Microsporidian xenomas in fish seen in wider perspective

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Abstract. The history of understanding xenoparasitic complexes or xenomas provoked in the host cell by various protists and especially by microsporidia is outlined. Microsporidia have been known to produce xenomas in oligochaetes (e.g., genera *Bacillidium*, *Burkea*, *Hrabyeia*, *Jirovecia*, species of the collective group *Microsporidium*), crustaceans (e.g., *Abelspora*, *Mrazekia*), insects (e.g., *Polydispyrenia*, *Thelohania*) and poikilothermic vertebrates, mostly fish (*Alloglugea*, *Amazonspora*, *Glugea*, *Ichthyosporidium*, *Loma*, *Microfilum*, *Microgemma*, *Neonosemoides*, *Pseudoloma*, *Spraguea*, *Tetramicra*). An overview of characters of xenomas caused by species of these genera is presented. The study of microsporidia causing xenomas in fish offers an insight into cell pathology and is of interest since many of these species are important agents of diseases in commercial fish. Xenomas produced from a few types of target cell display a complete change of organisation of the host cell and differ, according to the agent, in their structure. Recent data show that proliferation of the parasite may have already started in the cells transporting the parasites to the final site of xenoma formation. However, these are preliminary revelations and most of the facets of the life cycle are still to be clarified. Curiously, xenoma-forming microsporidia do not seem to be strictly host specific. The salient features of fish microsporidian xenomas are discussed, such as role of the xenoma, whether its features are host- or microsporidium-dependent, development and demise of the xenoma in the course of time, and host reaction phenomena. The need of further research is emphasised.

HISTORICAL INTRODUCTION

One of the most interesting features of microsporidian biology is the capacity to stimulate hypertrophic growth of the invaded cell of the host animal. A symbiotic co-existence develops between the host cell and its microsporidian parasites and both partners turn into a well-organized xenoparasitic complex. It was Moniez (1887) describing what we know now as *Glugea anomala* (Moniez, 1887) Gurley, 1983 who clarified the parasitic nature of the *Glugea* "tumours". Twelve years later, Mrázek (1899) was the first to recognise that infection with what we now term *Spraguea lophii* (Doflein, 1898) Vávra et Sprague, 1976 turns the ganglion cell of *Lophius piscatorius* into a huge, cyst-like structure.

Xenoparasitic complex (XC) is actually the term ("complexe xénoparasitaire") used by Chatton (1920), who coined it for the unit involving the parasitic dinoflagellate *Sphaeripara catenata* and the oikoplast (a huge gland cell) of the appendicularian, *Fritillaria pellucida*. The host cell undergoes hypertrophy and has many, mostly polyploid, nuclei. The dinoflagellate develops within the cell, forms a thick-walled, disc-shaped hyposome from which long branched rhizoids extend into the host cytoplasm, serving for nutrient absorption. In a later paper, Chatton and Courrier (1923) described a microsporidium now termed *Microsporidium cotti*

(Chatton et Courrier, 1923) Canning et Lom, 1986, forming XC in the testes of *Taurulus bubalis*. The hypertrophic host cell residing in a fluid-filled cavity was equipped with a dense microvillous cover.

In Chatton's definition, the XC 1) displays hypertrophy of the host cell provoked by the action of the parasite in the cell, 2) preserves the host cell nucleus and 3) has a cover of absorptive microvilli, which may be missing in some cases.

In 1922, Weissenberg coined the term "xenon" for the XC due to *Glugea anomala* infecting sticklebacks but later, realising that this term was preoccupied for a chemical element, he changed it to "xenom" or "xenoma" (Weissenberg 1949) and still later redefined the phenomenon (Weissenberg 1968). The term xenoma is now currently used for microsporidian XCs. The xenoma is presently understood as the host cell with a completely changed structure and the parasites proliferating inside it, both components being morphologically and physiologically integrated to form a separate entity with its own development in the host at the expense of which it grows.

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In fact, hypertrophic growth of host cells and their nuclei due to protistan infection has been observed since about the beginning of the twentieth century. Siedlecki (1901, 1911) observed cell hypertrophy in enterocytes of the tunicate *Ciona intestinalis*, where it is due to trophonts of the gregarine *Lankesteria ascidiae.* The hypertrophic cell becomes a mere envelope around the parasite and eventually dies. Hesse (1909) described trophonts of the gregarine *Nematocystis magna* inducing hypertrophy in seminal cells of earthworms in which it lives; the hypertrophic cell extends as outgrowths into neighbouring cells.

Siedlecki (1902) described meronts of the coccidian *Caryotropha mesnili* eliciting hypertrophy in spermatogonia of the polychaete *Polymnia nebulosa*; the affected cell undergoes hypertrophy together with the uninfected neighbours, forming what could be called a syncytial xenoma. Merozoites of several species of the coccidian genus *Eimeria*, formerly assigned to a separate genus *Globidium*, induce enormous hypertrophy of infected cells. Thus *Eimeria gilruthi* produces a xenoma up to 6 mm in size, with a central nucleus and with a microvillous cover for better nutrient absorption (Chatton 1910). Similarly, *E. navillei* induces a syncytial xenoma in subepithelial connective tissue cells of the intestine of *Natrix viperinus* (Guyénot et al. 1922). Merozoites of *Aggregata octopiana* stimulate hypertrophy of connective tissue cells of intestinal submucosa of octopuses (Wurmbach 1935). A similar species, *A. eberthi*, however, does nothing similar in its cuttlefish host.

In coccidians of the genus *Sarcocystis*, the invasion of merozoite released from the liver produces in the muscle cell a special type of xenoma, in which the parasite develops inside a peculiar cyst delimiting it from the sarcoplasm proper (e.g., *S. cruzi, S. hirsuta, S. arieticanis, S. tenella* – see Mehlhorn et al. 1976, Eckert et al. 1992).

A quite different protist, *Coelomycidium simulii* (Phycomycetes, Chytridiales) developing in adipose cells of simuliid larvae, also produces cell hypertrophy reminiscent of xenoma formation (Weiser 1966). The infected cell and its nucleus increase in volume, then the cell loses its contact with neighbouring cells and is disengaged from the fat body into the haemolymph.

