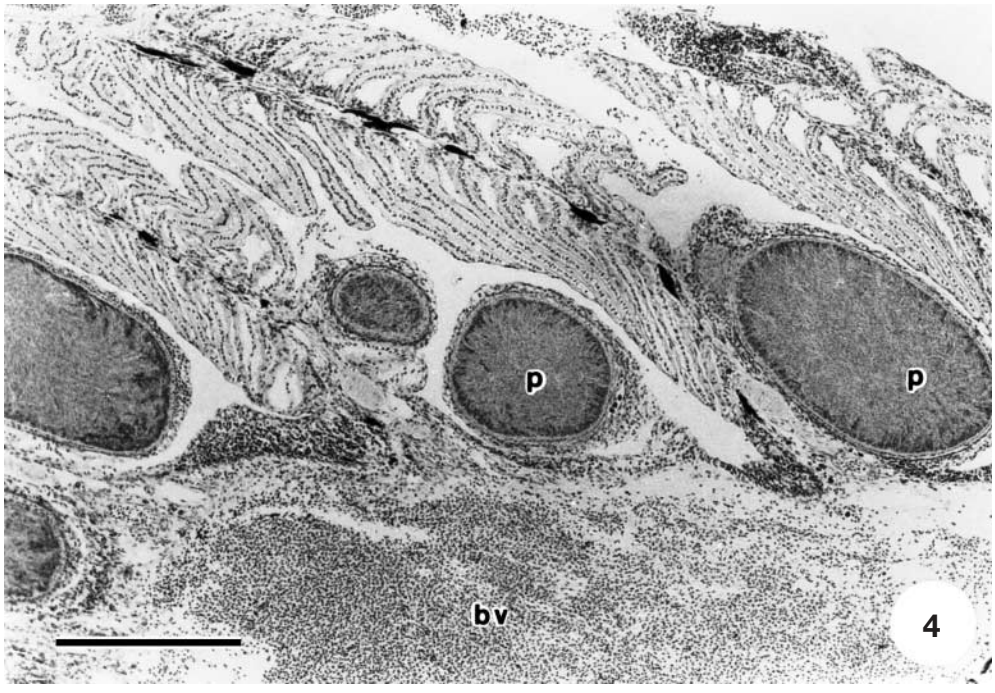

Fig. 4. Section through pseudobranch, showing several *Henneguya lesteri* plasmodia (p) adjoining a pseudobranchial blood vessel (bv) (hematoxylin and eosin stain, scale bar = 250 µm)

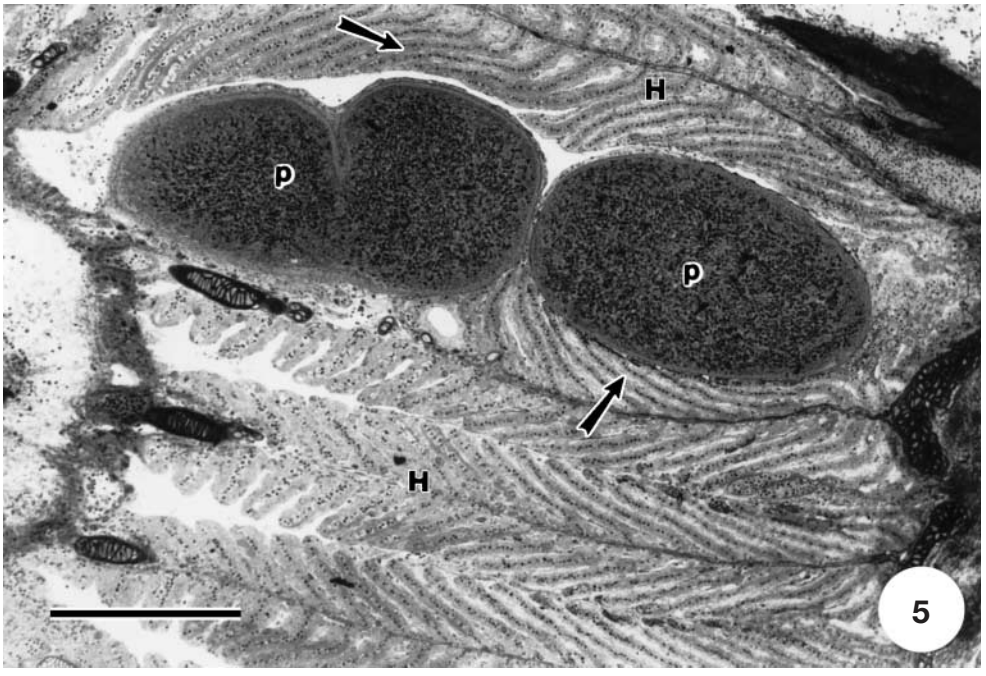

Fig. 5. Tangential section through 2 *Henneguya lesteri* plasmodia (p) wedged in between 2 pseudobranch filaments. The lamellae (H) have been pushed aside (arrows) (toluidine blue stain, scale bar = 250 µm)

branch artery and associated blood vessels (Fig. 4), their bulk displacing the adjoining filaments and disrupting their normal array (Fig. 5).

Gills

Plasmodia in the gills were located on the inner hemibranch margin, lodged in the filament crypts (Fig. 6). The plasmodia, some appearing in pairs, were subspherical and measured 175 to 335 µm (mean 240) in diameter (n = 15). Each plasmodium was surrounded by a finely granulated, weakly eosinophilic layer measuring 3 to 5 µm. This was covered by a layer of stratified, flattened, hyperplastic branchial epithelium of varying thickness, often 10 to 25 µm but reaching 50 µm.

In summary, the features of *Henneguya lesteri* n. sp. are as follows: its host is sand whiting, *Sillago analis* Whitley 1943; its sites of infection are the pseudobranchs and gills; prevalence of infection is 9 in 23 (39.1%); and its locality is Moreton Bay, Queensland.

Diagnosis

Lom & Dyková (1992) reported approximately 120 species belonging to the genus *Henneguya*, the majority from freshwater hosts. Among the marine species, only a few are from subtropical or tropical regions. In fact, no *Henneguya* spp. are documented in the list of 580 parasite species found in fishes of Heron Island, Great Barrier Reef (Lester & Sewell 1989, Moser et al. 1989). Roubal (1994) documented a species of *Henneguya* in yellowfin bream

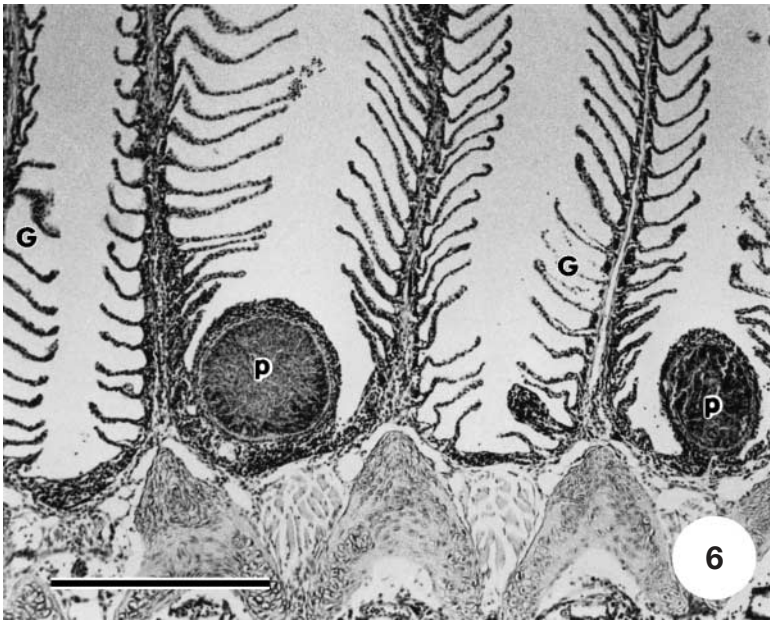

Fig. 6. Section through gill arch, exhibiting 2 *Henneguya lesteri* plasmodia (p) attached to the epithelium at the base of the filaments (G) (hematoxylin and eosin stain, scale bar = 250 µm)

Acanthopagrus australis in Moreton Bay, Queensland. Although this species remains unidentified, the author provided detailed measurements and photographs, which indicate that the spore is more rounded and considerably smaller, and has thicker valves than the present species. Details on an unidentified species of *Henneguya* from *Mugil cephalus* were not provided (Lom et al. 1992).

