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Occurrence of microsporidians *Glugea hertwigi* and *Pleistophora ladogensis*, in smelt *Osmerus eperlanus* from two German rivers, North Sea coast

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ABSTRACT: Monthly samples of smelt *Osmerus eperlanus* (Linnaeus, 1758) were collected from July 1985 to May 1986, in the river Elbe (Germany), and examined for infections with microsporidians. Two microsporidians were found: *Glugea hertwigi* Weissenberg, 1911, infecting the digestive tract and *Pleistophora ladogenis* Voronin, 1978, infecting the skeletal musculature. *G. hertwigi* infection led to the formation of xenomas, whereas *P. ladogensis* was characterized by diffuse infections, with the production of macroscopic visible thread-like or oval-shaped infection foci. Development of *G. hertwigi* in the host cells showed characteristics typical of the genus *Glugea*. The ultrastructural development of *P. ladogensis* showed features typical of the genus *Pleistophora*, without evidence of the production of 2 types of spores. Host reaction consisted of inflammatory tissue surrounding some of the infection foci as well as phagocytosis of spores. *G. hertwigi* was only found in juvenile smelt (<10 cm in length), whereas *P. ladogensis* infected smelts from 6 to 26 cm in length. Prevalence increased with fish length to a maximum value of 9.6%. Seasonal fluctuations in prevalence of infection were also found, with the lowest value in the winter months (2.5% in January 1986) and the highest in summer (11.8% in July 1985). The differences in prevalence of infection with fish length and date of sampling were significant. Additionally, samples of smelt caught in April 1986 from the rivers Eider and Ems revealed infections with *P. ladogensis* in the first river system only.

KEY WORDS: Microsporidia · *Glugea hertwigi* · *Pleistophora ladogensis* · Smelt · *Osmerus eperlanus*

INTRODUCTION

The European smelt *Osmerus eperlanus* (Linnaeus, 1758) is a small anadromous fish species belonging to the family Osmeridae (Pisces: Osmeridae) (see Froese & Pauly 2011). They rarely exceed 20 cm in length and are divided into 2 distinct forms: the euryhaline smelt, which is found in coastal waters and spawns in the rivers, and the freshwater form, which remains in the rivers. European smelt is found in the Northeastern Atlantic from the White Sea to the western coasts of France, in the Baltic Sea and the southern North Sea, and in lakes of coastal areas of the North, Baltic, White, and Barents Seas. In the river Elbe, they suffer from several diseases of viral and bacterial etiology (Möller 1984, Möller & Anders 1986), microsporidian infections (Weissenberg 1911), and infections with other endoparasites (Sprengel & Lüchtenberg 1991). Microsporidians are unicellular obligate intracellular parasites, infecting many species of hosts, particularly arthropods and fishes (Lom & Dyková 1992, Lom 2002). To date, >31 species of *Glugea* and >26 species of *Pleistophora* have been reported from fish (see Lom 2002). Two species of microsporidians — *Glugea hertwigi* Weissenberg, 1911 and *Pleistophora ladogensis* Voronin, 1978 — were described infecting smelt *O. eperlanus* in the Baltic Sea and Lake Ladoga (Voronin 1978, Horppila et al.

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1996, Pekcan-Hekim et al. 2005). *G. hertwigi* also infects the American smelt *Osmerus mordax* (Mitchill, 1814) (see Delisle 1972, Nepzy & Dechtiar 1972). In the American smelt, this microsporidian has been found to cause severe mortalities due to disintegration of intestinal tissue and occlusion of the intestinal lumen by large xenomas (Legault & Delisle 1967, Nepszy & Dechtiar 1972).

P. ladogensis was found to produce whitish infection foci in the musculature of smelt and burbot *Lota lota* Linnaeus, 1758 (Gadiformes, Lotidae) from lakes Vrevo and Ladoga (Russia) (Voronin 1978, 1981). However, the pathological effect of this parasite was not studied, and little is known about the spore ultrastructure. *P. ladogensis* has received little attention since its first description by Voronin (1978, 1981), and no further studies have been conducted on this microsporidian. This fact encouraged us to review and publish our data collected from the survey done in 1985 to 1986 from smelt caught in rivers in northern Germany.

In the present survey, the occurrence and the temporal and size-related changes in infections with both microsporidians were studied during a 1 yr period, with observations of the spatial distribution and ultrastructural development of these parasites.