Rather recently, several myxozoans, presently considered to be metazoans, have been found to induce xenoma-like formation in vertebrates, e.g., *Myxidium lieberkuehni* in renal corpuscles of pike, *Esox lucius* (Lom et al. 1989), *Thelohanellus pyriformis* in gill endothelial cells of tench, *Tinca tinca* (Dyková and Lom 1987), *Ortholinea* sp. in the kidney of *Scatophagus argus* (unpublished) and a myxosporean-like parasite in the brain of moles, *Talpa europaea* (Friedrich et al. 2000). However, in spite of similarity of all these XCs to microsporidian xenomas, there is one essential difference. These XCs harbour cells of just one part of the life cycle of the parasite and the rest takes place elsewhere. In microsporidian xenomas the whole cycle, merogony and sporogony, is confined to the xenoma, apart from the stages developing *en route* from the portal of entry to the final site of xenoma implantation.

CHANGES ELICITED BY MICROSPORIDIA IN SOME INVERTEBRATE HOST CELLS

In simple cases of microsporidian infection, the parasite proliferates within the infected cell and the mass of its stages replaces the host cell cytoplasm and distends the cell to various degrees (as e.g., in *Nosema apis*). Simple hypertrophy of infected insect cells can be exemplified by *Microsporidium chaetogastris* (Schröder, 1909) Sprague, 1977. This species infects connective and muscle tissue cells of *Chaetogaster diaphanus*, turning them into hypertrophic multinucleate cells (up to 100 µm in size) full of parasites in various stages of development (Schröder 1909). *Thelohania tipulae* Weissenberg, 1926 causes hypertrophy of infected adipose cells and their nuclei so that eventually, only the nucleus and cell membrane of the infected cell replete with mature spores are left (Weissenberg 1926). Lange and Sokolova (2005) reported formation of xenomas —which they do not specify— from single adipose cells of *Locusta migratoria* by the microsporidian *Johenrea locustae* Lange, Becnel et Razafindratiana, 1996.

Special cases are so-called syncytial xenomas caused by microsporidia of the genera *Polydispyrenia* Canning et Hazard, 1982 and *Stempellia* Léger et Hesse, 1910 in adipocytes of the fat body of simuliid larvae. These cells undergo hypertrophy, usually including nuclear hypertrophy, fragmentation of nucleoli and appearance of polytenic chromosomes. The whole fat body assumes a syncytial nature and is encased with a PAS-positive basal membrane. Sometimes (in *Stempellia*) this membrane has a lamellar structure reminiscent of the wall of a *Glugea* xenoma. It covers syncytial tissue, which arose from dedifferentiated fat body with microsporidian developing stages. The stages are stratified and mature spores concentrate in the middle of the xenoma. At the end of this development, there is a mass of spores in a common cavity enveloped by a basal membrane (Maurand and Manier 1967, Maurand 1973).

Microsporidian xenomas comparable with those of fish occur also in several crustaceans. In *Asellus aquaticus*, the species *Mrazekia argoisi* Léger et Hesse, 1916 induces xenomas with a hypertrophic nucleus from fat cells around the stomach (Debaisieux 1931). *Microsporidium cyclopis* (Vávra, 1962) Sprague, 1977 has no such effect in its copepod host (Vávra 1962). *Abelspora portucalensis* Azevedo, 1987 infects *Carcinus maenas*. What was described as a xenoma (Azevedo 1987) is in fact an assemblage of hypertrophic cells each with a large parasitophorous vacuole where the parasites proliferate. In the parasitic copepod *Lepeophtheirus*
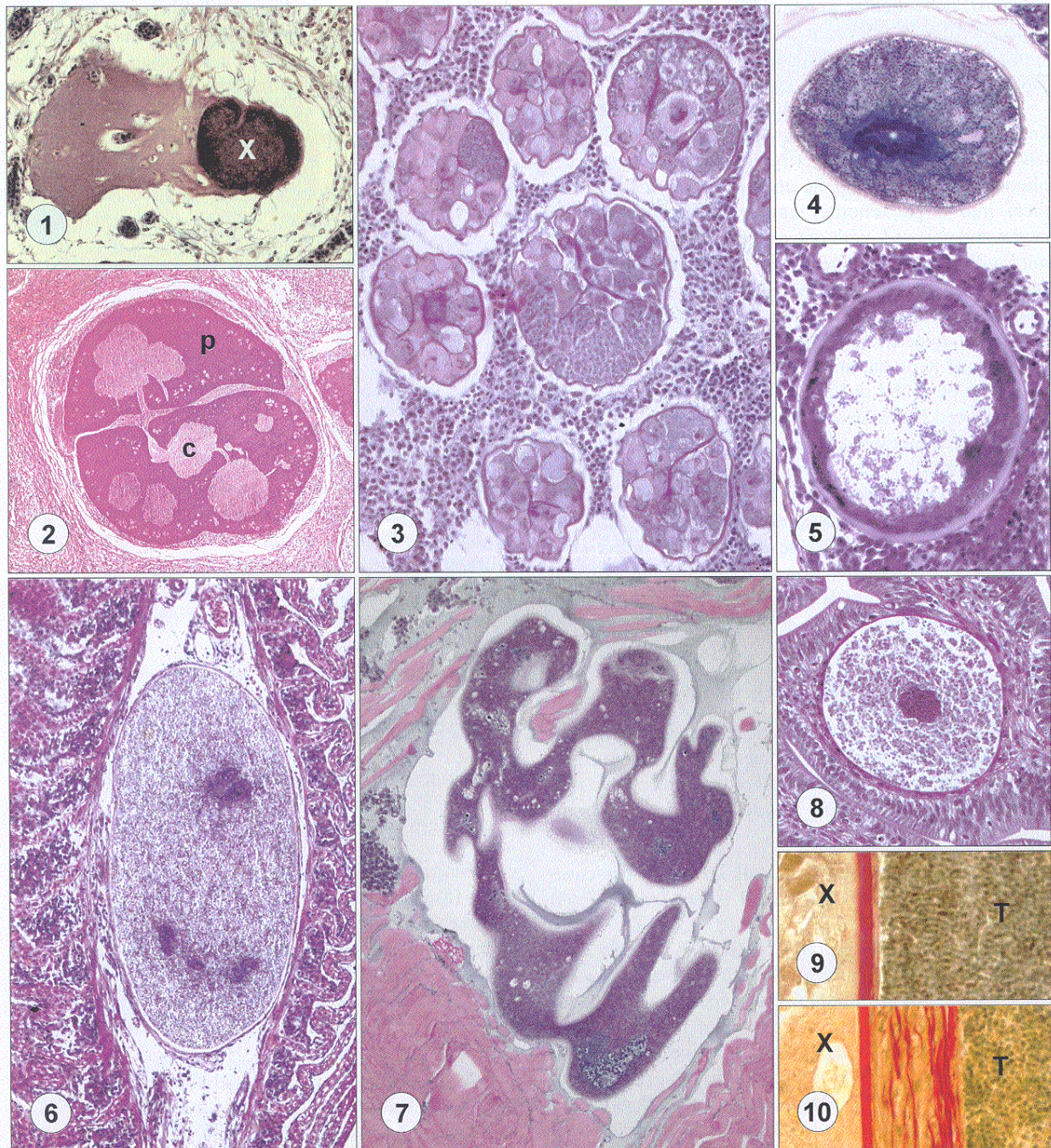
Figs. 1–10. Different types of xenomas of fish microsporidia. **Fig. 1.** Early stage of *Spraguea lophii* xenoma; the parasite mass (X) occupies only part of the ganglion cell of *Lophius piscatorius*. Bodian, × 620. **Fig. 2.** Advanced stage of *S. lophii* xenoma in the ganglion of *L. piscatorius*. Note the different staining of parasite mass at the periphery (p) with *Nosemoides*-type spores and in the centre (c) with *Nosema*-type spores. H&E, × 70. **Fig. 3.** "Cystic" stages preceding formation of huge xenomas of *Ichthyosporidium giganteum*. Compartments contain different stages of merogonial proliferation. H&E, × 225. **Fig. 4.** Xenoma of *Tetramicra brevifilum*, in a liquid-filled cavity in liver parenchyma of *Scophthalmus maximus*. H&E, × 200. **Fig. 5.** Mature xenoma of *Glugea anomala* in the body cavity of *Nothobranchius* sp. H&E, × 225. **Fig. 6.** Xenoma of *Loma branchialis* in the gills of *Melanogrammus aeglefinus*. H&E, × 130. **Fig. 7.** Xenoma of *Tetramicra brevifilum* in folded-over shape in the muscle tissue of *Scophthalmus maximus*. H&E, × 160. **Fig. 8.** *Loma acerinae* xenoma with a centrally located host cell nucleus in the subepithelial connective tissue of the intestine of *Gymnocephalus cernuus*. H&E, × 260. **Figs. 9, 10.** Parts of the wall of similar, mature *Glugea plecoglossi* xenomas (X), localised in testes (T) of *Plecoglossus altivelis*. Xenoma wall and mature encircling connective tissue (present in Fig. 10) are stained red. Van Gieson, × 1,500.