Henneguya vitiensis Laird 1950 from the Fiji Islands was described from the heart tissue of *Leiognathus fasciatus*. The spore of this species is more rounded and considerably larger, and has thicker valves than our species (see figures in Laird 1950). *H. latesa* Zahoe & Jianping 1994 from the gills of *Lates calcarifer* from the South China Sea, although within the same size range, has a less elongated spore and polar capsules, thicker valves and a longer, bifurcated tail. *H. otolithi* Ganapati 1941, which infects the bulbous arteriosus of *Otolithus* spp. from the coast of India, has a larger, more rounded spore and polar capsules and a much longer tail (35 to 40 µm).

Two marine species have been described from marine fish in Florida: *Henneguya lagodon* Hall & Iversen 1967 from pinfish *Lagodon rhomboides* and *H. ocellata* Iversen & Yokel 1963 from red drum *Sciaenops ocellatus*. Both species differ considerably in spore shape and size from our species.

Records from Australian freshwater fish that warrant consideration are *Henneguya gracilis* from black bream *Therapon hillii* (= *Scortum hillii*) and *H. australis* from golden perch, *Plectroplites ambiguus* (= *Macquaria ambigua*), that inhabit freshwater of central Queensland (Johnston & Bancroft 1918). Nevertheless, the spore body of our *Henneguya* from whiting is smaller and has smaller polar capsules than these 2 latter species.

Henneguya santae Guimaraes & Bergamin 1934, from the gills and branchial arches of the freshwater *Tetragnopterus santae* from Brazil, has spore morphometrics very similar to our species (Guimaraes & Bergamin 1934). However, the slightly smaller polar capsules, shorter and bifurcated caudal process, remote geographic locality and different host in a freshwater habitat of the Brazilian specimen suggest that the species are different. We therefore consider that the Australian specimen is new to science and propose the name *Henneguya lesteri* n. sp.

Etymology

The species is named after Prof. Robert J. G. Lester, University of Queensland, in recognition of his significant contribution to the knowledge of marine fish parasitology. Synypes (2 slides) are deposited at the International Protozoan Type Collection, Smithsonian Institution, Washington, DC, Accession Number USNM 51546.

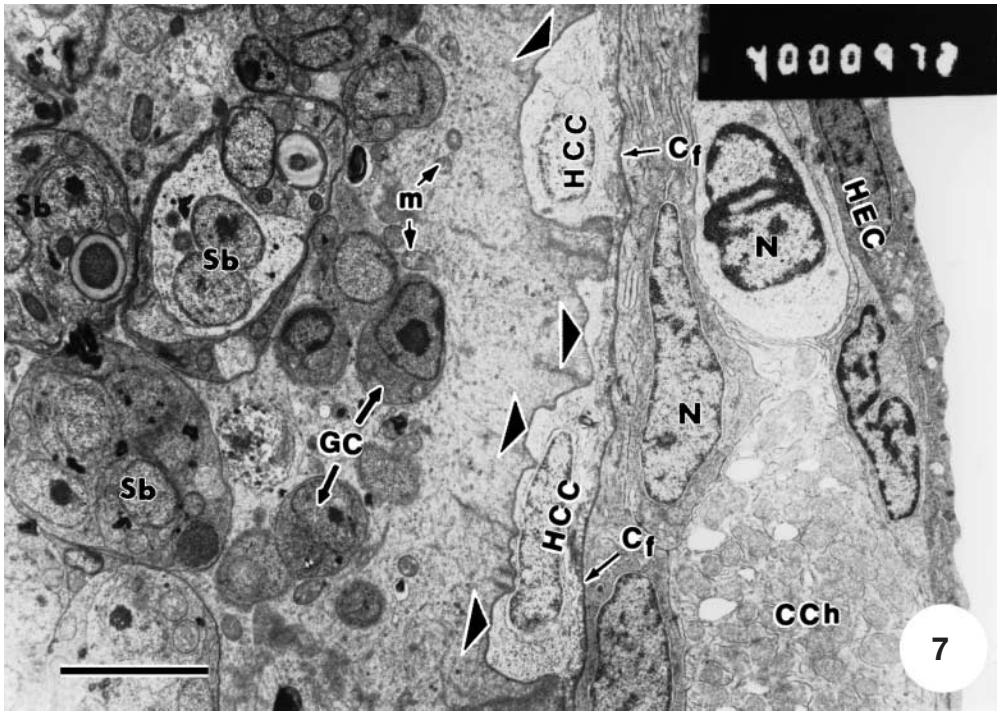

Fig. 7. Transmission electron microsopic image (TEM) of *Henneguya lesteri* plasmodium sections in *Sillago analis* pseudobranch. Section through periphery of plasmodium and surrounding host tissue. The boundary between host and plasmodium is a convoluted, single unit membrane (arrowheads). Flattened host cells rich in microfibrils (HCC) form a thin capsule over the plasmodium wall. The capsule is overlaid by a stratum of branchial tissue that includes epithelial (HEC) and chloride (CCh) cells. Generative cells (GC), sporoblasts (Sb) and mitochondria (m) are seen at left. N: nucleus; C_f: collagen fibres (scale bar = 3 µm)

Ultrastructure

Plasmodium

The outermost layer of the plasmodium consisted of several strata of host cells, including epithelial cells, lymphocytes, granulocytes, rodlet cells, mucus cells, mitochondria-rich chloride cells and pseudobranchial cells. Below this was a layer, 1 to 2 cells in thickness, of flattened host cells, forming an encapsulation around the plasmodium. The cytoplasm of these cells contained cisternae of smooth endoplasmic reticulum (ER), free ribosomes, mitochondria and bundles of microfibrils. Sandwiched in between these 2 host cell layers was a thin stratum of collagen fibers. The plasmodium outer membrane fitted tightly against the encapsulating cell membrane and extended finger-like extensions into it (Fig. 7). The plasmodium inner envelope—a convoluted, single unit membrane—extended perpendicular pinocytotic channels, approximately 400 nm in diameter, inward into the ectoplasm, which ramified into an intricate complex system of secondary canals (Fig. 8). Below this zone was a region of reduced density that contained scattered vacuoles, vegetative nuclei, membrane-bound pinocytotic vesicles, mitochondria and generative cells (Figs 8 & 9). An interface junction between 2 adjacent *Henneguya lesteri* plasmodia is shown in Fig. 10.

Sporogenesis

The full range of developmental stages—sporonts, sporoblasts and spores—was found in all examined plasmodia. Aggregations of early developing sporogonic cells were located at the plasmodium peripheral ectoplasm (Figs 10 & 11). In the central parts of the plasmodium were more advanced stages of developing sporoblasts and immature and ripe spores. The polar capsules of the latter were often degraded or missing due to poor fixative penetration (Fig. 11).

Generative cells were small, spherical and bounded with a double membrane, and measured 2 to 3.5 µm in diameter (Fig. 8). The nucleus was large with a prominent nucleolus and narrow peripheral zone of electron-dense chromatin. The relatively dense ribosome-rich cytoplasm contained no vacuoles and had several large mitochondria with tubular cristae. A Golgi apparatus was often seen in the cytoplasm bordering the nucleus.

The earliest stage of sporogenesis appeared as pairs of generative cells consisting of an outer enveloping cell and inner sporogonic cell. The latter contained more ribosomes but fewer membrane stacks (smooth ER) than the envelope cell.

Aggregations of 3, 4 and 5 cells, presumably a product of sporont division, were common (Fig. 11). Further development was characterized by concomitant differentiation of 2 valvogenic cells and 2 capsulogenic cells, and the maturation of a binucleated sporoplasm. The

