Description of *Enteromyxum scophthalmi* gen. nov., sp. nov. (Myxozoa), an intestinal parasite of turbot (*Scophthalmus maximus* L.) using morphological and ribosomal RNA sequence data

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SUMMARY

A new Myxozoa species causing enteritis and death in cultured turbot, *Scophthalmus maximus*, is described at light and electron microscope levels. In addition, small subunit ribosomal RNA gene sequences (SSU rDNA) from the new species and from similar myxozoans were obtained and used for phylogenetic inference, as complementary criteria to resolve its taxonomic classification. The new parasite is closely related to *Myxidium leei*, another enteric histozoic species from marine fish. However, the ascription of *M. leei* to the genus *Myxidium* was based on weak morphological evidence and is not supported by our rDNA data analysis. A close relationship with *Zschokkella*, the other morphologically related myxozoan genus is also not supported. The combined morphological and molecular study results in the establishment of the new genus *Enteromyxum* to accommodate the new species *E. scophthalmi*, and the former *M. leei*, which is transferred to the new genus as *Enteromyxum leei* (Diamant, Lom & Dyková 1994) n. comb. This genus of marine, histozoic and enteric myxozoans includes significant parasite species for marine finfish culture.

Key words: fish parasites, Myxozoa, turbot, ultrastructure, molecular systematics.

INTRODUCTION

An enteric myxozoan infection responsible for important losses in cultured turbot (*Scophthalmus maximus*) was recently reported (Branson, Riaza & Álvarez-Pellitero, 1999). The parasite resembled *Myxidium leei*, described from *Sparus aurata* (Diamant, Lom & Dyková, 1994) and also reported from other marine fish species (Le Breton & Marques 1995; Diamant, 1998; Padrós *et al*. 2001). However, the turbot myxozoan showed differences from *M. leei* and it was preliminarily considered a distinct, although closely related organism (Branson *et al*. 1999). We have conducted light and electron microscope studies of this parasite, in order to describe it formally as a new species.

In the original description of *M. leei*, Diamant *et al*. (1994) recognized that the parasite could be assigned either to *Myxidium* or *Zschokkella*, but in both cases with some reservations. These genera present overlapping morphological characters and it is difficult to assign some species to either of them (Lom & Noble, 1984; Lom & Dyková, 1992). In fact, a possible synonymy of *Myxidium* and *Zschokkella* was used as an argument to assign *M. leei* to this genus, according to the rules for priority (Diamant *et al*. 1994). This situation is a good example of the limitations of morphological criteria for systematic classification and is not an unusual occurrence in Myxosporea, whose taxonomy is largely based on the morphology of mature spores making it very difficult to differentiate some species (Andree *et al*. 1997).

Molecular techniques are increasingly gaining relevance in addressing questions of identity and phylogeny of parasites. The small subunit ribosomal RNA gene (SSU rDNA) is nowadays widely used for phylogenetic inference because it embodies many of the desirable features for these studies (Hillis & Dixon, 1991). Comparative studies of rDNA are specially useful for the study of parasitic microorganisms whose taxonomy is based on morphological characters of particular life-stages. A few recent studies have used rDNA data from myxozoans to address important taxonomic questions (reviewed by Kent *et al*. 2001). According to some of these studies, the traditional criteria for myxozoan classification, mainly based on spore morphology but also on the fish host and life-cycle, have been proven to show important limitations and to correlate poorly with rDNA-based phylogenetic inference (Andree *et al*. 1997; Kent & Palenzuela, 2002).

In order to clarify the classification of the new species from turbot, we complemented the morphological and ultrastructural study with molecular phylogeny analyses. Nuclear rDNA sequences were obtained from the turbot parasite, from the closely

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related species *M. leei* and from a typical and well-defined member of the genus *Zschokkella*, *Z. mugilis* Sitjà-Bobadilla & Álvarez-Pellitero, 1993. These sequences were combined with a larger data set including several myxozoans, representing the 3 main clades within the group (Kent & Palenzuela, 2002; Kent *et al*. 2001). As a result of the analyses a new genus is erected to accommodate the new myxozoan from turbot and *M. leei*.

MATERIALS AND METHODS

Fish

Turbots were obtained from a farm located in NW Spain, while sampling periodically from 1997 to 2000, for the study of the enteric disease. They were cultured in a pump ashore site with a flow through seawater supply. Fish were held in 160 m^2 raceways at a stocking density of about 25 kg/m^2. Animals were killed by overexposure to the anaesthetic MS-222 (Sigma, St Louis, MO, USA). Fresh smears of intestine were obtained and examined under the microscope. Measurements were taken from fresh material using a calibrated eyepiece.

Histological procedure

For light microscopy (LM), fish tissues were routinely fixed in 10% neutral buffered formalin and embedded in Technovit 7100 resin (Kulzer, Heraeus, Germany). Thin sections (1–3 μm) were stained with toluidine blue or Giemsa's stain. For transmission electron microscopy (TEM), small pieces of the tissues were fixed in 2·5% (v/v) glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2). Samples were washed several times with the same buffer, post-fixed in 1% (w/v) cacodylic OsO_4, dehydrated through a graded ethanol series and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were double-stained with uranyl acetate and lead citrate (Reynolds, 1963). Some sections were mounted on gold grids and stained by the Thiéry reaction for carbohydrates (Thiéry, 1967). The material was studied in Philips CM-10 and Hitachi 800 TEMs, operating at 80–100 kV.