salmonis, subcuticular xenoma-like cysts are due to a microsporidian similar to members of the genus *Nucleospora* Hedrick, Groff et Baxa, 1991 (see Freeman et al. 2004).

Microsporidian xenomas in oligochaetes have been known since Mrázek (1898). Species of the genus *Jirovecia* Weiser, 1977 infect lymphocytes of freshwater oligochaetes (chloragogene cells in one case) and turn them into large xenomas having numerous host cell nuclei and covered, with one exception, with densely set microvilli. Similarly, species of the genus *Bacillidium* Janda, 1928 turn infected lymphocytes (in one case the cells of pharyngeal glands) into large xenomas with one or several hypertrophic nuclei. A review on the fine structure of xenomas in oligochaetes and further references can be found in Larsson (1986). Similar xenomas are produced by *Hrabyeia xerkophora* Lom et Dyková, 1990 in coelomocytes of *Nais christinae* (see Lom and Dyková 1990), while *Burkea gatesi* de Puytorac et Tourret, 1963 was reported to develop xenomas in muscle cells of *Pheretima hawayana* (de Puytorac and Tourret 1963). There are also several microsporidia with *Nosema*-like spores, infecting oligochaetes and assigned to the collective group *Microsporidium* Balbiani, 1884. Some of them induce host cell hypertrophy, some not (Oumouna et al. 2000).

MICROSPORIDIAN XENOMAS IN FISH

According to the structure of xenomas, genera that comprise xenoma-forming species can be grouped in several categories. (References following the text pertaining to each genus give sources of xenoma description.)

a) Xenomas without a thick wall, in which the complete volume of the original cell is not transformed into xenoma

Spraguea Vávra et Sprague, 1976: the infected zone of a ganglion cell is grossly hypertrophic and covered by a simple plasmalemma. The hypertrophic nucleus (HN) resides in the uninfected part of the cell. In the infected part of the cell, the stages at the periphery of the parasite mass differ (*Nosemoides* type of spores) from those in the centre (*Nosema* type) (Figs. 1, 2). Type and only species *S. lophii* in *Lophius piscatorius*. (Mrázek 1899, Loubès et al. 1979, Takvorian and Cali 1986).

b) Xenomas without a thick wall, with the complete volume of the original cell transformed into xenoma

Ichthyosporidium Caullery et Mesnil, 1905: in the course of the still insufficiently known life cycle of the type species there are two types of xenomas: 1) "cystic" ones, each representing a hypertrophic fibroblast coalescing to form a rounded "syncytial" xenoma (up to only 20 μm in size) harbouring immature developmental stages and only rarely producing spores (Fig. 3), and 2) large lobose xenomas (up to 4 mm) with intermingled developmental stages and spores, having an ectoplasmic layer covered with a simple plasmalemma and raised into villous projections. It is not known whether the "cystic xenomas" develop into the large xenomas. Type species: *I. giganteum* in *Crenilabrus melops*. (Sprague and Vernick 1974, Sprague and Hussey 1980).

Microfilum Faye, Toguebaye et Bouix, 1991: xenomas with a microvillous surface, multiple HNs distributed throughout xenoma, and developmental stages intermingled. Type and only species: *M. lutjani* in *Lutjanus fulgens* (Faye et al. 1991).

Microgemma Ralphs et Matthews, 1986: xenomas with plasmalemma raised into surface villosities, reticulate HN lies between a peripheral band of mitochondria and the cell centre occupied by intermingled stages of the parasite. Type species: *M. hepaticus* in *Chelon labrosus*. (Ralphs and Matthews 1986, Amigó et al. 1996, Leiro et al. 1999, Lores et al. 2003).

Microsporidium cotti (Chatton et Courrier, 1923) Canning et Lom, 1986: xenoma invested with a brush border; HN forms a peripheral net and the centre is filled with intermingled stages. Chatton and Courrier (1923) found it floating in a fluid-filled cavity in the testis of *Taurulus bubalis*. Warrants further study, may belong to the genus *Microgemma*.