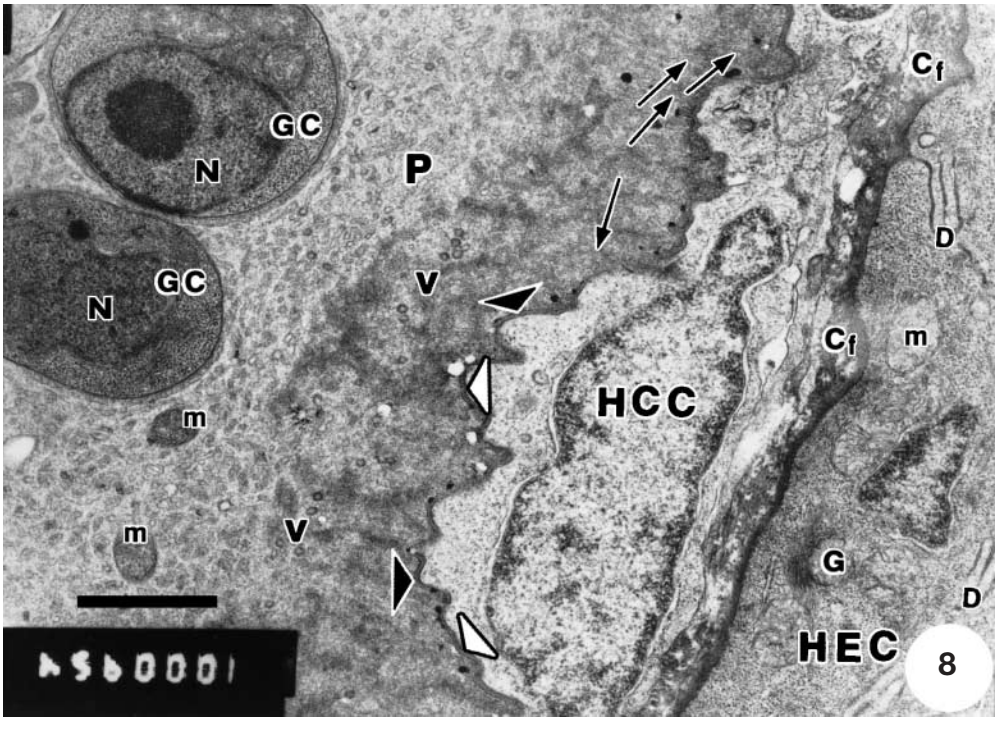

Fig. 8. TEM of *Henneguya lesteri* plasmodium sections in *Sillago analis* pseudobranch. Section of *H. lesteri* plasmodium (P) with 2 generative cells (GC). Underlying the plasmodium membrane is a band of ectoplasm containing numerous perpendicular pinocytotic channels, seen in tangential and longitudinal sections (small arrows). The plasmodium wall is convoluted and consists of plasmodial (black arrowheads) and host (white arrowheads) membranes. C_f: collagen fibres; D: desmosome; G: golgi apparatus; HCC: capsular cell of host origin; HEC: host epithelial cell; m: mitochondria; N: nucleus; v: pinocytic vesicles (scale bar = 1 µm)

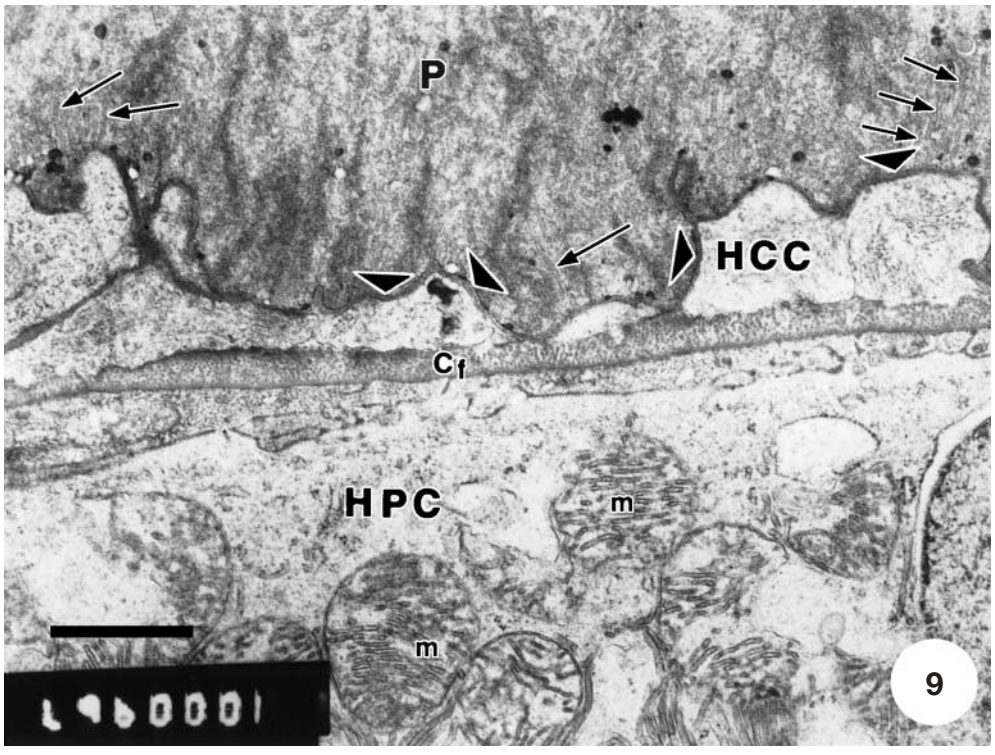

Fig. 9. Transmission electron microsopic image of *Henneguya lesteri* plasmodium sections in *Sillago analis* pseudobranch. Section of plasmodium (P) interface with host tissue. Arrowheads indicate plasmodium outer membrane; small arrows indicate pinocytotic channels. C_f: collagen fibres; HCC: capsular cell of host origin; HPC: pseudobranchial cell; m: mitochondria (scale bar = 1 µm)

valvogenic cells appeared as thin cytoplasmic protractions that enveloped the sporoblast and extended to the sutural ridge, filament discharge channels and posterior valve processes. Developing capsulogenic cells had a dense cytoplasm, mitochondria with tubular cristae, Golgi apparatus and nuclei with no nucleoli (Fig. 12). The amorphic capsular primordium matured to produce 4 coils. The sporoplasm contained charac-

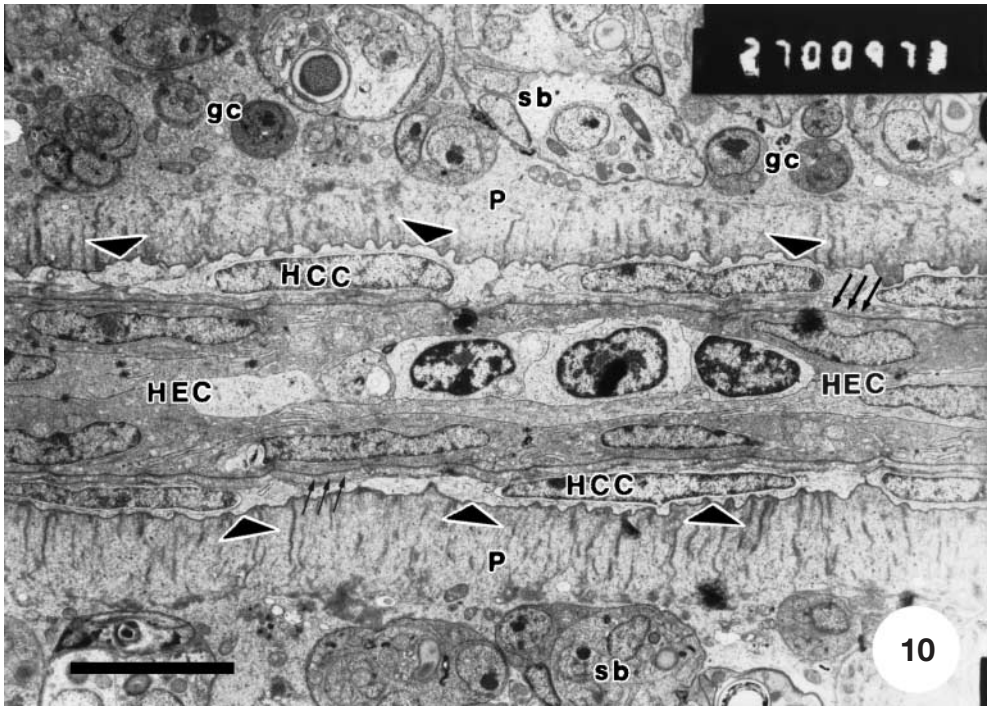

Fig. 10. TEM of *Henneguya lesteri* plasmodium sections in *Sillago analis* pseudobranch. Interface between 2 adjacent *H. lesteri* pseudobranchial plasmodia (P). The plasmodium outer membrane is indicated by arrowheads. Flattened host cells (HEC) of several types comprise the thin cellular layer sandwiched in between the 2 plasmodia, bounded by thin layer of collagen fibers (small arrows) and capsular cells of host origin (HCC). gc: germinative cells; sb: sporoblast (scale bar = 250 µm)