Molecular studies

Parasites. Suspensions of the enteric myxozoans were obtained from heavily infected intestinal tissues preserved in 80% ethanol. *M. leei* material was obtained from cage-cultured sharpsnout sea bream (*Diplodus puntazzo*) and it was kindly provided by Dr Effie Athanassopoulou-Irvine (Athens Veterinary Research Center, Greek National Agricultural Research Foundation). Spores of *Z. mugilis* were purified from the bile of infected mullets (Sitjà-Bobadilla & Álvarez-Pellitero, 1993) by centrifugation and washings with sterile Hanks' Balanced Salt Solution.

DNA cloning and sequencing. The parasites were suspended in DNA extraction buffer (100 mM NaCl, 10 mM Tris–HCl, 25 mM EDTA, 1% (w/v) SDS, pH 8). The DNA was obtained by proteinase K digestion followed by phenol–chloroform protein extraction and ethanol precipitation (Palenzuela, Trobridge & Bartholomew, 1999). Myxozoan nuclear SSU rDNA was amplified by the polymerase chain reaction using subterminal primers MM18Sf (ctggttgattctgccagtggtc) and MM18Sr (cggtactagcgacgggcg). The products were ligated into a vector (pGEM–T Easy Vector system II, Promega, Madison, WI, USA), which was used to transform competent *Escherichia coli* cells (JM109, Promega). Transformants were selected by the blue/white screening method on plates containing X-Gal and the presence of the inserts of the expected size was confirmed by restriction digestion analysis with *Eco*RI enzyme. Both strands of the DNA were sequenced from at least 2 different clones containing each myxozoan gene, using an ABI 377 automated sequencer (Applied Biosystems Inc., Branchburg, NJ, USA). New primers were designed to complete the sequencing of the products. Sequence segments assemblage and primer design were aided by the software packages Sequencher v 4.05 (Gene Codes Corporation) and Gene Runner v 3.02 (Hastings Software).

Phylogenetic inference. Sequences were aligned by hand with the homologous sequences from other myxozoans available on public databases. The myxozoan alignment was further refined according to the universal model of eukaryotic SSU rRNA secondary structure (Wuyts *et al*. 2000) using the 'Dedicated Comparative Sequence Editor', DCSE v 2.6 (De Rijk & De Watcher, 1993). Unambiguously aligned positions were sampled for phylogenetic inference using 3 major procedures: the neighbour-joining (NJ) method (Saitou & Nei, 1987) applied to distances corrected according to Kimura's two-parameter model (Kimura, 1980); the maximum-parsimony (MP) method as implemented by DNApars, included in the Phylip package (Felsenstein, 1993); and the maximum likelihood (ML) method performed with Tree-Puzzle 5.0 (Strimmer & von Haeseler, 1996) using the HKY 85 model of nucleotide substitution (Hasegawa, Kishino & Yano, 1985) and calculating rate heterogeneity among sites from the data set. Support for the internal branches of the trees was assessed by bootstrap resamplings of the data set (Felsenstein, 1985) in NJ and MP methods, and by the quartet puzzling support values in ML trees.

RESULTS

Description of the species

Enteromyxum scophthalmi n. g., n. sp.

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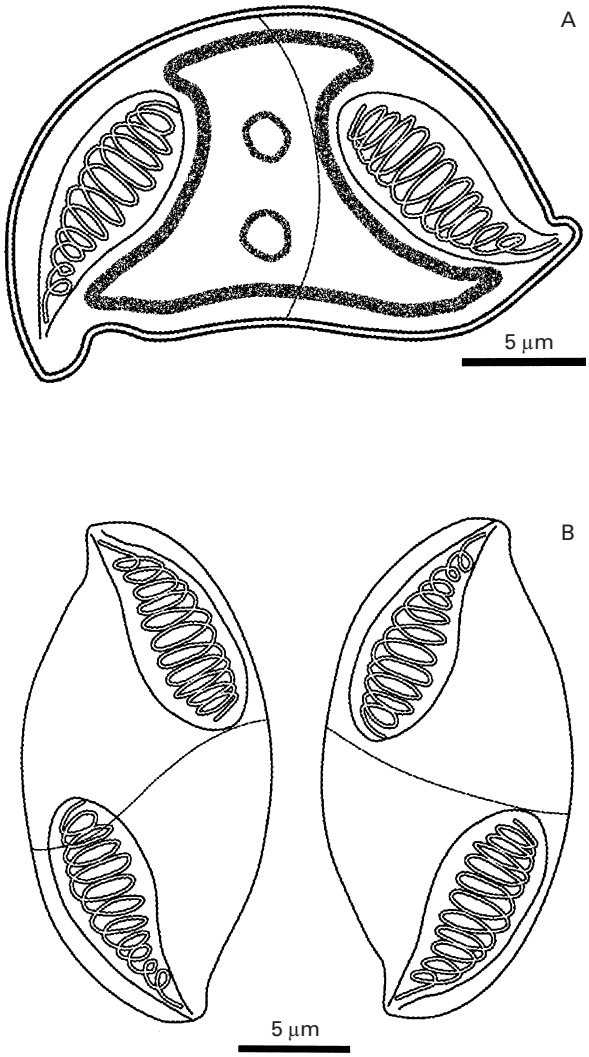

Fig. 1. Line drawings of *Enteromyxum scophthalmi* spore. (A) Frontal view. (B) Superior and inferior views.

Type host: *Scophthalmus maximus* L.
Location in the host: Histozoic. Digestive tract epithelia. Mainly the intestine, also oesophagus or stomach. Occasionally in the gall bladder.
Type locality: Northern Galicia (NW Spain)
Type material: Holotype (histological section of a heavily infected turbot intestine containing varied developmental stages) deposited in the Museo Nacional de Ciencias Naturales (Madrid, Spain): Colección Invertebrados, with acquisition number MNCN 37.01/11.