Tetramicra Matthews et Matthews, 1980: xenoma has microvillous, membrane-bounded projections, by which several xenomas may interlock to form a composite "cyst"; a single reticulate HN, developmental stages intermingled. Type and only species: *T. brevifilum* in *Scophthalmus maximus* (Matthews and Matthews 1980) (Figs. 4, 7, 11, 12).

c) Xenomas with plasmalemma covered by host collagen fibrils

Amazonspora Azevedo et Matos, 2003: plasmalemma raised into anastomosing microvilli is covered with up to 22 layers of collagen fibrils; HN is deeply branched, surrounded by intermingled parasite stages. Type and only species: *A. hassar* in *Hassar orestis* (Azevedo and Matos 2003).

Neonosemoides Faye, Toguebaye et Bouix, 1996: xenoma covered with a simple evenly spread plasmalemma covered with a thin glycocalyx, overlaid by host collagen fibrils. Peripheral part is intensively vacuolised, interior containing intermingled stages of the parasite. Type and only species: *N. tilapiae* in *Tilapia zillii* (Sakiti and Bouix 1987, Faye et al. 1996).

Nosemoides syacii Faye, Toguebaye et Bouix, 1992: xenoma wall with cell plasma membrane covered with collagen fibres, HN broken into several parts; developmental stages of the parasite are intermingled. In *Syacium micrurum* (Faye et al. 1994). The generic assignment is most probably wrong, as well as in *N. zeusi* Faye, 1992 and *N. brachydeuteri* Faye, 1992 (Faye 1992).
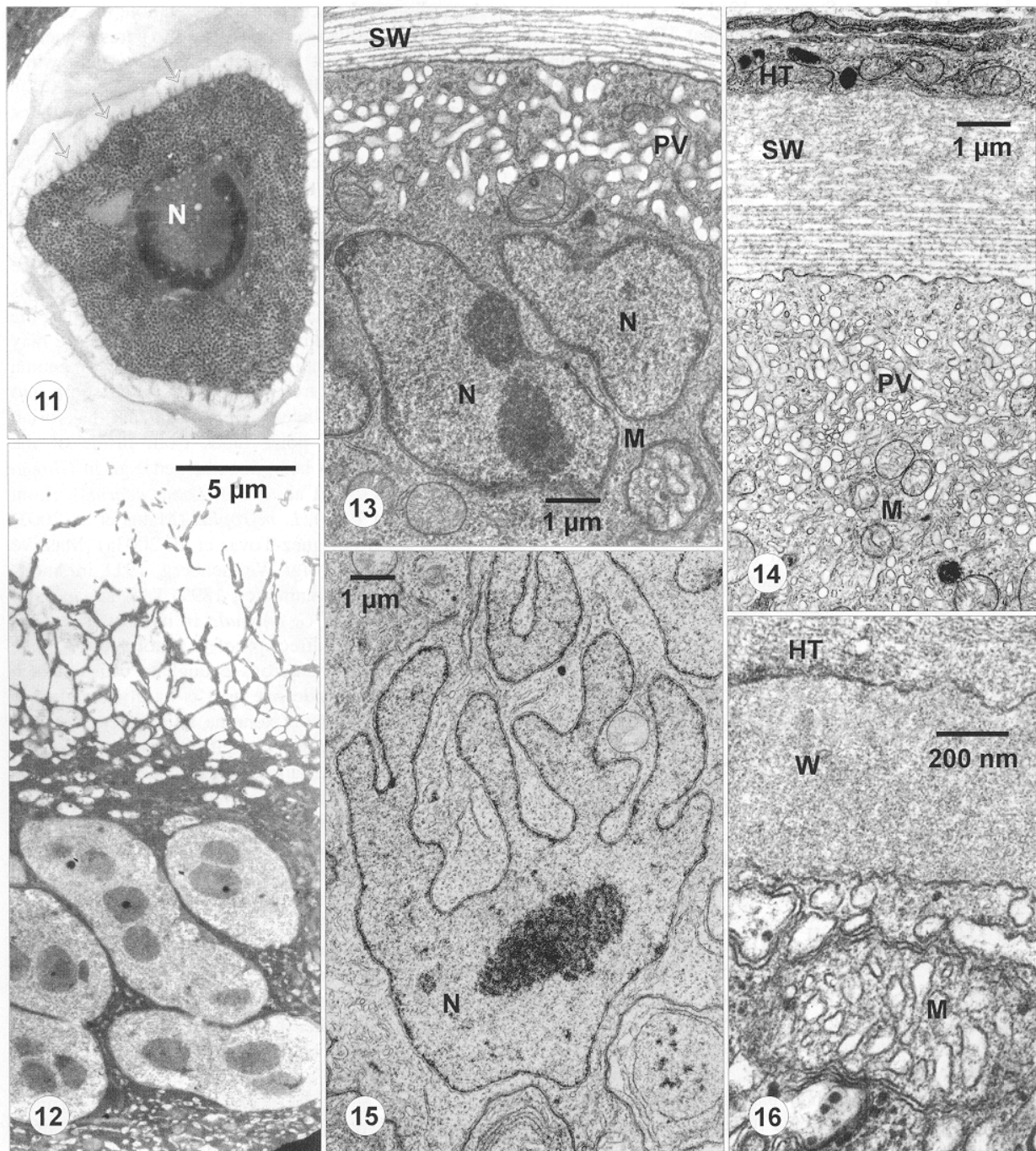
Figs. 11–16. Features of fish xenomas. **Fig. 11.** Surface villosities (arrows) and centrally located hypertrophic nucleus (N) of *Tetramicra brevifilum* xenoma. Toluidine blue-stained semithin section, × 220. **Fig. 12.** Meshwork of surface microvilli of *T. brevifilum* xenoma. **Figs. 13, 14.** Periphery of an early (Fig. 13) and advanced (Fig. 14) xenoma of *Glugea anomala*. SW – stratified xenoma wall; PV – pinocytotic vesicles; N – nucleus of the host cell; M – mitochondrion; HT – host tissue. **Fig. 15.** Branched segment of the hypertrophic nucleus (N) of *G. anomala* xenoma. **Fig. 16.** Thick wall (W) of *Loma acerinae* xenoma. M – mitochondrion; HT – host tissue. Figs. 12–16. Transmission electron micrographs (TEM).

d) Xenomas with a thick wall

Glugea Thélohan, 1891: laminar layers of sloughed-off cell coat form the wall outside the plasma membrane, the central HN is highly branched, and developmental stages are stratified. Type species: *G. anomala* in *Gasterosteus aculeatus*. (Weidner 1976, Canning et al. 1982, Takvorian and Cali 1983, Morrison et al. 1985) (Figs. 5, 9, 10, 13–15).