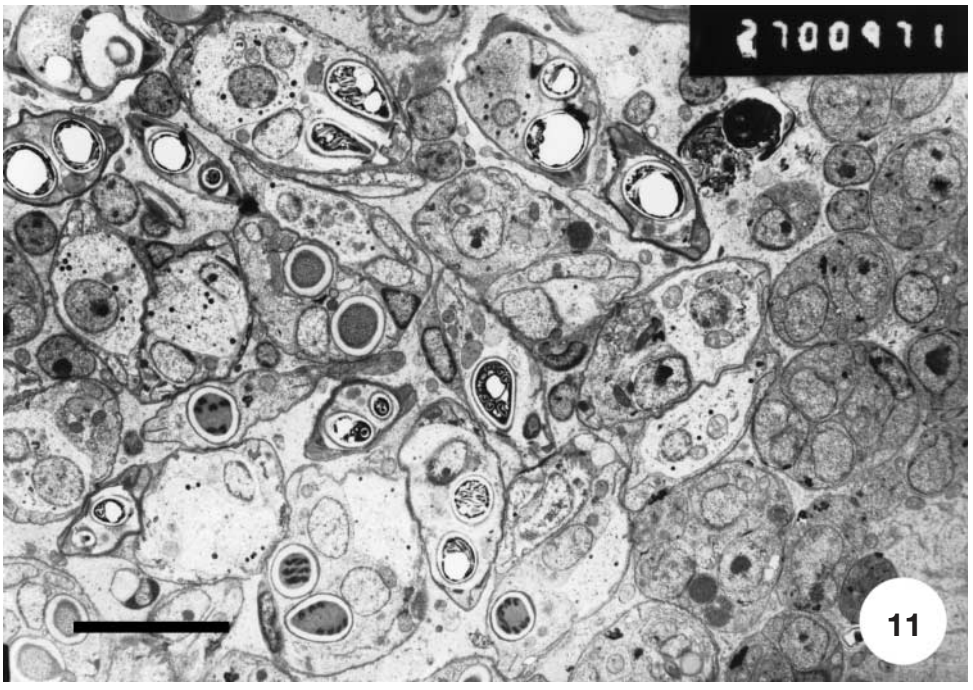

Fig. 11. TEM of peripheral section of *Henneguya lesteri* plasmodium sections in *Sillago analis* pseudobranch. Numerous, closely packed developing early stage (right) and advanced stage (left) sporoblasts are seen (scale bar = 5 µm)

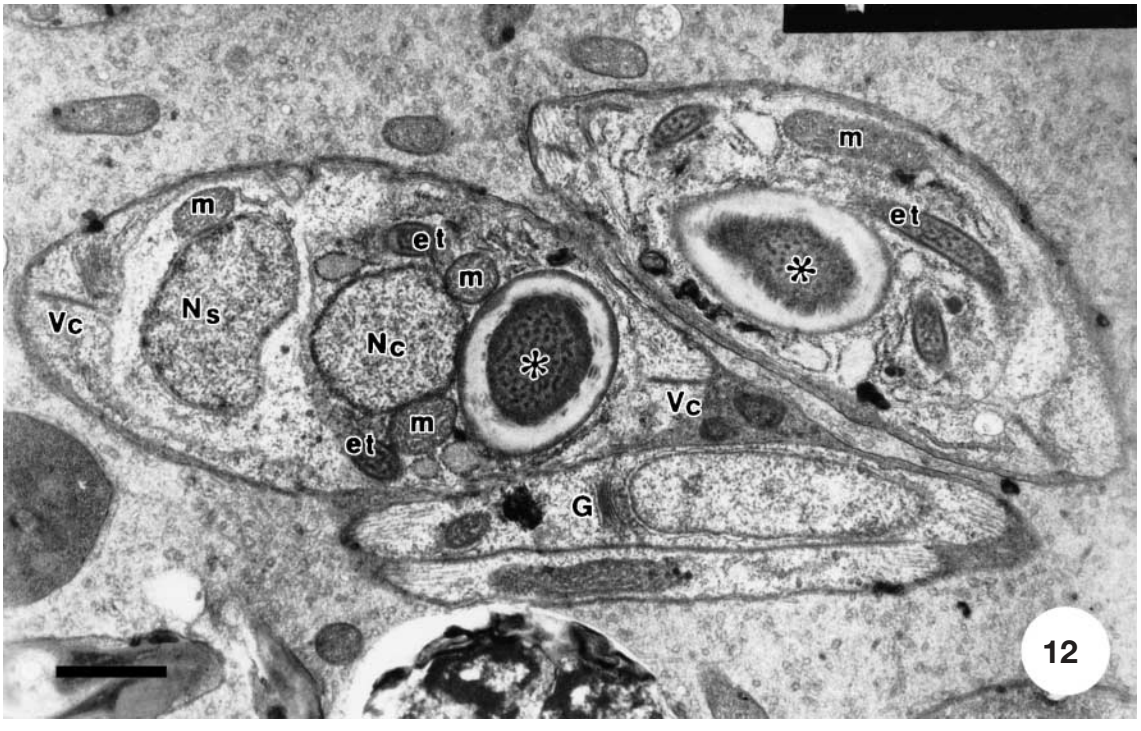

Fig. 12. TEM of *Henneguya lesteri* plasmodium sections in *Sillago analis* pseudobranch. Developing sporoblasts of *H. lesteri* display early stages of capsulogenesis, with cross sections of the external tube (et) surrounding the capsular primordium (✻); the capsular cell nucleus (Nc) and mitochondria with dense cristae (m) adjoin the capsular primordium. The sporoplasm, with 1 of its 2 nuclei (Ns), is seen at left. Vc: valvular cell; G: golgi apparatus (scale bar = 1 µm)

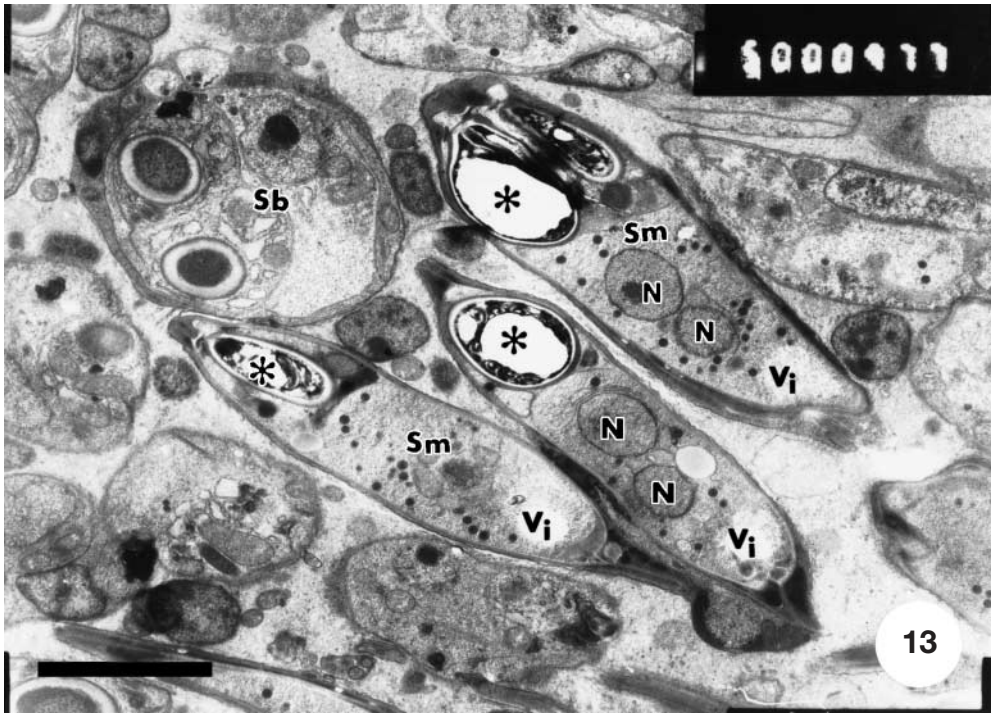

Fig. 13. TEM of peripheral section of *Henneguya lesteri* plasmodium in *Sillago analis* pseudobranch. Young spores showing binucleated sporoplasm (Sm) with electron dense sporoplasmosomes and mitochondriae; V_i: spherical polysaccharide inclusion (= iodinophilous vacuole); N: nucleus; ✻: polar capsules; Sb: earlier stage of developing sporoblast (scale bar = 3 µm)

Table 2. Similarities (%) between the nucleotide sequences (1987 characters) of *Henneguya lesteri* and 5 freshwater *Henneguya* species from 18S rDNA data

	H. exilis	*Henneguya* sp.	*H. zschokkei*	*H. salminicola*	*H. lesteri* n.sp.
H. doori	82.8	93.7	62.4	68.3	77.1
H. exilis		85.5	64.5	70.1	78.5
Henneguya sp.			64.7	70.3	78.6
H. zschokkei				92.2	63.1
H. salminicola					69.1

teristic electron-dense sporoplasmasomes measuring 100 to 150 nm, several large mitochondria, 2 nuclei containing large nucleoli, a Golgi apparatus, numerous ribosomes and stacks of rough ER. The maturing sporoplasm contained a region of polysaccharide-like matter, corresponding to the 'iodinophilous vacuole' of light microscope taxonomy (Fig. 13). The 2 elongated valvular elements gradually tapered off and fused to form the spore's caudal process.