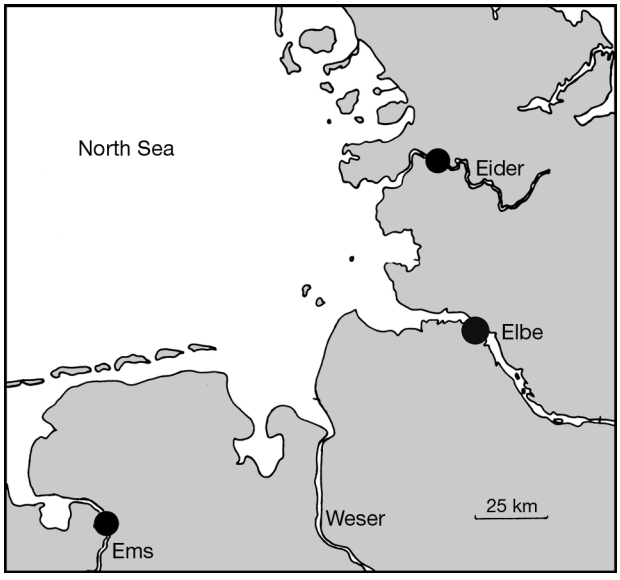

Fig. 1. North Germany showing the sampling locations on the rivers Elbe, Eider and Ems. The river Weser is also shown

MATERIALS AND METHODS

Smelt were caught from the river Elbe (Northern Germany) (Fig. 1) every month from July 1985 to May 1986, except in August 1985 and in February and April 1986. They were examined either fresh on board the fishing vessel or frozen and later defrosted and examined in the laboratory. A total of 11 118 smelts (total length [TL] ranging from 3 to 26 cm) were examined for the presence of microsporidians in the digestive tract and skeletal muscles. The fish were measured to the nearest centimeter (TL), rounded down. In April 1986, additional samples of 279 and 269 smelt were obtained from the Eider and Ems rivers, respectively. For histological study, pieces of skeletal muscle showing typical lesions and xenomas found attached to the digestive tract were fixed in Bouin's fluid for 24 h, dehydrated in ascending alcohol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm obtained with a rotary microtome were stained with Mayer's haematoxylin and eosin, dehydrated in ascending alcohol series, cleared in xylene, and mounted in Entellan. For transmission electron microscopy, pieces of xenomas and pieces of skeletal muscle showing typical lesions were fixed in 2.5% glutaraldehyde in phosphate buffer for 2 h at room temperature, washed in phosphate buffer for 2 h, post-fixed with 2% osmium tetroxide for 2 h at 4°C, dehydrated in ascending ethanol series and propylene oxide, and embedded in Araldite resin (Merck). Semithin sections for light microscopy study were stained with methylene blue and examined with a Zeiss light microscope, whereas ultra-thin sections were contrasted with uranyl acetate and lead citrate. The latter were observed with a Zeiss EM 9A transmission electron microscope. Prevalence of infection was calculated according to Bush et al. (1997). The statistical significance of the relationship between prevalence and fish length was studied using binomial logistic regression analysis. Differences in prevalence in relation to season and sampling locations were compared using a contingency table analysis. All statistical analysis was performed using SPSS 11.0.

RESULTS

Light and transmission electron microscope observations

Whitish xenomas of *Glugea hertwigi* were found attached to the subepithelial connective tissue of the digestive tract of juvenile smelt *Osmerus eperlanus* (Fig. 2a,b). Individual xenomas measured 1 to 2 mm in diameter (n = 12), but they could coalesce forming a large whitish 'knot' which attained up to

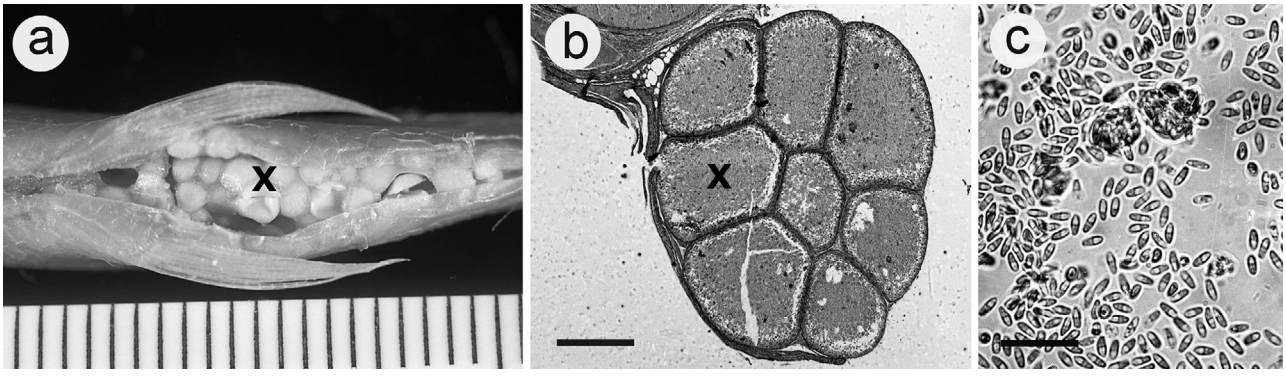

Fig. 2. (a) Macroscopic view of the xenomas of *Glugea hertwigi* in the visceral cavity of smelt *Osmerus eperlanus*. Ruler divisions = 1 mm. (b) Histological section showing group of xenomas (**X**) of *G. hertwigi* from the intestinal wall (scale bar = 400 µm). (c) Light micrograph showing fresh spores of *G. hertwigi* (scale bar = 16 µm)