Loma Morrison et Sprague, 1981: the wall consists of a thick, granular amorphous cell coat, HN is centrally located, and various developmental stages are intermingled throughout the xenoma. Type species *L. branchialis* in *Melanogrammus aeglefinus*. (Morrison and Sprague 1981a, b, 1983, Bekhti 1984, Lom and Pekkarinen 1999) (Figs. 6, 8, 16).

Loma myrophis Azevedo et Matos, 2001: unlike in the type species and perhaps jeopardising the generic assignment, the wall of the xenoma was reported to consist of a layer of fibrous material surrounded by fibroblasts. In *Myrophis platyrhynchus* (Azevedo and Matos 2002).

Pseudoloma Matthews, Brown, Larison, Bishop-Stewart et Kent, 2001: detailed data on the xenoma are not available.

MICROSPORIDIAN XENOMAS IN VERTEBRATES OTHER THAN FISH

Alloglugea Paperna et Lainson, 1995: xenomas with a simple folded plasmalemma coated with a layer of host fibroblasts and a central (sometimes fragmented) HN surrounded by stages of the parasite. Type and only species: *A. bufonis* in tadpoles of *Bufo marinus* (Paperna and Lainson 1995).

In cultured green monkey kidney (E6) cells infected by the human pathogen *Vittaforma corneae* (Shadduck, Meccoli, Davis et Font, 1990), a strange type of response of the host cell was described (Leitch et al. 2005), remotely reminding of xenoma organisation. Inhibition of cytokinesis resulted in a cell complex of up to 200 μm in size, with a central focus of infection of parasite stages and a single central large microtubule-organizing centre and peripherally located multiple host cell nuclei.

CHARACTERS OF FISH XENOMAS

Infection of the host cell involves its complete restructuring. The structure of grown xenomas in fish, compared with the original host cell, in many of them supposedly a leucocyte, is highly varied. The xenomas reveal various surface structures, e.g., microvilli with pinocytotic vesicles at their base and a thick layer of ectoplasm. Inside the xenoma there may be bundles of microfibrils, sometimes annulate membranes, various vesicles or fat globules, modified endoplasmic reticulum, which envelops the developing stages of the parasite, and various tubular structures. The nucleus, always hypertrophic, may be centrally located, branched or lobed, or amitotically divided into a number of fragments sometimes forming a peripheral network. The parasite's capacity to produce xenomas of different structure from a supposedly identical or similar type of host cells seems itself to testify that xenoma structure reflects the nature of the microsporidian and not that of the host.

According to the accepted interpretation, the xenoma offers optimal growth conditions for the parasite including protection against the host immune system, while confining it to one cell and preventing its free spread in the host organism. This is not quite accurate, since spores may discharge their sporoplasms through the xenoma wall and infect the cells that surround it. The newly infected cells may then distribute the infection further in the organism and perpetuate it. Sometimes, "secondary xenomas" may form inside the "primary" one (Fig. 19). It has not been resolved yet whether the secondary xenomas originate in connective tissue cells or macrophages that have broken through the wall of the old xenoma. The stimulus for polar tube discharge may be increased hydrostatic pressure inside the xenoma and/or catabolism of trehalose stored in the spore into smaller molecules (Undeen 1990, Cali and Takvorian 1999). Discharge of polar tubes from inside of the xenoma (Fig. 21) has been documented e.g., in *Glugea capverdensis* (Lom et al. 1980), *Loma acerinae* (Lom and Pekkarinen 1999), *L. myrophis* (Matos et al. 2003) and *Loma* sp. (Rodríguez-Tovar et al. 2003a). Massive infections of *G. hertwigi* Weissenberg, 1911 in smelts and *G. stephani* (Hagenmüller, 1899) Woodcock, 1904 in flatfish or even of *G. anomala* in its hosts (Fig. 32) can be used as an indirect proof of autoinfection since ingestion of spores numerous enough to cause such a mass of xenomas inside one host is hardly imaginable and ingestion of a whole xenoma is unlikely. Xenoma only protects the parasite when it is young or growing. As soon as the wall of a grown xenoma has lost its integrity, it is pervaded by granulation tissue and the spores are digested by macrophages (Fig. 20) (Dyková and Lom 1980, Leiro et al. 1999). The spores may also be set free by rupture of xenomas located on the body surface or by decay of the perished host.