Description. *Spores*. (Figs 1 and 2A–E). Slightly crescent shaped, with somewhat sharp points. Polar capsules large, very elongated and tapering to their distal ends, with openings at the ends of the spore and oriented in opposite directions relative to a longitudinal plane bisecting the spore in front view (Figs 1 and 2A, B, E). Polar filament with 10–13 coils. One sporoplasm, binucleated (Fig. 3A), filling nearly all the space between the polar capsules. Suture line rather inconspicuous, nearly transversal in front and back views (Figs 1A and 2B–C) and somewhat sinuous in apical or inferior views (Fig. 1B). No ornamentation on the valve surface was observed. The dimensions of the mature spores are presented in Table 1. Spores were present only in very advanced infections, usually in symptomatic fish. Even when present, the proportion of mature spores to developmental stages was extremely low.

Developmental stages. Spores developed in disporic pansporoblasts usually located in the intestinal epithelium or detached together with epithelial debris (Fig. 2D–F). Other developmental stages could be found in the same location (Fig. 2G–L) and they included primary (P) cells harbouring 1 or more secondary (S) cells, which could also harbour tertiary (T) cells. In severe infections abundant stages appeared in the intestinal lumen among the remnants of the detached epithelial cells (Fig. 2G, L). Amoeboid motile stages harbouring 1 or more S cells (Fig. 2H) were quite frequent in the liquid filling the intestinal lumen of heavily infected fish. Some large plasmodia (40–70 μm) harbouring or releasing several small stages were also found free in the lumen, among the epithelial debris (Fig. 2I–K).

Ultrastructural observations

Spores. Fully mature spores were not detected in ultrathin sections, but some immature or rather advanced spores were observed (Fig. 3A, B). Valvogenic cells, still with some organelles in immature spores, became flattened as the spore matured. The remnants of their nuclei appeared in the posterior part of the spore (Fig. 3A). At the sutural line, the sutural border of one valve overlapped the other (Fig. 3B, inset). Capsulogenic cells occupied an important part of the immature spore (Fig. 3A). They contained a nucleus, abundant rough endoplasmic reticulum (rER), mitochondria, ribosomes, as well as electron-dense bodies probably lipidic. The formation of the polar capsule seems to take place as usual in myxosporeans. A capsular primordium and an external tube were observed in some developing spores (Figs 3A and 4A, B). In more advanced spores, the polar filament was already coiled inside the capsule (Figs 3B and 4C). The polar capsule wall showed 2 electron-dense and 2 electron-lucent alternating layers (Fig. 4C). The sporoplasm contained 2 nuclei, usually surrounded by rER (Fig. 3A). It was densely packed with ribosomes and contained sporoplasmosomes, mitochondria, β-glycogen granules and some lipidic droplets (Fig. 3A, B). Electron-dense inclusions and sporoplasmosomes were more abundant in mature sporoplasms (Fig. 3B). The plasmodium harbouring the spores appeared rather vacuolated and showed some β-glycogen granules, lipidic inclusions and electron-dense residual bodies, probably degenerated mitochondria (Fig. 3A, B).

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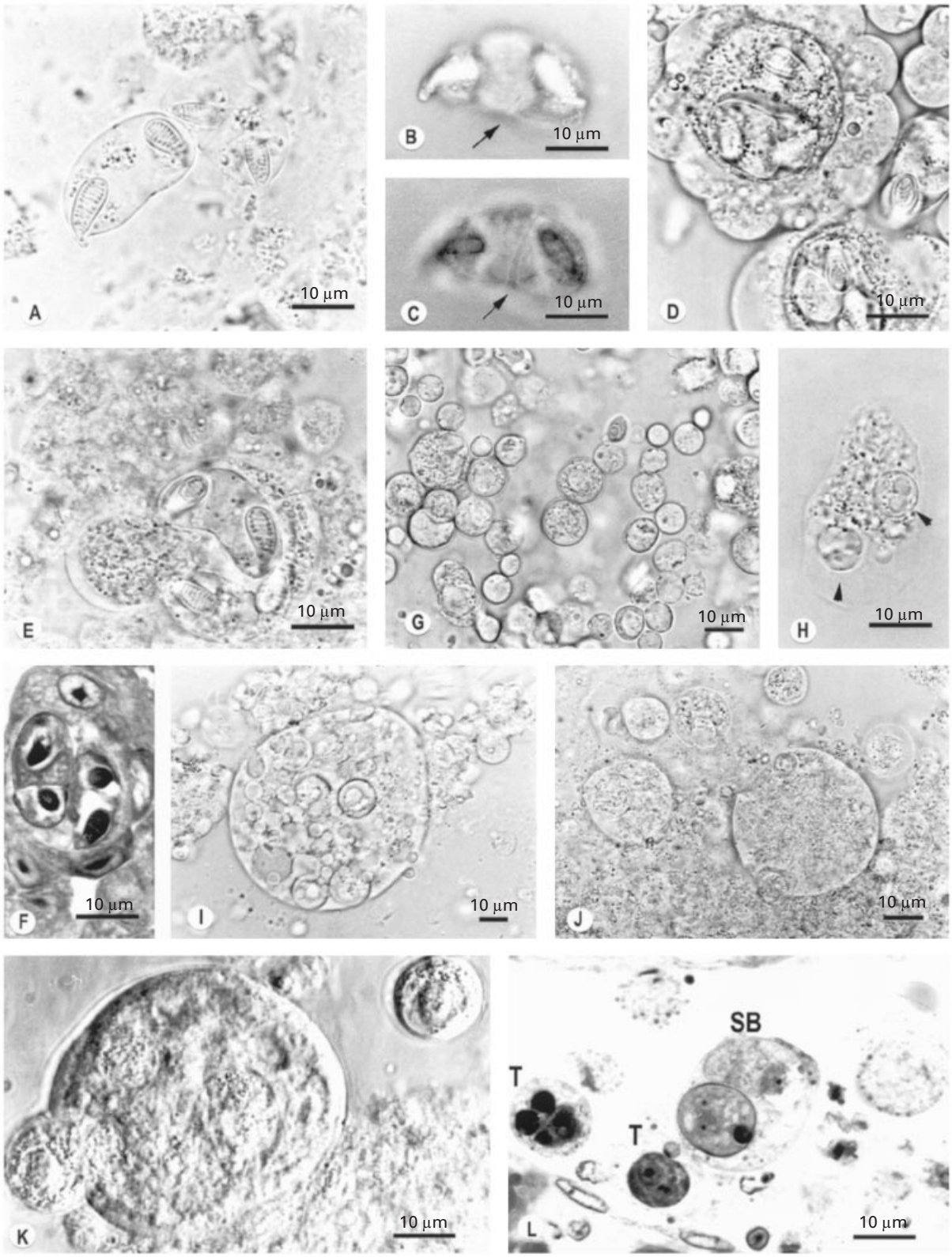