6 mm in maximum diameter, leading to pronounced swelling of the body cavity. Many free spores were seen in the centre of the xenoma, with a mean size of 2.0 × 5.1 µm (range from 2.0 × 4.3 to 2.2 × 5.4 µm, n = 14) (Fig. 2c). Ultrastructural study revealed that the xenoma was delimited by a layer of apposed connective tissue measuring 32 to 50 µm, followed by the xenoma wall of laminated structure (Fig. 3a,b). In the cytoplasm of the xenoma, merogonial stages were observed at the periphery,

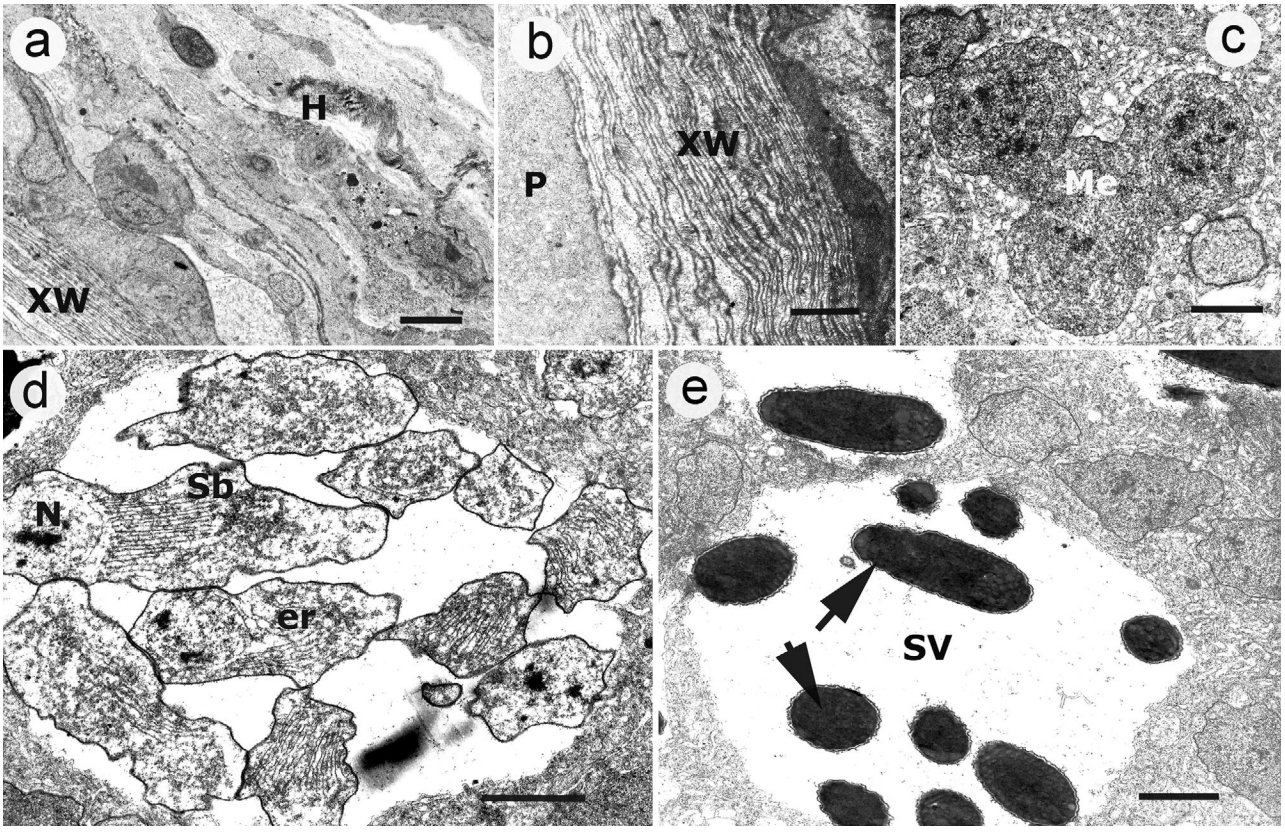

Fig. 3. Ultra-thin sections showing the ultrastruture of the xenoma wall, the merogonial stages and the formation of spores of *G. hertwigi*: (a) xenoma of *G. hertwigi* showing peripheric capsule of connective tissue (H) and xenoma wall (XW) (scale bar = 4 µm); (b) xenoma wall (XW) and parasite cytoplasm (P) (scale bar = 2 µm); (c) dividing meront (Me) in the cytoplasm of the xenoma of *G. hertwigi* (scale bar = 1 µm); (d) sporoblasts (Sb) in the sporophorous vesicle, showing nucleus (N) and endoplasmic reticulum (er) (scale bar = 1.4 µm); (e) Spores of *G. hertwigi* (arrowed) within the sporophorous vesicle (SV) (scale bar = 2.3 µm)

whereas the sporophorous vesicles, measuring 11.2 to 18.3 µm in diameter and filled with the egg-shaped spores, were located in the central area of the xenoma. The meronts (Fig. 3c) and vegetative plasmodia, located at the periphery of the xenoma, measured 1.6 × 2.5 µm (n = 7) and 1.9 × 6.2 µm (n = 5), respectively. The sporogonial plasmodia divided into uninucleate sporonts (sporoblast mother cells) inside the sporophorous vesicles (Fig. 3d). Mature spores, seen inside the sporophorous vesicles, showed very electron-dense cytoplasm, but 12 to 16 coils of the polar tube were observed (Fig. 3e).