There is a long-standing question, whether the xenoma formation and its nature depend on the innate qualities of the parasite or of the host. Thus far no xenoma-forming microsporidian is available in culture to show *in vitro* whether the microsporidian could transform into a xenoma when the cell is relieved from the influence of the host organism. This would decide the question. Lores et al. (2003) cultured a xenoma-forming microsporidian of uncertain identity (*Glugea*?) in a mosquito cell line. They observed hypertrophy of nucleus and cytoplasm but no true xenoma formation. Insect cells might not be the proper environments for a fish xenoma to develop. Even if using well-established fish cell culture, the parasite might not find proper conditions for developing its special capacity for xenoma formation. Pending further experiments, this question can only be approached resorting to comparisons. For examples, there are microsporidia infecting tubificids, which do not elicit xenoma formation unlike species of the genus *Jirovecia* or *Bacillidium*, e.g., *Microsporidium epithelialis* (Oumouna et al. 2000). In addition,
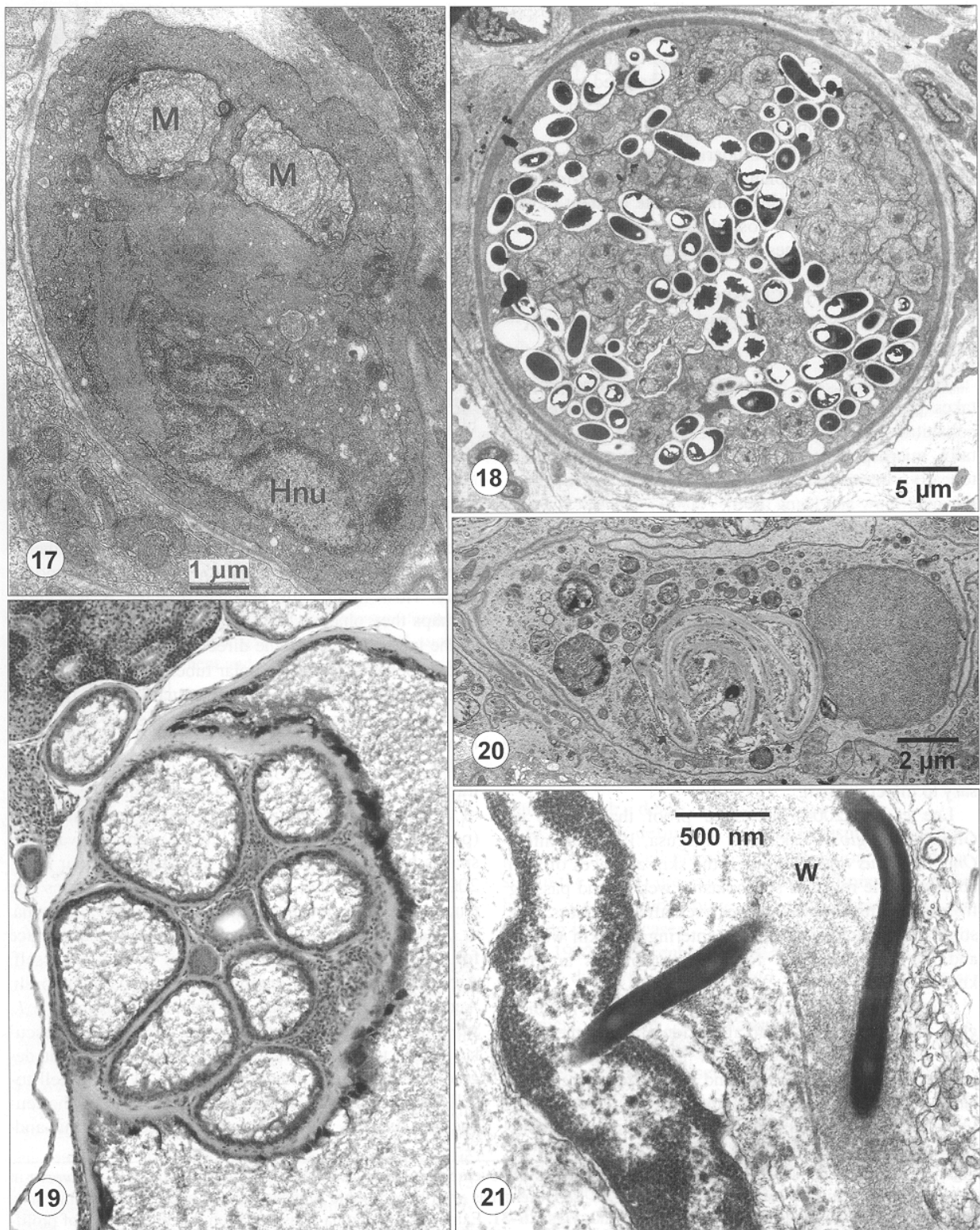
Figs. 17, 18. Growth stages of *Loma acerinae* xenomas. TEM. **Fig. 17.** Early stage of development in a slightly transformed neutrophile, day 6 post infection. M – merozoite; Hnu – host cell nucleus. **Fig. 18.** A grown xenoma with a thick wall and inter-mingled developmental stages. Host cell nucleus is beyond the level of the section. **Fig. 19.** A group of secondary *Glugea anomala* xenomas developing within the old one. H&E, × 280. **Fig. 20.** Chitinous spore shells, the last remnants to be digested from phagocytosed microsporidian spores. TEM. **Fig. 21.** Discharged polar tubes of *Loma acerinae* piercing the xenoma wall (W) and (at left) the nucleus of an adjacent fibroblast. TEM.

there are several xenoma ("cysts")-forming sarcosporidia infecting the same hosts (e.g., sheep, cattle) and yet the structure of their cysts is entirely different from each other. Some *Eimeria* (formerly assigned to a separate genus "*Globidium*") elicit xenomas in the same host, e.g., sheep, while the others do not.

A remote but helpful comparison can be drawn from the action of aphids (plant lice) or other gall-forming insects. They are thought to manipulate a latent developmental programme of host plants to produce parasite-specific xenoparasitomes or galls (Stern 1995).

ROUTE OF FISH MICROSPORIDIA FROM THE PORTAL OF ENTRY TO THE SITE OF XENOMA FORMATION

Transmission of xenoma-forming microsporidia takes place generally *per os*, which is facilitated by cohabitation of fish with the diseased ones. Experimentally, microsporidia can easily be transmitted intraperitoneally, intramuscularly, intravascularly or by anal gavage (Shaw and Kent 1999).

Glugea spp. are easily transmitted via crustaceans acting as transport hosts. Olson (1976, 1981) found that spores of *Glugea stephani* elicited heavier infections after passage through crustacean digestive tract than when produced by intraperitoneal injection. He even suggested that amphipods might represent a natural route of transmission for *G. stephani*. Figueras et al. (1992) failed to infect turbots with *Tetramicra brevifilum* intraperitoneally or by exposure to waterborne spores and concluded that eating aquatic crustaceans —copepods, mysids and decapod larvae— was necessary to infect the fish.

Lee et al. (2004) presented proof that spores of *Glugea plecoglossi* Takahashi et Egusa, 1977 can infect *Oncorhynchus mykiss* through the skin at places of skin abrasion. The released sporoplasms were found passing from epidermis to muscle layer even after six hours. The stimuli for hatching of spores entering the skin wound and the transport cells for the sporoplasms are not known.

Pleshinger and Weidner (1985) proved that in *Spraguea lophii* a shift to the alkaline side of pH in the presence of polyanions (mucines or polyglutamates) may induce polar tube discharge and hence the spores may hatch in the mucous coat of the intestinal epithelium. Lee et al. (2003) presumed that, after ingestion, mucous cells are the initial sites of entry of *G. plecoglossi* and that pepsin and trypsin may activate hatching in the gastrointestinal tract. Interaction mediated by lectins may be the stimulating factor for this species.