Molecular analysis

The combination of myxozoan specific primers with the more general primers 18e and 18g enabled the sequencing in both directions of 1966 bp of 18S rDNA of *Henneguya lesteri*. Initially, a 1900 bp fragment was amplified using the primers MYX1f and 18g and sequenced using the primers MYX1f, ACT1f, ACT2f, 18g, ACT1r and MYX4r. A second fragment of 600 bp was amplified using ACT2f and 18g, which these 2 primers were used to sequence. A third product 1000 bp in size was amplified and sequenced with MYX1f and ACT1r. A final fragment of 1150 bp was generated using 18e and ACT1r, and the primers 18e, ACT1fr, MYX1f and ACT3f were used in the subsequent sequencing reactions.

Henneguya lesteri was compared with 5 freshwater species deposited in GenBank (Fig. 14 & Table 2). It differed genetically from all 5, resembling most closely

Fig. 14. Alignment of partial small-subunit ribosomal gene sequence comparing the marine *Henneguya lesteri* with 5 freshwater *Henneguya* species. Homologous nucleotide-bases are represented by dots, gaps by dashes and unknown bases by 'N'. Horizontal numbers denote base position relative to first sequence

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                        10         20         30         40         50
H. lesteri        CATGTGCCA- G-TTCATACA TTAAAAATGT GAGACTGCGG ACGGCTCAGT
H. doori          ..G....T.- .C........ .ATGT..... .......... ..........
H. exilis         ..A....T.- .-........ .ATTT..... .......... ..........
Henneguya sp.     ..G....T.- .-........ .ATGT..... .......... ..........
H. zschokkei      ..G....T.C AG.A.....G .G..TT-C.. .......... .A........
H. salminicola    ..G....T.C AC.A.....G .G..TT-C.. .......... .A........

                        60         70         80         90        100
H. lesteri        ATATCAGTTA TAATCTGCTC GATTGTTAAG GTTATTGGAT AACCGTGGGA
H. doori          .......... .......... .......G.. ....G..... ..........
H. exilis         .......... .......... .......... .......... ..........
Henneguya sp.     .......... .......... .......C.. .......... ..........
H. zschokkei      .A........ .C....AT.T ......CT.. CCC....... ..........
H. salminicola    .A........ .C....AT.T ......CT.. CCC....... ..........

                        110        120        130        140        150
H. lesteri        AATCTAGAGC TAATACATGC AGT-TTGCGG T--GGTTGCT TTCGG-GCGA
H. doori          .......... .......... .ACGCCT.A. .--T-.GCGG ....CC....
H. exilis         .......... .......... .-CC.CT..- .--C.CGAAG C.T.CTTTTC
Henneguya sp.     .......... .......... .-CGCCT... .--TACG.T- .C..CC.T..
H. zschokkei      .......... .........T ...TANNT.. .GGT..A.TA ..TATATT.T
H. salminicola    .......... .........T ...TAATT.. .GGT..A.TA ..TATATT.T

                        160        170        180        190        200
H. lesteri        CTGGCGTG-G CATTTATTAG AG-AATACCA ATGGCACGTA CGAG----AG
H. doori          GCT.AA..-- .......... ..CG...... .CCATG.ACG T..-------
H. exilis         G.C.AT..-- .......... ..CG...... .C--.GT..G .A.-------
Henneguya sp.     ACC.AA..-- .......... ..CG...... ...ATGTACG T..-------
H. zschokkei      ACCA.CAAN. .......... N-TG.AG... .CT..TT..T G.G.GTGC.A
H. salminicola    ACCA.CAAT. .......... .-TG.AG... .CT..TT..T G.G.GTGC.A

                        210        220        230        240        250
H. lesteri        ATCGT--GCG AGCGGGGTGA ATCTAGATAA CT-GTGCAGA TCGCAT-GGC
H. doori          ----.--..A ---------. .......... ..C....... ...T..-...
H. exilis         -------A.A --..T..C.. .......... ..-....... ...T..-...
Henneguya sp.     ----.--A.A --T.T..... .......... ..-....... ...T..-...
H. zschokkei      ...TCCAA.T T.TAA.N... G......... .CGT...... ...T..T...
H. salminicola    ...TCCAA.T T.TAA..... G......... ..GT-..... ...T..-...

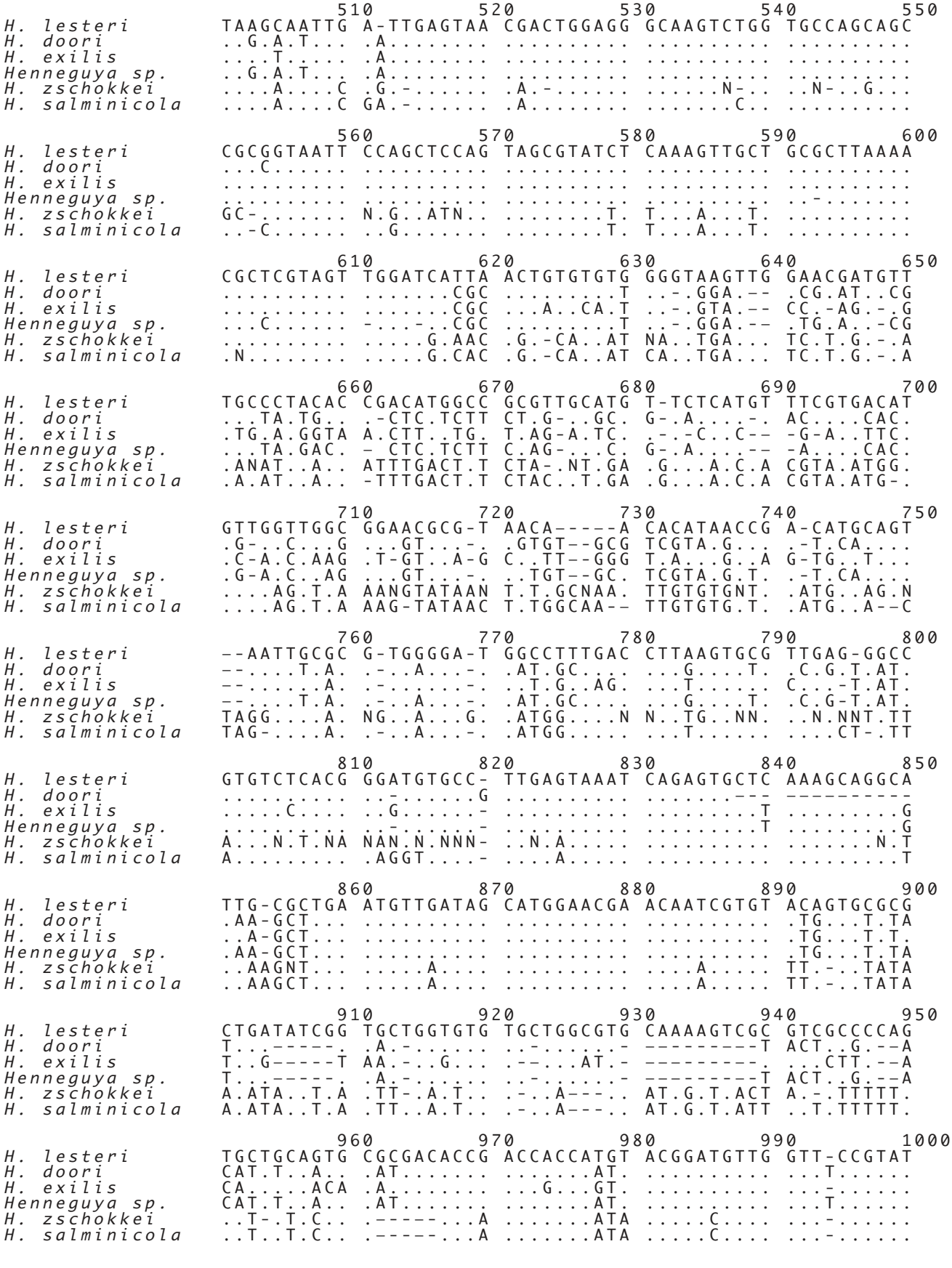
                        260        270        280        290        300
H. lesteri        CTT-GAGC-C GGCGACATTT CGATTGAGTT TCTGCCCTAT CA-CCTA-GA
H. doori          ...-....-T .A....G... .......A.. .......... ..A.TC.T.T
H. exilis         ...-....-T .A....... .......A.. .......... ..A.TC-T.T
Henneguya sp.     ...-....-T .A....G... .......A.. .......... ..A.T.-T.T
H. zschokkei      ...T.T..CT .......G... .A...C.A.. C..C..... ..A.TA--.T
H. salminicola    ...-.T..-T .A....G... .A.....A.. .......... ..A.TA--.T

                        310        320        330        340        350
H. lesteri        TGCAAGTGTA TTGTACTTGC ATGGGGGTCA CGGGTGACGG AGGATCAGGG
H. doori          C.AC..... G..G...GC. GA..TTT.T. .....A.... G.A.......
H. exilis         ..GC..... G..G...GC. .A..TTT.T. .....A.... G.A.......
Henneguya sp.     C.AC..... G..G...GC. GA..TTT.T. .....A.... G.A.......
H. zschokkei      ..GT.AG... GATCCTAAC. .A..TT..G. .....A.... G.A.......
H. salminicola    ..GT.AG... G.AGT.AAC. .A..TT..G. .....A.... G.A.......