Whitish, oval, and thread-like shaped infection foci of *Pleistophora ladogensis* were found infecting the skeletal muscles of smelt (Fig. 4a). They had a mean size of 1.3 mm (1.0–1.7 mm, n = 20). These infection foci were packed with spores, within sporophorous vesicles or free, and a few developmental stages. Spores were egg-shaped, measuring 3.0 × 5.3 µm (range 2.9 × 5.0 to 3.0 × 5.8 µm, n = 21) (Fig. 4b). More than 40 spores could be counted in the sporophorous vesicles, in the semi-thin sections (exact number not known). Histological study of infected skeletal muscles showed the infection foci packed with sporophorous vesicles filled with spores and some sporonts (Fig. 4c). This deformed the muscle fibers (Fig. 4d) and elicited in some cases a pronounced inflammatory response surrounding the infection foci, with occasional necrosis and destruction of muscle tissue. However, in other sections of infected tissue, no host reaction against the parasite was observed. At the ultrastructural level, phagocytized spores were seen inside macrophages, with spores showing degenerative changes, with degradation of the spore envelopes and destruction of its cytoplasm. Merogonial and early sporogonial stages could not be studied by electron microscopy due to unsatisfactory fixation, which had been carried out at room temperature. Sporophorous vesicles, containing large numbers of spores (>40 spores, exact number not counted), were thick-walled and measured 11 to 36 µm (n = 10) (Fig. 4e). Multinucleated sporogonial plasmodia, delimited by a thick electron-dense membrane, were dividing to give rise to uninucleate sporoblasts