It has been generally assumed that macrophages (Weissenberg 1968) or neutrophils (Bekhti and Bouix 1985, Canning and Lom 1986, Pekkarinen and Lom 1999) are the first sites of infection for *Glugea* spp. after inoculation of sporoplasms released in the intestine. Their further fate has not been explicitly described. However, merogonial proliferation may presumably start in the cells that were initially infected. Sánchez et al. (2001), using *in situ* hybridisation technique, have found that *Loma salmonae* migrates from mucosal epithelium to the lamina propria of the intestine before reaching the final destination in the gills. The dividing merogony stages were then detected within infected blood cells in the heart as early as day 2 post exposure (p.e.), thus proving unequivocally the haematogenous spread by infected blood cells. Transportation and dissemination via blood cells has also been documented for *Tetramicra brevifilum* (Matthews and Matthews 1980). The transport cells were suggested to be intraepithelial lymphocytes, T cells or migratory cells such as monocytes. How these cells become infected is not clear. Perhaps they phagocytize the parasite in lamina propria of the intestine, or become directly infected by injection of the sporoplasm via the polar tube. To what extent the infected transport cell may eventually turn into the xenoma in different microsporidian species is still not known.

In *Loma salmonae*, merogony is initiated in the transport cells prior to xenoma formation. The journey of the already dividing merozoites of *L. salmonae* ends (perhaps attracted by high O_2 levels) in the gill vascular spaces between the pillar cells (Rodríguez-Tovar et al. 2003b). Then, either the pillar cell phagocytizes the parasite from the leucocyte and converts into a xenoma, or the extensions of the pillar cells retract to make space for the leucocyte, which turns into a xenoma itself. Around it, a new basement membrane is then built. Another possibility is that the leucocyte hosting the *L. salmonae* merozoites transmigrates through extravascular spaces using enzymes (metalloproteinases) that degrade the basement membrane and/or by using cell-to-cell interaction with endothelial cells. Some of the leucocytes succeed in reaching the connective tissue and

Figs. 22–25. Xenomas of *Glugea anomala* in early stages of development. **Fig. 22.** A spontaneous infection of *G. anomala* in *Austrolebias nigripinnis*. H&E, × 70. **Figs. 23–25.** Early xenomas with hypertrophic branched nuclei and cylindrical meronts, which predominate in Figs. 24 and 25. H&E, × 450. **Figs. 26–31.** Examples of xenoma transformation due to the onset of proliferative inflammation of the host. **Fig. 26.** *Glugea plecoglossi* infection in ovaries of *Plecoglossus altivelis*. H&E, × 60. **Fig. 27.** Proliferation of granulation tissue in *Loma acerinae* visualised by Masson's trichrome staining, × 120. **Fig. 28.** Xenoma of *Tetramicra brevifilum* transformed into granuloma in the liver of *Scophthalmus maximus*. H&E, × 150. **Fig. 29.** Granulomatous lesion at the site of *Glugea anomala* xenoma in the glandular part of the stomach wall in *Gasterosteus aculeatus*. H&E, × 220. **Fig. 30.** Granuloma in the ovary of *Nothobranchius rubripinnis* replacing *G. anomala* xenoma. H&E, × 250. **Fig. 31.** *Spraguea lophii* xenoma partly transformed into a granuloma. H&E, × 220. **Fig. 32.** Overview of a massive spontaneous infection of *G. anomala* as seen in the intestine of *Gasterosteus aculeatus*. H&E, × 70.
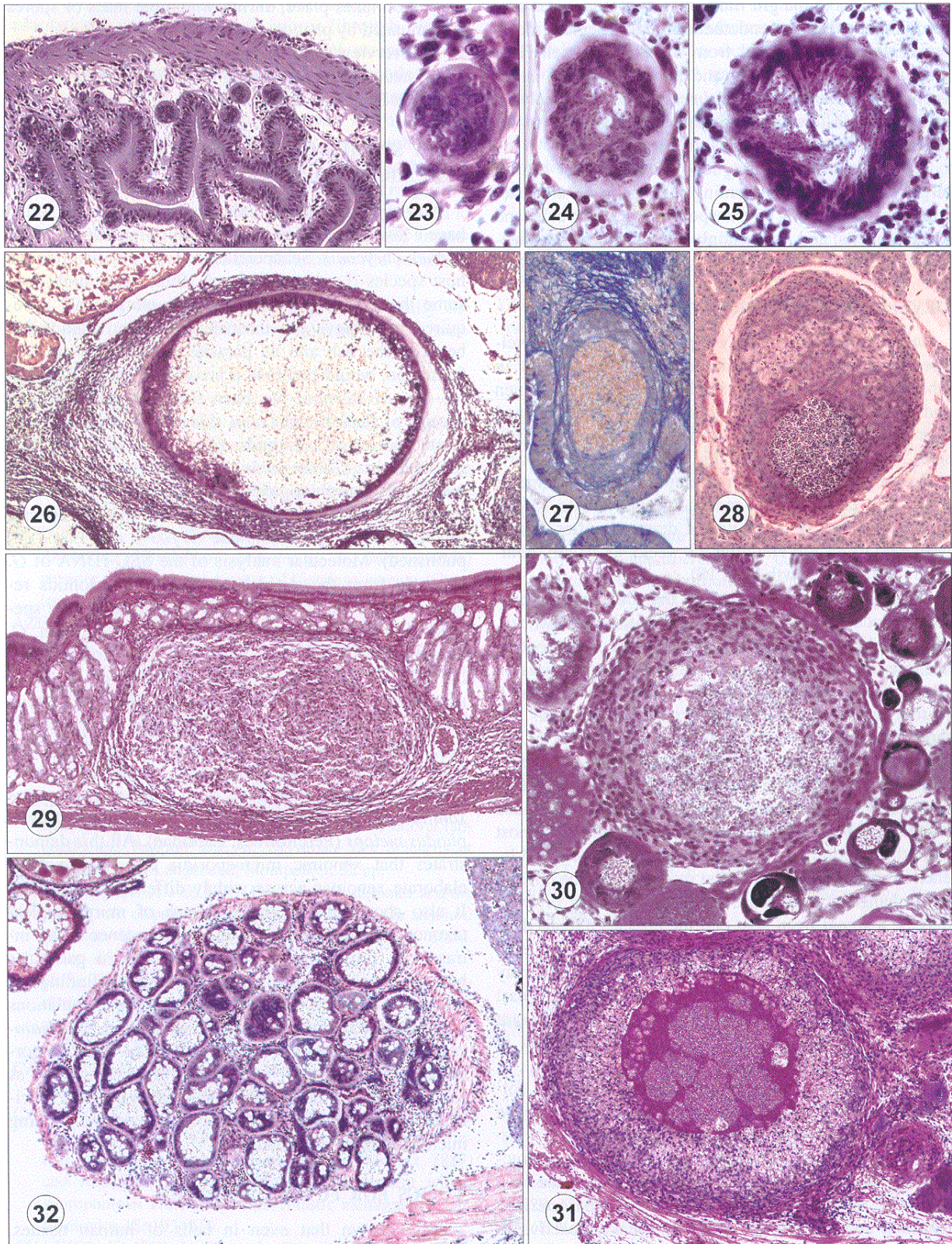
form xenomas in the gill filament. Other leucocytes stay confined between the endothelium and basement membrane after having exited from the blood vessel. How much of tissue specificity and parasite tropism is involved in the case of *L. salmonae* and especially in other xenoma-forming microsporidia has still to be investigated (Rodríguez-Tovar 2003b).