                        360        370        380        390        400
H. lesteri        TTCGATCCCG GAGAGGGAGC CTGAGAAACG GCTACCACAT CCAAGGAAGG
H. doori          ......T... .......... .......... .......... ..........
H. exilis         ......T... .......... .......... .......... ..........
Henneguya sp.     ......T... .......... .......... .......... ..........
H. zschokkei      ......T... -.........N ..T....TT. .......... .-........
H. salminicola    ......T... .......... T......TT. .......... ..........

                        410        420        430        440        450
H. lesteri        CAGCAGGCGC GCAAATTACC CAATCCAGAC AATGGGAGGT GGTG-ACGAA
H. doori          .......... .......... .......... G......... ...A-.....
H. exilis         .......... .......... .......... .......... ....-.....
Henneguya sp.     .......... .......... .......... .....-.... ....-.....
H. zschokkei      T......... .........A. .C.ATT.... .GA.T....G ....T....G
H. salminicola    .......... .......... ....T..... .G..A..... ....-....G

                        460        470        480        490        500
H. lesteri        AAGTACCGAA TAACG-CCAA ATGGTTGTTA GTTCAGAATG GGAATCAATT
H. doori          .......A.. .G...C..T. .C..G...C. ...TG..... AA..-.....
H. exilis         .......... .GG.AG.TT. T..T-..CC. ...TG..... AA..-.....
Henneguya sp.     .......A.. .G...C..T. .C..G...C. ...TG..... AA..-.....
H. zschokkei      ..T....A.G .GGT.A...T .A.....CC. A..TG..... AACG.-....
H. salminicola    ..T....A.G .GGT.A...T .A.....CC. A..TG..... AACG.-....
```


510 520 530 540 550
H. lesteri TAAGCAATTG A-TTGAGTAA CGACTGGAGG GCAAGTCTGG TGCCAGCAGC
H. doori ..G.A.T... .A..... ....
H. exilis ....T..... .A..... ....
Henneguya sp. ..G.A.T... .A..... ....
H. zschokkei ....A....C .G.-..... .A.-..... .....N-.. ..N-.G...
H. salminicola ....A....C GA.-..... .A..... .....C.. .....
560 570 580 590 600
H. lesteri CGCGGTAATT CCAGCTCCAG TAGCGTATCT CAAAGTTGCT GCGCTTAAAA
H. doori ...C..... ....
H. exilis ....
Henneguya sp. ....-.....
H. zschokkei GC-..... N.G..ATN.. .....T. T...A...T. .....
H. salminicola ..-C..... ..G..... .....T. T...A...T. .....
610 620 630 640 650
H. lesteri CGCTCGTAGT TGGATCATTA ACTGTGTGTG GGGTAAGTTG GAACGATGTT
H. doori ....CGC .....T ..-.GGA.-- .CG.AT..CG
H. exilis ....CGC ...A..CA.T ..-.GTA.-- CC.-AG.-.G
Henneguya sp. ...C..... -...-..CGC .....T ..-.GGA.-- .TG.A..-CG
H. zschokkei ....G.AAC .G.-CA..AT NA..TGA... TC.T.G.-.A
H. salminicola .N..... .....G.CAC .G.-CA..AT CA..TGA... TC.T.G.-.A
660 670 680 690 700
H. lesteri TGCCCTACAC CGACATGGCC GCGTTGCATG T-TCTCATGT TTCGTGACAT
H. doori ...TA.TG.. .-CTC.TCTT CT.G-..GC. G-.A....-. AC....CAC.
H. exilis .TG.A.GGTA A.CTT..TG. T.AG-A.TC. .-.-C..C-- -G-A..TTC.
Henneguya sp. ...TA.GAC. - CTC.TCTT C.AG-...C. G-.A....-- -A....CAC.
H. zschokkei .ANAT..A.. ATTTGACT.T CTA-.NT.GA .G...A.C.A CGTA.ATGG.
H. salminicola .A.AT..A.. -TTTGACT.T CTAC..T.GA .G...A.C.A CGTA.ATG-.
710 720 730 740 750
H. lesteri GTTGGTTGGC GGAACGCG-T AACA-----A CACATAACCG A-CATGCAGT
H. doori .G-..C...G ...GT...-. .GTGT--GCG TCGTA.G... .-T.CA....
H. exilis .C-A.C.AAG .T-GT..A-G C..TT--GGG T.A...G..A G-TG..T...
Henneguya sp. .G-A.C..AG ...GT...-. ..TGT--GC. TCGTA.G.T. .-T.CA....
H. zschokkei ....AG.T.A AANGTATAAN T.T.GCNAA. TTGTGTGNT. .ATG..AG.N
H. salminicola ....AG.T.A AAG-TATAAC T.TGGCAA-- TTGTGTG.T. .ATG..A--C
760 770 780 790 800
H. lesteri --AATTGCGC G-TGGGGA-T GGCCTTTGAC CTTAAGTGCG TTGAG-GGCC
H. doori --....T.A. .-..A...-. .AT.GC.... ...G....T. .C.G.T.AT.
H. exilis --.....A. .-.....-. ..T.G..AG. ...T..... C...-T.AT.
Henneguya sp. --....T.A. .-..A...-. .AT.GC.... ...G...T. .C.G-T.AT.
H. zschokkei TAGG....A. NG..A...G. .ATGG....N N..TG..NN. ..N.NNT.TT
H. salminicola TAG-....A. .-..A...-. .ATGG..... ...T..... ....CT-.TT
810 820 830 840 850
H. lesteri GTGTCTCACG GGATGTGCC- TTGAGTAAAT CAGAGTGCTC AAAGCAGGCA
H. doori ....-.....G ....--- ----------
H. exilis .....C.... ..G.....- ....T .....G
Henneguya sp. ....-.....- ....T .....G
H. zschokkei A...N.T.NA NAN.N.NNN- ..N.A..... ....N.T
H. salminicola A..... .AGGT....- ....A..... ....T
860 870 880 890 900
H. lesteri TTG-CGCTGA ATGTTGATAG CATGGAACGA ACAATCGTGT ACAGTGCGCG
H. doori .AA-GCT... .... .TG...T.TA
H. exilis ..A-GCT... .... .TG...T.T.
Henneguya sp. .AA-GCT... .... .TG...T.TA
H. zschokkei ..AAGNT... .....A.... ....A..... TT.-..TATA
H. salminicola ..AAGCT... .....A.... ....A..... TT.-..TATA
910 920 930 940 950
H. lesteri CTGATATCGG TGCTGGTGTG TGCTGGCGTG CAAAAGTCGC GTCGCCCCAG
H. doori T..-----. .A.-..... ..-.....- ---------T ACT..G.--A
H. exilis T..G-----T AA.-..G... .-...AT.- ---------. ...CTT.--A
Henneguya sp. T..-----. .A.-..... ..-.....- ---------T ACT..G.--A
H. zschokkei A.ATA..T.A .TT-.A.T.. .-..A----.. AT.G.T.ACT A.-.TTTTT.
H. salminicola A.ATA..T.A .TT..A.T.. .-..A----.. AT.G.T.ATT ..T.TTTTT.
960 970 980 990 1000
H. lesteri TGCTGCAGTG CGCGACACCG ACCACCATGT ACGGATGTTG GTT-CCGTAT
H. doori CAT.T..A.. .AT..... ....AT. ....T.....
H. exilis CA.....ACA .A..... ...G...GT. ....-.....
Henneguya sp. CAT.T..A.. .AT..... ....AT. ....T.....
H. zschokkei ..T-.T.C.. .-------...A ....ATA .....C.... ...-.....
H. salminicola ..T..T.C.. .-------...A ....ATA .....C.... ...-.....

```
                        1010        1020        1030        1040        1050
H. lesteri              TGGGGTACTG ATTAAGAGGA GCGGTTGGGG GCATTGGTAT TTGGCAGCGA
H. doori                .......... .......... .......... A......... ..........
H. exilis               .......T.. .......... .......... .......... ..........
Henneguya sp.           .......... .......... .......... A......... ..........
H. zschokkei            ......GA.. .....A.A.. .......... ...G...-.. ...T.C..A.
H. salminicola          ......GA.. .....A.A.. .......... .......... ...T.C....