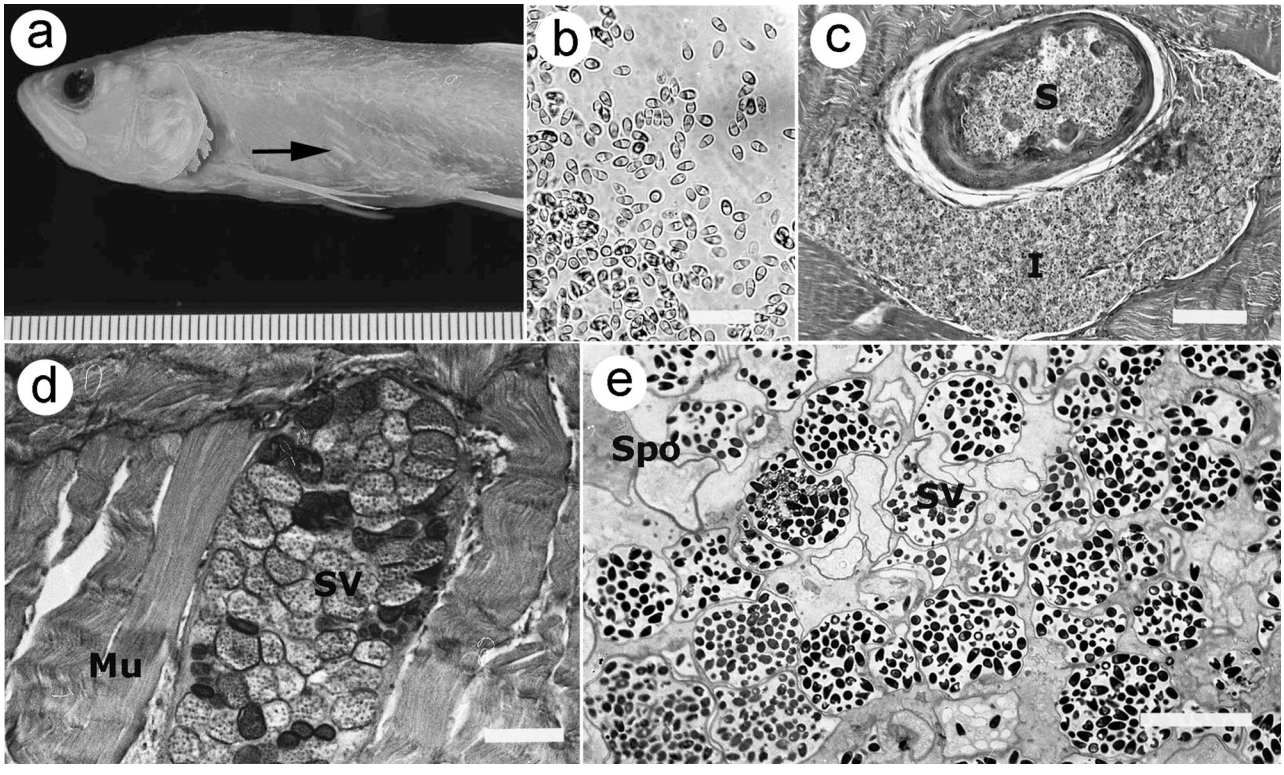

Fig. 4. (a) Macroscopic view of 1 infection focus (indicated with the arrow) of *Pleistophora ladogensis* in the muscles of smelt *Osmerus eperlanus* (ruler divisions = 1 mm). (b) Light micrograph showing fresh spores of *P. ladogensis* (scale bar = 20 µm). (c) Histological section of infected muscles showing 1 infection focus filled with spores of *P. ladogensis* (S) surrounded by inflammatory cells (I) (scale bar = 235 µm). (d) Histological section of infected muscles (Mu), with 1 infection focus of *P. ladogensis*, packed with sporophorous vesicles (SV) filled with spores and early developmental stages (scale bar = 50 µm). (e) Semi-thin section of 1 infection focus of *P. ladogensis* showing sporophorous vesicles with spores (SV) and sporogonial plasmodia (Spo) (scale bar = 26 µm)

(Fig. 5a) inside sporophorous vesicles. The uninucleate spore had a posterior vacuole, which occupied about half of the spore. The spore was surrounded by a double-layered membrane, composed of an electron-dense exospore and an electron-lucent endospore (Fig. 5b). The anchoring disk was sub-terminal and showed vesicular structures. The polar filament had 13 to 17 coils forming 1 row (Fig. 5b,c). The diameter of the polar filament was about 92 nm and was formed of several different concentric layers (Fig. 5c). Immature spores were seen with 2 to 3 rows of the polar filament, a corrugated exospore and the more visible vesicular and lamellar parts of the polaroplast (Fig. 5d).

Prevalence of infection

Infections with *G. hertwigi* were only found in some juvenile smelt (3 to 6 cm in total length) (p = 0.7%, n = 3653), whereas *P. ladogensis* was found in smelts ranging in length from 6 to 26 cm long (Fig. 6a). Prevalence of the latter showed a positive relationship with fish length, with juveniles from 3 to 5 cm long being apparently uninfected, whereas an increasing trend of prevalence was observed in fish from 6 to 26 cm, reaching a maximum of 9.6% in length class 18 cm. A logistic regression showed a significant effect of length on the prevalence of infection with *P. ladogensis* (Wald test = 4.022, p = 0.045). Nevertheless, length only explained 0.3% of the variation (R^2 = 0.003). Fish up to 11 cm in length showed only a few foci (<10) of infection, whereas older fish had many foci of infection (>50) throughout the muscles. Prevalence of infection with *P. ladogensis* in fish from 11 cm to 26 cm long was higher in summer (11.8%, in July 1985), decreasing to 6.5% in autumn (September and October 1985; n = 522) and to 3.3% in winter (November and December 1985, January and March 1986; n = 2177) (Fig. 6b). The lowest prevalence value was found in January 1986 (p = 2.5%, n = 654), at a water temperature of 1.8°C. There was a statistically significant association between prevalence of infection and season, with observed frequencies of infected fish being higher than expected ones in summer and autumn (χ^2 = 76.78, p = 0.000). Regional differences in prevalence of *P. ladogensis* were found between the river Elbe and 2 other freshwater systems, the rivers Eider and the Ems (Table 1). Eider smelt from 13 to 16 cm had a prevalence of 2.5% and 5.9% respectively, with the highest prevalence of 16.7% in the length group 20–22 cm, but smelt of the length classes 17 and 18 cm were not infected. In contrast, in the river Elbe,