As evident from existing reports, the thus far proven target cells of xenoma-forming microsporidia are macrophages (also acting as transport cells), pillar cells and ganglion cells, and we certainly cannot exclude connective tissue cells.

DEVELOPMENT OF XENOMAS IN FISH

In *Loma acerinae*, at day 6 p.e. only meronts enveloped by rough endoplasmic reticulum are present in what was originally the neutrophil (Fig. 17) (Pekkarinen and Lom 1999). Three weeks p.e., merogony and sporogony have progressed and mature spores are present. Xenoma wall, still of thin consistency, only starts to be formed while the cytoskeleton of microfilaments in the host cells is being reduced. By days 6 to 13, the xenoma reaches up to 8 μm in diameter, after 3 to 4 weeks up to 14 μm and after 11 weeks to 20 μm, demonstrating slow growth (Fig. 18).

In *Loma salmonae*, during the third week p.e., meronts occupied the marginal area within the host cell. This localisation is associated with host mitochondria because of the need of active parasite cell division (Rodríguez-Tovar et al. 2003b) but by weeks 5 and 6 mature spores have already occupied that area. Although on week 5 and 6 p.e. the plasmalemma of the xenoma did not seem injured, the proximity of inflammatory cells indicated that an inflammatory signal of some kind was generated but not so strong as to induce leucocyte attack. Some signals may be emitted almost from the beginning of xenoma formation, as testified by encircling fibroblasts. The host response may be elicited by a change of antigens on the plasmalemma or there may be a signal from host cell membranes damaged by toxic metabolites from the parasite. Nevertheless, even xenomas with integral, undamaged cell membrane may become covered by fibroblasts from the local fibroblast population rearranged due to pressure atrophy. Relevant data on immunogenicity of xenomas can be found in Shaw and Kent (1999).

The progress of xenoma growth can easily be followed in heavy spontaneous infections of *Glugea anomala* in cyprinodontid hosts (Figs. 22–25).

None of the xenomas, however, escape final destruction by the host (Figs. 26–31). The stages of the host response towards xenoma have been characterized (Dyková and Lom 1978, 1980) as weakly reactive in young and developing xenomas, and productive in fully developed xenomas when proliferative inflammation transforms xenomas in granulomas. Finally, granuloma involution takes place, during which the mass of spores is eliminated by phagocytosis.

An overview of some papers on immune phenomena associated with granuloma growth and demise is presented in Shaw and Kent (1999).

HOST SPECIFICITY OF THE XENOMA-FORMING FISH MICROSPORIDIA

Non-xenoma-forming microsporidian species often have a low degree of host specificity. Thus *Pleistophora hyphessobryconis* Schäperclaus, 1941 infects over 18 host species (Lom and Dyková 1992). One might presume that the degree of close co-evolution that is required to achieve the intricate symbiotic relationship between the fish and its parasite, reflected in xenoma formation, would preclude a broad host range. It is not so. *Glugea stephani* has been found in nine different species of flatfish and *Loma salmonae* infects nine different species of salmonids. *Glugea anomala* was first reported in *Gasterosteus aculeatus* and *Pungitius pungitius*; morphologically indistinguishable microsporidian populations have been found in eight species of the family Cyprinodontidae (Dyková and Lom, unpublished). Molecular analysis of the SSU rDNA of *G. anomala* from the stickleback and cyprinodontids revealed only a slight degree of difference below the species level (Frank Nilsen, pers. comm.). In addition, *G. stephani* and *G. atherinae* have been found to be identical with *G. anomala* (Pomport-Castillon et al. 1998) according to SSU rDNA analysis. In addition to the type host *Psetta maxima* (Pleuronectiformes, Scophthalmidae), *Tetramicra brevifilum* has been found also in *Lophius budegassa* (Lophiiformes, Lophiidae). *Ichthyosporidium giganteum* has been found in *Leiostomus xanthurus* (Perciformes, Sciaenidae) in addition to *Symphodus melops* (Perciformes, Labridae). All this demonstrates that xenomic microsporidia are able to form elaborate xenomas across widely different host taxons. It also shows clearly the problems of morphological taxonomy of microsporidia and the existence of an intraspecies polymorphism associated with a particular host. This has been again confirmed by the findings of Freeman et al. (2004) that *Spraguea lophii* populations in species of the genus *Lophius*, other than *L. piscatorius* and *L. budegassa*, may not display spore dimorphism ("nosema" and "nosemoides" type of spores) as found in the type host. Further studies on the host specificity and intraspecific variation of xenoma-forming microsporidia is warranted.

TOPICS FOR FUTURE RESEARCH

It is known that even in cells of human tissues, mainly myocard and muscles, there is a plethora of agents, which can induce cell hypertrophy, including various chemicals and products of cells of the organism

itself. It is also known that cell hypertrophy is one of the adaptational responses to cell injury (through viral or rickettsial infection, physical, chemical or mechanical factors), as an adaptation to heightened demands. These are, however, by no means so elaborate hypertrophies as encountered in microsporidian xenomas. Might there be an inducing factor common to these hypertrophies and to the intricate structures of xenomas? Also, might there be xenoma-inducing agents common in microsporidia infecting various fish and other hosts? Closely related to these questions might be investigation into the immunomodulation potential of the xenoma in the course of its development.

In most of the microsporidian species, a really detailed knowledge of the course of infection is still miss-

ing. Precise site of the portal of infection, first-station cells, transport cells, transformation of the original cell cytoskeleton, target cells, exact site of xenoma formation, duration of separate stages of development, way of spreading in the host organism, autoinfection and pathogenicity still await a due scrutiny. Development of *in vitro* culture techniques for xenoma-inducing microsporidia may help in disclosing relevant characters of xenoma formation.

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