Fig. 2. Light microscope images of *Enteromyxum scophthalmi* stages. (A–C) Fresh smears of spores. The suture line (arrows) is focused in frontal (B) and back (C) views. (D–F) Disporous plasmodia in fresh smears (D, E) and in a toluidine-blue stained histology section (F). (G–K) Fresh smears of parasitic stages released to the intestinal lumen. Trophozoites (G). Amoeboid stage (H) containing 2 secondary cells (arrowheads). (I–K) Large stages containing or releasing inner cells. (L) Toluidine blue-stained section of stages in the lumen. Trophozoites (T) and a disporous pansporoblast (SB) with immature spores are present.

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Table 1. Comparison of spore measurements of *Enteromyxum scophthalmi* and related myxozoans

(Mean, S.D. and/or range in μm given as available. (F*): Formalin-fixed spores; (H**): Histological sections.)

		Spore dimensions			Polar capsules			
Myxozoan	Host	Length	Width	Thickness	Length	Width	Coils	Report
	Sparus aurata	14·7 (13·2–15·2)	6·9 (5·6–7·8)	6	7·4 (6·2–8·8)	3·2 (2·8–3·8)	7 (6–8)	Diamant *et al.* (1994) (F*)
	Sparus aurata	16·2 (15·2–17·7)	7·8 (7·5–8·6)	?	8·2 (6·9–9·8)	3·6 (3·1–4·3)	?	Diamant (1998)
Myxidium leei	*Sparus aurata*	16·7 ± 1 (15–18)	9·7 ± 1 (8–11)	?	8·5 ± 1·3 (8–9)	2·7 ± 0·7 (2·5–4)	6–8	Sakiti *et al.* (1996)
	Sparus aurata	15–18	7–8	?	7	3	?	Diamant (1992) (F*)
	Diplodus puntazzo	15–19	5–7	?	6·5–9	2·5–4	?	LeBreton & Marques (1995)
	Sciaenops ocellatus	17·5 (15·5–19·5)	7·4 (7·0–8·7)	?	8·4 (7·0–9·8)	3·8 (3·3–4·5)	?	Diamant (1998)
Unidentified	*Amphiprion frenatus*	11	6·6	?	4·5	2·5	?	Kent (1999) (H**)
Enteromyxum scophthalmi	*Scophthalmus maximus*	22·2 ± 1·4 (20–25)	11·7 ± 1·2 (19·2–14·1)	14	10·4 ± 1·1 (8·6–13)	4·6 ± 0·6 (3·6–6)	11 (10–13)	Current study

Developmental stages. Various stages were found in the intestinal epithelium and free in the lumen, the latter present mainly in severe infections. Stages within the epithelium were usually in an intercellular position, very closely joined to the epithelial cell's membranes with which they formed sinuous interfaces (Fig. 4D). Intracellular stages were also confirmed (data not shown). The most common stage found in the epithelium was a P cell harbouring 2 or more S cells, which could also harbour 1 or more T cells (Fig. 4D). The P cell displayed some mitochondria with scarce tubular cristae, abundant β-glycogen granules, vacuoles, residual electron-dense bodies and scarce lipidic inclusions. One or 2 nuclei could also be observed. S and T cells were conspicuously more electron dense than the P cell due to the abundant, densely packed ribosomes. They also showed some mitochondria and rER.

Developmental stages also appeared in the intestinal lumen, sometimes similar to the amoeboid stages observed by light microscopy. The predominant stage in the lumen was nearly spherical, 9–15 μm in size in the sections, and consisted of a P cell with one or more S cells, some of them also harbouring T cells (Fig. 4E). The P cell was quite similar to that of tissue stages except for the presence of scarce β-glycogen granules and abundant lipidic granules in the lumen stages. S cells harbouring T cells contained cytoplasm similar to that of P cells, but with more abundant rER and fewer residual bodies and vacuoles. Other S cells and the T cells were very electron dense and contained abundant membranes. In advanced parasite stages, vacuoles as well as some electron-dense bodies, lysosomes and membranous debris accumulated in the cytoplasm of P cells. Some of the stages present in the lumen appeared to be releasing the inner stages, and many showed evident signs of degeneration.

Analysis of SSU rRNA gene sequences

In *Myxidium leei*, *Zschokkella mugilis* and the species from turbot, almost the entire length of the SSU rRNA gene was amplified, cloned and sequenced. The sequences have been deposited in GenBank as follows: *Enteromyxum leei* 1589 bp, accession number AF411334; *Enteromyxum scophthalmi* 1609 bp, AF411335; *Zschokkella mugilis* 1632 bp, AF411336.