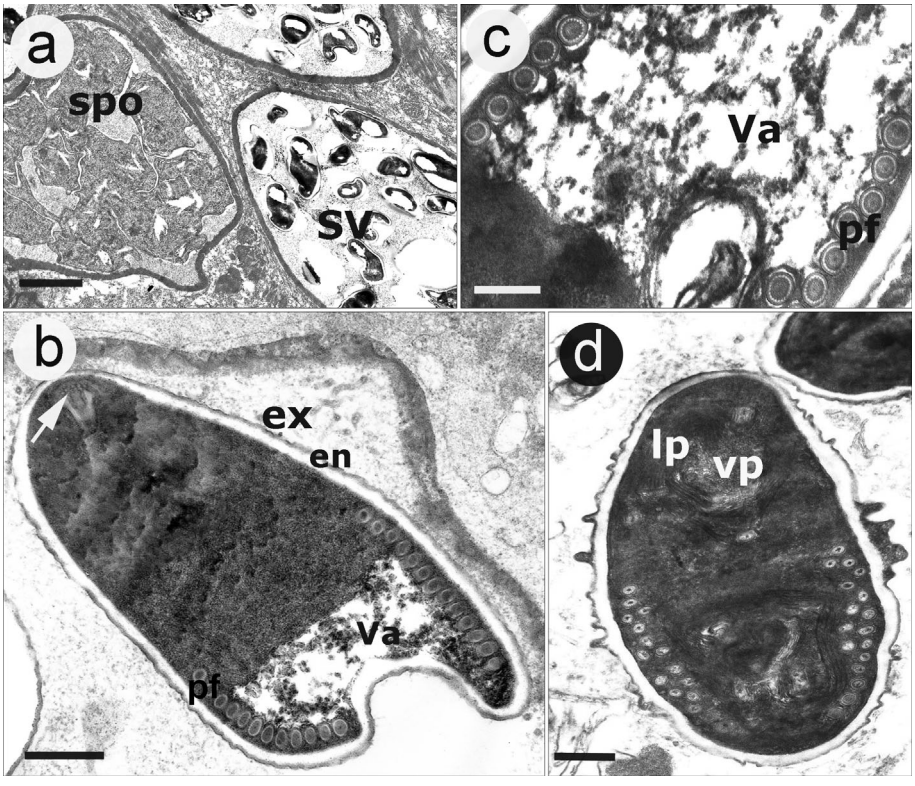

Fig. 5. Ultra-thin sections of sporophorous vesicles with sporogonial plasmodia and spores of *P. ladogensis*: (a) dividing sporogonial plasmodia (spo) giving rise to sporoblasts, and spores both inside sporophorous vesicles (scale bar = 5 µm); (b) microspore of *P. ladogensis,* inside sporophorous vesicle, showing exospore (ex), endospore (en), anchoring disc with vesicles (arrowed), posterior vacuole (Va), and polar filament (pf) (scale bar = 0.7 µm); (c) detail of the polar filament (pf) of the spore of *P. ladogensis*, evidencing different concentric layers (scale bar = 0.2 µm); (d) microspore of *P. ladogensis* showing vesicular (vp) and lamellar portion (lp) of the polaroplast (scale bar = 0.8 µm)

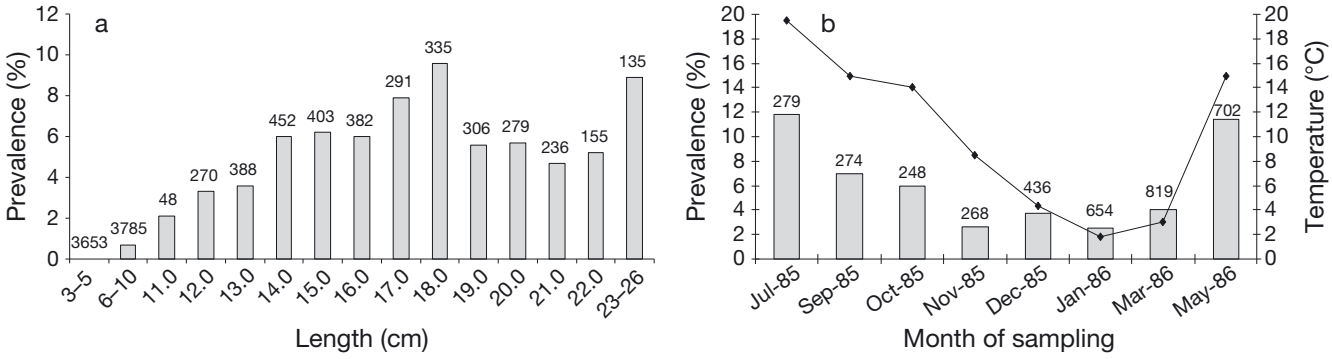

Fig. 6. Prevalence of *P. ladogensis* in smelt from the river Elbe in relation to (a) fish length (cm) and (b) fish sampling date (for smelt length of 11 to 26 cm). Numbers above bars represent the number of fish examined. Line indicates water temperature

Table 1. Prevalence of *P. ladogensis* in smelt *Osmerus eperlanus* from 3 different river systems, in relation to fish length (cm). The numbers of fish examined are in parentheses

Fish length (cm)	Prevalence (%) (no. examined) Eider	Elbe	Ems
12	0 (17)	3.3 (270)	0 (2)
13	2.5 (40)	3.6 (388)	0 (9)
14	2.9 (34)	6.0 (452)	0 (17)
15	2.6 (39)	6.2 (403)	0 (30)
16	5.9 (34)	6.0 (382)	0 (30)
17	0 (40)	7.9 (291)	0 (30)
18	0 (38)	9.6 (335)	0 (30)
19	8.0 (25)	5.6 (306)	0 (30)
20–22	16.7 (12)	8.2 (670)	0 (77)

prevalence values of 7.9 and 9.6% were observed for those length groups. Smelt from the river Ems were not infected with *P. ladogensis*. The differences in the prevalence of *P. ladogensis* in smelt from the 2 river systems, Elbe and Eider, were weakly significant ($\chi^2 = 3.88$; $p = 0.049$).