                        1060        1070        1080        1090        1100
H. lesteri              GAGGTGAAAT TCTTGGACCT GCCAAGGACT AACAAATGCG AAGGCATCTG
H. doori                .......... .......... .T........ .......... ..........
H. exilis               .......... .......... .......... .......... ..........
Henneguya sp.           .......... .......... .T........ .......... ..........
H. zschokkei            .GN.G.TG.A AT..------. TNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
H. salminicola          .......... .........G .A........ .......... .......T..

                        1110        1120        1130        1140        1150
H. lesteri              TCCAGACCGT ATCC-ATTAA TC-AAGAACG A-TAGTGAGA GGTTCGAAGA
H. doori                .......... ....G..... ..G..... .A........ ..........
H. exilis               .......... ....-..... .-C..... .A-..A.... ..........
Henneguya sp.           .......... .-..-..... .TC..... .T-..... ..........
H. zschokkei            NNNNNNNNNN NNNN-NNNNN NN-NNNNNNN NNNNNNNNNN NNNNNNNNNN
H. salminicola          C.T....... C..T-..... ..-....... .TAGAG..AG .A.N......

                        1160        1170        1180        1190        1200
H. lesteri              --CGA-TCAG ATACCGTCCT AGTTCTCACT GT-AAACTA- -TGC-CGACC
H. doori                GA...G.... .......... .........C ..-.......- -...-.....
H. exilis               CG-A--.... .......... .......T.. ..-.......- -...-.....
Henneguya sp.           CG-..T.... .......... .........C ..-.......- -...-.....
H. zschokkei            ---NNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
H. salminicola          ---.GA.... .....C..G. ....TC.T.C ..T.....CA C...G.A...

                        1210        1220        1230        1240        1250
H. lesteri              CGGGATCAGC TTGGAGTTAT ATACTCATGC TCGAGGTTGG TCCCCCTGGG
H. doori                .........T ......CAG. G..TC..C.. .......... ..........
H. exilis               .........T .......... ...TC..C.. .......... ..........
Henneguya sp.           .........T ......CAG. G..TC..C.. .......... ..........
H. zschokkei            NNNNNNNNNN NNNNNNNNNN NNNNN-NNNN NNNNNNNNNN NNNNNNNNNN
H. salminicola          ......G..T ..A...C..A T..TA-.C.. ..T....... ........N.

                        1260        1270        1280        1290        1300
H. lesteri              AAA--CCTGA AGTTTTTCGG TTGCGGGGGG AGTATGG--T CGCAAGGCTG
H. doori                ...--...-. .......... .......... .......--. ..........
H. exilis               ...--..... .......... .......... .......--. ..........
Henneguya sp.           ...--....A. .......... .......... .......--. ..........
H. zschokkei            NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
H. salminicola          .G.TA..... .......... .......... .......GG. T....A....

                        1310        1320        1330        1340        1350
H. lesteri              AAACTTTAAA GGAATTGACG GAAGGGCACC ACCCAAGGGT GGAGCCTGCG
H. doori                ......-... .......... ..........- ....-.G.... ..CATG-...
H. exilis               ......-... .......... .......... ....-.G.... ..........
Henneguya sp.           ......-... .......... .N........ ....-.G.... .....-....
H. zschokkei            NNNNNN-NNN NNNNNNNNNN NNNNNNNNNN NNN-NNNNNN NNNNNNNNNN
H. salminicola          .......-... .......... .......... ....-.G.A.. .....--....

                        1360        1370        1380        1390        1400
H. lesteri              GCTTAATTTG ACTCAACACG GGGAAACTTA CCTGGTCCGG ACATCCGAAG
H. doori                .......... .......... ..A....... .......... .....GA...
H. exilis               .......... .......... ..A....... .......... .....AA...
Henneguya sp.           .......... .......... ..A....... .......... .....GA...
H. zschokkei            NNNNNNNNNN NNNNNNNNNN NNN.------ .TC...T... ....TGAC..
H. salminicola          .......... .......... ..A....... .TC...T... ....TGAC..

                        1410        1420        1430        1440        1450
H. lesteri              GATACTCAGA CC-TAAGATC TTATTTGATC TGGTGATTGG TGGTGCATGG
H. doori                ....AA.C.. ..AA...... G.T......A C.A....... ..........
H. exilis               ....GA.... .TGCC..... ..TC.....A .......... ..........
Henneguya sp.           ....AA.C.. ..AA...... G.T......A C.A....... ..........
H. zschokkei            ....AA...T TTGAT...A. ..T......A C.....A... ..........
H. salminicola          ....AA...T TTGAT..NA. ..T......A C.....A... ..........

                        1460        1470        1480        1490        1500
H. lesteri              CCGTTCTT-A GTTCGTGAAG TGATTTGTCA GGTTTATTCC GGTAACGGAC
H. doori                ........-. .......G.. ....C..... ..C.A..C.. .......A..
H. exilis               ........-. .......G.. ....C..... ..CCA..... .......A..
Henneguya sp.           ........-. .......G.. ....C..... ..C.A..C.. .......A..
H. zschokkei            .....T..T. .......G.. .A..C..... ..C.A.CC.. .......A..
H. salminicola          .....T..-. .......G.. .A..C..... ..C.A.CC.. .......A..
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```
                    1510        1520        1530        1540        1550
H. lesteri          GAGACTGCGT TCTCCATTTG ACGAGC-TGA AGTTTGTGC- -GTT-CGCAC
H. doori            .....CA.A. .......... ......-C.T ..AAG.C.TT G.C.AT.AGT
H. exilis           .....CA.T. .......... ......-C.G ..AAG.C.TT G..ATT.AGG
Henneguya sp.       .....CA.A. .......... ......-C.T ..AAG.C.TT G.C.AT.A.A
H. zschokkei        ...NTCAN.C G......... GG.T.TACA. GAAAA.C.ATC G..AGT..-T
H. salminicola      ....TCAGCG .......... GG.T.TACA. GAAA...ATC G..AGT..-T

                    1560        1570        1580        1590        1600
H. lesteri          ACAGTCATTG GCAACGGTGA CTGC-GTTGT G-ACCGTATG GCTGCCGGTG
H. doori            G.TC..G--- --G.GA.A.T T..TG..CAA C-GT..A.G. TT.AAT.AGA
H. exilis           .TTT.TGC.T ....G...CC .C.GT.C.AA T-T.T...G. TACTGT..G.
Henneguya sp.       GTTT.TC..C .GG.GA.A.T T..TG..CAA C-GT..A.G. TT.AAT.AGA
H. zschokkei        G..T.GT..C .AC.GT..C. .ATTTA...A TAGTTAG.G. CTGTGT..G-
H. salminicola      G..T.GT..C .AC.GT..C. .ATTTA...A TAGTTA..G. CTGTGT.AG-

                    1610        1620        1630        1640        1650
H. lesteri          -GTGGTGGGC GTTGT--TGT GCGGCTCAAG GTT-ACCTTT CGGGGTGCCT
H. doori            C.G..-A..T .CC.AGA... TT.ATGGG.A .GC-.A.GAC TT.C.GA.TA
H. exilis           AAGCTCA..G .GCA.TGGC. .A.ATCGC.A ..C-.A-GGC T..T.CA.T.
Henneguya sp.       C.G..-A..T .CC.AGA... TT.ATGGG.A .GC-.A.GAC TT.C.GA.TA
H. zschokkei        T...CCT.TT .CC.ATGGTA AT.T.AACTC ...A.AAGG. T.ATA.TG..
H. salminicola      T...C-T.TT ....ATGGT. .TAC.AATT. ...T..TAG. T..T...G..

                    1660        1670        1680        1690        1700
H. lesteri          TCAGTCGTAA GGATGGCGTC GCATGCTGCT GGCAAGCCTT CAGCCAGACA
H. doori            CA..-.....  T..C----.T ..GA.T.C.C TA.GGT.GAA ...T.GTGA.
H. exilis           .G..--T..T C--.----C. A..A--.C.C TC.GGTTTAA ...T-GCGTG
Henneguya sp.       CA..-.....  T..C----.T ..GA.T.C.C TA.GGT.GAA ...T.GTGA.
H. zschokkei        .A..GT.GCT .TT.A..-.T ...--..C.C TTGTCC.AAA ...T-.TGAC
H. salminicola      .A.AGT.GCT .CT.A..-.T ...--..C.C TTGT.C.AAA ...T-.T-AC

                    1710        1720        1730        1740        1750
H. lesteri          GTGCTTCGTT TGGAGTGCTG TATCATGGA- -G-AGAC-AA CGG-AACATA
H. doori            TCCG.GA.AG .TCC...... .........- -.G.....-.- ...-..A.--
H. exilis           C.CGCAA.AG ...-..AT.. .........- -.-.....-.. ...-..T...
Henneguya sp.       TCCG.GA.AG .TCC...... .........- -.G.....-.. ...-..A.AT
H. zschokkei        AGTG.AA-AC ..CTA..... AC......GC G.AG...CGG T..TGGG.AT
H. salminicola      AGTG.AA-AC ..-TA..... .C.......- --G....GGG T..-----AT

                    1760        1770        1780        1790        1800
H. lesteri          TTA-CAAAAT CCGAGGAA-G TGTGGCTATA ACAGGTCTGT GATGCCCTTA
H. doori            -..A.C.... .NAG---.A. ....-..... .......... ...-......
H. exilis           C..AA..... .........-. .......... .......... ..........
Henneguya sp.       A..AG..... .........A. .......... .......... ..........
H. zschokkei        A..ATC...C .AC....CG. C-....A.A. -......... _.........
H. salminicola      A..ATC...C .AC....CG. C-....A.A. .......... T.........