Phylogenetic trees constructed with NJ, MP and ML analysis of the 1299 positions of the alignment used, agreed on the grouping of *M. leei* and *E. scophthalmi* as a monophyletic group, with maximum bootstrap support (Fig. 5). This branch was also consistently placed far apart from the clade containing all the species of the genus *Myxidium* sequenced up to date. The position of *M. leei* and *E. scophthalmi* was firmly established within the clade containing the marine myxozoans plus *Ceratomyxa shasta*. Within this clade, the branching order and

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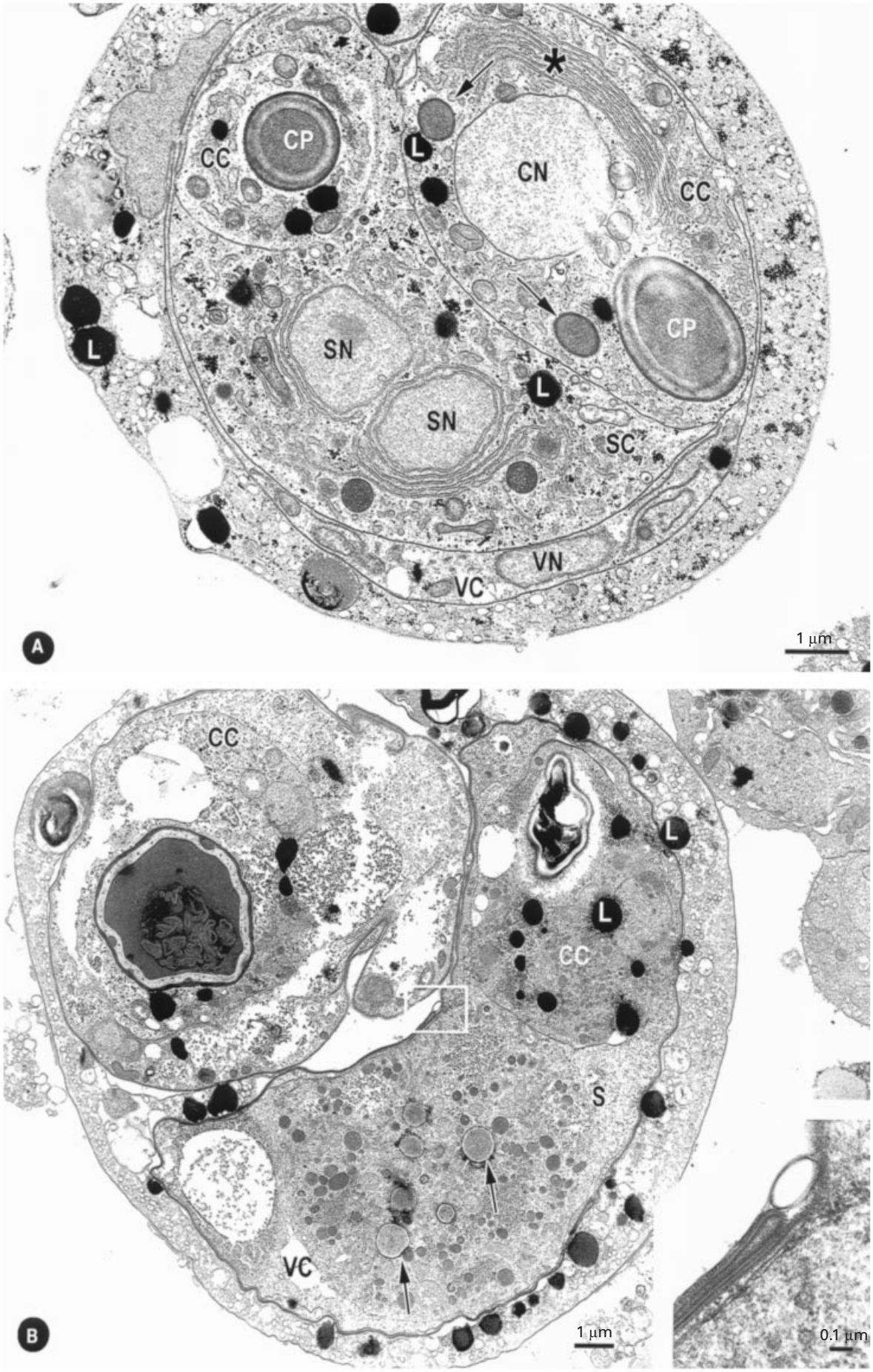

Fig. 3. For legend see opposite.

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the support for the different phylogenetic hypotheses was somewhat dependent on taxon sampling, method of reconstruction, and use of more or less conservative alignment masks (data not shown). This instability mainly affected the position of *Z. mugilis* and *C. shasta*, although neither of them grouped with *M. leei* and *E. scophthalmi* in a monophyletic group. In maximum likelihood trees, the branching of *Z. mugilis*, *C. shasta* and *Enteromyxum* was frequently resolved in a multifurcating node (data not shown).

DISCUSSION

Among the species of Myxosporea described to date the most similar to the turbot parasite is *M. leei*, an enteric species originally described from *Sparus aurata* (Diamant *et al*. 1994). This similarity is not limited to the spore stage, but also to the target tissue and the general pathology caused in the fish. Though there are some differences in the spore size of *M. leei* from different hosts (Le Breton & Marques, 1995; Diamant, 1998; Sakiti *et al*. 1996; Padrós *et al*. 2001), the spores of the turbot parasite are clearly larger than those of *M. leei*. In addition, *E. scophthalmi* presents more elongated spores and polar capsules, more coils of the polar filament, and lacks valve ornamentation. The polar capsules of *E. scophthalmi* clearly open to opposite sides of a plane bisecting the spore longitudinally in frontal or apical view, and actually the distal ends of the spores have that orientation giving the spores a characteristic twisted shape. According to our own observations of *M. leei* (Padrós *et al*. 2001 and unpublished data), this species also has the particular orientation of the spore ends and polar capsules, although this detail was not specifically stressed in the species description (Diamant *et al*. 1994).