DISCUSSION

In the present study, the 2 microsporidians found infecting the digestive tract and skeletal muscles of smelt *Osmerus eperlanus*, from 2 river systems in northern Germany, were identified as *Glugea hertiwigi* and *Pleistophora ladogensis* respectively, based on light and transmission electron microscopy.

The development of *G. hertwigi* has previously been described based on light microscopy observations from smelt caught near Berlin, Germany (Weissenberg 1911), and complies in general with the typical development for *Glugea* species (see Lom & Dyková 1992). Later, this parasite was found infecting smelt *O. mordax* from North American freshwater systems (Schrader 1921) and *Coregonus lavaretus* and *Hypomesus olidus* from northern Russia (Akmerov 1946, Shulman & Shulman-Albova 1953). Ultrastructural observations of the mature spores of *G. hertwigi* from *O. mordax* showed the same difficulties we faced in the visualization of the spore structure, due to the spores having very electron-dense cytoplasm (Lovy et al. 2009). Nevertheless, we were able to observe meronts, dividing meronts, sporoblast mother cells, and sporoblasts, which were not previously examined at the ultrastructural level for this species.

Glugea hertwigi in smelt from Canadian lakes reached prevalences of up to 100% (Nepszy & Dechtiar 1972), whereas prevalence in smelt from German freshwater systems reached only 2% (Weissenberg 1911). In our study, infections were confined to a few juvenile fish, in which prevalence was below 1%, which suggests that heavily infected fish could die due to debilitation or to increased susceptibility to predation. A decrease in the prevalence of *G. hertwigi* in American smelt was observed, with juveniles heavily infected and older fish having a lower prevalence of infection, indicating a high mortality rate of infected smelt (Legault & Delisle 1967, Nepszy & Dechtiar 1972). In contrast to our low value of prevalence of *G. hertwigi* in the Elbe smelt, recent surveys of microsporidian infections in smelt from lake systems in Finland found a much higher prevalence of *G. hertwigi* (Horppila et al. 1996, Pekcan-Hekim et al. 2005). Mortalities of heavily infected young-of-the-year smelt occurred in Canada (Legault & Delisle 1967) and presumably also in Finland, where fish were also found heavily infected (Horppila et al. 1996).

P. ladogensis was first observed by Voronin (1978) infecting the skeletal muscles of *O. eperlanus* and burbot *Lota lota* from Lake Ladoga and Lake Vrevo

(Russia). Description of the developmental stages and spores was based on light microscopy observations. According to Voronin (1978), the size of the sporophorous vesicles, filled with more than 16 spores, was 43 µm (18–60), and the size of spores was 2.9 (2.7–3.3) × 5.4 (5.0–5.8) µm. In our study, sporophorous vesicles measured 26 to 36 µm in diameter and contained >40 spores (exact number not counted), and the spores were of the same size as reported by Voronin (1978). *Pleistophora* species are characterized by yielding large numbers of spores (Canning & Nicholas 1980, Leiro et al. 1996). We were unable to obtain good transmission electron micrographs of the developmental stages, meronts, and sporogonial plasmodia, probably due to the fact that fixation of material was done at room temperature (see Leiro et al. 1996). In some *Pleistophora* species, 2 types of spore (macrospores and microspores) are produced, as described for *P. littoralis*, *P. typicalis*, *P. mirandellae* (now *Ovipleistophora mirandellae*), *P. duodecimae*, and *P. priacanthusis* (Canning & Nicholas 1980, Lom et al. 1980, Hua & Dong 1983, Maurand et al. 1988, Lom 2002). In our study, only microspores were observed in the fresh mounts and in several ultrathin sections. Some other *Pleistophora* species do not produce 2 types of spores, for example *P. hyphessobryconis*, *P. hippoglossoideos*, and *P. senegalensis* (Lom & Corliss 1967, Morrison et al. 1984, Faye et al. 1990), suggesting that this feature may not be relevant for the diagnosis of the genus *Pleistophora* (Faye et al. 1990).