                    1810        1820        1830        1840        1850
H. lesteri          GATGTTC-AG GGCTGCACGC GCGCTACAAT GGCAGCGACA GC-GAGTGTT
H. doori            .......G.. .......... .......... ..-------. ..-....T..
H. exilis           .......-.. .......... .......... .A.TA..... ..-....C.C
Henneguya sp.       ....N-.-.. .......... .......... ...G...... ..-.....C
H. zschokkei        ...A-C.G.. .-.G...... .......... .AT.A..... ..-....T.C
H. salminicola      ...A.C.G.. .-.G...... .......... .AT.A..... ..A....T.C

                    1860        1870        1880        1890        1900
H. lesteri          TGAATCGAAA GATTCGGGTA ATCTTGTAAT CG-TTGCCGT GATGGGGATT
H. doori            ..G....... ......A... .......... ..GCC..... ..........C
H. exilis           ..G....... .......... .......... ..-.A.T... ..........C
Henneguya sp.       ..G....... ....T..... .C........ ..-CC..... ..........C
H. zschokkei        .AGG.T.... ..CCT..... .....N.G.A TCG..AT... ....A.....
H. salminicola      .AGG.T.... ..CCT..... .....T-G.A TCG..AT... ....A.....

                    1910        1920        1930        1940        1950
H. lesteri          GACGTTTGTA ACT--CC-TC ATGAAAGAGG AATCCCTAGT ATGTGCGATT
H. doori            .......... .A.--A.G.. G......... .......... .C........
H. exilis           .......... .T.--A.G.. G......... .......... .C........
Henneguya sp.       .......... .A.--A.G.. G......... ...N....N. .C........
H. zschokkei        ....G..... .T.TT..G.. ......T... ...T...C.. .....TACG.
H. salminicola      ....G..... .T.TT..G.. ......T... ...T...C.. .....TACG.

                    1960        1970        1980    1987
H. lesteri          CA-TGAGATC -CCCAAATTA CGTCCCTGCC CTTTG-A
H. doori            ..A...A... G.G.GG...T A......... .....T.
H. exilis           ..-.T.A... G.G.GG...T A......... .....T.
Henneguya sp.       ..-...A... G.G.GG...T A......... .....T.
H. zschokkei        ..-.T..CGT GTA..G.A.. A......... .....T.
H. salminicola      ..-.T..CGT GTA..G.A.. A......... .....T.
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Henneguya sp. (78.6% homology) and *H. exilis* (78.5%). Determined pairwise distances (based on 1816 bp) between *H. lesteri* and the 4 marine actinosporeans, *Sphaeractinomyxon ersei*, *Tetraspora discoidea*, *Endocapsa rosulata* and *Triactinomyxon* sp., were 76.9%, 75.7%, 75.2% and 76.9% similar, respectively.

DISCUSSION

Two distinct branchial configurations of *Henneguya* infections of the gills, manifested as intra- or interlamellar, have been identified and previously studied in freshwater fish. The interlamellar form is considered to be the more pathogenic of the 2 types (Minchew 1977, Current & Janovy 1978). The present species, *Henneguya lesteri*, is of the interlamellar type. It displays an affinity to the host vascular system, similar to that observed in *H. creplini* plasmodia from the gills of *Perca fluviatilis*, which position themselves in the lamellar crypts juxtaposed to branchial blood vessels (Haaparanta et al. 1994). The latter authors suggested that this positioning ensures developmental success of the plasmodium by providing it with the necessary supply of nutrients for growth.

Although the plasmodium wall of cyst-forming myxosporeans displays some variation among the genera and species that have been studied, the basic structure shows numerous similarities. For example, the plasmodium wall of *Thelohanellus nikolskii*, although infecting the skin of a freshwater host (Desser et al. 1983), does not differ significantly from that of *Henneguya lesteri*.

Henneguya lesteri is a marine species, and it is reasonable to assume that the fine structure of the plasmodium wall may, at least in part, reflect the increased osmotic ambience of the gills and the host's habitat. The wall structure of gill-infecting *Henneguya* spp. has been shown to be a manifestation also of the infection site of the plasmodium, depending whether it inhabits an intra- or inter-lamellar site (see Haaparanta et al. 1994). Current & Janovy (1978) and Current (1979) described the detailed fine structure of the host-parasite interface in 2 freshwater *Henneguya* species. The wall in *H. lesteri* is similar to 1 of these, *H. adiposa*, in that it is highly convoluted; in contrast, the wall of *H. exilis* is smooth (Current & Janovy 1978). Current (1979) suggested that such differences in the plasmodium wall might determine, at least in part, the degree of pathogenicity of the parasite.

The *Henneguya lesteri* plasmodium wall clearly functions as a nutrient transport system, with pinocytotic channels leading inward from the outer limiting membrane and branching to form a network of finer ducts. At the base of this area, the nutrients appear to be absorbed into the ectoplasm. This is in agreement with previous observations of Obiekezie & Schmahl (1993) and Haaparanta et al. (1994), who described ectoplasmic inclusions of fibrous material and arrays of minute vesicles at the interface of the peripheral pinocytic zone and adjoining ectoplasm in freshwater *Henneguya* spp.

Myxosporean spore development in a given plasmodium may be either synchronous or asynchronous (Lom & Dyková 1992). In 'synchronous' species, such as *Henneguya waithairensis*, *H. doori* and *H. creplini* (Narasimhamurti & Kalavati 1975, Cone 1994, Haaparanta et al. 1994), the plasmodium contains a single developmental stage at any given time. In asynchronous species such as *H. diversis*, *H. longicauda* and *H. adiposa* (Minchew 1977), the plasmodium exhibits the entire range of developmental stages: generative cells, sporonts, sporoblasts and spores. *H. lesteri* has an asynchronous spore development, and the fine structural details of sporogenesis are remarkably similar not only to freshwater *Henneguya* species (Schubert 1968, Current & Janovy 1977, Current 1979), but also to other myxosporean genera: pairs of generative cells, dividing sporont cells within the envelope cell, and development of two 5 cell sporoblasts, each consisting of a single bi-nucleate sporoplasm, 2 capsulogenic cells and 2 valvogenic cells (e.g., Desser et al. 1983).

The molecular homology of *Henneguya lesteri* with the freshwater *Henneguya* (excluding *H. zschokkei*, which contains over 300 unknown bases) ranged from 69.1 to 78.6% compared with 68.3 to 93.7% among only the freshwater *Henneguya*. These differences are greater than those among other myxosporean groups in other genetic studies. Andree et al. (1997) found 3 species of *Myxobolus* to be 77.8 to 89.9% similar, while 4 species of *Kudoa* were 90.9 to 97.7% similar (Hervio et al. 1997). Hervio et al. (1997) also determined that *Kudoa* compared with 3 other myxosporean genera were 63 to 70.8% similar and *Kudoa* compared with their fish host were 57.3 to 63.3% similar.

The complete life cycle of any *Henneguya* species, freshwater or marine, has not yet been elucidated experimentally. However, molecular methods have identified an aurantiactinosporean as the alternate life stage of *Henneguya exilis* (freshwater) based on their 18S rRNA sequences (Lin et al. 1999). 18S rDNA is known for 4 marine actinosporeans from oligochaetes from Moreton Bay, where *H. lesteri* was described (S. L. Hallett unpubl. data). Comparison of their sequence data, however, indicates that none is an alternate stage of *H. lesteri*, with base similarities in the range of 75.2 to 76.9%.

Myxosporean infections, although quite common in the gills (e.g., Current & Janovy 1978, Haaparanta et

al. 1994, Roubal 1994), appear to be quite rare in the pseudobranchs. In fact, only 1 other case has been reported, of *Myxobolus centropomi* from snook *Centropomus undecimalis* pseudobranch in Florida, USA (Landsberg 1993).

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