Ultrastructural information on *M. leei* is very scarce and does not stand a thorough comparison with our data. Only a TEM image of a sporoblast was provided in the species description by Diamant *et al*. (1994), and some scanning electron microscope studies were presented with another TEM image by Sakiti *et al*. (1996). The sporoblast illustrated by Diamant *et al*. (1994), however, contains capsulogenic cells similar to those found in our material, including abundant rER. The electron-dense vacuoles described by those authors within the capsulogenic cells and primary cell correspond to lipidic inclusions in our material.

As in the case of *M. leei*, the general morphology of the turbot enteric myxozoan partly resembles that of the genera *Myxidium* or *Zschokkella* according to the diagnosis of Lom & Noble (1984), but they do not properly fit in either of these genera. Morphologically, the distinction of *Myxidium* and *Zschokkella* is rather precarious (Diamant *et al*. 1994) because of the overlapping characters and criteria and this situation is exacerbated by many incomplete and poorly documented species descriptions. The most characteristic features of *Enteromyxum* spores are the polar capsule size, shape and orientation, which allow easy distinction of *Zschokkella* spp. (with almost spherical ones). Nevertheless, in *Myxidium* the polar capsules are pyriform and in some species they can also be rather large and elongated, although unlike *Enteromyxum* they usually discharge in the same plane. Furthermore, most *Myxidium* spp. display a suture line bisecting the spore longitudinally, whereas in *E. scophthalmi* it is nearly transversal in front view but somewhat sinuous in top and bottom views, and it partly resembles the curving line that can be noticed in some *Zschokkella* spp. when observed from particular perspectives. Despite these differentiating characters, their diagnostic value is limited given the diversity of patterns reported for both *Myxidium* and *Zschokkella*.

Ultrastructural data do not provide support for choosing one or other of these genera. In *Myxidium*, ultrastructural studies of a few of the more than 150 known species have shown important diversity of structures and developments, suggesting that this genus may be a heterogenous collection of species (see Canning *et al*. 1999). Ultrastructural data are equally fragmentary for *Zschokkella* spp., with only a few thorough studies available and also considerable heterogeneity (Davies & Sienkowski, 1988; Diamant & Paperna, 1992; Lom & Puytorac, 1965; Lom & Dyková, 1996; Sitjà-Bobadilla & Álvarez-Pellitero, 1993).

There are some characteristic biological features of the enteric myxozoan from turbot that are atypical for either *Myxidium* or *Zschokkella*. First, like *M. leei* it is a true histozoic parasite, a condition that has rarely been reported for *Zschokkella* spp. (Diamant *et al*. 1994) and is also rather unusual in *Myxidium*. *M. rhodei*, and some stages of *M. lieberkuehni* and

Fig. 3. Transmission electron microscope images of *Enteromyxum scophthalmi*. (A) Sporoblast showing an immature spore. Remnants of the nucleus (VN) and mitochondria are displayed in the flattened valvogenic cells (VC). Notice the capsular primordium (CP), the external tube (arrows), the nucleus (CN) and the abundant endoplasmic reticulum (*) in the capsulogenic cells (CC). The sporoplasmic cell (SC) contains 2 nuclei (SN). L: lipidic inclusions. (B) Disporous plasmodium containing 2 nearly mature spores. Notice the abundant electron-dense granules and sporoplasmosomes (arrows) in the sporoplasm (S), and the lipidic inclusions (L) in the enveloping primary cell and capsulogenic cells (CC). The remnants of the valvogenic cell (VC) are also visible. Inset: detail of the overlapping at the valves at the suture line.

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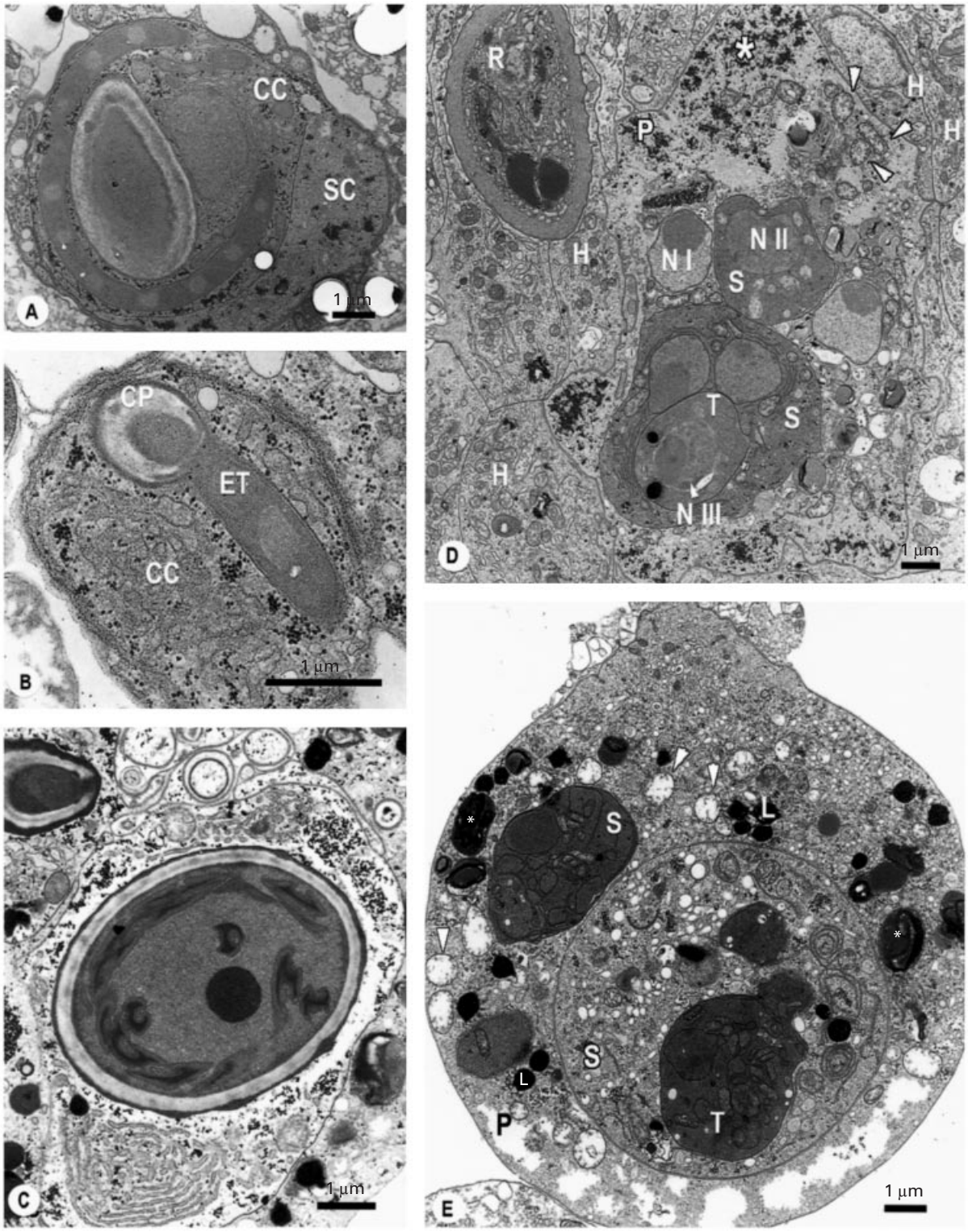