As in our study, Voronin (1981) found that prevalence of *P. ladogensis* in smelt from 11 to 22 cm long was 5 to 8%, in contrast to 32 to 50% in burbot *L. lota*, suggesting that smelt is a secondary host of *P. ladogensis*, and its typical host is burbot. Microsporidians are not necessarily host-specific. Two examples of microsporidians with a broad range of hosts, belonging to different fish families, are *P. hyphessobryconis*, which occurs in 18 different fish hosts, and *G. stephani,* infecting 10 different fish hosts (Olson & Pratt 1973, Olson 1976, Canning et al. 1986). Nevertheless another survey of parasites of smelt conducted in the Elbe, Eider, and Weser rivers, 3 yr later, found much higher prevalence values of *P. ladogensis* (Kerstan 1992). Furthermore, this study determined that prevalence of this microscoporidian was age-related rather than length-related, increasing with age up to 30% (see Kerstan 1992). Although infection with *P. ladogensis* produced histopathological effects, expressed as degeneration and disorganization of some of the infected muscles fibers, it appeared that the remaining skeletal musculature was preserved, and so the pathological effect of this microsporidian infection may not pose a threat to the survival of the host. Nevertheless, heavily infected fish could have their swimming capability reduced and may become more susceptible to predation (Sprengel & Lüchtenberg 1991). Some *Pleistophora* species, such as *P. hyphessobryconis,* do induce severe pathological effects on infected hosts, while others, such as *P. typicalis,* do not contribute to severe pathology (Lom & Corliss 1967, Canning & Nicholas 1980, Lom & Dyková 1992, Sanders et al. 2010). The observed increase in prevalence with length, in the present work, and with age (Kerstan 1992) supports the hypothesis that the pathogenic effect of this microsporidian does not impair host survival. Little is known about the transmission of *P. ladogensis*, in particular to its fish hosts, or of the transmission of other microsporidians to their fish hosts (Lom & Dyková 1992, Lom 2002). Some microsporidians have direct infection patterns, whereas others need intermediate transport hosts to reach their final hosts (Delisle 1972, Scarborough & Weidner 1979, Lom & Dyková 1992).

The age and length of smelt at the first infection was not determined because juvenile smelt were only examined in July 1985 and in March and May 1986. However, young smelt of 3 to 5 cm in length (July 1985) were not infected with *P. ladogensis*, whereas smelt of 6 to 10 cm were already infected (March and May 1986). Young fish migrate from the river in September to October to the coastal areas of the North Sea, where they stay until December, when they swim back to the river (Lillelund 1961). Infection could take place during their residence in the coastal areas. The observed differences in prevalence of *P. ladogensis* with season of the year could be the result of a combination of the effect of water temperature and spawning stress. Spawning takes place in March and April (Lillelund 1961). In May, just after the spawning period, a high prevalence of infection was found (11.4%). The water temperature in September 1985 and May 1986 was similar (15°C), although the prevalence found in September was much lower (7.0%). Furthermore, prevalence reached lower values in the winter months. This appears to be the trend for *P. ladogensis* infection in smelt from the river Elbe. In contrast, for smelt of the river Eider, higher prevalence was found in autumn and winter (Kerstan 1992). Water temperature is an important factor for the development of microsporidians, with lower temperatures inhibiting their development (McVicar 1975, Olson 1981, Beaman et al. 1999, Pekcan-Hekim et al.

2005). Prevalence values of *P. ladogensis* in the rivers Eider and Elbe showed a similar related pattern with fish length, whereas no infected smelt were found in the sample collected from the river Ems. Because the Elbe and Eider rivers empty into the southeastern North Sea (see Fig. 1) and are connected through the Kiel Canal, it appears that smelt from these rivers belong to the same population, or at least some degree of population mixing occurs, whereas fish from the river Ems, which empties into the southwestern North Sea, appear to represent a different population. However, this assumption must be treated with caution as only 1 single sample each was taken from the Eider and Ems rivers. Some years later, Kerstan (1992) found smelt from the river Ems infected with *P. ladogensis,* albeit with low prevalence. Nevertheless, significant differences in prevalence between regions can still be used as indicators of different populations (see MacKenzie 2002).

Glugea hertwigi infects 4 different fish species, 3 osmerids, and 1 salmonid, whereas *P. ladogensis* infects only 2 fish hosts of different families (Osmeridae and Lotidae). Recent advances in molecular characterization of microsporidians have revealed that *G. hertwigi* is closely related to *G. plecoglossi*, *G. anomala*, *G. atherinae*, and *G. stephani* — all species characterized by the production of xenomas (Lovy et al. 2009). The close relationship of *G. hertwigi* and *G. anomala* could support the claim by Weissenberg (1968) concerning the successful transmission of *G. hertwigi* to a non-osmerid fish, *Gasterosteus aculeatus*, which is the type host of *G. anomala* (Weissenberg 1968, Lom 2002).

It would be worthwhile to re-examine smelt for infections with *P. ladogensis* in order to obtain material for molecular analysis, to assess the phylogenetic relationships within the genus *Pleistophora*. Although in general, *Pleistophora* does not induce xenoma formation, but instead foci of infection (see Lom & Dyková 1992, Lom 2002), at least 1 species has produced xenomas, namely *P. senegalensis* infecting the digestive tract of the gilthead sea bream *Sparus aurata* (Faye et al. 1990). It thus appears that 2 features of the genus need to be revised: the production of 2 types of spores (macro- and microspores), which does not occur in all described *Pleistophora* species, and the expression of the infection as diffuse infection foci with no xenoma formation. Nevertheless, before revising the taxonomical criteria, it is necessary to complement the known microscopically based descriptions with molecular characterization of the parasites.

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