Fig. 4. Transmission electron microscope images of *Enteromyxum scophthalmi*. (A and B) Details of developing spores stained with the Thiéry reaction. Notice the positively stained β-glycogen granules in the capsulogenic (CC) and sporoplasmic (SC) cells. (B) The external tube (ET) is being internalized in the capsular primordium (CP). (C) A more mature capsulogenic cell. (D) Parasite stage in the intestinal epithelium. The primary (P) cell harbours two secondary (S) cells, one of them containing 1 tertiary (T) cell. Their nuclei are labelled as NI, NII and NIII, respectively. Notice the abundant glycogen granules (*) and some mitochondria (arrowheads) in the P cell and the high electron density of S and T cells. Also note a neighbouring rodlet cell (R) and other host cells (H). (E) Parasitic stage released to the intestine lumen. The P cell contains 2 S cells, and one of these harbours a T cell. Notice the high electron density and abundance of membranes in the T cell and in 1 of the S cells, as well as the accumulation

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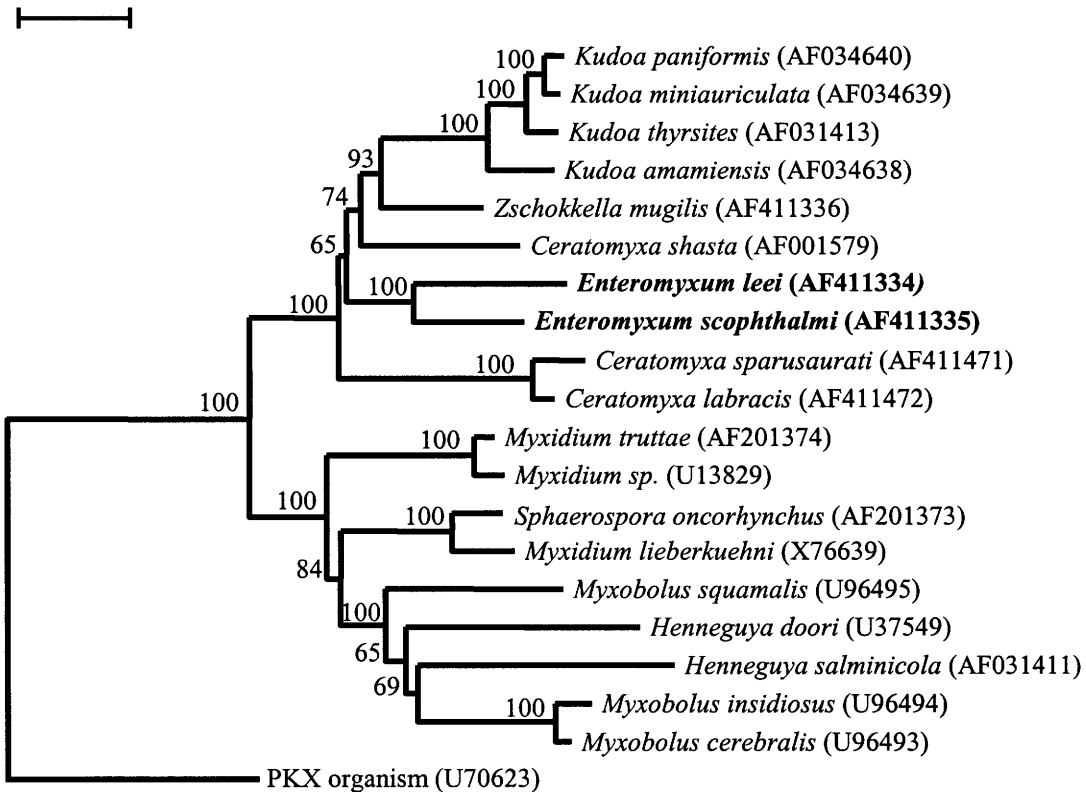

Fig. 5. Phylogenetic tree inferred from neighbour-joining analysis of the SSU rRNA gene from *Enteromyxum scophthalmi* and related myxozoans. Numbers at the forks represent the percentage of replicates in which the given branch was obtained out of 100 bootstrap samplings. Scale bar at the top represents a rate of 0·05 substitutions per site.

M. giardi are found in histozoic situations (Lom & Dyková, 1992), although these species are morphologically and biologically very different to *E. scophthalmi*. Two other histozoic, enteric myxozoans have recently been reported as similar or related to *M. leei* (Kent, 1999; Tun *et al*. 2000), although their ascription to *Myxidium* was only presumptive. Secondly, in *E. scophthalmi* infections there are abundant stages, present in the digestive epithelium or free in the lumen, which harbour S or S and T cells. These stages seem to play an important role in the biology of the parasite as they can release their inner cells, which, in turn, can migrate from the lumen to the epithelium or through the interstitial spaces inside the epithelium, and can eventually penetrate into the host cells. They represent a proliferative phase in the life-cycle of the parasite and substantially contribute to the severe pathological effect produced, leading to the cachectic and emaciated condition typical of the disease and ending with the fish death (Branson *et al*. 1999). The high activity of these proliferative stages is evidenced by the ultrastructure of their inner S and T cells, very rich in membranes and densely packed ribosomes, whereas mitochondria are more abundant in the P cells or more mature S cells. The proliferative stages releasing infective cells could possibly be responsible for the existence of a direct, spontaneous fish-to-fish transmission of the disease, as it was suggested by the epizootiology of the parasitosis in farmed turbot (Branson *et al*. 1999). In this respect, *M. leei* is considered a unique case among myxosporeans because it readily and spontaneously transmits to other fish (Diamant *et al*. 1998; Padrós *et al*. 2001). Further studies on the transmission and life-cycle of *E. scophthalmi* are currently under way and will be published elsewhere.

The need for additional data to resolve this *Myxidium vs. Zschokkella* ambiguity led us to use molecular phylogenetic analyses of SSU rDNA sequences, including members of both genera and the 2 enteric species, *E. scophthalmi* and *M. leei*. These studies have greatly contributed to clarification of their systematics. First, rDNA data confirmed that the new parasite from turbot is not *M. leei*, though the closeness between both species indicates that they should be grouped in the same genus. Secondly, both *M. leei* and the turbot myxozoan fit into a branch of the tree which firmly groups all the marine species plus *Ceratomyxa shasta*, but consistently excludes members of the genera *Myxidium*,

of lipidic inclusions (L) in the P cell. Mitochondria at different stages of degeneration (arrowheads) and residual bodies (*) are abundant in the P cell.

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Sphaerospora, *Myxobolus* and *Henneguya*. The former group has previously been identified as a primarily marine clade, although it includes species which have been secondarily adapted to freshwater, such as *C. shasta* (Kent & Palenzuela, 2002, Kent *et al*. 2001). In contrast, the *Myxidium* spp. whose sequence data are available to date (including the type species *M. lieberkuehni*) are always positioned in a primarily freshwater clade in rDNA phylogenies. This arrangement does not support the assignment of either the species from turbot or *M. leei* to the genus *Myxidium*. Thirdly, although the sequence of *Zschokkella mugilis* places this species among the primarily marine myxozoans, there is no support for the grouping of *Z. mugilis* with the enteric species from turbot in a monophyletic clade. The position of *Z. mugilis* in this group also questions whether there is a close relationship between *Myxidium* and *Zschokkella*, currently included in the same family (Myxidiidae), and the suggestion that they may be synonymous taxa (Lom & Dyková, 1992; Diamant *et al*. 1994). The lack of unambiguous diagnostic criteria and the morphological similarities between *Zschokkella* and *Myxidium* spores might only reflect some convergence due to similar ecological niches, while there could be profound differences in their phylogenies as suggested by the results of our analyses with *Z. mugilis*. Further analyses including other *Zschokkella* spp. would be necessary to clarify these aspects.

The relationships between *Zschokkella*, *Enteromyxum*, and *C. shasta* were not firmly resolved in the present study. The alignment of sequences from species of the primarily marine clade with those from the other myxozoans was achieved by alignment of many positions of uncertain homology, because of the important differences observed between the sequences of both groups. Further molecular phylogenetic analyses limited to the marine group and including other taxa could result in a more reliable finer topology and are currently under way. Nevertheless, the molecular analyses confirm that *M. leei* and the species from turbot do not properly fit into either *Myxidium* or *Zschokkella*. Furthermore, they do not show clear affinity with any other known myxozoan genus at morphological or molecular levels and thus we erect the genus *Enteromyxum* to accommodate these species of marine, histozoic and enteric myxozoans. Other recent reports of *M. leei*-like species from exotic fish (Kent, 1999; Tun *et al*. 2000) might actually correspond to distinct species belonging to this new genus.

Diagnosis. *Enteromyxum* n.g. Myxozoa: Myxosporea. Spores with slightly crescent shape, very large and elongated polar capsules tapering to their distal side and opening at the ends of the spore, but discharging in opposite directions relative to a longitudinal plane bisecting the spore in top or bottom view. One binucleated sporoplasm. Spores develop in disporic pansporoblasts. Histozoic parasites in the epithelia of the digestive system of marine fish.

Type species. *Enteromyxum scophthalmi*

Type host. *Scophthalmus maximus*

Other species: *Enteromyxum leei* (Diamant, Lom & Dyková, 1994) n. comb.

Remarks. Includes highly pathogenic species, usually causing acute enteritis, cachexia, emaciation and death, in susceptible fish. Direct transmission and wide host-specificity demonstrated in *E. leei*.

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